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Performance of variable climate waste stabilisation pond systems during the critical spring warm-up period

by

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This thesis is dedicated to my Nan

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS
SCHOOL OF CIVIL ENGINEERING & THE ENVIRONMENT

Doctor of Philosophy

PERFORMANCE OF VARIABLE CLIMATE WASTE STABILISATION POND SYSTEMS DURING THE CRITICAL SPRING WARM-UP PERIOD

by Caroline Whalley

The research investigated some of the factors influencing the rate of stabilisation of wastewater in the spring period in variable climate waste stabilisation ponds (WSP): in particular, the potential for bringing forward the discharge date by optimising storage capacity and dilution. Experiments consisted of batch-fed systems under both controlled and natural conditions at the University of Southampton, UK; semi-continuous fed units in Almaty, Kazakhstan; and a trial WSP at Lockerley, Hampshire, UK.

Batch-fed experiments revealed a three-fold reduction in BOD load produced a three-fold increase in overall BOD removal rate. Significance of dilution in improving overall removal rates with changing COD concentration increased when light availability was reduced as the photosynthetic process was adversely affected at higher initial loads. The onset of an algal bloom and recovery to net oxygen production were delayed by up to 16 days with the increase in COD, leading to odorous conditions for longer.

Semi-continuous fed 780-l tanks were operated as maturation ponds with storage dates from 1 December-1 May and 1 January-1 June. Both appeared to reach summer steady-state COD values of 25-40 mg l^{-1} well before their nominal discharge dates, with little difference between them. Five tanks were operated at HRT of 15-60 days and surface loading rates of 33-133 kg BOD ha^{-1} d^{-1}. No long-term adverse effects of reducing the HRT were shown though delays of 4-6 weeks in the rise in both COD and nutrient removal efficiency were apparent in the range 15-20 days. Separate dilution experiments in 25-l units which included a period of ice cover supported findings of the 780-l tanks whereby an earlier improvement in removal efficiency is possible when the starting load is reduced. Differences observed in the bucket experiments that experienced a period of ice cover and those conducted under a period of 'open water' suggest improvement possibilities through the dilution of the winter load.

Lockerley pond system demonstrated that intermittent discharge for seasonal communities could provide a cost-effective and reliable alternative to wastewater treatment in a UK climate. Both modes of operation were reasonably successful; the long retention time in Phase 1 produced suitable effluent quality by November; in Phase 2, BOD and suspended solids were able to meet discharge requirements throughout summer but further treatment is required for nutrients to meet discharge standards.

Results indicated that there may be scope for alternative operating protocols designed to maximise performance and economic potential of variable climate pond systems with respect to making wastewater available early in the spring.

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NOTATION

A	pond area
b	beam attenuation coefficient
BOD	biochemical oxygen demand
BOD_5	biochemical oxygen demand (5 day)
BOD_7	biochemical oxygen demand (7 day)
c	beam attenuation coefficient
CH_4	methane
cm	centimetres
CO_2	carbon dioxide
COD	chemical oxygen demand
d	day
D	pond depth
DO	dissolved oxygen
FP	facultative pond
g	grams
h	hour
ha	hectare
HRT	hydraulic retention time
k	irradiance attenuation coefficient
k	kelvin
K_1	reaction rate constant
kg	kilograms
l	litres
L	ultimate BOD
m	metres
mg	milligrams
MJ	megajoules
ml	millilitres
mm	millimetres
mV	millivolts
μ	apparent net growth
µg	micrograms
µm	micrometre
µmol	micromoles
n	sample size
nm	nanometres
N	nitrogen
N_2	molecular nitrogen
O_2	oxygen
°C	degrees celsius
°E	degrees east
°N	degrees north
°W	degrees west
Q	wastewater flow
s	seconds
SS	suspended solids
SMP	storage maturation pond
sqm	square metre
UK	United Kingdom

US	United States
V	pond volume
W	watts
WSP	waste stabilisation pond/s
y_{BD}	visual clarity
z_{eu}	euphotic depth

"Just because something doesn't do what you planned it to do doesn't mean it's useless"

THOMAS EDISON

1 INTRODUCTION

1.1 Background

Waste stabilisation ponds (WSP) are a well-established method of biological wastewater treatment. Wherever suitable land is available at low cost, they are usually significantly cheaper than other treatment processes and can produce high quality effluents. WSP can provide either full or partial treatment of domestic sewage, and are widely used in both developed and developing countries. Treatment arises solely from the natural processes of biological purification that occur in any water body, and no external energy other than that derived from sunlight is required for their operation (Tilsworth and Smith, 1984).

1.2 Sewage treatment in variable climate pond systems

WSP are often the wastewater treatment process of first choice in warm climates, but can also be an appropriate and effective means of treatment in other parts of the world. Ponds in these regions may show significant variations in effluent quality through the year, due primarily to seasonal changes. In continental regions, the difference between average summer and winter temperatures may be as much as 40°C, and ponds have two distinct operating modes: ice-covered in winter and ice-free in summer (Prince *et al.*, 1995b). Conditions of this type occur across Siberia and central Asia and much of North America, including Canada and the northern states of the US. While continental regions provide the most extreme example, similar effects are also apparent in temperate areas like the UK and northern Europe, where different processes are dominant in the pond in winter and midsummer leading to variations in effluent quality. For the purpose of this research, climates where seasonal factors induce significant variations in pond performance are collectively termed 'variable climates'.

The pond types used in WSP systems in variable climates are essentially the same as in tropical areas: one common classification is anaerobic, facultative and maturation ponds. The main difference from warm climate systems is in the discharge mode. Most warm climate WSP operate as continuous discharge systems, where treated effluent discharges into a watercourse all year round at a rate dependent mainly on the inflow. In variable climates, intermittent discharge systems are common, in which the wastewater is retained for long periods in a storage/maturation pond and is released either once or twice a year, often over a short period in spring and/or autumn. Some of the advantages and disadvantages of pond systems in variable climates are summarised in Table 1.1.

Table 1.1 Advantages and disadvantages of ponds in variable climates

Advantages	Disadvantages
• Low operating costs	• Extensive land requirement
• Normally lower capital costs compared to other methods	• Lack of control of process
• Easy to maintain and operate	• Odours, especially in spring*
• Minimal requirement for mechanical equipment	• Excessive suspended solids in the effluent during periods of high algal growth *
• Able to achieve primary or secondary treatment objectives, including natural disinfection	• Performance susceptible to seasonal climatic variations *
• Wide fluctuations in influent quality/quantity do not impact treatment performance as severely as in other systems (* true for all WSP but particularly for long-storage intermittent discharge systems)	
• May have periods of exceptionally high effluent quality at certain times of year e.g. before the onset of winter*	

NB. Unmarked factors apply to most WSP systems; those marked with an asterisk * are particularly relevant to variable climates

In all variable climate regions, lower temperatures and light intensities in winter and early spring produce a reduction in biological/photosynthetic activity (Banks *et al.*, 2003). Where ponds experience freezing, light penetration is further reduced by ice and snow cover. In extreme cases, ponds may have ice cover for several months of the year which can lead to anaerobic conditions in the entire water column. Under these conditions the pond will develop an over-winter accumulation of pollutants that, following ice-thaw, will produce a spring peak, possibly accompanied by objectionable odours. It has been suggested that a key factor which determines the performance of a pond with pollutants accumulated after winter storage is the size of the pollutant peak at the time the ice melts. For effective operation this peak must be rapidly reduced to a low level (Heaven *et al.*, in review).

It is possible that innovative operating protocols could be designed that would improve pond performance in spring and allow the wastewater to meet a standard suitable for discharge earlier in the year, thus reducing storage costs and increasing the potential for economic reuse. Continental climates, where the effects of seasonal change on pond performance are most acute, are also in general relatively dry, making water a precious resource. The possibility of wastewater reuse may therefore be a significant factor in choice of technology and design, making this an important area for further research.

1.3 Research aims

The general aim of this research is to contribute to an improved understanding of the behaviour of WSP in variable climates where there are significant changes in treatment performance and effluent quality throughout the year. The specific aim was to investigate how a range of operational and environmental factors can affect the performance of the system during transient conditions, particularly the spring warm-up period.

The output from this work could then be used in the design of refined operating protocols to improve pond performance in variable climates.

1.4 Objectives

In order to achieve the research aims, controlled laboratory experiments and pilot-scale trials were used to derive data on system performance under a range of environmental and operational conditions, with the following objectives:

- To determine the effects of dilution and load on the rate of removal of the pollutant load under controlled and natural conditions.
- To evaluate the effect of environmental conditions on the significance of dilution and load in improving pollutant removal rates.
- To use a number of parameters, including the oxygen balance and treatment efficiency to evaluate system performance.

1.5 The thesis

The thesis is arranged as follows:

Chapter 2 contains a literature review of the theory of WSP design and treatment, focusing on pond systems which are subject to significant seasonal variation in temperature and other climate-related parameters. The key factors involved in reducing the spring pollutant peak are discussed and their relative importance is considered. As the algal population plays a key role in determining pond performance, relevant research on algal behaviour at low temperature and different light intensities is evaluated.

Chapter 3 summarises the materials and methods relevant to all experiments.

Chapter 4 presents the methods and results of work aimed at developing a synthetic wastewater suitable as feed for the experimental WSP and for general laboratory use. An extensive range of parameters were evaluated against the expected values for domestic sewage and against two existing synthetic wastewaters.

Chapter 5 presents work with associated methods carried out using batch-fed WSP systems based at the University of Southampton, UK. The work consisted of laboratory based one litre flask experiments and the use of two 1000 litre tanks all under controlled conditions. Temperature was adjusted in the tanks to simulate spring warm-up in a continental climate and were operated under different conditions of organic load and light intensity. Two sets of outdoor experiments were also carried out to utilise natural conditions.

Chapter 6 presents the methods and work carried out using semi-continuous fed 780 l tanks and 25 l units in Almaty, Kazakhstan. The research focused on the development of a design and operational concept to make accumulated water available for discharge as early in the spring as possible. The concept involved using dilution as a mechanism to reduce the initial load on the storage maturation pond, and testing reduced hydraulic retention times in the facultative pond.

Chapter 7 reports on the monitoring of a trial facultative WSP based at Lockerley Water Farm, UK operated under two different regimes over consecutive years. This work provides an example of a temperate pond that is subject to milder seasonal changes than more extreme climates but still has a varying wastewater quality due to changes in loading throughout the year.

Chapter 8 presents an overall discussion of the findings.

Chapter 9 presents conclusions followed by recommendations for further work.

2 LITERATURE REVIEW

2.1 Waste stabilisation ponds

2.1.1 Introduction

Waste stabilisation ponds (WSP) are constructed impoundments where sewage is treated and discharged after a specified retention period. Treatment occurs as a result of natural purification processes which take place at their own natural rates (Abis and Mara, 2004). Although WSP are simply constructed, their effectiveness depends upon a complex interaction of physical, chemical and biological processes. Treatment is optimised by selecting appropriate organic loadings, retention periods and pond depths, to promote the maximum growth of organisms beneficial to the treatment process (Mara *et al.*, 1992).

The main advantage of these systems is their simplicity to build and operate. Although these systems are often termed 'low tech', the mechanisms involved in the way they treat and stabilise pollution are as numerous and complex as those in conventional 'concrete and steel' technologies (Shilton, 2001).

WSP rely on heterotrophic bacteria in a mutualistic relationship with photosynthetic algae. Organic matter in the wastewater is oxidised by bacteria using oxygen produced by algal growth, releasing nutrients and CO_2, which in turn are assimilated by algae to produce biomass. The algae are then settled out or removed by physical or chemical means prior to discharge. In terms of nutrient levels, nutrient transformations, algal growth and taxa, stabilisation ponds resemble highly eutrophic lakes (Middlebrooks *et al.*, 1983).

Used on their own or in series they can be operated as either anaerobic or aerobic based systems that can be loosely classified into three groups: anaerobic ponds and lagoons, oxidation ponds and aeration lagoons (Metcalf and Eddy, 2002). Ponds can also be classified by the treatment objective: primary, secondary, tertiary, maturation, or polishing; or even by their hydraulic regime: continuous discharge, intermittent discharge, total containment. There are a number of variations in the way ponds are designed and applied to the task of wastewater treatment, though they are normally arranged in a series with an anaerobic pond preceding a facultative pond that feeds into one or more maturation ponds.

2.1.2 Anaerobic ponds

Anaerobic ponds are used for the preliminary treatment of strong organic wastes. They are devoid of oxygen and designed to reduce the wastewater biochemical oxygen demand (BOD) and solids concentrations by sedimentation and anaerobic digestion. Anaerobic digestion principally occurs in the sludge at the bottom of the pond, converting the organic load to methane and carbon dioxide and releasing some soluble by-products into the water column (e.g. organic acids, ammonia) (Abis, 2002). It is thought that the waste gases carry anaerobic bacteria into the water column, thus perpetuating anaerobic digestion throughout the pond. At temperatures below 15°C, the digestion processes slow down and the dominant process is thought to be sedimentation (Pearson, 1990). The depth of anaerobic ponds is in the range 2-5 metres deep and the hydraulic retention time (HRT) depends on the volumetric BOD loading required (g m^{-3} d^{-1}) for the climate and can be up to 20 days (WEF, 1998). Anaerobic ponds work extremely well in warm climates: for example a properly designed pond will achieve around 60% BOD removal at 20°C and over 70% at 25°C and above (Pena and Mara, 2004). They also reduce the problems associated with sludge accumulation and solids feedback in a following facultative pond. The main disadvantages of anaerobic ponds are the risk of odour and the increase in ammonia and sulphide concentrations caused by the anaerobic processes (Pearson *et al.*, 1987).

2.1.3 Storage maturation ponds

Maturation ponds are primarily designed as a tertiary treatment process for improving the effluent quality from secondary biological processes. Their primary function is to remove pathogens, but they can also achieve significant nutrient removal (Mara *et al.*, 1992). Total nitrogen removal in a whole pond system is often above 80% and ammonia removal is generally more than 90% (though these figures depend on the number of maturation ponds included in the pond system). Phosphorus removal is somewhat lower (usually about 50%) (Pena and Mara, 2004). The organic loading to the pond is low, as a result of substrate removal in previous treatment ponds. They may be shallower than facultative ponds at 1.0 to 1.5 metres in depth to allow light penetration to the bottom and aerobic conditions throughout the whole depth. Algal populations are much more diverse than that in facultative ponds as algal diversity tends to increase from pond to pond along the series. The main mechanisms of faecal bacterial and viral decay are driven by the algal activity along with photo-oxidation. The number of maturation ponds required is determined by the retention time necessary to achieve a specified effluent pathogen concentration. Retention times are generally long, usually 12 months in colder regions until the appropriate time for discharge, ideally in the autumn. Effluents meeting all regulatory requirements are discharged to receiving waters during periods of no ice cover.

2.1.4 Facultative ponds

Facultative ponds are the most common type of pond and perform both primary and secondary treatment. A primary pond receives raw wastewater, while a secondary pond follows primary treatment i.e. an anaerobic pond and so has already undergone some form of prior treatment. Facultative ponds are characterised by a permanent anaerobic layer at the bottom where the sludge accumulates and an aerobic layer on the surface oxygenated by the photosynthetic action of algae and wind aeration. The treatment effected by the facultative pond results from the complex mutualistic association between algae in the upper euphotic zone and bacteria in the lower layers that bring about an ecological pattern different from that of pure culture behaviour in which the respiratory oxygen requirements of the pond is met by photosynthetic oxygen (Kayombo *et al.*, 2003). In the water column of a facultative pond aerobic and anaerobic conditions may alternate. The rate at which the pond fluctuates between these conditions depends on the balance between the respiratory and photosynthetic rates, which in turn depend on temperature, light availability, presence of algae and bacteria and the availability of nutrients.

Facultative ponds must be relatively shallow in order to have a sufficient area-to-volume ratio to enable good algal growth and therefore these ponds are designed on the basis of surface organic loading. Mara *et al.* (1992) stated that the surface organic loading on a facultative pond in a cold climate should not exceed 100 kg ha^{-1} d^{-1}, so that at depths of 1.5-2.0 m, retention periods are 20-50 days depending on strength.

BOD removal in primary facultative ponds is about 70% on unfiltered samples and more than 90% on filtered samples (filtering the sample before BOD analysis excludes the BOD due to the algae present in the sample). Over 90% of suspended solids leaving the facultative pond during the summer can be due to algae. Mara *et al.* (1992) reported that the algal contribution to suspended solids concentration can reach 40-100 mg^{-1} in temperate climates.

2.1.4.1 Algae

The algae that tend to predominate in WSP are the motile genera as these can optimise their vertical position in the water column in relation to incident light intensity and temperature than non-motile forms (Table 2.1). The concentration of algae in a facultative pond, as measured by the chlorophyll-a concentration, depends on loading and temperature, but is usually in the range of 500-2000 μg chlorophyll-a per litre (Mara and Pearson, 1987). Data collected in Brazil and Portugal suggests that a consistent mean water column chlorophyll-a concentration below 300 μg^{-1} indicates an unstable system which may be heading towards failure (Pearson, 1996).

Table 2.1 Examples of algal genera present in waste stabilisation ponds (Mara and Pearson, 1987)

Algae	Facultative Ponds	Maturation Ponds
Euglenophyta		
Euglena (motile)	+	+
Phacus (motile)	+	+
Chlorophyta		
Chlamydomonas (motile)	+	+
Chlorogonium	+	+
Eudorina	+	+
Pandorina	+	+
Pyrobotrys	+	+
Ankistrodesmus	-	+
Chlorella (non-motile)	+	+
Micractinium	-	+
Scenedesmus (non-motile)	-	+
Selenastrum	-	+
Carteria	+	+
Coelastrum	-	+
Dictyosphaerium	-	+
Oocystis	-	+
Rhodomonas	-	+
Volvox	+	-
Chrysophyta		
Navicula	+	+
Cyclotella	-	+
Cyanophyta		
Oscillatoria	+	+
Anabaena	+	+

+ = present; - = absent

Most WSP are designed on the basis of BOD loading or pathogen removal rates, taking the algal activity for granted. Algae play several key roles: providing oxygen for efficient bacterial degradation of organic load and minimisation of odour, and providing conditions for enhanced pathogen reduction and ammonia nitrogen removal. Poor performance or pond failure can occur if the algal population is insufficient. The ability to predict and ultimately control algal concentration would therefore be a useful tool for designers and operators (Weatherell *et al.*, 2003).

Pond failure occurs when the reoxygenation processes are insufficient to generate aerobic conditions during daylight hours. Failure is indicated by changes in biology: the first stages are accompanied by a reduction in algal diversity and eventually only motile algae such as *Chlamdymonas* and *Euglena* may remain (Almasi and Pescod, 1996). When the algae disappear completely, anoxic or anaerobic conditions prevail and the purple photosynthetic bacteria become visible at the surface (Li *et al.*, 1991; Lai and Lam, 1997). The pond may become fully anaerobic, indicated by a black colouration, elemental sulphur deposits (from the oxidation of hydrogen sulphide by anaerobic photosynthetic bacteria), and possibly hydrogen sulphide odour from anaerobic sulphate reduction (Abis, 2002).

Seasons have a strong influence on algal populations in temperate climates; the lower temperatures and shorter day length experienced in winter may be insufficient to support an algal population at all (Cauchie *et al.*, 2000).

2.1.4.2 Bacteria

Bacteria are the primary degraders of organic wastes. The bacteria found in the aerobic layer of WSP are typically the same type as those found in other aerobic forms of sewage treatment. Most obtain their energy for growth by the oxidation of organic compounds, but a few are able to use inorganic compounds for this purpose. Most heterotrophic bacteria have a wide range in environmental tolerance and can function effectively in BOD removal over a wide range in pH and temperature. In temperate climates, mesophilic (optimum temperatures between 20-45°C) and psychrophilic (optimum temperature below 20°C) bacteria dominate in summer and psychrophilic and psychrotrophic (grow below 0°C) bacteria dominate in winter (Townshend and Knoll, 1987). Aerobic BOD removal generally proceeds well from pH 6.5 to 9.0 and at temperatures from 3-4°C to 60-70°C (mesophilic bacteria are replaced by thermophilic bacteria at temperatures above 35°C). BOD removal generally declines rapidly below 3-

4°C and ceases at 1-2°C. Anaerobic, heterotrophic bacteria that commonly occur in ponds are involved in methane formation (acid-forming and methane bacteria) and in sulphate reduction (sulphate-reducing bacteria) which is a major cause of odours in ponds.

In general, microbes such as bacteria and viruses are the most resistant organisms to freezing. Torrella *et al.* (2003) examined the survival dynamics of bacterial and viral indicators of pollution present in raw wastewater under freezing stress for periods of up to two months. The samples were kept at temperatures of -14°C. On freezing of the wastewater, it was found that presumptive, total and faecal coliforms showed a first rapid phase (days) of inactivation followed by a slower second phase (up to four weeks) and then stabilisation at between 1-10% of the initial population size.

2.2 Ponds in variable climates

Pond treatment in regions where seasonal ice cover occurs requires special operational strategies to avoid the discharge of poor-quality effluents to receiving waters with low assimilative capacities (Prince *et al.* 1995b). The factors that affect pond processes can be classified into two categories: factors related to climatic conditions and factors that are controlled by the design or operational strategy of pond systems (Prince *et al.* 1995b). Both of these factors are discussed, in particular, how operating protocols can be used to optimise pond performance in extreme climates. These climates can be divided into cold, continental and temperate but for the purpose of this research these types of climates are collectively termed 'variable climates'. The Köppen climate classification is one of the most widely used climate classification systems and has been recently updated by Kottek *et al.* (2006). It combines average annual and monthly temperatures and precipitation, and the seasonality of precipitation. In this classification, the term 'variable climate' covers areas referred to as 'group c': temperate/mesothermal climates and 'group d': continental/microthermal climates. The former climates have an average temperature above 10°C (50°F) in their warmest months, and a coldest month average between –3°C and 18°C, while the latter climates have an average temperature above 10°C in their warmest months, and a coldest month average below –3°C. For much of this discussion, climatic conditions found in continental regions will be used and although it is recognised that there are significant differences between these and cold regions, a common theme exists whereby seasonal factors induce significant variations in pond performance and final effluent quality.

A typical variable climate WSP system consists of three types of pond in series: anaerobic, facultative and storage/maturation. Most warm climate ponds operate as continuous discharge systems, however in variable climate systems, intermittent discharge is more common. There is a single discharge in the autumn as the temperature cools causing the algae to settle out and the water body becomes free of suspended solids. During the winter, wastewater continues to flow through the anaerobic pond where solids settle out and into the facultative pond. The cleaner water from the facultative pond is then diluted out into the storage pond (Heaven and Banks, 2005). In variable climates, WSP can experience two distinct seasons: winter and ice free (Prince *et al.*, 1995a). Ice cover isolates the water surface from the mixing forces of the wind. Ice formation occurs at 0°C at the surface, but the temperature in the rest of the water body is generally considered to remain between 0 and 4°C, although these temperatures may be affected by the concentration of impurities. The winter season is usually characterised by the predominance of anaerobic conditions in the pond system. In spring, when the ice melts, the organic load in the system is at its highest and dissolved oxygen levels are at a minimum whilst the summer is characterised by the establishment of steady state conditions in the pond.

Heaven and Banks (2005) qualitatively described the series of events in a variable climate facultative pond over a 12-month period (Figure 2.1). The fate of key parameters is divided into three phases: 'accumulation' where BOD load is added but not destroyed; 'non steady state' where BOD load is progressively destroyed as temperature and microbial numbers (algal and bacterial) increase; and 'steady state' where BOD is consistently low, microbial numbers are in equilibrium, and summer temperatures are more or less constant.

Research on extreme climate WSP has been carried out predominantly in Canada and Alaska; (Dawson and Grainge, 1969; Schneiter *et al.*, 1983; Tilsworth and Smith, 1984; Henry and Prasad, 1986; Heinke *et al.*, 1991; Prince *et al.*, 1995b). Heaven *et al.* (2003) examined the construction and operational aspects of extreme climate WSP, comparing North American, North European and Russian standards. It was concluded that an exchange of design models and operational experience between regions of similar climate would be likely to lead to improved performance and uptake of WSP in all areas. However many uncertainties remain concerning processes in extreme climate ponds; design methods are not well defined and this would suggest that a wide variability in performance and efficiency exists. Designs are normally a combination of several factors, including (i) organic loading, (ii) retention time, (iii) depth, (iv) temperature and (v) light intensity. Design equations that have been developed for facultative ponds including those of Gloyna (1976), Marais and Shaw (1961) and Thirumurthi (1974) and are for the most part not applicable to cold regions because of the temperature limitations and light intensity restrictions (Tilsworth and Smith, 1984).

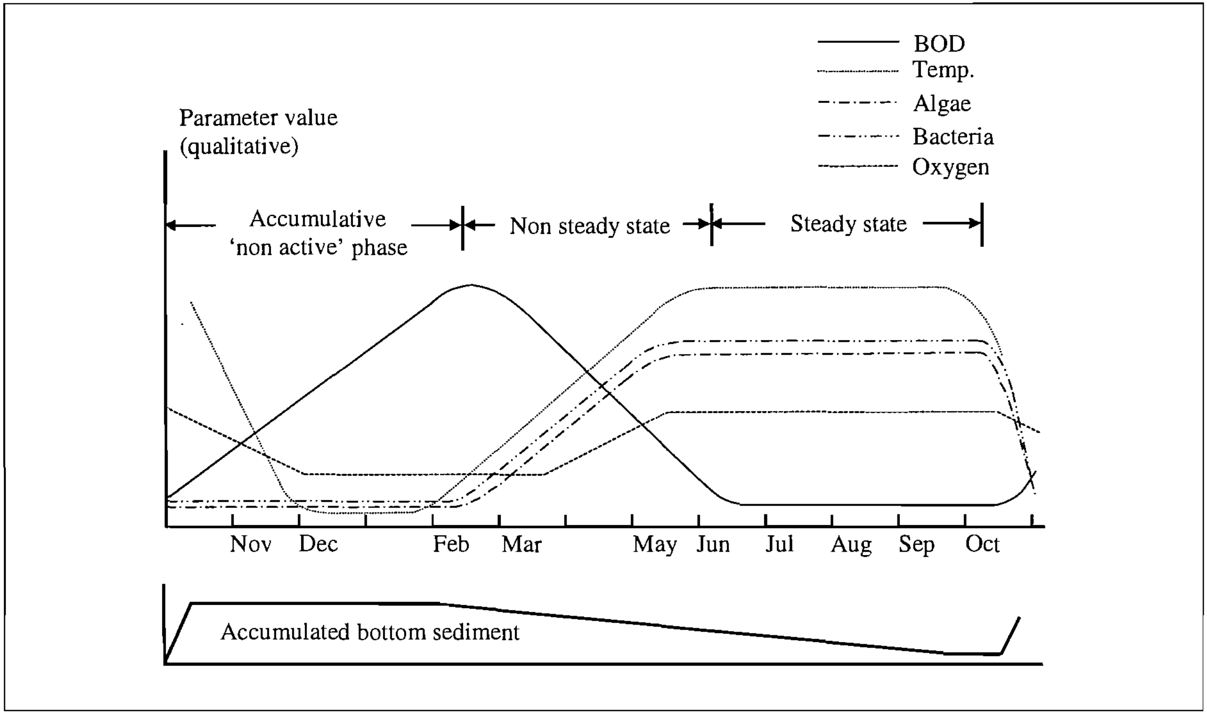


Figure 2.1 Seasonal fluctuations in key parameters in a variable climate pond in the northern hemisphere working in batch mode with a once-yearly discharge (Heaven and Banks, 2005)

Prince *et al.* (1995b) provided a detailed evaluation of pond performance in a region that experiences seasonal ice cover and determined the design factors that influence the performance. This was achieved by analysing a database of historical effluent quality from operating facilities in Alberta, Canada. The samples were collected and analysed and a total of 28 parameters were listed in the database. The database of pond effluent indicated that season of discharge, storage time and pond configurations are important factors in determining effluent quality. The analysis of the data demonstrated that pond facilities consisting of four anaerobic ponds, one facultative and one storage pond (4S, 1T, 1L) (S = sedimentation, T = treatment, L = lagoon storage) with an autumn discharge and 12 months storage is superior because of the longer ice-free treatment period preceding discharge and traditional rapid drop in temperature that kills and removes most of the algae. They concluded that properly designed intermittent discharge ponds are capable of producing effluent quality that is superior to the effluent produced by mechanical treatment facilities in the same region.

Banks *et al.* (2003) considered the factors which determine oxygen balance in extreme climate WSP during the critical spring warm-up period. The paper also describes the operation of a typical continental climate WSP and the events leading to a balanced steady state system as spring develops into summer. A mathematical model was used to simulate conditions within a semi-continuously fed experimental pond over the transient period. Bacterial growth was simulated by a Monod kinetic model in which growth rate depends on initial substrate concentration. However, values for the initial numbers of both bacteria and algae were found to be difficult to ascertain as the viability of organisms remaining after ice formation is as yet unknown. The model was found to agree with existing data and could be used to simulate parameters in experimental ponds recovering from ice cover. Banks *et al.* (2003) stated that relatively little is known about the survival of microorganisms in WSP during the period of ice cover. The model depicts what might be happening, but it is clearly necessary to determine through experimental work the viability of these organisms after exposure to low temperatures.

Mackenthun and McNabb (1961) investigated the behaviour of ponds in Wisconsin, USA, an area that experiences extreme climates. Pond performance was measured through sampling at regular intervals during 24 hour periods of normal operation when ice covered the pond and again in the spring when the pond was ice free. They found that light was the principal factor causing vertical differences in the rate of oxygen production and that this decreased with depth. They also noted that ice cover prevented the occurrence of surface aeration, which would indicate that any oxygen present at these times originated solely through photosynthesis. Sub-ice photosynthetic aeration was found in the Junction City WSP system, Wisconsin, with substantial quantities of dissolved oxygen confined to the narrow stratum just below the ice. Concentration increased during daylight hours when the percentage of light reached 4-7%. Poor light intensity and low water temperature were expected to have a limiting effect on photosynthesis, but were not completely inhibitory. The study provided an opportunity to observe the effects of pond loading on the stabilisation mechanism from winter to summer and an evaluation of pond recovery after winter stagnation. The results are also of significance as it was clear that formation of ice over the pond surface does not kill all micro-organisms and there is continuing microbial activity within both the ice and unfrozen water mass.

2.3 Variable climate WSP during the spring warm-up period

The critical phase in variable climate WSP systems is in spring during the 'non steady state' period because the initial organic load is high, the oxygen production potential (algal numbers) is at its lowest, and the potential for bacterial growth (aerobically or anaerobically) is high. If the initial load is such that the rate of oxygen depletion is greater than the rate of replenishment then the system will be predominantly anaerobic during this phase, leading to odours and lower rates of reaction. If the initial load and the continuing input are low, then the rate of oxygen replenishment may exceed the oxygen demand and the system will become oxygen saturated. In terms of treatment, this latter state does no harm but it does represent process inefficiency as the system is larger than it needs to be (Heaven and Banks, 2005).

Freezing temperatures and minimal light penetration reduce the levels of microbial and photosynthetic activity, leading to an accumulation of pollutants that peaks around the time of ice thaw. Algal cells that are respiring without light also augment the BOD in the water column. Heaven *et al.* (in review) reported on the changes that take place in an extreme climate WSP during the critical spring period where there is a transition between ice cover and free water. At each of the loading rates tested, the experimental ponds demonstrated the capacity to produce a rapid reduction in high chemical oxygen demand (COD), BOD and nutrient concentrations over a period of a few weeks in spring, followed by sustained low levels throughout the summer period. This recovery from winter conditions is due to a variety of factors promoting the rapid development of a spring bloom, followed by the hot summer typical of a continental climate. The timing of this will depend on local climatic conditions, but monitoring of pilot-scale facultative ponds suggests that in south-east Kazakhstan this usually occurs by June or July. This is too late in the growing season to maximise the usefulness of the water for irrigation or other purposes. Ideally, water availability in late April or early May best matches demand from the agricultural sector.

If the effluent is intended for use in irrigation then the potential for continuous discharge from early summer is an advantage, and BOD and nutrients are unlikely to be a problem at the levels observed. The key parameter in this case is the level of pathogens present. There is considerable evidence indicating the potential for extended survival of pathogens in cold conditions in WSP (Torrella *et al.*, 2003) but unfortunately little guidance on appropriate design parameters. In continental climates with their typically low precipitation, however, the possibility of wastewater reuse may be an important factor in choice of technology and design, making this an important area for further research. In Canada and the northern United States the recommended design and operating protocol for a pond system includes one or more facultative ponds, followed by a storage/maturation pond with a 12-month retention period, which is discharged once a year in autumn. From the viewpoint of maximising environmental protection this system has many attractive features. It does not, however, consider water reuse as part of the design strategy and may therefore not be optimal for more sharply continental climatic conditions. In large areas of central Asia, for example, the climate is characterised by a short transition between cold winters and hot summers, and very low rainfall. In this situation WSP could be used to provide water for agriculture or river and aquifer replenishment, provided that the required quality standards can be guaranteed (Heaven *et al.* 2005).

The possibility of innovative operating protocols to optimise spring performance in ponds has already been recommended by Heaven *et al.* (in review). They suggested the possibility of using a multi-pond system where there may be benefits from alternating the maturation and facultative ponds: in one year a pond is more heavily loaded and builds up sediments that will release nutrients during the following spring, while in the next it is lightly loaded. This could aim to increase the overall loading rate and efficiency of the treatment system. By replacing the single storage/maturation pond with two ponds in parallel that store and discharge water in alternate years, effluent that is treated to a high standard by the end of the summer can be stored over winter without any further addition of incoming wastewater. Although an extra storage/maturation pond is needed, the overall costs would be less since the ponds could handle considerably increased hydraulic and organic loadings. The stored water could become available for irrigation from early spring, thus maximising its economic usefulness.

2.4 Reduction of the spring pollutant peak

A number of factors will affect how rapidly the pollutant peak in spring is reduced. These include temperature, light, organic loading, nutrient status, size and type/condition of algal inoculum, many of which are interdependent. In general, light and temperature are considered to be the major factors determining yield of microalgae biomass (Holmgren, 1984) and numerous studies have been carried out to measure the response of algae to different temperatures and light. Gronlund *et al.* (2001) however stated that from a cold climate perspective the role of light and temperature in wastewater treatment is not very clear. These parameters are also difficult to control or influence in large-scale outdoor ponds, making them less suitable as a basis for practical engineering interventions. Nutrient status and algal inoculum are also important but as WSP influent contains large amounts of nutrients relative to those found in lakes, streams and oceans, these factors are less likely to be limiting. The factor of main interest to operating protocols is therefore organic loading, although the relevance of each is discussed.

In practice there are only a few ways organic loading can be varied; by altering the influent strength of wastewater by dilution and by changing pond dimensions, i.e. surface area and depth and therefore hydraulic retention time.

2.4.1 Temperature

2.4.1.1 The role of temperature in variable climate WSP

Temperature is highly important in the functioning of WSP because it affects the rate of biochemical degradation. The average temperature, daily fluctuations, and yearly variations all influence the biological, physical and chemical processes in the pond. The temperature-growth range of an alga is important ecologically because it defines the range over which the alga can be metabolically active (Brock, 1991). Algae experience temperature fluctuations of many different types, including diurnal changes, seasonal changes and long-term inter-annual variability associated with natural climatic cycles. Temperature sets an upper limit on the rate of photosynthesis and on the phytoplankton growth rate through its influence on the enzymatic activity of cells. Photosynthesis is suppressed by low temperature, but the decline in photosynthetic rate occurs at lower temperatures than the decline in bacterial growth and metabolism. Thus between +1°C and -1°C, photosynthesis is substantial but rates of bacterial growth and metabolism are low. This is potentially important where water temperature is in that critical range during the spring bloom period when much of the annual primary production occurs (Pomeroy and Deibel, 1986).

Pond water temperatures respond to meteorological conditions which act on a pond through the water surface. In cold regions, heat transfer processes which normally occur through an open water surface are substantially altered by ice and snow covers (Fang and Stefan, 1996). The fraction of incident radiation transmitted through the ice and snow cover depends in part on the depth of these covers and on the albedo of the snow and/or ice (Henneman and Stefan, 1999). The amount of photosynthetically active radiation (400-700 nm) and ultraviolet radiation (280-400 nm) transmitted through the ice will strongly affect the biological activity in and under it. A major requirement for the maintenance of algae under ice is that enough light be able to pass through the ice to fuel algal growth in the water below. For this to be the case snow cover must be thin, since snow shades much more effectively than ice (0.05 m of snow atop one metre of clear ice reduces light transmission by a factor of 50) (Kelley, 1997). Therefore, growth can proceed readily only if snow cover is minimal (Kelley, 1997). This is often the case in large WSP that are swept clear of snow by winds extending over long fetches.

Ice affects phytoplankton communities in various ways. It provides a specific light climate in the water column depending on the thickness and the condition of the ice cover and the presence or absence of snow. Turbulence is markedly reduced, leading to vertical gradients in temperature, oxygen and nutrients. Therefore it is possible that conditions during the winter determine the 'inoculum' of species in spring (Adrian *et al.*, 1999).

In spring, heat is required not only to raise the temperature of snow and ice on the pond to the melting point and to warm up the water but also to effect the change of state of the frozen cover. Adrian *et al.* (1999) found the timing of maximal algal biomass in spring to be positively correlated with the ice duration in shallow, highly eutrophic lakes. Overall, a one day increase in ice duration corresponded to a 0.4-day delay in the maximum total algal biomass. Since ice formation has substantial effects on light, temperature and turbulence gradients in the water column, the starting conditions for the spring succession (i.e. inocula and water temperature) are likely to differ depending on the length of the ice cover period.

2.4.1.2 Effects of freezing on algae

The effects of freezing on the survival of algal cells are important as there are many problems concerned with the ability of algae to survive low temperatures in their natural habitat. Low temperatures and ice cover greatly reduce the metabolic activities of micro-organisms and photosynthetic activities are also reduced, which presents operational problems. It is therefore necessary to consider some of the key work on the viability of algae after exposure to low temperatures. The effects of subzero temperatures and freezing on algae has been the subject of relatively few reports, and the majority of these focus on cryopreservation involving storing the algae at -196°C. A range of freezing protocols have been developed but the more conventional method uses liquid nitrogen at -196°C to complete the freezing process. Ponds in variable climates though are unlikely to reach temperatures much lower than -40°C in the ice layer, and remain close to zero in the unfrozen water. Storage at non-liquid N_2 temperatures has also been investigated. Holm-Hansen (1963) reported viabilities of up to 100% in microalgae frozen to a range of temperatures between -10°C and -70°C. Samples were frozen for only a short time period though, in this case experiencing only a momentary exposure to the end temperature.

Cotelle and Ferard (1996) examined the effects on algae of freezing at different temperatures. Four different temperatures were chosen: 4°C (refrigerator), -20°C or -80°C (freezers) and -196°C (liquid nitrogen system). Different algal parameters were evaluated after a storage time of 7 days. Aliquots of frozen algae were thawed on the day of use at 40°C until ice was not apparent. The study indicated that freezing had no effect on cell concentration as there was no significant difference between the four groups. There was also no significant difference in optical density between the groups corresponding to different temperatures of freezing. However, experiments with growth on solid medium showed an absence of growth at -20°C and -196°C. They concluded that algae frozen at this temperature seems to be the better choice as a cryopreservation treatment, though the results indicated that the different treatments for freezing did give rise to some stresses on algal viability.

Little is known about the temperature growth limitations of algae living in temperate freshwaters during colder seasons. Seaburg and Parker (1983) compared the temperature ranges of growth of algal isolates from temperate habitats in Virginia during both warm and cold seasons to other studies of algae collected from habitats exposed to different temperature regimes. Of the 115 strains of microalgae studied, 63 were isolated from $\leq$ 6°C waters and 52 were isolated from $\geq$ 20°C waters. The coldest test temperature of 2°C inhibited growth of 61% of all isolates indicating that the capability of temperate climate algae to grow at near freezing temperature is not common. However, a much larger percentage of isolates from cold waters were capable of growth at 2°C than those from warm waters (59% vs. 15%). Seaburg *et al.* (1981) isolated 128 strains (4 algal taxa) of Antarctic algae from various habitats to assay them for growth over a temperature range of 2-34°C. Three of the four isolates tested could grow at -1°C, just above the freezing point for the medium. Seaburg and Parker (1983) compared the results from this previous study to the results they obtained from the Virginia algal isolates. It is clear that the

Virginia cold-group isolates were nearly as well adapted to low temperatures as the Antarctic isolates. At very low temperatures only a slightly higher percentage of Antarctic isolates grew than the Virginia cold-group isolates. Thus, both groups from cold habitats grew at low in situ temperatures.

Tang *et al.* (1997) compared polar cyanobacteria and green algae (a Chlorococcalean assemblage) specifically for tertiary wastewater treatment. They reported that cyanobacteria isolated from the Arctic and the Antarctic offer an interesting alternative to temperate algae for wastewater treatment. Although numerous isolates of polar cyanobacteria have an optimal temperature for growth at 15°C or above, they remain active at lower temperatures, thereby extending the operation of the solar bioreactor into early spring and late autumn (Tang *et al.*, 1997). These organisms resist freezing (Davey, 1989) and they can retain a large overwintering biomass, thus maintaining a large inoculum for the next growing season and allowing treatment to recommence once temperature becomes milder in late winter and early spring (Tang *et al.*, 1997). Results showed that polar cyanobacteria are well adapted for wastewater treatment in cold climates because they can grow better and take up nitrogen and phosphorus more efficiently than chlorophytes at low temperature.

Work by Bartosh (2004) showed agreement with earlier work, finding that typical WSP algae can survive, photosynthesise and grow at very low light intensities and temperatures. Experiments on survival showed that a proportion of cells of *Chlorella vulgaris* (Chlorophyta) could survive long periods (22 weeks) of dormancy in complete darkness and low temperatures (+4 and –20°C). Although the work was limited to just two WSP species that are likely to be present in a WSP, it does have significant implications for over-winter survival and recovery in spring. A well-designed WSP is unlikely to freeze completely and there is potential for survival in water close to 0°C; there is also potential for survival within the frozen ice layer. The experimental results indicated that survival in cold water and ice are both possible but are likely to show species differentiation. While light and temperature are particularly difficult environmental parameters to control, it is clear that species can survive in sufficient numbers to provide an inoculum for the spring bloom (Bartosh, 2004).

2.4.2 Light

2.4.2.1 Light penetration

Light penetration is of fundamental importance to the functioning of facultative ponds affecting both pathogen survival (Curtis *et al.*, 1992) and the concentration and productivity of the algal population (Kirk, 1983). Light attenuation influences photosynthetic activity throughout the pond depth. High surface irradiances and temperatures may inhibit photosynthesis and thereby oxygen production. Surface incident irradiance varies seasonally and is further modulated by weather conditions (cloud cover) which are responsible for marked inter-annual variations within each season. Algae present in WSP are exposed to a range of irradiances mediated by attenuation, climate and hydrodynamic conditions. Light-dark cycles are known to occur in pond and lake systems. Light-dark cycles of the order of seconds to minutes can be caused by cloud passage, surface waves, floating macrophytes and edge shadows (Kirk, 1983). Furthermore, the amount of light available for phytoplankton also varies as a function of water transparency (Townsend *et al.*, 1994).

There is only one of two things that can happen to a photon of light in a WSP: it can be scattered or it can be absorbed (Curtis *et al.* 1994). The light absorbtion properties of natural waters are attributable to four components: water, gilvin (dissolved yellow matter or humic substances), algae and tripton (inanimate particulate matter). Algae, being photosynthetic, have large quantities of pigments which also impede light penetration. Curtis *et al.* (1994) stated that in productive waters such as WSP, light absorbance of light by algae may often limit the growth of algae themselves. Light penetration in WSP is dependent on the absorbative properties of the water and changes in turbidity will not influence the optical properties of pond water (Kirk, 1983). Curtis *et al.* (1994) characterised the fundamental aspects of light penetration in WSP. Results showed that the attenuation of light into the ponds was dominated by light absorbtion by gilvin and pond-to-pond variation was mainly attributable to algal biomass. They found that light scattering processes (turbidity) was of no importance.

2.4.2.2 Photosynthesis

Photosynthesis is the process whereby organisms are able to grow utilising the sun's radiant energy to power the fixation of atmospheric CO_2 and subsequently provide the reducing power to convert the CO_2 to organic compounds. Respiration is a physiological process in which organic compounds are oxidised mainly to carbon dioxide and water. In the presence of light, respiration and photosynthesis can occur simultaneously in algae. However, the respiration rate is low compared with the photosynthesis rate, resulting in a net consumption of carbon dioxide and production of oxygen. In the absence of light, algal respiration continues while photosynthesis stops, resulting in a net consumption of oxygen and production of carbon dioxide.

Heyman and Lundgren (1988) stated that there are three different types of dynamics of algae in terms of light and photosynthesis: (i) low light conditions where light is more or less limiting either due to low incident light as in winter or low average light due to deep mixing, (ii) conditions where light is not limiting and the biomass is much lower than the carrying capacity, and (iii) bloom conditions (biomass is close to carrying capacity) where the algal cells are truly nutrient-deficient and/or light-limited due to self-shading. In low light conditions (i) the cells are characterized by high nutrient content (Ahlgren, 1988) and low growth rate. When light is not limiting (ii), it does not seem to have any direct effect on growth and biomass. Instead temperature determines the maximum growth rate and nutrients determine the carrying capacity (Berman and Pollingher, 1974; Kalff and Knoechel, 1978). When the phytoplankton biomass is close to carrying capacity (iii) the growth rate is low (Persson, 1985) because of lack of nutrients or self-shading. If self-shading is determining the biomass, a change in the light climate will have large effects on the biomass itself but light does not seem to have any direct effect (Heyman and Lundgren, 1988). Light is, however, a very important factor for terminating a bloom when, for example, light decreases below the limit for sustaining the biomass (Reynolds, 1984).

Three physiological regions, all of which influence WSP performance, are recognised in the algal photosynthesis/irradiance (P/I) response curve, firstly, where light limited and secondly, light saturated photosynthesis proceeds and thirdly where photoinhibition occurs (Ratchford and Fallowfield, 2003). The light levels required to induce photoinhibition vary with the experimental conditions. The onset of photoinhibition for the green algae that predominate in WSP may occur at irradiances >200 μmol m^{-2} s^{-1}, significantly lower than the incident surface irradiances of 1,500-2,000 μmol m^{-2} s^{-1} typical for many WSP (Ratchford and Fallowfield, 2003). Environmental stresses may sensitise the photosynthetic apparatus to photoinhibition (Falk *et al.*, 1990). This is evident for example under nutrient (Prezelin *et al.*, 1986) and low temperature stress (Van Hasselt and Van Berlo, 1980). In the most favourable conditions algae are able to utilise up to 2.6% (1.5% average) of sunlight during summer months (Goldman, 1979).

During winter months the efficiency of light utilisation is higher, which may be explained by the absence of photoinhibition periods.

As well as changing light intensity, algae in most natural environments also experience significant seasonal changes in daylength. Experimental studies show that daylength affects growth and photosynthesis of many groups of algae and these effects may be species-specific (Litchman *et al.*, 2003). Shorter daylength has shown to decrease growth rates of cyanobacteria and diatoms (Foy and Gibson, 1993). Litchman *et al.* (2003) measured the rates of growth and photosynthesis of a freshwater diatom, green alga and cyanobacterium under contrasting daylengths (18:6h versus 6:18h light-dark cycles) and phosphorus regimes. The growth rates of all three species declined under short daylength. Phosphorus limitation decreased the maximum rates of photosynthesis in all three species, though the green alga was the most sensitive to phosphorus limitation and this was more pronounced under longer daylength. Under the conditions of shorter daylength and sufficient phosphorus, the green algae showed an increase in chlorophyll-a concentration. In spring therefore, the effects of phosphorus limitation would be less pronounced due to both the shorter daylength and the sufficient nutrients present in the ponds post winter freeze. A study by Humphrey (1979) found that when eight species of marine unicellular algae were grown under a 12:12h light-dark regime, they had a higher photosynthetic rate and photosynthesis : respiration ratio than when grown under constant illumination. The author suggested this was due to an increase in metabolism to compensate for the reduced total illumination. However, as with the findings of Litchman *et al.*, (2003), higher growth rates were observed under constant illumination.

2.4.2.3 Oxygen balance

Previous studies have generally been directed towards hydraulic, design and sanitary aspects. Despite being the key to the optimization of performance, to date, the biological community – the engine of wastewater self-regeneration – has been the focus of very little research in treatment ponds (Soler *et al.*, 1991; Arauzo *et al.*, 2000). It is known that in pond systems, algae stimulate bacteria, and bacteria stimulate algae. In this way, the chemical units that comprise the organic waste eventually become incorporated into the algae as stable organic components of living cells. The death and decomposition of large numbers of algae lead to undesirable condition similar to those caused by the original wastewater in the pond. Consequently, this needs to be avoided to keep the trophic structure well balanced for optimum performance.

Microbial processes can significantly influence water quality through effects on dissolved oxygen, ammonium, and labile (i.e. readily biodegradable) organic matter, indirectly measured as BOD. The heterotrophic microbial community uses oxygen while feeding on unconsumed feed, photosynthetic products, and waste products. Nitrogen waste products and carbon dioxide are used in phytoplankton growth, which often includes the release of labile organic matter. Labile organics, along with oxygen, are consumed by water column bacteria, thereby recycling carbon and nitrogen in a microbial loop with continual oxygen demand (Bratvold and Browdy, 1998).

Daytime respiration in planktonic communities is an important component of metabolic and ecosystem materials budgets because it is critical to the distinction between gross and net primary production (Bender *et al.*, 1987). Respiration can also be used to index microbial biomass (Costa-Pierce *et al.*, 1984) or general water quality (as BOD) (Szyper *et al.*, 1992).

In WSP, oxygen tension is an operational parameter that shows a great deal of daily and hourly variation. The two natural sources of dissolved oxygen in ponds are surface re-aeration and photosynthetic oxygenation. Observation has shown that dissolved oxygen in wastewater ponds varies almost directly with the level of photosynthetic activity, being low at night and early morning and rising during daylight hours to a peak in the early afternoon (Kayombo *et al.*, 2000). The growth of algae is light and temperature-dependent; hence the rate of oxygen production (photosynthetic) follows the same pattern. Temperature is a parameter that shows marked seasonal and daily variation in WSP. It influences photosynthesis, growth of microorganisms and bio-decomposition of organic carbon in the system. The fluctuation of pH influences the kinetics of microbial growth, species competition and product formations in the pond (Fritz *et al.*, 1979). Thus the combined effect of change in temperature, pH and light intensity may have a more marked effect on the microbial activities in the pond rather than when one factor is considered. Fundamental studies have been done on the influence of individual forcing functions on the processes taking place in WSP. In reality changes of these forcing functions occurs simultaneously and thus their influence on the processes ought to be determined at this level. Kayombo *et al.* (2000) determined the manner in which, pH, temperature and light intensity influence the production and utilisation of dissolved oxygen in secondary facultative ponds. Using a dissolved oxygen model comprising of one state variable (dissolved oxygen) and three forcing functions, they concluded that all forcing functions simultaneously affects the rate of photosynthesis based on the multiplicative function. They also suggested that for a balanced system, the amount of dissolved oxygen produced by the photosynthesis process is enough to keep the system healthy.

The oxygen balance in WSP is influenced by three factors: the utilisation of oxygen by bacteria during destruction of BOD; the production of oxygen by the algae during photosynthesis and the utilisation of oxygen by algae in the process of turning glucose produced during photosynthesis into algal cells. Determining the oxygen balance within a pond can provide a measure of performance throughout the year. During the winter, although evidence shows that it is possible to maintain continuous microbial activity at or near freezing point, rates of reaction for both oxygen production and respiration are sub-optimal with respect to wastewater stabilisation. Only a minimal amount of treatment can be attributed to biological mechanisms in WSP during the winter months (Heaven and Banks, 2005). During the spring warm-up period, an understanding of the oxygen balance in the system could provide an indication of whether oxygen production is exceeding demand thereby creating the aerobic conditions required for the rapid reduction of the winter pollutant peak.

2.4.2.4 Measuring photosynthetic activity

Methods for measuring photosynthesis and general activity from changes in dissolved oxygen concentration have not changed since Gaarder and Gran (1927) published their account of 'investigations of the production of plankton in the Oslo fjord'. Water samples containing the phytoplankton are suspended in bottles in the water (or in an incubator) and changes in carbon and oxygen concentrations resulting from photosynthetic activity are measured. Photosynthesis measurements are often carried out using the Winkler titration method (Hepher, 1962; Bender *et al.*, 1987). This method however is not applicable to many industrial and domestic wastewaters or to field testing and cannot be adapted easily for continuous monitoring of dissolved oxygen *in situ*. Czapleweski and Parker (1973) suggested that by using an oxygen probe instead of Winkler titrations, many more samples can be processed each time with no loss in accuracy. The electrometric method of using a dissolved oxygen meter is an alternative technique previously tested by Bratvold and Browdy (1998) in hypereutrophic aquaculture ponds. Activity measurements tested by Bratvold and Browdy (1998) included net photosynthesis based on changes in dissolved oxygen concentrations under light and dark conditions and microbial activity as indicated by oxygen consumption rates in the dark.

They were able to provide general measurements of pond microbial activity. A study by Reeder and Binion (2001), compared a number of commonly accepted methods of measuring water column activity in a highly productive freshwater wetland. These were diurnal oxygen changes; light and dark bottle incubations; chlorophyll-a concentration; daily changes in pH and algal volume. Productivity from diurnal oxygen changes calculated at 0.25, 0.5, 1, 2, 3, and 4 hour intervals gave similar estimates. Net productivity in the bottles was found to be slightly lower than that indicated by diurnal oxygen changes and gross productivity in bottles was much lower than diurnal changes. These results show that processes occurring during bottle incubations can never exactly match those of populations *in situ*.

Light and dark bottle experiments have been applied for a long time (Talling, 1984), but are not devoid of errors, such as bacterial respiration and increase in algal population in the light bottles during the experiment. Most of these errors, however, result from long exposure time. Although primary production is considered a major component in the functioning of ecosystems, little is known about the relative merits of different methods used to measure aquatic productivity in shallow water bodies. The technique for measuring production and respiration during *in vitro* incubations gives results which are often ambiguous and which fail to give a complete description of community metabolic rates. Bender *et al.* (1987) found production rates in glass bottles were being significantly lowered by bottle effects, showing that bottles may have an adverse effect on community production. 'Bottle effects' can arise from the glass acting as a substrate for bacterial growth leading to erroneous values for phytoplankton respiration and net photosynthesis. Results from the work by Bender *et al.* (1987) however did demonstrate excellent reproducibility showing that the effects of containment in bottles are uniform. Work carried out using photobioreactors for algal culture has shown that dissolved oxygen concentrations exceeding about 120% can inhibit photosynthesis and otherwise damage the culture (Sanchez Miron *et al.*, 2000; Acien Fernandez *et al.*, 2001). Therefore when there is an accumulation of oxygen in a closed system, such as during light and dark bottle experiments, the inhibition of photosynthesis will lead to an underestimation of the photosynthetic rate. The super-saturation capacity of the sample may occasionally be exceeded, and gaseous oxygen will evolve in the bottle, essentially being lost to fixation.

In choosing to measure the rate of photosynthesis in the laboratory rather than in the natural state means that the populations are exposed to irradiance regimes and temperatures different to those which they might have experienced in the natural environment. Light, temperature, and available nutrients have the greatest effect on photosynthetic oxygen production. As light and temperature are controlled when conducting laboratory primary production experiments, further consideration is needed regarding these factors. It is virtually impossible to reproduce field conditions precisely in the laboratory; the intensity, spectrum, angle, and polarization of incident light change during the day, with small concomitant changes in temperatures. Furthermore, the organisms may show varying responses in accordance with their light history (Goldman, 1967). However, field experiments are difficult to control adequately because the environmental variables are so numerous causing experimental error to be significantly high. Laboratory experiments have the advantage of allowing a higher frequency of measurements of the photosynthetic rate and because artificial light is used to maintain constant saturation irradiance, the measurement can therefore be performed at any time of the day.

With very low light intensity, the rate of photosynthesis may be less than that of respiration. As the intensity increases, the photosynthetic rate reaches the respiration rate and then exceeds it. The initial correlation is linear with the rate of photosynthesis increasing in direct proportion to the increase in light intensity. Photosynthesis can become saturated by light and the rate reaches a plateau which is the maximum photosynthetic rate. Figure 2.2 shows the three regions that can be depicted in the relationship as a light-limited or direct response portion, a light saturated portion and a light-inhibited portion where photo-oxidative destruction of enzymes occur (Wetzel, 2001).

Light saturation in lakes has been suggested to occur as low as 100 μmol m^{-2} s^{-1} and inhibition as reported to begin at 510-770 μmol m^{-2} s^{-1} (Welch, 1992). As stated earlier, the onset of photoinhibition for the green algae that predominate in WSP may occur at irradiances >200 μmol m^{-2} s^{-1} (Ratchford and Fallowfield, 2003). This is significantly lower than the irradiances reported for lakes and shows how the relationship between light intensity and photosynthesis will vary with species, temperature and adaptation.

There are numerous difficulties also involved in finding a relevant reference temperature for the comparison of photosynthesis of cultures acclimated at different temperatures. The response of photosynthesis to temperature is dependent upon the amount of light available, with the response at subsaturating light levels being very different from that at saturating light levels (Davison, 1991). Although the initial photochemical reactions are independent of temperature, many associated aspects of photosynthesis are temperature-dependent (Raven and Geider, 1988).

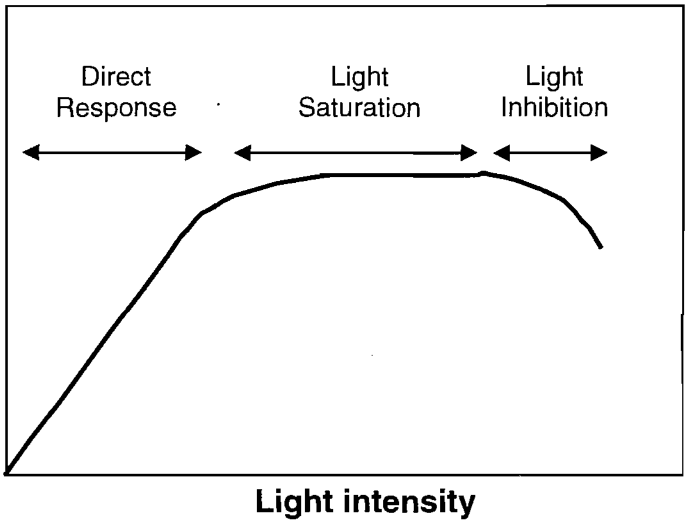


Figure 2.2 Hypothetical relationship between photosynthesis and light intensity

In a study by Falk *et al.*, (1990) experiments were conducted to assess the susceptibility of photosynthesis to photoinhibition and its recovery in the unicellular green alga *Chlamydomonas reinhardtii* acclimated at 12 and 27°C. The temperature during the photosynthesis measurements were kept at the cultivation temperature of the sample, 12 and 27°C, respectively. Findings suggested that there was very little difference in the rate of recovery of gross oxygen evolution in both light and darkness between algae grown at 12 and 27°C under growth temperature conditions. When the inhibitory light and temperature conditions were interchanged between the algae, the algae grown at 12°C were inhibited less than those grown at 27°C, showing that *C. reinhardtii* had an increased resistance to photoinhibition both at low and high temperatures when acclimated to a low temperature regime. Choosing to define the photosynthetic characteristics under growth temperature conditions, they stated that an alternative method would have been to make comparisons at the optimum temperature of photosynthesis of the cultures grown at 12 and 27°C. Davison (1987) conducted a study into the adaptation of photosynthesis in the brown alga *Laminaria saccharina* to changes in growth temperature. Plants were grown for 4 weeks at either 5°C or 15°C and photosynthesis measurements taken under a range of temperatures. Results showed that dark respiration generally increased with the assay temperature, though no significant change was observed between 10 and 25°C for the algae grown at 15°C and between 15 and 25°C for the algae grown at 5°C. A second observation was the rate of net photosynthesis measured at subsaturating light levels, decreased with increasing temperature. A similar study was carried out by Eggert and Wiencke (2000) using Antarctic red algae. Respiratory rates were also shown to increase with increasing temperatures. The temperature optima of photosynthesis was also found to be higher than the growth rate. In general, temperature optima for photosynthesis are generally higher than the optimum temperature for growth, and positive net photosynthesis can occur at temperatures well above the upper thermal limit for long-term survival (Davison and Davison, 1987). Studies have shown that optimum temperatures for photosynthesis are highest (25-35°C) in warm-temperate/tropical species (Terrados and Ros, 1992), intermediate (20-25°C) in cold-temperate to Arctic species and lowest (10-20°C) in Antarctic macroalgae (Eggert and Wiencke, 2000). The observed differences in these studies show the importance of using standard conditions during laboratory based experiments for photosynthesis and respiration measurements.

2.4.3 Nutrients

In addition to carbon, hydrogen, and oxygen, algae require some 13-15 additional elements to grow and reproduce. The growth rate of an alga will decline if the concentration and/or supply rate of a given nutrient drops below that required to maintain the existing growth rate. Nutrients can compensate, within limits, for physical limitations e.g. light and temperature. Most of these nutrients are usually present in sufficient amounts in WSP, relative to the alga's needs, so as not to be potential limiting factors for growth. In variable climate ponds, there is an accumulation of nutrients over the winter period coupled with the release of nutrients from benthic sediments in the spring turnover. The ability of algae to accumulate these nutrients is likely to reduce them to growth-limiting levels even when the growth limiting factor may be light limitation due to algal self-shading. Nutrients locked up in algal cells are eventually released, and in a cold climate pond system there is substantial evidence to show that this occurs in winter and early spring. The degree and timescale of recycling of nutrients in a real pond system is unknown on a micro-scale, but results from Bartosh (2004), Powell *et al.* (2006) and other work in batch culture suggest that this may be quite rapid, even over a period of hours.

Nitrogen removal is a key component in WSP technology. The three mechanisms for nitrogen/ammonia removal in ponds are gaseous ammonia removal or volatilisation, ammonia assimilation into algal biomass (conversion to organic nitrogen) followed by subsequent sedimentation and retention in the benthic sludge and biological nitrification coupled to denitrification (Middlebrooks *et al.*, 1982). The major route is considered to be via volatilisation, with some reports of >90% nitrogen removal by this mechanism (Pano and Middlebrooks, 1982) as the pH in ponds increases above 7, however there is disagreement regarding the relative importance of ammonia volatilisation and algal uptake. Rockne and Brezonik (2006) evaluated the fate and removal efficiency of the nutrient elements nitrogen, phosphorus and organic carbon in a three stage cold weather WSP system experiencing four months of ice cover. To achieve this, a mass balance model was developed to determine fluxes of these elements through each pond. The dominant sink for nitrogen from the system was found to be volatilisation of un-ionised ammonia during the early summer when the pH rose above 9, however they did propose that both ammonia volatilisation and algal uptake probably have the potential to be the dominant nitrogen sink under the appropriate conditions. The results showed that the onset of mid-depth aerobic conditions in the pond following the ice thaw in spring coincided with the time of peak ammonia volatilisation rates. A recent study by Camargo Valero and Mara (2007) showed disagreement with the study by Rockne and Brezonik (2006). They used ^{15}N-labelled ammonia to track ammonium transformations on an experimental pilot-scale WSP system in Bradford, UK. Under summer conditions, results showed that a more feasible route for ammonia removal predominates from the uptake of ammonia by pond algal biomass. They concluded that ammonia volatilisation can be discounted as a major pathway for nitrogen removal even when pH and temperature are favourable.

Phosphorus is often the limiting nutrient in fresh water systems and the major nutrient contributing to the increased eutrophication of lakes and other natural waters. For this reason, phosphorus removal from wastewater is increasingly becoming a mandatory discharge requirement. Algae appear to offer the most easily exploited biological system for extracting phosphorus from sewage. Phosphorus removal is generally low in WSP, usually between 15 and 50% (Picot *et al.*, 1992; Racault *et al.*, 1995). Removal is a result of both chemical and biological mechanisms. Precipitation occurs due to an elevated pH resulting from algal growth and it is also removed due to organism growth. Powell *et al.* (2006) investigated the effects of different variables on biological phosphorus removal in

WSP. They conducted one litre batch experiments under varying light intensities, temperature and diurnal effects. Initial phosphate concentration, light intensity and temperature were shown to significantly affect phosphorus removal. Growth of the algae alone, however, was shown not to be responsible for the removal. The unexplained phosphorus removal was suggested to be a consequence of luxury uptake as growth continued without an external phosphate source and at higher phosphate concentrations more phosphate was removed but it did not result in higher growth. Virtually all the phosphorus in a system may be inside living organisms at any given time, yet it may be overturning every hour with the result that there will be a constant supply of phosphate for organisms able to concentrate it from a very dilute solution. Such systems may remain stable biologically and chemically for considerable periods in the apparent absence of available phosphate (Pomeroy, 1960).

Tang *et al.* (1997) evaluated the nutrient uptake abilities of cyanobacteria isolated from a high Arctic lake and green algae from Canada to screen for their potential use in outdoor wastewater treatment systems in colder climates. The relationship between phosphate concentration as a function of time in the presence of the two organisms took the form of an exponential decay function. High phosphate uptake despite low biomass was observed at the beginning of the experiment and was likely to be the result of luxury uptake by the algae. Both cyanobacteria and green algae are known to take up surplus phosphate and store it as polyphosphate (Reynolds, 1984).

2.4.4 Organic loading

The design of WSP is part rational and part empirical. The actual design of a facultative pond depends on a great variety of local conditions, but a number of useful and rational design procedures are available. Climatic extremes of temperature, humidity and solar radiation greatly affect the design and operation of ponds, therefore no single design criterion can be applied. The most commonly used criterion is organic loading. Owing to the importance of sunlight to provide oxygen from photosynthesis, organic loading is expressed as a surface loading rate either as kg BOD ha^{-1} d^{-1} or g BOD m^{-2} d^{-1} that varies significantly with temperature (Gray, 2002). An example of design values for surface BOD loading rate at various temperatures is shown in Table 2.2. Many WSP have been designed with inappropriate BOD loadings. Inappropriately high loadings lead to odour and pond failure, whereas loadings that are too low, especially on anaerobic ponds, lead to under-performance and overall costs are increased as the land area used is greater than necessary.

Table 2.2 Design values for the surface BOD loading rate on facultative ponds at various temperatures (Mara, 1996)

Temperatures (°C)	Surface loading rate (kg BOD ha^{-1} d^{-1}
≤10	110
11	112
12	124
13	137
14	152
15	167
16	183
17	199
18	217
19	235
20	253
21	272
22	292
23	311
24	331
25	350
26	369
27	389
28	406
29	424

Sewage strength (including characteristics) plays an important role in influencing symbiotic activities between bacteria and algae in WSP as reported by (Kayombo *et al.*, 2002). Investigations into diurnal fluctuations of pH, dissolved oxygen and light intensity in WSP showed that the primary facultative pond receiving the highest organic loading had relatively low diurnal variations. Carbon dioxide in the pond was assumed to limit algal activity when the rate of oxidation of organic matter is preceded by high uptake of carbon dioxide by algae. Bartsch & Allum (1957) studied biological factors affecting five different WSP in the northern plains area of the USA. They evaluated the photosynthetic system in relation to the sewage load. Results showed that oxygen production and total respiration were more intense in response to higher loading. They also found that during winter, clarity of the ice varied with loading. Regardless of ice thickness, the contrast in light transmission between ponds was such that clear ice was found in the lower loaded ponds (55.5% light transmission) and opalescent ice at the heavier loaded pond (1.2%

light transmission). Ice quality therefore appears to be more important than thickness in determining the amount of light received by the ponds during the winter months. This 'cloudiness' of the ice caused by organic matter could possibly be improved with a reduction in BOD loading.

Abis and Mara (2004) reported on the performance of facultative ponds in the UK operated at different loadings over a two-year period. The UK has a cold temperate climate: the average temperature during the coldest month is 2-4°C. Experiments showed that at loadings of 60 and 80 kg BOD ha^{-1} d^{-1}, performance was the same. The pond loaded at 110 kg BOD ha^{-1} d^{-1} had good BOD removal, but was characterised by a prolonged period in winter when the pond was devoid of algae and therefore very low concentrations of dissolved oxygen at the surface. In a second trial, Abis and Mara (2005) monitored the effect of surface BOD loading on the algal populations during the winter months in an experimental WSP in the UK. Two loadings were tested: 50 and 80 kg BOD ha^{-1} d^{-1}. The results of the trial showed that the algal population was more stable and the dissolved oxygen concentration generally higher in the pond loaded at 50 kg BOD ha^{-1} d^{-1} than at 80 kg ha^{-1} d^{-1}. However, no significant effect on pond performance was observed. Results from both ponds suggest that in the UK climate, land saving could be significant, as a full-scale pond loaded at 80 kg BOD ha^{-1} d^{-1} would be for example 75% of the size of a pond loaded at 60 kg ha^{-1} d^{-1}.

Travieso *et al.*, (2006a) reported on the use of a laboratory stabilisation pond for the tertiary treatment of distillery waste. The effect of the hydraulic retention time and the influent total chemical oxygen demand (TCOD) were evaluated. Removal efficiency appeared to be influenced by the influent concentration. An increase in the influent strength brought about a decrease in the removal efficiency. The removal efficiency also increased with the increase in HRT. The effect of HRT and influent strength on the development of photosynthetic organisms was also evaluated. The concentration of chlorophyll-a in the effluent was used as measure of photosynthetic organisms. The results showed that the growth of photosynthetic organisms may be limited by a low influent strength concentration but also inhibited by high influent strength concentration with a low HRT. This increase in organic loading produced a considerable decrease in the process performance. It is therefore important to establish an optimum load to avoid a negative effect on the process efficiency.

At moderate to high loadings, nutrients are usually provided in excess from the wastewater (domestic wastewater usually has high concentrations of nitrogen, phosphorus and carbon compounds), so the algae are usually light-limited. Racault *et al.* (1995) studied three ponds in SW France which failed during the winter. The three ponds failed at the same time after the death of the algae in late autumn; insufficient surface aeration during the calm winter led to odour problems. The loss of the algae may be due to a number of factors, but was mainly due to the overloading of solids, either from the wastewater or the sludge, which block out light (BOD in itself does not retard algal growth) (Parker and Skerry, 1968). The algae may also be destroyed at low or moderate loadings by other factors such as grazing, ammonia and sulphide toxicity (Shillinglaw and Pieterse, 1977; Konig *et al.*, 1987) or algal parasites (Lawty *et al.*, 1996). Climate also has a very important part to play. Solids blocking out the sunlight seriously affect the algae; the sources of these solids include excess colloidal solids in influent wastewater; solids feeding back from the sludge; and other algae (self-shading). Increasing the organic load can stimulate gas production in the sludge which causes the eruption of solids and ammonia into the upper layers (Parker and Skerry, 1968). At lower loadings however, *Daphnia*, rotifers and large protozoan blooms can consume the algal population within days (Cauchie *et al.* 2000).

2.4.5 Hydraulic retention time

The mean hydraulic retention time (HRT) (θ, days) in an individual WSP is given by: $\theta = V/Q$ (or AD/Q) where V is the pond volume (m^3), Q the wastewater flow through the pond ($m^3\ d^{-1}$), A is the pond area (m^2) and D is the pond working liquid depth (m) (Pena and Mara, 2004). The design of anaerobic ponds is usually based on HRT and depth, although surface or volumetric loading rates are sometimes quoted (Gray, 1999). The design of the storage maturation pond for an intermittent discharge system is also based on HRT, determined by climatic conditions and the required frequency of discharge.

Abis and Mara, (2005) conducted a study to assess the effects of a reduced HRT (20-60 days) on primary facultative pond performance in the UK. They used a fixed BOD loading of 80 kg $ha^{-1}\ d^{-1}$ which was achieved by diluting the raw wastewater with tap water before it entered each of the three ponds. The HRT was reduced by increasing the flow of tap water. The results of the experiment showed that there was no loss in performance for BOD and ammonia removal on reducing the HRT to 20 days. There was some loss in performance between 20 and 30 days for suspended solids removal, though due to algal solids present in the effluent, this effect was not conclusive. From these results, it is possible to suggest that at a loading of 80 kg BOD ha^{-1} d^{-1}, facultative ponds in temperate climates such as the UK could be maintained at a HRT of 20 days. It is unknown whether such a HRT could be possible for facultative ponds in more extreme climates.

2.5 Conclusions

The review indicates there may be scope for improving the performance of WSP in variable climates. One way of achieving this might be by gaining a better understanding of what factors reduce the pollutant peak in spring. Design guidelines are needed for variable climates to avoid inefficient system design by avoiding the need for prolonged storage and making water available for re-use in the spring.

3 MATERIALS & METHODS

3.1 Experimental plan

The research addressed three main areas and was conducted at three separate locations: (i) Batch-fed small-scale systems at the University of Southampton, UK conducted under natural and temperature and light controlled conditions to test the effect of dilution on removal rates and performance; (ii) Semi-continuous fed units based at Almaty, Kazakhstan designed to test the effect of dilution and different HRT on removal rates and pond performance under extreme variable climate conditions; (iii) Experimental waste stabilisation ponds at Lockerley Water Farm, near Southampton, UK. This was a larger-scale system operated using two different regimes in a variable climate. Detailed descriptions of the individual experimental plans are given in each section.

3.2 Sampling and laboratory analytical methods

Duplicate samples and their filtrates (where appropriate) were analysed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998) unless otherwise stated, and the final result expressed as means. Total biochemical oxygen demand over 5 days (BOD_5) (Method 5210 B) was analysed in duplicate whilst chemical oxygen demand (COD) (Method 5220 C) and total suspended solids (TSS) (Method 2540 D) were measured in triplicate. Measurements of ammonia, nitrate and orthophosphate were made using colorimetric standard methods (Method 4500-NH_3 F; Method 4500-NO_3 B; Method 4500-P E). A Jenway 3010 pH meter (Jenway, Essex, UK) standardised with buffers at pH 7 and 9 was used to measure the pH on all samples and their filtrates. Dissolved oxygen concentrations were measured using a YSI 5000 DO meter with a YSI 5010 BOD probe (Yellow Springs Instruments, Yellow Springs, OH). The meter was calibrated and operated according to the manufacturer recommendations.

Chlorophyll-a was determined by filtering through a 0.45 μm GF/C filter (Whatman, UK) previously dosed with 0.2 ml of a saturated solution of $MgSO_4$. Extraction was by grinding followed by treatment overnight with acetone. Centrifugation the following day was for 15 minutes at 2000 *g*. The supernatant was measured at 750 and 664 nm, then acidified with 0.1 ml of 0.1 M HCl to convert chlorophyll-a to pheophytin and measured at 665 nm and 750 nm, using a 3000 series Cecil Instruments spectrophotometer (Cecil

Instruments, Cambridge, UK). Values at 750 nm were subtracted from those at 664 and 665 nm to correct for turbidity.

Net growth

The apparent net growth (μ) of the algae during the exponential phase was calculated from changes in the chlorophyll-a biomass (Pedersen and Borum, 1996). This approach was also used for periods of steady growth that were assumed to be exponential, and therefore provided a minimum estimate for μ in this case (equation *3.1*):

$$\mu = \left(\ln B_l - B_0\right) t^{-1} \tag{3.1}$$

where: B_0 = initial chlorophyll-a biomass

B_l = end chlorophyll-a biomass at the extremes of linear growth

t = number of days during this exponential phase

Absorbance

For measurements of absorbance samples were filtered through a 0.45 μm GF/C filter (Whatman, UK). The resultant colour of both filtered (true colour estimates) and unfiltered samples (apparent colour) were measured relative to distilled water at 440 nm (Cuthbert and del Giorgio, 1992) and 678 nm (Bartosh, 2004) using a 3000 series Cecil Instruments spectrophotometer (Cecil Instruments, Cambridge, UK). The absorption coefficient at 440 nm (g_{440} m^{-1}) was determined using equation *3.2* (Kirk, 1976):

$$g_{440} = \frac{2.303A}{l} \tag{3.2}$$

where: A = absorbance at 440 nm

l = cuvette path length (m)

Oxygen production

Gross and net photosynthesis were determined by calculating the difference in rates of change of dissolved oxygen concentration in closed samples incubated under light and dark conditions. Net oxygen production was determined from changes in dissolved oxygen in the light and gross oxygen production determined from the rate of change in the light minus rate of change in the dark. Samples were vigorously shaken to ensure initial DO measurements between 90 and 100% saturation, and placed into a purpose-built 250 ml black PVC BOD bottle with a transparent acrylic base that light could penetrate. Temperature in the sample was controlled using an external heating/cooling coil with water circulating through it. Optimum oxygen production for many species is reported to occur at about 20°C (Gloyna, 1971), hence this temperature was used as the standard condition. A PAR 36 light with a sealed beam 30 W halogen lamp (General Electric 4515) was positioned below the sample to give a light intensity of 250 μmol m^{-2} s^{-1}. The DO probe was placed into the sample bottle and dissolved oxygen concentrations were recorded every 10 seconds and averaged. Both dark and light phases were run in duplicate for periods of 60 minutes. According to Lieth & Whittaker (1975), shorter time periods are considered to give more accurate estimates, but increase the importance of analytic error.

3.2.1 Algal inoculum

Typical species of algae normally found in WSP were obtained from the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, UK. Cultures of *Scenedesmus subspicatus* (CCAP 276/20), *Chlorella vulgaris* (CCAP 276/20), *Chlamydomonas reinhardtii* (CCAP 11/32b) were initially grown on Jaworski's medium (CCAP JM recipe) in 2-litre flasks. They were then transferred to synthetic wastewater medium, as described in Chapter 4, at 1:100 dilution in 20-litre flasks. Growth was at 20 ± 1°C under constant illumination of 55 μmol m^{-2} s^{-1} (2950 K) provided by warm white fluorescent lamps. The cultures were agitated daily, as no agitation through aeration was provided. Chlorophyll-a concentrations at the time of use as an inoculum were typically in the range of 0.5-0.8 mg l^{-1}.

3.3 Removal rates and efficiencies

Removal rates were calculated on a 'rolling basis' using equation *3.3*:

$$R = \frac{[(C_i - C_{i+t})/C_i]}{t} \qquad (3.3)$$

where: R = removal rate (g g^{-1} d^{-1})

$C_i =$ concentration on day i (mg l^{-1})

$C_{i+t} =$ concentration on day $i+t$ (mg l^{-1})

$t =$ number of days between C_i and C_{i+t}

Overall removal rates were calculated using equation *3.4*:

$$R = \frac{[(C_o - C_e)/C_o]}{t} \tag{3.4}$$

where: $R =$ removal rate (g g^{-1} d^{-1})

$C_o =$ concentration on day 0 (mg l^{-1})

$C_e =$ steady state, final or lowest concentration (mg l^{-1})

$t =$ number of days between C_o and C_e

Removal efficiencies were calculated using equation *3.5*:

$$E = [(C_i - C_{i+1})/C_i] \cdot 100 \tag{3.5}$$

where: $E =$ removal efficiency (%)

$C_i =$ concentration on day i (mg l^{-1})

$C_{i+t} =$ concentration on day $i+t$ (mg l^{-1})

Overall removal efficiencies were calculated using equation *3.6*:

$$E = [(C_o - C_e)/C_o] \cdot 100 \tag{3.6}$$

where: $E =$ removal efficiency (%)

$C_o =$ initial or influent concentration (mg l^{-1})

$C_e =$ steady state, lowest or final concentration (mg l^{-1})

3.4 Statistical analysis

Regression analysis was employed to compare experimental values between two parameters. ANOVA variance and t-test (two-sample assuming equal variances) were used to determine the statistical significance of paired variables. A critical p-value of < 0.05 was set.

4 FORMULATION OF A SYNTHETIC SEWAGE FOR USE IN LABORATORY-SCALE BIOLOGICAL TREATMENT STUDIES

4.1 Introduction

Domestic sewage is a complex mixture of materials resulting from everyday activities such as personal hygiene and sanitation, food preparation, laundering and cleaning, together with a small proportion from less common activities that may introduce a range of synthetic substances into the wastewater stream. In the UK, the relative proportions from these activities are approximately one third from the toilet, one third from personal washing, and one third from cleaning, laundry, food and drink preparation (Mann, 1979). While the basic content is similar, the characteristics of sewage vary from one country to another and even within a country. Factors influencing the volume of water used per capita and the strength and composition of the sewage produced include water availability, climatic conditions, economic status and social customs (Gloyna, 1971). Composition and volume are also affected by diurnal water usage patterns, whether or not rain water enters the collection system, the time the sewage remains within the collection system and the physicochemical conditions therein. For example, urea is abundant in fresh sewage but is rapidly converted to ammonia under both aerobic and anaerobic conditions: the rate of conversion is temperature dependent and has been estimated at 3 mg N $hour^{-1}$ at $12^{o}C$ (Painter, 1958). In a collection system with a mean retention time of 12 hours, not uncommon for a city, most of the urea will therefore have vanished before the sewage arrives at the treatment plant. It is therefore difficult or impossible to give a chemical definition of the composition of sewage, and even from a single collection system there will be significant variations in strength. In some cases this variability can make it difficult to carry out controlled experiments to simulate treatment processes for the purposes of understanding and development. For wastewater treatment plant design, however, it is important to have an approximate characterisation of domestic wastewater, and various text books provide this. Table 4.1 shows a typical composition given in Metcalf and Eddy (1999) and used for comparative purposes in the current study. The use of raw sewage in the laboratory is further complicated by health and safety considerations, due to the presence of viruses and pathogenic bacteria within the sample and the potential inclusion of toxic components. The composition of sewage also changes rapidly on storage as bacterial action converts sugars to organic acids and increases the acidity.

Table 4.1 Typical composition of domestic sewage (mg l^{-1} except settable solids and COD:BOD ratio). Adapted from Metcalf and Eddy, (1999)

Constituent	Concentration		
	Strong	**Medium**	**Weak**
Solids, total	1200	720	350
Dissolved, total	850	500	250
Fixed	525	300	145
Volatile	325	200	105
Suspended, total	350	220	100
Fixed	75	55	20
Volatile	275	165	80
Settleable solids (ml/l)	20	10	5
Biochemical oxygen demand, 5-day, 20°C (BOD_5)	400	220	110
Total organic carbon (TOC)	290	160	80
Chemical Oxygen Demand (COD)	1000	500	250
COD:BOD ratio	2.5	2.3	2.3
Nitrogen	85	40	20
Organic	35	15	8
Free ammonia	50	25	12
Nitrites	0	0	0
Nitrates	0	0	0
Phosphorus	15	8	4
Organic	5	3	1
Inorganic	10	5	3
Chlorides	100	50	30
Alkalinity (as $CaCO_3$)	200	100	50
Grease	150	100	50

Depending on the aims of the research the use of a synthetic sewage can have advantages, although it can also be criticised as being untypical or non representative. A large number of different types of synthetic sewage have been formulated depending on the specific research requirements: the ISI Web of Knowledge Service for UK Education (http://wok.mimas.ac.uk/, accessed 14 April 2008) has over 6500 references to 'synthetic sewage' and 'synthetic wastewater', ranging from simple dilutions of a particular pollutant to formulations characteristic of specific industries and those designed to imitate typical domestic sewage.

The Organisation for Economic Cooperation and Development (OECD) protocol 303A prescribes a synthetic sewage for use in testing for biodegradability: its composition is shown in Table 4.2. The sewage is prepared from laboratory-grade chemicals, and has been criticised in some applications as not providing an accurate reflection of real domestic sewage due to its unbalanced composition (Kaiser *et al.*, 1997). It was, however, designed for a specific purpose and to provide an international basis for a testing regime, and was not proposed for other purposes to which it has been subsequently applied.

Table 4.2 Composition of OECD synthetic wastewater (diluted 1:100 to give the OECD synthetic sewage)

Component	Quantity per litre in concentrate (g)	Final concentration in synthetic sewage (mg l^{-1})
Peptone	16	160
Meat extract	11	110
Urea	3	30
NaCl	0.7	7
$CaCl_2.2H_2O$	0.4	4
$MgSO_4.7H_2O$	0.2	2
K_2HPO_4	2.8	28
Distilled water	to make up to 1 litre	-

Aiyuk and Verstraete (2004) developed a synthetic sewage called 'SYNTHES' for experiments to test the performance and application of an upflow anaerobic sludge blanket (UASB) reactor. They formulated SYNTHES particularly to compare with actual domestic sewage in terms of the percentage soluble COD and COD:N:P ratio. A compositional breakdown of the concentrate used to prepare the working strength sewage is given in Table 4.3. Concentrated SYNTHES is intended to have a total COD of 8000 mg l^{-1}, a soluble COD of 2500 mg l^{-1} and a pH of 7.1.

The synthetic sewage developed as part of this research was formulated to mimic used water from domestic sources, which may include institutional wastewaters from schools, hospitals and commercial buildings without a manufacturing capacity. The synthetic sewages proposed by OECD (1976) and Aiyuk and Verstaete (2004) were compared to the formulation devised as part of the current research and also to the typical values suggested in Table 4.1.

Table 4.3 Composition of concentrated SYNTHES wastewater (diluted 1:100 to give the SYNTHES synthetic sewage).

Component	Quantity per litre in concentrate (g)	Final concentration in synthetic sewage (mg l^{-1})
Chemical compounds		
Urea	12	120
NH_4Cl	1.5	15
Na-acetate.$3H_2O$	16.875	168.75
Peptone	2.25	22.5
$MgHPO_4.3H_2O$	3.75	37.5
$K_2HPO_4.3H_2O$	3	30
$FeSO_4.7H_2O$	0.75	7.5
$CaCl_2$	0.75	7.5
Food ingredients		
Starch	15.75	157.5
Milk powder	15	150
Dried yeast	6.75	67.5
Soy oil	3.75	37.5
Trace metals		
$Cr(NO_3)_3.9H_2O$	0.1125	1.125
$CuCl_2.2H_2O$	0.075	0.75
$MnSO_4.H_2O$	0.015	0.15
$NiSO_4.6H_2O$	0.0375	0.375
$PbCl_2$	0.015	0.15
$ZnCl_2$	0.0375	0.375
Tap water	to make up to 1 litre	-

4.2 Materials and methods

4.2.1 Selection and formulation of ingredients

The selection of the ingredients for the synthetic sewage was based on readily available commercial materials and aimed to provide a mixture of soluble, colloidal and settleable organic components with a balance between carbon, nitrogen and phosphorus similar to that found in domestic sewage. In addition a trace element solution was added to ensure that the mix was not deficient in essential elements to promote microbial growth. The materials were also chosen to minimise the cost of preparation of the synthetic sewage so that it could be used up to a semi-technical or pilot scale if required. The formulation was based on earlier formulations that had been used successfully in algal waste stabilisation pond experiments (Heaven *et al.*, in review). The concentrate from which a working solution is prepared by a 100-fold dilution is shown in Table 4.4.

Table 4.4 Composition of synthetic sewage concentrate (diluted 1:100 to give a working solution).

Component	Quantity		Preparation
Trace element solution	1	ml	Added directly
Yeast (block bakers form)	23	g	Dissolved in 0.23 l of tap water and autoclaved for 15 min
Urea	2.14	g	Added directly
Full cream milk (UHT sterilised)	144	ml	Added directly
Sugar (granulated white)	11.5	g	Added directly
Blood (Freeze dried)	5.75	g	Homogenised with 0.2 l of water
Ammonium phosphate $(NH_4)_2HPO_4$	3.4	g	Added directly
Tap water	to make up to 1 litre		-

The trace element solution was prepared according to the formulation of Pfennig *et al.* (1981) as shown in Table 4.5.

Table 4.5 Composition of trace element solution (Pfennig *et al.* 1981)

Component	Quantity	
HCl (36%)	5.1	ml
$FeCl_2.4H_2O$	1.5	g
H_3BO_3	60	mg
$MnCl_2.4H_2O$	100	mg
$CoCl_2.6H_2O$	120	mg
$ZnCl_2$	70	mg
$NiCl_2.6H_2O$	25	mg
$CuCl_2.2H_2O$	15	mg
$Na_2MoO_4.2H_2O$	25	mg
DI water	to make up to 1 litre	

Fresh pressed bakers yeast was chosen to represent solids such as faecal material by providing a source of microbial cellular material that contained both a settleable and colloidal fraction on dilution. Urea provided a source of nitrogen as is typically present in urine. Full cream milk was used as a source of fats, lactose and protein. Sugar provided a soluble readily degradable and fermentable carbon source. Dried blood (Scobies Direct, UK) contains complex proteins and fats and on suspension contains soluble, colloidal and separable fractions; it is also rich in iron. Ammonium phosphate was added to balance the nutrient composition and to ensure that ammonia was present from the outset without the need for hydrolysis of urea or protein. In all cases tap water was used as the dilutant. The synthetic sewage was prepared 5 times to test the variability in its preparation. Once prepared, samples were stored frozen and thawed at room temperature.

Both SYNTHES and the OECD synthetic sewages were prepared using the instructions provided in the original publications (OECD, 1976; Aiyuk and Verstraete, 2004).

4.2.2 Chemical analysis

Unless otherwise stated all analyses were carried out at least in triplicate. 5-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total dissolved solids (TDS) and volatile dissolved solids (VDS) were all measured according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Settleable solids were measured after settling for 1, 2, 3, 4, 6, 24 hours and 5 days in an Imhoff cone (Metcalf and Eddy, 2002). After one hour's settlement a sample of supernatant liquid was removed and analysed for BOD_5, COD and SS. Total Kjeldahl Nitrogen (TKN) was measured using the Kjeltec System 1002 (Foss, Warrington, UK) with steam distillation and titration for ammonia according to the manufacturer's instructions. Total nitrogen, total carbon and total organic carbon (TOC) were measured using a Dohrmann DC 190 analyser (Dohrmann, Santa Clara, USA) with infrared and ultraviolet detectors. Soluble ammonia, nitrate and orthophosphate were measured on samples filtered through a GF/C grade glass fibre filter (Whatman, UK) and analysed photo-colorimetrically using a Bran and Luebbe AA 3 auto analyser (Bran and Luebbe, Norderstedt, Germany) following the manufacturer's APHA-approved method. Total phosphorus was measured on unfiltered samples by persulfate oxidation followed by the orthophosphate colorimetric method. Alkalinity was measured by titration with 0.25*N* H_2SO_4 to an endpoint of pH 4.0. Sulphates and chlorides were measured with a PF11 portable filter photometer (Macherey-Nagel, Duren, Germany) using the Nanocolour and Visicolor techniques. Heavy metals were analysed with the Varian Spectra AA 200 flame atomic absorption spectrophotometer (Varian, Palo Alto, USA). A Jenway 3010 pH meter (Jenway, Essex, UK) standardised with buffers at pH 7 and 9 was used to measure pH. Fats and oils and anionic surfactants were measured in triplicate by Southern Water, UK using the partition-gravitation Standard Method (5520 B) and the Methylene Blue active substances Standard Method (5540 C) respectively (APHA 2005).

4.2.3 BOD removal kinetics

The rate of oxygen removal during the BOD test was determined using the Oxitop (WTW, Wilheim, Germany) CO_2 adsorption system with pressure measurement in sealed bottles. Standard BOD dilution water (APHA, 2005) was used with nitrification inhibited by allylthiourea, and a seed inoculum from a secondary sedimentation tank at a municipal wastewater treatment plant was added. The manufacturer's instructions concerning the volume of the sample were followed. This meant in all cases using a 164 ml sample of each of the synthetic sewages; these were compared to a control containing only BOD dilution water with inoculum. The test was carried out over a period of 30 days in a temperature controlled incubator (Gallenkamp, UK) at 20 ± 0.2°C. During this period the bottles were stirred on the Oxitop proprietary magnetic stirring unit. The data were interpreted using the graphical method of Thomas (1950) in Ramalho (1977), which allows calculation of the ultimate BOD by plotting $(t/y)^{1/3}$ as a function of time t. The slope $K_1^{2/3}(3.43\,L)^{1/3}$ and the intercept $(2.3\,K_1 L)^{-1/3}$ of the line of best fit of the data was used to calculate K_1 (reaction rate constant) and L (the ultimate BOD). The test was carried out in duplicate.

4.2.4 Optical characteristics

Optical characteristics of the synthetic wastewater were determined because of its potential use as a feed for experimental WSP and other algal systems, where light penetration is an important characteristic. The optical properties of the synthetic wastewater were tested in 1.2 m deep tanks containing 1000 litres of diluted synthetic sewage. Illumination of the water column was provided by fluorescent lighting giving a surface illumination of 100 μmol $m^{-2}\,s^{-1}$ (48 W m^{-2}). The irradiance attenuation coefficient (k), an index of the penetration of light (Kirk, 1994) into water was determined using an array of type BPW 21 photodiodes (RS components, Corby, UK) measuring irradiance (E) at different values of depth (z). The value of k was calculated from the slope of the plots of ln E versus z. The euphotic depth (the depth at which photosynthetically available radiation (PAR) is reduced to 1% of the surface value) was then calculated from $z_{eu} = 4.6/k$ (PAR) (Kirk, 1994). To correct for a non-parallel light source and to account for the contribution of water itself to the attenuation of light, the vertical extinction coefficient for pure water 0.1 m^{-1} (Kirk, 1994) was subtracted from both the irradiance attenuation coefficient and euphotic depth. Visual clarity was measured using a 20 cm diameter horizontally-mounted black disk. This yields a direct estimate of the beam attenuation coefficient c = $4.8/y_{BD}$ where y_{BD} = maximum horizontal visual range in water (m) (Davies-Colley and Smith, 1992). Absorbance of membrane-filtered (0.2 μm) samples was measured using a Cecil 3000 series scanning spectrophotometer (Cecil Instruments, Cambridge, UK), and g_{440}, the dissolved humic absorption coefficient at 440 nm, was calculated from $g = 2.303D/l$ where D is the absorbance and l is cuvette path length (Davies-Colley *et al.*, 2005).

4.3 Results and discussion

4.3.1 Comparison of chemical constituents of 3 synthetic wastewaters

The average characteristics for the three synthetic wastewaters are shown in Table 4.6 with the same parameters measured for the SYNTHES and OECD synthetic sewages. These in turn can be compared to typical values for weak, medium and strong wastewater as shown in Table 4.1.

Table 4.6 Typical chemical characteristics of synthetic sewages

Parameter	Unit	Synthetic sewage		SYNTHES formula	OECD formula
		average	range (n=5)	average	average
TS	mg l^{-1}	772	746-813	675	593
VS	mg l^{-1}	498	449-541	374	326
TSS	mg l^{-1}	170	140-202	143	23
VSS	mg l^{-1}	118	83-138	129	17
Fixed SS	mg l^{-1}	52	-	14	6
TDS	mg l^{-1}	556	522-587	461	550
VDS	mg l^{-1}	329	310-349	211	278
Fixed DS	mg l^{-1}	227	-	250	272
	ml	1hr = 0.1	0.1-0.1	1hr = 0.0	1hr = 0.0
	ml	2hr = 0.2	0.2-0.2	2hr = 0.0	2hr = 0.0
Settleable solids	ml	3hr = 0.3	0.2-0.3	3hr = 0.0	3hr = 0.0
(ml/l)	ml	4hr = 0.4	0.3-0.3	4hr = 0.0	4hr = 0.0
	ml	6hr = 0.4	0.4-0.4	6hr = 0.0	6hr = 0.0
	ml	24hr = 0.5	0.5-0.5	24hr = 0.0	24hr = 0.0
	ml	5d = 1.4	1.3-1.5	-	-
TC	mg l^{-1}	221	213-238	129	173
TOC	mg l^{-1}	195	175-221	125	135.4
COD	mg l^{-1}	460	450-474	547	315
BOD	mg l^{-1}	220	187-247	230	140
COD:BOD	ratio	2.1	-	2.4	2.25
Settled COD	mg l^{-1}	345	-	-	-
Settled BOD	mg l^{-1}	195	-	-	-
TN	mg l^{-1}	33	29-36	62.3	51

Table 4.6 Typical chemical characteristics of synthetic sewages

Parameter	Unit	Synthetic sewage		SYNTHES formula	OECD formula
		average	range (n=5)	average	average
TKN	mg l^{-1}	24	21.8-26	53.3	35
Nitrate	mg l^{-1}	0.38	0.21-0.56	0.05	0.06
Ammonia	mg l^{-1}	9.8	9.1-10.6	4.77	0.27
Orthophosphate	mg l^{-1}	5.2	4.8-5.5	15.2	4.1
Total Phosphorus	mg l^{-1}	7.0	6.9-7.2	22.2	5.2
Alkalinity	mg $CaCO_3$ l^{-1}	147	127-164	384	270
pH		7.34	7.32-7.36	7.5	7.6
Chloride	mg l^{-1}	49	44-54	10.9	53.7
Sulphate	mg l^{-1}	43	37-47	26	56
Copper	mg l^{-1}	0.161	0.158-0.162	0.257	0.001
Zinc	mg l^{-1}	0.066	0.06-0.07	0.02	0.01
Lead	mg l^{-1}	0.043	0.038-0.047	0.72	0.5
Iron	mg l^{-1}	0.285	0.278-0.296	0.88	0.12
Fats & oils	mg l^{-1}	44.0	41.5-47.5	-	-
Anionic detergents	mg l^{-1}	0.21	0.21-0.21	-	-

Organic strength

The average COD of 460 mg l^{-1} and the BOD_5 of 220 mg l^{-1} categorise the synthetic sewage as medium strength while the SYNTHES and the OECD formulas are classed as medium and weak respectively. The ratio of COD:BOD provides a useful guide to the proportion of organic material present in wastewater that is biodegradable. Typical values for a domestic wastewater are in the range 2.0 - 2.5 for raw sewage; this increases with each stage of biological treatment as biodegradable matter is consumed and non-biodegradable organics remain (Gray, 2002). The COD:BOD ratio for the synthetic sewage was found to be approximately 2:1 and this relationship remains fairly constant for the five batches tested; the other two synthetic sewages also showed ratios similar to a domestic wastewater (Table 4.6).

Solids

The total solids of the synthetic wastewater were in the range of a medium strength sewage with a volatile solids content of 65%, which is slightly higher than typically found in domestic sewage. Suspended solids comprised 22% of the total solids, similar to the values reported in Table 4.1 for domestic wastewater (30%) but again with a higher volatile content. Both the other synthetic sewages showed a lower proportion of suspended solids relative to their respective total solids and both showed a high VS component within this although the volatile fraction of the total solids was 55% in both cases, indicating a higher inorganic dissolved salt content than is typical of a real sewage. The amount of settleable solids in the synthetic wastewater was 0.1 ml l^{-1} which is much lower than typically found in domestic wastewater. Although the total solids content was in the range of medium strength wastewater, the solids were very slow to settle to the base of the Imhoff cone. The material on the other synthetic sewages appeared to be more finely divided and colloidal, and therefore after a period of 24 hours no measurable solids had settled.

Macro nutrients

The total carbon (TC) content in wastewater is expressed as the sum of the organic and inorganic carbon while the total organic carbon (TOC) indicates the amount of carbon covalently bonded in organic molecules. In the synthetic wastewater and SYNTHES, a high proportion of the total carbon (88% and 97% respectively) was made up from organic carbon with the ratio of COD:TOC being 2.35 and 4.4 respectively, whilst the typical value for domestic wastewater is around 3.1. All three synthetic sewages could be classified as medium strength based on their TOC. Total Kjeldahl nitrogen (TKN) is the sum of organic nitrogen and ammonia. Approximately 73% of the total nitrogen in the synthetic sewage is in the form of TKN, and a similar pattern was observed for the other designed wastewaters. Orthophosphate was approximately 5 mg l^{-1} and total phosphorus was found to be 7 mg l^{-1} in the new wastewater and these figures would normally be found in a medium strength wastewater. Values were slightly lower for OECD synthetic sewage and three times higher in SYNTHES with orthophosphate values of 15 mg l^{-1} and total phosphorus at 22 mg l^{-1}. In sewage the concentrations of nitrogen and phosphorus are in excess of the requirements for microbial growth, which are generally recognised to be a BOD:N:P ratio of 100:5:1 for aerobic suspended growth systems (Cheremisinoff, 1997; Gray, 2002) and 100:0.5:0.1 for anaerobic systems. In aerobic treatment systems, however, when the sewage is depleted of its organic carbon through microbial growth and respiration a large proportion of nitrogen and phosphorus is still in solution. Most aerobic treatment systems subsequently aim to remove these components or convert them to the least environmentally damaging form. In anaerobic treatment systems although the microbial requirement of nitrogen and phosphorus for growth is much lower, the nitrogen component is important as ammonia provides much of the buffering in the system but at high concentrations can be toxic to methanogens. Typically domestic sewage has a BOD:N:P ratio of 100:17:5 for unsettled sewage and 100:23:7 for settled sewage (Lin and Lee, 2001) although this is highly variable. The synthetic sewage tested gave an approximate BOD:N:P ratio of 100:15:3.2 which provides an excess over the microbial growth requirements for both aerobic and anaerobic systems. The BOD:N:P of SYNTHES was found to be 100:27:9.6 and of the OECD wastewater approximately 100:36:3.7. Both ratios are in excess for adequate biological treatment and in both the nitrogen component is likely to be higher than found in most domestic sewages. Both the SYNTHES and OECD synthetic sewages include peptone as a nutrient source (160 mg l^{-1} and 300 mg l^{-1}, respectively), which has been implicated in inhibition of the growth of nitrifying bacteria (Sato *et al.*, 1988). Christofi *et al.* (2003) confirmed this for OECD synthetic sewage: they found the OECD feed was indeed inhibitory to nitrifying microorganisms and switched to using a formulation of Novak *et al.* (1994) which contains both particulate and soluble constituents and a higher organic strength.

Micro and trace elements

The concentration of soluble sulphates was similar in the synthetic sewage and the OECD formulation but lower in SYNTHES. In all cases concentrations were not sufficiently high to be considered problematic in either aerobic or aerobic treatment systems. The lower concentration in the SYNTHES sewage may have been a reflection of the design of this sewage for use in anaerobic treatment, where high sulphates can be more problematical. Chloride levels in the synthetic sewage were indicative of a medium strength wastewater and similar concentrations were found in the OECD sewage but much lower levels in SYNTHES.

Trace elements were added to both the synthetic sewage and SYNTHES, whereas the OECD formula does not include these as part of the recipe. The heavy metal concentration indicators analysed were lead, zinc and copper. All were less than 1 mg l^{-1} in all three formulations and were therefore unlikely to cause any operational difficulties in aerobic or anaerobic treatment systems. The level of soluble iron in the synthetic sewage was above the threshold of 0.1 mg l^{-1} suggested by Foster (2004) as the minimal requirement for aerobic treatment. SYNTHES has a much higher level than this, which again may reflect the design for anaerobic treatment systems.

Fats and oils

These were only measured in the synthetic wastewater, at a concentration of approximately 44 mg l^{-1} which is lower than found in most domestic sewages. Although the SYNTHES sewage was not analysed the formulation reflects a higher fat and oil content as soy oil is one of the ingredients used in its preparation. The synthetic sewage relies upon the contribution from full cream milk and blood constituents as the source of this component. If necessary the fat and oil content could be increased by adding between 5-6 g of soy oil to the concentrate: this would raise the fat and oil content of the working strength sewage to that representative of a medium strength sewage.

4.3.2 BOD kinetics

Figure 4.1, Figure 4.2 and Figure 4.3 (a) show the BOD curves for each wastewater as well as the graphical plots obtained from the Thomas method in (b). Although nitrifying inhibitor allythiourea was added to the samples, the BOD curve obtained for OECD wastewater showed that nitrification began to occur after 14 days which suggests that not enough inhibitor was added. The oxygen consumption data recorded continuously in the Oxitop BOD measuring system was plotted in accordance with the Thomas graphical method in order to determine the BOD rate constant (K_1) and ultimate BOD (L) for each synthetic wastewater. The data was calculated from the first 14 days as this proved to give the highest correlation coefficients and did not include the nitrogenous oxidation phase.

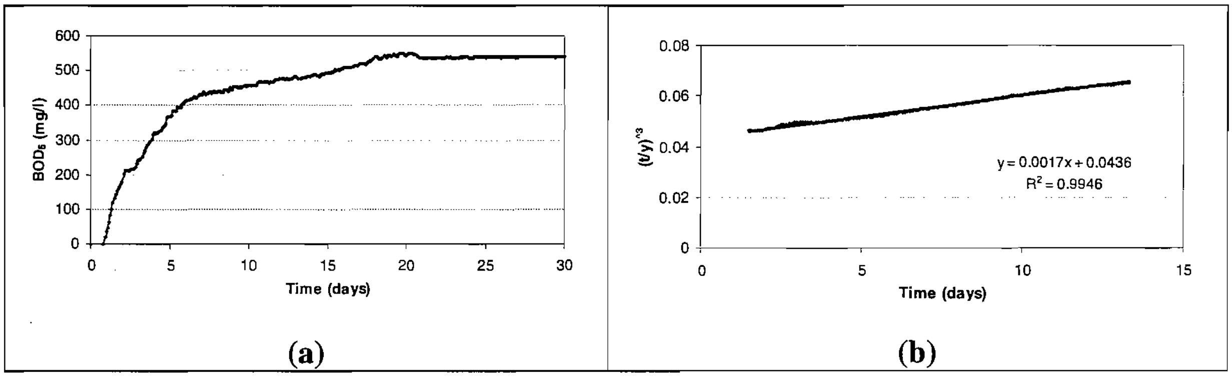


Figure 4.1 (a) Synthetic BOD curve and (b) determination of the BOD constant and ultimate BOD using the Thomas graphical method

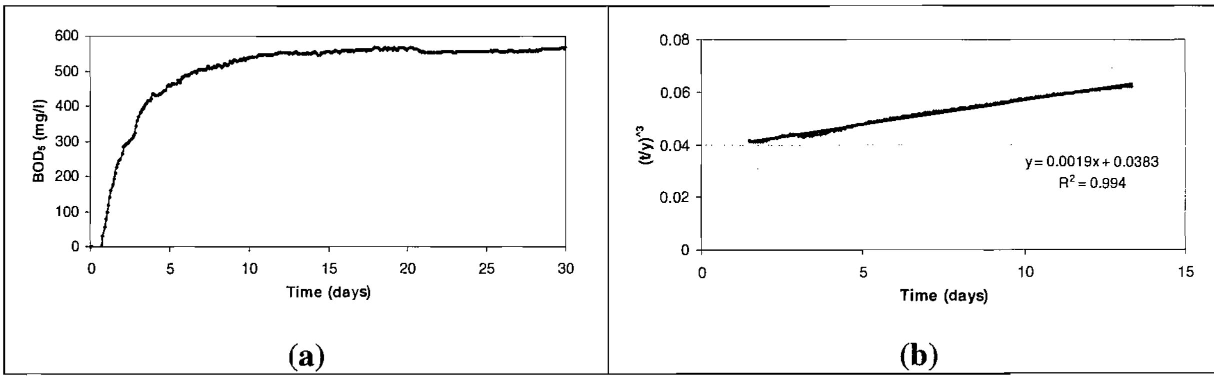


Figure 4.2 (a) SYNTHES BOD curve and (b) determination of the BOD constant and ultimate BOD using the Thomas graphical method

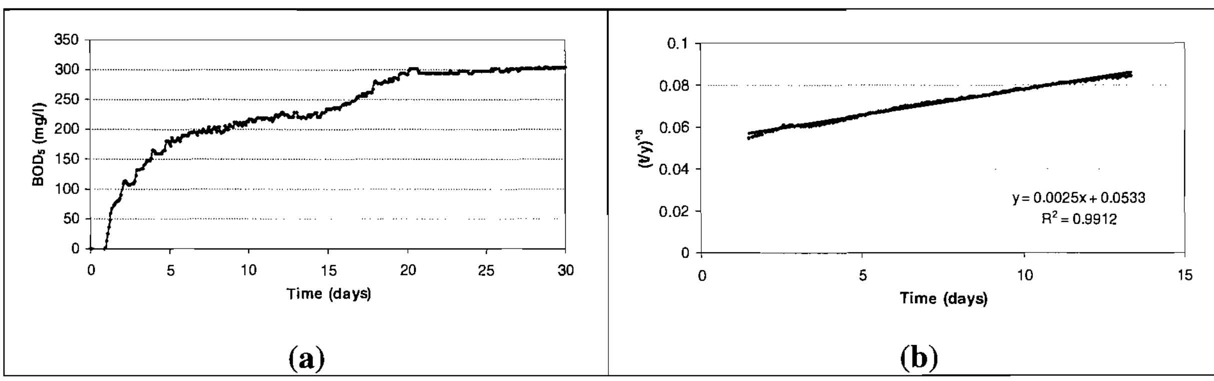


Figure 4.3 (a) OECD BOD curve and (b) determination of the BOD constant and ultimate BOD using the Thomas graphical method

The BOD curves show that oxygen consumption was most rapid during the first five days in SYNTHES wastewater and stabilisation of the material took about ten days. The process was slower in both the synthetic wastewater and the OECD sewage and by day 5, there was still substantial organic material to be stabilised. Stabilisation did not occur until about day 20 in both wastewaters which is typical of real wastewater that can take up to 3 weeks to be fully degraded at 20°C. Nitrification occurred in the OECD wastewater from day 14 and it is possible that this was also the case for the synthetic wastewater as the graph shows a slight upwards curve at the same time. In raw wastewaters, nitrification is a significant source of oxygen demand after 8-10 days, while in partially treated effluents nitrification can dominate the oxygen demand after just a few days (Gray, 2002). The occurrence of nitrification can increase the ultimate BOD due to the oxidation of non-carbonaceous matter which was apparent when using the full 30 day BOD data as opposed to just results from the first 14 days.

The BOD kinetics determined from the Thomas method and also a simple curve fitting method are given in Table 4.7. The curve fitting procedure gave similar results to the

Thomas method for the ultimate BOD (BOD_u) although slightly higher for the SYNTHES wastewater. The Thomas method has been described as an approximate method which is justified since precision of the experimental results is often limited (Ramalho, 1977). von Sperling and Chernicharo (2005) presented typical ranges of the conversion factor for BOD_5 to BOD_u but stated that various authors have adopted the ratio BOD_u/BOD_5 equal to 1.46. The ultimate BOD calculated for both methods is much higher than what would be expected for the synthetic and SYNTHES wastewater if using the ratio value of 1.46 with the results obtained for BOD_5 in Table 4.6. The ratios calculated for all synthetic wastewaters using the BOD curves are in the range 1.2-1.4 which according to the data published in von Sperling and Chernicharo (2005) is typical of primary effluent. The BOD measured after 5 days during the Oxitop experiments revealed higher values than those obtained during the standard BOD_5 test which would have lowered the value of each ratio. Higher BOD_u/BOD_5 ratios in the range 1.5-3.0 are more typical of secondary effluent.

SYNTHES wastewater showed the highest reaction rate out of the three wastewaters though all demonstrated typical oxygen consumption rates as the value of K_1 (base e) for untreated wastewater is generally about 0.12 to 0.46 d^{-1}, with a typical value of 0.23 d^{-1} (Metcalf and Eddy, 2002). The values in Table 4.7 for K_1 show both methods for establishing BOD kinetics gave similar results for the rate constant. The synthetic wastewater demonstrated a reaction rate constant most consistent with typical wastewater and polluted water values.

Table 4.7 Average K_1 (reaction rate constant) given as base e values and L (the ultimate BOD) for each synthetic wastewater by curve-fitting and the Thomas method

	Synthetic		**SYNTHES**		**OECD**	
	Curve-fitting method	**Thomas method**	**Curve-fitting method**	**Thomas method**	**Curve-fitting method**	**Thomas method**
K_1 (d^{-1})	0.22	0.23	0.30	0.30	0.25	0.28
L (mg l^{-1})	510	515	560	598	250	235

4.3.3 Optical properties

Coefficient values for a number of key parameters are shown in Table 4.8.

Table 4.8 Optical characteristics of the new synthetic wastewater

Parameter	Unit	Notation	Synthetic sewage	SYNTHES	OECD
Visual clarity	m	y_{BD}	0.13	-	-
Beam attenuation coefficient	m^{-1}	c	37	-	-
Euphotic depth	m	z_{eu}	0.63	-	-
Irradiance attenuation coefficient	m^{-1}	k	6.32	-	-
Humic matter attenuation coefficient	m^{-1}	g_{440}	17.3	1.0	5.3

The irradiance attenuation coefficient, k, had a value of approximately 6.3 m^{-1}. No k values for wastewater have been found in the literature, but an attenuation coefficient of 11 m^{-1} was recorded for sewage waste stabilisation ponds in New Zealand where organic material and algal biomass both contribute to the restricted light penetration (Davies-Colley *et al.*, 1995a). Weatherell (2003) reported values of between 3.16 and 14.97 m^{-1} for a three-pond system in Dar es Salaam, Tanzania. The euphotic depth (z_{eu}) was 0.63 m and therefore if the synthetic sewage was used in experimental waste stabilisation ponds with a depth of 1.2 m, about half of the water column would lack sufficient light for algal growth. A previous reported value for sewage of 0.35 m (Davies-Colley *et al.*, 2005) indicates greater light attenuation and a more restrictive euphotic depth. The visual clarity, y_{BD} was low at 0.13 m, but compares well with typical values obtained from waste stabilisation ponds in New Zealand of around 0.12 m (Davies-Colley *et al.*, 1995a). The beam attenuation coefficient, c was 37 m^{-1} demonstrating the very turbid character of the synthetic wastewater. Effluent quality from ten waste stabilisation ponds gave an average beam attenuation coefficient of 42 m^{-1} (Davies-Colley *et al.*, 1995a). The absorption coefficient for dissolved humic matter (g_{440}) was 17 m^{-1} which confirms the highly coloured nature of the wastewater compared to the less coloured SYNTHES and OECD sewages, which had coefficients of 1 m^{-1} and 5 m^{-1} respectively. These can be compared with values for sewage in New Zealand which has been found to have a dissolved humic absorption coefficient of 6.5 m^{-1}, whilst waste stabilisation ponds treating dairy cow wastes in New Zealand were found to have an absorption coefficient of 41 m^{-1} (Davies-Colley *et al.*, 2005).

4.4 Discussion and conclusions

The starting point for the development of the synthetic sewage was as a controlled, easily prepared and low cost medium for use with pilot-scale waste stabilisation ponds. For this reason it was desirable that the sewage developed should have a settleable organic fraction that would yield up some of its nutrients slowly as part of the bottom sediments of a facultative pond. This is not necessarily an important characteristic in other studies, however, and this settleable fraction could be settled out before use, as would happen with real sewage where a primary sedimentation tank is used. The concentrations of total and suspended solids within the synthetic sewage were typical of those of a medium strength domestic sewage although the organic fraction of the total solids was higher and the dissolved salt fraction lower. The volatile suspended solids were, however, at a typical concentration for a medium strength wastewater. The nutrient balance within the synthetic wastewater was similar to that found in domestic wastewater, with levels of both N and P in excess of microbial growth requirements. Chloride, sulphate and heavy metals were at a concentration unlikely to cause any operational difficulties with aerobic or anaerobic treatment systems. BOD curves suggested that oxygen consumption was most rapid in SYNTHES wastewater with stabilisation occurring in half the time of the synthetic and OECD wastewaters. Nitrification occurred in the OECD wastewater and results suggest that nitrogenous oxidation also took place in the synthetic wastewater from day 14. BOD kinetics obtained for each wastewater gave similar reaction rate constants in the range of real wastewater, in particular, the newly developed synthetic wastewater showed a rate constant of 0.23 d^{-1} (base *e*) reported to be typical of domestic wastewater. For experiments with algal-based systems where oxygen production is light-dependent, the optical properties of the wastewater are of importance. In this respect the synthetic sewage was found to mimic the optical properties of real sewage and in practice it has been used extensively in work on these systems (Heaven *et al.*, in review). Overall the synthetic sewage compared well with the anticipated characteristics of a medium strength domestic sewage. The cost of the ingredients to prepare it was calculated as £0.07 per litre of concentrate (UK-based prices, Q3 2007) which is sufficient to make 100 litres of working strength synthetic sewage.

5 EFFECTS OF DILUTION ON REMOVAL RATES AND PERFORMANCE OF BATCH-FED WASTE STABILISATION SYSTEMS

5.1 Introduction

The literature review discussed a number of factors that affect how rapidly the pollutant peak in spring is reduced in a variable climate WSP. These include temperature, light, organic loading, and nutrient status. The research presented concerns whether the rate of pollutant breakdown in spring can be increased by changing the organic load through dilution, and looks at some effects of light and temperature on the rate.

5.2 Experimental design

To assess the potential of dilution for promoting early purification, experiments were carried out on batch-fed systems based at the University of Southampton, UK (Table 5.1). To ensure a feedstock that was both reasonably consistent and at the same time resembled a natural wastewater, the experiments were carried out using the synthetic sewage formulated as described in Chapter 4. In the first experiment, 2-litre bottles containing synthetic wastewater at different concentrations were set up under natural light inside a glasshouse. This was followed by an experiment using 25-litre buckets, positioned outside and operated at a slightly wider range of loading rates, to replicate more closely the depth effects associated with WSP. Experiments in 1-litre flasks were carried out under controlled conditions to test the effect of a second variable of light input. 1000-litre tank experiments then followed operating under variable temperature but constant light intensity. Using this experimental set-up it was possible to simulate temperature change during the spring warm-up in a seasonally variable climate.

Experiments were operated in batch mode so that each run started with a specific COD and the relative rate of removal over time could be evaluated. The dilutions used were designed to imitate a potential operating protocol in large-scale storage maturation ponds where untreated accumulated winter wastewater could be diluted by a volume of stored pond water at the start of spring. Removal rates were calculated on a rolling basis at 2, 4, 6 and 8 days or 5, 10, 15 and 20 days using equation *3.3*. Overall removal rates were calculated based on the initial and the lowest stable concentration using equation *3.4*.

Table 5.1 Summary of experimental design for batch-fed experiments

Experiment	Experimental conditions	Dilution	Filtered COD (mg/l)	Replicates	Controls
Bottles	Glasshouse	1:50	491 ± 28	3	2
	Variable : load (altered	1:100	251 ± 18	3	2
	by dilution);	1:200	134 ± 3	3	2
	Runs x 3	1:400	84 ± 9	3	2
Buckets	Outside	1:50	868 ± 5	2	0
	Variable: load (increased	1:100	450 ± 3	2	0
	initial concentration)	1:200	202 ± 2	2	0
	with depth effects	1:400	118 ± 7	2	0
Flasks	Controlled conditions	1:50	483 ± 32	6	3
	Variable: load and light;	1:100	240 ± 22	6	3
	(24.0h, 12:12h, 6:18h,	1:200	143 ± 11	6	3
	0:24h daylengths x 2)	1:400	86 ± 12	6	3
Tanks	Controlled conditions	1:50	424 ± 7	2	0
	Variable : load whilst	1:100	227 ± 17	2	0
	simulating spring warm-	1:200	145 ± 20	3	0
	up (temperature only)	1:400	91 ± 3	2	0

5.3 Bottle experiments

As an initial experiment to determine whether dilution had an effect on COD removal rate and other parameters related to treatment performance, 20 clear 2-litre polyethylene terephthalate (PET) batch-fed bottles were set up in a glasshouse under natural light. The height and open surface area of the bottles were 27 cm and 65 cm^2 respectively. Each bottle had the top section removed leaving a working volume of 1750 ml. Four of the bottles were covered with black bin liners and a cardboard box and four with just the cardboard box to provide dark variants: light was still recorded in these, at a mean intensity of approximately 5 and 15 μmol m^{-2} s^{-1} respectively. Three experimental runs were carried out during July and August 2007, each over a period of 10 days. Bottles were started in triplicate using dilutions 1:400, 1:200, 1:100 and 1:50 which gave mean filtered COD concentrations of 84 mg l^{-1}, 134 mg l^{-1}, 251 mg l^{-1} and 491 mg l^{-1}. An algal inoculum of 35 ml was used representing 2% of the total volume. Light intensity and temperature was monitored every five and ten minutes respectively. The light intensity was measured using a type BPW 21 photodiode (RS components, Corby, UK) calibrated against a RC/0308 standard photovoltaic cell (PV Systems, Cardiff, UK). Photodiode outputs were sampled at one second intervals and readings were averaged over a five minute period using an EasyLogger (Lascar Electronics, Salisbury, UK). Output was measured in volts and converted into W m^{-2}. The air temperature was recorded every 10 minutes by a Thermochron iButton (Maxim Integrated Products, California, USA). Samples were taken without shaking between 09:00 and 10:00 on alternate days for a period of 10 days and analysed for filtered COD, chlorophyll-a, pH, absorbance at 440 nm and 678 nm and dissolved oxygen levels in mg l^{-1}.

5.3.1 Light intensity and temperature

Table 5.2 shows the mean, maximum and minimum light intensity and temperature observed during each run which varied only marginally with each run. Mean light intensities were calculated from daylight hours only on data from both outside and inside the glasshouse. Average daylength is also shown. Using the mean inside light intensity data and average daylength it was possible to calculate an approximate total daily light input for each run. Results for each run were very similar, although Run 2 recorded the highest amount of light energy per day with a total daily light input of 7.61 mol m^{-2} d^{-1}.

Table 5.2 Environmental conditions during each run

	Inside light intensity (µmol m^{-2} s^{-1})	Outside light intensity (µmol m^{-2} s^{-1})		Average daylength (hours)	Total daily light input (mol m^{-2} d^{-1})	Temperature (°C)		
Run	**Mean**	**Mean**	**Max**			**Mean**	**Max**	**Min**
1	144	540	1995	14.5	7.51	23.5	45	14.5
2	150	583	1918	14.1	7.61	22.9	49	10.5
3	149	591	1817	13.5	7.24	22.7	49	11.0

5.3.2 COD removal

Figure 5.1 shows COD concentration against time, while Table 5.3 and Figure 5.2 show filtered COD removal rates for day 2, 4, 6 and 8 during each experiment.

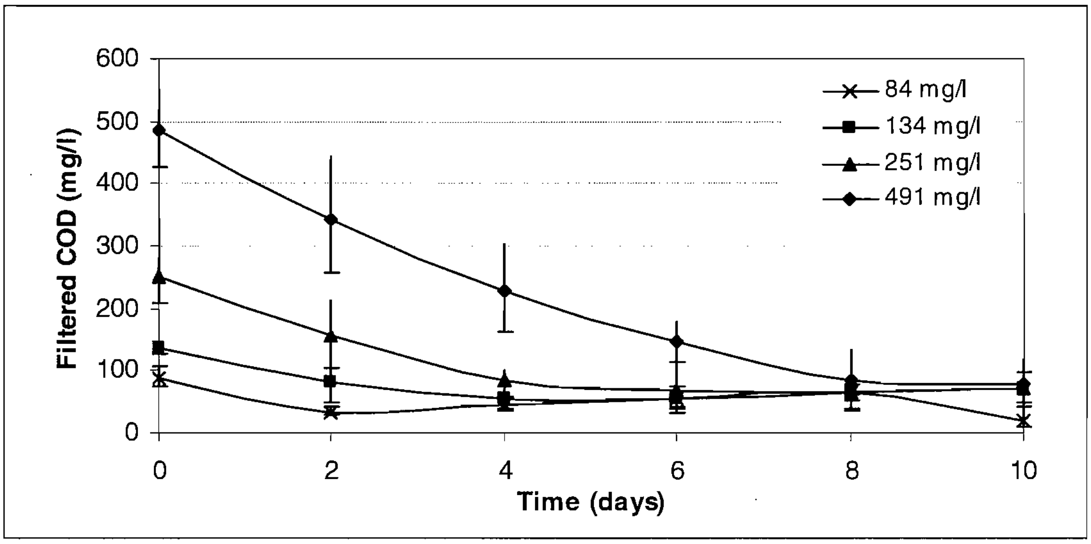


Figure 5.1 Decrease in filtered COD concentration over time for different initial COD concentrations

Results were similar for all runs reflecting consistency in environmental conditions. Removal rates on day 2 show a pattern of increase with decreasing concentration. After day 2, removal rates start to fall and negative values to appear, at first in low initial concentrations and then in successively higher concentrations. This is considered to be due firstly to the available substrate being used up; and secondly to the growth then decline of an algal population leading to release of soluble products. Removal rates for 491 mg COD l^{-1} remained consistent during the experiment and showed the least variation between runs. Negative removal rates were not observed for the highest initial concentration.

Table 5.3 Mean filtered COD removal rate (g g^{-1} d^{-1}) with standard error* for days 2, 4, 6 and 8 for the different initial concentrations

Mean initial concentration (mg COD l^{-1})	**Day 2**	**Day 4**	**Day 6**	**Day 8**
84	**0.30**	**-0.22**	**-0.10**	**-0.20**
S.E	(0.01)	(0.04)	(0.06)	(0.05)
134	**0.27**	**0.06**	**-0.04**	**-0.15**
S.E	(0.02)	(0.03)	(0.06)	(0.04)
251	**0.26**	**0.24**	**-0.03**	**-0.06**
S.E	(0.02)	(0.03)	(0.04)	(0.04)
491	**0.20**	**0.19**	**0.21**	**0.11**
S.E	(0.01)	(0.02)	(0.03)	(0.04)

*NB. Standard error in brackets (n = 9)

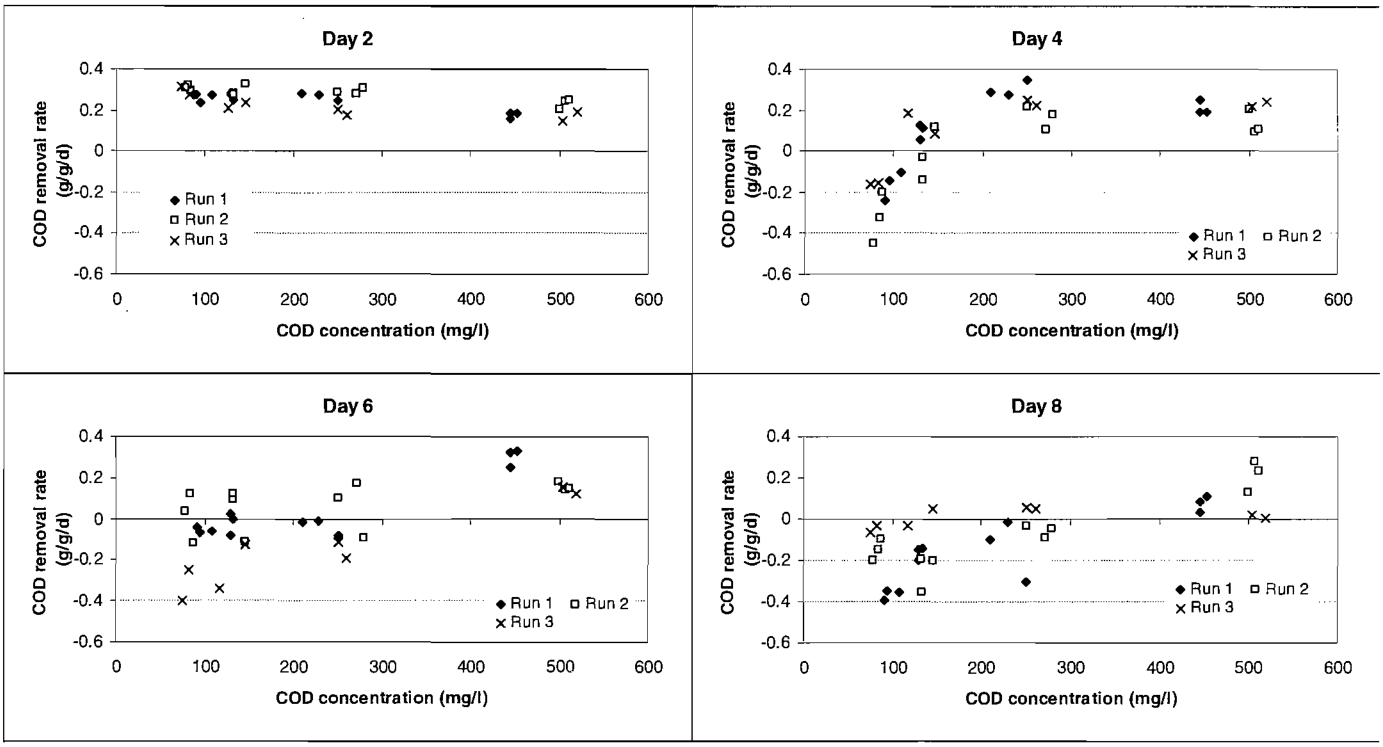


Figure 5.2 2, 4, 6, and 8-day COD removal rates for different initial COD concentrations

Figure 5.3 shows COD removal rates on day 2 with regression lines for each run. Table 5.4 shows results of the regression analysis. This varied little between experiments with Run 1 showing the highest rate of change of COD removal at 0.0003 g g^{-1} d^{-1} per mg l^{-1} and an overall average of all runs of 0.00023 g g^{-1} d^{-1} per mg l^{-1}.

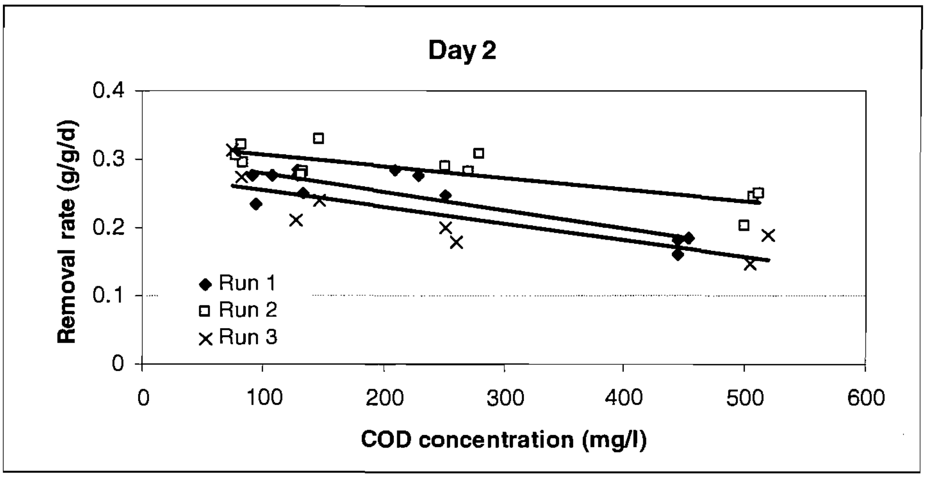


Figure 5.3 COD removal rate in all bottles on day 2 for each run (with regression lines)

Table 5.4 Regression analysis for COD removal on day 2

Run no.	Slope*	R^2	p-value
1	-0.0003	0.74	<0.001
2	-0.0002	0.66	0.001
3	-0.0002	0.62	0.02

*Rate of change of removal ($g\ g^{-1}\ d^{-1}$ per $mg\ l^{-1}$)

Considering the regression analysis results for day 2, it can be seen that concentration does have an effect on removal rate e.g. it accounts for 62 – 74% of variation, and the rate of change in removal rate with concentration is similar in the 3 runs. Results indicate that COD concentration does affect removal, but under these conditions (batch experiments with limited initial input of substrate, transparent bottles that allows light to penetrate from all directions into a small volume of water, and an advantageous time of year) the practical effect is small as they all clean up very quickly; but it might be more evident in real/continuous systems under more adverse conditions.

5.3.3 Chlorophyll-a

Figure 5.4 shows mean chlorophyll-a concentration during all bottle experiments. As load increased, maximum recorded chlorophyll-a levels decreased. Maximum concentration was reached on day 4 for 84 and 134 mg COD l^{-1} with levels between 1 and 1.8 mg l^{-1} attained in bottles receiving 84 mg COD l^{-1}. Initial concentration 491 mg COD l^{-1} showed a steady rise in chlorophyll-a stabilising at an average of 0.5 mg l^{-1} for the first two runs. Higher chlorophyll-a levels up to 0.9 mg l^{-1} were observed for Run 3. The variation was high for all COD concentrations on day 4 and 6 when maximum chlorophyll-a levels were obtained: slight variation in light intensity may have been responsible for this (see Table 5.2) Chlorophyll-a concentration gradually declined from day 4 in three of the four initial concentrations used. This coincided with the algal content settling to the bottom.

Nutrient limitation may have accounted for the decline seen in substrate concentrations 84, 134 and 251 mg COD l^{-1} as light was not a limiting factor. Decline of the algal population appeared to occur at a fairly constant rate which is consistent with the phase of exponential decay associated with batch-growth curves when availability of substrate in the medium is reduced (Gaudy and Gaudy, 1980). Some of the organic suspended solids representing the algal population would have been converted into soluble organic matter by hydrolysis. This coincided with the rise in filtered COD and the inherent negative removal rates noted above. Chlorophyll-a levels for 491 mg COD l^{-1} stabilised without the subsequent decline that occurred during the other COD concentrations. This may have been due to the lack of initial rapid growth in algae and consequently the availability of sufficient nutrients, as well as having a greater concentration of nutrients at the start.

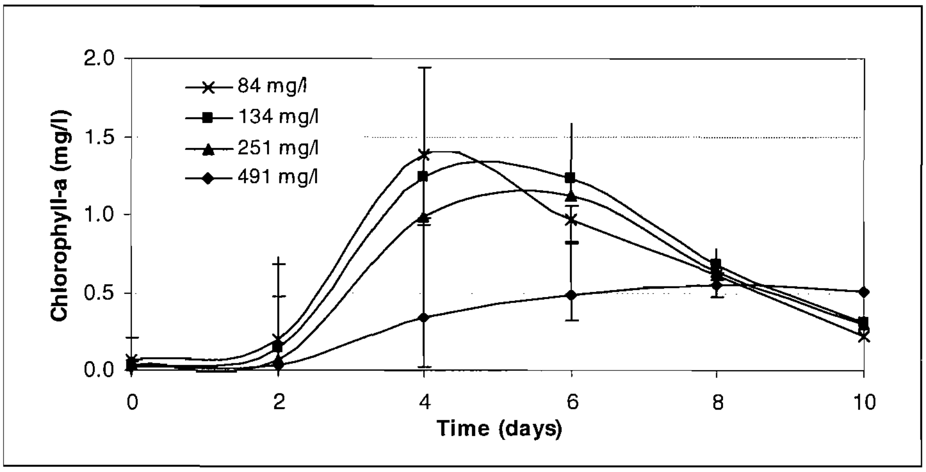


Figure 5.4 Change in mean chlorophyll-a levels over time for each initial COD concentration (with range bars)

The apparent growth rate of algae as measured by chlorophyll-a concentration was calculated for each substrate concentration for every two day's incubation (Table 5.5). The experiment was not specifically designed to establish algal growth rates and there are insufficient points clearly to identify the start and end of the exponential phase, therefore where growth rate was most rapid between days 2 and 4 in all bottles an estimate of the minimum apparent growth rate could be made. Apparent growth rate was highest in the bottles receiving a mean initial concentration of 134 mg l^{-1} up to day 2 with the higher COD of 251 mg l^{-1} having the greatest specific growth rate of 0.055 h^{-1} between days 2 and 4. Mean chlorophyll-a levels were highest in the lowest strength wastewater, although apparent growth rates were the lowest (0.040 h^{-1}) between days 2 and 4. By day 2 however, chlorophyll-a in the flasks had already started to rise.

Table 5.5 Effect of initial concentration on apparent algal growth rate

Mean initial concentration (mg filtered COD l^{-1})	Mean estimated growth rate (h^{-1})				
	Day 2	Day 4	Day 6	Day 8	Day 10
84	0.024	0.040	-0.007	-0.009	-0.022
134	0.031	0.045	0.000	-0.012	-0.017
251	0.018	0.055	0.003	-0.012	-0.016
491	0.008	0.046	0.007	0.003	-0.002

5.3.4 pH

Figure 5.5 shows mean pH values for all filtered samples. Variation between runs was low except on day 4 when the highest variation in chlorophyll-a levels was also observed. pH values showed an initial decrease on day 2 falling below pH 7 for all loads except 84 mg COD l^{-1}. This could be a result of the higher load leading to increased bacterial growth resulting in a decrease in pH through the release of acidic metabolites (e.g. organic acids, H_2SO_4) (Bitton, 2005). On day 4, pH levels rose to between 8 and 9 for lowest loads corresponding to the rise in chlorophyll-a levels and the presence of measurable oxygen. pH values above 8 were recorded on day 6 for initial concentrations 251 and 491 mg COD l^{-1}. This reflected the subsequent rise to maximum chlorophyll-a and dissolved oxygen levels observed in the higher loaded systems.

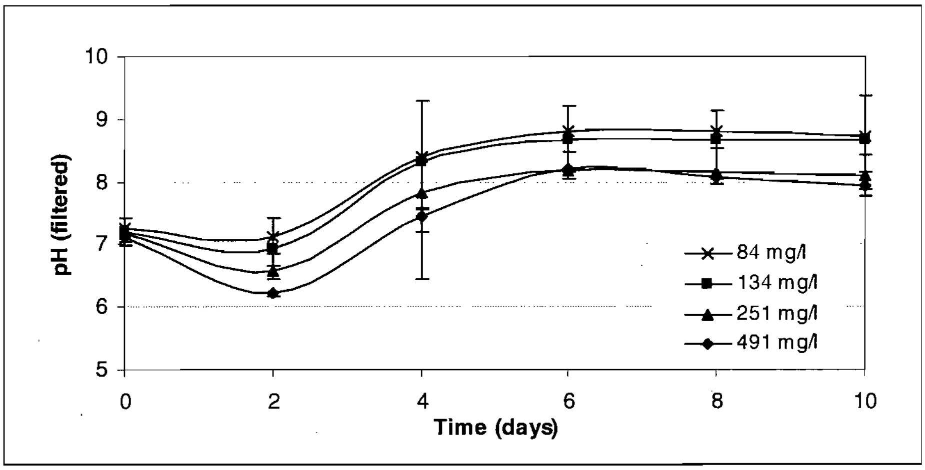


Figure 5.5 Change in mean filtered pH values over time for each initial COD concentration

5.3.5 Dissolved oxygen

Dissolved oxygen concentrations (Figure 5.6) followed the same trend as chlorophyll-a levels with highest levels measured for initial concentration 84 and 134 mg COD l^{-1}. Both these systems were oxygen saturated by day 4. 251 mg COD l^{-1} peaked at between 7 and 8 mg l^{-1} on day 6.

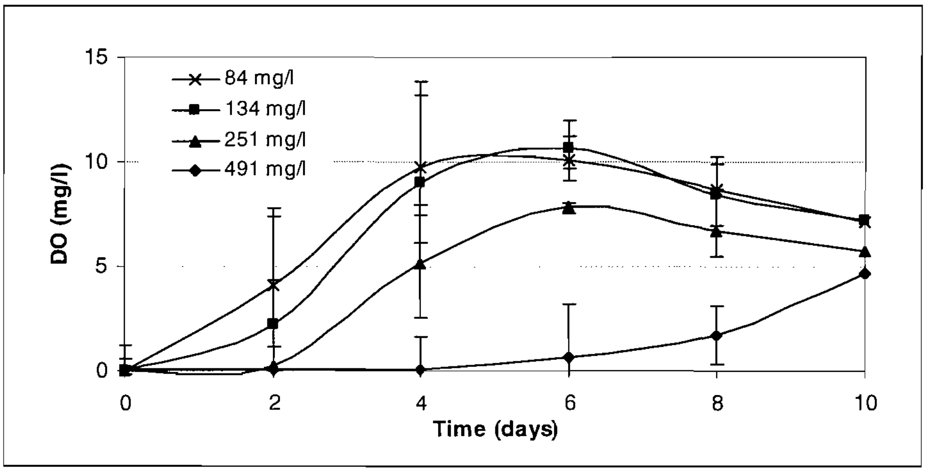


Figure 5.6 Change in mean dissolved oxygen levels over time for each initial COD concentration (with range bars)

Corresponding to the fall in chlorophyll-a levels, oxygen levels in all three substrate concentrations subsequently declined. In contrast to this, 491 mg COD l^{-1} showed a gradual increase in dissolved oxygen peaking on the final day at 5 mg l^{-1}. This is reflective of the steady rise in algal growth observed at the highest load. The results for chlorophyll-a, pH and dissolved oxygen all suggest that the algal population consumed the pollutants, bloomed and subsequently died and was itself contributing to COD concentration and creating negative removal, thereby supporting the interpretation of the COD data.

5.3.6 Absorption

Figure 5.7 shows the relationship between chlorophyll-a and ratio of fluorescence induced at 440 nm and 678 nm for each initial COD concentration. By convention, 440 nm is used as a reference wavelength for coloured dissolved organic matter and non-algal particulate absorption and often 676 nm for chlorophyll-a (Kirk, 1994). To obtain an estimate of any increase in chlorophyll-a, samples were measured at 678 nm following the work of Bartosh (2004).

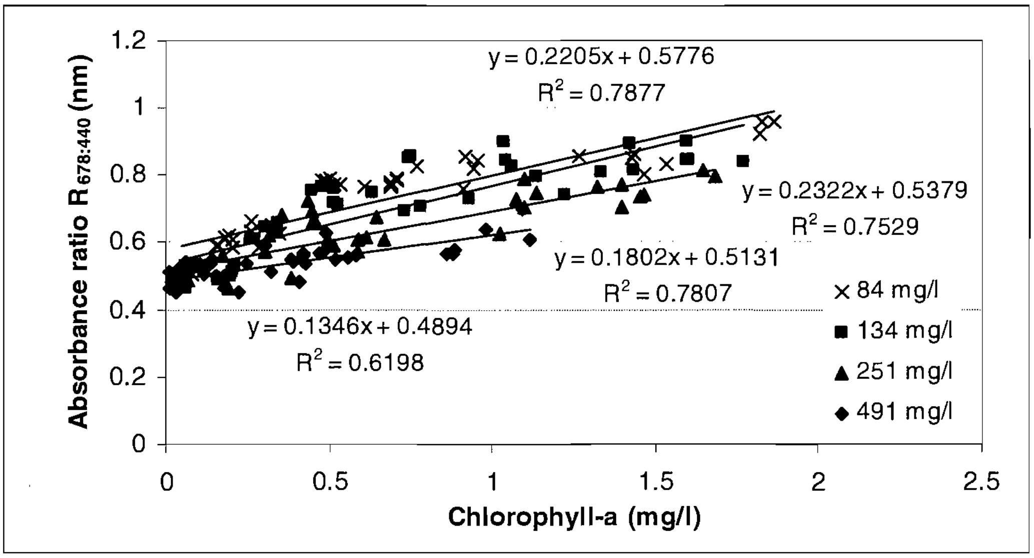


Figure 5.7 Relationship between chlorophyll-a and absorbance ratio ($R_{678/440}$) for each initial COD concentration

Results showed significant relationships between chlorophyll-a levels and unfiltered absorbance ratio $R_{678:440}$ ($p < 0.001$). Ratio $R_{678:440}$ was at its minimum between 0.4 and 0.5 at the start of each experiment and increased with the rise in chlorophyll-a levels. The associated decrease in organic matter utilised by bacterial activity reduced the absorption spectra at 440 nm. This was most apparent in the bottles with the lowest load where $R_{678:440}$ rose to 0.96. In contrast to this, the highest ratio calculated for the highest load was 0.7. The results support the effect of dilution and reduced load on the capacity for algal growth and reduction in humic content. Figure 5.7 also suggests that the rate of change of absorbance ratio with chlorophyll-a concentration depends on the concentration of organic matter as indicated by initial COD concentration, with values ranging from 0.13 to 0.23 nm^{-1} although initial concentration 84 mg COD l^{-1} had a slope of 0.22 nm^{-1}, slightly less than that recorded for 134 mg COD l^{-1}. The increase in absorbance ratio $R_{678:440}$ from the beginning of the experiment to the end emphasises the shift from a system low in chlorophyll-a biomass to one with a high algal content and low in humic matter.

5.3.7 Dark variant bottles

5.3.7.1 COD removal

Removal rates for dark variant bottles are shown in Table 5.6 which seem to show the same pattern of declining and eventually negative removal rate with time. Reducing light penetration into the vessels had a slight negative effect on removal although unlike the bottles in full light there was no clear relationship between removal rate and concentration on day 2. It is interesting that there is removal even at these very low light intensities; some of this may be due to simple physical methods like sedimentation but results below on chlorophyll-a, pH and dissolved oxygen suggest removal taking place is due to algal oxygen production too.

Table 5.6 Mean filtered COD removal rate (g g^{-1} d^{-1}) for days 2, 4, 6 and 8 for the different initial concentrations in the dark variant bottles

Mean initial concentration (mg COD l^{-1})	Day 2	Day 4	Day 6	Day 8
70 (1)*	0.21	0.09	-0.03	-0.11
*66 (2**)*	0.21	0.14	0.02	-0.03
155 (1)	0.20	0.16	0.08	-0.07
134 (2)	0.21	0.17	0.12	-0.02
268 (1)	0.20	0.20	0.15	-0.04
264 (2)	0.20	0.19	0.18	-0.03
523 (1)	0.16	0.18	0.16	0.14
513 (2)	0.17	0.18	0.16	0.16

*15 μmol m^{-2} s^{-1}; ** 5 μmol m^{-2} s^{-1}

Figure 5.8 shows the overall mean removal rates for both dark variant bottles and those in full light as calculated using the lowest recorded filtered COD values during each run. As removal rates were very similar between the two dark variant bottle conditions, averages were taken using both results.

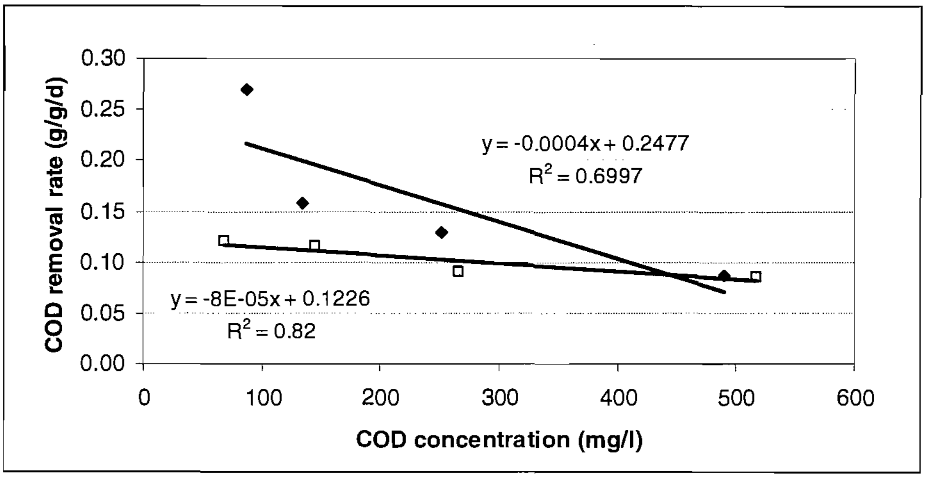


Figure 5.8 Overall mean COD removal rate in light (♦) (p = 0.163); dark variant bottles (□) (p = 0.017) (with regression lines)

Results show that the mean overall removal rate was affected by the reduction in light intensity. This was most apparent in bottles containing the lowest initial concentration where maximum removal rates were 0.27 g g^{-1} d^{-1} in full light compared to 0.12 g g^{-1} d^{-1} for the dark bottles. Use of a t-test to compare the rate of change in removal rate with respect to concentration for the light and dark conditions showed no statistically significant difference ($p > 0.05$). This may be due to the decrease in variation between removal rates with increasing COD concentration. For example, results for the highest initial concentration remained the same (0.09 g g^{-1} d^{-1}) for both the dark variant and light bottles.

5.3.7.2 Chlorophyll-a

Figure 5.9 shows the chlorophyll-a concentration for the two sets of dark variant experiments. The difference in chlorophyll-a levels from these and the bottles positioned in full light is apparent, with values reaching just a quarter of the concentration in full light. A light intensity of 15 μmol m^{-2} s^{-1} gave a chlorophyll-a concentration of 0.35 mg l^{-1} for the mean initial COD of 145 mg l^{-1}, however, with values of 0.27 and 0.25 mg l^{-1} for 68 and 266 mg COD l^{-1} respectively: these values are as high as observed in some WSP providing treatment, which therefore helps to confirm the idea that some removal in the dark variant bottles is due to algal oxygenation. Chlorophyll-a concentration in the highest COD remained below 0.10 mg l^{-1}. Chlorophyll-a levels were somewhat lower under a light intensity of 5 μmol m^{-2} s^{-1} with all initial concentrations measuring not more than 0.10 mg l^{-1}. The pattern of growth also differs from the bottles under full light. Figure 5.4 shows a growth pattern that appears to include an exponential phase followed by a declining phase, whereas the algae receiving less light exhibited a much slower growth rate, only increasing more rapidly from day 8 in the bottles receiving the three lowest initial COD concentrations. The inhibited growth could have been a symptom of reduced nutrient uptake caused by low light intensity which was not in evidence in the bottles under full light. This suggestion is supported by the work of Viner (1984) who carried out laboratory batch experiments to investigate the effect of sustained light and temperature on the uptake of phosphate, nitrate and ammonia and on the biomass of the phytoplankton of Lake Rotongaio in New Zealand. Given sufficient light (14.4 to 68 μmol m^{-2} s^{-1}) nitrogen and phosphate nutrients were rapidly consumed with nitrate and ammonia being reduced to very low levels in a period of two to five days. Below this light level, nutrient uptake was severely delayed. Growth rates were also shown to increase with light intensity. The findings of Viner (1983) also support the argument that nutrient limitation was the predominant factor in the rapid decline in algal growth and onset of a death phase.

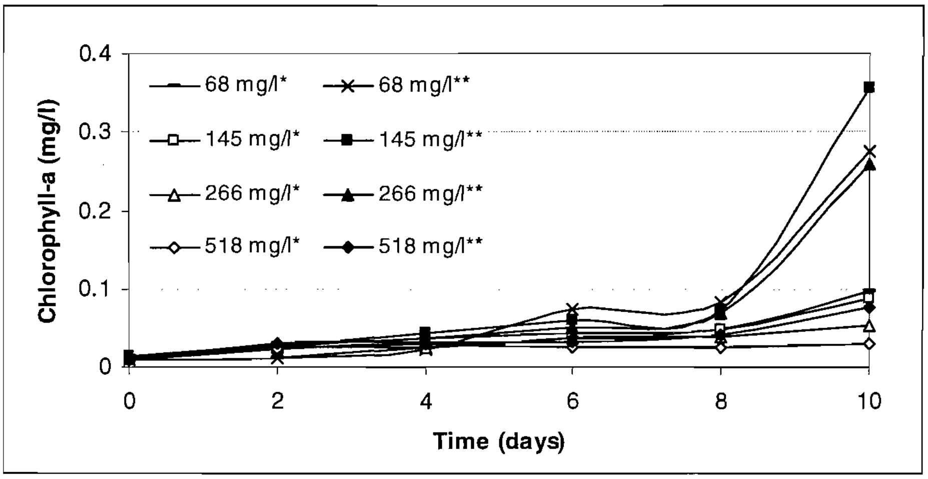


Figure 5.9 Change in mean chlorophyll-a levels for dark variant bottles (*5 μmol m^{-2} s^{-1}; **15 μmol m^{-2} s^{-1})

5.3.7.3 Dissolved oxygen

Figure 5.10 shows mean dissolved oxygen levels in the control bottles for each initial concentration corresponding to light intensities of 5 and 15 μmol m^{-2} s^{-1}. There was a clear difference in the level of measurable oxygen influenced both by light intensity and COD concentration. Comparing Figure 5.10 with Figure 5.6 shows that the decrease in light intensity from full light conditions results in a clear reduction in the oxygen within the system. A reduction in light intensity from 15 to 5 μmol m^{-2} s^{-1} also reduced the oxygen and this is most apparent in the higher loaded systems where the rate of oxygen consumption imposed by the heavier load was too high to be satisfied by insufficient light energy driving the photosynthetic process.

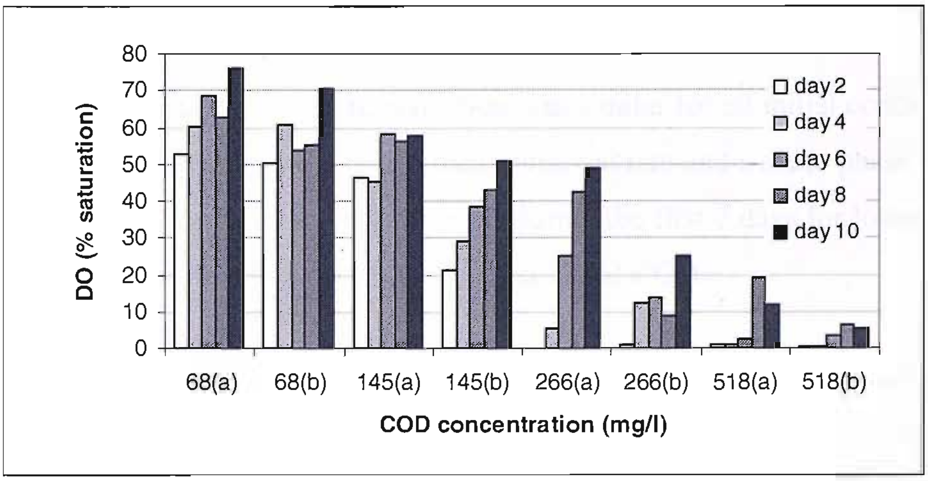


Figure 5.10 Change in dissolved oxygen levels for different initial COD concentrations in the dark variant bottles ([a]15 μmol m^{-2} s^{-1}; [b]5 μmol m^{-2} s^{-1})

5.4 Bucket experiments

Following the bottle tests, bucket experiments were carried out to better simulate small-scale depth effects found in WSP. They were run at a slightly wider range of initial concentrations as the results from the bottle experiment showed the purification process at low loadings was very rapid. Eight buckets (Solent Plastics, UK) were placed outside in Southampton near a south-facing wall free from shadow between 10:00 and 16:00. The height, diameter and surface area of the buckets were 40 cm, 34.5 cm and 935 cm^2 respectively, giving a total volume of 27.5 l and an effective volume of 25 l with a water depth of 36 cm. One experimental run was carried out at the end of April-May 2007. Buckets were batch-fed in duplicate using synthetic wastewater with mean COD concentrations of 118 mg l^{-1}, 202 mg l^{-1}, 450 mg l^{-1} and 868 mg l^{-1}. A volume of 500 ml of algal inoculum was used, which represented 2% of the total volume. Duplicate samples were taken as static dips between 09:00 and 10:00 on alternate days and analysed for filtered COD, suspended solids, ammonia, nitrate, orthophosphate, chlorophyll-a, pH and absorbance at 440 nm. Net and gross photosynthetic oxygen production were determined. During the experimental period, air temperature varied from a minimum of 3.7°C at night to a maximum of 27.5°C during the day with a mean value of 14.9°C. Solar irradiance was at continually high levels throughout the experiment reaching 1400 μmol m^{-2} s^{-1} every day except for three days in the middle of the run, with a mean daily irradiance of 705 μmol m^{-2} s^{-1}.

5.4.1 COD removal

Figure 5.11 shows the pattern of COD reduction was similar for all initial concentrations tested, consisting of a phase with a fairly steady removal rate and a static phase with no further removal. COD removal mainly occurred during the first 7 days for lower initial concentrations and the first 15 days for the highest initial COD.

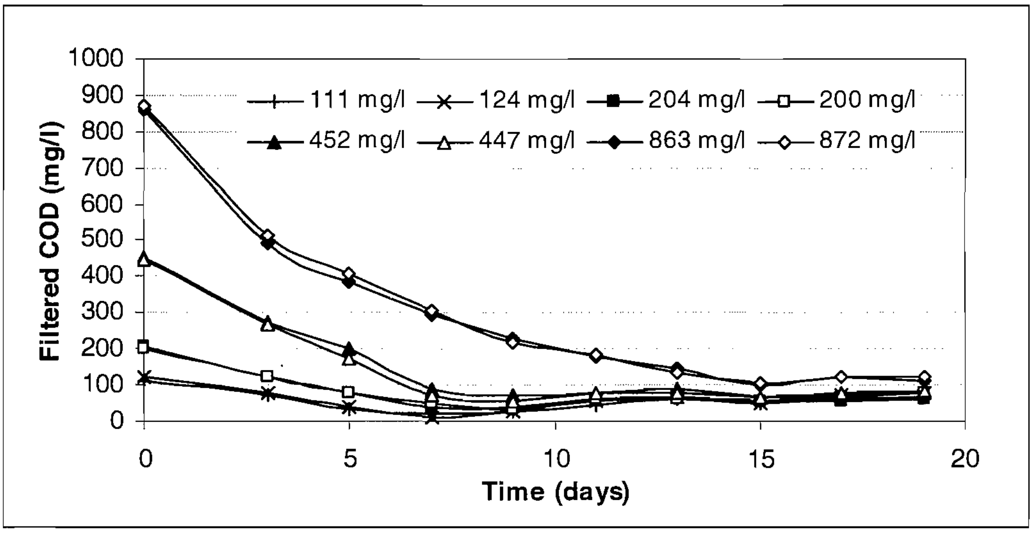


Figure 5.11 Decrease in filtered COD concentration over time for different initial COD concentrations

Results of the batch experiment showed that COD removal was achieved in all buckets, with removal efficiencies in the range 75-90%. Overall removal efficiencies were calculated based on the number of days required for COD concentration to reach a stable value (see Table 5.7).

Table 5.7 Overall percentage COD removal efficiencies for all buckets

Bucket No.	Initial concentration (mg filtered COD l^{-1})	Overall % COD removal*
1	111	88% (15)
2	123	88% (15)
3	204	80% (7)
4	200	84% (7)
5	452	80% (7)
6	447	75% (7)
7	863	77% (7)
8	872	90% (7)

* Figure in brackets gives total number of days over which % removal calculated

Figure 5.12 shows the relationship between COD removal rate and initial concentration on days 5, 10 and 15 as well as the overall removal rate. On day 5, although the removal rate of 0.14 g g^{-1} d^{-1} was calculated for the buckets with the mean initial concentration of 118 mg l^{-1}, a removal rate of 0.11 g g^{-1} d^{-1} was also observed for the buckets with the much greater mean concentration of 868 mg l^{-1}. The difference between removal rates for different initial concentrations was not large and this may have been due in part to the high light intensities observed during the run. Negative removal rates were seen on day 10 for the lowest initial concentration and by day 15 in the buckets with 202 mg COD l^{-1}. The appearance of negative removal rates occurred much later than in the bottle experiments; the difference may be due to higher initial concentrations and reduced light penetration into the buckets. The overall COD removal rate in the buckets showed a linear relationship and an inverse correlation with initial COD concentration ($R^2 = 0.77$; $p = 0.004$). Overall removal rates for 118 mg COD l^{-1} were 0.012 g g^{-1} d^{-1} compared to 0.06 g g^{-1} d^{-1} for 868 mg COD l^{-1} showing only a 2-fold increase in the rate of removal for a 7-fold reduction in filtered COD.

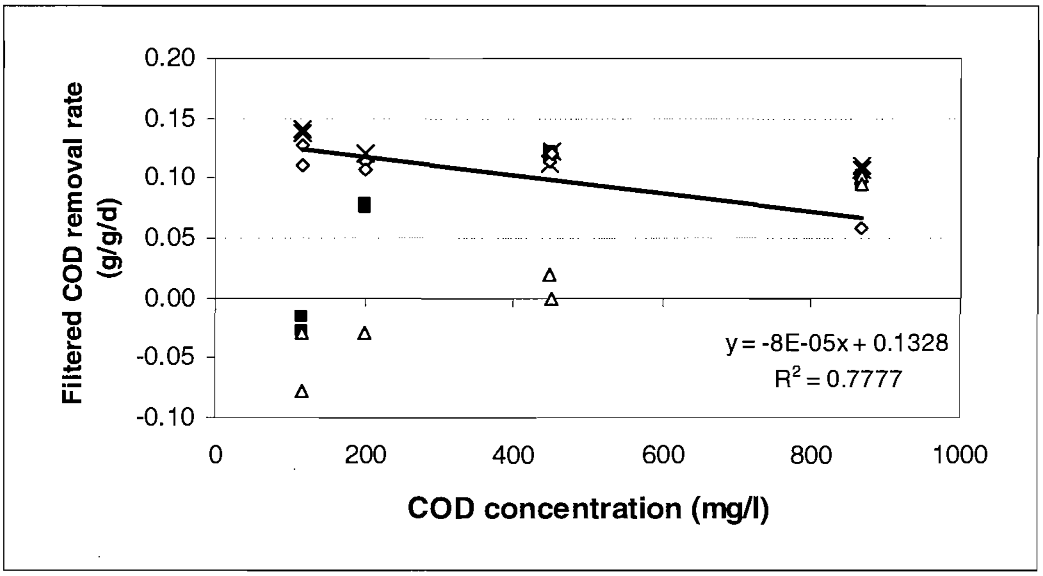


Figure 5.12 5-d (×), 10-d (■), 15-d (△) and overall (◊) COD removal rate (with regression line)

Table 5.8 shows the output of regression analyses from the results in Figure 5.12. Regression coefficients showed the poorest correlation between COD removal rate and initial concentration on day 10 ($R^2 = 0.40$; $p = 0.095$). This was due to the transition between positive to negative removal rates in the lower loaded systems. By day 15, removal rates had reduced to zero or had become negative in three of the four substrate concentrations. Only removal rates for the highest loaded buckets remained positive, creating a strong relationship between removal rate and initial COD concentration (R^2 = 0.95; $p < 0.001$). Although the overall rate in the bottles was found to be not very linear, the actual rate of change of overall COD removal rate with respect to concentration was five times less in the buckets than that calculated for the bottle run, at 0.00008 g g^{-1} d^{-1} per mg l^{-1} compared to 0.0004 g g^{-1} d^{-1} per mg l^{-1}. This may be due to depth effects and reduced light penetration in the buckets.

Table 5.8 Regression analysis for COD removal rate on days 5, 10, 15 and overall

Days	Slope*	R^2	p-value
5-d	-0.00003	0.67	0.012
10-d	0.0001	0.40	0.095
15-d	0.0002	0.95	<0.001
overall	-0.00008	0.77	0.004

*Rate of change of removal (g g^{-1} d^{-1} per mg l^{-1})

5.4.2 Chlorophyll-a

Figure 5.13 shows chlorophyll-a levels in all buckets. A lag was observed until around day 5-6 in all of the buckets except those with the highest load, which had a slightly longer lag phase to day 8-10. The highest chlorophyll-a concentration was measured in the buckets receiving the mean initial load of 202 mg COD l^{-1} peaking at 1.3 mg l^{-1} on day 13. Levels in buckets with 450 mg COD l^{-1} also peaked on the same day at a slighter lower concentration. Chlorophyll-a concentrations peaked at 0.75 mg l^{-1}on day 11 in the buckets that started with the lowest load. Buckets with the highest starting load showed a rapid rise from 0.3 mg l^{-1} to between 1 and 1.2 mg l^{-1} from days 11 and 13. The algal population then showed a decline in all buckets. A second bloom was observed in all four buckets receiving the highest dilutions which could have been due to organic matter contributions being augmented by nutrients released from the decay of former plankton populations. This behaviour was not seen in the shorter-duration bottle and flask experiments where only a reduction in chlorophyll-a was observed following the initial growth phase; thereby supporting the idea that there was algal die-off after the first peak in the previous experiments. As pond biology was not assessed in detail it is impossible to determine whether any algal predators such as rotifers, *Culex* and *Paramecium* were present. As a similar pattern of chlorophyll-a growth was observed for each experiment it was unlikely to be a cause of the decline in algae.

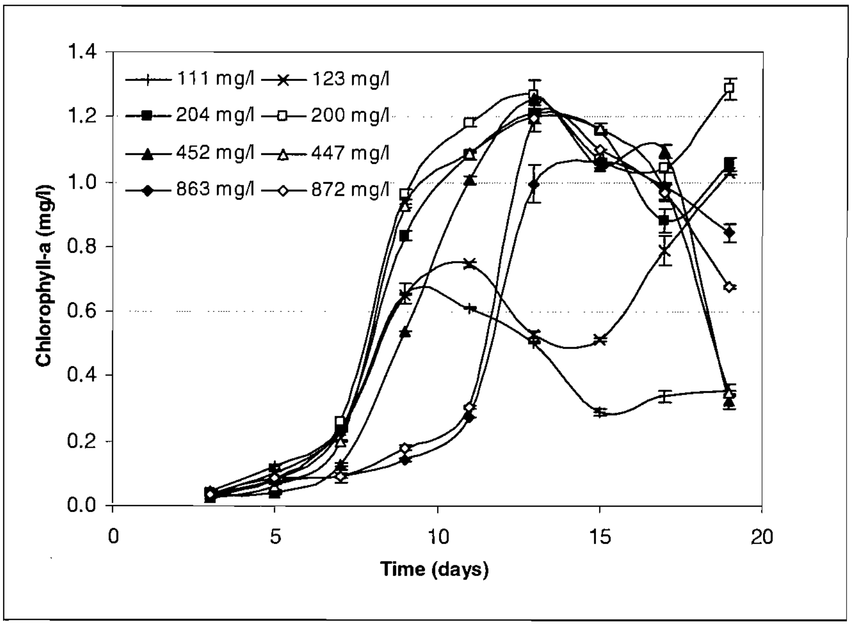


Figure 5.13 Change in chlorophyll-a concentration over time for different initial COD concentrations

The apparent growth rate of algae was calculated for each substrate concentration over the course of the experiment (Figure 5.14) and during the most rapid phase of algal growth (Table 5.9) which was between days 7 and 9 for the three lowest starting COD concentration and between days 11 and 13 for the highest concentration. A lag phase for the highest load can be seen clearly in Figure 5.14. Maximum apparent growth rates were however similar between loads with three of the four initial concentrations ranging from 0.027 to 0.031 h^{-1}. Although the experiment was not designed to determine specific growth rates, it gave indicative results. The lowest apparent growth rate calculated between days 7 and 9 was exhibited for the mean COD of 118 mg l^{-1} but this may be because the most rapid phase of growth occurred earlier. The apparent specific growth rate measured from the rapid growth phase shows only a marginal difference at different initial COD concentrations. It can be concluded from these results that the substrate concentration appears to have little effect on the growth rate of algae but lengthens the lag phase.

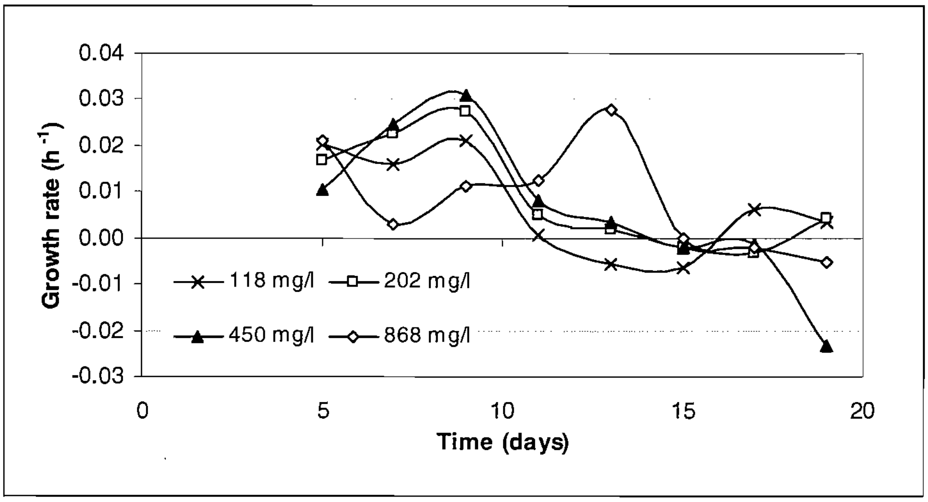


Figure 5.14 Effect of mean initial concentration on algal growth rate

Apparent growth rates measured for the bottle experiments were approximately 1.7 times higher than those calculated for the bucket experiment. They also showed a similar pattern of growth rate increasing with initial COD concentration up to dilution 1:100, then a decline in growth rate for 1:50 dilution. Kayombo *et al.*, (2003) studied the effect of substrate concentration on the growth of a mixed culture of algae and bacteria from secondary facultative ponds using COD concentrations from 200 to 800 mg l^{-1}. The experiments were carried out in batch reactors under fluorescent lighting at an intensity of 80 μmol m^{-2} s^{-1}. Results showed slightly lower specific growth rates to those obtained in this experiment possibly attributed to reduced light intensity but with maximum growth rates also observed between 400–500 mg l^{-1}.

Table 5.9 Effect of initial concentration on apparent algal growth rate

Mean initial concentration (mg filtered COD l^{-1})	**Apparent growth rate (h^{-1})***
111	0.021
123	0.021
204	0.027
200	0.027
452	0.030
447	0.032
863	0.027
872	0.028

*Calculated from the most rapid phase of growth

Based on the data of algal growth and substrate uptake in the period up to the end of the rapid growth phase, the values of the observed microalgae yield given in mg chlorophyll-a produced/mg COD consumed were obtained. The data was plotted against initial COD concentration and are shown in Figure 5.15. The value of the observed yield increased with decreasing substrate concentration and the relationship between the two parameters gave a regression coefficient of 0.80 and a p-value of 0.002. A COD concentration of about 202 mg l^{-1} produced the highest observed yield at about 0.009 mg chlorophyll-a/mg COD. A mean initial concentration of 868 mg l^{-1} produced a six-fold reduction in the observed microalgae yield of 0.0015 mg chlorophyll-a/mg COD. Although chlorophyll-a levels were lowest in the buckets containing the lowest strength wastewater, the quantity of algal growth per amount of COD consumed was highest when the starting substrate concentration was low. The highest observed microalgae yield in the current study was a factor of 10 less than that obtained in a study by Travieso *et al.* (2006b) but this was probably due to the difference in experimental conditions with Travieso *et al.* (2006b) utilising only *Chlorella* which has a high chlorophyll-a content and piggery wastes which are characterised by high concentrations of organic compounds and a good balance of carbon and nutrients. The higher observed yield obtained from a lower starting COD concentration in the current study supports the findings of greater COD reduction earlier in the experiment and an increase in treatment efficiency from dilution.

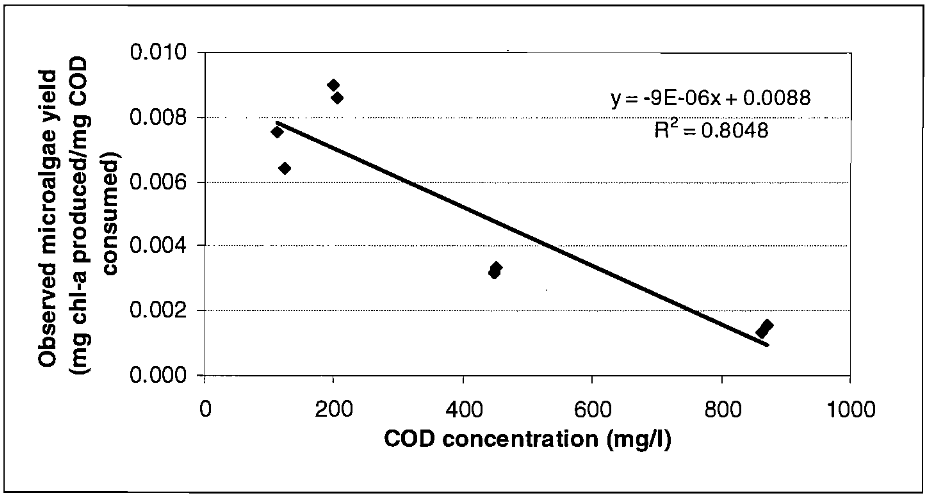


Figure 5.15 Observed microalgae yield for each substrate concentration

5.4.3 Suspended solids

Figure 5.16 shows removal of suspended solids during the run. A 75% reduction was observed for the highest initial concentration during the first five days with values declining from 250 mg l^{-1} to 60 mg l^{-1}. The mean initial concentration 450 mg COD l^{-1} showed a 50% reduction and 202 and 118 mg COD l^{-1} showed an average removal of 35% over five days. In Chapter 4, characterisation of the wastewater showed that settlement of wastewater solids continued up until the test finished on day 5. Suspended solids removal in facultative ponds is a complex subject due to the capacity of the system to produce solids in the form of algal cells. Chlorophyll-a levels increased more quickly in the buckets receiving the lowest load, suggesting higher levels of algal content, and the same rapid reduction in suspended solids concentration was not observed within the experimental period. The growth of an algal population in all buckets is evident in Figure 5.16 where an increase in suspended solids between day 5 and day 10 can be seen. Regression analysis on suspended solids with chlorophyll-a concentration revealed significant relationships. The relationships for each mean COD concentration are shown as equations *5.1*, *5.2*, *5.3* and *5.4*.

$$118 \text{ mg COD } l^{-1}; \quad SS_{out} = 24.29 + 60.61 \text{ Chl-a} \quad (R^2 = 0.69;\ p < 0.001) \tag{5.1}$$

$$202 \text{ mg COD } l^{-1}; \quad SS_{out} = 36.46 + 58.22 \text{ Chl-a} \quad (R^2 = 0.67;\ p < 0.001) \tag{5.2}$$

$$450 \text{ mg COD } l^{-1}; \quad SS_{out} = 55.03 + 36.69 \text{ Chl-a} \quad (R^2 = 0.50;\ p = 0.002) \tag{5.3}$$

$$868 \text{ mg COD } l^{-1}; \quad SS_{out} = 80.56 + 68.59 \text{ Chl-a} \quad (R^2 = 0.60;\ p < 0.001) \tag{5.4}$$

The results suggest that the concentration of effluent suspended solids probably due to algal solids has a linear relationship with initial concentration when plotted for each dilution ($R^2 = 0.98$; $p = 0.009$). For example, effluent suspended solids concentrations in excess of 80 mg l^{-1} in the highest loaded buckets would be due to the contribution of algal content. In comparison, an initial concentration of 118 mg COD l^{-1} demonstrated that only an excess of 24 mg l^{-1} would be due to algal solids. This is in agreement with the results in Figure 5.16 which clearly shows the initial fall in suspended solids followed by the subsequent increase. At the end of the experiment, the rise in suspended solids is notably higher in the buckets containing the highest substrate concentration.

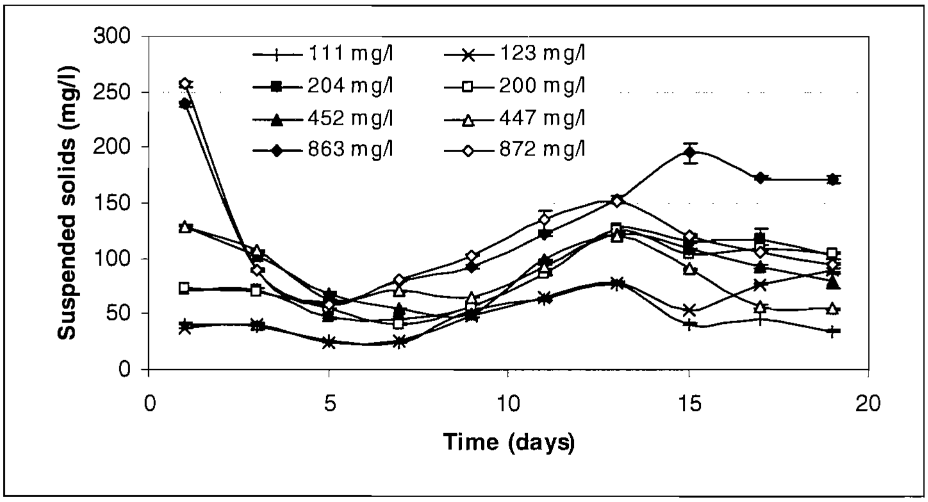


Figure 5.16 Change in suspended solids concentration for each initial COD concentration

Figure 5.17 shows the relationship between chlorophyll-a concentration and ratio of suspended solids to COD concentration. The quotient between suspended solids and COD concentration is a possible measure of the amount of biomass present and therefore correlates strongly with chlorophyll-a content ($p \leq 0.006$). The chlorophyll-a concentration increases linearly with the quotient between suspended solids and COD concentration in all buckets reflecting the increase in biomass as COD is removed. The results indicate that the system is working as expected and further analysis of the parameters measured is capable of revealing its behaviour.

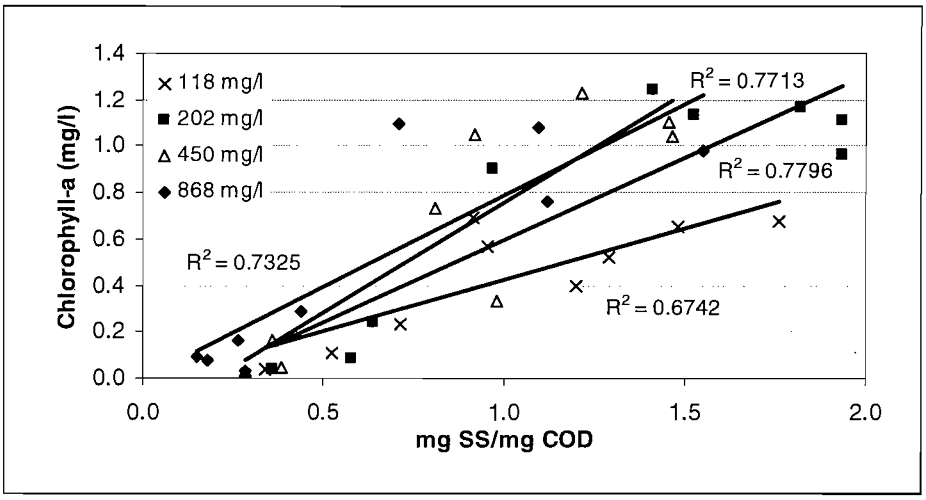


Figure 5.17 Relationship between mean chlorophyll-a levels and ratio of suspended solids to COD concentration

Figure 5.18 shows the positive correlation between suspended solids and absorbance coefficient at 440 nm for all dilutions. This is not unexpected as the suspended particulate matter is the source of the spectral absorbance. R^2 values of 0.82 to 0.95 indicated that about 90% of the variability of the particulate specific absorption coefficient was caused by biogenic matter. p-values <0.001 suggest a good level of correlation between the spectral absorbance at 440 nm on unfiltered samples and suspended solids. Gallegos (2005) reported measurements of absorption with standard water quality parameters in an estuarine environment. Absorption by non-algal particulate matter at 440 nm was plotted against suspended solids, though this was using filtered samples. Unfiltered samples give estimates of 'apparent' colour whereas the values for filtered samples represent 'true' colour. Dilution with treated wastewater is likely to reduce not only suspended solids concentration but could also reduce apparent colour. As this has important consequences on the amount of light energy reaching pond microbial communities, it is likely to be one of the mechanisms producing an effect on pond performance.

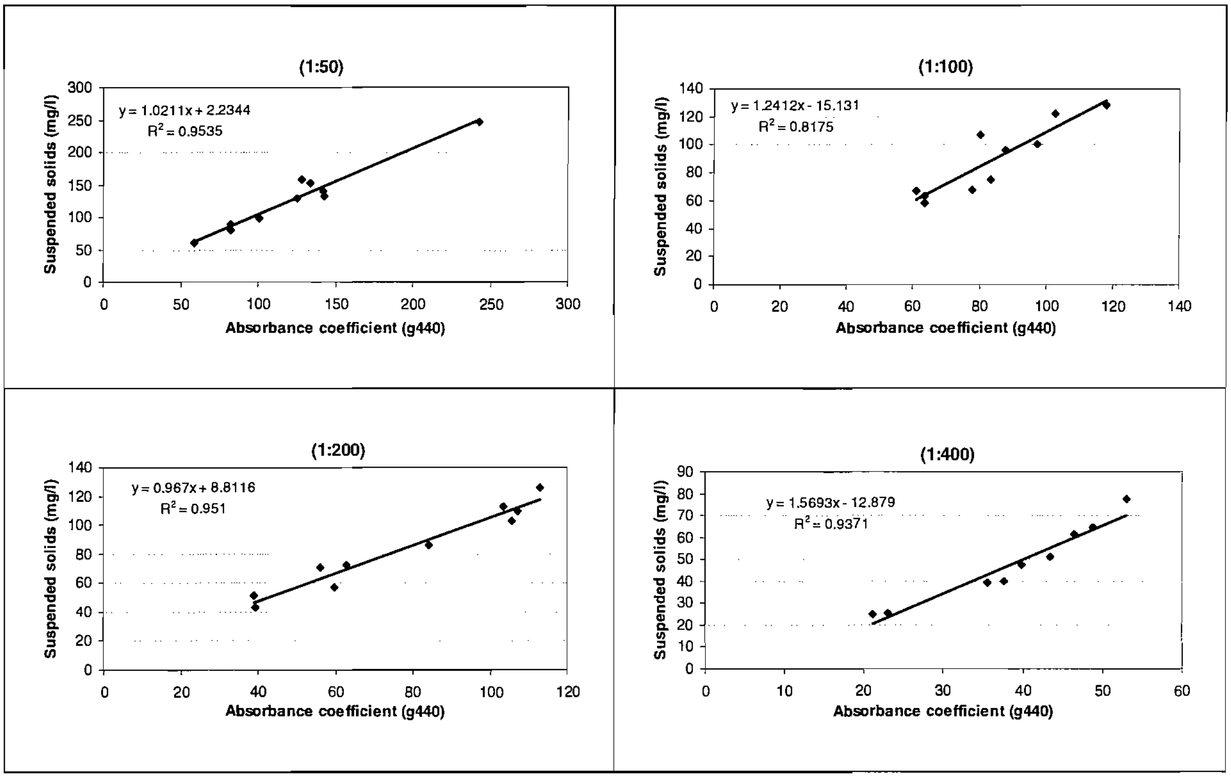


Figure 5.18 Relationship between unfiltered absorbance at 440 nm and suspended solids for each initial COD concentration

5.4.4 Oxygen production

The relationship between gross oxygen production and chlorophyll-a levels is shown in Table 5.10. Strong positive correlations ($R^2 = 0.68$-0.99) existed between the two parameters. As expected, gross oxygen production correlated more strongly with chlorophyll-a than net oxygen production ($R^2 = 0.52$-0.97). Similar results were found in a study by Copeland *et al.* (1964).

Table 5.10 Regression analysis of chlorophyll-a concentration with gross oxygen production

Initial substrate concentration (mg l^{-1})	R^2	p-value
111	0.68	0.005
123	0.90	<0.001
204	0.90	<0.001
200	0.82	<0.001
452	0.79	0.001
447	0.74	0.003
863	0.97	<0.001
872	0.99	<0.001

Figure 5.19 and Figure 5.20 show the gross and net oxygen production rates during the experiment. Gross oxygen production accounts for both oxygen production and consumption by respiration. The difference between values in Figure 5.19 and Figure 5.20 is therefore attributable to respiration processes by the microbial community. The most noticeable difference between gross and net oxygen production was apparent in bottles that received the highest load, where the increased COD breakdown led to a higher O_2 demand. The smallest difference was therefore observed in the lower loaded systems. The reduction of organic matter to low levels by day 15 was accompanied with a decline in respiration in all buckets. Net oxygen production was negative in all buckets at the start of the experiment reflecting the addition of organic matter to each system and the subsequent excess of oxygen consumption over production. The change from negative to positive net oxygen production was first observed in buckets receiving the lowest initial concentration on day 5. Positive net oxygen production was then apparent on day 7 for the buckets with 202 and 204 mg COD l^{-1} and day 9 for the average initial concentration of 450 mg l^{-1}. Not until day 13 was net oxygen measured in the buckets receiving the highest strength wastewater. The change to positive net oxygen production coincided with the reduction in COD to low levels and shows another beneficial effect of

dilution. The net oxygen production rate stabilised at 2-3 mg O_2 l^{-1} hr^{-1} for buckets with the lowest initial concentration. This increased to approximately 5 mg O_2 l^{-1} hr^{-1} for mean concentrations 202 and 450 mg l^{-1} and although there was an initial delay for 868 mg l^{-1}, the highest rate of net oxygen production was measured in these buckets peaking at approximately 10-11 mg O_2 l^{-1} hr^{-1} between day 13 and 15. After a period of 5 days this value had dropped to 6 mg O_2 l^{-1} hr^{-1}.

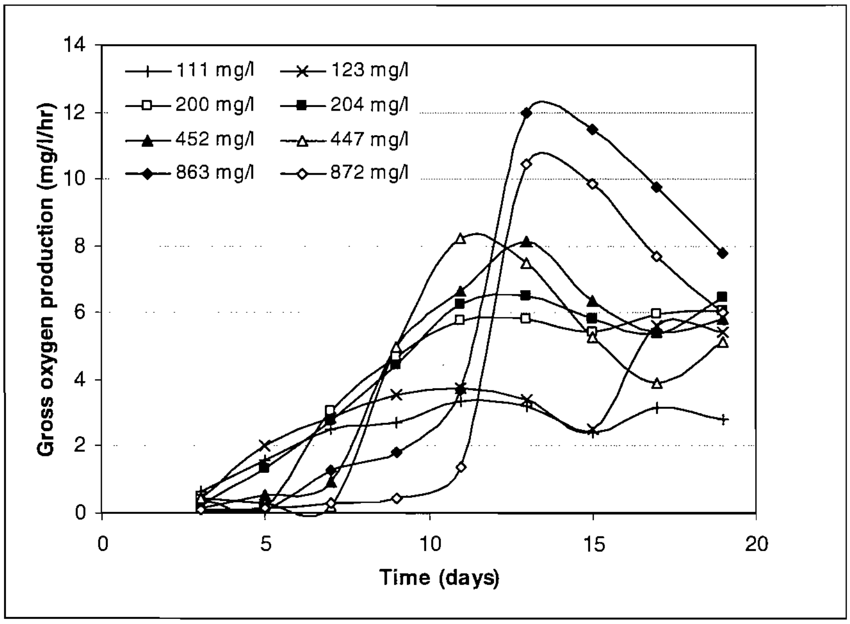


Figure 5.19 Change in gross oxygen production for each initial COD concentration

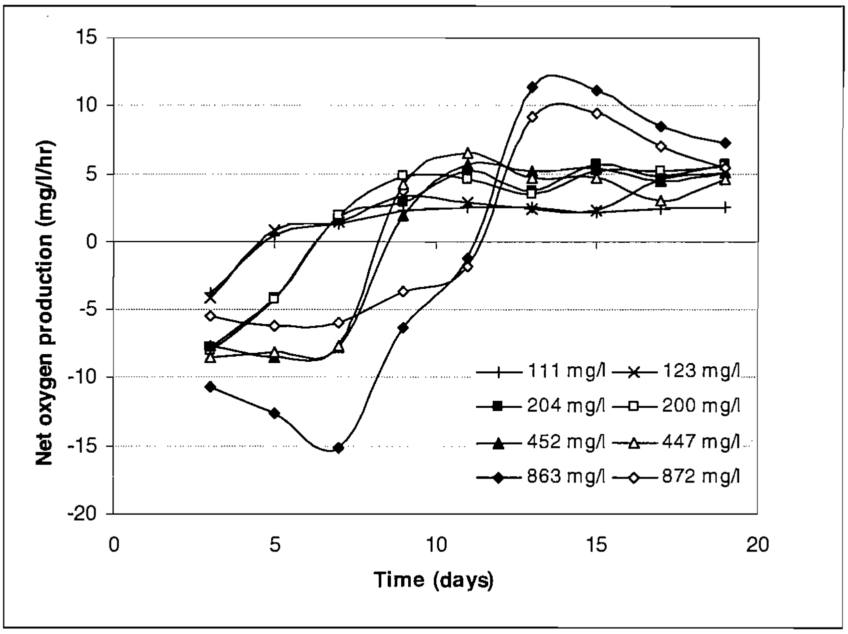


Figure 5.20 Change in net oxygen production for each initial COD concentration

Estimated total gross oxygen produced between sampling intervals was compared with COD removal between sampling days and the results of regression analysis between the two parameters is shown in Table 5.11. COD removal in the buckets containing lower strength wastewater was more closely correlated with gross oxygen production ($p \leq 0.002$) than those containing the highest COD load which showed a poor correlation between the amount of COD removed over time and the estimated amount of gross oxygen produced in the same period ($p > 0.05$).

Table 5.11 Regression analysis of total gross oxygen production with amount of COD removed

Initial substrate concentration ($mg\ l^{-1}$)	R^2	p-value
111	0.89	<0.001
123	0.86	<0.001
204	0.76	0.002
200	0.80	0.001
452	0.73	0.004
447	0.65	0.009
863	0.38	0.079
872	0.35	0.090

The amount of oxygen utilised over time for each substrate concentration was calculated by multiplying the average gross oxygen production between sampling days by the time interval. The oxygen uptake in one interval was added to that in the previous interval and the accumulated oxygen uptake curve is shown in Figure 5.21. The results show different slopes for the initial concentration and this indicates different substrate consumption rates. In the buckets receiving the highest strength wastewater there was a lag phase up to day 11 followed by a more rapid phase in oxygen uptake until day 15. The lag phase was most pronounced in the buckets with substrate 872 mg COD l^{-1} and represented minimal oxygen consumption prior to biodegradation. Following the lag phase, oxygen uptake increased rapidly coinciding with rate of biomass growth and reaching a maximum oxygen uptake of between 490 and 560 mg l^{-1} on day 15. At this time, soluble COD was at its minimum. The oxygen uptake curves for mean substrate concentrations 118 and 202 mg l^{-1} reached a plateau by day 11 and for 450 mg l^{-1} by day 13. At that time, the oxygen uptake for mean initial concentrations 202 and 450 mg l^{-1} was approximately 280 and 365 mg l^{-1} respectively. The buckets containing the lowest strength wastewater reached a mean maximum of 175 mg l^{-1}. The oxygen plateau is known to occur at the time of exhaustion of exogenous carbon sources (Bhatla and Gaudy, 1965). The COD removed should be equal to the sum of the accumulated oxygen uptake and the oxygen accounted for by the amount of cells produced. Values for oxygen production in the lowest strength wastewater appear high compared to COD removed: this may be due to losses due to saturation or errors in extrapolation of instantaneous values to longer periods.

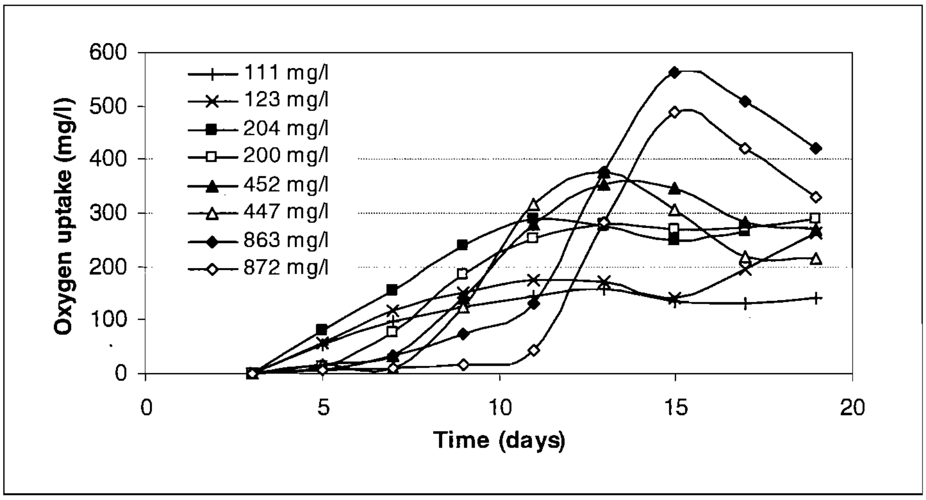


Figure 5.21 Oxygen uptake curves for each initial COD concentration

5.4.5 pH

Figure 5.22 shows initial COD concentration had a marked effect on pH during the course of the run. As in the bottle experiments, there was an initial drop in pH probably due to bacterial growth, and then a steady rise associated with the onset of photosynthesis from the growth of algae. Values above pH 8 were first measured in the buckets with lowest loaded flasks on day 7. At this stage, values for unfiltered pH in the highest COD concentration were 6.5 and finally rose to pH 8 around day 13-15.

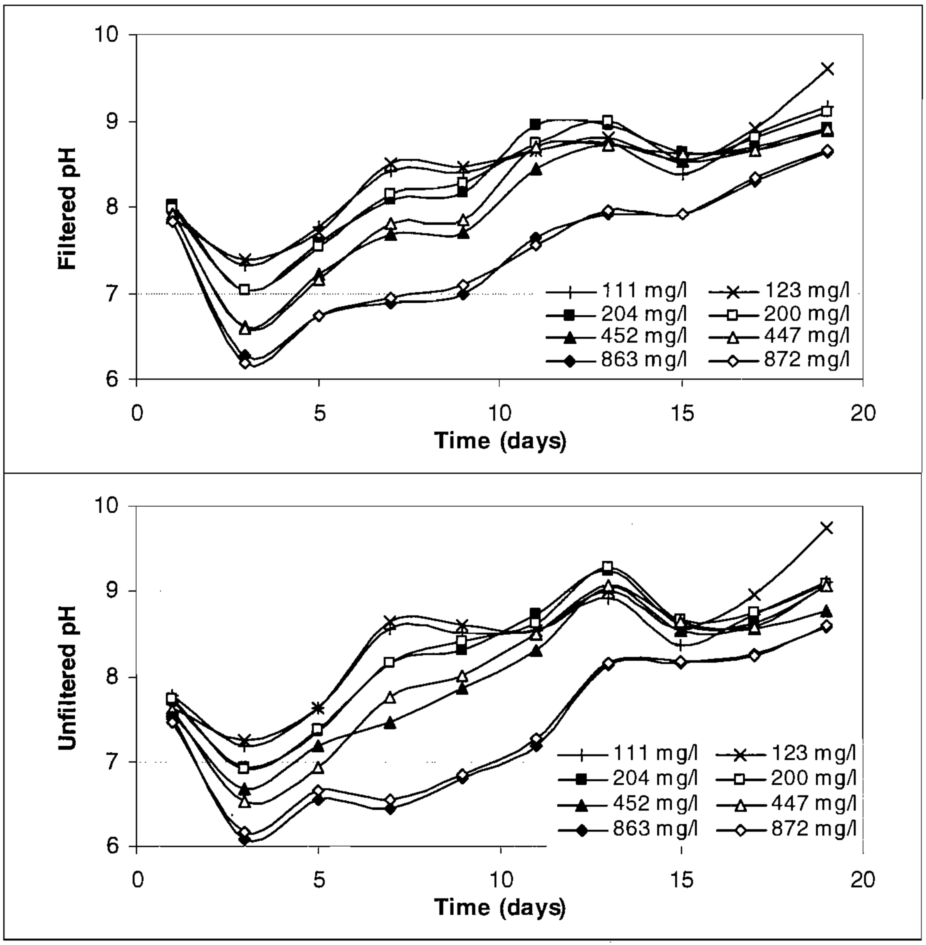


Figure 5.22 Change in pH values over time for each initial COD concentration

Using the t-test there were no significant differences between filtered and unfiltered values however, pH in filtered samples was always higher than unfiltered samples until a substantial rise in chlorophyll-a was observed. This is shown in Figure 5.23 with an example from each dilution. Although filtered samples would have also contained the hydroxyl ions know to cause the rise in pH through photosynthesis, higher pH values in the unfiltered samples were possibly due to the presence of algal solids. Figure 5.23 also shows the change in net oxygen production which links the rise in pH associated with the increase in photosynthetic oxygen production. This is best shown for the highest load (863 mg COD l^{-1}) where pH values above 8 occur with the rise in chlorophyll-a and also with the change from negative to positive net oxygen production.

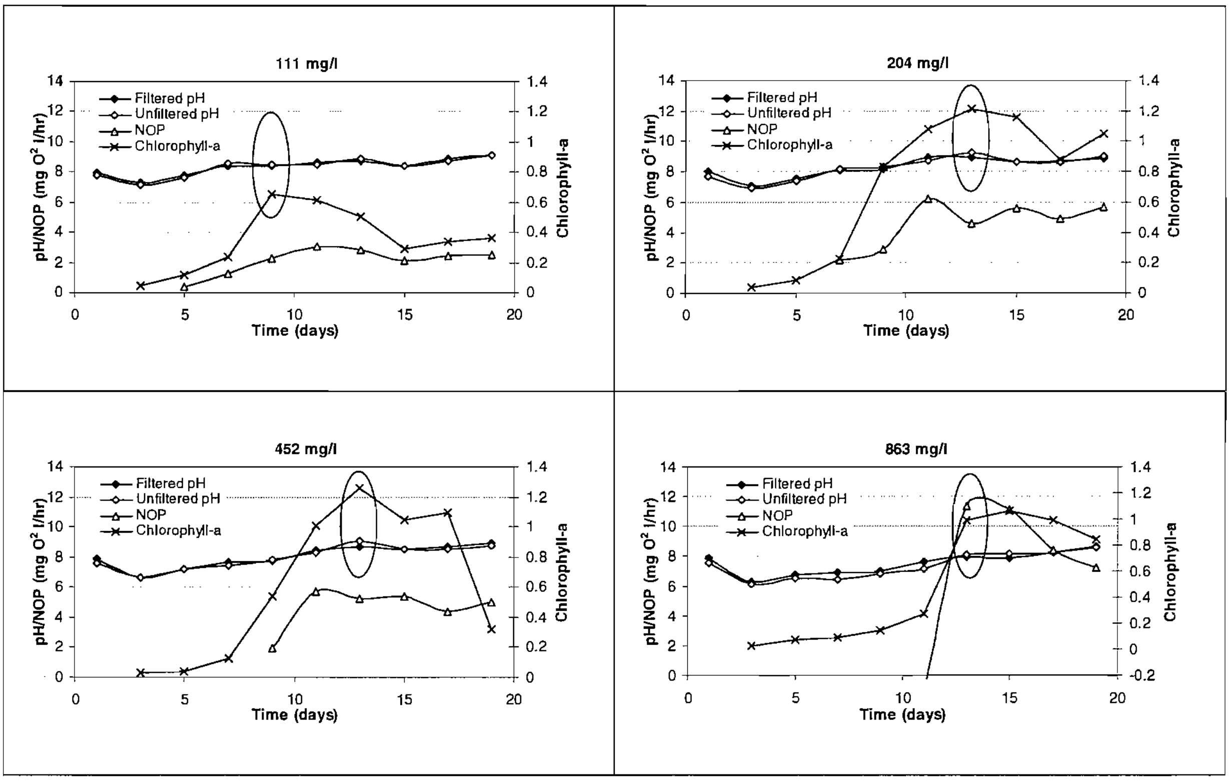


Figure 5.23 Changes in filtered and unfiltered pH in response to rises in chlorophyll-a and net oxygen production

O = where unfiltered pH became higher than filtered pH

5.4.6 Nutrients

The 3 and 5-day removal rates for ammonia and orthophosphate are shown in Figure 5.24 and Figure 5.25. The 3-day ammonia removal rates showed a strong negative correlation with initial COD concentration ($p = 0.007$) whereas by day 5, ammonia removal showed a positive relationship with COD concentration ($p < 0.001$). Ammonia removal has previously been correlated with chlorophyll-a concentration (Fallowfield *et al.*, 1999; Abis, 2002) providing evidence in support of algal uptake. Increased pH levels from algal growth are also known to be a factor in ammonia removal. Ammonia volatilisation arises from the conversion of ionised to free ammonia which can be lost to the atmosphere as a gas and this is more likely when the pH is high. At pH 9.2, the two forms are equal in concentration and by pH 12, all the ammonia is in the free form (Reed, 1985). As pH levels were still below pH 9 by day 5, it is assumed that algal uptake was the predominant pathway for ammonia removal and as chlorophyll-a levels increased more quickly in the buckets with the lowest strength wastewater, algal growth may have accounted for the higher initial removal rate. However, overall ammonia removal efficiency was lowest in these buckets, which is possibly due to the feedback mechanism augmenting the existing ammonia concentration (Abis and Mara, 2004). This may have accounted for the second algal bloom seen in the buckets receiving the lower loads.

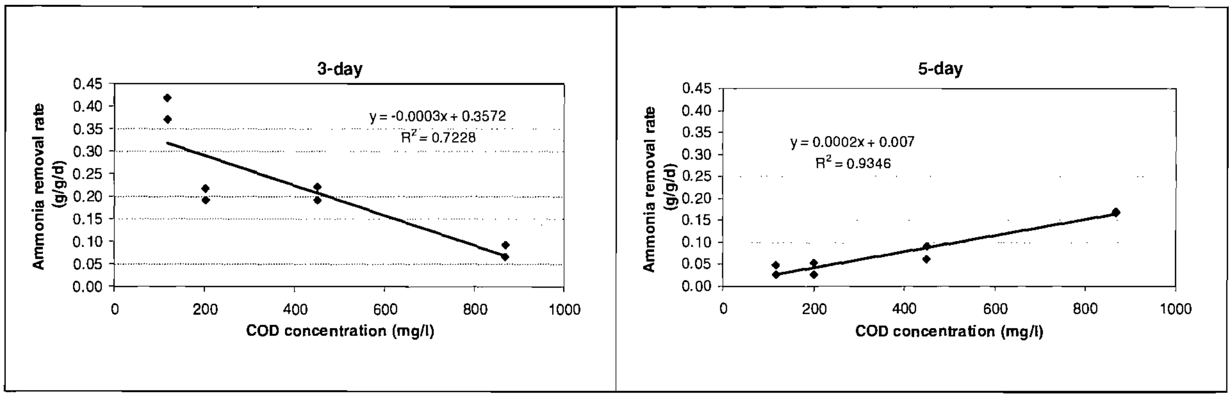


Figure 5.24 3 and 5-day ammonia removal rate

Both the 3 and 5-day orthophosphate removal rates were correlated positively with initial COD concentration ($p \leq 0.003$) (Figure 5.25). Similar findings were discussed by Powell *et al.*, (2006) who used batch experiments to assess the factors affecting phosphorus removal. Phosphorus removal in WSP is a result of both chemical and biological mechanisms. Removal can occur through precipitation of hydroxyapatite ($Ca_5(PO_4)_3OH$) at above pH 8.2 (Mara *et al.*, 1992). By day 5 however, pH levels were still below 8. Algal uptake was therefore the most likely source of orthophosphate removal.

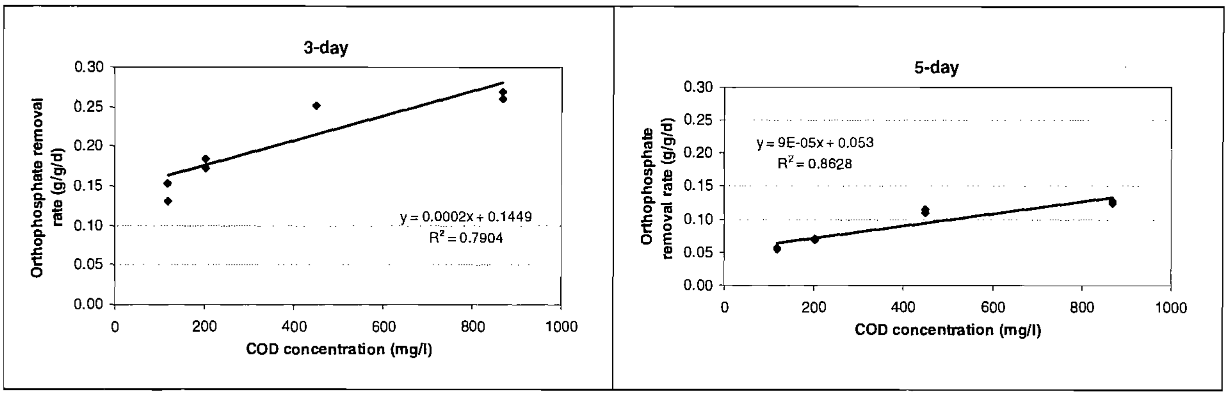


Figure 5.25 3 and 5-day orthophosphate removal rate

A mechanism known as luxury uptake whereby some microalgae are capable of storing phosphorus might account for the phosphate being removed by low levels of algae in the higher loaded buckets but no increase in growth (Morris, 1980). It might also be responsible for the positive linear relationship between COD concentration and orthophosphate removal although overall removal efficiency was highest in the buckets receiving the lowest initial concentration.

5.5 Flask Experiments

It was considered desirable to investigate the effect of the input light energy on removal rates for two reasons: a) because this varies in the spring warm-up period in seasonal climates; and b) because if you want to do experiments under more controlled conditions this will involve using artificial light and it is necessary to know whether you can supply sufficient light energy to provide simulation of natural conditions/saturation. It was also important to deduce whether any effect of input light energy varies with organic loading. To do this, flask experiments were set up with different total daily light inputs, achieved by providing a constant illumination over different daylengths (Meseck *et al.*, 2005).

Twelve one-litre batch-fed conical flasks were set up in triplicate under different light-dark regimes with mean COD concentrations of 86 mg l^{-1}, 143 mg l^{-1}, 240 mg l^{-1} and 483 mg l^{-1}. The flasks were placed in a Gallenkamp orbital incubator (SANYO, Japan) and kept stationary at a constant illumination of 160 μmol $m^{-2} s^{-1}$ and at 20°C with light-dark regimes of 6:18h, 12:12h and 24:0h carried out twice during six consecutive 8-day runs. Total daily light input was calculated for each daylength (Table 5.12). One dark control run consisted of each dilution in triplicate (1 μmol $m^{-2} s^{-1}$). 12:12h and 6:18h runs were set so lights turned on at 06:00 to provide consistency between the runs. Settled samples were taken between 09:00 and 10:00 on alternate days for COD, chlorophyll-a, pH, absorbance at 440 nm and 678 nm and dissolved oxygen levels in mg l^{-1}.

Table 5.12 Quantity of light at each daylength

Daylength (hours)	Light intensity (μmol $m^{-2} s^{-1}$)	Total daily light input (mol $m^{-2} d^{-1}$)
0:24h	1	<0.1
6:18h	160	3.46
12:12h	160	6.91
24:0h	160	13.82

5.5.1 COD removal

Table 5.13 shows the average overall COD removal efficiencies, which ranged from 67-88% after 4-8 days depending on daylength and initial concentration.

Table 5.13 Average overall COD removal efficiencies (%)

		Daylength (light – dark regime)			
Dilution	**Initial COD concentration ($mg\ l^{-1}$)**	**0:24 (control)**	**6:18**	**12:12**	**24:0**
1:400	**86**	75 (8)	68 (4)	68 (4)	80 (4)
1:200	**143**	78 (8)	86 (8)	76 (6)	67 (4)
1:100	**240**	74 (8)	85 (8)	87 (8)	82 (4)
1:50	**483**	54 (8)	80 (8)	88 (8)	75 (8)

Figure in brackets gives total number of days % removal calculated

Table 5.14 shows mean COD removal rate with time for each initial COD concentration. Removal rates showed very little difference between 0:24h and 6:18h on day 2 and for all daylengths for the lowest strength wastewater. As the strength of wastewater increased so did the difference in removal rates between 0:24h and flasks with a higher daily light input. For a 24:0h light-dark regime and initial concentrations 86 mg $l^{-1}$and 143 mg l^{-1} relative COD removal rate was at its maximum on day 2, averaging 0.25 and 0.28 g g^{-1} d^{-1} respectively, and then by day 8 became negative. Once again the change to negative removal rate represents the addition of soluble COD to the system, probably due to algal cell lysis occurring in the flasks and augmenting the dissolved and colloidal organic matter.

Maximum removal rates for COD concentrations 240 mg l^{-1} and 483 mg l^{-1} were found on day 4, averaging 0.25 and 0.33 g g^{-1} d^{-1} respectively, and the removal rate for 240 mg l^{-1} then became negative by day 8. The 12:12h regime produced a similar relationship to 24:0h on day 2 but with a lower removal rate at all substrate concentrations. By day 8 removal rate had declined in the flasks with the three lowest initial concentrations, but the flask with the highest load demonstrated an increase in COD removal rate with its maximum reached on day 8. For the 6:18h regime, the removal rate for the flasks containing the lowest load again showed the same pattern of decrease with time. A similar pattern was observed for flasks with 143 mg COD l^{-1} but with a much slower decline. 240 mg l^{-1} and 483 mg l^{-1} demonstrated a gradual increase in removal rate with time.

Table 5.14 Mean filtered COD removal rate (g g^{-1} d^{-1}) with standard error* for days 2, 4 and 8 for each daylength

Initial COD concentration (mg l^{-1})	**Daylength**					
	0:24h			**6:18h**		
	Day 2	**Day 4**	**Day 8**	**Day 2**	**Day 4**	**Day 8**
86	0.28	0.20	0.03	0.23	0.19	-0.02
	(0.010)	*(0.006)*	*(0.030)*	*(0.020)*	*(0.020)*	*(0.020)*
143	0.15	0.17	0.12	0.17	0.17	0.13
	(0.006)	*(0.030)*	*(0.020)*	*(0.009)*	*(0.009)*	*(0.010)*
240	0.13	0.21	0.19	0.15	0.15	0.17
	(0.010)	*(0.020)*	*(0.010)*	*(0.005)*	*(0.010)*	*(0.008)*
483	0.08	0.07	0.09	0.1	0.08	0.18
	(0.010)	*(0.020)*	*(0.016)*	*(0.002)*	*(0.008)*	*(0.004)*
	12:12h			**24:0h**		
	Day 2	**Day 4**	**Day 8**	**Day 2**	**Day 4**	**Day 8**
86	0.25	0.13	0.01	0.28	0.26	-0.37
	(0.010)	*(0.010)*	*(0.010)*	*(0.010)*	*(0.006)*	*(0.080)*
143	0.24	0.19	0.06	0.25	0.16	-0.06
	(0.003)	*(0.020)*	*(0.090)*	*(0.006)*	*(0.030)*	*(0.010)*
240	0.21	0.25	0.13	0.23	0.33	-0.12
	(0.009)	*(0.010)*	*(0.008)*	*(0.010)*	*(0.020)*	*(0.009)*
483	0.17	0.09	0.2	0.17	0.25	0.06
	(0.003)	*(0.020)*	*(0.006)*	*(0.010)*	*(0.020)*	*(0.010)*

*Standard error in brackets (n = 6)

Figure 5.26, Figure 5.27 and Figure 5.28 show an increase in daylength had a noticeable effect on COD removal rate. This was most apparent on day 2 (Figure 5.26) which demonstrated that a longer daylength increased the removal rate and this in turn varied with initial concentration. The removal rate at 12:12h and 24:0h (6.91 and 13.82 mol m^{-2} d^{-1}) was approximately 1.5 times as high for 86 mg COD l^{-1} as it was for 483 mg COD l^{-1} and twice as high at 6:18h (3.46 mol m^{-2} d^{-1}). The four-fold reduction in light energy therefore reduced the removal rate by two-fold when the initial COD concentration in this case was approximately six times as much.

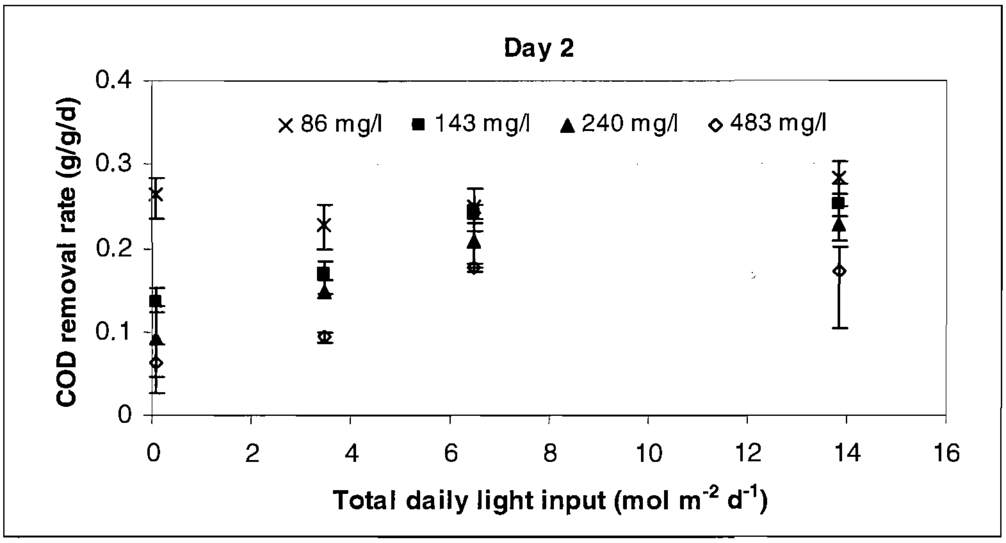


Figure 5.26 COD removal rate on day 2

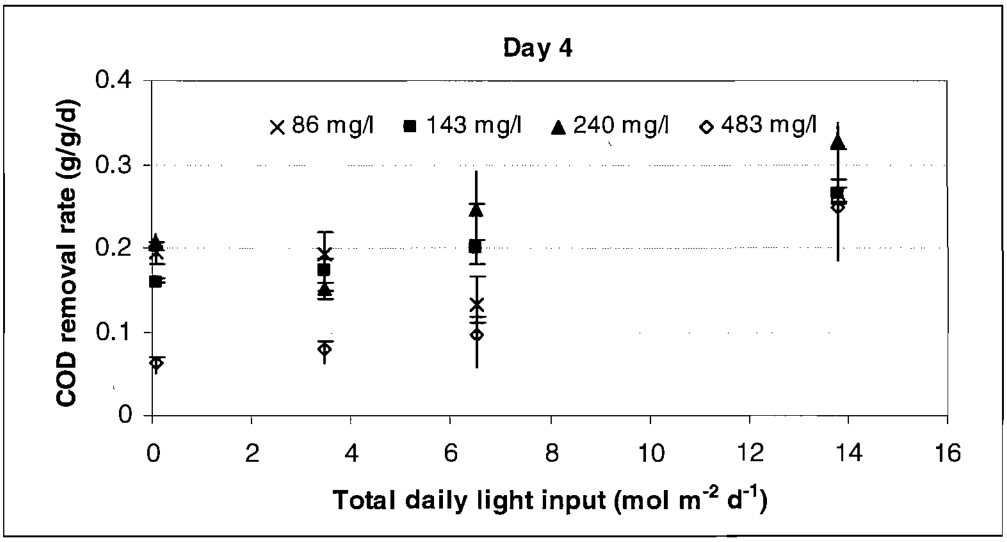


Figure 5.27 COD removal rate on day 4

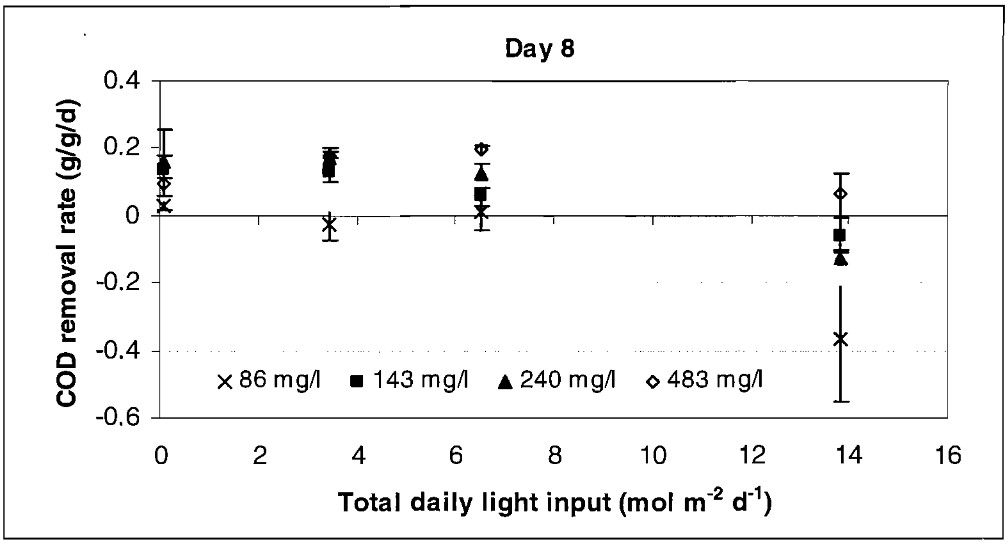


Figure 5.28 COD removal rate on day 8

Figure 5.29 shows the results from day 2 for the effect of total daily light input calculated from daylength on COD removal rate for each initial concentration including regression lines. Table 5.15 shows the output statistics of the regression lines for each COD load. Results were statistically significant ($p \leq 0.016$). Figure 5.29 shows that there is a marked difference in actual removal rates with the highest rate calculated for the lowest loaded flasks during 24:0h daylength (0.3 g g^{-1} d^{-1}) and a moderate difference in the rate of change of COD removal for each initial concentration. These varied from 0.0064 g g^{-1} d^{-1} per mol m^{-2} d^{-1} for 86 mg COD l^{-1} to 0.0097 g g^{-1} d^{-1} per mol m^{-2} d^{-1} for 483 mg COD l^{-1}. The slope gradually increases to a maximum for flasks containing 483 mg COD l^{-1} because when the light input was low the removal rate was affected most when the starting concentration was high.

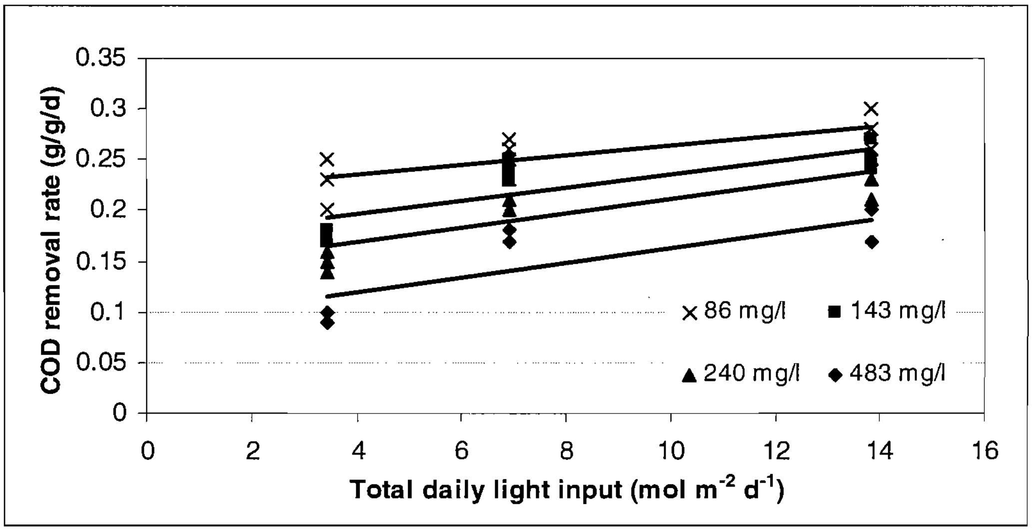


Figure 5.29 COD removal rate with change in total daily light input for each initial COD concentration (day 2)

Table 5.15 Regression analysis for COD removal rate with total daily light input (day 2)

Initial COD concentration (mg l^{-1})	Slope*	R^2	p-value
86	0.0064	0.59	0.016
143	0.0088	0.68	0.006
240	0.0093	0.67	0.007
483	0.0097	0.61	0.013

*Rate of change of removal (g g^{-1} d^{-1} per mol m^{-2} d^{-1})

The t-test was used to establish whether there was any significant difference between rate of change of COD removal for each initial COD concentration and the change in total daily light input from Figure 5.29. Table 5.16 shows a matrix of each starting load. Values in italics represent no significant difference between paired substrate concentrations, i.e. p-value of 0.172 showed that for COD concentrations 143 and 240 mg l^{-1}, the rate of change of removal on day 2 was not significantly different. The same was true for 86 and 143 mg l^{-1} ($p = 0.055$). This would be expected as the strength of wastewater between the pairs was relatively close. Where the starting COD concentration was much greater between pairs i.e. 86 and 483 mg l^{-1} rates of change of COD removal showed significant differences ($p < 0.001$).

Table 5.16 p-values for paired initial concentrations for the rate of change of COD removal on day 2 (using the t-test)

Initial COD concentration (mg/l)	**86**	**143**	**240**	**483**
86	-	*0.055*	0.002	<0.001
143	*0.055*	-	*0.172*	0.001
240	0.002	*0.172*	-	0.022
483	<0.001	0.001	0.022	-

Figure 5.30 shows day 2 results of COD removal against initial COD concentration and includes the control data and regression lines. Table 5.17 shows the results of these regression lines for each daylength. Results proved to be statistically very strong ($p \leq 0.005$). The slope was calculated to determine the rate of change of COD removal for each daylength. These varied from 0.00021 g g^{-1} d^{-1} per mg l^{-1} for 12:12h daylength to 0.00033 g g^{-1} d^{-1} per mg l^{-1} for the control with an overall average of 0.00025 g g^{-1} d^{-1} per mg l^{-1} ($p < 0.001$). The difference in rate of change of COD removal between daylengths was due primarily to variation between removal rates in the highest loaded flasks. For example, average removal rates for initial concentration 483 mg l^{-1} were 0.17 g g^{-1} d^{-1} for 24:0h and 0.08 g g^{-1} d^{-1} for 0:24h showing that a reduction in input light energy had a negative effect and this was most apparent in the flasks containing the highest strength wastewater.

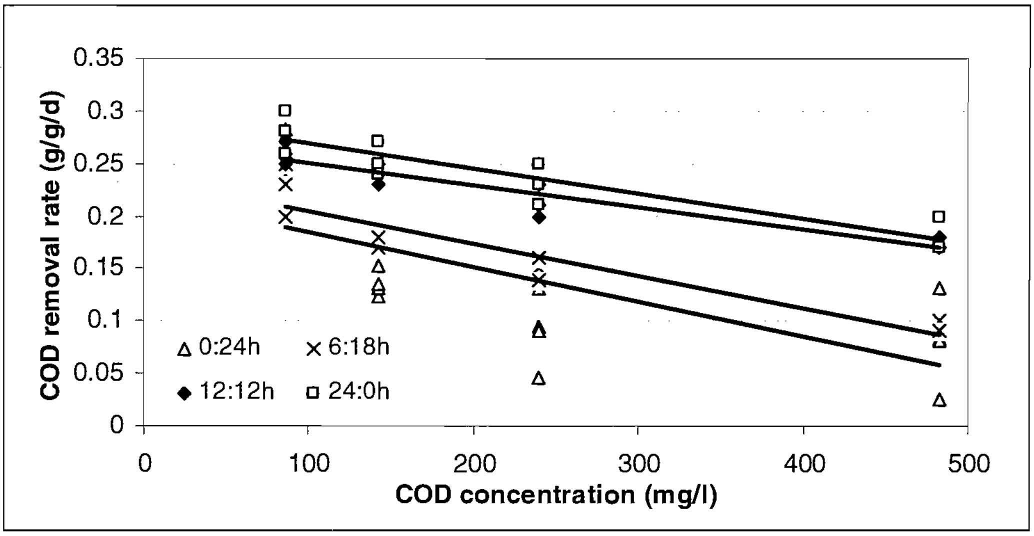


Figure 5.30 COD removal rate at different initial COD concentrations for each daylength on day 2 (with regression lines)

Table 5.17 Regression analysis for COD removal rate with daylength (on day 2)

Daylength	Slope*	R^2	p-value
0:24h	-0.00033	0.46	0.005
6:18h	-0.00030	0.87	<0.001
12:12h	-0.00021	0.91	<0.001
24:0h	-0.00024	0.84	<0.001

*Rate of change of removal ($g\ g^{-1}\ d^{-1}$ per $mg\ l^{-1}$)

Using t-test, it was possible to determine whether there was any statistically significant difference between the rate of change of removal between daylengths on day 2 from Figure 5.30. Table 5.18 shows a matrix of each daylength including the control results. Values in italics represent no significant difference between paired daylengths, i.e. p-value of 0.376 confirmed that for daylengths 12:12h and 24:0h, the rate of change of removal on day 2 did not vary. The same outcome applied to the results from 6:18h daylength and the control (p = 0.310). However, variations between the remaining pairs of daylength showed significant differences. Although no noticeable difference was observed between daylengths 12:12h and 24:0h, results demonstrated that by dramatically reducing the light energy or having a daylength of 6:18h the process of removal did slow down to some extent.

Table 5.18 p-values for paired daylengths for the rate of change of COD removal on day 2 (using the t-test)

Daylength	0:24h	6:18h	12:12h	24:0h
0:24h	-	*0.310*	0.001	<0.001
6:18h	*0.310*	-	0.003	<0.001
12:12h	0.001	0.003	-	*0.376*
24:0h	<0.001	<0.001	*0.376*	-

The individual effects of total daily light input and COD concentration on COD removal rate and the combined effects of light with starting COD concentration on COD removal rate were calculated in a regression analysis for the results with and without dark variants (Table 5.19). All results demonstrated significant relationships between parameters and COD removal ($p < 0.001$) although closest relationships were found when the parameters of light and COD concentration were combined suggesting that the two parameters are dependent in their effect. Performing the analysis without results for dark variants reduced the residual variation further.

Table 5.19 Regression analysis for COD removal with total daily light input, starting COD concentration and the combined effect of both parameters on day 2 (with and without dark variants)

	With dark variants		**Without dark variants**	
Parameter	**R^2**	**p-value**	**R^2**	**p-value**
Total daily light input (mol m^{-2} d^{-1})	0.30	< 0.001	0.28	< 0.001
Starting COD concentration (mg l^{-1})	0.41	< 0.001	0.53	< 0.001
Both light and COD concentration	0.70	< 0.001	0.82	< 0.001

Dark variants

As shown in Table 5.13, the dark variant flasks provided to account for the rate of removal in the dark, achieved quite high removals (54-78% after 8 days). This could have been achieved by heterotrophic bacteria that would have obtained their energy from the oxidation of organic matter or by sedimentation. Figure 5.34 also shows that measurable dissolved oxygen was present in the three lowest loaded flasks which may indicate the presence of photosynthetic algae, although only very low levels of chlorophyll-a were found. Many algae are known to grow not only photosynthetically but also by using organic substrates for biosynthesis and cell maintenance (Martinez *et al.*, 1997). Therefore in situations of light limitation such as this, organic compounds could have supplied the necessary additional energy. This perhaps explains the fact that results from this and previous experiments demonstrated highest removal rates were predominantly achieved before algae started to grow exponentially. The presence of oxygen in the systems with mean substrate concentrations 86 mg l^{-1}, 143 mg l^{-1} and 240 mg l^{-1} may have accounted for the overall higher removal efficiencies compared to 483 mg l^{-1} which was marginally less.

5.5.2 Chlorophyll-a and pH

A time-course variation of chlorophyll-a and pH for each daylength is shown in Figure 5.31 and Figure 5.32. The pattern that emerges from Figure 5.31 is that a reduced daylength delays the rise in chlorophyll-a levels. Peaks occurred earlier under the longer daylength: for example in COD load 143 mg l^{-1}, chlorophyll-a concentration peaked at 0.31 mg l^{-1} on day 4 for 24:0h daylength, at 0.25 mg l^{-1} on day 6 for 12:12h daylength and at 0.26 mg l^{-1} on day 8 for 6:18h daylength. Chlorophyll-a generally peaked between 0.2 and 0.3 mg l^{-1} at initial concentrations 86 and 143 mg l^{-1} for each daylength with higher values obtained in the flasks containing 143 mg l^{-1}. Flasks containing more wastewater therefore increased chlorophyll-a levels but this was only apparent between 86 and 143 mg COD l^{-1}. As the starting concentration increased above 143 mg COD l^{-1}, chlorophyll-a levels did not rise accordingly, which may have been a consequence of light limitation or inhibition by the higher initial substrate concentration. Results for 24:0h showed lower values for initial concentration 240 mg l^{-1} than expected when compared to 12:12h; one possible explanation might be a difference in the state/health of the inoculum used. Results for 86 mg COD l^{-1} also appear to show a slower response under the longer daylength when compared with the results for the other daylengths. For the highest initial concentration at under a 24:0h daylength chlorophyll-a levels rose to over 0.1 mg l^{-1}, but showed little or no increase at this dilution at under daylengths of 12:12h and 6:18h. The shorter daylength of 6:18h also had an adverse effect in flasks containing 240 mg l^{-1} where chlorophyll-a levels showed no increase by day 4 and were still less than 0.06 mg l^{-1} on day 8. In contrast, flasks containing the lowest strength wastewater showed comparatively high peak chlorophyll-a concentrations for all daylengths. Although results are not shown for the control flasks, there was no change in chlorophyll-a levels with values remaining less than 0.03 mg l^{-1}.

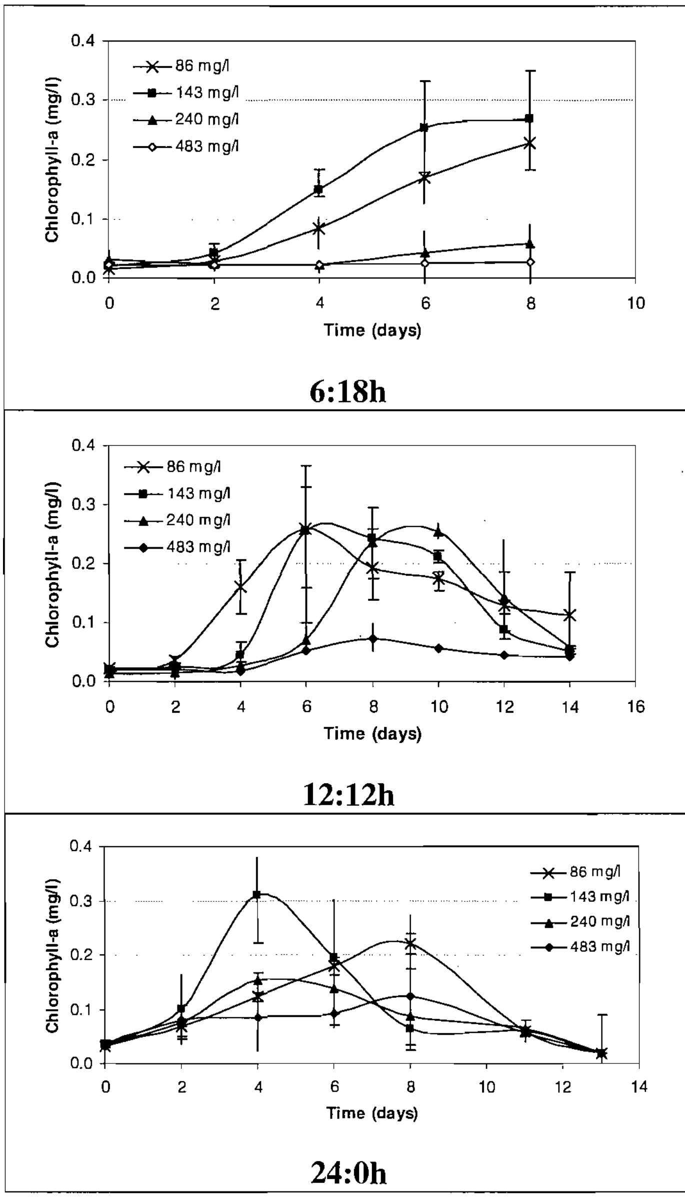


Figure 5.31 Change in chlorophyll-a concentration for each initial COD concentration for all daylengths

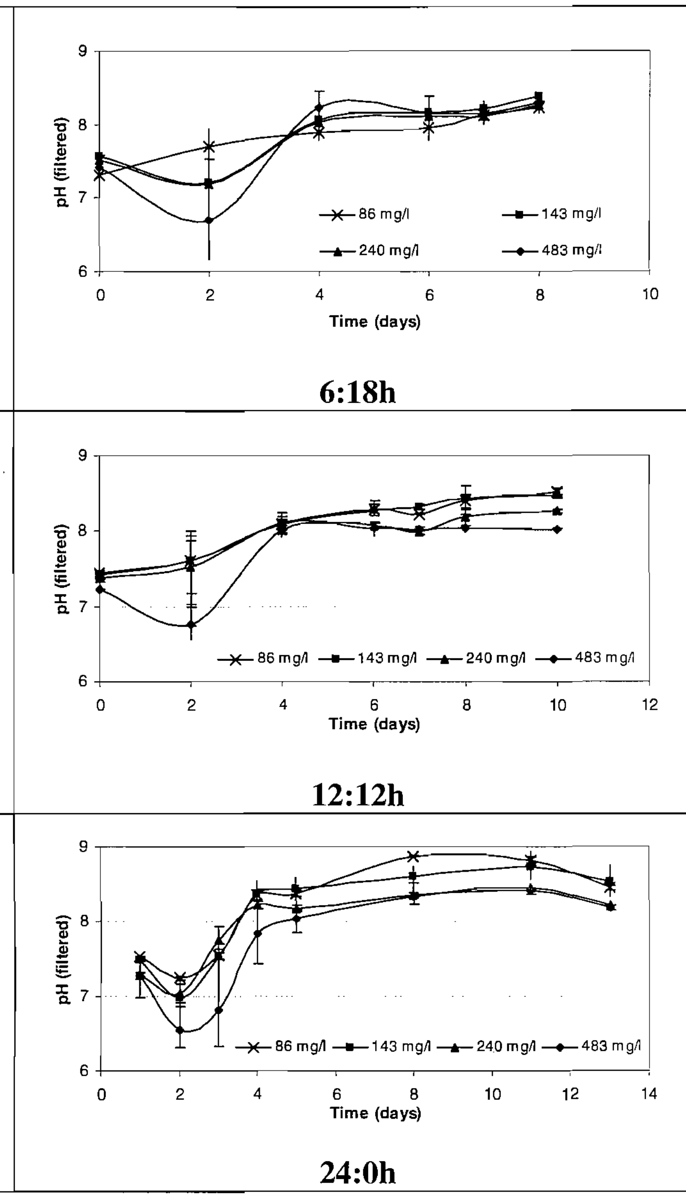


Figure 5.32 Change in filtered pH levels for each initial COD concentration for all daylengths

Chlorophyll-a levels declined quickly after initial peaks under daylengths at 12:12h and 24:0h and this was most pronounced in the flasks receiving the lower strength wastewater. Chlorophyll-a levels however remained stable during 6:18h. It is suggested that this reduction in chlorophyll-a attributed to algal cell death (lysis) was caused by nutrient limitation enhanced by longer daylengths. A study by Litchman *et al.* (2003) found that the effect of phosphorus limitation was more pronounced with a longer daylength (18:6h) than a shorter one (6:18h) on the growth responses of three freshwater algae. They concluded that by allowing faster growth with an increased photoperiod, longer daylength leads to a stronger phosphorus limitation and a decline in the growth and photosynthetic rates. Meseck *et al.* (2005) found that longer daylength resulted in higher biomass production in the marine microalga *Testraselmis chui* and complete utilisation of nitrate and phosphate in less time. It is therefore possible that the longer period of darkness with daylength 6:18h could have resulted in the slower uptake and assimilation of nitrogen and phosphorus and prevent the decline in algal biomass observed under longer daylength.

pH values showed a similar pattern over time for each daylength, with a reduction in pH between day 0 and day 2: this was most marked in the flasks receiving the highest load where the pH dropped below 7 on day 2, corresponding to an assumed rise in bacterial activity. pH values then rose in all flasks to above pH 8 with slightly higher values and an earlier increase for the longer daylength. This behaviour was comparable with the results from the bottle experiments where an early dip in pH was seen followed by a rise to similar pH values. pH values in the control flasks remained between 7 and 8 from day 4 onwards. Values above pH 8 usually signify photosynthetic processes are taking place, this was therefore not apparent in the flasks under 0:24h daylength.

The apparent growth rate of algae was calculated for each substrate concentration over the course of the experiment (Figure 5.33) and clearly shows that the highest growth rate for each initial concentration was observed for daylength 12:12h with a maximum on day 6 for 143 mg COD l^{-1} of 0.036 h^{-1}. The initial concentration of 86 mg COD l^{-1} showed growth rates peaked earlier in the experiment and steadily decreased from day 4 for all daylengths. This may have been a consequence of nutrient limitation which was further enhanced under a daylength of 24:0h where growth rates for all substrate concentrations declined from day 4. Daylength 12:12h showed a steady rise in growth rates from the start of the experiment reaching maximums on day 4, 6 and 8. A reduced light input of 6:18h produced the lowest growth rates at the higher initial COD concentrations, for example, 483 mg l^{-1} had a maximum growth rate of 0.002 h^{-1} compared to 0.022 h^{-1} for 86 mg COD l^{-1} suggesting that low light levels combined with the higher substrate concentration had a negative effect on algal growth.

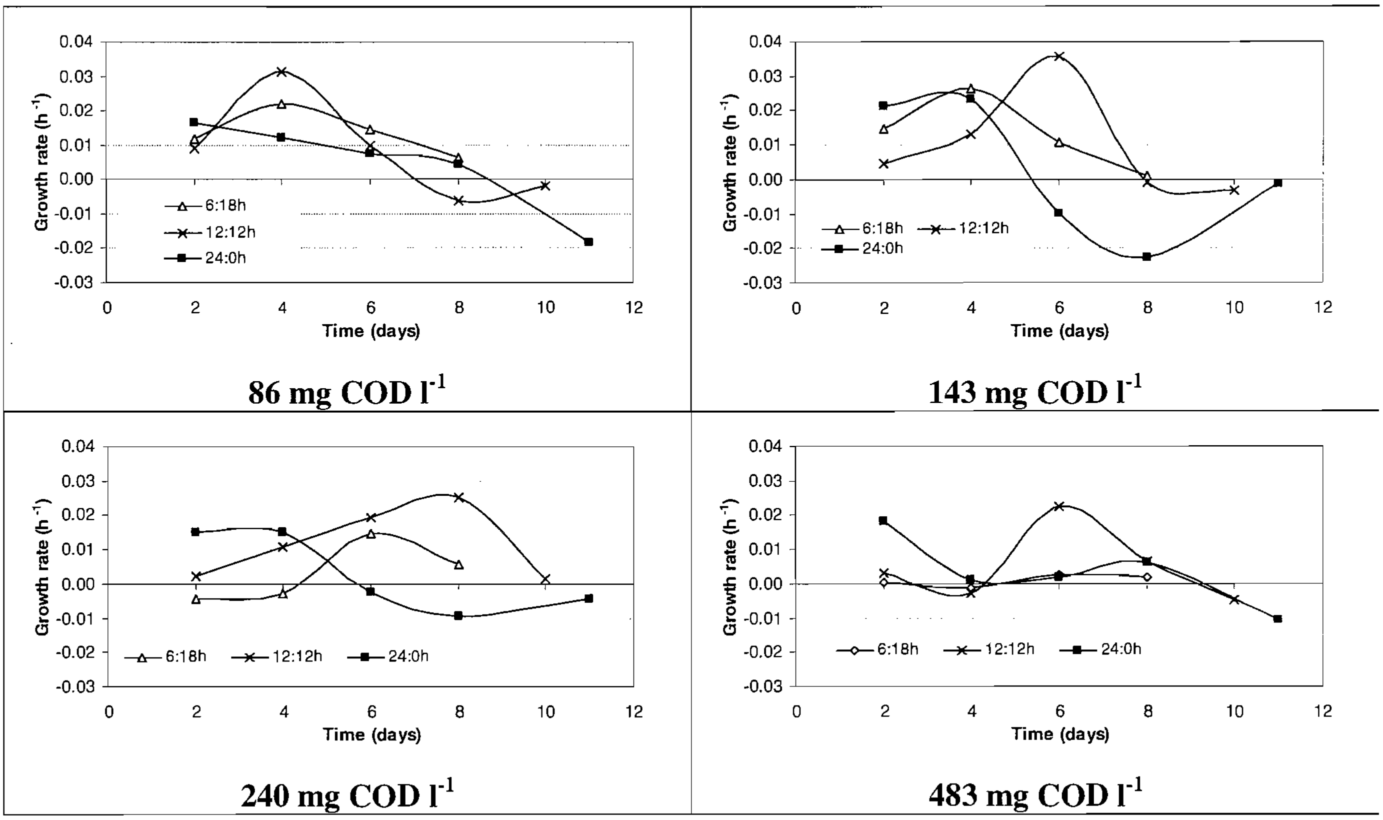


Figure 5.33 Effect of daylength on apparent growth rate for each initial concentration

5.5.3 Dissolved oxygen

A time-course variation of dissolved oxygen levels is shown in Figure 5.34 for each daylength. Dissolved oxygen showed fairly similar patterns to chlorophyll-a concentration in that levels were slowest to rise under the shortest daylength and increased more rapidly as the daylength became longer. Initial concentration of 86 mg COD l^{-1} demonstrated the capacity for net oxygen production earliest under all conditions. The presence of oxygen was observed in both of the lower loaded flasks under 0:24h daylength from day 4 and 6 respectively. 2.5 mg l^{-1} of oxygen was measured in flasks containing 86 mg COD l^{-1}. A study by Bartosh (2004) found that algal species likely to occur in WSP were capable of photosynthetic activity at light intensities as low as 7.8 μmol m^{-2} s^{-1}. *Scenedesmus* species have a critical light intensity of about 6 μmol m^{-2} s^{-1} and *Chlorella* species even lower (Huisman et al., 2002). This may account for the presence of oxygen at such low light intensities. For loads of 86 and 143 mg COD l^{-1} and a daylength of 24:0h, dissolved oxygen concentrations reached 10 mg l^{-1} by day 3, and remained between 10-15 mg l^{-1} until day 11 when levels began to decline, reflecting the fall in chlorophyll-a. For daylengths 6:18h and 12:12h although dissolved oxygen was initially higher in 86 mg COD l^{-1}, levels then rose higher in flasks containing 143 mg

COD l^{-1} which was concurrent with the results for chlorophyll-a. Oxygen levels in 240 mg COD l^{-1} stabilised at 5 mg l^{-1} from day 3 whereas dissolved oxygen levels for 483 mg COD l^{-1} showed a gradual rise to just 1 mg l^{-1} by day 5 and then to 3 mg l^{-1} on day 13. There was no recovery in oxygen levels observed for the highest substrate concentration under daylengths of 12:12h and 6:18h.

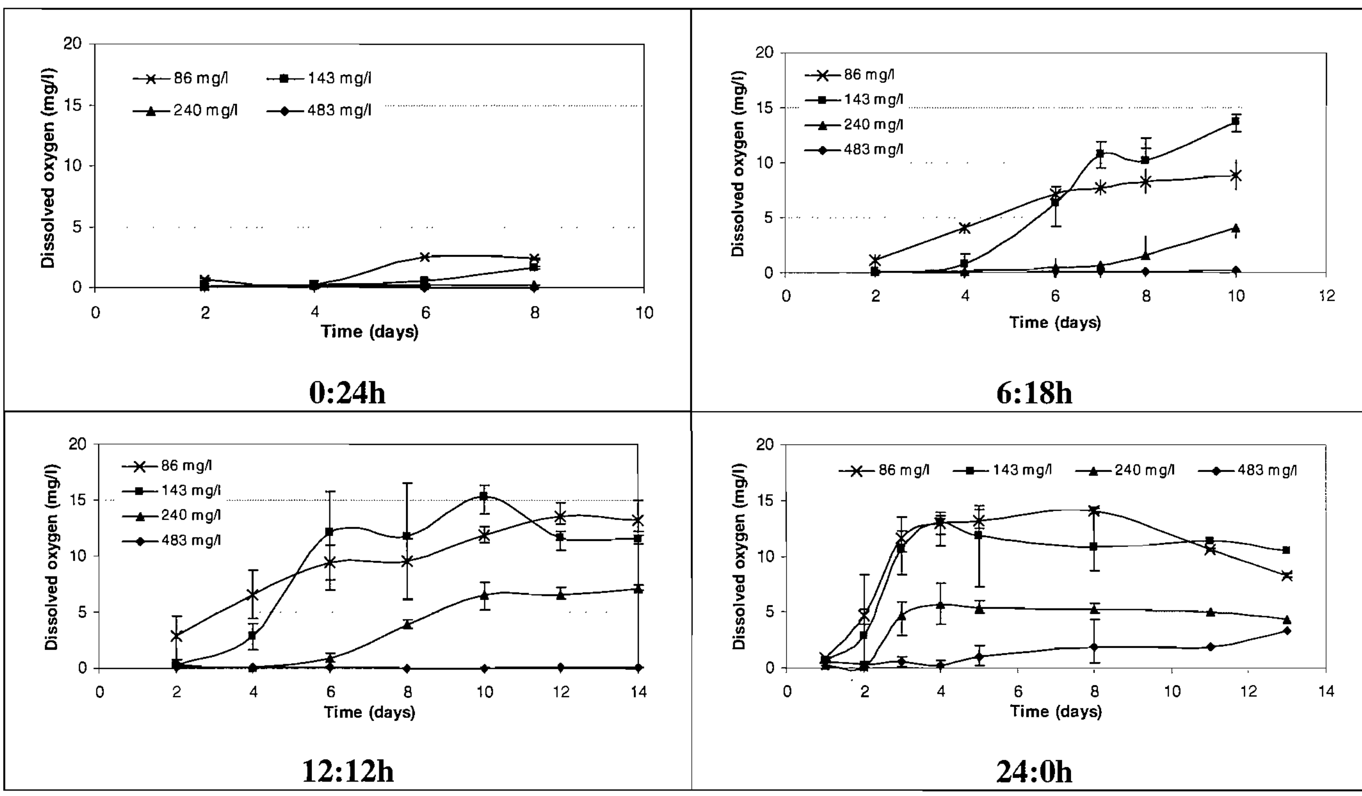


Figure 5.34 Change in dissolved oxygen levels for each initial COD concentration for all daylengths

5.5.4 Absorption

The ratio $R_{678:440}$ increased over time as algal growth occurred and humic substances in the samples declined. This was most apparent in flasks containing the lowest strength wastewater where chlorophyll-a levels of 0.2 to 0.3 mg l^{-1} were observed. Values for unfiltered samples (apparent colour) were used to determine whether there was a significant relationship between chlorophyll-a and absorbance ratio $R_{678:440}$. Figure 5.35 shows the relationship between the two parameters ($R^2 = 0.79$; $p < 0.001$).

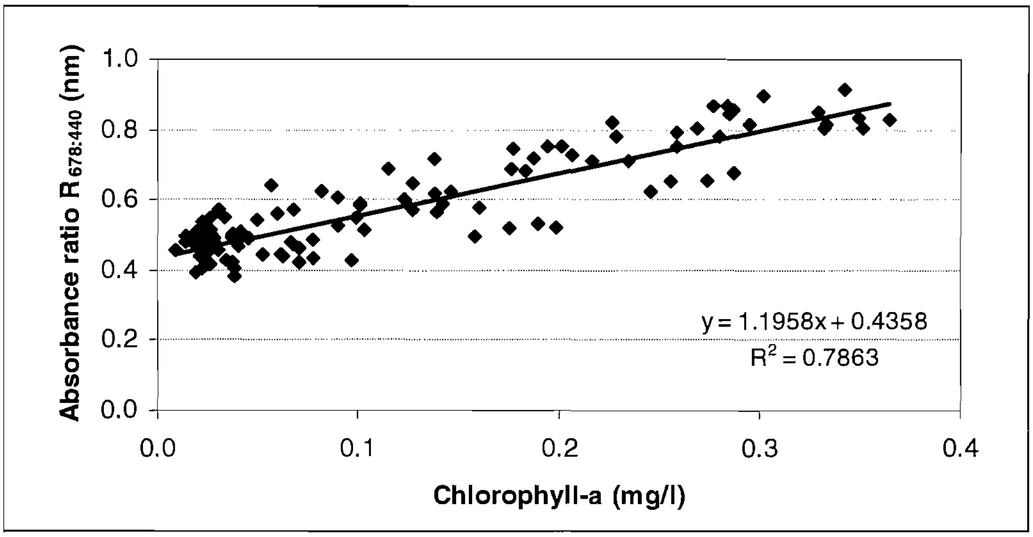


Figure 5.35 Relationship between chlorophyll-a and absorbance ratio $R_{678/440}$

As expected, results confirm that as chlorophyll-a levels increase so does the absorbance ratio. As a number of flasks during the experiment showed very low levels of algal growth, there are a high number of cluster points at absorbance ratios between 0.4 and 0.5 which affected the significance of the results. The absorbance ratio $R_{678:440}$ on unfiltered samples was a quicker alternative to establish chlorophyll-a levels and provide an indication of the optical effects of a transition from a system high in humic matter and low in chlorophyll-a to one experiencing a chlorophyll-a bloom and with moderate to low humic matter.

5.6 Tank experiments

The previous experiments provided results on the effects of light availability and dilution. The tank experiments were carried out to test the effect of dilution at a larger scale, similar to WSP with regard to depth, and under variable temperature conditions similar to those encountered in seasonal transitions.

5.6.1 Experimental tank construction

Two tanks, each 1.2m deep with 1000 l capacity, located in a temperature controlled room at the University of Southampton, UK, were used to simulate pilot-scale facultative ponds. The tanks (Mailbox Mouldings, Stockport, UK) were made from semi-translucent medium density polypropylene and insulated with 75 mm of insulation with a thermal conductivity of 0.02 W m^{-1} k^{-1} (Celotex, Hadleigh, UK), surrounded in a 12 mm plywood housing preventing any light entering through the tank walls. Each tank was illuminated by 27 fluorescent lights (15 x 30W, 6 x 16W and 6 x 20W) providing a maximum surface illumination of 100 μmol $m^{-2} s^{-1}$ (Figure 5.36). Light intensity in the tanks was altered by changing the height of the light unit using a winch system. Two systems were used to control the temperature of the water within the tanks. To reduce the temperature, coolant from a chiller unit (ICS, New Milton, UK) was passed through copper coils wound around the outer surface of the tank. To simulate natural heating the original plan was to raise the tank temperature by a combination of rising ambient temperature and heat radiated from the light units. This was found to be insufficient for controlled raising of the tank temperature so to achieve this, water was passed through hosepipe coils at the bottom of the tank using a Techne C-400 thermocirculator (Techne, Cambridge, UK). Air temperature of the room was controlled through the use of a portable air conditioning unit (Homebase, UK).

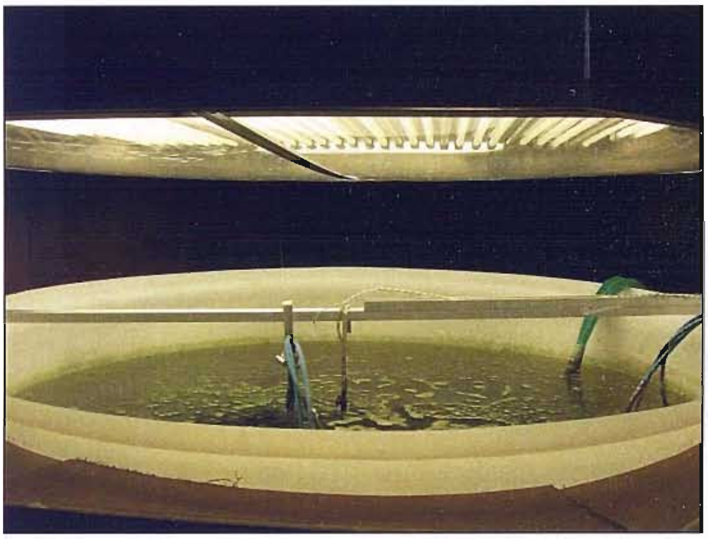


Figure 5.36 University of Southampton experimental tank

5.6.2 Instrumentation and calibration

Light sensors

Light intensity in the experimental tanks was measured using type BPW 21 photodiodes (RS components, Corby, UK). These were calibrated against a LI-210SA photometric sensor (LiCor, Lincoln, USA) and a RC/0308 standard photovoltaic cell (PV Systems, Cardiff, UK). The photodiode outputs were sampled at one second intervals using a datalogger (DataTaker D500) and expansion unit. The readings were averaged over a 10-

minute period and recorded in milliamps. These values were then converted into micromoles per square metre per second. An array of photodiodes was constructed to take measurements at the surface and at depths of 0.15, 0.3, 0.45, 0.6, 0.75, 0.9 and 1.05 m, and was moved between the tanks on alternate days. Using the light measurements it was possible to calculate the percentage transmission of surface incident light, based on the irradiance at a given depth compared with irradiance at the pond surface (Wetzel, 2001). The vertical extinction coefficient was then calculated from the percentile absorption of surface light through depth using equation *5.5* (Wetzel, 2001):

$$\text{Vertical extinction coefficient} = \frac{-(\ln l_z - \ln l_0)}{depth\ at\ z} \qquad (5.5)$$

where: l_0 = irradiance at surface
l_z = irradiance at depth z

DO probes

Dissolved oxygen concentration in the tanks was measured using oxygen probes (Dryden Aqua Ltd, Edinburgh, Scotland). These were calibrated by leaving them in air above the tank surface for a period of one hour. The output was measured in mV and the concentration in mg l^{-1} for each probe at 100% saturation was established using a calibration table provided by the manufacturers. Three oxygen probes were placed in each tank at depths of 0.15 m, 0.57 m and 0.99 m. Readings were taken every minute and averaged over a 10-minute period.

Temperature sensors

Temperature sensors (K-type thermocouples) were constructed to monitor the temperature just above the surface (ambient temperature) and at depths of 0.15 m, 0.57 m and 0.99 m in each tank. Readings were taken every minute and averaged over a 10-minute period.

5.6.3 Wastewater samples

Wastewater grab samples were collected using 1-litre bottles from a depth of 0.25 m. After collection, samples were taken to the laboratory and analyses performed on the same day.

5.6.4 Preliminary run

The temperature in the experimental tanks was adjusted to simulate the rapid rise in temperatures experienced during the spring warm-up in a continental climate, over a period of one month. Conditions in Almaty, Kazakhstan were used as an initial guideline for experimental parameters during the tank experiments. Almaty has a typically sharply continental climate for which some experimental data on pond performance is available. Daylength was originally set to simulate the daylight hours experienced in Almaty during the spring warm-up period. Almaty is located at latitude 43°N and the monthly average of the maximum possible hours of bright sunshine (without cloud) i.e. daylength of the average day of the month and the daily solar radiation (averaged by months) experienced at this latitude are shown in Figure 5.37 and Figure 5.38 respectively.

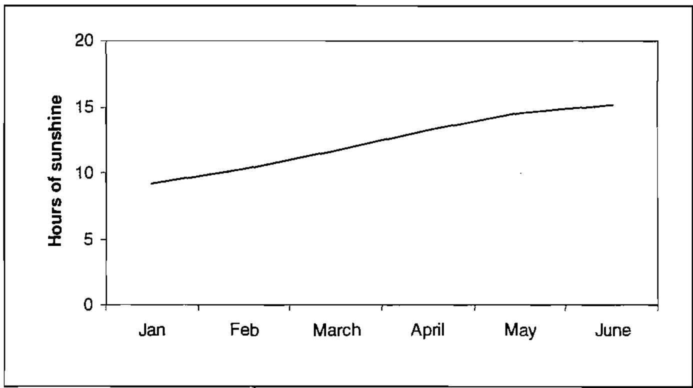


Figure 5.37 Monthly average of the maximum possible daily hours of bright sunshine at 43°N (Duffie and Beckman, 1980)

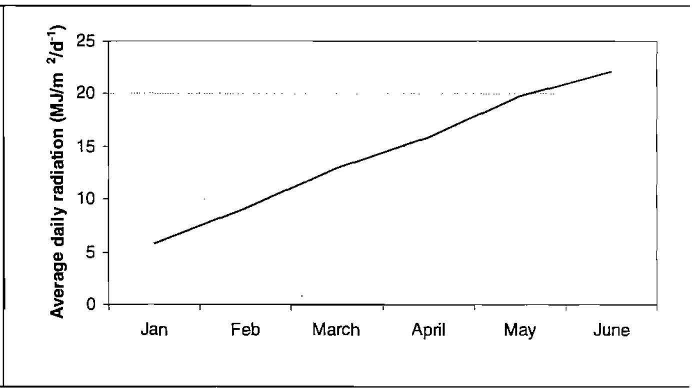


Figure 5.38 Average daily potential solar radiation (on a horizontal surface) at 43°N (Klein *et al.*, 1978)

A light-dark regime of 10:14h was initially trialled to imitate the shorter daylength experienced at the start of spring. The maximum light intensity achieved with the available light source was 100 μmol m^{-2} s^{-1}. A preliminary experiment was conducted using an initial concentration of 240 mg filtered COD l^{-1} and after a period of more than two months no algal bloom had developed. On day 70, the light-dark regime was changed to 24:0h and within days the algal population as indicated by chlorophyll-a concentration had increased significantly, as had dissolved oxygen levels (Figure 5.39).

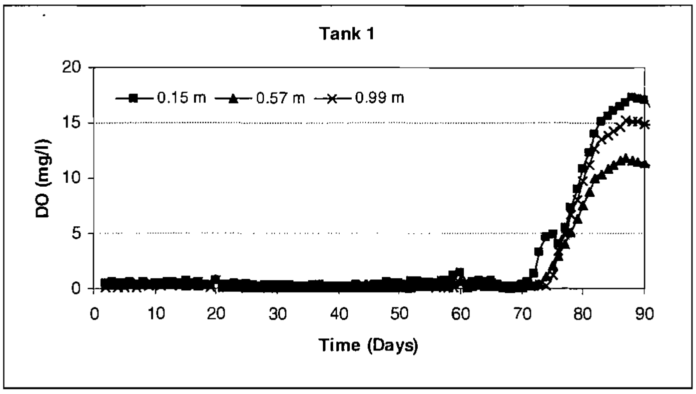


Figure 5.39 Dissolved oxygen levels during the preliminary run

5.6.5 Revised run parameters

5.6.5.1 Light

Using the data from Figure 5.37 and Figure 5.38, the total daily light input for a pond system located in Almaty was calculated. These values are shown in Table 5.20.

Table 5.20 Total daily light input during the spring at latitude 43°N

Month in Spring	Daylength (hours)	Average daily radiation (MJ m^{-2} d^{-1})	Average daily radiation (µmol m^{-2} s^{-1})	Total daily light input (mol m^{-2} d^{-1})
March	11.7	12.9	310.6	13.08
April	13.2	15.9	382.8	18.19
May	14.5	19.8	476.7	24.88
June	15.2	22.1	532.0	29.11

Potential values for total daily light input to the tanks were calculated based on the maximum light intensity and daylengths, and are shown in Table 5.21.

Table 5.21 Total daily light input for a range of daylengths and light intensities for UK batch-fed tanks

Daylength (hours)	Average daily radiation (MJ m^{-2} d^{-1})	Average daily radiation (µmol m^{-2} s^{-1})	Total daily light input (mol m^{-2} d^{-1})
10	4.15	100	3.24
12	4.15	100	4.32
24	4.15	100	8.64
24	2.28	55	4.75

With a daylength of 10 hours, the amount of light reaching the tanks was a quarter of what would be received in March at the start of the spring warm-up in a pond in Almaty, and approximately equivalent to the value used in the 6:18h flask experiments which also showed some inhibition; thus explaining the lack of an algal bloom during the preliminary run. Continuous illumination was instead opted for to increase the light input. This differs from nature where the light regime is discontinuous and the intensity varies daily. A study by Toro (1989) found, however, that phytoplankton growth is a function of the total amount of light per day, not the photoperiod. Toro (1989) observed the growth rate of two species of marine microalgae cultured under 12:12h light-dark and continuous illumination using different light intensities. Both light regimes provided equal amounts of light per day. After 14 days of growth, the numbers of cells per unit of volume showed no significant differences between the two light regimes. Meseck *et al.* (2005) detailed a study designed to examine whether growth and uptake of nutrients were affected by the amount of light energy provided to a culture per day (i.e. total daily light input) rather than the photoperiod response (i.e. duration). It was concluded that the microalga *Tetraselmis chui* has a minimal photoperiod requirement for maximal growth, but once that minimum photoperiod is met, growth rate is a function of the total daily light input.

Although working with cells grown under a light-dark regime gives the advantage of a closer approach to most natural conditions, it brings the need for a consistency in sampling time to offset variation in biochemical response caused by rhythmic changes in metabolic characteristics (Humphrey, 1979). Running the experiment at maximum light intensity for 24 hours would have been possible, but could have led to operational problems: for example the lights generate heat, making temperature control difficult at low temperatures. It was therefore decided to reduce the light intensity from 100 μmol $m^{-2} s^{-1}$ to 55 μmol $m^{-2} s^{-1}$ and operate at 24:0h, giving a total daily light input of 4.75 mol $m^{-2} d^{-1}$. This was the intensity used for growing the algal inoculum under 24:0h in the laboratory. It is also within the range of saturating light characteristics of algae normally found in WSP (Lehman *et al.*, 1975; Canale *et al.*, 1976; Morris, 1980; Huisman, 1999a; Ratchford and Fallowfield, 2003). Bartosh (2004) carried out a study concerning the physiological and growth responses of two algal species *Chlorella vulgaris* and *Scenedesmus subspicatus*. Results showed that both species reached maximum light specific growth rates at 47 μmol $m^{-2} s^{-1}$ at temperatures from 5 to 20°C.

5.6.5.2 Organic load and dilution

The tanks were operated for 5 runs at different starting COD concentrations with the details of each run shown in Table 5.22. The tanks were filled with tap water and the appropriate amount of concentrated feed was added to make up the volume to 1000 l. The volume of algal inoculum added to each tank was dependent on the measured chlorophyll-a concentration prior to the start of each run. Average values of 0.01 mg l^{-1} are observed in large-scale ponds in Almaty, Kazakhstan at the beginning of March and therefore the volume of algae added was adjusted to obtain a similar value. Following recommendations by Torrella, *pers. comm.* (2006), the algal inoculum was acclimated first to the experimental start-up temperature of 4°C by first transferring the 20-litre flasks outside for 48 hours. This gave a drop in temperature averaging about 10°C, though this was dependent on the time of year and outside temperature. The flasks were then emptied into the tanks 48 hours prior to the start of each run to reach an approximate temperature of 4°C. The length of each run varied depending on the starting COD concentration and the rate of removal achieved.

Table 5.22 Organic load and dilution used in each tank during each run

	Tank 1		**Tank 2**	
	Dilution	**Filtered COD concentration (mg l^{-1})**	**Dilution**	**Filtered COD concentration (mg l^{-1})**
Run 1	1:100	210	-	-
Run 2	1:200	164	1:100	244
Run 3	1:200	125	1:400	94
Run 4	1:50	417	1:50	431
Run 5	1:400	87	1:200	128

5.6.6 Results

Results from Run 1; Tank 2 are not presented or included in the discussion because of problems with the light system.

5.6.6.1 COD removal

Figure 5.40 shows COD removal rates on filtrates from day 5, 10, 15 and 20. COD removal rates for day 10 and 15 showed an inverse correlation with initial concentration (R^2 = 0.77; 0.76; $p \leq 0.01$). By day 20 this correlation was much weaker. COD removal rate in the tanks with the lowest initial concentration showed a decline in removal rate due to the reduction of organic matter to very low levels. Although COD removal rate remained low in the tanks that received the highest load, the rate did show an increase from day 15 to day 20. The results are not fundamentally different from the outcome of previous experiments, except that because of slower removal processes there is no relationship at 5 days. The effect of initial concentration is visible at days 10 and 15 but the rate was starting to fall in the lowest initial concentration by day 15 due to lack of material left to break down. By day 20, the absence of organic matter at higher dilutions is much clearer. The results suggest a slowed-down version of previous experiments attributable to less light or reduced temperatures or both.

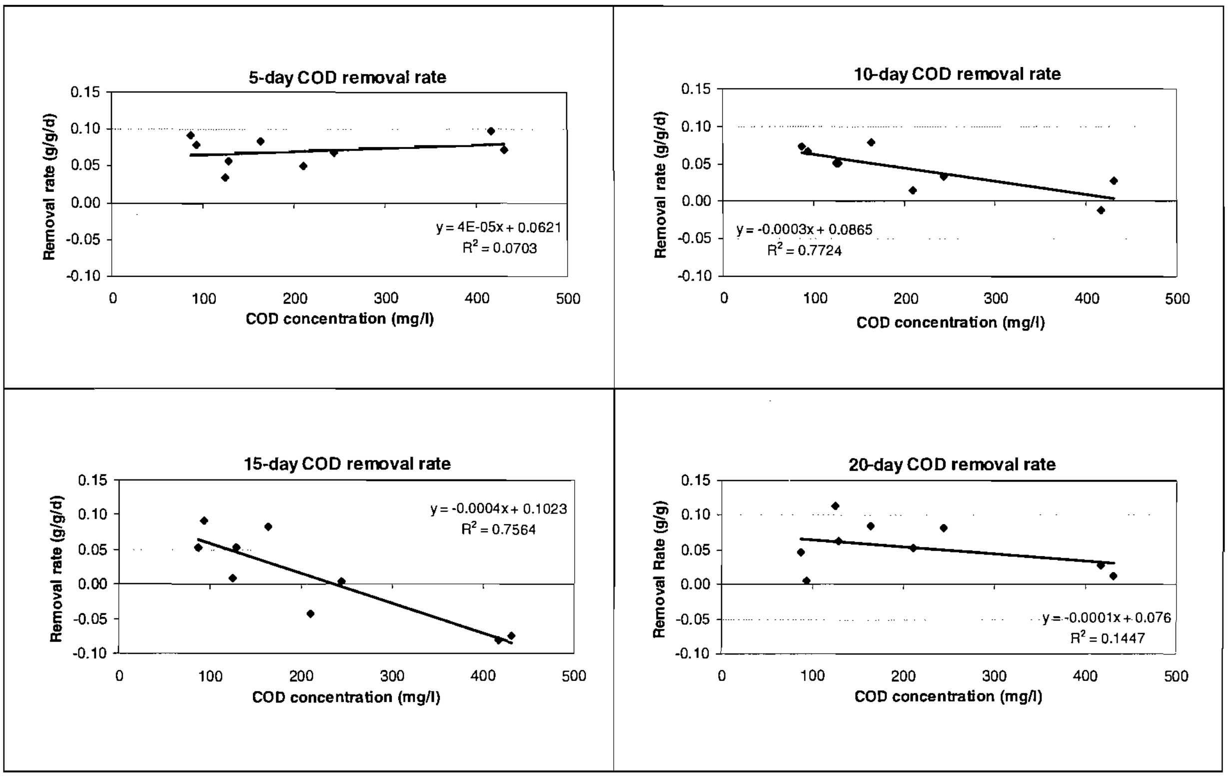


Figure 5.40 5, 10, 15 and 20-day filtered COD removal rates for each initial COD concentration

Figure 5.41 shows overall removal rate for COD during the experiment. A linear regression analysis of the overall COD removal rate with initial COD concentration gave an R^2 value of 0.69 and a p-value of 0.005, with a value of 0.0001 g g^{-1} d^{-1} per mg l^{-1} for the rate of change. Overall removal rates ranged from 0.064 to 0.020 g g^{-1} d^{-1} for initial concentrations 94 and 417 mg COD l^{-1} respectively. This suggests that an approximate four-fold reduction in the initial COD could increase the removal rate by three-fold.

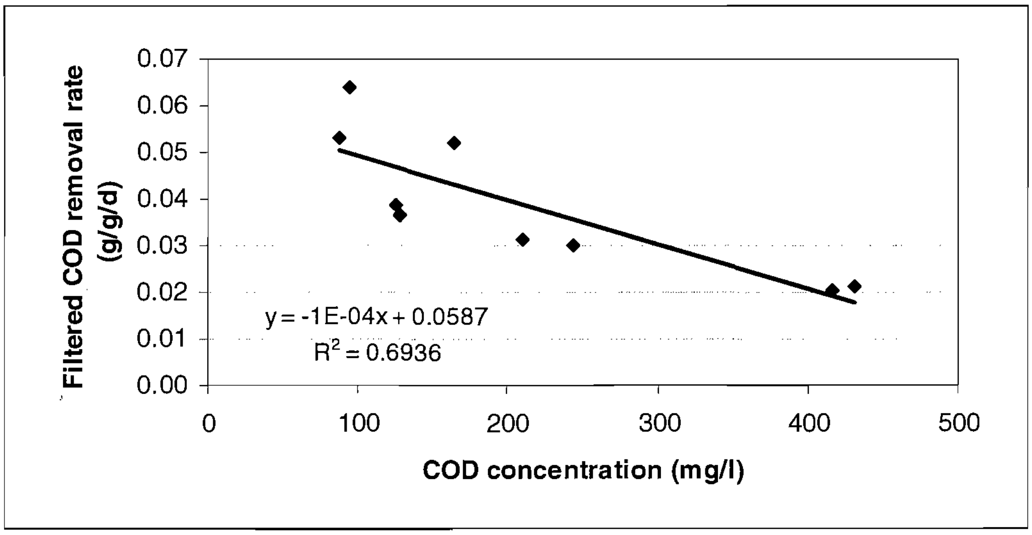


Figure 5.41 Overall COD removal rate for each initial COD concentration

5.6.6.2 BOD_5 removal

Filtered BOD_5 removal rates were calculated for each run. BOD_5 removal rates for day 5, 10, 15 and 20 are shown in Figure 5.42. The pattern of removal rates was similar to those observed for COD. The 5-day regression shows BOD_5 removal rates in the tanks were not significantly affected by the initial BOD_5 concentration ($R^2 = 0.34$; $p = 0.099$). Results for day 10 showed a change from a slight positive correlation for day 5 to a negative correlation with an increase in significance ($R^2 = 0.63$; $p = 0.01$). The negative correlation continued until day 15 for removal rate with initial BOD_5 load. The tanks receiving BOD_5 concentrations between 36 mg l^{-1} and 61 mg l^{-1} showed a BOD_5 removal rate of between 0.04 and 0.12 g g^{-1} d^{-1} between day 5 and 15. By day 20, removal rate in these tanks had reduced, due to the very small quantities of organic matter present at this stage. Tanks with a BOD_5 100 mg l^{-1} had removal rates of between 0.01 and 0.04 g g^{-1} d^{-1} and the tanks that had the highest load had very low BOD_5 removal rates (0.01 g g^{-1} d^{-1}) throughout the experiment. A negative removal rate between day 10 and 15 could have been associated with a sludge layer on the surface of the water that was accompanied by hydrogen sulphide odours. This may have added to the soluble organic matter in the tanks thereby decreasing the removal rate.

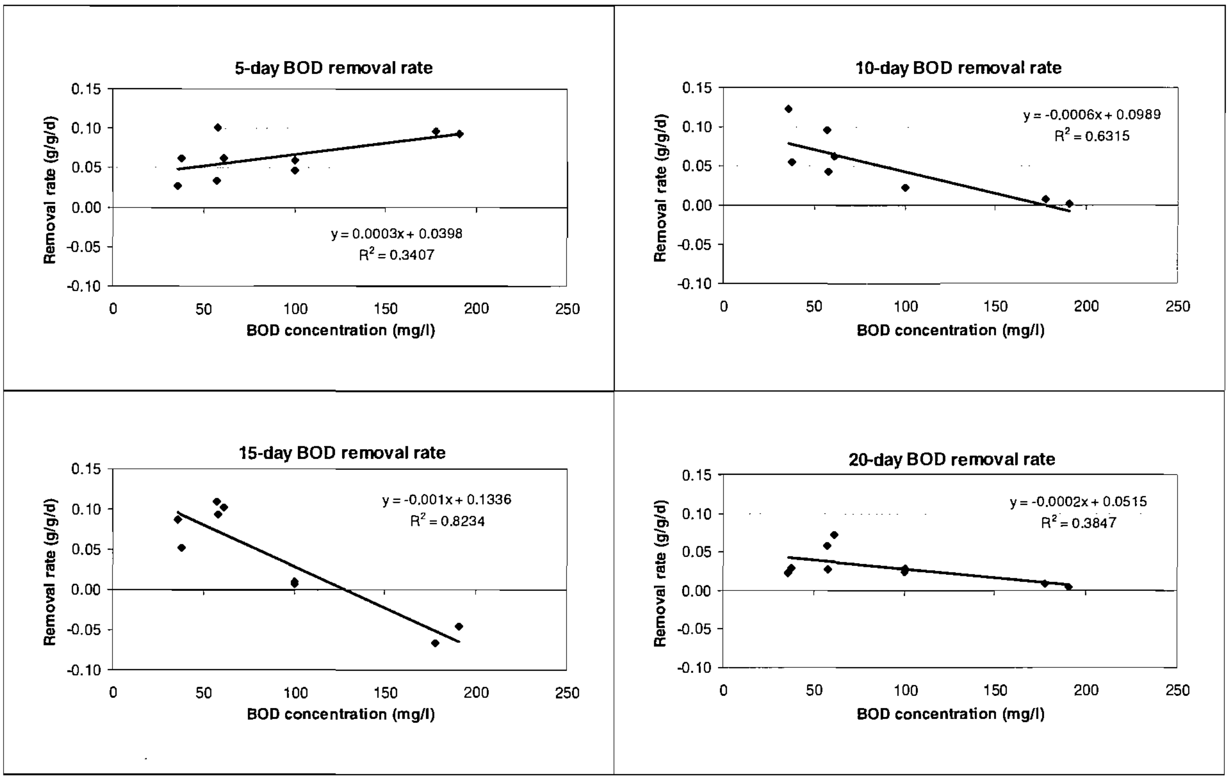


Figure 5.42 5, 10, 15 and 20-day filtered BOD_5 removal rates for initial BOD_5 concentration

The overall filtered BOD_5 removal rate for each dilution is shown in Figure 5.43. A regression analysis of the overall BOD_5 removal rate with BOD_5 concentration found a strong negative correlation ($R^2 = 0.80$; $p = 0.001$). The results are similar to those for COD removal except an approximate three-fold reduction in BOD_5 concentration produced a three-fold increase in overall removal rate.

This outcome can be expressed in practical terms to suggest whether improving the removal rate by dilution has potential in real applications. If an initial BOD_5 concentration of 191 mg l^{-1} gives an overall removal rate of 0.02 g g^{-1} d^{-1} and 57 mg l^{-1} a removal rate of 0.061 g g^{-1} d^{-1}, then dilution by 1:2 i.e. tripling the pond volume is required to obtain this 3-fold increase in rate. For a sizeable increase in volume, the increase in rate does not appear worthwhile. But if it is assumed these rates are constant, then a rate of 0.02 g g^{-1} d^{-1} implies a requirement for a 50-day hydraulic retention time (HRT) for 100% removal and a rate of 0.06 g g^{-1} d^{-1} implies a 17-day HRT. If this is applied to an incoming wastewater flow of 1000 m^{-3} d^{-1} at 180 mg BOD_5 l^{-1}, a pond volume of 50,000 m^3 would be required. Diluting this flow 1:2 to give a BOD_5 of 60 mg l^{-1} would require a pond of (1000 + 2000) m^3 day^{-1} x 17 days = 51,000 m^3. Although this means a 2% increase in the required pond volume, this may be offset by a 33-day reduction in retention time and the likelihood of no accompanying odours. This is of course an oversimplification, as removal rates did not remain constant throughout the experiment, and WSP are generally fed systems with mixing; the result therefore cannot be applied directly in design, but the calculation provides a numerical example to assess whether the increased removal rates have any practical application.

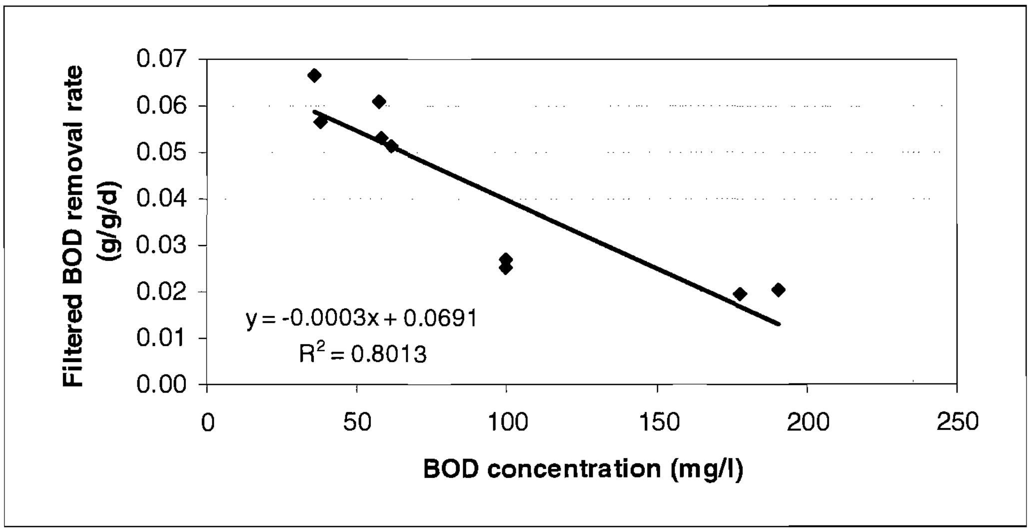


Figure 5.43 Overall BOD_5 removal rate for each initial BOD_5 concentration

5.6.6.3 Light intensity

Figure 5.44 shows the percentage transmission of incident light with depth in the tanks in relation to dilution/initial COD concentration, based on the irradiance at a given depth compared with irradiance at the pond surface. The results clearly show rapid increase in attenuation of light in the tanks by order of initial load. For a 1:50 dilution at 0.46 m, just 3% transmission of surface light is remaining. This compares to 18% which remained for dilution 1:400. At a depth of 0.91 m, less than 5% transmission of light was apparent in all tanks. This reflects the turbid nature of the synthetic wastewater which dilution naturally reduced.

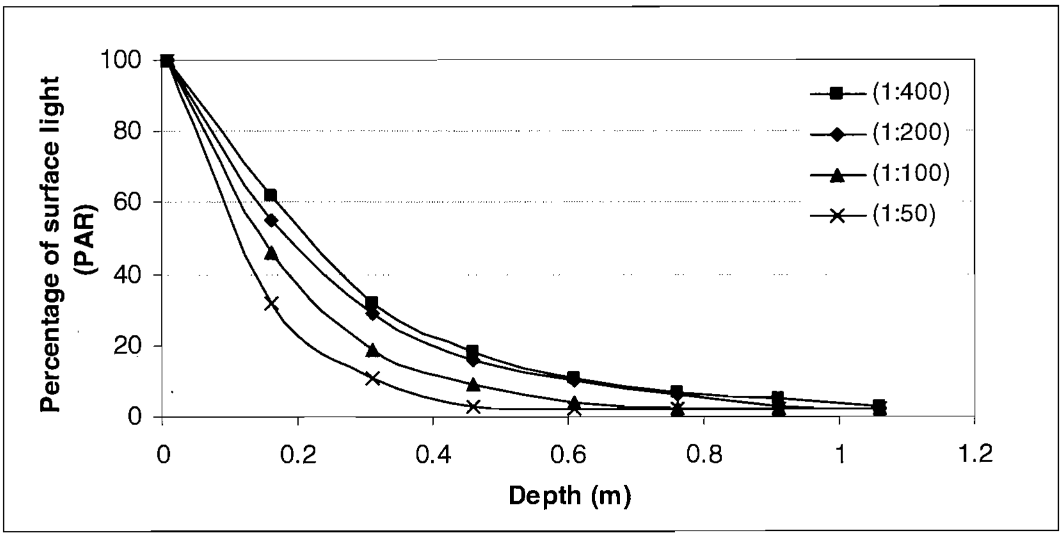


Figure 5.44 Percentage transmission of incident light with depth in the tanks in relation to dilution

The vertical extinction coefficient for each COD concentration was calculated using equation *5.5* and correcting for the non-parallel nature of the light source by deducting the vertical extinction coefficient for pure water 0.1 m^{-1} (Kirk, 1994). A vertical extinction coefficient of 3.58 m^{-1} was measured for mean COD concentration 94 mg l^{-1} whilst initial concentration 145 mg l^{-1} was only marginally more with a coefficient of 3.74 m^{-1}. Reflecting the increase in particulate and dissolved organic matter, vertical extinction coefficients of 5.26 m^{-1} and 6.42 m^{-1} were obtained for substrate concentrations 227 and 424 mg l^{-1} respectively. Values of 4.0 m^{-1} indicate water with very high biogenic turbidity whilst values in excess of 10 m^{-1} as quoted by Wetzel (2001) indicate environments where turbidity is extremely high. Regression analysis of the vertical extinction coefficient with initial COD concentration gave a strong linear relationship ($R^2 = 0.94$; $p = 0.032$).

5.6.6.4 Temperature effects on organic removal

The average surface water temperature for all runs over time in each tank is shown in Figure 5.45. Range bars show the variation between runs. Difficulties with the chiller unit and thermocirculator during early runs caused this variation. In general at the start of the run the temperature at 0.57 m and 0.99 m was 4°C and at the top (0.15 m) was 6°C. After approximately 25 days, temperature at all depths was >20°C.

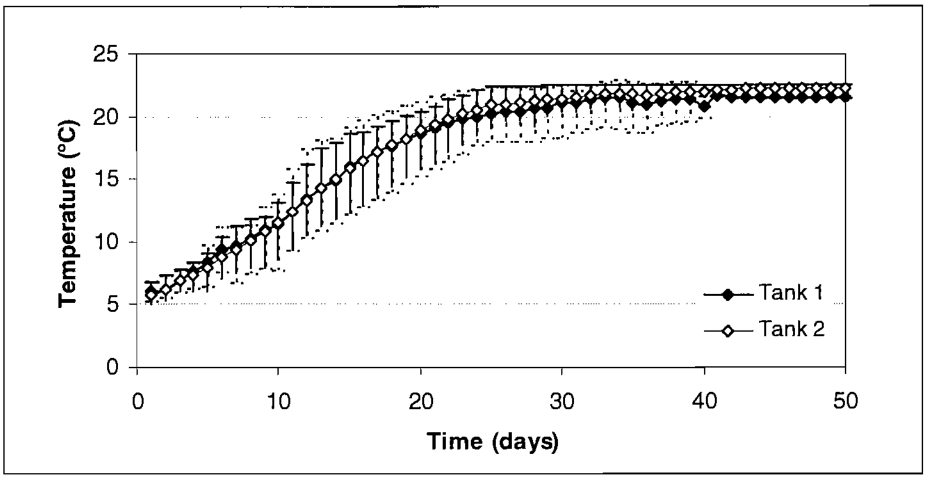


Figure 5.45 Average water temperature (0.15 m) in the tanks
(--Tank 1 – Tank 2)

Figure 5.46 shows the relationship between temperature and light intensity on COD and BOD removal rate. The increase in temperature over the course of the experiment had no apparent effect on removal rate of organic matter. This result was surprising as the rate of any chemical reaction increases with temperature provided that this increase in temperature does not produce alterations in the reagents or the catalyst (von Sperling and Chernicharo, 2005). Anomalies may have occurred with early removal rates when temperatures were low and a high proportion of removal was likely to be due to sedimentation; this may have masked any effect on biologically-mediated removal. Removal rates were also lower than in previous experiments, and this could have been due to temperature effects. Including constant surface light intensity with temperature in the regression only marginally reduced the residual variation compared with regression of temperature data alone (Figure 5.46).

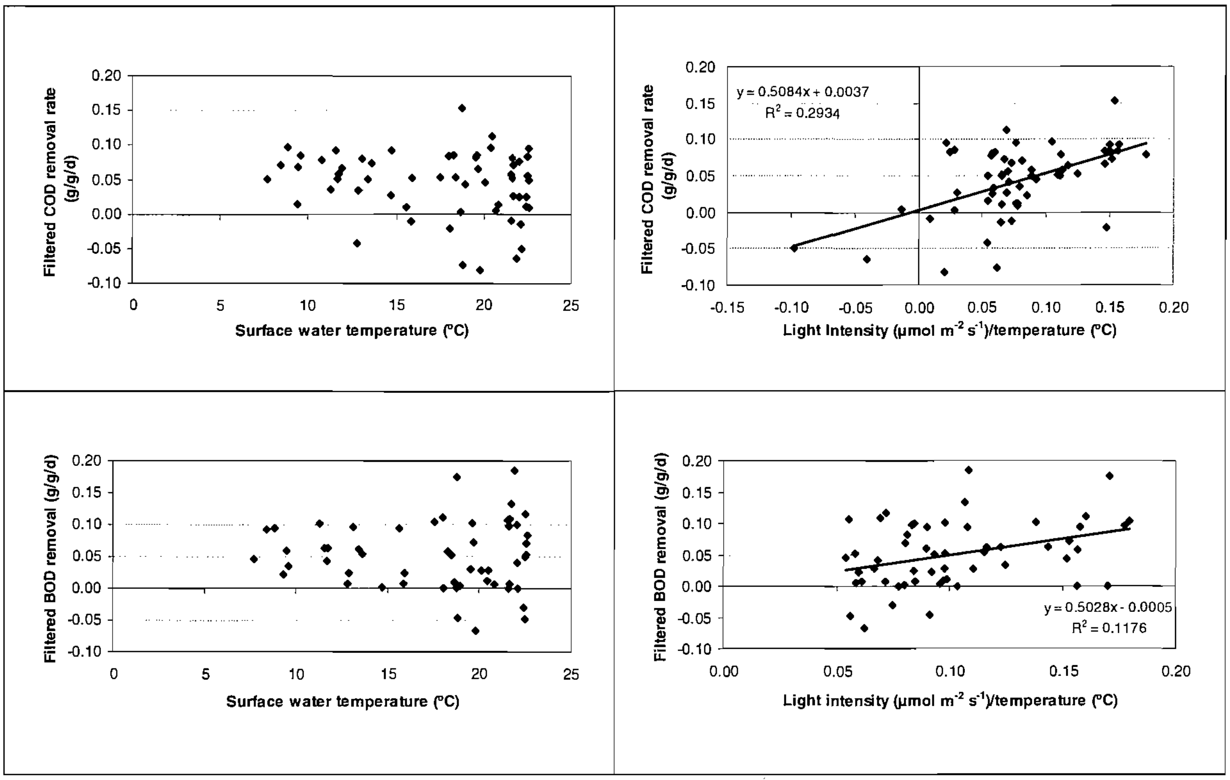


Figure 5.46 COD and BOD_5 removal rate and temperature and temperature with light intensity during tank experiments

For each run, temperature gradient was then compared with the change in COD concentration rather than removal rates and the results are shown in Table 5.23. There was a good level of significance between parameters for all runs except the two highest initial concentrations and the starting concentration 94 mg COD l^{-1} ($p > 0.05$). According to the regression analyses, only 11 and 24% of the variability in COD concentration can be explained by temperature for the two highest loads. This poor correlation is a likely consequence of the length of time the experiments ran; temperature remained at 20+ 2°C for half the duration which would have affected the correlation coefficient. The weak relationship between temperature and COD concentration for 94 mg COD l^{-1} might be due to the speed at which removal processes took place. By day 15 more than 75% of the initial soluble COD had been removed showing stabilisation had occurred irrespective of a major rise in temperature. The slopes from each regression plot are also shown in Table 5.23.

Table 5.23 Regression analysis of the effect of temperature on change in COD concentration

Run	Initial concentration (mg COD l^{-1})	R^2	Slope (mg COD removed d^{-1} °C^{-1})	p-value
1	210	0.72	-6.18	0.031
2	164	0.95	-8.58	<0.001
2	244	0.70	-10.21	0.018
3	125	0.93	-6.03	0.002
3	94	0.42	-1.72	0.166
4	417	0.11	-17.50	0.378
4	431	0.24	-11.31	0.166
5	87	0.89	-3.09	0.059
5	128	0.96	-6.52	0.022

Results show that temperature had more of an influence on the rate at which COD concentration was reduced when the initial COD concentration was higher. With an initial COD of 94 mg l^{-1} for example, results suggest there was a reduction of 1.72 mg COD l^{-1} for every 1°C increase in temperature. The higher initial concentration of 417 mg COD l^{-1} saw a reduction of 17.5 mg COD l^{-1} for every 1°C rise in temperature. This again is a reflection of the length of time at which the experiment was run at 20°C and much of the removal of COD took place during the latter period. Regression analysis on the slope values versus the initial COD concentration proved significant ($R^2 = 0.77$; $p = 0.002$). The temperature at which chlorophyll-a levels began to rise during each run was then plotted against initial COD concentration (Figure 5.47). The temperature required to kick-start an algal bloom increased linearly with the increase in initial load. An R^2 of 0.85 and a p-value of < 0.001 indicated a strong relationship between the two parameters.

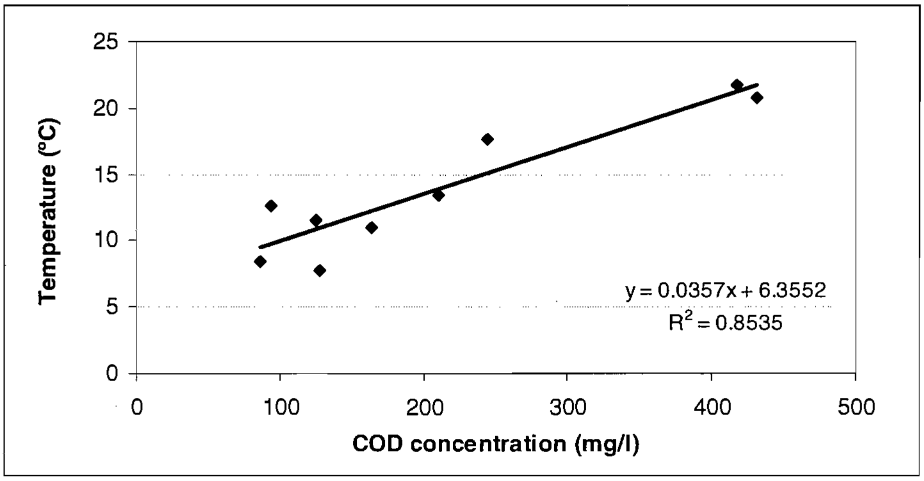


Figure 5.47 Temperature at which chlorophyll-a levels rose in relation to initial COD concentration

5.6.6.5 Chlorophyll-a

Figure 5.48 shows chlorophyll-a concentrations obtained in each run. The lower initial concentrations resulted in the earliest and most rapid growth of algae. Substrate concentrations 87 and 128 mg COD mg l^{-1} showed chlorophyll-a increased from as early as day 5 and no later than day 15. The highest chlorophyll-a level (0.8 mg l^{-1}) was for 164 mg COD l^{-1} on day 23 during Run 2. Initial concentrations 244 and 210 mg COD l^{-1} showed a rise in chlorophyll-a levels from day 15 and 17, and peaked around day 23 and 25, in runs 2 and 1 respectively. The peak concentrations were slightly different at 0.36 and 0.48 mg l^{-1}, the cause of which may be due to variations in the temperature regime during different runs. For the highest initial concentration growth in algae was minimal until day 21 when chlorophyll-a levels in both tanks rose and peaked at 0.6 mg l^{-1} on day 33. Following the peak of algal growth during all runs, chlorophyll-a levels then rapidly declined to 0.1 mg l^{-1} or less.

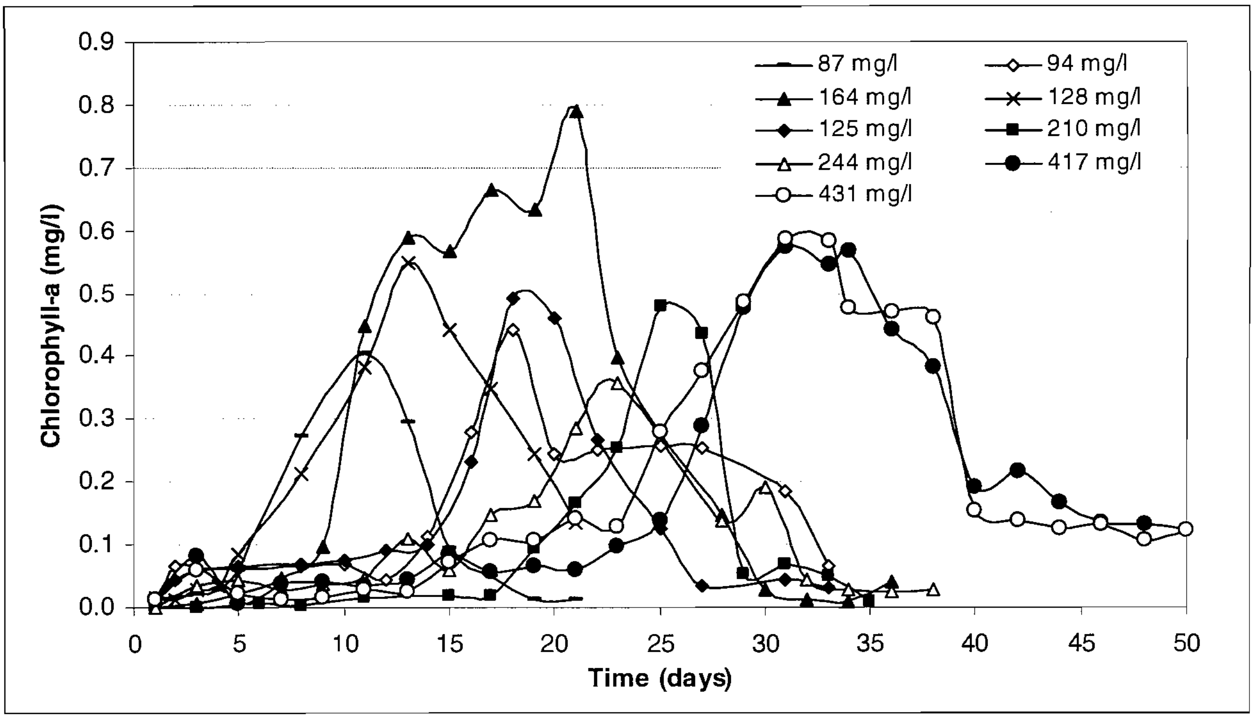


Figure 5.48 Change in chlorophyll-a levels over time for each initial COD concentration (same symbols represent the same run; open symbols tank 1; closed symbols tank 2)

Table 5.24 shows the approximate number of days to the end of the lag phase and/or the start of algal bloom. Although there is some variation between runs, it is clear that there are differences between initial concentrations. The onset of an algal bloom was delayed in the tanks that received the highest load. As shown in Figure 5.48 the time for each lag phase appeared to be correlated with temperature but the results in Table 5.24 suggest that is was also influenced by the quantity of organic matter present in the tanks and its effect on light penetration. Although there is an appreciable effect, a rather variable difference between 5 and 21 days lag is not huge for an 8-fold increase in dilution.

Table 5.24 Effect of initial concentration on the duration of lag phase prior to an algal bloom

Mean initial concentration (mg filtered COD l^{-1})	**Duration of lag phase (days)**
94	11
125	11
128	5
164	8
210	17
244	15
417	21
431	21

Table 5.25 shows apparent growth rates at different substrate concentrations calculated from the most rapid phase of growth. 128 mg COD l^{-1} showed the highest apparent growth rate at 0.020 h^{-1} and the lowest growth rate at 0.008 h^{-1} was calculated for initial concentration 431 mg l^{-1}. Apparent growth rate varied between 0.013 and 0.020 h^{-1} for COD concentration between 87 and 210 mg l^{-1} and declined at substrate concentrations higher than this. This behaviour suggests that the growth of algae were in some way inhibited by the high initial substrate concentration possibly aided by the low temperatures and reduced light. Regression analysis of apparent growth rate with initial concentration revealed 59% variability in growth rates was explained by the change in substrate concentration with a p-value of 0.01. These findings are not too dissimilar to those reported by Travieso *et al.* (2006b) who studied the effect of initial concentration of settled piggery wastewater on a mixed culture of *Chlorella vulgaris* and bacteria using one litre batch culture bottles. 250 and 400 mg COD l^{-1} both produced a specific growth rate of 0.016 h^{-1}. This rose to 0.023 h^{-1} for 800 mg COD l^{-1} and dropped to 0.011 h^{-1} for the highest COD of 1100 mg l^{-1}. Growth rates were marginally higher for increased substrate concentrations but this is a likely consequence of enhanced environmental conditions utilising summer temperatures and natural light.

Table 5.25 Effect of initial concentration on apparent algal growth rate

Mean initial concentration (mg filtered COD l^{-1})	**Apparent growth rate (h^{-1})***
87	0.016
94	0.016
125	0.017
128	0.020
164	0.013
210	0.017
244	0.011
417	0.009
431	0.008

* Calculated from most rapid growth phase only

Based on the data of algal growth and substrate uptake in the period up to the end of the exponential phase, the values of the observed microalgae yield given in mg chlorophyll-a produced/mg COD consumed were obtained. The data was plotted against initial COD concentration and is shown in Figure 5.49. The value of the observed yield increased with decreasing substrate concentration and the relationship between the two parameters gave a regression coefficient of 0.68 and a p-value of 0.006. The biomass yield was slightly higher in the bucket experiments than in the tanks when comparing similar initial COD concentrations; however the y-intercept of the equation was 0.008 in this experiment and 0.0088 for the buckets. Biomass yields were more similar between experiments when the strength of wastewater was lower. For example, an initial COD concentration of 128 mg l^{-1} in the tanks produced an observed yield of about 0.0074 mg chlorophyll-a/mg COD whereas an initial COD concentration of 111 mg l^{-1} in the buckets produced an observed yield of about 0.0075 mg chlorophyll-a/mg COD. At higher initial loads (+ 400 mg l^{-1}) there was an approximate three-fold reduction in the observed microalgae yield and this was 1.5 times lower than those found in the buckets at the same concentration. The difference between experiments is likely to be a consequence of environmental conditions which were less favourable to algal growth in the tanks and this was more pronounced at higher initial substrate concentrations.

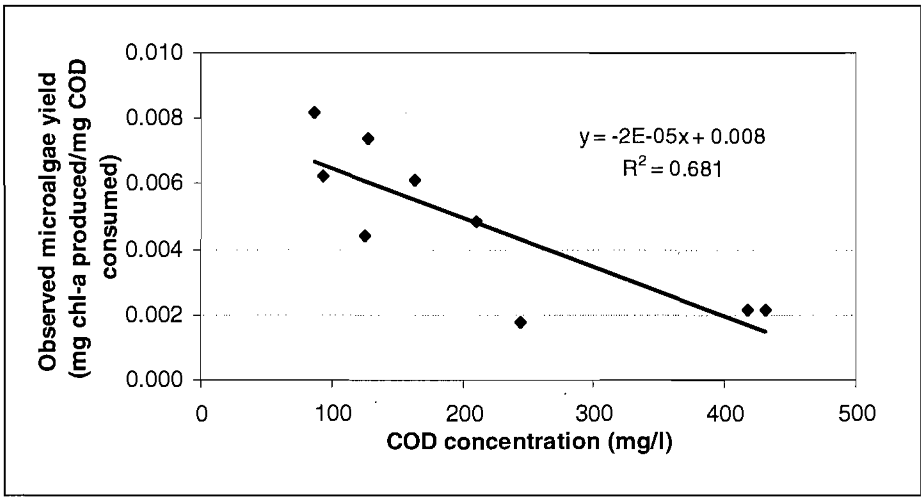


Figure 5.49 Observed microalgae yield for each substrate concentration

5.6.6.6 Suspended solids

Suspended solids concentration decreased substantially during the first 5 days for all runs (Figure 5.50). Suspended solids removal can be affected by the settleable solids in the initial concentration, accumulation of sludge and solids feedback into the water column. The main cause of reduction of suspended solids in the tanks is likely to be the settleable solids content of the synthetic wastewater. These results also correspond to a reduction in unfiltered BOD_5/COD during this period reflected in the removal rates calculated for day 5. Suspended solids then fluctuate and rise with the corresponding concentrations in chlorophyll-a. For example, values for 210 mg COD l^{-1} during Run 1 increased to 75 mg l^{-1} on day 25 coinciding with the peak in chlorophyll-a. A rise in suspended solids occurred in both tanks at dilution 1:50 (417-431 mg l^{-1}) between days 15 and 22 during Run 4. Chlorophyll-a levels were still relatively low at this point and therefore do not account for the increase. During this period, however, sludge mats had risen to the surface of the pond due to anaerobic processes in the sediments. Increasing load and temperature stimulates gas production which creates motion in the sludge that becomes less dense and may erupt solids into the upper layers of the pond (Parker and Skerry, 1968). Break-up of this sludge layer during sampling would have elevated levels of suspended solids in the sample.

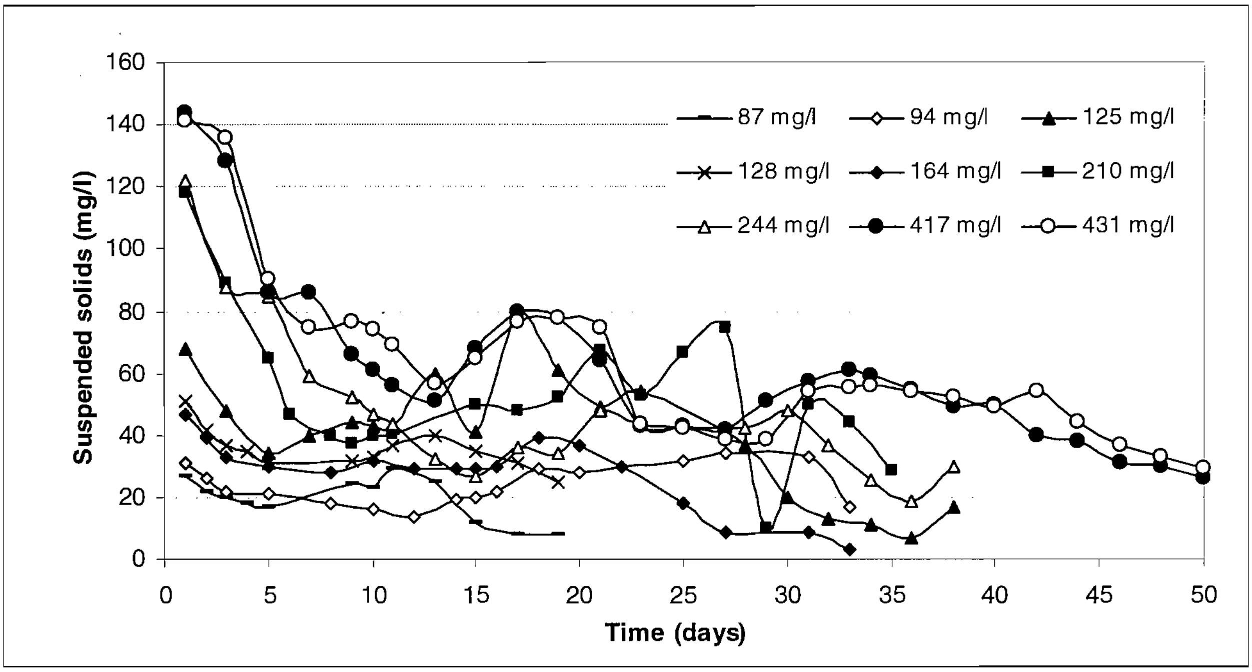


Figure 5.50 Change in suspended solids concentrations over time for each initial substrate concentration (same symbols represent the same run; open symbols represent tank 1; closed symbols tank 2)

Figure 5.51 show the relationship between suspended solids removal rate and initial COD concentration. During the first 5 days, removal rate ranged from 0.08 to 0.12 g g^{-1} d^{-1}; this small variation might indicate removal was primarily due to sedimentation. Rates for 5-day suspended solids removal were slighter higher than 5-day COD removal rates. Regression analyses both resulted in high residual variation between parameters (see Figure 5.51). By day 10 a relationship between the two parameters was apparent from the change in R^2 value from 0.008 to 0.26, however the correlation remained weak and showed some residual variance particularly for the tanks with the lowest initial concentration. The decrease in removal rate would have been due to the growth of algae increasing the suspended concentration and this too would account for the variation in rates.

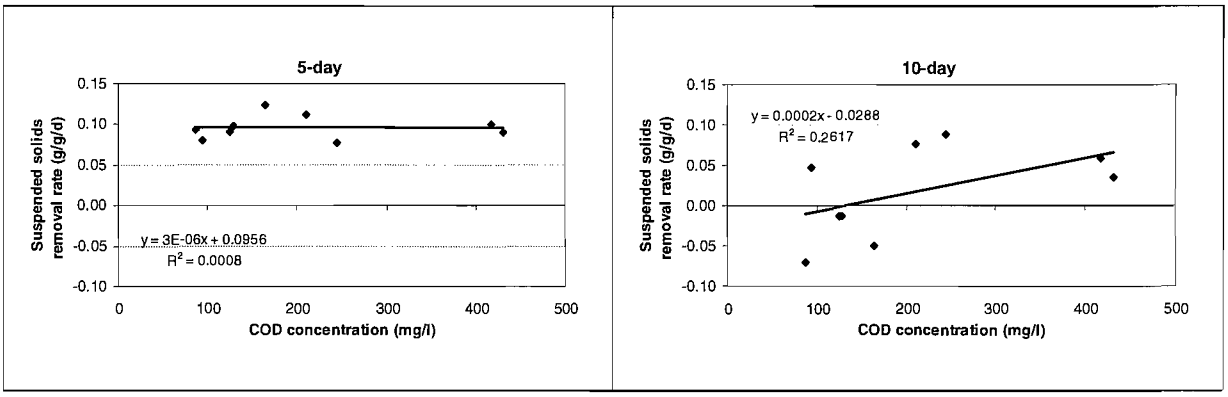


Figure 5.51 5 and 10-day suspended solids removal rate for each initial COD concentration

5.6.6.7 Nutrients

Figure 5.52 shows the rate of ammonia removal against initial COD concentration. The 5-day removal rate shows that the tanks receiving the lowest substrate concentration had a higher removal rate than the tanks with the higher load. No significant difference was observed in ammonia removal between dilution 1:200 and 1:400 ($p = 0.63$). By day 10 ammonia removal rates had dropped in the tanks with the lowest substrate concentration and had increased in the tanks with COD concentration above 400 mg l^{-1} to an average of 0.024 g g^{-1} d^{-1}. Ammonia levels are known to reduce more rapidly during the spring warm-up period due to the rapid uptake of ammonium by growing algae, coupled with volatilisation of any residual ammonia at the high pH during this period. Rockne and Brezonik (2006) measured nutrient removal in a cold region WSP in a region with 4 months ice cover. Their results demonstrated the importance of ammonia volatilisation as a nitrogen sink in ponds that experience ice cover, setting up conditions for both high primary production and high ammonia levels in the spring. This is unlikely here as by day 5, pH levels were below 8 in all tanks and therefore algal uptake would be the primary sink for ammonia removal. The rise in chlorophyll-a earlier in the tanks with the reduced load would also support this argument. The possibility of earlier ammonia removal (aided by the incorporation into biomass) from diluting wastewater would be advantageous for an earlier discharge in the spring. Nitrification was not a likely cause of ammonia removal as nitrate levels remained low following an initial drop in levels at the start of each experiment. During this phase any oxygen initially present in the tanks would have been used up due to the oxygen demand imposed by the addition of organic matter and the system would have become anoxic. Heterotrophic organisms present could have begun oxidation of the initial load through the use of nitrate as an alternative electron acceptor. Analysis of the wastewater for nitrate showed 90-95% removal in all tanks during the first 5 days. There is little evidence for denitrification in WSP as nitrate is usually found at very low levels in domestic wastewater. During this experiment, however, large quantities of tap water were used which contained relatively high levels of nitrate[1].

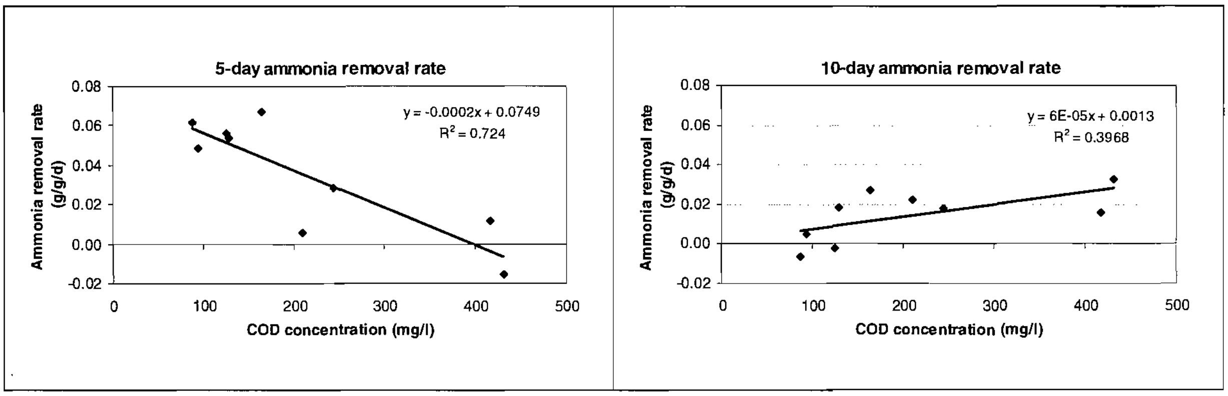


Figure 5.52 5 and 10-day ammonia removal rate for each initial COD concentration

Figure 5.53 shows orthophosphate removal in all tanks. By day 5, removal was slower for orthophosphate than ammonia removal but results reflected a similar trend towards a negative correlation between orthophosphate removal and initial concentration. Removal of phosphorus is known to be less effective in facultative ponds than ammonia removal as there are more mechanisms and pathways of removal ammonia, although these are the subject of much debate (Yamamoto *et al.*, 2006; Babu *et al.*, 2007; Camargo Valero and Mara, 2007). By day 10, there was no relationship between removal rate and dilution and there was considerable variation between rates. The reduction in orthophosphate would have been due to algal uptake and this would have occurred at different rates affecting the residual variance. Regression analysis of chlorophyll-a with reduction in orthophosphate using data points from the start of the experiment to the end of the chlorophyll-a peak for each run were calculated (see Table 5.26). Results show a good level of significance between parameters for all runs ($p < 0.05$) however the variability differs more between similar initial COD concentrations than between different dilutions during the same run. In fact, the variability shown between chlorophyll-a and orthophosphate for the same run are quite similar regardless of dilution. This may be a reflection of the variation in temperature regime between runs. Orthophosphate never

[1] Mean nitrate levels: 27.54 mg l^{-1} (Southern Water Report for year 2004)

became limiting for algal growth as concentrations in the tanks did not reach significantly low levels.

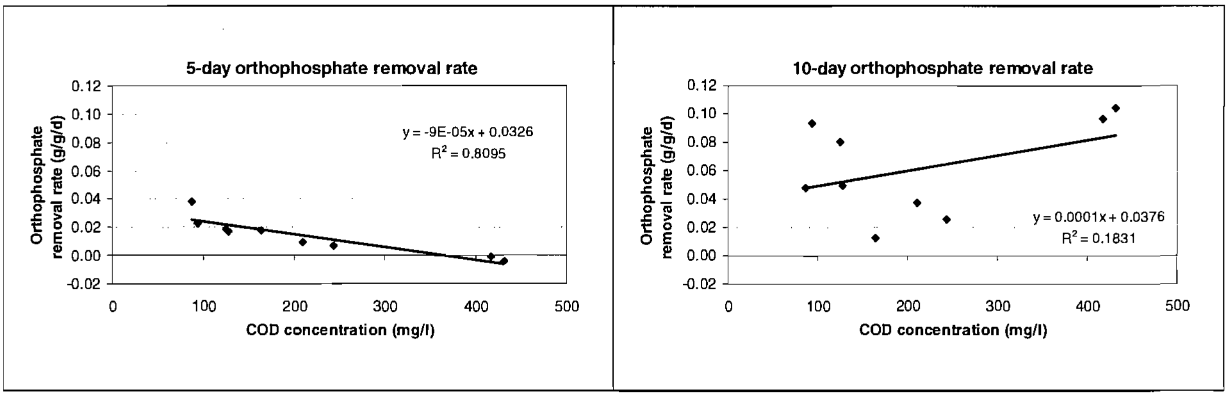


Figure 5.53 5 and 10-day orthophosphate removal rate for each initial COD concentration

Table 5.26 Relationship between chlorophyll-a and orthophosphate removal for each initial COD concentration

Run	Initial concentration (mg COD l^{-1})	R^2	p-value
1	210	0.72	<0.001
2	164	0.67	0.002
2	244	0.61	0.002
3	125	0.41	0.03
3	94	0.43	0.01
4	417	0.69	<0.001
4	431	0.71	<0.001
5	87	0.82	0.005
5	128	0.93	<0.001

5.6.6.8 Dissolved oxygen

Figure 5.54 shows dissolved oxygen concentrations in all tanks during the experimental period. The average temperature is also shown. Dissolved oxygen levels in all ponds were approximately 10 mg l^{-1} at the start of each experiment when the algal inoculum was added, and subsequently declined as the oxygen demand quickly reduced the available dissolved oxygen leading to anoxic conditions. The tanks with the lowest initial concentration produced larger rises in dissolved oxygen during periods when the algal blooms occurred, reaching saturation during peak chlorophyll-a levels. COD concentrations of 87 and 94 mg l^{-1} saw oxygen levels rise to 20 mg l^{-1} compared to the rise of 15 mg l^{-1} and 13 mg l^{-1} observed for substrate concentrations 128 and 164 mg l^{-1} respectively. There was an anomaly during Run 3 which saw oxygen levels in the tank rise to only 3.5 mg l^{-1} for 125 mg COD l^{-1}. Very little measurable oxygen was observed during the runs with wastewater diluted 1:100 (210 and 244 mg COD l^{-1}). Slight increases up to 2-3 mg l^{-1} were recorded at the end of the experiment. In the tanks with the highest initial load, DO levels remained below 1 mg l^{-1} throughout the experimental period. Although chlorophyll-a concentrations did increase, oxygen produced by the algae was constantly utilised by respiring organisms keeping dissolved oxygen levels in the tanks at a minimum. There would have been some oxygenation from the air as in outdoor ponds where wind mixing increases the transfer of atmospheric oxygen, but the major source of free oxygen is generally considered to be from algal photosynthesis as noted in the literature review. The absence of DO is a problem for highly loaded WSP in spring as this leads to odour complaints, so a reduction in the period without it is potentially important.

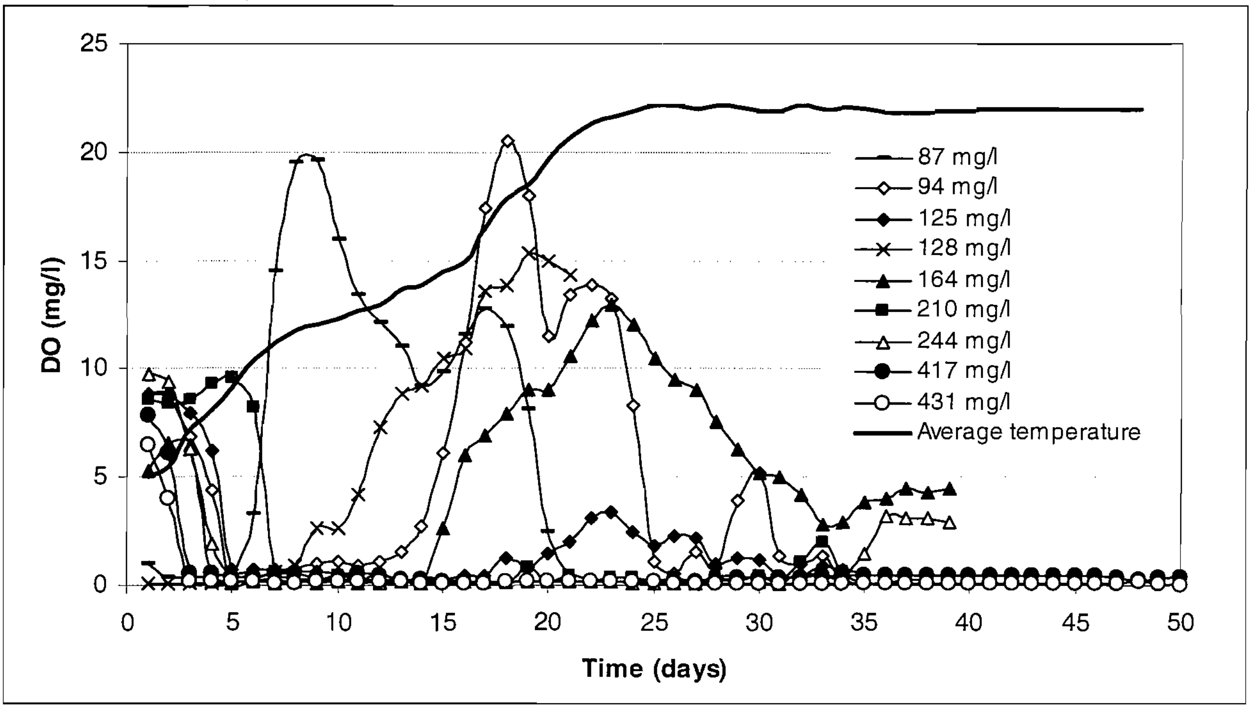


Figure 5.54 Change in surface dissolved oxygen levels for each initial COD concentration (same symbols represent the same run; open symbols represent tank 1; closed symbols tank 2)

A scatterplot shown in Figure 5.55 shows an example of a clear relationship between chlorophyll-a levels and dissolved oxygen for initial COD 94 mg l^{-1}, whereas Figure 5.56

shows no relationship between chlorophyll-a and dissolved oxygen where the higher load 417 mg l^{-1} was used. This same observation was reported by Abis (2002) regarding results from pilot-scale ponds based in West Yorskshire, UK. Abis (2002) found no clear relationship between these two parameters in ponds receiving high loadings. However there was a relationship using results from the pond with the lowest BOD_5 loading. It was suggested that this was due to the difference in dissolved oxygen uptake rate which is related to BOD_5 loading.

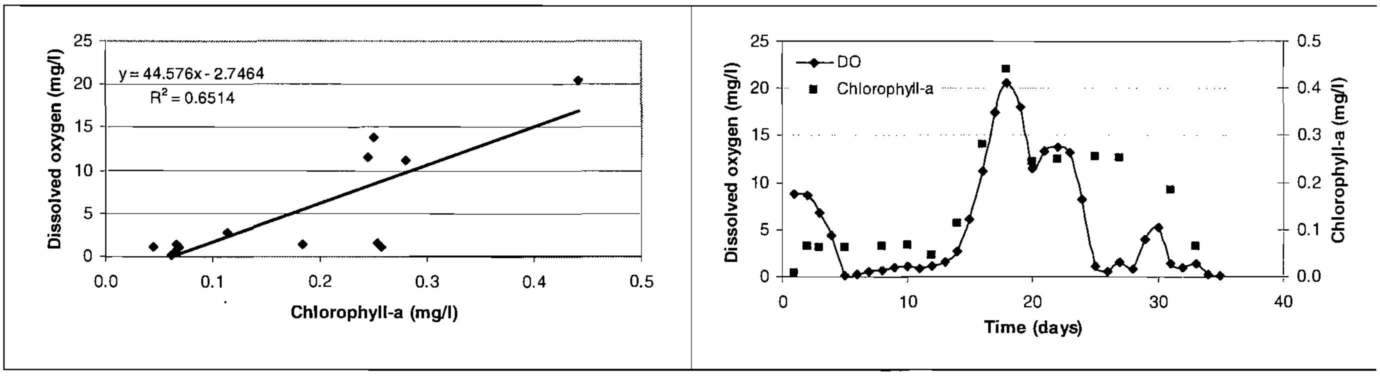


Figure 5.55 Relationship between surface DO concentration and chlorophyll-a concentration for COD 94 mg l^{-1}

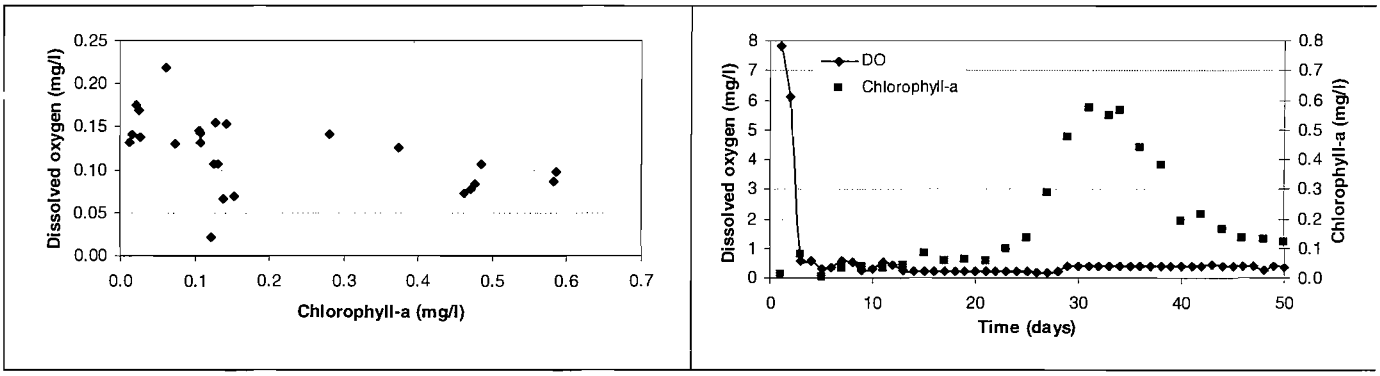


Figure 5.56 Relationship between surface DO concentration and chlorophyll-a concentration for COD 417 mg l^{-1}

The increase in chlorophyll-a concentration shown in Figure 5.56 for the highest initial concentration was most likely due to the availability of nutrients and sufficient light to stimulate growth. Because the oxygen demand of the wastewater exceeded the oxygen produced by algal photosynthesis, however, conditions remained anoxic. The rise in chlorophyll-a levels was preceded by the water turning pinkish/red in colour accompanied by an odour of hydrogen sulphide, further indicating the potential of dilution for avoiding odour problems. Figure 5.57 shows a sample of the wastewater which was sent for identification to the Department of Genetics and Microbiology at the University of Murcia, Spain, and kindly examined by Professor Francisco Torrella. On isolation the group of bacteria was identified as purple non-sulphur bacteria. The bacterium only bloomed in the tanks receiving the highest initial concentration indicating that these tanks were overloaded. The bloom in purple non-sulphur bacteria coincided with both a rise in suspended solids (Figure 5.50) and negative COD removal rates (Figure 5.40), and was possibly responsible for the increased COD concentration rather than the sludge layer as previously stated. Dark respiration and net oxygen production rates were also low during this time (Figure 5.58). Chlorophyll-a rise was observed following the decline in the purple non-sulphur bacteria and this coincided with a change from negative to positive gross oxygen production. Honda *et al.* (2006) reported a similar phenomenon using an anaerobic pond to treat noodle processing wastewater. They measured the levels of both bacteriochlorophyll-a, photosynthetic pigments found in most purple non-sulphur bacteria, and chlorophyll-a and found bacteriochlorophyll-a content dropped sharply when chlorophyll-a increased accompanying the algal growth. They suggested that this was probably due to oxygen generation from algal growth, which is known to suppress the synthesis of bacteriochlorophyll-a formation (Barnes, 1998).

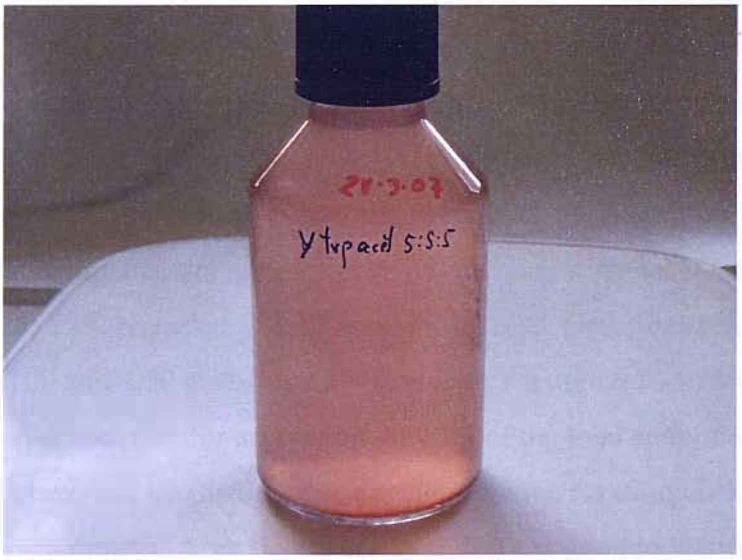


Figure 5.57 Sample of phototrophic purple non sulphur bacteria isolated from dilution 1:50 (Torrella, 2007)

5.6.6.9 Oxygen production

Figure 5.58 shows an example for each dilution of the rate of net and gross oxygen production and respiration over the course of a run. For dilution 1:400/lowest initial concentration, gross production increased 3-fold to a maximum of 2.96 mg O_2 l^{-1} hr^{-1} from day 12 to day 18. A change from negative to positive net production occurred on day 10 leading to a subsequent increase in free oxygen in the system as shown in Figure 5.58. Maximum net production rates (2.0-2.3 mg O_2 l^{-1} hr^{-1}) occurred between day 18 and 27. Dark community respiration rates remained low throughout the run. On day 10 and 12, results of the light and dark test showed an increase in dissolved oxygen resulting in positive respiration rates in both the light and dark bottles. Many studies have shown similar results and number of possible explanations were provided but without testing (Dugdale and Wallace, 1960; Vollenweider, 1969). Pamatmat (1997) however, presented experimental evidence for the increase instead of decrease in oxygen when incubating water in dark bottles to measure plankton respiration. The findings were explained by testing a theory of H_2O_2[2] production and decomposition. When plankton respiration was poisoned without inhibiting catalase[3] activity in the dark, there was an increase in oxygen resulting from H_2O_2 decomposition. Catalase in the presence of H_2O_2 results in the production of oxygen in dark bottles. Pamatmat (1997) stated that there is no other known process that leads to oxygen production in the dark and that H_2O_2 decomposition and O_2 production in the dark are one and the same process. No investigations of this type were carried out during this study, thus it is only possible to speculate on the cause. Oxygen increase in the dark only occurred in the lowest initial concentration. Dilution 1:200 showed a rise in net oxygen production on day 11 reaching a maximum of 2.56 mg O_2 l^{-1} hr^{-1}on day 17. Respiration values accounted for 37% of the total sum of oxygen measurements (including net and gross oxygen production) when taken as absolute values compared to 23% recorded at the lowest initial load. Gross oxygen production from dilutions 1:100 and 1:50 gave values of less than 1.6 mg O_2 l^{-1} with contributions from gross oxygen accounting for an average of 12% of the total sum of oxygen measurements. Net oxygen production was always negative for dilution 1:50 reflecting the increased oxygen demand from the higher load. Net oxygen production for dilution 1:100 showed a gradual increase from negative values on day 34 recording a maximum

[2]Hydrogen peroxide (H_2O_2) is a ubiquitous natural substance in aquatic ecosystems (Pamatmat, 1997). In sunlight, H_2O_2 is generated by photolytic reduction of O_2 by humic acids at rates depending on the dissolved organic matter and solar radiation (Cooper and Zika, 1983).

[3]Catalase decomposes H_2O_2 to O_2 and H_2O and is also ubiquitous (Pamatmat, 1988)

of 0.88 mg O_2 l^{-1} hr^{-1} on the final day. Both observations are reflected in the dissolved oxygen levels recorded during each run (Figure 5.54). Similar to the bucket experiments, gross oxygen production showed a strong correlation with chlorophyll-a concentration ($p \leq 0.001$).

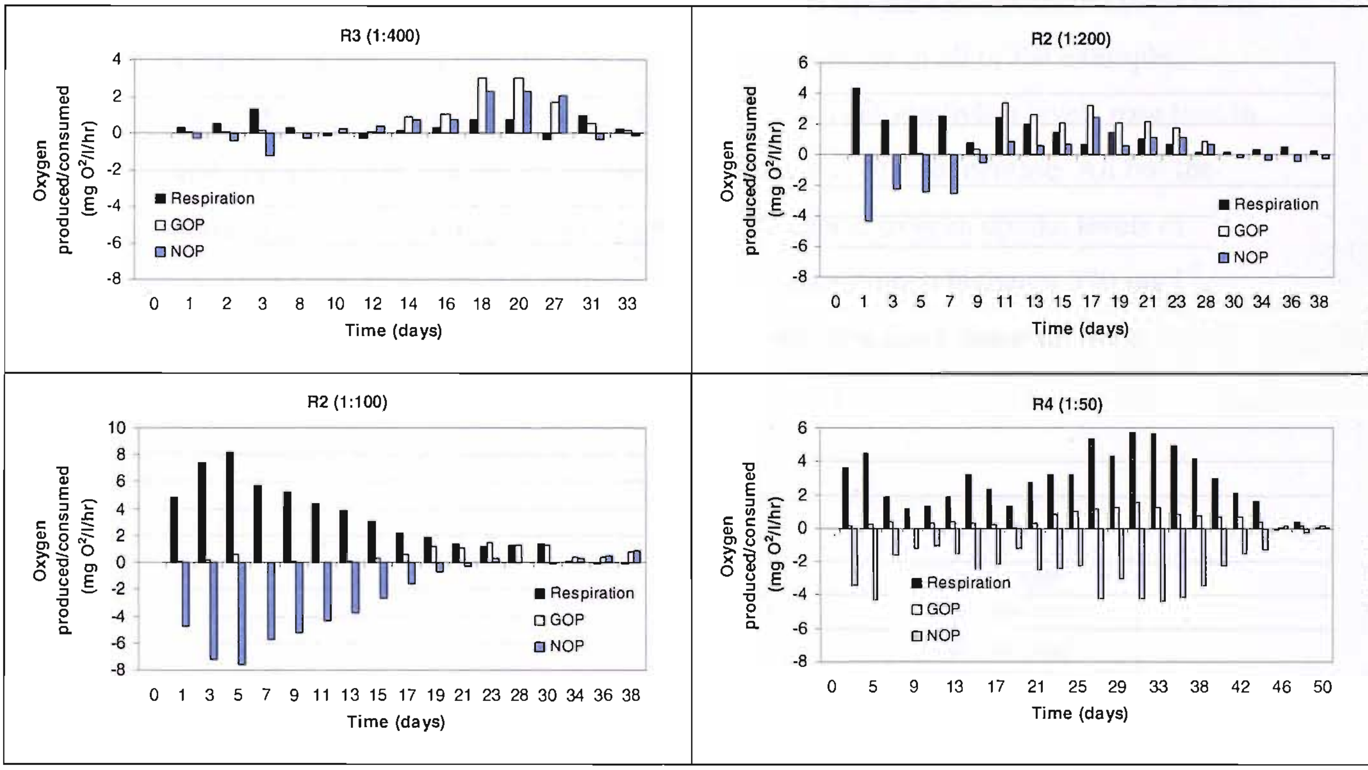


Figure 5.58 Examples of community respiration, net and gross photosynthesis (mg O_2 l^{-1} hr^{-1}) for each dilution

The estimated total gross oxygen produced between sampling intervals was compared with COD removal between sampling days and evaluated with regression analysis. COD removal over time was poorly correlated with gross oxygen production in the same period ($p > 0.05$). Results for the bucket experiment showed a better correlation between the two parameters. A reason for the poor correlation was possibly due to the temperature profile, which may have delayed the rise in gross oxygen but did not prevent a substantial amount of COD being removed in the tanks. As oxygen production then increased and COD removal slowed, there was less COD being removed than could be accounted for.

The amount of oxygen utilised over time was then calculated using average gross oxygen production between sampling days multiplied by the time interval, for an example from each dilution. The accumulated oxygen uptake curve is shown in Figure 5.59. For dilutions 1:400 (94 mg l^{-1}), 1:100 (244 mg l^{-1}) and 1:50 (417 mg l^{-1}) there was a lag phase up to days 14, 15 and 21 respectively followed by a more rapid phase in oxygen uptake until day 27 for dilution 1:400. For the higher strength wastewaters, there was a steady increase in oxygen uptake reaching 150 mg l^{-1} and 75 mg l^{-1} for 1:100 and 1:50. The lag phase represented minimal oxygen consumption prior to biodegradation possibly due to the low initial temperatures. For dilution 1:200 (164 mg l^{-1}), the lag phase was shortened to just 9 days and reached 143 mg l^{-1} on day 13. Oxygen uptake remained consistent until day 28 when oxygen utilisation then declined. Oxygen uptake in all of the examples coincided with rate of biomass growth in Figure 5.48. As chlorophyll-a levels rose first in the initial concentration 164 mg l^{-1}, oxygen utilisation was first to increase. All but the lowest strength wastewater appeared to peak then plateau at oxygen uptake levels of between 50 and 150 mg l^{-1}. 1:400 dilution however peaked much higher at 390 mg l^{-1}, possibly as a result of the assumption made that the rate remained linear between sampling days.

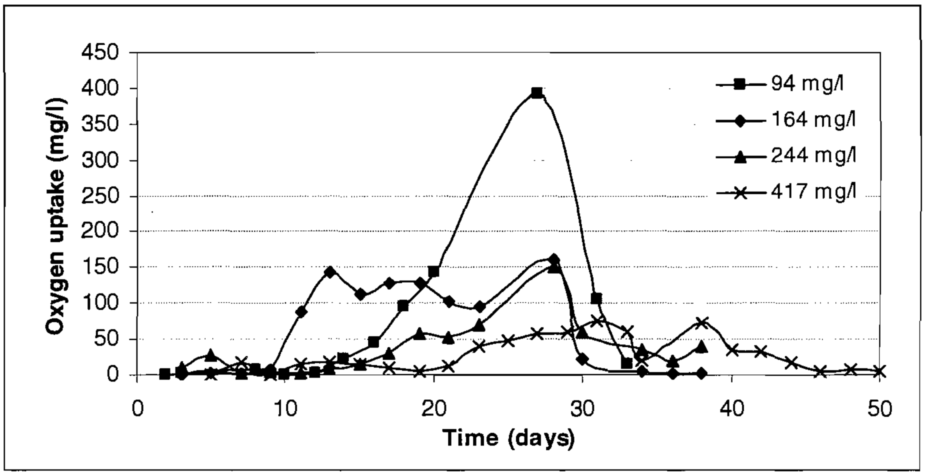


Figure 5.59 Oxygen uptake curves for different initial substrate concentrations

5.7 Discussion and conclusions

5.7.1 Effect of initial COD concentration on removal rate and O_2 production

Batch culture experiments were used and although WSP are frequently continuous flow systems, the design of the experiment has allowed for the rate of stabilisation to be monitored. The use of dilution to alter the strength of wastewater had a significant effect on the relative BOD_5 and COD removal rates, with negative correlations between initial organic load and removal rate. A similar result was found by Queiroz *et al.*, (2007), who reported on the kinetics of organic matter removal from parboiled rice effluent by cyanobacteria in a stirred batch reactor. Results showed a dependence of the reaction order on the substrate concentration, with highest orders obtained at minimum COD values. In the current study, monitoring of batch-fed systems demonstrated a pattern whereby the highest removal rates were found in systems receiving lower strength wastewater. Removal rates were calculated over a series of days and where possible overall removal rates were determined. These varied from 0.020 to 0.063 g g^{-1} d^{-1} in the tanks and from 0.17 to 0.28 g g^{-1} d^{-1} during the first two days in the flasks under a daylength of 24:0h. Using results from the tank experiments which were the closest replicate to a WSP, removal rates suggested that for a 2% increase in the required pond volume, there is the possibility for a 33-day reduction in retention time. Furthermore, improvement in pond oxygen relations might prevent the occurrence of unpleasant odours: this is an important factor since these are one of the chief causes of complaint against WSP systems in seasonal climates and their replacement by other methods of wastewater treatment.

Despite the wide range of COD removal rates under the different experimental conditions, the response of removal rate to changing concentrations was fairly similar. In particular, results were comparable between bottles and flasks on day 2. The bottles gave an average for all runs of 0.00023 g g^{-1} d^{-1} per mg l^{-1} whilst although the rate of change in the flasks varied with changing total daily light input the result for 12:12h daylength was 0.00021 g g^{-1} d^{-1} per mg l^{-1} and 0.00024 g g^{-1} d^{-1} per mg l^{-1} for 24:0h daylength. It is likely that results were similar as both experiments displayed conditions involving small volumes and high light exposure. Results for rate of change of removal rate with respect to COD concentration in the bucket and tank experiments were possible to compare on day 5 due to their larger volumes incorporating depth effects. These however gave more differing results at 0.00003 g g^{-1} d^{-1} per mg l^{-1} for buckets and 0.00004 g g^{-1} d^{-1} per mg l^{-1} for tanks. Overall removal rates with changing COD concentration were closer at 0.00008 g g^{-1} d^{-1} per mg l^{-1} for buckets and 0.0001 g g^{-1} d^{-1} per mg l^{-1} for tanks. It is possible that the differences observed between these experiments were due to both depth and temperature effects. The addition of reduced light penetration is likely to account for the more notable difference between overall removal rates obtained in the bucket and tank experiments and those obtained for the bottle experiment which was much higher at

0.0004 g g^{-1} d^{-1} per mg l^{-1}. As rates quickly become negative in the flask experiments, overall removal rates were not calculated.

Negative removal rates reflected the rapid reduction in organic matter, and the establishment of an algal population with its own BOD. Organic matter in suspension is converted into soluble matter through the mechanism of enyzmatic hydrolysis, thereby increasing the soluble content before further stabilisation (von Sperling and Chernicharo, 2005). Algae are also known to release a large number of extracellular organic compounds. These include glycolic acid, carbohydrates, amino acids, volatile substances and toxins (Wetzel, 2001) and could equally have been responsible for an increase in filtered COD levels. At higher COD concentrations above 400 mg l^{-1}, removal rates showed a gradual increase before decreasing. Negative values were not obtained at these concentrations as organic matter was still being degraded in the system and a significant increase in algal solids had not occurred during the period of the experimental run.

From all results on removal rate, it is observed that the highest rates were achieved before algae started to grow exponentially. Tarlan *et al.*, (2002) observed a similar phenomenon and attributed this to the effects of enhanced heterotrophic and mixotrophic growth. Time-course data of Chen *et al.*, (1997) showed that cells of *Haematococcus lacustri* could grow in both heterotrophic and mixotrophic modes, although growth was much better in mixotrophic culture. Kobayashi *et al.*, (1992) suggested that chlorophyll-a concentration in *Chlorella* species under heterotrophic conditions increases rapidly after the organic carbon source has been depleted to some extent. These findings might help to explain the pattern of significant early COD removal, aided by sedimentation that would also have been a factor during the first few days, followed by the exponential growth of biomass. COD removal was substantial in dark-variant flasks and bottles, which also suggests the occurrence of heterotrophic activity.

As a result of both the lower loading and the increased removal rate, recovery to oxygenated conditions occurred more quickly in systems receiving lower organic loads than those where the starting COD load was high. During the flask experiments there was a clear relationship between dissolved oxygen and substrate concentration in each flask. Oxygen levels remained low (< 0.1 mg l^{-1}) for the highest strength wastewater during light-dark regimes 12:12h and 6:18h but were able to recover to 3 mg l^{-1} by the end of the run for 24:0h. In contrast to this, supersaturation was reached during all experiments for the two lowest initial substrate concentrations and the speed at which this occurred was dependent on the light energy available during the course of each run. An absence of recovery to facultative conditions in the higher loaded systems could also have been a consequence of limited light penetration and higher toxicity due to sulphide, ammonia, etc., which may have inhibited the development of phototrophs. In parallel to dissolved oxygen measurements, light and dark bottle experiments were conducted to establish oxygen exchange in some of the systems. As expected, gross oxygen production correlated strongly with chlorophyll-a levels. The amount of oxygen utilised over time for each substrate concentration could also be calculated using gross oxygen production. Where the initial load was high a lag phase represented minimal oxygen consumption prior to biodegradation. Oxygen uptake coincided with rate of biomass growth in both the tank and bucket experiments.

5.7.2 Temperature and organic matter removal

Almasi and Pescod (1996) found that temperature had a positive effect on BOD_5 reduction in WSP operating under conditions between fully anaerobic and facultative. The higher temperature resulted in lower effluent BOD_5 concentration and the anoxic ponds performed more efficiently. This is due to the effect of water temperature on oxidation and degradation of biodegradable substrate by bacterial activities. The removal rate is therefore a reflection of bacterial activity, so temperature should have an effect on the rate. The results of the tank experiment showed no clear relationship between temperature and rate of removal. Relationships were however established between temperature and change in COD concentration over time for each initial substrate concentration. The relationship was less significant in the tanks that received COD concentrations above 400 mg l^{-1} as half the duration of the experiment was run at the same temperature (20+ 2°C), due to the longer time period required for stabilisation. Temperature was also shown to have an effect on the timing of an algal bloom and this was linearly correlated with initial COD concentration. Results demonstrated that the temperature required to kick-start the rise in chlorophyll-a concentration increased accordingly with the increase in initial load.

Although temperature has an effect on photosynthesis and the growth of algae, the ecological effects of light and temperature are difficult to separate because of the interrelationships between metabolism and light saturation. The intensity of light required to saturate algal photosynthesis commonly increases as water temperature increases. Below light saturation, photochemical reactions limit photosynthesis, and these reactions are relatively temperature-independent except at very low temperatures (< 5°C) (Wilhelm, 1990). Separate temperature experiments were not conducted during this study but previous studies have evaluated the effect of temperature on pond treatment. Thirumurthi (1974) evaluated the work of Suwannakarn (1963) who ran four laboratory-scale ponds under identical conditions, except that they were maintained at temperatures of 35, 24, 20 and 9°C. Results showed that after 15 days, removal efficiencies were 98% for the pond at 35°C, 95% for 24°C, 85% for 20°C and 80% for the pond at 9°C; there is thus little difference in pond efficiency between temperatures 9 and 20°C. Temperatures much lower than 9°C are likely to result in the suppression of photosynthesis and the retardation of aerobic bacterial activity, therefore affecting pond treatment. Temperature and organic matter removal are discussed further in Section 5.7.3.

5.7.3 Light and organic matter removal

The effects of light intensity and total daily light input on COD removal were evaluated by comparing the results from each experiment. Similar removal rates were recorded for the bottle and flask experiments, with the highest rates at an average of 0.27 g g^{-1} d^{-1} for the lowest strength wastewater. Both experiments were run in containers with high surface area to volume ratios. Facultative ponds are designed to be relatively shallow to have a sufficient surface area to volume ratio to enable good algal growth and increased oxygen production via photosynthesis. Oxygen levels reached saturation during both experiments for the lowest strength wastewater although chlorophyll-a levels were up to 5 times higher in the bottles than the flasks. This may have been a consequence of different light intensities and natural light as opposed to fluorescent. The spectral quality of artificial lighting and natural solar radiation is known to differ in the PAR region which is important for photosynthetic efficiency. Thimijan and Heins (1983) published ratios for radiation (400-700 nm) per unit of luminous output (W m^{-2}) for different light sources and reported figures of 2.81 for warm white fluorescent tubing and 4.02 for daylight. This equates to a reduction of 30% of the total PAR from artificial lighting compared to that expected from natural sunlight, however this is an approximation as data came from typical lamps in the USA where spectral difference may vary.

Lowest removal rates were observed in the tanks under simulated conditions where the lowest light intensity was recorded (55 μmol m^{-1} s^{-1}). Flask experiments were also conducted under simulated conditions but with three times the intensity of light. The overall removal rate was 4-6 times that calculated for the tanks for all substrate concentrations. This increase may be due to the vessels used in the flask tests which allowed light through the entire surface area, and/or the different temperatures imposed on the systems. Bucket experiments showed lower removal rates than the bottle and flask tests but higher than the tank experiments, with an average of 0.12 g g^{-1} d^{-1}. The highest light intensity in all experiments was achieved during the bucket experiments with an average of 705 μmol m^{-1} s^{-1} and an approximate total daily light input of 15 mol m^{-2} d^{-1}. The high level of light input was not reflected in the removal rates, but the use of sunlight rather than fluorescent lighting may have accounted for removal rates being twice as high in the buckets as the tanks. Both the buckets and the tanks incorporated depth effects similar to those in WSP. Therefore the surface area exposed to light was limited to the top surface which although reflects normal pond operation did reduce the surface area to volume ratio compared with the smaller flask and bottle tests.

During the flask experiments daylength rather than light intensity was used to vary the total daily light input. Results demonstrated that the input light energy did have an effect on the rate of COD removal. This was most evident on day 2 where removal rates for all substrate concentrations were 0.05-0.07 g g^{-1} d^{-1} higher in the flasks that received four times the amount of light energy (6:18h and 24:0h). Difference in removal rates for different daylengths was highest in the flasks receiving the lowest strength wastewater; a four-fold reduction in light energy reduced the removal rate by two-fold. Statistical analysis was used to determine whether there was any difference between the rate of change of removal between daylengths on day 2. No noticeable difference was observed between daylengths 12:12h and 24:0h but results did demonstrate that by dramatically reducing the light energy using 6:18h daylength, the process of removal was slowed down to some extent. This difference was relevant where removal rates were measured on set days, but daylength showed little effect on overall removal rates when calculated using end values. This is likely to be a result of the growth of algae and its subsequent die-off that occurred in the flasks under 24:0h daylength, but that was not apparent during 6:18h light regime. Regression analysis of overall removal rates with total daily light input and also light intensity for all experiments revealed no correlation between the two parameters. This is unsurprising as there were numerous other variables that would have had an effect on removal processes in each experiment, i.e. temperature and surface area to volume ratio.

The most important physical feature of diluting the substrate concentration and its effect on the removal rate was the amount of light reaching the water. The depth at which maximum rates of photosynthesis occur varies with the transparency of the water, which is governed by the concentration of dissolved and particulate organic matter and abiotic turbidity. When densities of phytoplankton are high, self-shading effects can also greatly reduce light penetration and the trophogenic zone is reduced accordingly. A study by Fallowfield *et al.* (1999) on the effects of seasonal and diel climatic variation and operational parameters on the proportion of biomass in batch-fed high-rate algal ponds found no direct correlation between chlorophyll-a and incident daily irradiance. These results contrast with those obtained for continuous cultured ponds where biomass was correlated with daily irradiance (Cromar *et al.*, 1996). Fallowfield *et al.* (1999) suggested that this may be due to the non-steady state conditions occurring in ponds operated as batch cultures compared with the quasi-steady state which is established in outdoor continuous cultures. Fallowfield *et al.* (1999) also stated that since batch cultures are often in exponential phase for much of their duration, it is unlikely that sufficient biomass is attained for the cultures to be light-limited due to self-shading. The dissolved and particulate organic matter present in the wastewater might therefore have been the main factor reducing light intensity, at least at the start of the experiment as shown in Figure 5.35. Absorption experiments using unfiltered samples at wavelength 440 nm correlated strongly with suspended solids and the ratio $R_{678:440}$ provided a good measure of chlorophyll-a levels in the system. The absorbance ratio $R_{678:440}$ tended to increase as each experiment proceeded and this increase was more apparent in the systems receiving the lowest initial COD concentration. It provided an indication of the transition from a system high in humic matter and low in chlorophyll-a to one experiencing a chlorophyll-a bloom and with moderate to low humic matter. Kirk (1994) suggested that the *in situ* absorption due to particulate matter at 440 nm is a convenient general measure of particulate colour in any water but is not as good a guide to particulate colour as filtrate samples are to soluble colour. Nevertheless, Kirk (1994) did conclude that in waters with a substantial particulate humic component, it is a useful parameter. The results suggested that useful information about organic matter and chlorophyll-a state could be retrieved from optical properties on unfiltered samples.

In the tanks, algal concentrations increased sooner where the substrate concentration was lower. In the highest strength wastewater there was a lag phase of 21 days before the algal bloom, compared with as little as 5 days for the lowest initial concentration. The highest biomass yield was generally obtained at the lowest load with values ranging from 0.007 mg chlorophyll-a mg^{-1} COD at initial substrate concentrations of about 100 mg l^{-1}

to 0.0025 mg chlorophyll-a mg^{-1} COD at COD concentrations of about 400 mg l^{-1}. This finding is supported by the work of Martinez *et al.* (1997) who used glucose as a substrate to monitor the influence of light intensity on *Chlorella pyrenoidosa*. Greater yields were reached at an initial glucose concentration of 0.1 g l^{-1} and these increased as light intensified. In the current study, the microalgae yield was marginally higher in the bucket experiments when compared with the tank experiments possibly due to increased light intensity.

Under light limited conditions, COD reduction was shown to still be significant. COD removal efficiency in the set of control flasks ranged from 54-78% suggesting that the lack of sufficient light did not completely prevent stabilisation processes occurring. Several authors studying the kinetics of organic matter removal by cyanobacterial species reported evidence of a great reduction in COD in the absence of light with low conversion to biomass (Tam and Wong, 2000; Queiroz *et al.*, 2007). The results suggested the existence of a cyanobacterial metabolism capable of assuring slow growth in the dark. Although microalgal species were utilised in this study, the same result was obtained which may suggest that some microalgal species have a similar capability or may suggest the presence of some cyanobacteria. Some WSP algae are capable of chemo-organotrophic growth (i.e. growth in the dark on organic substrates). Pearson (2006) stated that *Chlamydomonas*, *Chlorella* and *Euglena* could all grow in the dark on acetate under aerobic conditions. Martinez *et al.*, (1997) examined the influence of light intensity on the growth of *Chlorella pyrenoidosa* using different concentrations of glucose. Results showed that during the exponential mixotrophic growth phase, the specific growth rate did not depend on light, since the algae were able to use glucose as an energy source. It would explain the high COD removal, however with low conversion to biomass reflected by very low chlorophyll-a levels.

Comparing results for measurable oxygen levels between the tank and the bucket experiments showed quite a difference between their capacities for net oxygen production. No significant rise in oxygen levels were recorded for the tanks with the highest load (dilution 1:50) under simulated 24 hour light conditions, but there was a change from negative to positive net oxygen production measured in the buckets even though they received a higher initial substrate concentration. The buckets were however exposed to a greater light intensity. A similar phenomenon was apparent in a study by Neel *et al.* (1961) who conducted research on five identical experimental WSP in Fayette, Missouri. Each pond had an area of 0.31 ha and depths of 0.76 m. BOD_5

loadings were 22, 45, 67, 90, 112 kg ha^{-1}d^{-1}. Figure 5.60 and Figure 5.61 show BOD removal efficiencies and dissolved oxygen concentration as a function of available light energy and air temperature. BOD removal efficiency was found to decrease with progressively higher loadings. As loading increased, temperature appeared to have an influence on BOD removal particularly at temperatures less than 5°C. When the load was above 45 kg ha^{-1}d^{-1} (BOD_5 of 250 mg l^{-1}), light intensity fairly well dictated the level of the photosynthetic process. Ponds receiving the lowest loadings were less influenced by both environmental parameters; light effects were subordinate to those exercised by changes in available algal nutrients.

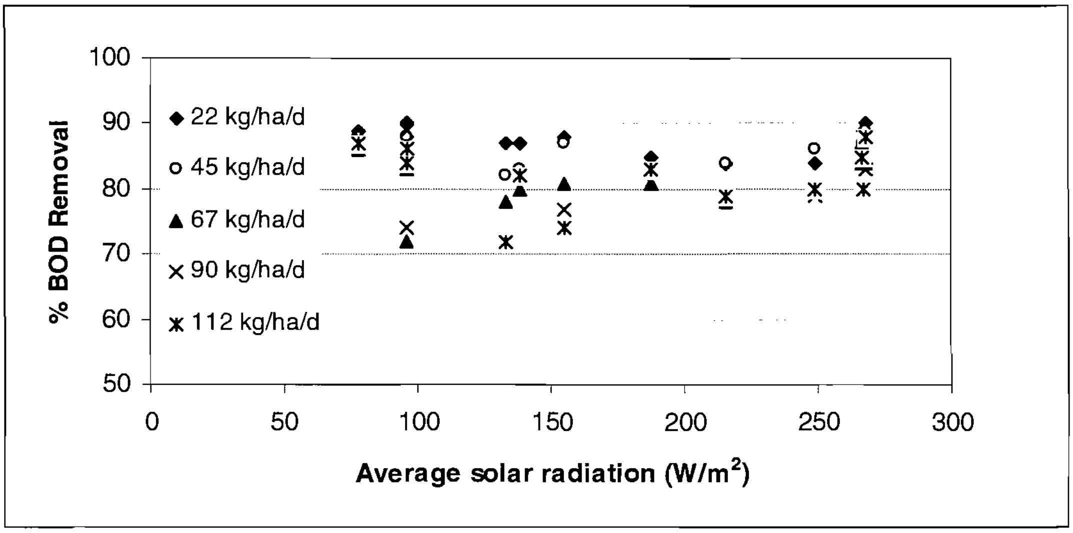


Figure 5.60 BOD_5 removal as a function of light intensity in Fayette ponds (Neel *et al.*, 1961)

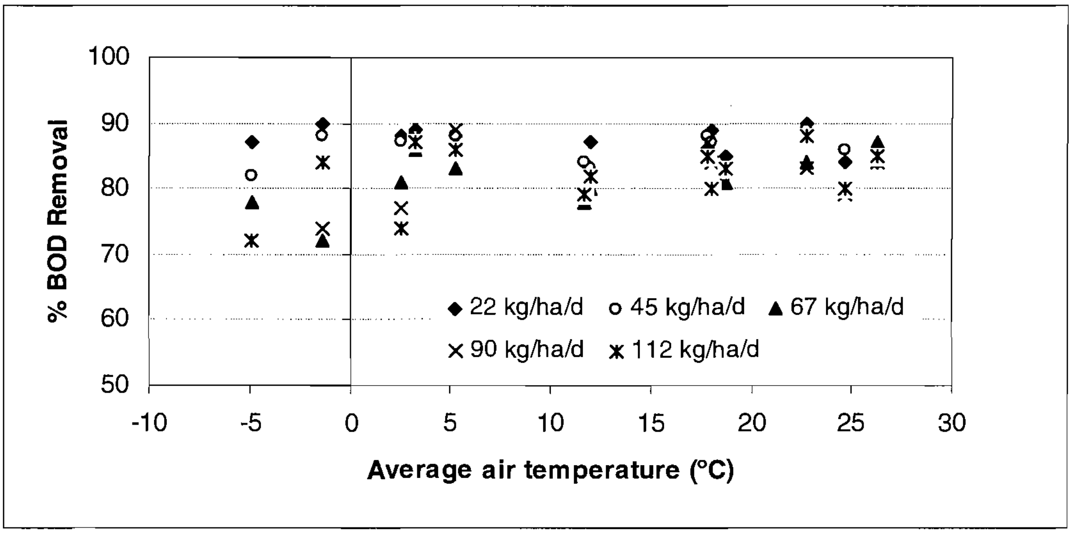


Figure 5.61 BOD_5 removal as a function of temperature in Fayette ponds (Neel *et al.*, 1961)

Results for Pond 2 which received approximately 45 kg $ha^{-1}d^{-1}$ are shown in Figure 5.62. Dissolved oxygen concentrations declined when visible solar radiation dropped, whereas BOD removal efficiency did not decrease accordingly. Temperature had little effect on dissolved oxygen levels and removal efficiency varied even less with temperature than with light intensity. Although the ponds in the study by Neel *et al.* (1961) were fed, the results agree with the findings of the current study where light intensity was responsible for the dissolved oxygen levels in the systems and became more important when the levels of organic matter present were high. At lower loadings, high light intensities appeared to be less important to system performance and algal growth was controlled by the availability of nutrients. This was shown in the bucket experiments where a second bloom was observed in the buckets with mean substrate concentration 118 mg and 202 mg COD l^{-1}. This was probably due to organic matter contributions being augmented by nutrients released from the decay of former plankton populations and not the availability of bright sunlight.

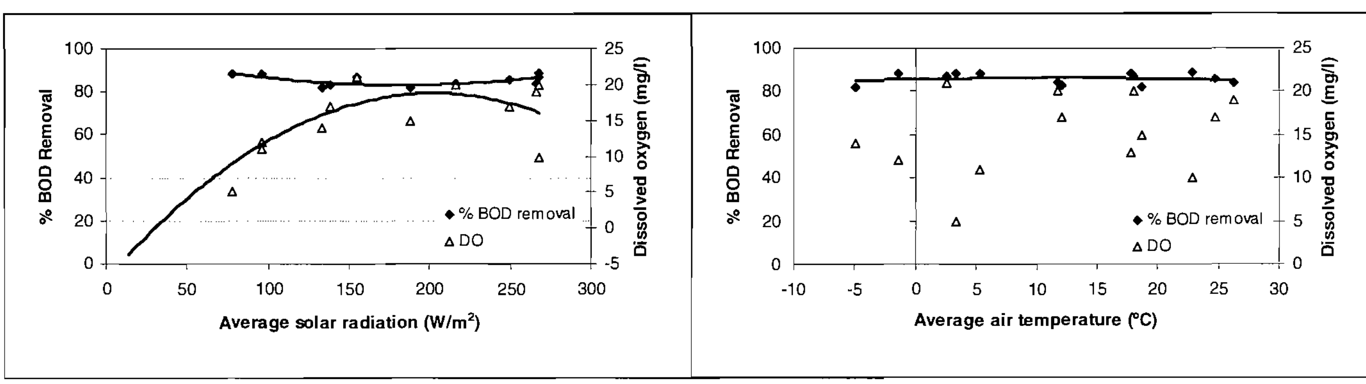


Figure 5.62 Dissolved oxygen concentration and BOD removal as a function of temperature and light intensity in Fayette, Missouri (loading 45 kg BOD ha/d)
NB. Results taken from work by Neel *et al.* (1961)

Laboratory studies were conducted by Thirumurthi (1974) on two identical 0.267 m deep tanks with a mean influent BOD_5 of 164 mg l^{-1} subjected to light intensities of 2 and 58 $\mu mol\ m^{-2}\ s^{-1}$. Results showed similar findings to Neel *et al.*, (1961). A reduction in BOD removal efficiency from 90 to 81% was observed and although the difference was not large, Thirumurthi (1974) concluded that this was caused by insufficient light energy to support the required photosynthesis. Dissolved oxygen in the effluent was zero under 2 $\mu mol\ m^{-2}\ s^{-1}$ of light. The findings by Thirumurthi (1974) support the current work whereby a reduction in BOD removal rate was a likely cause of inadequate light energy needed to support the photosynthetic process in the units receiving the highest loads. The lower loaded systems with high dilutions showed excellent performance at all light intensities. Measurable oxygen occurred even in the controls under very low light intensities where light was still adequate to support detectable oxygen production with the lowest initial load. The rate of oxygen consumption imposed by heavier loads was too high to be satisfied by deficient oxygen production under low light intensities.

6 EFFECTS OF DILUTION AND LOADING ON THE PERFORMANCE OF SEMI-CONTINUOUS FED EXPERIMENTAL WASTE STABILISATION SYSTEMS IN KAZAKHSTAN

6.1 Introduction

The batch experiments described in the previous chapter showed the potential and limitations of dilution as a way of increasing removal rates and/or bringing forward the date at which water reaches a discharge standard; the experiments in this chapter look at more complex fed systems in real climate conditions. The research presented concerns the development of a design and operational concept aiming firstly to reduce the initial accumulated spring load on the storage/maturation pond; and secondly to buffer the storage/maturation pond from short-circuiting of raw sewage by using a short retention, high load, primary facultative pond. Reduction of the initial load can be achieved by dilution with treated wastewater, which is retained in the storage/maturation pond from the previous year. At first sight this appears to require an increase in pond size, but this is offset by the ability to discharge rather than accumulate over the summer period, thus reducing the overall volume or allowing retention of treated water.

The concept is illustrated in a simplified model (Figure 6.1) put forward by Whalley *et al.* (2007) that compares the standard North American design with some different discharge and retention scenarios. One alternative (variant 1) is to begin discharge as soon as effluent quality is acceptable; this has the advantage of reducing the pond volume, but as there is no dilution in the maturation pond the predicted discharge date is late in the season. A second alternative (variant 2) is to maintain the same pond volume while storing water for 6 months and discharging over 6 months. This allows approximately 50% carry-over of treated water from the previous year, diluting the spring load on the maturation pond and possibly speeding up purification. Variant 3 shows a discharge that starts and finishes earlier, resulting in a reduced pond volume.

A wide range of options is possible, but the most useful require early discharge and are likely to be achieved by carry-over of water. To assess the potential of dilution for promoting early purification, eight 25-l fed systems were set-up to investigate the effect of partial effluent retention from the previous year. 780-l tank experiments then provided larger systems that were more realistic in terms of long operating times/histories and attempted to simulate operation of a pond system e.g. facultative and maturation stages in a region with a winter period typically lasting from late November until late March. Year 1 determined whether the hydraulic retention time (HRT) in a pilot-scale facultative tank could be reduced from 30 to 20 days whilst still providing adequate buffering for the next stage; and to compare the effect of different start dates for a 5-month retention period in the storage/maturation tanks. Year 2 consisted of five tanks being operated as facultative ponds with different HRT and surface loading rates.

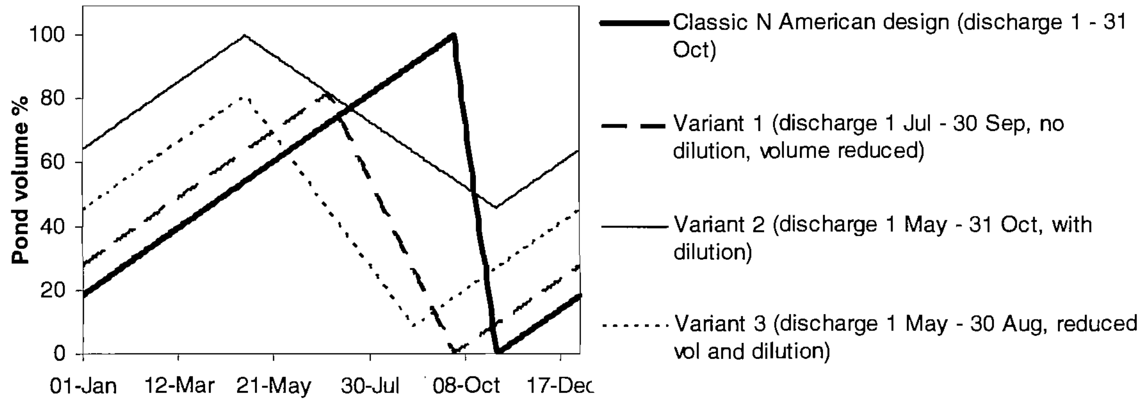


Figure 6.1 Simplified flow balance model with alternative time-dependent discharge and effluent retention scenarios

6.2 Materials and methods

The experimental plant and facilities used were based in Almaty, Kazakhstan. Operation of the plant, sampling and analysis were carried out by staff of the BG Chair of Environmental Technology at the Almaty Institute of Power Engineering and Telecommunications. Experimental design and analysis and interpretation of results were carried out in Southampton.

6.2.1 Small-scale experiments

Eight cylindrical polypropylene units with a working volume of 25 litres and a surface diameter of 25 cm were buried in sand to provide insulation and an approximation to soil temperatures. The units were run with different dilutions over two consecutive spring periods. Table 6.1 shows the experimental start-up conditions for the units. In Year 1, the units were filled after the spring thaw while in Year 2 they were filled at the start of winter. In Year 1, the units were initially filled on 27 March 2006 with a mixture of wastewater accumulated over winter in an experimental-scale facultative pond with a HRT of 20 days, and water held over from the previous year from another storage/maturation tank. The units were fed 5 days a week by removing 0.137 l of pond water using a pump at a depth of 25 cm and replacing it with the same volume of synthetic wastewater (see Chapter 4) with a COD of 460 mg l^{-1}. The flow rate was chosen to give a HRT of six months, with an average surface loading rate of 6.14 kg BOD_7 ha^{-1} d^{-1}. In Year 2, the units were initially filled on 7 December 2006 with synthetic wastewater diluted as required with tap water and left over the winter period before feeding began on 21 March 2007. Dilutions were altered slightly from those in Year 1 to change the starting COD concentration. The 0:100% ratio was designed to mimic the conventional North American design where the storage/maturation pond has been fully discharged in the autumn of the previous year leaving no treated water available for dilution. The 25:75 ratio simulates 3 months volume of dilution water remaining in the pond, 50:50 ratio mimics six months carry-over of treated water while the 75:25 ratio approximates 9 months of treated water available for dilution and three months of storage.

Table 6.1 25-l unit start-up conditions

Pond Number	**Year 1 (27 March 06-30 June 06)**	**Dilution Factor** *treated: untreated wastewater*	**Initial unfiltered COD concentration (mg l^{-1})**	**Year 2 (7 December 06 - 30 June 07)**	**Dilution Factor** *treated: untreated wastewater*	**Initial unfiltered COD concentration (mg l^{-1})**
1 & 2		0% : 100%	224		0% : 100%	1022
3 & 4		25% : 75%	182		20% : 80%	849
5 & 6		50% : 50%	177		50% : 50%	597
7 & 8		75% : 25%	172		80% : 20%	308

6.2.2 780-l tank experiments

Five experimental units based in Almaty, Kazakhstan were used to simulate waste stabilisation pond systems. The units were constructed from lined steel tanks with vertical sidewalls, each with a capacity of 780-l and a water depth of 1 m. Each of the tanks (numbered 1 – 5) was buried up to surface water depth in sand to provide natural insulation (Figure 6.2). Feeding was carried out by pumping out the appropriate volume of water from a depth of 50 cm during ice-free periods and 60-65 cm during periods of ice cover, and replacing it over approximately 1 hour by an equal volume of synthetic wastewater. The tank experiments were run over a period of 18 months as shown in Table 6.2.

Figure 6.2 780-l experimental tanks, Almaty, Kazakhstan

Table 6.2 Tank operation during each year

Tank	Year 1 (15 Jan 06 – 30 June 06)	Pond type*	HRT (days)	Surface loading rate (kg BOD_7 ha^{-1} day^{-1})	Simulated storage dates	Year 2 (30 Nov 06 - 30 June 07)	Pond type*	HRT (days)	Surface loading rate (kg BOD_7 ha^{-1} day^{-1})
1		FP	30	67	-		FP	30	67
2		FP	20	100	-		FP	20	100
3		SMP	-	-	1 Dec-1 May		FP	60	33
4		SMP	-	-	1 Jan-1 Jun		FP	40	50
5		-	-	-	-		FP	15	133

* FP = facultative pond; SMP = storage/maturation pond

During Year 1 which started on 15 January 2006 and ran until 30 June 2006, the 780-l tanks were operated as two facultative ponds and two storage/maturation ponds. The facultative tanks were run with hydraulic retention times of 30 days and 20 days giving surface loading rates of 67 and 100 kg BOD_7 ha^{-1} day^{-1} respectively. The two facultative tanks had both previously been run at 30-d HRT. The storage/maturation tanks were operated to simulate a storage time of 5 months with storage dates running from 1 December to 1 May and from 1 January to 1 June. These tanks were already running as storage/maturation tanks being fed from facultative tank effluent at an equivalent HRT of 365 days.

The storage/maturation tanks began operation on 15 January 2006. In order to simulate the required storage duration and end dates, 325 litres (equivalent to 5 months' storage) was removed from the 1 December - 1 May tank leaving 455 litres, and 97.5 litres of wastewater (equivalent to 1.5 months' inflow) was added from the facultative tank. For the 15 January - 1 June tank, 292.5 litres (equivalent to 4.5 months storage) was removed, without any addition. These initial conditions are summarised in Table 6.3. The storage/maturation tanks were then fed (without any removal from the tanks themselves) five days a week with 3 l taken from the 20-day facultative tank, so that by the time of the proposed discharge date the storage/maturation tanks were at maximum volume.

Table 6.3 Storage/maturation tank start-up (15 January 2006)

Period	Initial tank volume (l)	*Feeding days until discharge	Volume to fill until discharge (l)	Volume removed (l)	20-d HRT effluent added (l)	Tank volume (15 Jan) (l)
1 Dec-1 May	780	75	227.5	325	97.5	552.5
1 Jan-1 Jun	780	98	292.5	292.5	0	487.5

*NB. Tanks fed 5 days/wk

From 1 July to the start of Year 2 experiments, the tanks were run as described above and a fifth tank to be used in Year 2 was run as a facultative tank with a 30-d HRT. During Year 2 from 30 November 2006, all five of the tanks were operated as facultative tanks with different hydraulic retention times and surface loading rates as shown in Table 6.2.

6.2.3 Sampling and analysis

Two 50 ml samples were taken from all 25-l unit effluent and two 250 ml samples were taken from all tank effluent between 09:00 and 10:00 three times a week and analysed for filtered and unfiltered COD, suspended solids, ammonia, phosphate and chlorophyll-a.

Twice-weekly measurements of filtered and unfiltered BOD_5 and nitrate were also taken for the 25-l units, and net and gross photosynthetic oxygen production was determined. All samples were analysed in accordance with Standard Methods (APHA, 2005) except that BOD analysis was carried out over a 7-day test period. pH, air and water temperature were recorded at the time of sampling using an ETI 8000 pH meter (AT Instruments, UK). Ice thickness was measured using a long wooden ruler.

6.2.4 Removal rates and efficiencies

Removal efficiencies were calculated for a range of cases using equation *3.5* with the influent concentration as C_o and C_e as either the concentration of wastewater removed, the value following the initial fall in pollutants, or the concentration at the start of the steady-state period.

Removal rates were calculated using equation *3.3*, or equivalently by using equation *3.5* to determine removal efficiencies and then dividing E by the number of days taken to reach C_e.

Overall removal efficiencies were calculated for both units and tanks using equation *3.6* with C_o = influent concentration and C_e = the final concentration of the monitoring period (end of June).

Over-winter removal efficiencies were calculated for the 25-l units in Year 2 using equation *3.6* with C_o = concentration on 7 December 2006 (mg l^{-1}) and C_e = concentration on 21 March 2007 (mg l^{-1}).

Removal rates and efficiencies for nutrients were calculated from both the influent concentration and from early maximum values, using equations *3.4* and *3.6* with C_o = influent concentration or initial maximum values (mg l^{-1}) and C_e = lowest concentration (start of steady-state period) (mg l^{-1}).

Removal rates for the 780-l tanks in Year 2 were calculated using a mass balance approach as detailed in Table 6.4.

Table 6.4 Example of a spreadsheet used to calculate removal rate in the 780-l tanks

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*R*
Date	**Filtered COD**	**No. days tanks fed**	**Total days**	**Starting COD (mg to g)**	**COD removed**	**COD added**	**Actual COD**	**Final COD**	**COD destroyed**	**Removal rate**
	mg l^{-1}			**g**	**g**	**g**	**g**	**g**	**g**	**g $g^{-1}d^{-1}$**
07/02/07	170_x	5	7	122	20	36	138	133	5	0.005
14/02/07	181_y	5	7	133	22	36	147	141	6	0.006_z

Equation *6.1* was used to calculate the removal rate *R* for example *z* (see Table 6.4, 14/02/07) as follows:

$$R = \left[\frac{I}{G \cdot C}\right] \tag{6.1}$$

where: $R =$ removal rate (g g^{-1} d^{-1})

$D = (A_x$ [mg l^{-1}] · tank volume [l])/1000 [in g]

$E =$ (wastewater removed [l day^{-1}] · A_x· B [days])/1000 [in g]

$F =$ (wastewater added [l day^{-1}] * influent concentration [mg l^{-1}] · B [days])/1000 [in g]

$G = D - E + F$ [g]

$H = (A_y$ [mg l^{-1}] · tank volume [l])/1000 [in g]

$I = G - H$ [g]

$C =$ (no. of days elapsed [day])

6.3 Results and Discussion

6.3.1 Temperature

Figure 6.3 shows air temperature on during the experimental period. During both years, the spring thaw came considerably earlier than typical for the region (by approximately 20 days).

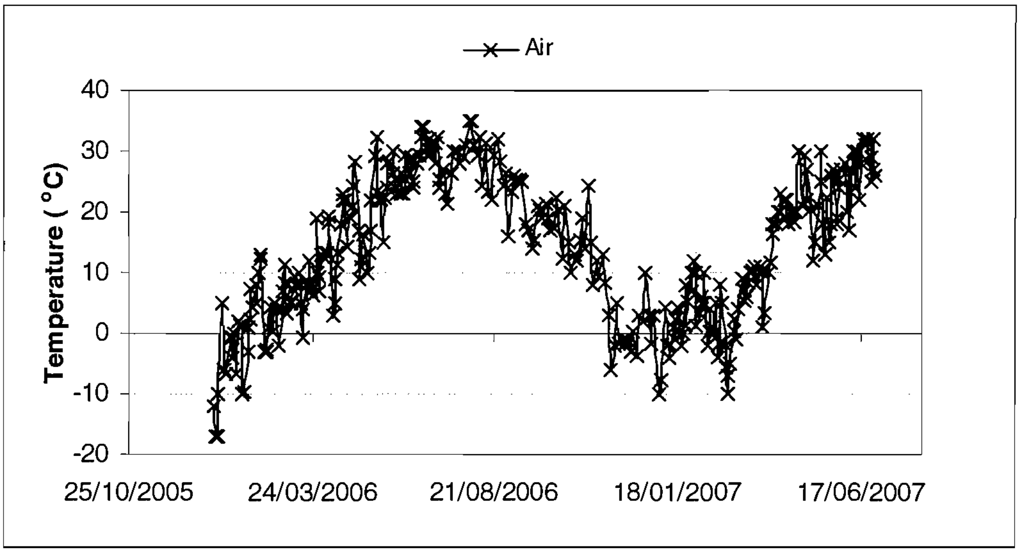


Figure 6.3 Air temperature measured on site at time of sampling

25-l units

Figure 6.4 shows surface water temperature in the 25-l units during Year 1 and 2. Both temperature profiles are similar with no notable difference between units. During Year 1, water temperature rose sharply from 4°C at the beginning of April to 17°C by the end of the month. The temperature then fluctuated falling to 10°C on the 8 May and then one week later rose to 21°C. Temperatures stabilised between 18 and 21°C from the end of May and June with the maximum temperature of 24°C recorded on the 22 June. In Year 2, temperature rose sharply from 4°C on the 30 March to 18°C by the 17 April. Similar to Year 2 there was then a decline to 11°C on the 10 May and one week later temperatures were up at 20°C. Temperatures for the remainder of the experiment were similar to those of Year 1.

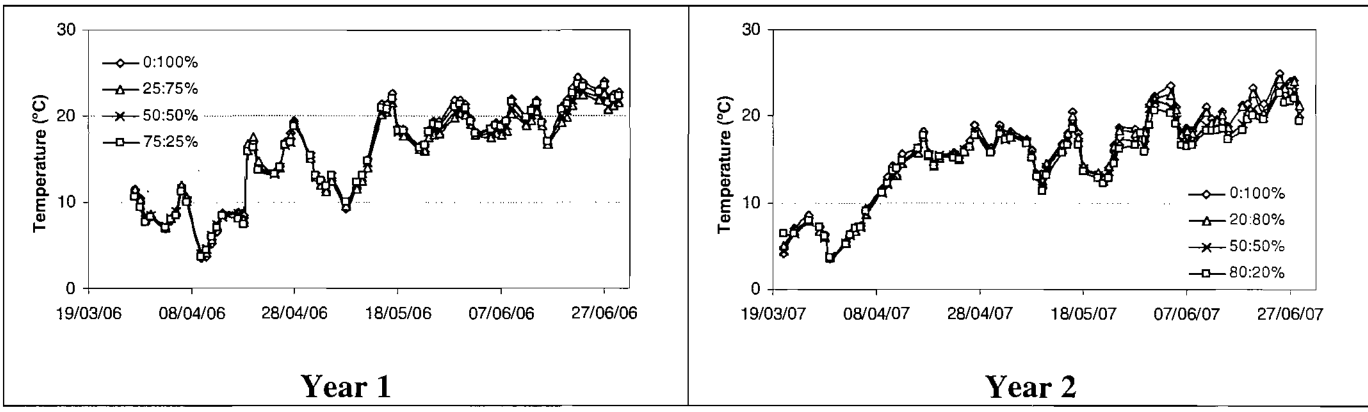


Figure 6.4 Average water temperature in the 25-l units during Year 1 and 2

780-litre tanks

Water temperature in the tanks in Year 1 rose sharply from zero in mid-March and stabilised at about 23°C by the middle of May. Water temperature fluctuated more during May in Year 2 with temperatures finally reaching 20°C by early June. Figure 6.5 shows the relationship between air and average surface water temperature; water temperature clearly follows the trend in air temperature although it fluctuates less and does not reach the same maxima. Air and water temperature correlated strongly in all tanks ($p < 0.001$) with an average R^2 of 85%. The relationship was slightly stronger when air temperatures < 0°C were not used as during the winter water temperature remained between -1.5 and 0°C even when air temperatures were -10 °C. *Rspaev, pers. comm.* (2005) confirmed the ability of the water temperature to fall below zero by tests in a cooling incubator, and was presumably due to the presence of alginates and colloidal materials. Abis and Mara (2006) reported that at air temperatures ≤ 3°C, the water-column temperature in a facultative pond in the UK remained constant at 2.8°C. The difference in temperature thresholds may be due to the extended period of lower temperatures in the current study.

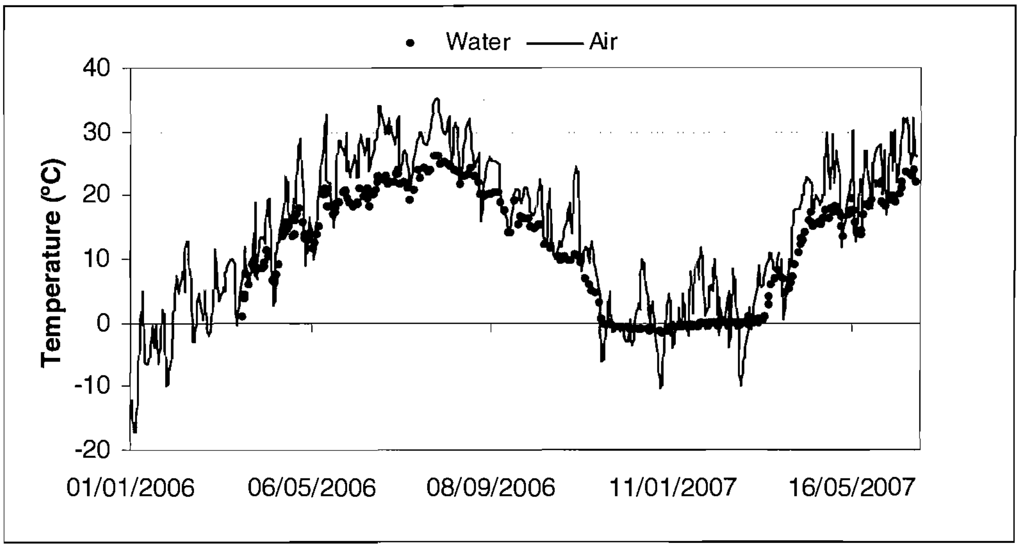


Figure 6.5 Air and average surface water temperature in the 780 l tanks

6.3.2 25-litre units - Year 1 (spring fill)

6.3.2.1 COD and BOD_7 removal

Figure 6.6 and Figure 6.7 show filtered and unfiltered COD and BOD_7 values in the units. For duplicate dilutions, the average range for filtered COD was 4 mg l^{-1} with a maximum of ± 25 mg l^{-1}; the average range for unfiltered COD was 9 mg l^{-1} with a maximum of ± 40 mg l^{-1}. For filtered BOD_7, the average range was 2 mg l^{-1} with a maximum of ± 9 mg l^{-1} and the average range for unfiltered BOD_7 was 4 mg l^{-1} with a maximum of ± 33 mg l^{-1}.

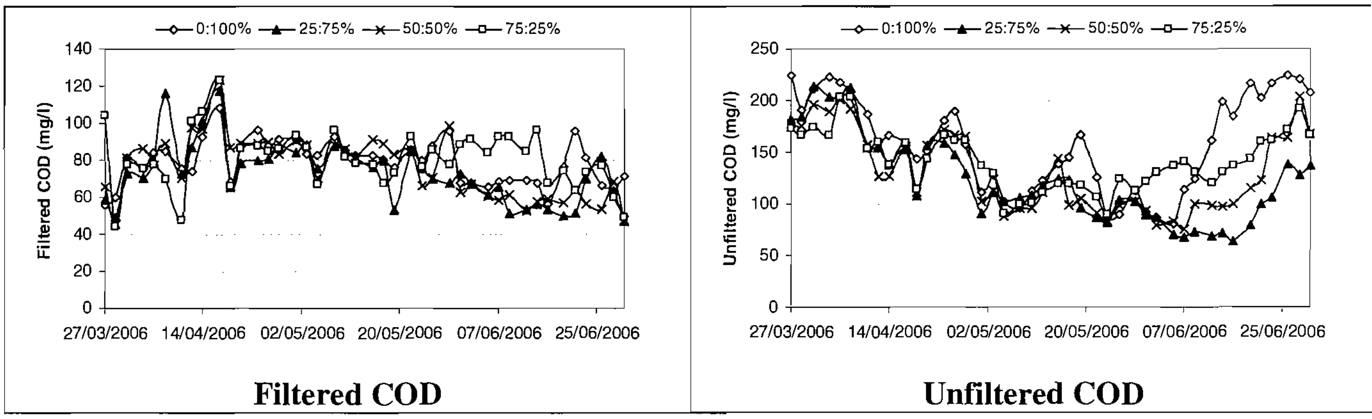


Figure 6.6 Mean changes in filtered and unfiltered COD concentration over time for each dilution (NB. different y-axis)

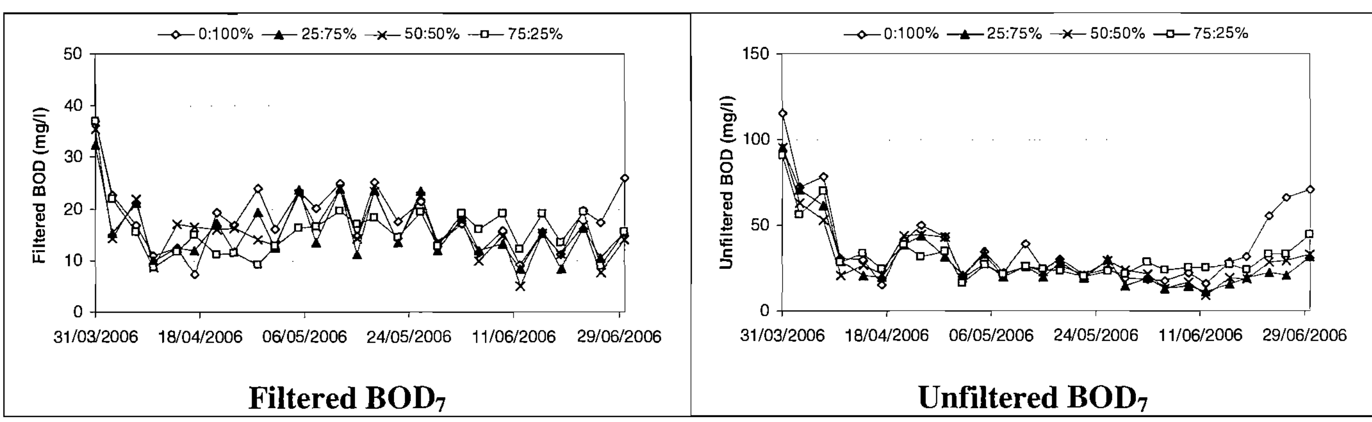


Figure 6.7 Mean changes in filtered and unfiltered BOD_7 concentration over time for each dilution (NB. different y-axis)

Initial fall

In Year 1 very little difference was observed between different initial concentrations in the trend for unfiltered COD and BOD_7 concentration.

Filtered COD and BOD_7 concentration on day 7 (3 April) and when steady-state conditions were reached, taken as 28 April and 1 May were used to calculate the removal efficiency and removal rate (Table 6.5). Removal efficiency was generally used for the fed tanks as opposed to removal rate (used in the previous chapter and where possible here), as it gives more consistent results and is the most common way of expressing performance in wastewater treatment for fed systems. Details of calculations for all removal efficiencies and rates can be found in Section 6.2.4. On day 7, removal efficiency and rate showed no trend in relation to dilution, with similar results for the highest and lowest diluted units. The highest removal rate of 0.122 g g^{-1} d^{-1} was determined for dilution 50:50%. During the initial fall in COD concentration there was also very little difference in removal between treatments, and minimum/steady-state values were reached on the same day for all dilutions. Comparisons with the UK batch bucket experiments from the previous chapter were hoped to be made as these were run in similar vessels to the 25-litre units. No samples were taken on day 5 therefore it is only possible to compare with results for day 4 and 7. COD removal rates shown in Table 6.5 were calculated on day 7 however, removal rates were also obtained for day 4 which ranged from 0.178 to 0.185 g g^{-1} d^{-1}. Removal rates for the UK bucket experiments were between 0.11 and 0.14 g g^{-1} d^{-1} after 5 days and as very little changed occurred in filtered COD levels between days 4 and 7 in the Almaty-based systems, removal rates for day 5 are assumed to be marginally higher than those carried out in the UK. Light intensity was not measured during the Almaty experiments, although values at the beginning of April would be expected to be higher than in the UK at the end of April and may therefore have been a factor in the increased removal rates. The initial load on the system was higher in the UK buckets, which were batch experiments as opposed to semi-continuous feeding, and these factors may also have affected removal rates. By the time steady-state conditions had been reached removal efficiency and removal rates for both filtered COD and BOD_7 varied even less between dilutions.

Table 6.5 Filtered BOD_7 and COD removal efficiency and rate

Dilution	Filtered COD removal efficiency (%)	Filtered COD removal rate (g g^{-1} d^{-1})	Filtered BOD_7 removal efficiency (%)	Filtered BOD_7 removal rate (g g^{-1} d^{-1})
	Day 7		**Day 7**	
0:100%	73	0.104	77	0.110
25:75%	75	0.107	85	0.121
50:50%	69	0.099	86	0.122
75:25%	73	0.104	78	0.112
	When steady-state reached (28 April)		**When steady-state reached (1 May)**	
0:100%	67	0.021	84	0.024
25:75%	69	0.021	87	0.025
50:50%	70	0.022	87	0.025
75:25%	69	0.022	69	0.025

Steady-state

By early April onwards filtered BOD_7 and COD remained between 10-25 mg l^{-1} and 50-80 mg l^{-1} respectively whilst unfiltered BOD_7 fell to 20-30 mg l^{-1} and unfiltered COD concentrations fell less rapidly reaching approximately 90 mg l^{-1} by late May. Both parameters began to increase again from mid-June, with highest values in the undiluted units (0:100%). Table 6.6 shows overall filtered BOD_7 and COD removal efficiencies for all dilutions. The effect of no dilution (0:100%) was apparent in the reduced efficiency compared with units that received some level of dilution; variation between these units was negligible.

Table 6.6 Overall filtered BOD_7 and COD removal efficiency

Dilution	Filtered BOD_7 removal efficiency (%)	Filtered COD removal efficiency (%)
0:100%	74	75
25:75%	85	83
50:50%	86	83
75:25%	85	82

6.3.2.2 Chlorophyll-a, suspended solids, pH and oxygen production

Chlorophyll-a levels are shown in Figure 6.8. Two peaks were observed, in both of which the order of chlorophyll-a concentration followed that of decreasing dilution. 0:100% dilution showed the highest concentrations, peaking at approximately 1.4 mg l^{-1} on 7 April and again on 24 April. The highest dilution (75:25%) had the lowest initial peak of 1 mg l^{-1}. While the units were untypical of a real WSP, the chlorophyll-a concentrations obtained were typical of the range expected in WSP systems in a hot climate as reported by Mara *et al.*, (1992). Although a healthy algal population was maintained during the first six weeks, from May onwards the fall in chlorophyll-a levels to < 0.3 mg l^{-1} indicated failure conditions according to the criterion suggested by Pearson (1996) but in fact COD and BOD_7 remain at or close to steady state values for a further six weeks before starting to rise again with chlorophyll-a in mid-June.

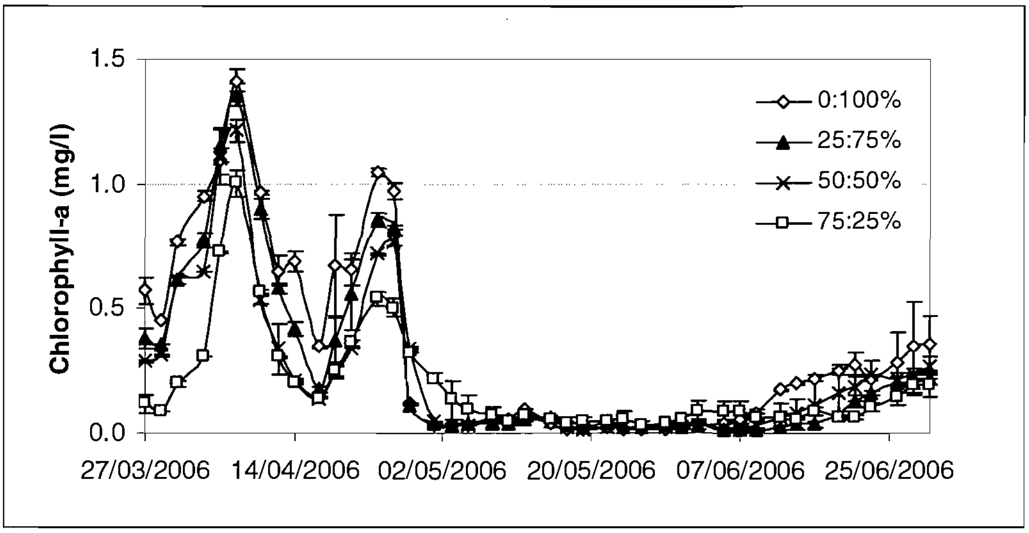


Figure 6.8 Change in chlorophyll-a concentration over time for each dilution

The calculation of apparent growth rates for each peak revealed that although chlorophyll-a concentration was highest in the units with the lowest dilution, growth rates during the first peak were highest in the most diluted units (Table 6.7). This was not true of the second peak although the growth rate remained lowest for the 0:100% dilution. Initial chlorophyll-a concentration at the start of the experiment increased with decreasing dilution which may have been responsible for the trend in chlorophyll-a concentration observed but not apparent algal growth rate.

Table 6.7 Apparent algal growth rates for both chlorophyll-a peaks

Dilution	Growth rate h^{-1} (peak 1)	Growth rate h^{-1} (peak 2)
0:100%	0.005	0.007
25:75%	0.006	0.009
50:50%	0.006	0.010
75:25%	0.012	0.008

Following the second peak, chlorophyll-a concentration fell as low as 0.04 mg l^{-1}. In 75:25% dilution this reduced more gradually and remained marginally higher than the other dilutions throughout May and the start of June. Chlorophyll-a began to rise again from the middle of June, possibly due to the augmentation of nutrients released from the decay of the previous algal bloom. The rise was most pronounced in the unit with the lowest dilution. The rise in COD coincided with a potential third algal bloom as shown by the rise in chlorophyll-a (Figure 6.8). COD concentration correlated positively with chlorophyll-a for each dilution throughout the run ($R^2 = 0.39-0.67$; $p < 0.001$). In contrast, BOD concentration was only weakly related to chlorophyll-a ($R^2 = 0.03-0.09$; $p > 0.05$).

Initial suspended solids concentrations reflected the loading in each unit, with values from 100 mg l^{-1} in dilution 0:100%, to 65 mg l^{-1} in the units with the highest dilution (Figure 6.9). The trend of initial reduction then peak corresponded to peaks in chlorophyll-a (Figure 6.8) and oxygen production (Figure 6.10 and Figure 6.11). Although chlorophyll-a concentration correlated weakly with suspended solids for each dilution ($R^2 = 0.15-0.36$; $p < 0.01$), with the weakest relationship in the most diluted units, it is likely that peaks were due to algal biomass. pH values also followed the trend of algal blooms with values ranging from 6.5-7.0 during periods of low chlorophyll-a concentration to 8.5-9.0 during peak levels.

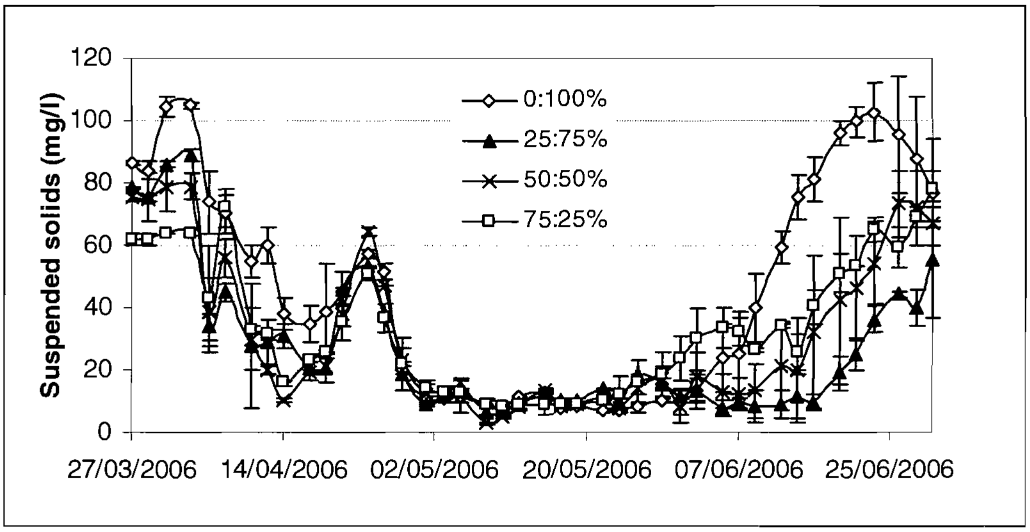


Figure 6.9 Change in suspended solids concentrations over time for each dilution

Figure 6.10 and Figure 6.11 show values of gross and net oxygen production. Dilutions behaved similarly, showing peaks in oxygen production during algal blooms. The oxygen production measurements began a week after the initial start-up and results suggest that data for the first peak in oxygen production associated with maximum chlorophyll-a were missed.

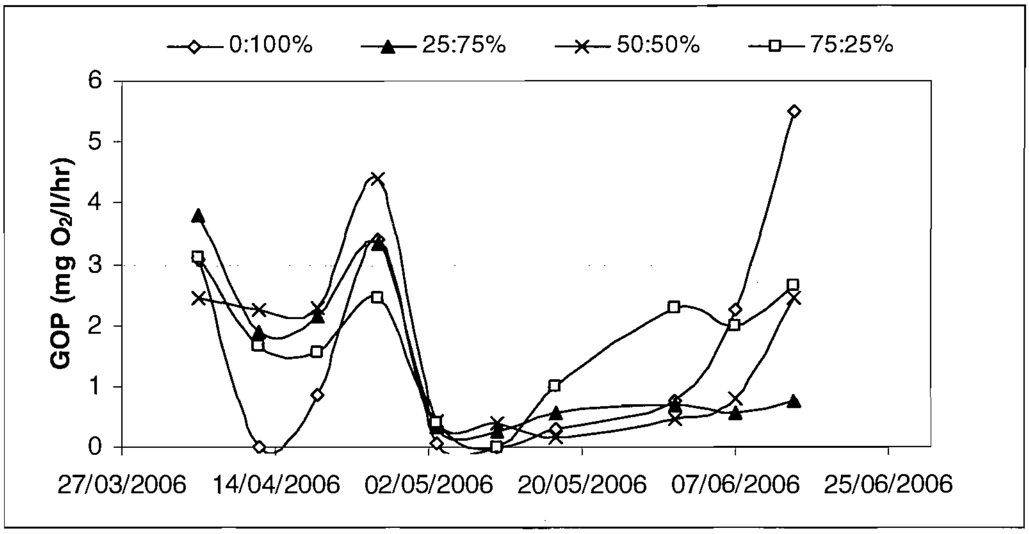


Figure 6.10 Change in gross oxygen production over time for each dilution

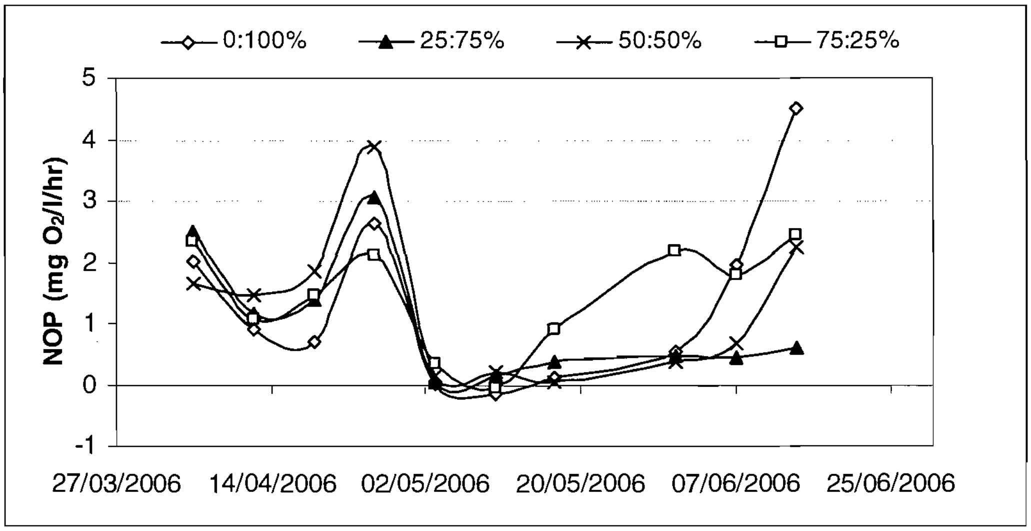


Figure 6.11 Change in net oxygen production over time for each dilution

Gross and net oxygen production fell shortly after the first potential peak with levels dropping to zero in 0:100% dilution; then peaked again on 26 April coinciding with the second peak in chlorophyll-a. Maximum gross oxygen production was 4.40 mg O_2 l^{-1} h^{-1} for 50:50% dilution compared with 2.45 mg O_2 l^{-1} h^{-1} for 75:25% dilution. Maximum net oxygen production was 3.88 mg O_2 l^{-1} h^{-1} for 50:50% dilution compared with 2.03 mg O_2 l^{-1} h^{-1} in the 75:25% units. By 3 May, net/gross oxygen production at all dilutions was less than 0.5 mg O_2 l^{-1} h^{-1}. From 10 May, a pronounced rise in net/gross oxygen production was then observed in the units with the highest dilution (75:25%) even though chlorophyll-a levels were at a minimum. By 14 June there was also a considerable rise in gross and net oxygen production in the remaining dilutions which coincided with a rise in chlorophyll-a. By this time, an average of 80% of BOD_7 had been removed creating conditions whereby oxygen produced by the rise in algae outweighed the oxygen consumed. The results for peak oxygen production were up to 3 times lower than those recorded during bucket experiments in the UK which used similar sized containers under natural conditions. The addition of BOD from semi-continuous feeding compared with a batch-fed system may have kept the maximum photosynthetic activity to a minimum. Gross ($R^2 = 0.58$-0.96; $p < 0.03$) and net oxygen ($R^2 = 0.49$-0.94; $p < 0.05$) correlated with chlorophyll-a concentration in the period to the end of May.

The ratio of oxygen production to chlorophyll-a, which is often referred to as the assimilation number, generally decreased as chlorophyll-a concentration increased showing higher values in June than April (Figure 6.12). The reason for this may be that with increasingly larger algal populations as observed in April, individual algae may come into contact with optimal sunlight less frequently. Copeland *et al.* (1964) who found a similar relationship in oil refinery effluent holding ponds proposed that the depletion of nutrients may also be a factor in low assimilation numbers when chlorophyll-a levels are high. Nutrients were reduced rapidly by mid-April (Figure 6.13 and Figure 6.14) suggesting that this may have been a cause of the low assimilation numbers. Variations in environmental conditions such as the increase in light and temperature in June may well have speeded up reactions and allowed faster regeneration rates that possibly caused higher assimilation numbers, although regression analysis of assimilation number with water temperature revealed a statistically weak relationship; average p-values ranged from 0.038 for dilution 75:25% to 0.224 for dilution 50:50%.

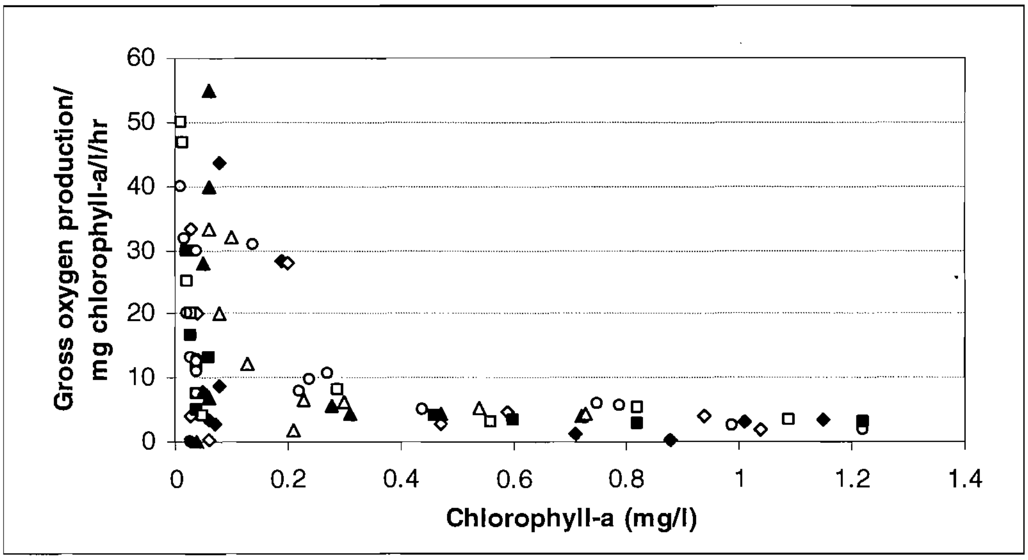


Figure 6.12 Assimilation numbers plotted against chlorophyll-a
(♦) 0:100% (■) 25:75% (○) 50:50% (△) 75:25%

6.3.2.3 Nutrients

Ammonia concentration is shown in Figure 6.13. Ammonia removal was rapid in all dilutions, falling from 18 mg l^{-1} to less than 0.5 mg l^{-1} by mid April. Nitrate concentrations remained low during the spring at around 0.2-0.3 mg l^{-1}, though dilution 75:25% showed a small increase in early June, coinciding with increases in COD and chlorophyll-a.

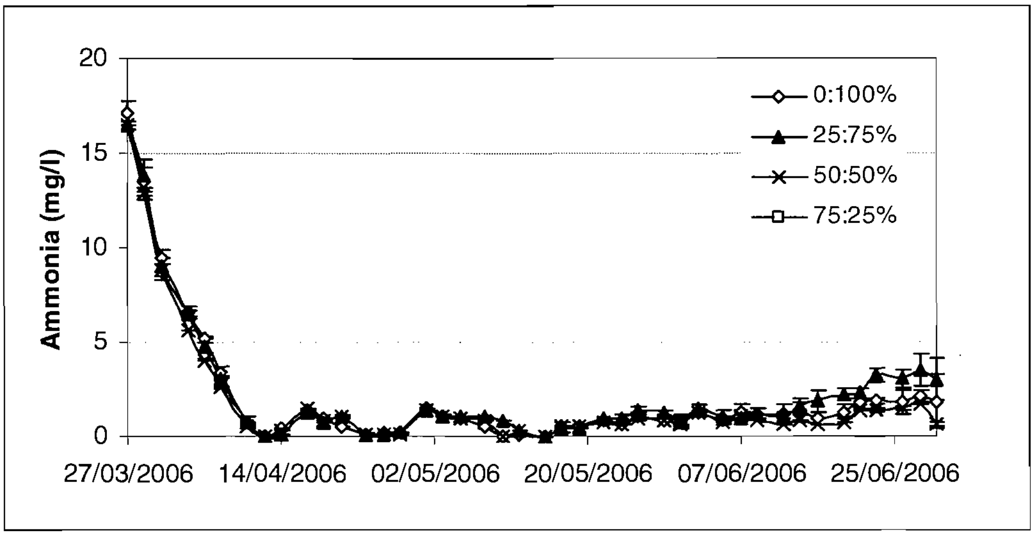


Figure 6.13 Change in ammonia concentration over time over time for each dilution

Average ammonia removal efficiency calculated using initial concentrations on 27 March and when ammonia levels reached their lowest values (14 April) exceeded 98% for all dilutions (Table 6.8). Removal rates after 18 days when ammonia was at a minimum gave an average of 0.055 g g^{-1} d^{-1}. These rates were high compared with those obtained in the UK batch bucket experiment where removal rates after 19 days (when ammonia was at its lowest level) gave an average of 0.037 g g^{-1} d^{-1}.

Table 6.8 Ammonia removal efficiency and removal rates

Dilution	Ammonia removal efficiency (from max to min values) (%)	Ammonia removal rate ($g\ g^{-1}\ d^{-1}$)
0:100%	98	0.054
25:75%	99	0.055
50:50%	98	0.054
75:25%	99	0.055

Phosphate concentration decreased from approximately 10 mg l^{-1} at the start of the period to an average of 2 mg l^{-1} in all dilutions (Figure 6.14). Values in 75:25% dilution were as high as 13 mg l^{-1} at the end of March but fell to the same concentration as the less diluted units by mid-April.

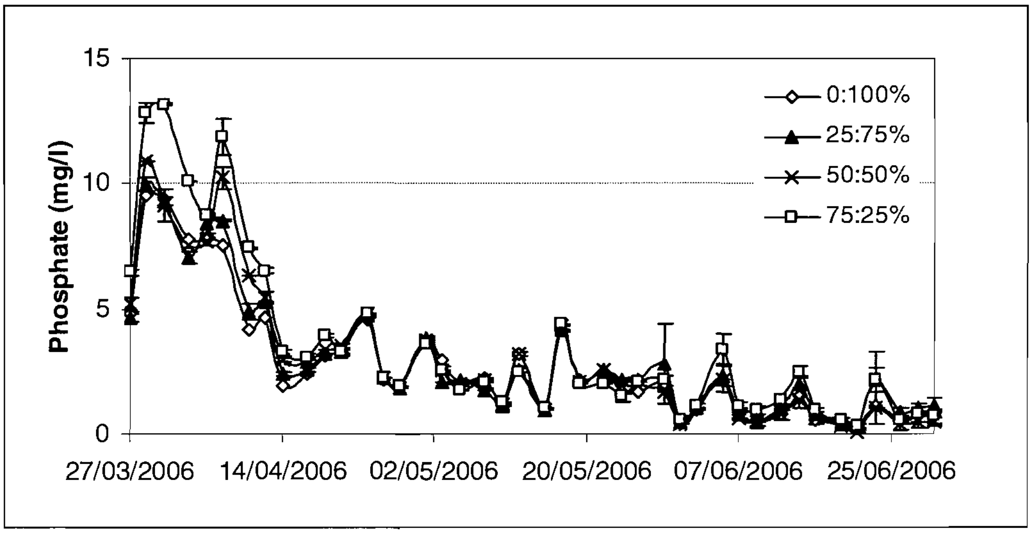


Figure 6.14 Change in phosphate concentration over time for each dilution

The fall from maximum values observed on 29 March to those measured on 14 April was calculated as removal efficiencies and rates and was highest in the undiluted units but varied little between dilutions (Table 6.9). Overall phosphate removal efficiency was higher, with all dilutions over 81%.

Table 6.9 Phosphate removal efficiency and removal rate

Dilution	Phosphate removal efficiency (from max values) (%)	Phosphate removal rate ($g\ g^{-1}\ d^{-1}$)	Overall phosphate removal efficiency (%)
0:100%	80	0.050	86
25:75%	76	0.048	81
50:50%	68	0.042	92
75:25%	75	0.047	89

Nutrients present at each dilution fell sufficiently to meet typical discharge requirements. The results, particularly for ammonia levels, suggest that each dilution was nutrient limited, possibly due to lack of nutrient return from the bottom sediments which would normally be present in a full-scale operational pond. Phosphate levels showed a peak once a chlorophyll-a bloom was over, presumably as a result of recycled algal material. The effect of nutrient limitation was evident both on chlorophyll-a levels and for a short period on gross/net oxygen production which showed a rapid decline following the drop in ammonia and phosphate concentration.

6.3.3 25-litre units - Year 2 (winter fill)

6.3.3.1 COD and BOD_7 removal

Problems with damage due to freezing over the winter period led to loss of liquid in three of the units in Year 2. The 0:100% dilutions were topped up using 5 l of wastewater from the facultative tanks kept from the previous year, but results showed this was too dilute. These results were therefore discounted. The same problem with one 20:80% unit but with a greater loss of liquid meant only one replicate for this dilution. Figure 6.15 and Figure 6.16 shows the results for filtered and unfiltered COD and BOD_7 concentration for the remaining dilutions. For duplicate dilutions, the average range for filtered COD was 6 mg l^{-1} with a maximum of ± 28 mg l^{-1}; the average range for unfiltered COD was 15 mg l^{-1} with a maximum of ± 42 mg l^{-1}. For filtered BOD_7, the average range was 3 mg l^{-1} with a maximum of ± 14 mg l^{-1} and the average range for unfiltered BOD_7 was 9 mg l^{-1} with a maximum of ± 31 mg l^{-1}.

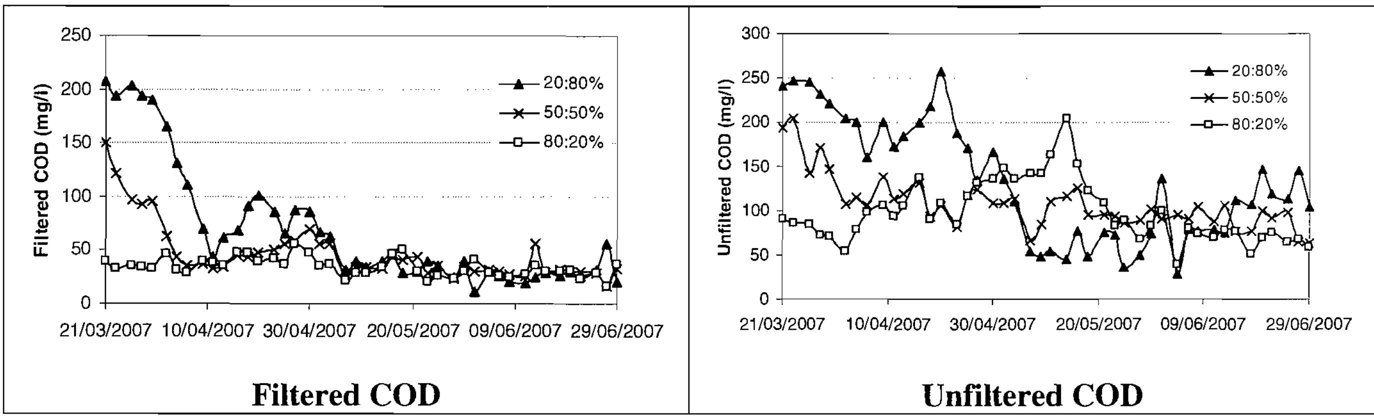


Figure 6.15 Change in mean filtered and unfiltered COD concentration over time for each dilution (NB. different y-axis)

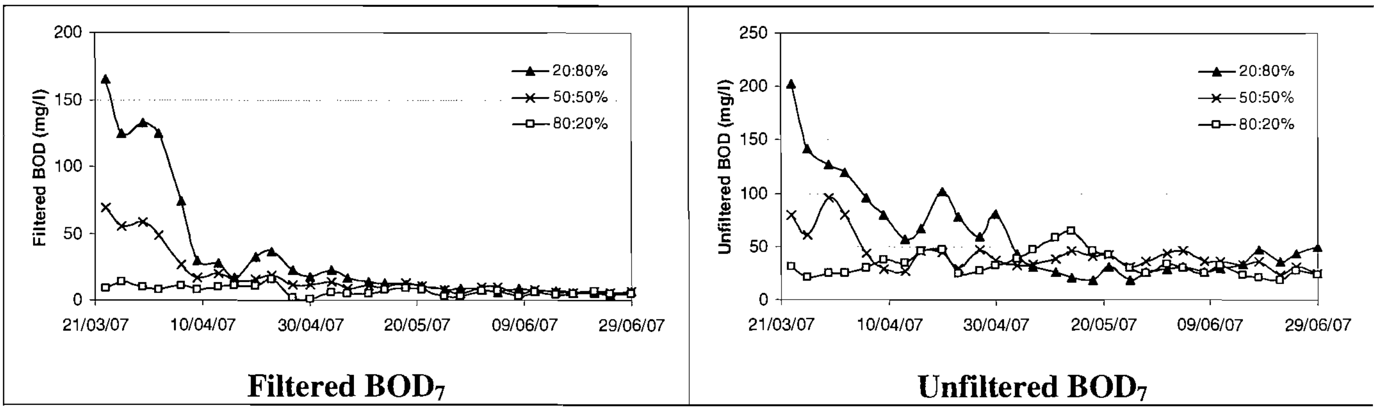


Figure 6.16 Change in mean filtered and unfiltered BOD_7 concentration over time for each dilution (NB. different y-axis)

Initial fall

Initial COD levels in the units prior to the winter period and the concentration on 27 March following the spring thaw were used to calculate removal efficiencies. Unfiltered COD concentrations at the start of the spring period were on average 240 mg l^{-1} in the 20:80% units compared with 90 mg l^{-1} in the 80:20% units. Filtered and unfiltered COD removal efficiency is shown in Table 6.10. The results demonstrate a good level of COD removal from sedimentation. Filtered COD removal varied most between dilutions with 80:20% dilution demonstrating the highest removal efficiency. Unfiltered COD removal showed a range of just 4% between efficiencies possibly reflecting dominant sedimentation processes in each dilution which can cause a reduction in BOD by 30-50% during ice-covered periods in ponds (Vinberg *et al.*, 1966).

Table 6.10 Average COD removal efficiency from winter start-up values to spring thaw

Dilution	Filtered COD removal efficiency (%)	Unfiltered COD removal efficiency (%)
20:80%	60	72
50:50%	58	68
80:20%	75	71

Where unfiltered BOD_7 was initially high, for example in the unit with 20:80% dilution, levels subsequently fell following the ice thaw from 203 mg l^{-1} reaching 57 mg l^{-1} by 13 April. Unfiltered BOD_7 in the 50:50% units showed an initial drop from 80 to 60 mg l^{-1} during the first three days and then rose again to 95 mg l^{-1} after a further four days. BOD_7 then declined and stabilised at concentrations between 30 and 45 mg l^{-1} by 6 April, one week prior to 20:80% dilution reaching steady-state values. The units containing the highest dilution had reached steady-state values from the start of the monitoring period with very low concentrations of pollutants averaging 30 mg BOD_7 l^{-1} which is what might be expected from a dilution factor of 1:4, some settlement and a winter period of no feeding. There was no increase in BOD levels when feeding began at the end of March and only a slight rise in BOD during mid-April and mid-May coinciding with algal blooms, but otherwise unfiltered BOD_7 remained between 20 and 30 mg l^{-1}. Unfiltered COD concentration fluctuated more than BOD_7 concentration particularly in dilution 20:80%. Unfiltered COD concentrations remained comparatively high in the units with 20:80% dilution until the algal bloom dissipated; COD then reached 54 mg l^{-1} at the beginning of May. In the other two dilutions, unfiltered COD concentrations fluctuated with chlorophyll-a concentration and remained fairly similar from the end of May ranging from 70 to 90 mg l^{-1}.

Steady-state

From the end of May there was very little difference between dilutions in the BOD_7 concentration. Filtered BOD_7 levels were at 15 mg l^{-1} by the middle of April and by the end of May were less than 10 mg l^{-1} in all cases. Filtered COD levels stabilised at between 20 and 40 mg l^{-1}. Using t-test, no difference was found between all results for 20:80% and 50:50% dilution but there was a significant difference ($p < 0.05$) found between unfiltered BOD_7/COD concentration in dilutions 20:80% and 80:20%. Figure 6.17 shows filtered BOD_7/COD removal efficiency during the run. The results confirm that dilution 80:20% had reached steady-state values at the start of the experiment with an immediate removal efficiency of 86% for both BOD_7/COD removal. Pollutant removal in the lowest diluted units was slower to reach efficiencies over 80%; 20:80% dilution began with negative removal efficiencies as material accumulated over the winter period settled out; then attained a BOD_7 removal efficiency of 80% 24 days after the beginning of the monitoring period when dilution 80:20% was already operating at an efficiency of 86%. 50:50% dilution reached a removal of over 80% one week prior to 20:80% dilution and 17 days from the start of the experiment. The results differ from those in Year 1 where no winter period was included and removal efficiencies were quite similar throughout the monitoring period. Starting the experiments before the winter freeze and including a spring warm-up period show that dilution affected system performance where in Year 1 no notable effect was observed. Removal efficiencies in the most diluted units were already high following the spring thaw and pollutant levels low enough to meet discharge requirements.

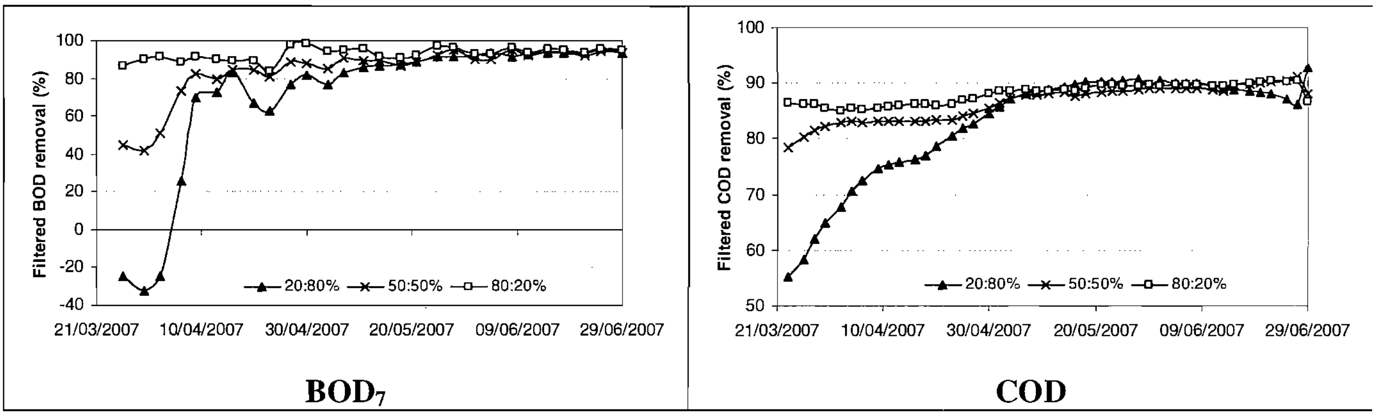


Figure 6.17 Filtered BOD_7 and COD removal efficiency for each dilution (NB. different y-axis)

COD removal and temperature

Water temperature correlated significantly ($p < 0.001$) with COD removal efficiency (Table 6.11); the correlation was strongest with the least diluted units suggesting that perhaps at a higher initial loading temperature was more important in removal efficiency than when loading was reduced.

Table 6.11 Regression output for the effect of surface water temperature on COD removal efficiency

Dilution	Surface water temperature	
	R^2	p-value
20:80%	0.67	< 0.001
50:50%	0.65	< 0.001
80:20%	0.57	< 0.001

6.3.3.2 Chlorophyll-a, suspended solids, pH and oxygen production

Following the ice melt, chlorophyll-a levels rose first in the units with the highest dilution; peak levels during the algal bloom in these units were the lowest reaching a maximum of 0.78 mg l^{-1} on 9 April (Figure 6.18). There was no significant difference ($p > 0.05$) in the rate of increase of chlorophyll-a from 2 April between the units containing dilutions 50:50% and 20:80%; peak values were 1.10 mg l^{-1} in the 50:50% units compared with 1.38 mg l^{-1} in the lowest dilution. Peak values were similar to those in Year 1 with the same increase in peak chlorophyll-a levels found with decreasing dilution. Following the decline in chlorophyll-a in the units with the lowest dilution algal content remained fairly minimal. In contrast, there appeared to be a second bloom in the units with 80:20% and 50:50% dilution, peaking at approximately 0.5 mg l^{-1} on the 16 May. This coincided with an increase in air temperature to 20°C following a dip to 11°C a week earlier. Chlorophyll-a concentrations in these units remained at sufficient levels to maintain oxygenated conditions throughout the experiment thereby preventing system failure. Unfiltered COD concentration correlated positively with chlorophyll-a for each dilution ($R^2 = 0.19$-0.56; $p \leq 0.003$) indicating that even in a fed system algal content was the major source of COD. Similar to Year 1, BOD concentration was weakly correlated with chlorophyll-a ($p > 0.05$).

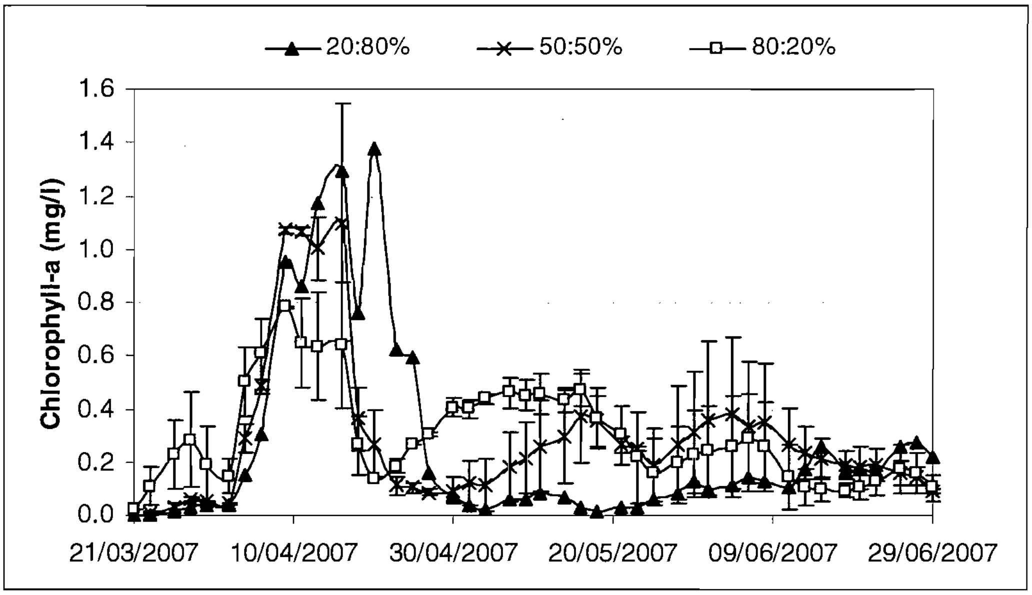


Figure 6.18 Change in chlorophyll-a concentration over time for each dilution

Apparent algal growth rates were calculated from the initial chlorophyll-a peak and shown in Table 6.12. Growth rates were lowest in the most diluted units and highest in the least diluted units which corresponded to minimum and maximum peak chlorophyll-a concentration. This was the reverse of Year 1 where highest growth rates during the first peak were found in the most diluted units. Unlike Year 1 however, an immediate rise in chlorophyll-a concentration was observed in the most diluted units when chlorophyll-a levels remained low in the other dilutions which may have reduced the growth rate in Table 6.12. Growth rates were three times as high in Year 2 than Year 1 for dilutions 20:80% and 50:50% possibly due to better light penetration if particulate matter was frozen out, but for the most diluted units the growth rate was slightly lower in Year 2. Growth rates for dilutions 20:80% and 50:50% were also similar to those observed in the UK batch bucket experiments with an average initial concentration of 118 mg filtered COD l^{-1}. Generally though, growth rates were lower than those found in the UK experiments which were conducted at higher temperatures. The exception to this are the results from the UK tank experiments which simulated a spring warm-up by slowly increasing the temperature; growth rates were very similar to the results found in this study which suggest the importance of environmental conditions, particularly temperature in regulating the growth of algae (Kayombo *et al.*, 2003; Meseck *et al.*, 2005).

Table 6.12 Apparent algal growth rates

Dilution	Growth rate h^{-1}
20:80%	0.020
50:50%	0.019
80:20%	0.010

Figure 6.19 shows suspended solids concentrations in the units during Year 2. Typically, the average range for duplicate dilutions was 10 mg l^{-1} with a maximum of ± 29 mg l^{-1}. On the first day of sampling, suspended solids were low reflecting the capacity for freezing to remove solids but as the sample was taken post ice thaw, there may have been some disruption to sediments or settling of solids that were trapped within the ice, although the tanks were not stirred before sampling. Suspended solids levels in 20:80% dilution rose in early April and allowing for fluctuations (which may be partly due to 5-day feeding regime) stayed high until the end of the month before falling until June; 80:20% rose slower and later, peaking in the first half of June while 50:50% remained unsteady and generally low. Concentrations fluctuated in all units in accordance with changes in chlorophyll-a content. Maximum suspended solids of 92 mg l^{-1} in dilution 80:20% were measured during the second bloom in mid-May whilst levels declined in the units with the least dilution falling to 10 mg l^{-1}. Suspended solids then increased again in June possibly due to the rise in algae and onset of a second bloom. Significant correlations were found with chlorophyll-a for all dilutions ($R^2 = 0.13$-0.58; $p \leq 0.02$) with the strongest relationship for 20:80% dilution ($R^2 = 0.58$; $p < 0.001$). pH values rose from 6-6.5 to between 7 and 9 in conjunction with the rise in algal biomass.

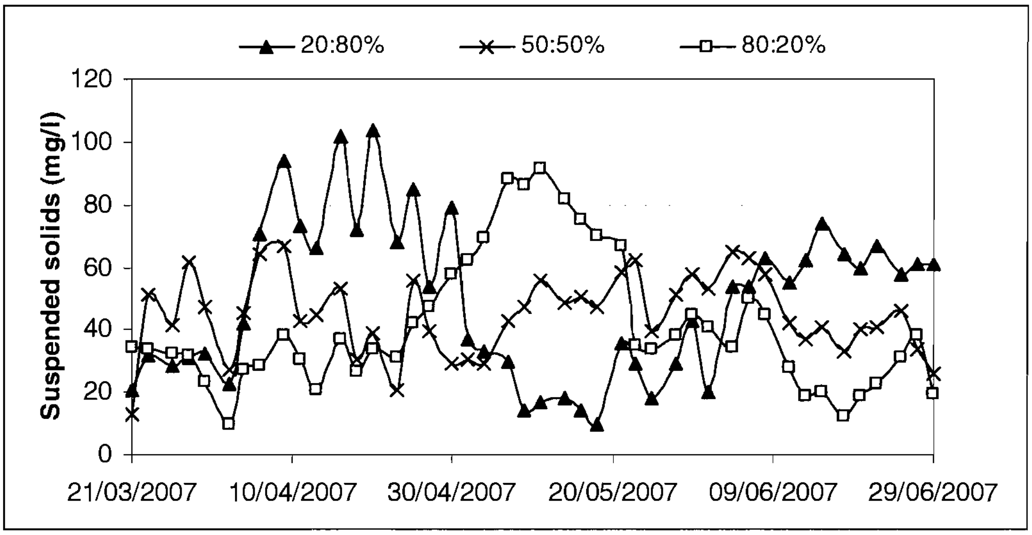


Figure 6.19 Change in suspended solids concentration over time for each dilution

Figure 6.20 and Figure 6.21 show both gross and net oxygen production in the units. Measurements were not started until 18 April but results immediately reflected the loading on each system with highest oxygen production in the most diluted units. Gross and net oxygen peaked on 16 May in the units containing dilutions 50:50% and 80:20% and coincided with peak algal content from the second bloom. Maximum gross oxygen production was 3.95 mg O_2 l^{-1} h^{-1} for 80:20% dilution compared with 2.90 mg O_2 l^{-1} h^{-1} for 50:50% dilution. Maximum net oxygen production was 6.53 mg O_2 l^{-1} h^{-1} for 80:20% dilution compared with 3.00 mg O_2 l^{-1} h^{-1} for 50:50% dilution. Oxygen production in the units containing 20:80% dilution fluctuated below 1.20 mg O_2 l^{-1} h^{-1} until maxima of 3.10 mg O_2 l^{-1} h^{-1} and 2.80 mg O_2 l^{-1} h^{-1} were recorded for gross and net oxygen production respectively on 13 June. By this time, approximately 85% of COD present at the start of the experiment had been removed thereby reducing the oxygen demand on the system in relation to the amount produced. Peaks in gross and net oxygen occurred at the same time as chlorophyll-a and suspended solids peaks for dilutions 50:50% and 20:80%, but there was no significant relationship between the values ($p > 0.05$). Chlorophyll-a levels in the highest dilution (80:20%) however, correlated well with gross ($R^2 = 0.59$; $p = 0.006$) and net ($R^2 = 0.61$; $p = 0.004$) oxygen production but suspended solids correlated more strongly with net oxygen production ($R^2 = 0.91$; $p < 0.001$). The strong correlation between oxygen production and suspended solids is possible as solids are able to provide a good indication of algal biomass, particularly at this stage in spring when the solids content is still mainly algal and indicated by the close relationship between chlorophyll-a and suspended solids. Year 1 demonstrated similar results where dilution was the same. For example dilution 50:50% showed peak levels of between 3 and 4 mg O_2 l^{-1} h^{-1} though the peaks were earlier in Year 1. Higher levels of gross and net oxygen production were recorded during Year 2 for the highest dilution even though chlorophyll-a levels were marginally lower. The reduction in pollutants even further through increased dilution may have increased the oxygen production to consumption ratio. Similar to Year 1, oxygen production in the least diluted units increased towards the end of the monitoring period. This could be a consequence of both a reduced demand on the system and also a rise in chlorophyll-a due to an algal bloom.

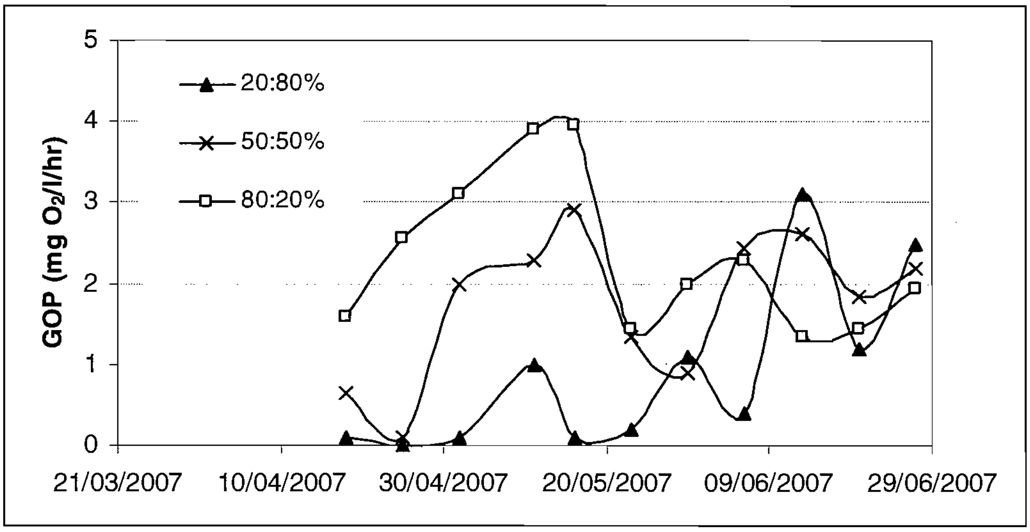


Figure 6.20 Change in gross oxygen production over time for each dilution

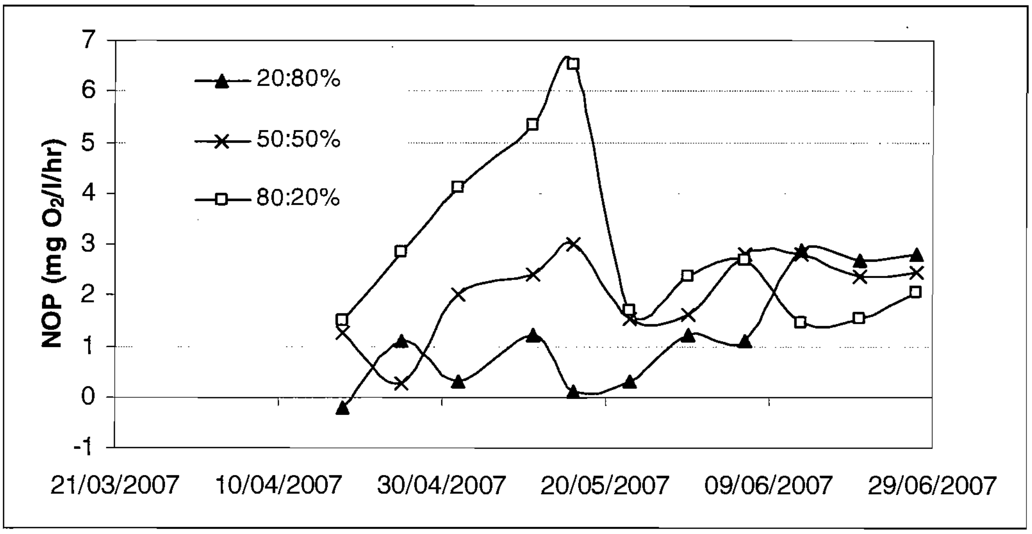


Figure 6.21 Change in net oxygen production over time for each dilution

The assimilation number was calculated for each dilution and as in Year 1 it generally decreased as chlorophyll-a concentration increased showing lower values in June than

April and May. Phosphate concentration was rapidly reduced by early April (Figure 6.22) which may have been a cause of the low assimilation numbers. The effect of temperature change on the assimilation number gave only weak relationships between the two parameters in all dilutions. The pattern of increasing assimilation number with decreasing chlorophyll-a concentration is likely to be a consequence of a number of factors, including nutrient availability and temperature and light intensity.

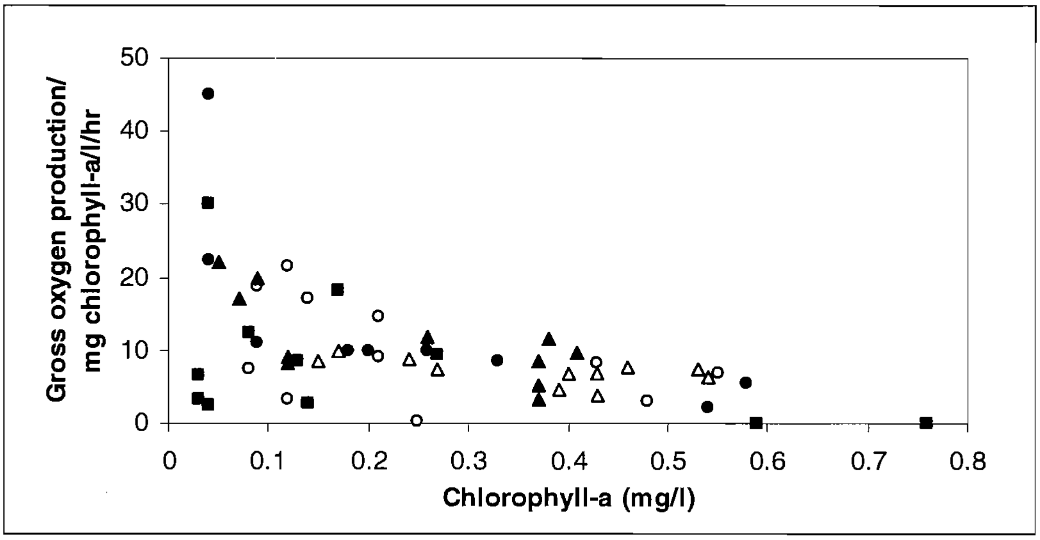


Figure 6.22 Assimilation numbers plotted against chlorophyll-a
(■) 20:80% (●) 50:50% (▲) 80:20%

6.3.3.3 Nutrients

The pattern of ammonia removal was similar in all dilutions showing an initial decline (except for 80:20% dilution where concentrations were stable) followed by a peak in levels on the 2 April to 24 mg l^{-1}, 20 mg l^{-1} and 16 mg l^{-1} corresponding to increasing dilution (Figure 6.23). The immediate trend for steady-state values in the most diluted units is consistent with the pattern of BOD_7 concentration and suggests that dilution may improve the rate of nutrient removal following the winter period. The subsequent increase in ammonia coincided with the day the algae started to bloom and may have been due to turnover of material deposited on the bottom. This was followed by a rapid decline in ammonia probably due to algal uptake required for growth. The fall in ammonia concentration was accompanied by a rise in pH above 8 supporting the argument for algal uptake. Nitrate concentrations remained low during the spring at around 0.2-0.3 mg l^{-1}, though slight increases were seen for all dilutions in late March, coinciding with increases in COD and chlorophyll-a.

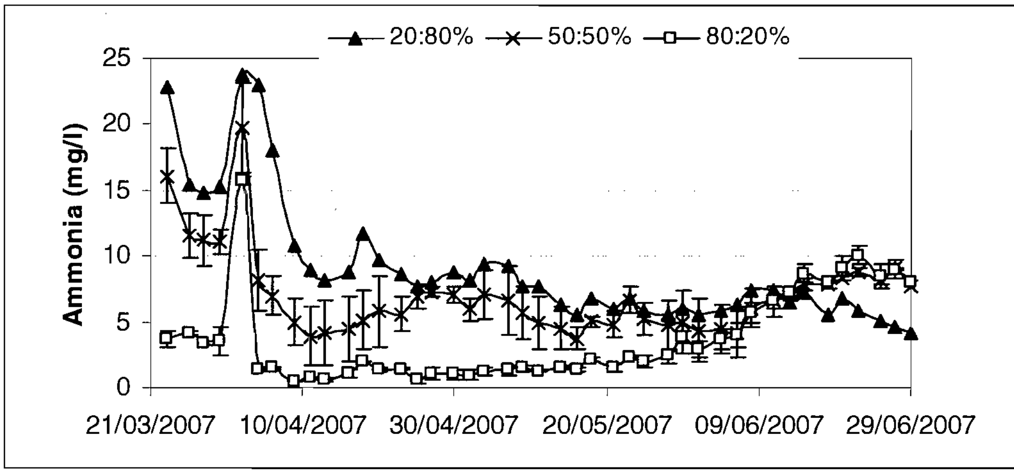


Figure 6.23 Change in ammonia concentration over time for each dilution

Ammonia removal efficiencies and removal rates were calculated for values measured on 13 April when ammonia levels dropped in all dilutions to steady-state values (Table 6.13). Efficiencies were calculated from both the influent ammonia concentration and from maximum values observed on 2 April. Both results demonstrated that the highest diluted units were most effective in ammonia removal with ammonia concentration falling to very low levels by mid-April. Ammonia removal was slowest in dilution 20:80% probably reflecting the initial loading and delayed removal over the winter months which resulted in high levels of ammonia being redissoluted in the spring. Ammonia removal rates calculated from maximum values to steady-state values were higher than in Year 1 because the removal process took a week less in Year 2 for levels to decline. This increase in removal rate may have been due to the higher algal growth rates observed in Year 2 compared with Year 1 supporting the argument for algal uptake as the primary mechanism for ammonia removal.

Table 6.13 Ammonia removal efficiency and removal rate

Dilution	Ammonia removal efficiency (from incoming concentration) (%)	Ammonia removal efficiency (from max values) (%)	Ammonia removal rate (from max values) ($g\ g^{-1}\ d^{-1}$)
20:80%	18	65	0.059
50:50%	58	79	0.072
80:20%	94	96	0.087

Phosphate levels decreased rapidly at the start of the period to less than 0.5 mg l^{-1} by the beginning of April in all units (Figure 6.24). Highest chlorophyll-a levels peaked on the day of lowest phosphate levels suggesting phosphate removal due to algal uptake. Each dilution appears to be phosphate limited or the phosphate may have been removed through luxury uptake by the algae as chlorophyll-a concentration remained at adequate levels following the decline in phosphate.

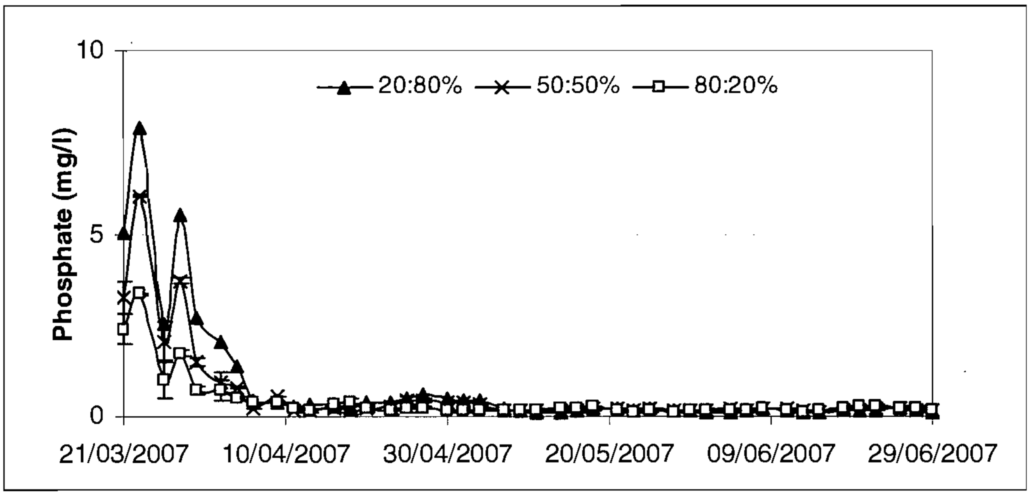


Figure 6.24 Change in phosphate concentration over time for each dilution

Phosphate removal efficiency and removal rates were calculated using maximum values on 23 March and minimum values on 6 April as well as overall efficiency using influent values and similar to Year 1 varied little between dilutions (Table 6.14). Phosphate removal rates comparable with ammonia removal were higher than in Year 1 perhaps reflecting the difference in algal growth rates. Overall phosphate removal efficiency was above 97% in all dilutions suggesting that although the units were perhaps phosphate limited all dilutions were able to satisfy discharge requirements early in the spring.

Table 6.14 Phosphate removal efficiency and removal rate

Dilution	Phosphate removal efficiency (from max values) (%)	Phosphate removal rate (g g^{-1} d^{-1})	Overall phosphate removal efficiency (%)
20:80%	94	0.067	98
50:50%	96	0.069	97
80:20%	88	0.063	97

6.3.4 25-litre units - Conclusions

Starting the 25-litre units both in the spring and before the winter freeze demonstrated some important differences between the behaviour of systems experiencing a winter period followed by a spring warm-up and those set up at the start of the spring, albeit at small scale. COD, BOD_7 and nutrient removal were affected by the addition of a winter period with delays in the increase of removal efficiencies at the start of spring. Steady-state values for BOD_7 concentration were immediate in the more diluted units but dilution 50:50% took 17 days from the start of the experiment to reach removal efficiencies above 80% and a further week was needed for dilution 20:80%. In Year 1, pollutant concentration and thus removal efficiencies were similar throughout the monitoring period. In Year 2 a delay in the onset of an algal bloom was observed in the least diluted units by 12 days compared with the highest diluted systems. In Year 1, no difference in the start date of an algal bloom was observed between dilutions. Findings of the oxygen production experiments supported the chlorophyll-a results whereby little difference was observed between the trend in oxygen production over time in Year 1 but during Year 2 the results showed notable differences between dilutions. Oxygen production reflected the loading on each system with the highest oxygen being produced in the most diluted units and the lowest production in the least diluted units. Unfortunately measurements were not conducted at the beginning of the monitoring period to establish whether net oxygen recovery came earliest in dilution 80:20% but the trend of oxygen production following changes in chlorophyll-a suggest this may have occurred. The importance of net oxygen conditions earlier in the spring is that it signifies the absence of odour following the break-up of ice and provides a measure of treatment quality.

6.3.5 780-litre facultative tanks - Year 1

6.3.5.1 COD removal

Unfiltered COD concentrations at both 20- and 30-d HRT peaked in late January between 450 and 480 mg l^{-1} and then started to fall as the melting ice layer began to dilute the sub-ice water. Filtered COD fell rapidly in the 30-d HRT tank as soon as the ice had melted. 20-d HRT initially lagged behind by about 10 days, but by early April filtered COD concentrations in both tanks were steady at around 65 mg l^{-1}. Unfiltered COD in both tanks stabilised at around 140 mg l^{-1} (Figure 6.25). Typically, the average range for filtered COD was 6 mg l^{-1} and 14 mg l^{-1} for unfiltered COD.

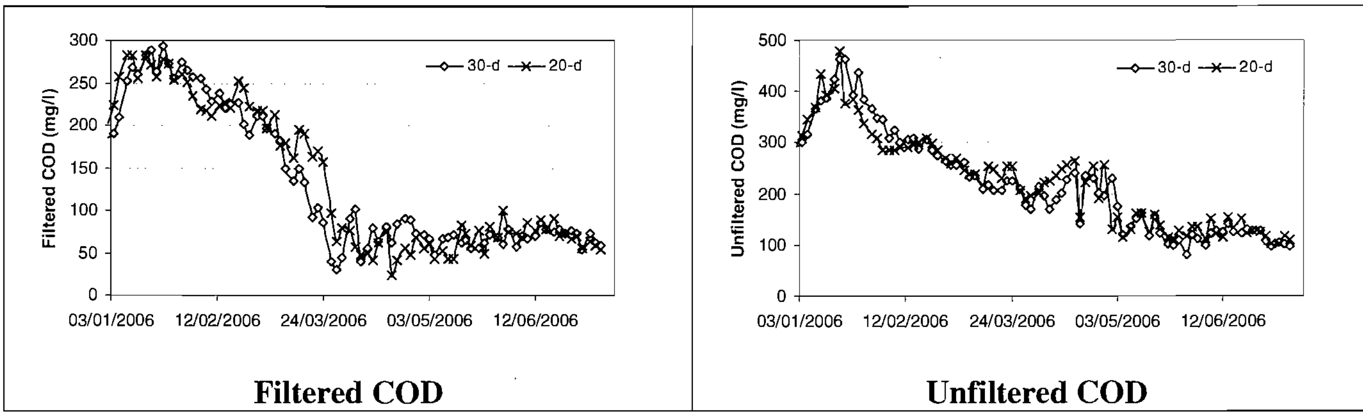


Figure 6.25 Change in mean filtered and unfiltered COD concentration for 20- and 30-d HRT (NB. different y-axis)

Figure 6.26 shows filtered COD removal efficiency in the 780-litre tanks during Year 1. COD removal in the 30-d tank started to rise from the beginning of February peaking at 90% removal efficiency at the end of March. From 10 April until the end of June removal efficiency stabilised at between 70 and 80%. COD removal in the 20-day HRT tank showed a more gradual increase in efficiency, reaching 80% at the end of March and then stabilising between 65 and 80%. The tank with the 20-d HRT showed higher COD levels than 30-d HRT during April, but after this the separation between the two reduced as similar steady-state values were reached reflected in more constant removal efficiencies. Removal efficiencies for filtered COD behaved similarly to the 25-litre units in Year 2 but with steady state values reached approximately one month earlier. In Year 1, steady state removal efficiencies in the 780-litre tanks had already been achieved by the beginning of the 25-l unit experiment, which then showed similar values: this again suggests that the absence of a winter period and no ice cover in the 25-l units created conditions which ensured there was no phase of unsteady-state removal efficiencies.

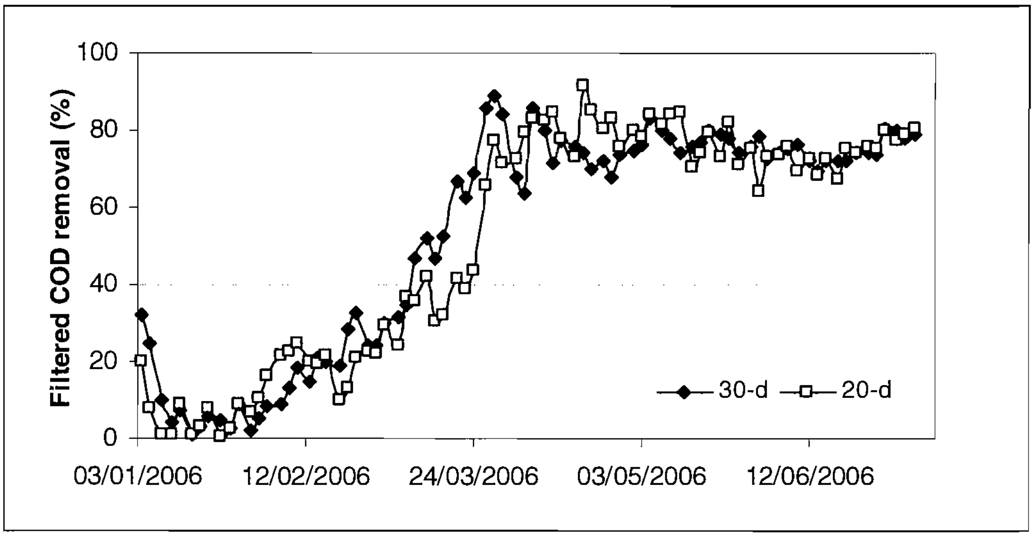


Figure 6.26 Filtered COD removal in facultative tanks with 20- and 30-d HRT

Regression analysis showed a strong relationship between COD removal efficiency and date from 30 January to 31 March for both the 30- and 20-d HRT ($R^2 = 0.92$ and 0.72 respectively, $p < 0.001$). T-test showed no significant difference between rate of change of removal efficiencies ($p = 0.299$). During the non-steady state period from the end of January until the end of March removal efficiencies were calculated for the fall in COD concentration from maximum to minimum values. These are shown in Table 6.15. From Figure 6.26 it can be seen that 20-d removal efficiency lagged up to 10 days behind the 30-day value for the period 30 January to 31 March. At the start of spring, reducing the HRT therefore seems to have a slight effect on the recovery of removal efficiency.

Table 6.15 Filtered COD removal efficiency during the non-steady state period in spring

HRT	Removal efficiency (%) non-steady state period
20-d	77
30-d	90

COD removal and temperature

Water temperature using all measurements correlated strongly ($R^2 = 0.75$; $p < 0.001$) with COD removal efficiency in both facultative tanks. When values of 0°C and below representing the period of ice cover were taken out, the relationships became weaker ($p \leq$

0.001) as shown in Figure 6.27. Removal efficiency was already over 30% when temperatures were just above zero and had more than doubled when the water temperature was still below 10°C. The consistency of removal efficiency in the temperature range 10-24°C reduced the correlation between the two parameters.

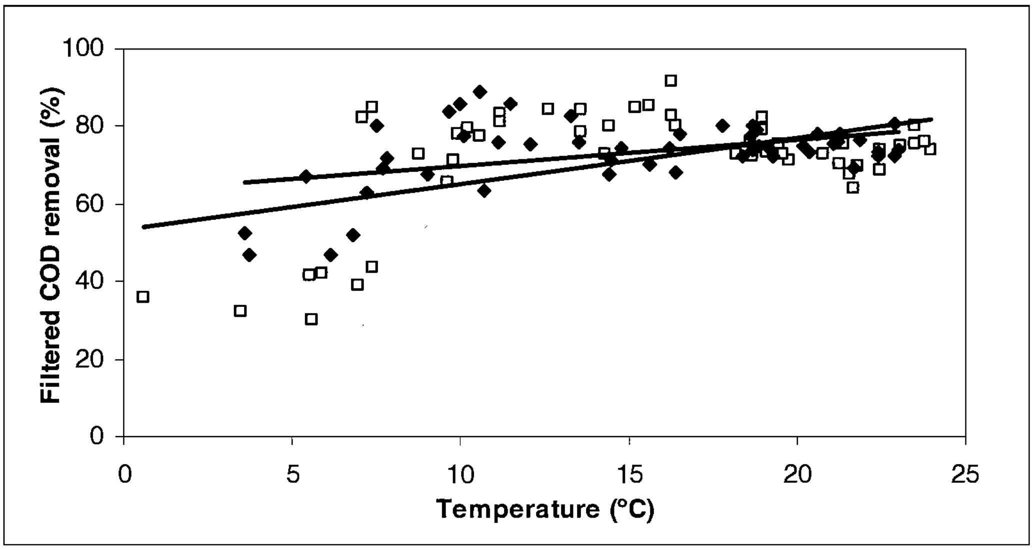


Figure 6.27 Relationship between filtered COD removal and water temperature in facultative tanks with 20- and 30-d HRT (♦) 30-d HRT ($R^2 = 0.19$); (□) 20-d HRT ($R^2 = 0.25$)

6.3.5.2 Chlorophyll-a, suspended solids and pH

The rise in algal population, indicated by chlorophyll-a and suspended solids concentrations, started from the day the ice melted in both tanks. Chlorophyll-a concentration in the 30-d HRT increased rapidly, whereas in the 20-d HRT it rose more slowly, blooming two weeks later (Figure 6.28). Figure 6.29 shows the slight delay in ice melt in the 20-d HRT tank which may have contributed to the later bloom. The peak chlorophyll-a concentration was also slightly greater for 30-d HRT (1.57 mg l^{-1}) than 20-d (1.27 mg l^{-1}).

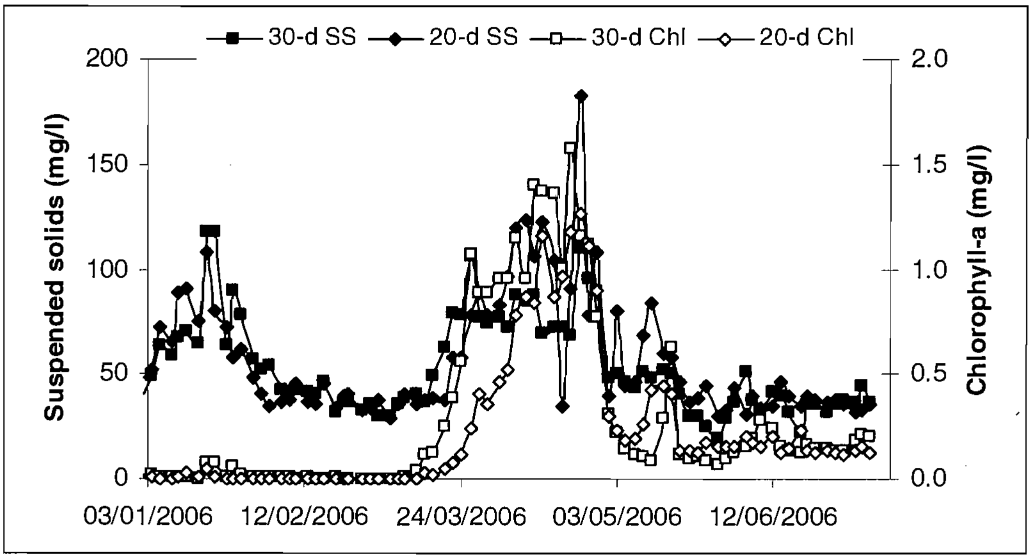


Figure 6.28 Chlorophyll-a and suspended solids concentration in facultative tanks with 20- and 30-d HRT

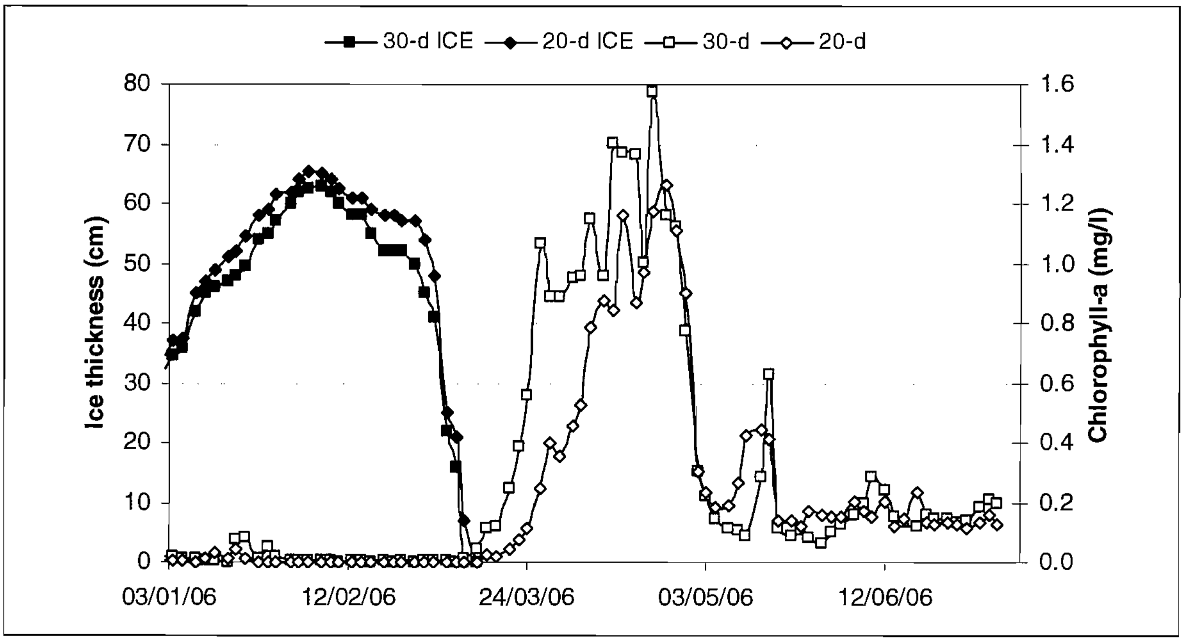


Figure 6.29 Change in chlorophyll-a concentration with ice thaw in facultative tanks with 20- and 30-d HRT

Chlorophyll-a values from the day of complete ice-out for each HRT were fitted to an exponential growth model to establish whether there was any difference in the rate of increase between retention times. Using actual start values for chlorophyll-a concentration and adjusting the rate to obtain a suitable growth curve, strong correlations were achieved for both HRT ($R^2 = 0.9961$). k values of 0.35 d^{-1} for both retention times showed the best fit and suggested the apparent lag phase and difference in maximum chlorophyll-a concentrations was more a consequence of algal removal, due to the shorter retention time than a result of COD concentration or time of ice-out. The model is very sensitive to starting values and therefore only provides an indication that HRT is the primary factor for the differences in chlorophyll-a, but as calculated growth rates were also very similar to each other at around 0.01 hr^{-1} (Table 6.16) it appears that the increased COD load was not critical. The observed growth rate for algae in ponds can range widely depending on temperature and other factors. The growth rate recorded in this study was mid-range of that found in previous UK studies 0.05-0.7 d^{-1} (Toms *et al.*, 1975).

Table 6.16 Apparent algal growth rates

Tank	Growth rates (h^{-1})
20-d	0.010
30-d	0.009

After the initial peak, chlorophyll-a values in both tanks declined from the end of April. Both tanks showed an increase in suspended solids from early March, reflecting the combination of loading and growth in algal biomass. Concentrations in 20-d HRT rose to 120 mg l^{-1} during April and in the 30-d HRT to 80-90 mg l^{-1} with both tanks stabilising at 30-50 mg l^{-1} in June. pH values in both tanks were fairly stable, rising from around 6.5 in March to 8 in mid-April.

6.3.5.3 Nutrients

Ammonia concentrations in both facultative tanks rose to 35-40 mg l^{-1} over winter (Figure 6.30), while phosphate reached between 10 and 15 mg l^{-1} (Figure 6.31). The rise in nutrients was probably due to freeze-out by the thickening ice and the accompanying low removal efficiencies associated with reduced biological activity. By mid-February there was no difference in ammonia concentration between HRT as levels fell to 8 mg l^{-1} by 7 April in association with the algal bloom. Ammonia started to rise again by the end of the month with slightly higher values in the 20-d tank. Phosphate levels in the tanks remained similar from the end of January onwards. The concentration of nutrients remained quite high relative to the results from the 25-l units which helped to confirm or support the view that the latter systems were nutrient limited. At these levels, meeting discharge requirements would pose a problem and further treatment would be necessary but it appears that a longer retention time has no benefit in terms of nutrient removal over a system running at 20-days. Abis and Mara (2005) conducted pond experiments in the UK at different HRT and found no loss in performance on reducing the HRT to 20 days.

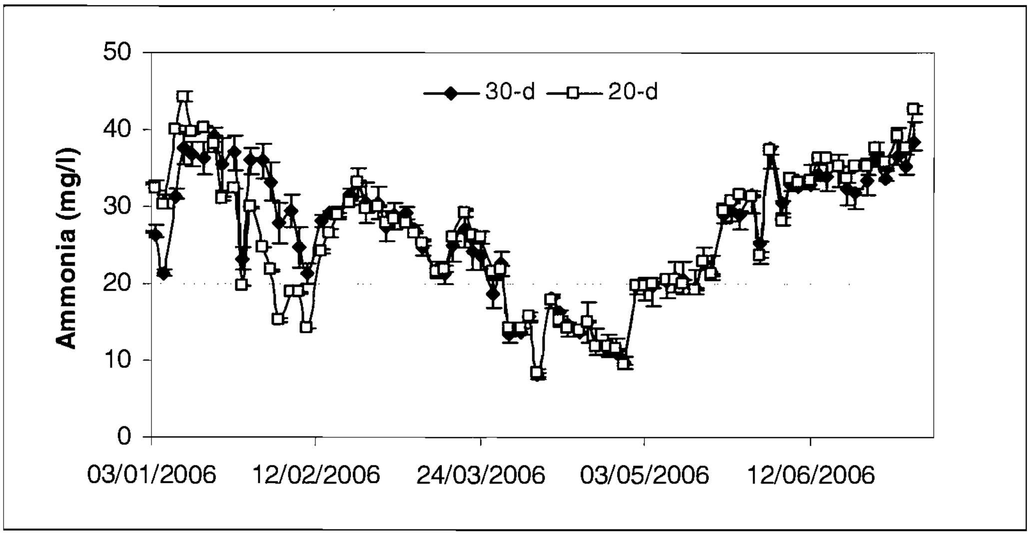


Figure 6.30 Change in ammonia concentration in facultative tanks with 20- and 30-d HRT

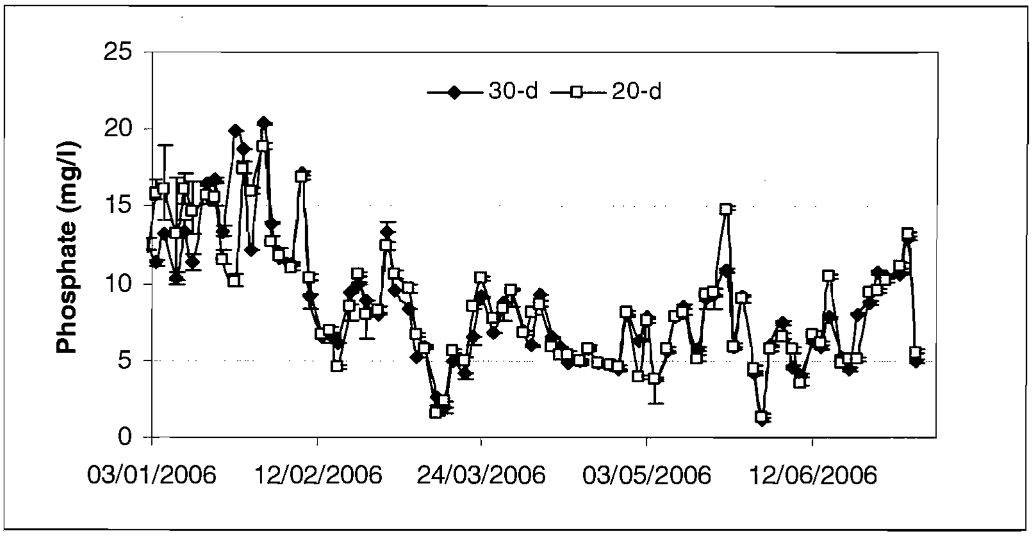


Figure 6.31 Change in phosphate concentration in facultative tanks with 20- and 30-d HRT

6.3.6 780-litre tanks - Year 2

6.3.6.1 COD removal

Figure 6.32 shows filtered COD concentration in all five facultative tanks during Year 2. From January onwards COD levels began to rise steadily in all tanks, peaking at around the end of February. The highest concentration of 293 mg l^{-1} was reached on 28

February in the 15-d HRT tank. In the other tanks peak values were between 190-230 mg l^{-1} with no apparent relationship to HRT. After this date the ice thickness on the ponds decreased over the next 12 days, corresponding with a reduction in concentration. From 12 March there was a much more rapid decrease in filtered COD in the tanks with a 40 and 60-day HRT, which reached their lowest values of around 20 mg l^{-1} by 23 and 28 March. The 30-day HRT tank showed a rapid decline after 12 March but took longer to reach its minimum value, which was achieved on 9 April. The 15 and 20-day HRT tanks showed a delay in the onset of the rapid decline in filtered COD. In the 20-day tank this began on 9 April and reached 46 mg l^{-1} on 27 April, while in the 15-day HRT tank the behaviour was more erratic but the final fall from high COD levels occurred place between 20 April and 9 May. Once steady state summer conditions were achieved from early May onwards the filtered COD concentration in all tanks was generally around 50-65 mg l^{-1} and unfiltered COD levels stabilised at around 110-140 mg l^{-1}. The results indicate that tanks with a shorter HRT were later in achieving steady-state summer COD concentrations. In the case of the 15-day HRT tank minimum COD concentrations were reached around 47 days after the 40-day HRT tank.

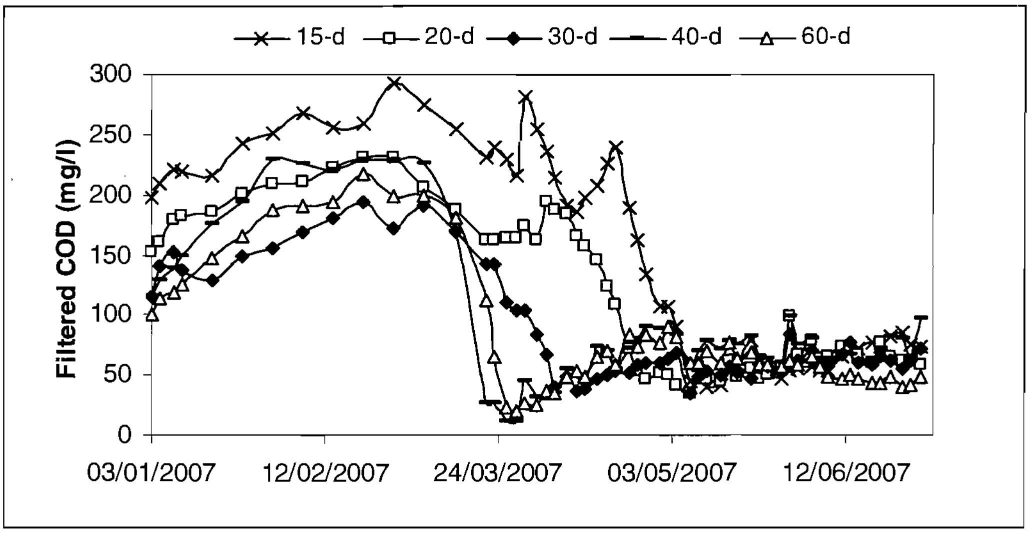


Figure 6.32 Change in filtered COD concentration over time in facultative tanks with different HRT

Figure 6.33 shows filtered COD removal efficiency in the large tanks during Year 2. Between 21 and 23 March, COD removal efficiency increased rapidly in the tanks in the range 30-60 days HRT. Removal efficiencies of over 90% occurred in the 40-d and 60-d tanks on the same day, then stabilised between 65% and 80%. Although COD removal efficiency in the 30-d tank began to increase on the same day as the tanks with the longer retention times, the increase was more gradual between 23 March and 6 April. The HRT in the range 15-20 days clearly had a negative effect delaying the increase by approximately 33 and 19 days respectively in comparison with the 40 and 60 day HRT tanks. COD removal efficiency fluctuated more in the tanks with lower retention times but eventually peaked at 86% at the end of April/beginning of May. Under summer conditions variations in removal efficiency were minor and all HRT achieved similar removals. In their study on ponds experiencing ice cover Neel *et al.* (1961) found that during open-water periods, percent reduction of sewage constituents was about the same at all loadings.

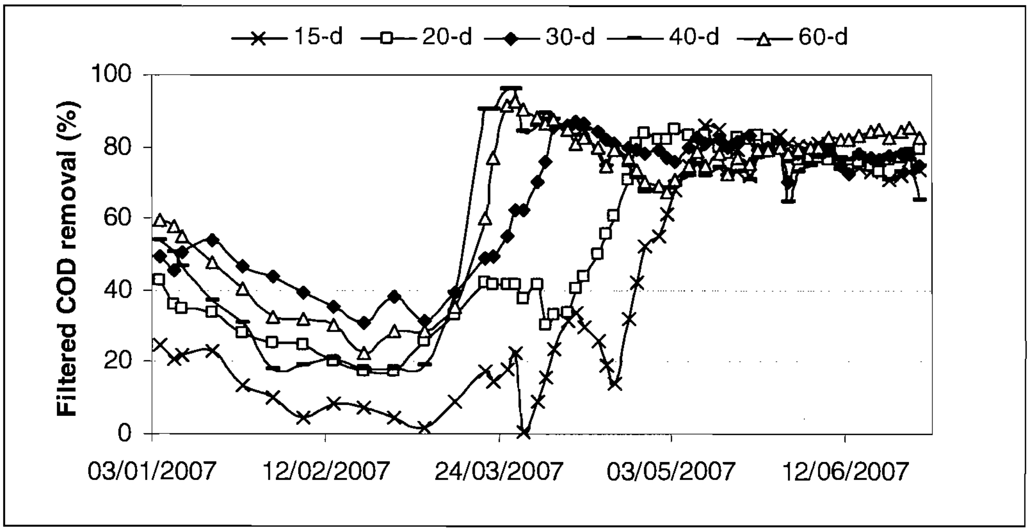


Figure 6.33 Change in filtered COD removal efficiency over time in facultative tanks with different HRT

Filtered COD removal rates were calculated taking into account the filtered COD added and destroyed (Equation *6.1*). Values for the spring period of transition between high and low COD concentrations are shown in Figure 6.34. The sequence of peak COD removal rates corresponds to that of decreasing HRT. The peak values ranged between 0.225-0.275 g g^{-1} d^{-1} with no apparent relation to HRT, although this may partly reflect the frequency of sampling which can affect the calculated rate in periods of rapid change. After the peak the observed removal rates declined rapidly in each case reflecting the achievement of steady state conditions and the low levels of available substrate.

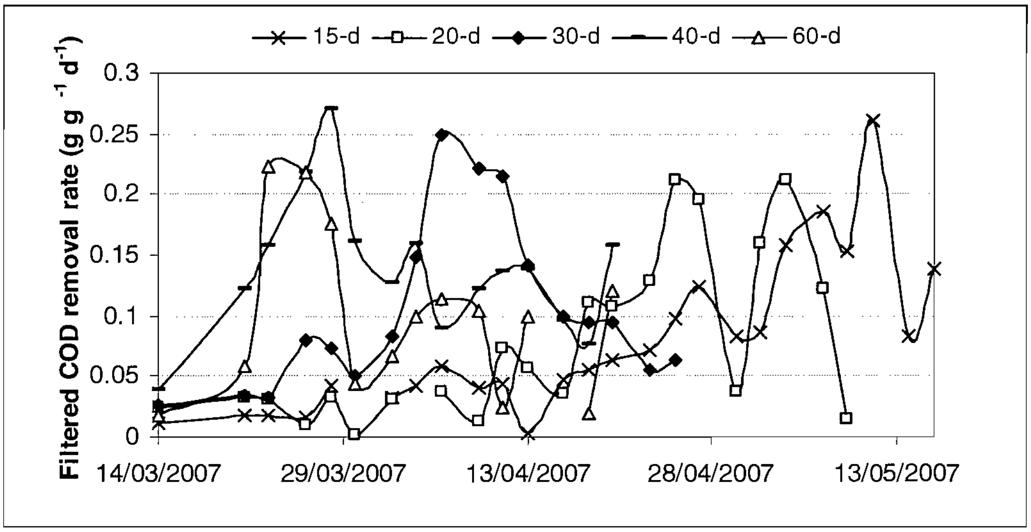


Figure 6.34 Change in filtered COD removal rate over time in facultative tanks with different HRT

COD removal data were then divided into winter and summer steady-state periods and spring non-steady state for each HRT, and typical removal rates calculated for each period. Typical winter and summer removal rates were obtained by averaging the daily values calculated for each steady state period. Typical BOD_5 reaction rate coefficients for Plug Flow systems operating at loading rates from 22 to 112 kg BOD_5 ha^{-1} d^{-1} have been published by the US EPA (USEPA, 1983). These were used to calculate predicted removal rates at the loadings used in the current study, using linear interpolation between US EPA loading rates and correcting for temperatures of 20°C and 0.5°C. Results for both COD removal rates in the current study and US EPA predicted BOD removal rates are shown in Table 6.17. Steady-state values for the two methods showed very similar behaviour with regard to loading, indicating that the results from the tank experiments were consistent with typical performance in full-scale plants.

Table 6.17 US EPA predicted BOD_5 reaction rates versus current study COD reaction rates for winter and summer steady-state periods

HRT	Loading rate (kg ha^{-1} d^{-1})	Current study (winter steady-state) (g d^{-1})	US EPA predicted rates at 0.5°C (g d^{-1})	Current study (summer steady-state) (g d^{-1})	US EPA predicted rates at 20°C (g d^{-1})
15-d	133	0.024	0.030	0.134	0.161
20-d	100	0.019	0.021	0.111	0.111
30-d	67	0.025	0.015	0.093	0.083
40-d	50	0.009	0.014	0.092	0.074
60-d	33	0.009	0.011	0.071	0.057

NB: US EPA values at a loading of 133 kg BOD ha^{-1} d^{-1} are based on linear extrapolation from the value at 112 kg BOD ha^{-1} d^{-1}. Other US EPA values are by linear interpolation.

Spring COD removal rates were obtained by carrying out a mass balance for the duration of the unsteady state period at each HRT. This was calculated over the time interval from when the COD started to fall sharply to when a low COD concentration was achieved, for each HRT. The results are given in Table 6.18 and show that at the shorter HRT the transition to low COD concentration took slightly longer as well as occurring at later date. The total COD removed was however higher at the short HRT. Volumetric and surface COD removal rates were similar in all cases, with the slightly higher rate at the 15-day HRT, probably reflecting the fact that this occurred much later in the season, with higher temperatures and total daily light input. In each case, the amount of COD removed per kg available for removal is similar, showing that the treatment capacity at the time of transition was similar in all ponds.

Table 6.18 Current study COD removal rates in the spring unsteady state period

Parameter	Units					
HRT	d	15	20	30	40	60
Surface loading rate	kg ha^{-1} d^{-1}	187	140	93	70	47
Volumetric loading rate	kg m^{-3} d^{-1}	0.019	0.014	0.009	0.007	0.005
Time taken to reach steady-state values	days	17	18	14	14	12
Initial COD concentration (winter)	mg l^{-1}	240	185	142	170	181
Final COD concentration (late spring)	mg l^{-1}	45	46	41	26	24
Average effluent COD concentration	mg l^{-1}	143	116	92	98	102
Total COD removed	kg	0.27	0.22	0.15	0.16	0.15
Surface removal rate	kg ha^{-1} d^{-1}	206	159	135	148	161
Volumetric removal rate	kg m^{-3} d^{-1}	0.021	0.016	0.014	0.015	0.016
g COD removed per g available for removal	g g^{-1}	0.886	0.861	0.823	0.889	0.891
g COD removed per g available for removal per day	g g^{-1} d^{-1}	0.052	0.048	0.059	0.063	0.074

A time-course variation of ice thickness, filtered COD and removal efficiency for each tank is shown in Figure 6.35. Soniassy and Lemon (1986) stated that during the formation of ice in a waste stabilisation pond, pure water crystallises out leaving the various constituents of sewage in a smaller volume of liquid, thereby increasing their concentrations. As the ice begins to melt, COD levels may fall as a result of dilution: this occurred from early March in the current study. At the same time addition of COD in the incoming wastewater continues, so to calculate the final concentration a mass balance approach is needed taking into account the amount of COD at the start of ice melt, the volume of ice and the amount of COD added and removed. As shown in Table 6.19 for 15, 20 and 30-day HRT the estimated COD concentration at the end of ice melt assuming no biologically-mediated removal had occurred was similar to the actual values measured. For the 40 and 60-day HRT the measured COD concentration after ice melt was 50-75% lower than expected, indicating that biological removal may have begun to occur even while the ice was still melting. In these two cases rapid removal of the remaining COD continued in the period immediately after ice melt, making it difficult to distinguish between the periods of partial ice cover and completely open water.

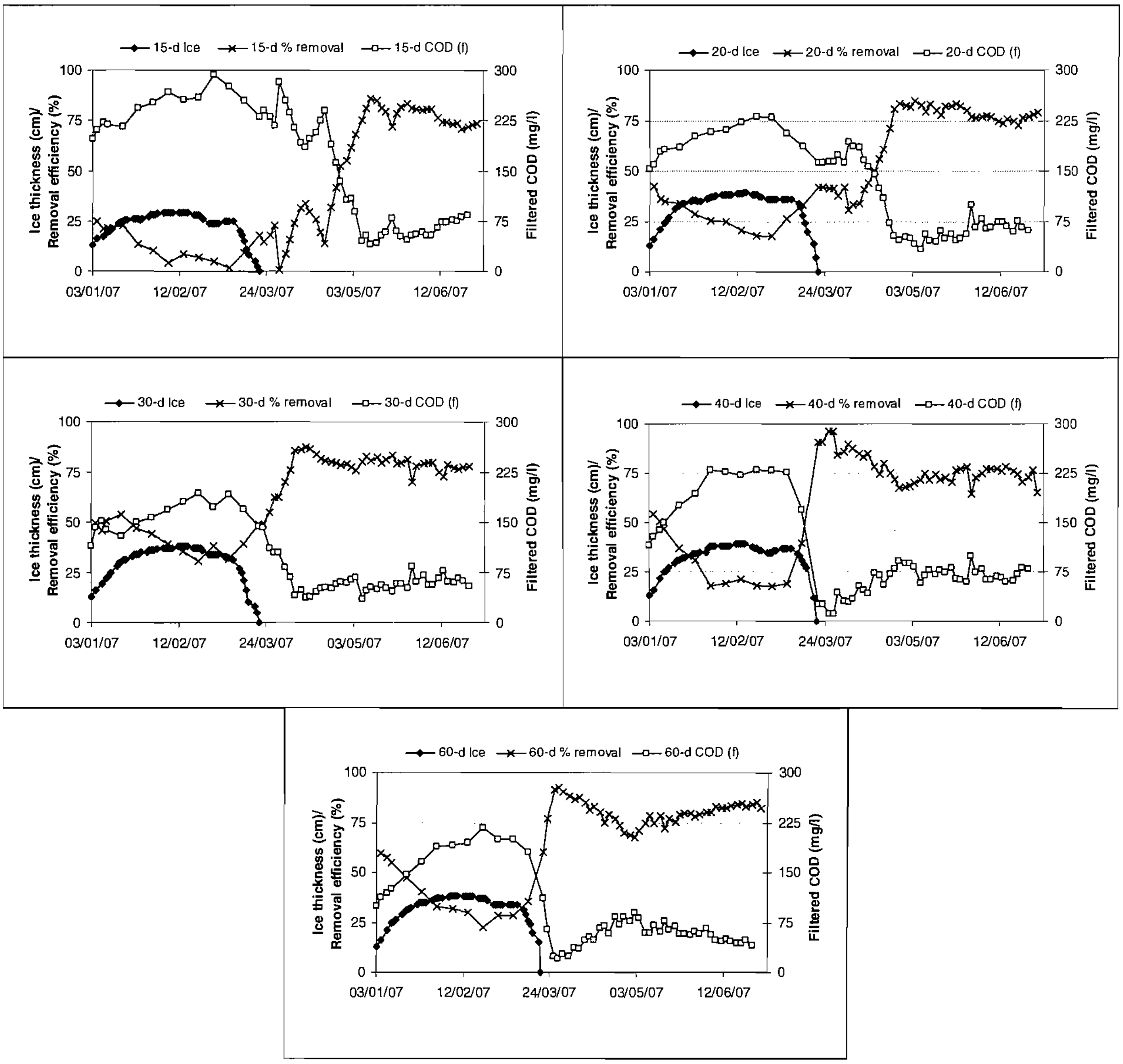


Figure 6.35 Time-course variation of ice thickness, filtered COD concentration and removal efficiency in all facultative tanks with different HRT

Table 6.19 Difference between expected and actual filtered COD concentration at each HRT during the period of ice thaw

HRT	d	15	20	30	40	60
Expected final COD concentration (without biological removal)	mg l^{-1}	196	171	158	175	153
Actual final COD concentration	mg l^{-1}	225	160	143	100	100

With the 60-d HRT data set excluded as the increase from 40 to 60 days showed little or no effect on spring recovery, there was a good correlation between HRT and the number of days from the start of ice thaw (taken as 1 March) to the first appearance of low COD concentrations. The result corresponds to approximately 1.9 days per day of HRT (R^2 = 0.996; $p < 0.05$), and since surface loading is inversely related to HRT in a given pond system, this means that doubling the surface loading approximately doubles the delay in onset of the rapid fall in COD concentration.

COD removal efficiency began to rise as the ice cover began to melt, which was most sharply in the tanks with the highest retention times; in addition to the effect of melting ice this is likely to have been due to the increased transmission of solar radiation influencing biological oxidation processes. Sub-ice light intensity was not measured, but ice cover reduces light penetration and photosynthetic aeration from the atmosphere. Ice itself has a very low albedo (approximately 2%) and in studies of the effects of ice cover on radiation transmittance in the 400-700 nm range through ice in the Great Lakes region, extinction coefficients varied from 0.006 cm^{-1} for clear ice to 0.059 cm^{-1} for a clear ice-refrozen slush combination (Bolsenga 1978). Ashton (1980) indicated that in the absence of measurements, reasonable estimates are in the order of 0.10 to 0.25 cm^{-1} for cloudy ice. These extinction coefficients are very low when it is considered that pure water is 0.03 m^{-1} but the presence of highly coloured water beneath the ice and sediments trapped within the ice itself would increase the coefficient further. The biggest effect on light penetration is the presence of snow on the surface of the ice. Environment-Canada (1985) reported on a study that found for a layer of ice 350 mm thick 30% of light was able to penetrate compared with only 0.3% through 100 mm of snow.

There was a good correlation (R^2 = 0.84; p = 0.028) between filtered COD concentration on the day of complete ice thaw (taken as 21 March) and the number of days taken to reach maximum removal efficiency. For example in the tank with a HRT of 15 days, the COD concentration on the day of ice thaw was 231 mg l^{-1}; it then took a total of 51 days to reach its maximum COD removal. This is compared with the HRT of 60 days, which took a total of 11 days to reach maximum COD removal efficiency; the COD concentration on the day of ice thaw was 111 mg l^{-1}.

COD removal and temperature

Using regression analysis water temperature showed significant relationships ($p < 0.001$) with COD removal efficiency at all HRT when all results were used. When values of 0°C and below were taken out as in Year 1 the relationships became weaker with 40-d and 60-d demonstrating no relationship between the two parameters (Table 6.20). Removal efficiency in the tanks with the longest retention times was already above 90% when temperatures were still below 10°C; suggesting that temperature was not as significant in process performance at the reduced loading. These results concur with those in Year 1 as well as from the 25-l units.

Table 6.20 Regression output for the effect of surface water temperature on COD removal efficiency

Tank	Surface water temperature (all results)		Surface water temperature (above 0°C)	
	R^2	p-value	R^2	p-value
15-d	0.72	< 0.001	0.63	< 0.001
20-d	0.81	< 0.001	0.63	< 0.001
30-d	0.70	< 0.001	0.37	< 0.001
40-d	0.50	< 0.001	0.08	0.052
60-d	0.59	< 0.001	0.04	0.199

6.3.6.2 Chlorophyll-a, suspended solids and pH

Chlorophyll-a concentrations started to rise from the day the ice melted in all tanks (Figure 6.36). The chlorophyll-a concentration in the 40-d and 60-d tanks increased rapidly, whereas the 15-d and 30-d HRT rose more slowly and the 20-d tank slower still. Chlorophyll-a levels peaked in the 40-d tank and 60-d tank on 6 and 9 April respectively. There was a delay of 10 days in algal growth in the 15-d and 30-d tanks. Algal concentrations remained low in the 20-d tank showing just one small peak at 0.79 mg l^{-1} on 27 April and averaging 0.30 mg l^{-1} from May onwards: the reason for this is unknown although it could represent the effect of inhibition, grazing or possibly picoplankton. After initial peaks, values in all tanks declined from the end of April with the onset of a possible second bloom at the end of June which was most pronounced in the 40-d tank. This may have been due to the death of the previous algal bloom, the greater availability of light from the end of the first bloom and the subsequent input of further nutrients from their decay.

Comparison of Figure 6.36 with Figure 6.32 shows that the time of chlorophyll-a build up was closely linked to a rapid early decline in COD concentration in the lower loaded 40 and 60-d HRT tanks. This relationship was less clear in the more heavily loaded tanks. A much closer relationship existed in the 15-30 d HRT tanks where algal peaks occurred later in the season but algal build up, COD loading rates and rate of algal washout interact to obscure any relationships.

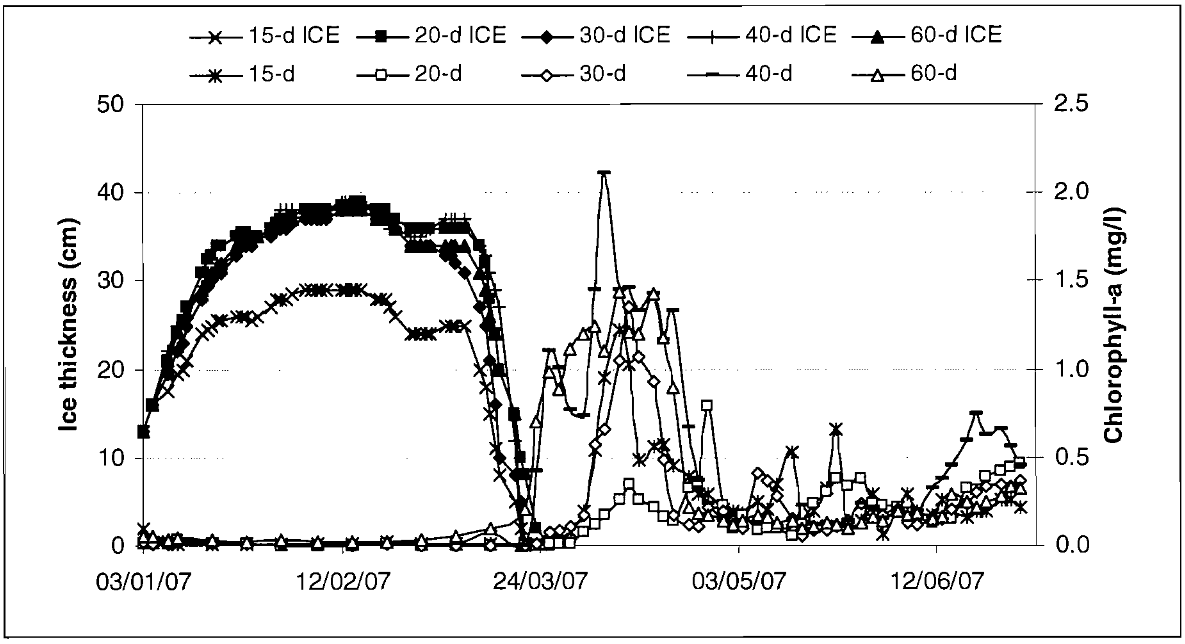


Figure 6.36 Change in chlorophyll-a concentration with ice thaw in facultative tanks with different HRT

Table 6.21 shows apparent algal growth rates calculated from the initial chlorophyll-a peak in Figure 6.36. Growth rate was highest in the 40-d tank corresponding to the high chlorophyll-a levels observed. In contrast, growth rate during the initial peak in the 20-d tank was over 5 times less than in the 40-d tank which was also consistent with the reduced chlorophyll-a concentration. Growth rate for the 20-d tank was slightly less than that observed in Year 1, but two and a half times higher in the 30-d tank in Year 2 compared with Year 1. In contrast, chlorophyll-a levels were slightly higher in the 30-d tank in Year 1 compared with Year 2 but the time taken to reach peak levels were longer in Year 1.

Table 6.21 Apparent algal growth rates

Tank	Growth rates (h^{-1})
15-d	0.016
20-d	0.007
30-d	0.024
40-d	0.039
60-d	0.026

All tanks showed an increase in suspended solids from the end of March (Figure 6.37). Concentrations in the 15-d HRT tank rose to 117 mg l^{-1} during April reflecting organic loading and rise in chlorophyll-a levels whilst in the 40-d HRT suspended solids rose to 114 mg l^{-1} reflecting the growth in algal biomass. Concurrent with the minimal growth of algae in the 20-d tank, suspended solids were the lowest recorded peaking at 78 mg l^{-1} on 27 April. Following a period where levels stabilised at between 20 and 60 mg l^{-1}, suspended solids concentrations in all tanks began to increase at the end of June reflecting the rise in chlorophyll-a levels. The most notable rise however was observed in the 60-d tank and was not reflective of algal biomass. This could have been attributed to other cells in the effluent or sludge feedback. pH values in all tanks were fairly stable, rising from around 6 at the end of March to between 7.5 and 8 by the middle of May. The highest value of pH 8 was recorded in the 40-d tank which is reflective of the higher concentrations of algal content.

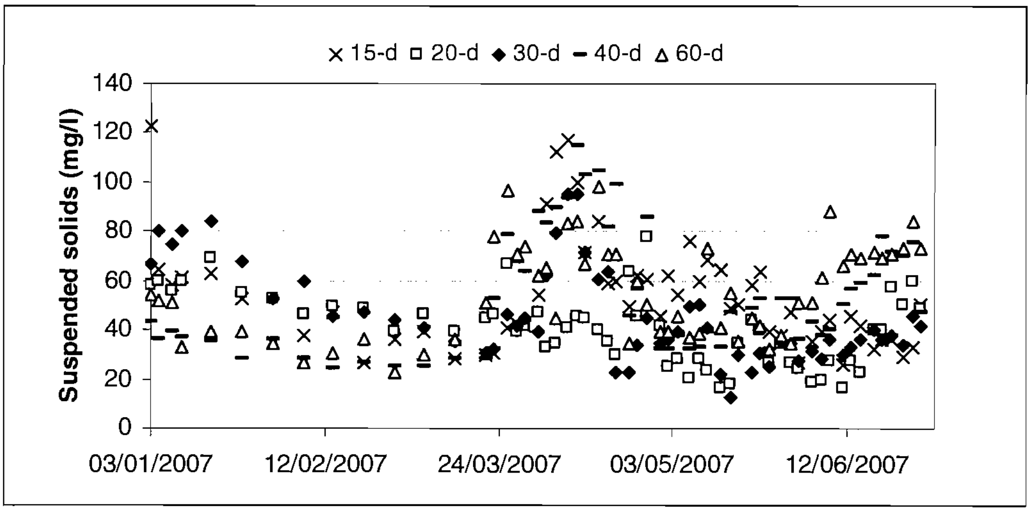


Figure 6.37 Change in suspended solids concentration over time in facultative tanks with different HRT

Regression analysis revealed significant relationships ($p < 0.001$) between suspended solids and chlorophyll-a concentration from the period of algal growth onwards. These findings suggest that in the spring effluent suspended solids are most likely due to the contribution of algal solids and not a consequence of organic loading. The correlation was weakest in the 60-d tank ($R^2 = 0.31$ compared to an average of 0.74) reflecting the high suspended solids relative to chlorophyll-a following the algal bloom.

6.3.6.3 Nutrients

Ammonia concentration for each HRT is shown in Figure 6.38. Winter values did not correspond exactly to HRT. The thickness of ice at its maximum in the 15-d tank was 10 cm less than in the other tanks, leaving a greater volume of sub-ice water available to dilute the various constituents of sewage. Calculating the volume of water remaining in the tank minus the volume taken up by ice and multiplying the concentration of ammonia found in this volume by the total tank volume revealed that the difference in ice thickness was responsible for the discrepancy between HRT. pH varied little between HRT during the winter and therefore did not influence ammonia concentrations. At the time of ice break-up ammonia concentrations began to decline in all tanks but most rapidly in the 40-d tank. Following the decline, there was a gradual rise before ammonia stabilised at levels more consistent with organic loading; although summer values appeared to separate into ammonia concentrations of around 20-25 mg l^{-1} for the two highest retention times and around 40-45 mg l^{-1} for HRT of between 20-30 days and 15-d a bit higher. The rise in levels was likely due to augmentation of nutrients from the breakdown of the algal bloom and subsequent release into the water column. The divide in ammonia concentration between retention times may also have been due to the experimental history of the tanks particularly from those run as facultative ponds in Year 1, which could have had an effect on the nutrients emerging from the redissolution of bottom sediments. High summer ammonia concentrations can result from the reduction in light availability due to the attenuation of increased chlorophyll-a levels and unused ammonia can remain (Welch, 1992). The high concentrations have implications for the storage maturation ponds following and their ability for its removal. The effect of HRT on ammonia concentration suggested that a significant relationship existed ($p < 0.001$) and a stronger correlation was apparent between HRT and ammonia levels during the spring ($R^2 = 0.77$) than the winter months ($R^2 = 0.40$).

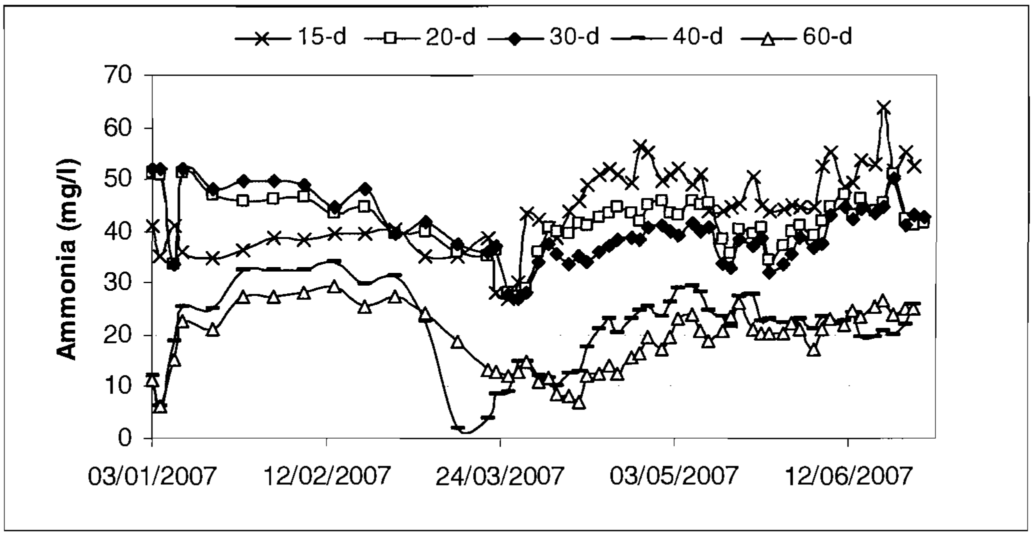


Figure 6.38 Change in ammonia concentration over time in facultative tanks with different HRT

Similar to ammonia concentration, winter phosphate levels were not exactly consistent with HRT with a divide between 40-60 days HRT at approximately 6 mg l^{-1} and 13 mg l^{-1} for 15-30 days. Values in all tanks reached between 12.9 to 14.46 mg l^{-1} by 7 February and then declined (Figure 6.39). By 14 February, phosphate had declined to 7 mg l^{-1} in all tanks. At this point ice cover over the pond was at its maximum thickness and chlorophyll-a concentration was negligible; therefore the reduction in phosphate does not appear to be attributable to algal uptake. One week later, phosphate had increased to between 11 and 14 mg l^{-1} in the tanks with 15-30 HRT, but a drop in phosphate levels was observed in the tanks with the longer retention times falling to less than 5 mg l^{-1}. The changing ice cover appeared to have no effect on phosphate levels with levels remaining stable in the 40-60 days tanks for a period of 40 days and a slight fluctuation in the remaining tanks between 9 and 15 mg l^{-1}. Phosphate concentration then became more consistent with HRT fluctuating between 5 to 16 mg l^{-1} from the end of April onwards. Phosphate concentration correlated reasonably with hydraulic retention time for both winter ($R^2 = 0.40$; $p < 0.001$) and spring ($R^2 = 0.41$; $p < 0.001$) values.

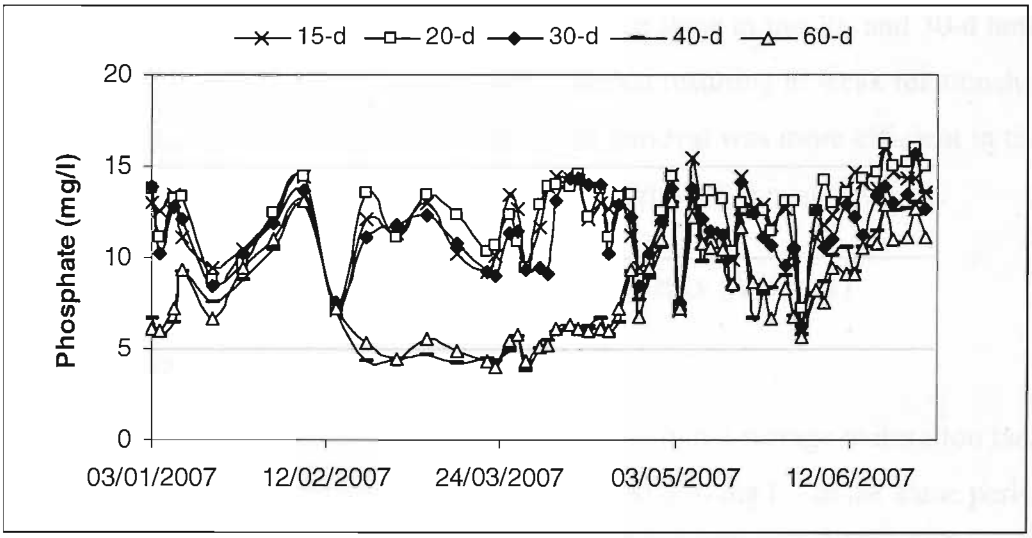


Figure 6.39 Change in phosphate concentration over time in facultative tanks with different HRT

Regression analysis was performed to determine whether any correlation existed between ammonia or phosphate concentration and chlorophyll-a levels in all five facultative tanks as shown in Table 6.22. Data sets were partitioned into winter and spring months to examine any seasonal effects on the relationship for each tank. Highlighted values indicate significant relationships between the two parameters.

Table 6.22 Relationship between nutrient concentration and chlorophyll-a content using winter and spring values in each tank

Tank (HRT)	Ammonia/chlorophyll-a				Phosphate/chlorophyll-a			
	Winter		Spring		Winter		Spring	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
15-d	0.03	0.466	0.17	0.009	0.04	0.446	0.07	0.096
20-d	0.01	0.635	0.00	0.784	0.00	0.989	0.02	0.542
30-d	0.40	0.005	0.06	0.130	0.00	0.876	0.19	0.005
40-d	0.22	0.050	0.64	<0.001	0.12	0.168	0.21	0.003
60-d	0.23	0.046	0.42	<0.001	0.18	0.082	0.38	<0.001

The findings suggest the most significant relationships between phosphate and ammonia with chlorophyll-a occurred during the spring months following the ice break-up in accordance with suggestions for algal uptake in the tanks with retention times 30-60 days for phosphate and 40-60 days HRT for ammonia. Chlorophyll-a levels remained comparatively low throughout the monitoring period in the 20-d tank and ammonia levels stayed predominantly over 40 mg l^{-1} suggesting poor removal efficiency. Ammonia levels remained comparably high during the time of ice thaw in the 20- and 30-d tanks signifying that algal uptake of the nutrient was minimal resulting in weak relationships between the two parameters. In contrast, phosphate removal was more efficient in the 40-d and 60-d tanks which were reflected in the high chlorophyll-a peaks in May.

6.3.7 780-litre tanks - Storage maturation tanks (Year 1)

6.3.7.1 COD removal

In January and February, unfiltered COD concentration in the storage maturation tank with a storage period from 1 December-1 May was 300-350 mg l^{-1}. In the same period the tank with storage dates from 1 January-1 June had an unfiltered COD of 250 mg l^{-1}. By 1 February, 1-December-1 May tank had received approximately 2 months' untreated wastewater from the 20-d facultative tank compared with just one month in the 1 January-1 June tank indicating that the former tank at this stage was less diluted and therefore the measured COD values would be expected from the dilution strategy. By mid-March unfiltered COD was reduced to less than 100 mg l^{-1}. Filtered COD concentrations showed a similar pattern, falling from around 250 mg l^{-1} in the 1 December-1 May tank and 170 mg l^{-1} in 1 January-1 June to 50 mg l^{-1} by mid-March. Both storage tanks appeared to reach summer steady-state values of 25-40 mg l^{-1} well before their nominal discharge dates and, once COD levels started to fall, the rate of decrease was such that there appeared to be little or no time lag or difference in performance between the two tanks. Figure 6.40 shows filtered COD removal efficiencies calculated using (20-d FP effluent concentration – SMP effluent concentration/20-d FP effluent concentration) were slightly higher in the 1 January-1 June tank than 1 December-1 May tank before both increased on the 1 March and remained very similar from the 8 March onwards in both storage tanks. Peak removal efficiencies occurred on 20 March between 87 and 94% when COD levels were at their lowest and then declined as COD levels gradually increased. Removal efficiencies fluctuated throughout May and June due to the incoming loading being so small that minor variations in measurement became significant. There was no relationship between water temperature and COD removal efficiency in the storage tanks ($p > 0.05$) compared with the facultative tanks where a significant relationship was found. The results support the findings from the previous experiments where at the higher loading environmental parameters such as temperature appear to be more important in removal efficiency than when loading is reduced as in the storage tanks.

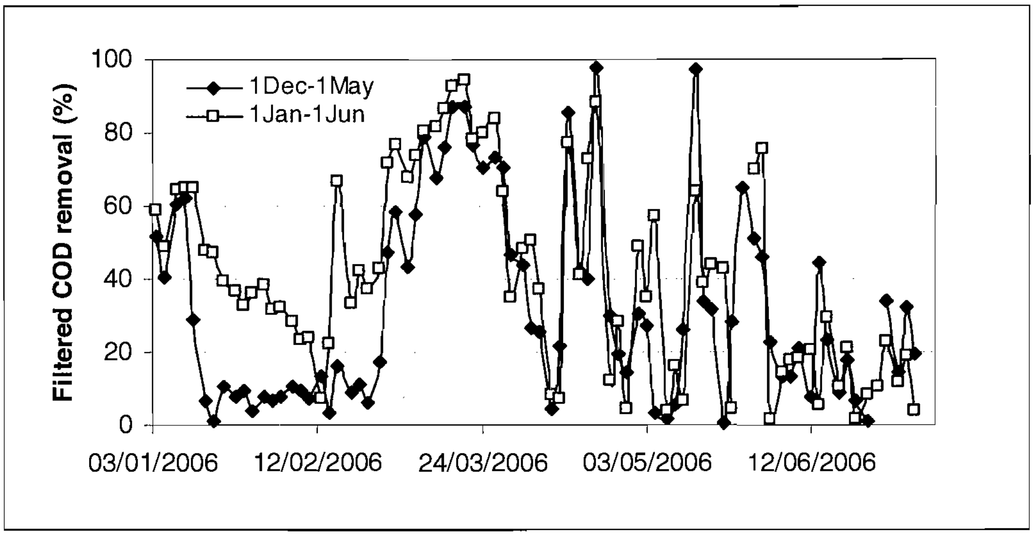


Figure 6.40 Change in filtered COD removal efficiency in storage maturation tanks with different storage periods and discharge dates

6.3.7.2 Chlorophyll-a, suspended solids and pH

Figure 6.41 shows the rise in chlorophyll-a concentration for the storage maturation tanks from 13 and 17 March with peak values of 1.27 mg l^{-1} and 1.87 mg l^{-1} observed on 22 and 27 March for 1 January-1 June tank and 1 December-1 May tank respectively. The less diluted tank therefore showed higher algal levels than the tank with the lower COD concentration. This corresponds to the results obtained from the smaller units where in both years greater biomass was obtained when the dilution factor was reduced. Suspended solids concentrations as shown in Figure 6.41 began to fall before the ice melt in conjunction with the fall in COD levels and then rose as chlorophyll-a levels increased. Chlorophyll-a and suspended solids concentration were strongly related when using values taken from the start of the spring bloom ($p < 0.001$). Values for pH rose to 8.5-9.0 soon after ice melt and showed more day-to-day variation than in the facultative tanks, reflecting the lower loading and higher chlorophyll-a concentrations. Both storage tanks showed similar variations in pH, with slightly higher values in 1 January-1 June tank throughout the spring.

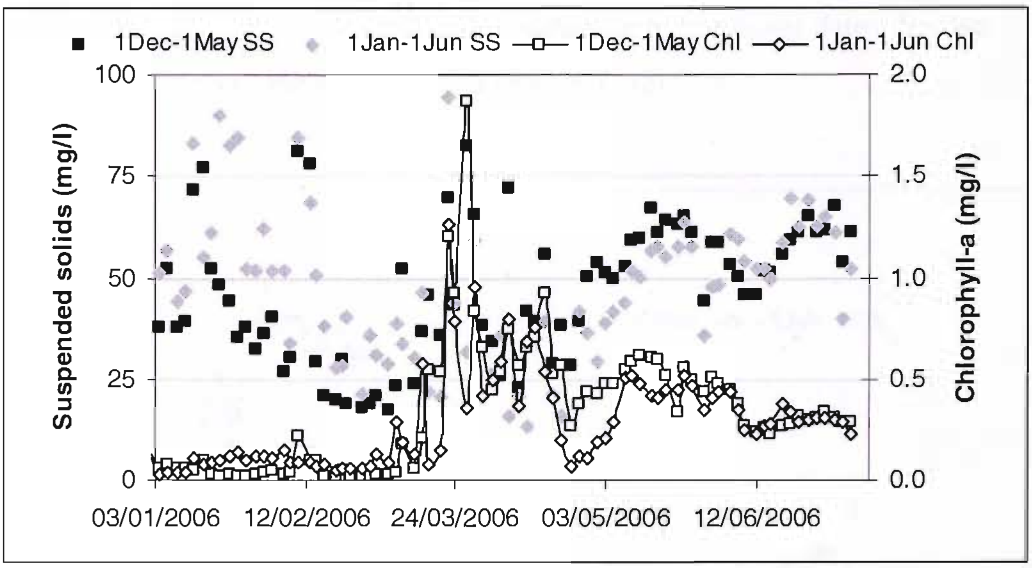


Figure 6.41 Chlorophyll-a and suspended solids concentration in storage maturation tanks with different storage periods and discharge dates

Apparent algal growth rates shown in Table 6.23 were calculated for the most rapid phase of the initial algal peak. The growth rate was marginally higher for the storage date 1 January-1 June and higher than those in the facultative tanks in the same year. The reduction in COD loading and suspended solids content would have increased the available light to encourage growth but also higher concentrations of nutrients were found to be present in these tanks which were perhaps responsible for the higher growth rates.

Table 6.23 Apparent algal growth rates

Tank	Growth rates (h^{-1})
1 Dec-1 May	0.017
1 Jan-1 Jun	0.023

6.3.7.3 Nutrients

In the storage tank with dates from 1 December-1 May, ammonia concentrations of 55 mg l^{-1} dropped in early March to between 5 and 10 mg l^{-1}. The concentration of ammonia in 1 January-1 June tank followed the changes observed in 1 December-1 May tank, but values were notably lower, with concentrations of between 20 and 30 mg l^{-1} in February falling to about 2 mg l^{-1} from March. The difference in winter values are consistent with

COD concentration resulting from dilution compatible with storage dates. Values remained low throughout the spring period (Figure 6.42).

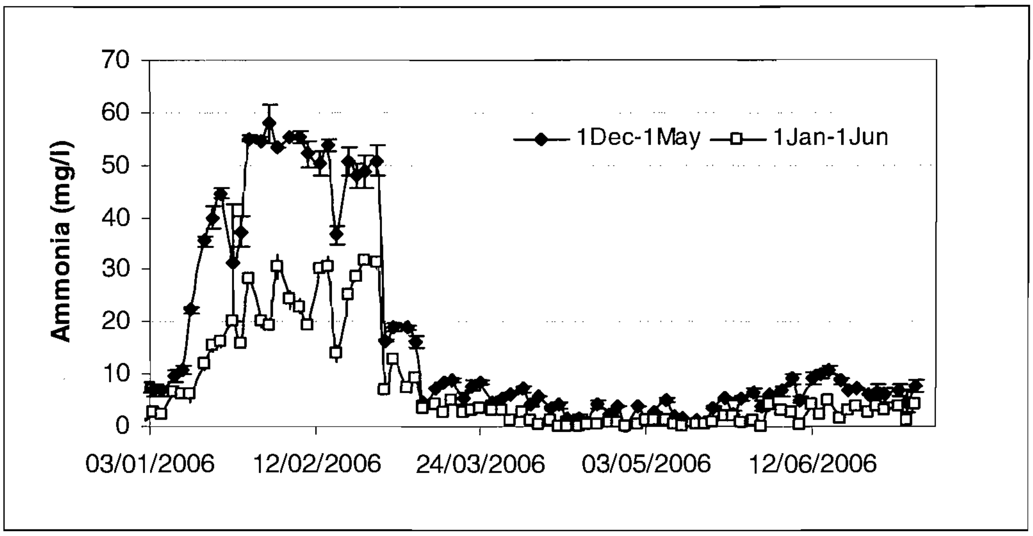


Figure 6.42 Ammonia concentration in storage maturation tanks with different storage periods and discharge dates

Table 6.24 shows ammonia removal efficiencies and removal rates calculated from maximum to steady-state values at the end of February. Removal efficiency was excellent in both tanks but the rate of removal varied between storage dates with the highest being demonstrated in the 1 December-1 May tank. The increase in removal rate may have been associated with the higher chlorophyll-a concentration observed in this tank. The removal rates were in the range of those observed in the 25-l units.

Table 6.24 Ammonia removal efficiency and removal rate

Tank	Ammonia removal efficiency (from max values) (%)	Ammonia removal rate ($g\ g^{-1}\ d^{-1}$)
1 Dec-1 May	91	0.082
1 Jan-1 Jun	91	0.057

Phosphate levels were higher in 1 December-1 May tank than 1 January-1 June tank with the concentration declining from 20 mg l^{-1} in February to less than 4 mg l^{-1} at the beginning of March (Figure 6.43). In 1 January-1 June tank for the month of February, phosphate was between 10-15 mg l^{-1} and fell to less than 2 mg l^{-1} in March. Phosphate levels in both tanks stabilised at less than 5 mg l^{-1} during the remainder of spring.

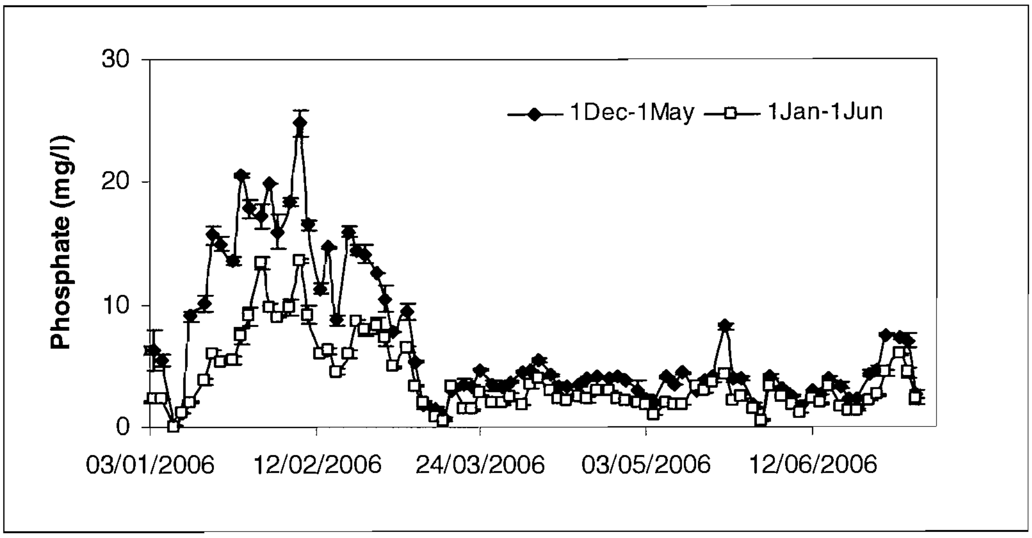


Figure 6.43 Phosphate concentration in storage maturation tanks with different storage periods and discharge dates

Phosphate removal efficiency and removal rates were calculated similar to those for ammonia. Removal efficiency was high following the spring thaw with no difference observed between storage dates (Table 6.25). Phosphate removal rates were much lower than ammonia removal rates as the phosphate concentration showed a decline over a longer period and unlike ammonia did not vary between tanks.

Table 6.25 Phosphate removal efficiency and removal rate

Tank	Phosphate removal efficiency (from max values) (%)	Phosphate removal rate ($g\ g^{-1}\ d^{-1}$)
1 Dec-1 May	97	0.028
1 Jan-1 Jun	96	0.027

The results for both ammonia and phosphate indicate that the earlier storage period may have had a very slight impact on residual nutrient concentrations into the early summer period but both were able to meet discharge requirement early in the spring.

6.3.8 780-l tanks - Conclusions

The results from the 780-l facultative tanks in Year 1 suggest that reducing the HRT from 30 to 20 days (increasing the surface loading rate from 67 to 100 kg BOD $ha^{-1}\ d^{-1}$) had a small impact in the earliest stage of spring, holding back COD removal and algal growth for up to two weeks after the ice had melted; but after this the tank coped well with the reduced retention time and increased loading. In Year 2, the wider range of HRT tested showed clear differences, with each 1-day reduction from a 40-day HRT leading to a delay of about 1.9 days in the establishment of peak COD removal and steady-state summer conditions. Increasing the HRT from 40 to 60 days had little effect in either the steady or unsteady state period. Filtered COD levels were approximately the same for all HRT by the beginning of May: this was slightly later than in Year 1, but indicates that although COD removal rates rose more slowly in the highest loaded tanks, they were still able to stabilise the influent wastewater. Abis and Mara (2005) found that HRT had no significant impact on BOD or ammonia removal in the range 20-60 days under UK climate conditions: only overall removal efficiencies were given, however, with no information on removal effects in spring. In the current study reductions in retention time seemed to have no long-term adverse effect on summer steady-state conditions, but obviously impact upon the loading applied to the storage maturation pond in the period immediately following ice melt.

In terms of nutrients, ammonia removal was more affected by decreasing the HRT than phosphate removal which demonstrated very little differences between effluent concentrations following the spring thaw. During the spring period only HRT of between 40-60 days were able to meet typical discharge requirements for ammonia concentration, as the lowest ammonia level recorded in the shorter retention times was approximately 27 mg l^{-1}; however if discharging into a storage pond, further nutrient removal would take place and nutrient levels would not be a concern.

The results of experiments in Year 1 replicating the effect of different storage times on the performance of storage maturation tanks fed with effluent from a 20-d HRT facultative tank showed excellent performance, with average COD removal efficiencies at 90% and nutrient removal efficiencies during the time of ice thaw over 90%. Both tanks were run simulating five months of storage but starting the storage period one month earlier in one tank enabled the effects of dilution to be observed. Removal efficiencies rose first in the most diluted tank (1 January - 1 June) but both storage tanks reached removals of over 85% well before their nominal discharge dates in mid-March suggesting that dilution at this range had little impact on process performance.

6.4 Discussion

6.4.1 COD/BOD$_7$ removal

Facultative tank experiments

It is clear from the results that the time taken from spring ice melt to achievement of an effluent with a low filtered COD concentration depends on the HRT of the system, with longer HRT reaching this much more quickly. The HRT itself is not considered to be the main factor influencing this, however, other than as a mechanism for controlling the organic load entering the pond.

In Year 2, the tanks operating at different HRT showed increasing COD concentrations over the winter period as cleaner water from the previous autumn was diluted out and low temperatures and ice cover limited the rate of COD degradation. At the time of ice melt, all of the tanks contained roughly similar concentrations of accumulated winter COD load. The dilution effect of the ice melt water can be explained by mass balance calculations, but the delay in the onset of rapid removal rates in the shorter HRT tanks needs further explanation.

The energy needed to bring about transformation of the accumulated winter load to a clean effluent in the spring is supplied by the sun, with increasing day lengths and decreasing angle of incidence. This raises the water temperature which increases the overall rate of biochemical reactions and microbial growth rates. More importantly, it leads to increasing photosynthetic activity and algal growth rates, which in turn supply the oxygen allowing the rapid aerobic respiration of organic matter compared to the energetically less efficient anoxic pathways.

The HRT controls both the washout or dilution rate and the loading rate. It is likely that at the time of ice melt the water temperature and degree of insolation do not allow high algal growth rates in the system. At the lower loadings growth rates may be sufficient for an algal population to develop that can supply sufficient oxygen to allow the aerobic degradation of the accumulated winter load. This is possible both as the long retention time implies very little washout of algae and as the associated low loading contributes little additional oxygen demand during the period of 7-14 days in which rapid removal of the winter COD load takes place.

At the shorter retention time there is evidence in Year 1 and Year 2 that algae are still growing in the system, but the increased dilution rate removes a greater proportion of the population and leaves insufficient numbers to meet the oxygen demand of both the accumulated winter load and the daily load of fresh COD being applied, which increases with decreasing HRT. If the oxygen demand is not met the system will remain anoxic, with less energy available for the growth of heterotrophic bacteria and consequently with a lower COD removal rate. It is not until more energy is supplied to the system by the increasing temperature and daily light input that the oxygen demand exerted by the initial load and the additional daily COD input can be matched by additional algal oxygen production, which reduces the oxygen deficit in the system and allows a switch to primarily aerobic metabolism. Once this happens a rapid decrease in COD concentrations can occur, starting at a later date for each loading rate and HRT.

From the above discussion and the results presented in Chapter 5 it is likely that the onset of rapid COD removal is controlled by a number of interacting factors. The qualitative explanation given above explains the observations made but to provide a full explanation of the system dynamics would require a kinetic modelling approach. This could be based on a simple Monod growth description both for the carbon consumption by heterotrophic bacteria and the oxygen production by autotrophic algae. In both cases the kinetic constants can be altered to reflect changes in temperature and light intensity. The overall aim of a model would be to predict net oxygen production and overall oxygen demand at any time to allow calculation of an oxygen balance and determination of when the changeover from anoxic to predominantly aerobic conditions will occur. To achieve this, the model would require values for kinetic constants in relation to the temperature and light intensities in each case.

Ideally a WSP system operating under variable climatic conditions should have enough capacity to retain the incoming wastewater when it cannot be treated to a standard suitable for discharge. In practice pond systems are often divided into multiple stages. In considering pond design it is preferable to have aerobic conditions as early as possible, whether this is to allow early discharge or simply to prevent the occurrence of odours. The results of the current research confirm that early oxygenation can be achieved by low loading rates. The optimum design for arid continental climates is therefore to have a series of ponds of sufficient total volume to store the incoming wastewater over winter, with each pond of sufficient area to maintain the loading rate (consisting of surface loading and accumulated winter load) below the critical threshold for rapid COD removal in spring. The ponds can then be sequentially drained by removal of treated wastewater at a rate faster than the inflow, providing a source of water for irrigation and other purposes through spring and summer and minimising the rate of water loss through evaporation from the water surface. Based on the parameters in the present study with a pond depth of 1 m and an influent filtered COD concentration of 280 mg l^{-1} the critical loading at the latitude of Almaty, Kazakhstan appears to be around 70-90 kg ha^{-1} d^{-1}, which compares favourably with the US EPA Areal Loading rate design recommendation of 40 kg BOD ha^{-1} day^{-1} loading on the first pond (US EPA, 1983). The design is strongly depending on local climatic conditions, however, reinforcing the importance of a modelling approach.

Batch experiments and storage/maturation tanks

In Year 1, although the 25-litre experiments were small-scale and simpler in that they only attempted to capture one loading rate and did not include bottom sediments, the performance in terms of final concentration in early spring was similar to the 780-l storage tanks. The 25-l units were run at a longer retention time of 6 months and therefore the favourable comparison with the storage tanks suggests that the scale difference did not appreciably alter the behaviour of the systems. Perhaps due to the unusually early spring thaw in relation to the start of the 25-l experiments, the fall in parameters such as BOD_7 and ammonia was sufficiently rapid in all units that dilution showed little effect, although peak chlorophyll-a concentrations increased slightly in less diluted ponds; a subsequent rise in COD, BOD_7 and suspended solids was slightly higher in undiluted units. It is apparent that under natural conditions and small loading rates pollutant removal was fairly rapid and minor variations in concentration were not significant. In Year 2, dilution showed more effect on COD and BOD_7 removal with highest removals observed in the most diluted units during the non-steady state period but in summer removal efficiencies between dilutions were very similar. In accordance with the tank results there was a delay in achieving maximum removal efficiency at the start of spring where the initial load was high. By the beginning of May, pollutant concentrations were similar in all units.

In the cases considered, a change in start and end dates of storage in the storage tanks showed little impact, and both tanks reached steady state conditions well before their nominal discharge dates. The results from Year 1 using effluent from the 20-d facultative tank to feed the storage tanks suggest that the short HRT had no adverse effect on the performance of the storage tanks. It would appear that although COD removal rates during the non-steady state period were lower in facultative tanks with shorter HRT in Year 2, the removal efficiency of the storage tanks was excellent and could deal with these minor variations. Therefore there is the possibility of using a short-retention pond as a buffer to the maturation pond, but as noted above this may not be the optimum solution for odour elimination.

6.4.2 The effect of ice thickness

Results suggest that during the winter as the thickness of ice increased, COD concentrations gradually increased as the volume of liquid was reduced by the thickening ice layer. With the continual addition of organic matter with little or no removal through biological activity due to a reduction in light availability and temperature from ice cover and freezing temperatures, sedimentation is likely to have been the dominant process responsible for pollutant removal during the winter months. During the period of ice melt, COD concentration reduced but the increase in removal efficiency did not appear to be due to dilution by the melting ice cover as results were consistent with the suggestion that the melt water is simply restoring the concentration to what it would have been without freezing. The exception to this was in the 40-d tank where COD levels dropped from 226 to 26 mg l^{-1} over a period of 2 weeks which was much lower than expected. The increase in solar radiation clearly had an affect on the pond system as algal growth was rapid from the day of ice melt. Unfortunately no light measurements were taken, but as the water temperature in the tanks rose slowly over a much longer period it is assumed that the increase in light intensity was the main factor in driving the onset of an algal bloom. Iriarte and Purdie (2004) studied the factors affecting the timing of spring blooms in estuarine waters over five years and stated that water column irradiance was the major factor in initiating a bloom whereas temperature did not function as a trigger of the bloom; temperature can however enhance growth rate (Eppley, 1972). Pechlaner (1970) studied the environment aspects of phytoplankton blooms in Lake Erken, Sweden; he found the availability of radiant energy was the key factor in the onset of blooms and two weeks following the ice-out, biomass levels decreased sharply with the depletion of nutrients. During the study, radiation levels entering the lake were found to be 10 times higher after ice-out compared with when the lake was ice-covered, which may have been due to increasing seasonal intensities but most likely to greater penetration of light following the ice melt.

6.4.3 Chlorophyll-a and oxygen production

Information regarding chlorophyll-a concentration and algal growth rates was of value as chlorophyll-a levels were found to correlate strongly with oxygen production which is necessary for the biological oxidation of waste and the prevention of odours; both particularly important factors in pond performance during the spring warm-up period. The 25-l units showed a pattern of algal growth in relation to loading different to the trend observed in the 780-l tanks. The highest initial loading in the units gave rise to the highest chlorophyll-a levels in both years, although in Year 2 chlorophyll-a levels began to rise as soon as the ice thawed in the most diluted units. Concurring with results from the larger systems, a reduced loading appears to result in the onset of algal growth more quickly following a period of dormancy under the ice. In contrast, less diluted units showed a clear lag phase following the winter period with peak levels of chlorophyll-a delayed by 10 days. This agrees with earlier work using the same tanks where an increase in loading produced by increasing the wastewater strength was also found to delay the onset of algal bloom, without major effect on steady-state summer values (Heaven *et al.*, in review). Using data points corresponding to the time of algal blooms oxygen production correlated well with chlorophyll-a, but following the initial bloom in Year 1 a rise in oxygen levels was observed although chlorophyll-a concentration was very low. COD concentration at this time had been reduced to low levels in all units and therefore the oxygen demand on the system was less leading to conditions of greater oxygen production compared with consumption. Recovery to net oxygen production from the onset of an algal bloom was reached earlier in the spring when the loading was reduced preventing problems of odour whilst creating conditions to enhance biological oxidation of the waste. Odour is the unpleasant outcome of a longer period taken to establish facultative conditions; although the extent of odour was not assessed it is a big problem in North America and the longer the period taken to establish facultative conditions the more likely odour will occur. This may therefore be a major consideration in evaluating the benefits of reducing the winter load through the possibility of dilution.

Apparent algal growth rates ranging from 0.005 to 0.020 h^{-1} in the 25-l units and 0.009 to 0.039 h^{-1} in the 780-l tanks were calculated during the most rapid phase of growth which occurred when water temperatures were approximately 10°C. Growth rates were calculated during the most rapid phase of growth which was assumed to be exponential; they can therefore only be seen as minimum estimates. However, although predominantly lower they were not too dissimilar from those obtained by Bartosh (2004);

algal growth rates typical of WSP were found to be 0.030 h^{-1} for *Chlorella vulgaris* and 0.022 h^{-1} for *Scenedesmus subpicatus* at 10°C and a light intensity of 78.3 $\mu mol\ m^{-2}\ s^{-1}$ when these species were grown on culture medium. The algal growth rates generally showed a trend of increasing when the systems were more dilute or with a higher retention time.

The timing of complete ice thaw initiated the onset of an algal bloom in all tanks during Years 1 and 2. In Year 1, the peak algal bloom in the 20-d tank was delayed by two weeks following the bloom in the 30-d tank. The ice-free period occurred three days later in the 20-d tank and the addition of an increase in light-attenuating organic matter were perhaps in part responsible for the lag between chlorophyll-a peaks but algal wash-out may also have been a factor as algal growth rates were very similar. Peak chlorophyll-a levels were highest in the tanks with the longest retention times. In Year 2, a HRT of 40 days showed highest chlorophyll-a levels at 2.1 mg l^{-1} whereas chlorophyll-a in the 20-d tank remained lower than 0.5 mg l^{-1}. The results for the 40-d tank are interesting as filtered COD levels were the most rapid to fall coinciding exactly with the melting ice cover. The reduction in COD may have increased light transmission into the tank thereby creating optimum conditions for algal growth. Ammonia removal was also most efficient in the 40-d tank with concentrations showing the most rapid fall around the time of ice melt, perhaps a combined result of dilution from the melting ice and algal uptake. Differences between chlorophyll-a levels and COD removal efficiencies in the 40- and 60-d HRT tanks were insignificant ($p > 0.05$) again suggesting that there was no performance improvement from retention times longer than 40 days.

6.4.4 Nutrient removal

Ammonia and phosphate removal showed the effects of a wider range of HRT in Year 2 with a divide in removal efficiency between HRT 40-60 days and 15-30 days. From the time of ice break-up onwards ammonia concentration remained highest in the 15-d tank and decreased by order of HRT. The difference in phosphate removal between retention times was similar to ammonia displaying greater removal efficiency in the tanks with the highest retention times. The difference between retention times may be due to the uptake of nutrients by algae which showed the highest chlorophyll-a peaks in the 40-60 day HRT. Phosphate concentration correlated less with HRT and loading than ammonia which may be due to the ability for luxury uptake without immediate growth; therefore the effect of loading might be less important in phosphate removal. The pathways for ammonia removal in WSP are more complex, depending on temperature, pH and chlorophyll-a. pH values were recorded in the morning and therefore did not necessarily represent maximum values in the tanks. Elevated pH levels can contribute to the mechanisms of removal for both ammonia and phosphate; therefore both algal uptake and volatilisation may have been pathways for ammonia removal. Ammonia concentration tended to increase in all facultative tanks following the ice melt and rise in algae. During a spring overturn, significant quantities of solids can be resuspended to the surface layers and as suspended solids concentrations rose sharply in the tanks following the spring thaw during this study, it is possible that some would have come from bottom sediments although growth of algae would have been responsible for much of the increase. Maximum correlation coefficients between chlorophyll-a concentration and suspended solids during this period were also less than 0.79 suggesting that solids from the sludge layer may well have augmented the concentrations of suspended solids. There is therefore the possibility that some ammonia may have been fed back into the water column following the spring turnover.

7 LOCKERLEY WASTE STABILISATION PONDS - NOVEL APPLICATIONS OF AN INTERMITTENT DISCHARGE SYSTEM

7.1 Introduction

The mean air temperature in the UK during the coldest month is 2-4 °C (Met Office, 2008b) similar to the temperature at the start of the spring warm-up in a continental climate. As a result, UK ponds experience both marked seasonal variations in performance, and occasional periods of ice cover. Experience with WSP systems in the UK is limited, but suggests that they can operate efficiently during the summer; satisfactory removal of nutrients may be a problem in winter, however (Mara *et al.*, 1998). Abis (2002) carried out detailed studies on three pilot-scale primary facultative ponds at Esholt wastewater treatment works, West Yorkshire, UK. The ponds were operated in parallel to test the effect of surface BOD loading on performance, the maintenance of facultative conditions and the accumulation of sludge. At a loading of 80 kg ha^{-1} d^{-1}, the pond produced an effluent quality that met the EC Urban Waste Water Treatment Directive (1991) standard of < 25 mg filtered BOD l^{-1} and < 150 mg suspended solids l^{-1} at all times. Anaerobic conditions were observed during the winter at loadings of > 100 kg ha^{-1} d^{-1} but only briefly at the loading of 80 kg ha^{-1} d^{-1} after the ice melted.

Lockerley Water Farm (51°2'23"N, 1°34'00"W) is a campsite owned by Hampshire Christian Trust, which accommodates an average of 80-100 campers each week during the period from May to early September. In addition to tents it has on-site kitchen facilities and mobile sanitation units containing showers and toilets. There are plans to increase the number of these units, and this in turn is likely to lead to increased wastewater volumes. At present wastewater from the campsite is collected in two underground storage tanks each approx 30 m^3 in capacity, which are emptied regularly during the summer by a tanker, at a cost of £3-4,000 per year. A study conducted by Boisseau (2005) explored the possibility of developing low-cost on-site wastewater treatment as an alternative to the current system of wastewater removal. The site was monitored during the summer of 2005, and was found to have a wastewater flow rate of 5.78 m^{-3} d^{-1} and an average influent BOD_5 of 277 mg l^{-1}. The study concluded that a

WSP system could offer a treatment solution. As the site only operates intermittently, one option would be for an intermittent discharge system, making use of the standard approach in colder climates. The wastewater could be stored until discharge over a relatively short period in the autumn or spring when the quality is highest; this is the classic operation of a cold-climate WSP. Alternatively, effluent could be discharged continuously as soon as it reaches adequate quality in summer, but this has important implications in terms of discharge consents. The Urban Waste Water Treatment Directive (UWWTD) (91/271/EEC) states that urban wastewater discharging to freshwater from agglomerations of less than 2000 p.e. requires 'appropriate treatment' which after discharge allows the receiving waters to meet the relevant quality objectives (CEC, 1991). Lockerley potentially has the option of land application or of discharging into the River Dun, a tributary of the River Test. The River Test has been classified as a Sensitive Area (Eutrophic) under UWWTD standards as phosphorus levels are considered to be elevated (Environment Agency 2006).

The best known and most widely applied effluent standard for river discharge in the UK is the '20/30 Royal Commission standard' (20 mg BOD_5 l^{-1} and 30 mg suspended solids l^{-1}), but the Environment Agency (EA) can impose its own more stringent quality levels. For the River Test, the EA sets objectives for river quality under the River Ecosystem (RE) scheme, to protect the ecology and fish stocks of the river which make the river famous; high quality game fisheries include salmon and brown trout. The highest objective in the scheme is RE1 (water of excellent quality) which is suitable for drinking water abstraction and supporting high class game and coarse fisheries. Altogether, 135km (84 miles) of the 139km (86.5 miles) of the River Test meet this objective. Current water quality results for the River Dun are BOD_5 at < 2 mg l^{-1} and ammonia at $<$ 0.1 mg l^{-1} (Source: Environment Agency).

The choice of discharge option also has an effect on system size because if wastewater is saved all year the pond has to be big enough to store additional rainfall; therefore another alternative might be to discharge over the winter period in accordance with incoming rainfall. While this site has its own special features, the situation of an intermittent or increased seasonal load is not unusual, for example in small rural or seaside places where there is an influx of summer visitors. A low-cost system that could deal with this type of loading may therefore have widespread applications.

7.2 Experimental design

Two modes of operation were trialled. In the first phase, wastewater was allowed to accumulate in a single pilot-scale facultative pond (Pond 1) over the summer filling period, and subsequently stored through the winter into spring, to ascertain the most suitable times for discharge over a relatively short period to land or water. In the second phase, another facultative pond (Pond 2) was added in series with the first and the system was allowed to discharge over the filling period to determine whether adequate quality could be reached for continuous summer discharge. Both options could potentially reduce operating costs and make treated water available for discharge or irrigation.

The trials were carried out over a period of 14 months. Phase 1 began in July 2006 and continued until the start of Phase 2 in July 2007. In Phase 2 the ponds were monitored until the second week in September 2007.

7.2.1 Pilot-scale facultative pond construction

The layout of the tanks and ponds and the connections between them are shown in Figure 7.1. Developments to the site in the second phase are shown with dotted lines. Both ponds were excavated in and lined with high density polyethylene membrane underlain with geotextile polyfelt.

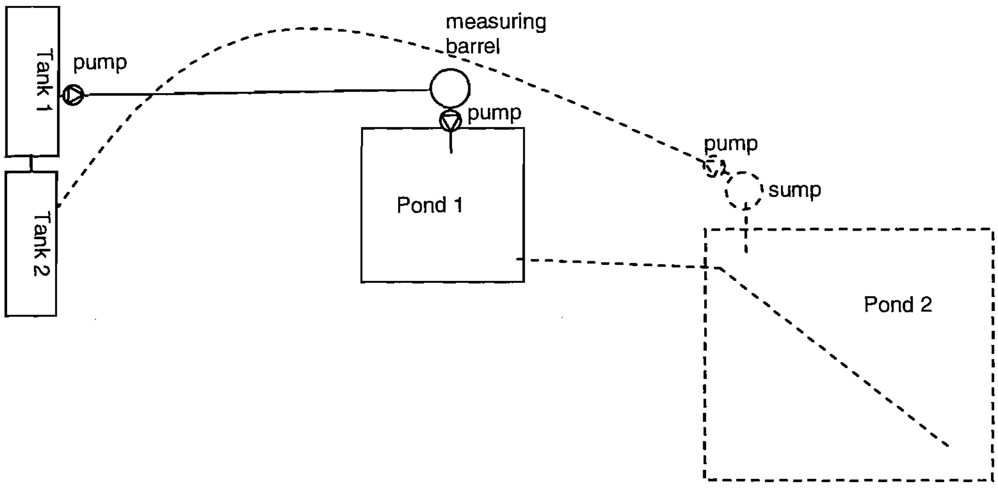


Figure 7.1 Layout of ponds and tanks

Phase 1

The first pilot-scale pond was constructed in May 2006, at approximately 1/10th scale assuming year-round storage of all wastewater from the site. Pond 1 had a maximum surface area of 40.96 m^2, a capacity of 30 m^3 and a maximum water depth of 1.2 m (Figure 7.2 and Figure 7.3). During this phase there was no outlet from the pond.

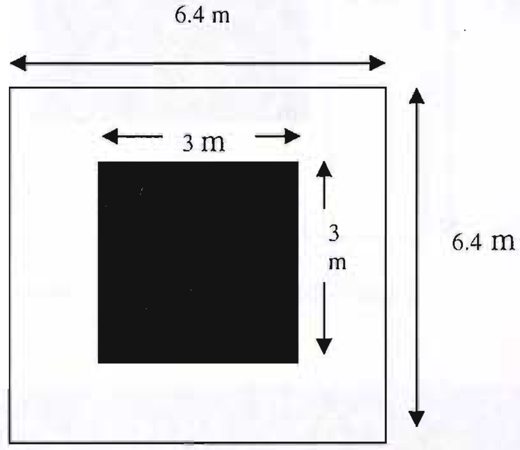


Figure 7.2 Dimensions of Pond 1

Figure 7.3 Pond 1 at start of filling period

Phase 2

The second facultative pond was constructed in April 2007. Pond 2 had a surface area of 72 m^2 and a maximum capacity of 73 m^3 at a depth of 1.7 m. (Figure 7.4 and Figure 7.5).

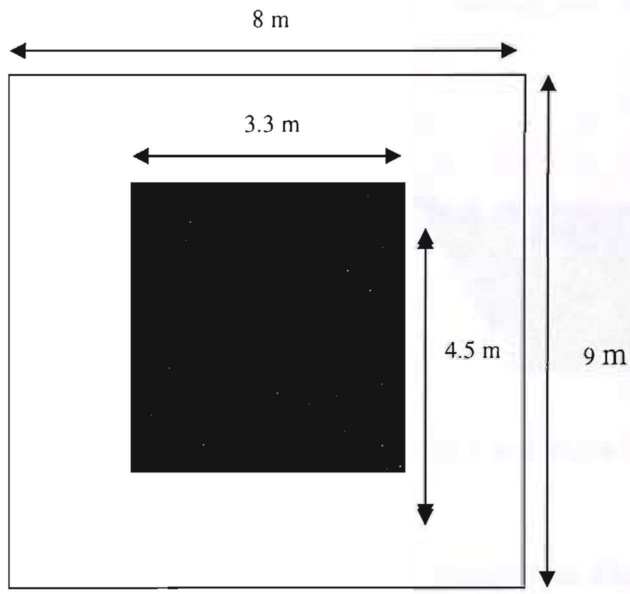


Figure 7.4 Dimensions of Pond 2

Figure 7.5 Pond 2 at approx half depth

70 mm diameter PVC "plastidrain" pipe was used to allow gravity feed of water from Pond 1 to Pond 2. A 'T' piece was fitted to the inlet end of the pipe (in Pond 1) to prevent any material from the surface of pond 1 i.e. leaves, floating mats of algae etc. flowing from Pond 1 to Pond 2 and to allow composite samples to be taken from the outflow. The pipe extended across the bottom of Pond 2 (as far as possible from the outlet in order to reduce short circuiting) with a short section (at the outlet) facing

vertically upwards, as shown in Figure 7.6. Wastewater flowing out of Pond 2 was collected in a sump and pumped back to tank 2.

Figure 7.6 Pipework connecting Pond 1 and Pond 2

Both ponds were instrumented to record air and water temperature, dissolved oxygen levels at intervals of 10 minutes using a datalogger (DataTaker D500). The probes were suspended from the end of a cantilevered pole which could be rotated to allow maintenance and calibration of the probes. The two poles were at different lengths of 1.5 m and 4 m to allow the probes to be placed on the flat bottom of the pond (Figure 7.7).

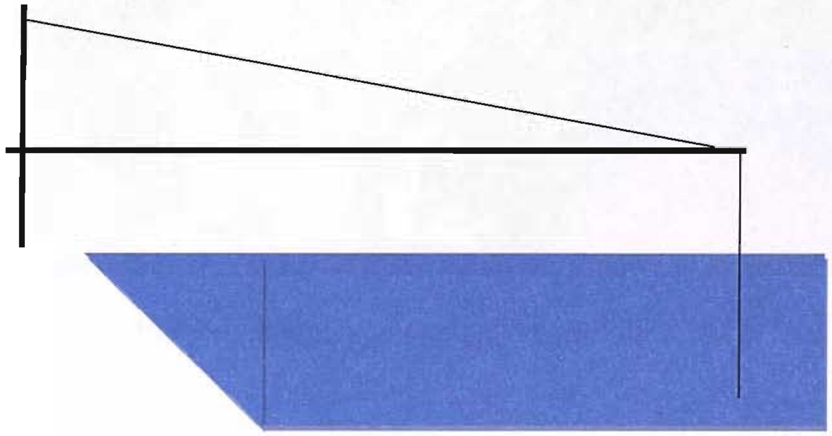


Figure 7.7 Suspension of the probes from a cantilevered pole

7.2.2 Start up and operation

In Phase 1, filling started on 28 July 2006 and ended on 10 September 2006 to coincide with the camping season. On the start day, 33 litres of algal inoculum with an approximate chlorophyll-a concentration of 27 $\mu g\ l^{-1}$ was added to the pond to ensure a rapid start up. Details of the algal inoculum are given in Section 3.2.1. During the camping season, a proportion of wastewater entering the storage tanks was pumped from storage tank 1 through a PVC pipe into a 250-litre barrel (Figure 7.8). When the required level was achieved in the barrel a float switch turned off the pump. The water was held in the barrel for 15 minutes to allow time for sampling, and then gravity fed into the pond 0.5 m below the surface to minimise disturbance of the sediment. All pumping was controlled by the datalogger which also controlled the sampling. Pond 1 received two additions of 160 l each day at a rate of 320 l^{-1} d^{-1} accounting for approximately 5% of the campsite's total wastewater produced. At the end of Phase 1 the pond was left full: exceptionally high rainfall in autumn and winter 2006 meant that approximately 13,770 litres of water over a period of three months from 5 December 2006 – 9 March 2007 had to be pumped out of the pond and back into the storage tanks to prevent overflow.

Figure 7.8 Operating unit and barrel for Pond 1

In Phase 2, filling started on 24 July 2007 and ended on 31 August 2007. As in Phase 1, a proportion of wastewater was pumped via the barrel into Pond 1. In Phase 2, the flow rate was increased to 2880 l^{-1} d^{-1} divided into 18 x 160 litres distributed uniformly over 24 hours thereby increasing the influent to 50% of the campsite's total wastewater. Wastewater flowing out of Pond 2 was collected in a sump and pumped back to tank 2 for disposing off site.

7.2.2.1 Influent and pond samples

During both phases influent samples were taken from the barrel by means of a peristaltic pump and stored in a refrigerated container to build a composite sample over a number of days. Sampling occurred twice a day (2 x 100ml) in Phase 1 and three times a day (3 x

100ml) in Phase 2. The pump was calibrated by measuring the flow using a 1 litre measuring cylinder and a stopwatch. During the sample period in Phase 1, grab samples were taken between 08:00 and 09:00 from each pond just below the surface using a 2-litre sample bottle. In Phase 2, grab samples were taken from both ponds. Composite samples were also taken; the outflow from Pond 1 was sampled (as the inflow to Pond 2) until pond 2 was full at which point samples were taken from the outflow of Pond 2. Composite samples of 300 ml a day (3 x 100 ml) were taken by means of a peristaltic pump and stored in refrigerated containers after sampling. Results given are an average of both grab and composite samples.

7.2.2.2 Air temperature

The air temperature was measured by two devices: a temperature sensor (K-type thermocouple) positioned above the pond surface and a Thermochron iButton. Thermochron iButtons log up to 2048 temperature data points and were programmed to record data every 10 minutes; the iButton was retrieved after approximately 14 days and the temperatures downloaded using iButton TMEX software. The iButton generally measured lower temperatures than the temperature sensor but the mean difference was not more than 1.4°C ($p < 0.001$) (Figure 7.9).

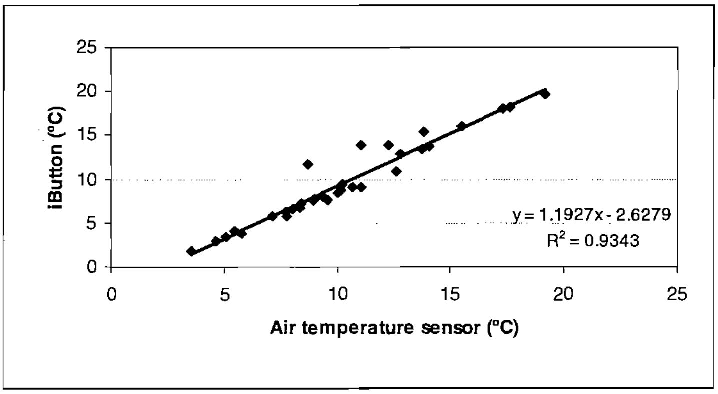


Figure 7.9 Relationship between the air temperature sensor and iButton readings (weekly average readings November-June)

7.2.2.3 Light intensity

Light intensity was measured using a pre-calibrated RC/0308 standard photovoltaic cell (PV Systems, Cardiff, UK). Measurements in mV were recorded every 10 minutes and converted into W m^{-2} by dividing the mV reading by 71.4 and multiplying by 1000 in accordance with the manufacturer's instructions.

7.2.2.4 Water temperature

The temperature in Pond 1 was measured by six temperature sensors (K type thermocouples) at 20 cm spacings. The bottom sensor was placed 10 cm from the bottom of the pond giving the top sensor a depth of 110 cm. In Pond 2, two temperature sensors (LM35CZ electronic thermocouples) were placed in the pond at 30 cm and 110 cm from the bottom.

7.2.2.5 DO probe calibration

Dissolved oxygen concentration in Phase 1 was measured using a single oxygen probe (Dryden Aqua Ltd, UK) suspended near the surface (approximately 10 cm below; adjusted as the pond was filled). In Phase 2, there were two probes in Pond 1 located 30 cm and 110 cm from the bottom and one probe in Pond 2 located 110 cm from the bottom. These were calibrated by leaving them for a period of one hour in air above the pond surface. The output was measured in mV and using a calibration table provided by Dryden Aqua, the concentration in mg l^{-1} for the probe at 100% saturation was established. This figure could then be used to determine the output from the oxygen probe in mg l^{-1} during the experiment. Readings recorded every five seconds were averaged over a 10 minute period.

7.2.2.6 Depth profile

Visual measurement of the depth of the water in each pond was provided by a 'stick' resting on the bottom and marked at regular intervals.

7.2.2.7 Rainfall measurements

Rainfall measurements were recorded every few days adjacent to Pond 1 using a gauge consisting of a 250 ml measuring cylinder and funnel with a diameter of 95 mm. Measurements in millilitres were converted to millimetres of rain by dividing the volume collected by the surface area of the funnel.

7.2.2.8 Removal efficiencies

Monthly removal efficiencies for Phase 1 were calculated using mean monthly values in equation *3.5*, e.g. for March-April C_i = mean monthly concentration in March (mg l^{-1}) and C_{i+t} = mean monthly concentration in April (mg l^{-1}).

In Phase 2, removal efficiencies for Pond 1 during the filling period were calculated using equation *3.6* with C_o = influent concentration (mg l^{-1}) and C_e = effluent concentration (mg l^{-1}). After the filling period, removal efficiencies were calculated using equation *3.5*, based on consecutive measurements. Removal efficiencies in Pond 2 were calculated using equation *3.6* with C_o = influent concentration from Pond 1 (mg l^{-1}) and C_e = effluent concentration from Pond 2 (mg l^{-1}).

7.3 Results

7.3.1 Influent composition

The wastewater at Lockerley is domestic in character and its composition is within the typical range of parameters as given by Metcalf and Eddy (1991) as shown in Table 7.1.

Table 7.1 Comparison of typical wastewater parameters with influent to Pond 1

	Typical wastewater range (Metcalf & Eddy, 1991)	Average value of influent to Pond 1 Phase 1 (n=8)	Average value of influent to Pond 1 Phase 2 (n=9)
BOD_5 (mg/l)	110-400	180	184
COD (mg/l)	250-1000	350	505
BOD:COD	0.4-0.8	0.51	0.36
SS (mg/l)	100-350	162	154
Ammonia (mg/l)	12-50	55	15
Nitrate (mg/l)	0	0.3	0.22
Phosphate (mg/l)	3-10	24	5
pH	-	7.66	7.42

From this classification, it is possible to characterise the pond influent as a weak to medium strength sewage. The only exception appeared to be in nutrient concentrations which in Phase 1 were characteristic of stronger sewage and in Phase 2 of weaker sewage. Nutrient levels were very different between Phase 1 and 2, with ammonia and phosphate concentrations 3-5 times higher in the first year than the second. BOD_5 remained similar during both phases, but COD was higher during Phase 2 which may reflect increased per capita water use in Phase 1. This is supported by the water meter readings that recorded a water consumption of approximately 6 m^{-3} d^{-1} (Figure 7.10) during both phases but total numbers were approximately 570 during Phase 1 and 580 in Phase 2, averaging 95 and 115 people per week respectively. Determining the population equivalent by adopting the reference value for per capita BOD load 54 g BOD $person^{-1}$ d^{-1} suggested that wastewater from the campsite had a polluting potential (in terms of BOD) equivalent to a population of 20 people, or approximately one fifth of the numbers actually on site. One reason for this is settlement in the tanks before entering the pond which may have been more than the usual 30-35%. There is a suggested equation in Metcalf and Eddy (2002) for BOD removal in sedimentation tanks; $R = t/(a + bt)$ where t = retention time, a and b constants of 0.018 and 0.020 respectively which with longer retention times gives BOD removal at 50% i.e. tank volume of 30 m^3 and a flow of 6 m^{-3} d^{-1} giving a retention time of 5 days, suggesting it is probably also acting as a septic tank and treating the waste too. The per capita BOD load may also be lower than average because the load is coming from a campsite and it is also possible that Hampshire Christian Trust arranges day trips so that not all of the BOD is deposited on site. Fenner *et al.* (2007) quoted a value of 27 g BOD $person^{-1}$ d^{-1} for a refugee camp which is half that of the reference value typically used.

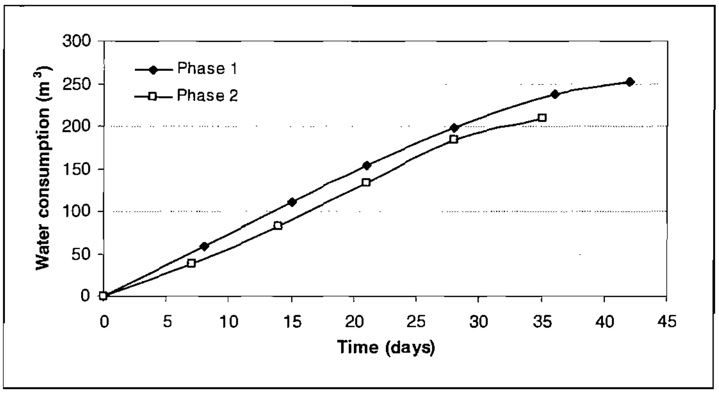


Figure 7.10 Cumulative water consumption during the camping season in Phase 1 and 2

7.3.2 Climatic and local conditions

Air temperature

Air temperature was not measured on site during the filling period in Phase 1, but weekly data obtained from a weather station based at the University of Southampton for this period are shown with recorded data from Lockerley in Figure 7.11. Although the weather station was 14 miles from Lockerley, the temperature sensor and iButton were clearly able to record reliable data that was not too dissimilar from the weather station data. Conditions during Phase 1 were noticeably warmer than in Phase 2.

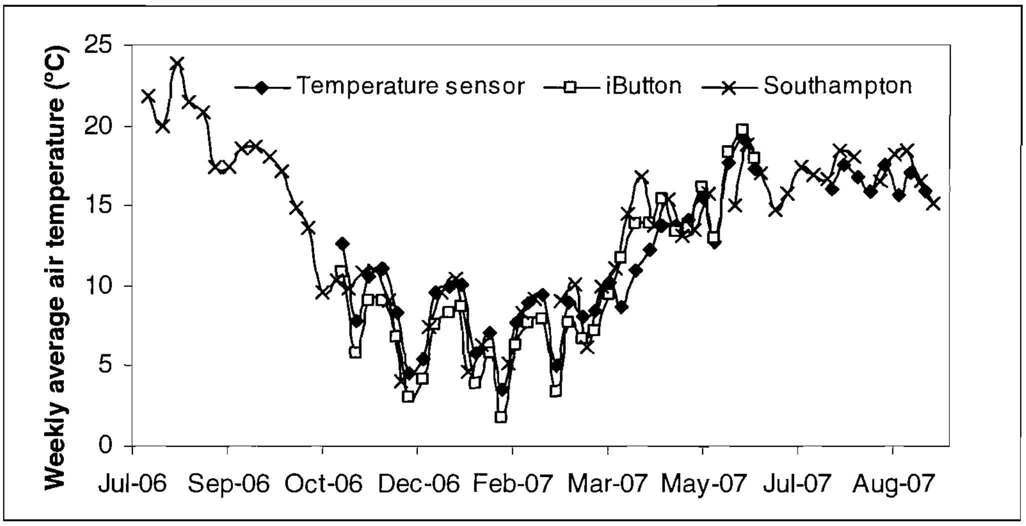


Figure 7.11 Weekly average air temperature at Lockerley

Figure 7.12 shows weekly maximum solar intensity at Lockerley from the middle of February until September 2007. During Phase 2, maximum light intensity was more variable than in Phase 1 presumably due to an exceptionally fine period followed by more unsettled/cloudy weather. There was a strong correlation between weekly average air temperature and weekly maximum light intensity ($R^2 = 0.67$; $p < 0.001$).

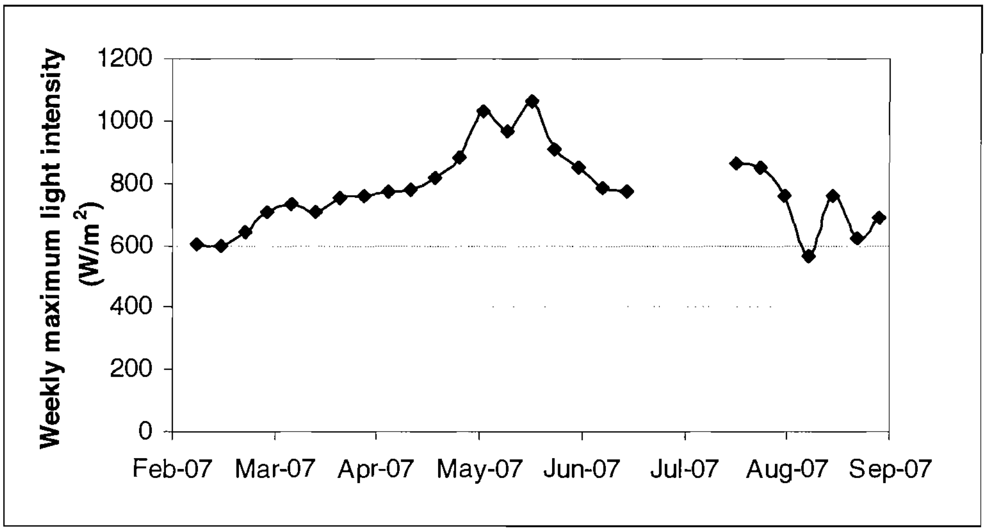


Figure 7.12 Weekly maximum light intensity

Water temperature

Figure 7.13 shows the weekly average water temperature at different depths in Pond 1 from August 2006 until June 2007. The pond surface temperature correlated strongly with air temperature from when monitoring started at the beginning of November (R^2 = 0.81, $p < 0.001$).

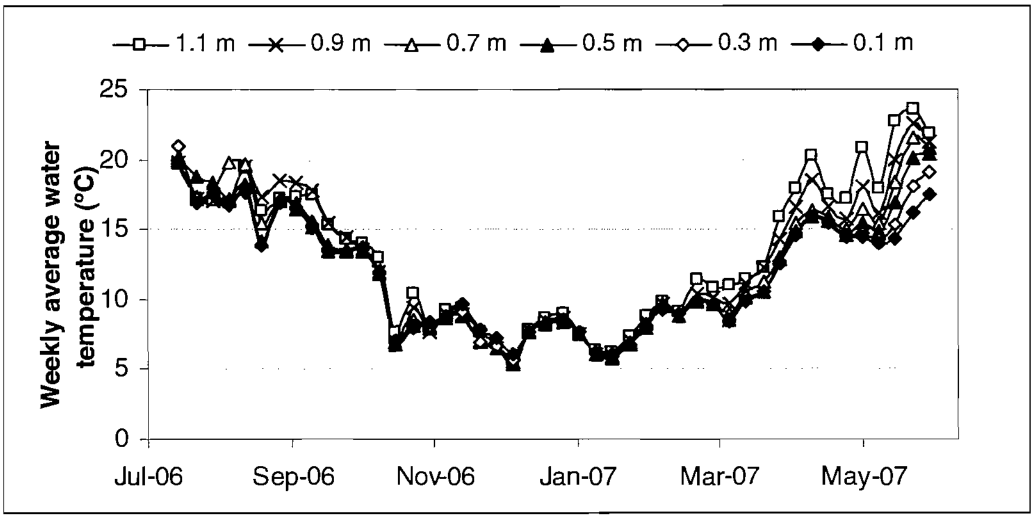


Figure 7.13 Weekly average pond temperature at all depths

The variation between air and water temperature was generated by fluctuations in air temperature that were not immediately reflected in the water temperature. Figure 7.14 shows an example over a period of one week in winter where air temperature fluctuated between -2°C and 11°C; although the pond temperature also varied changes were marginal and the response to increasing air temperature was much slower.

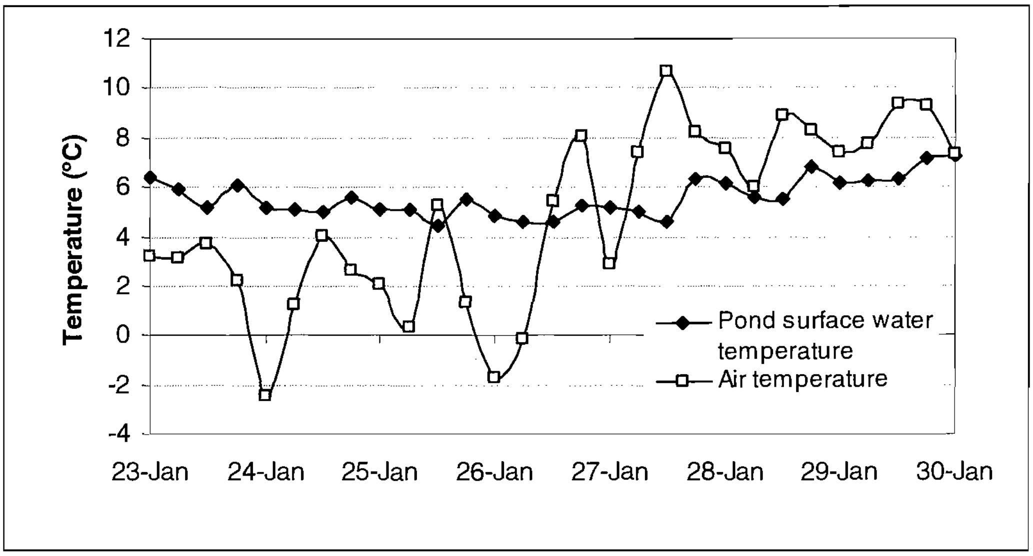


Figure 7.14 Air temperature and surface water temperature readings during one week in January in 2007 (average taken over six-hour period)

Weekly average water temperature for Ponds 1 and 2 during Phase 2 is shown in Figure 7.15. Water temperature was lower in Phase 2 than Phase 1, reflecting the higher air temperatures during the summer of 2006. The rapid increase and then decline in temperature in Pond 2 from 25/07/07 to 15/08/07 was due to the change in pond depth during the filling period. The surface temperature sensor was not covered until 15/08/07 as shown by the dashed line in Figure 7.15 and therefore the values during this time reflect air temperature.

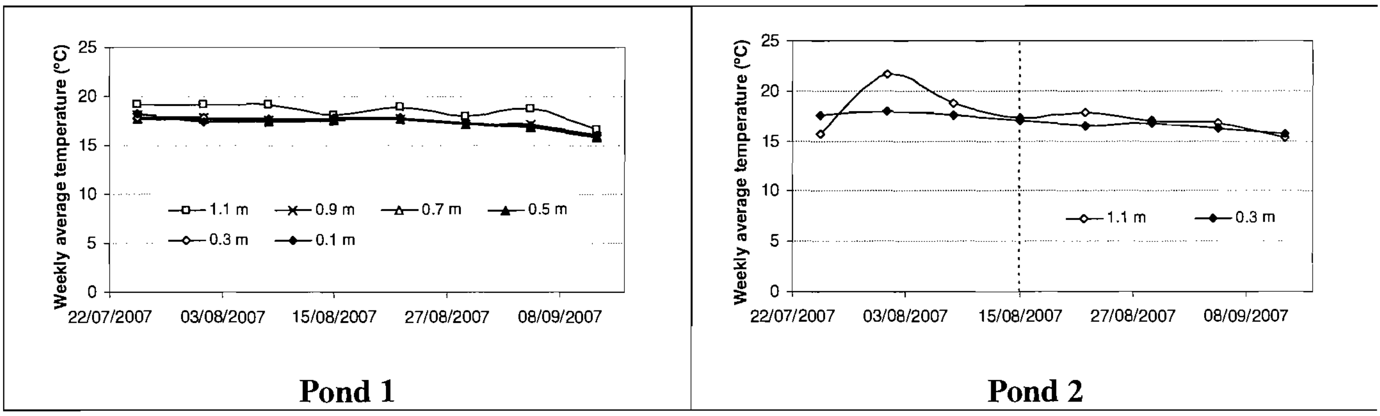


Figure 7.15 Weekly average water temperature for both ponds during Phase 2 (---) represents pond at maximum volume

Stratification was a significant feature in Pond 1 from March to June 2007 (Figure 7.13). The small surface area and absence of inflow to the system at this time would have reduced the in-pond mixing potential and therefore have contributed to the prolonged stratification. Stratification was also observed in Pond 1 during most of the filling period in Phase 2 except from 14/08/07 to 22/08/07 where for the most part stratification was not evident. Weather observations at this time recorded stormy conditions and high rainfall levels which would have prevented stratification occurring. It has been argued that stratification is undesirable in WSP because it causes a reduction in the effective volume of treatment and encourages hydraulic short-circuiting (Torres *et al.*, 1997; Kellner and Pires, 2002). Abis and Mara (2006) however found that the long periods of stratification observed in their ponds had no adverse effect on performance and suggested that stratification may even enhance the treatment process by reducing short-circuiting from bottom to top. Abis and Mara (2006) proposed that the stability of stratification helps to confine the sludge to the bottom of the pond, preventing chemical and physical disruption to the algal ecology at the surface and reducing the quantity of solids reaching the pond outlet. As the Lockerley pond was operating in intermittent discharge mode, reduced vertical short-circuiting has no benefit in terms of solids containment during periods without discharge, but may help to prevent odour release.

Precipitation and evaporation

Rainfall levels recorded at Lockerley were only approximate as very heavy rainfall meant the measuring cylinder used to collect rain occasionally overflowed. Figure 7.16 shows mean monthly rainfall data at Lockerley with averages for Southern England. Although winter 2006/07 was the second warmest in UK history it also had well-above average rainfall (Met Office, 2008a) which had implications for both pond volume and dilution. Evapotranspiration rates were calculated for the local area using long-term average values for Southampton and with these, it was possible to calculate net rainfall taking into account losses from evaporation. Figure 7.17 shows that October 2006 to February 2007 were very wet months with incoming net rainfall to the pond system totalling more than 3 m^3 each month. April 2007 was very dry with virtually no rainfall and a maximum air temperature above 20°C most days which resulted in negative net rainfall and a drop in the pond level from high levels of evaporation.

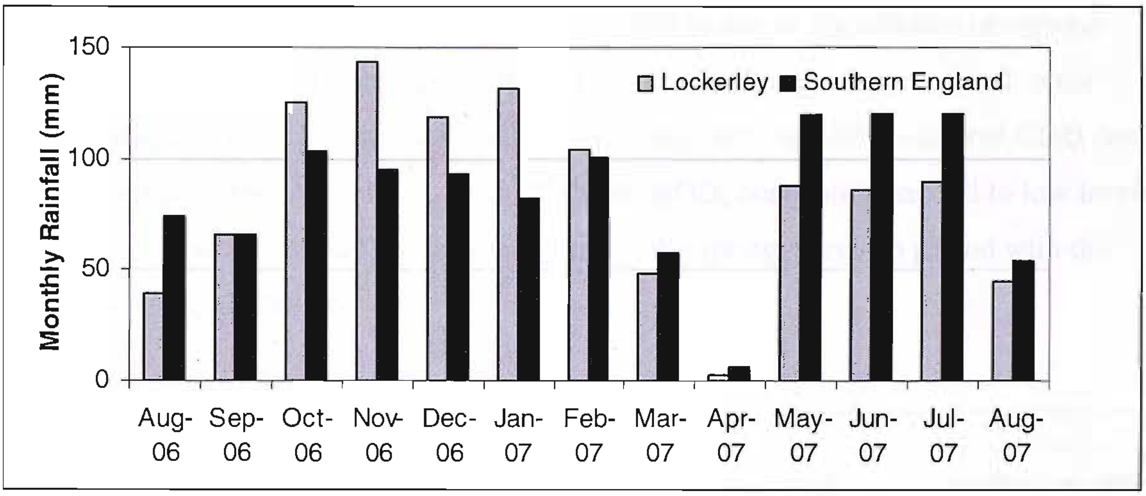


Figure 7.16 Monthly average rainfall recorded at Lockerley compared with Southern England (www.metoffice.gov.uk/climate/uk)

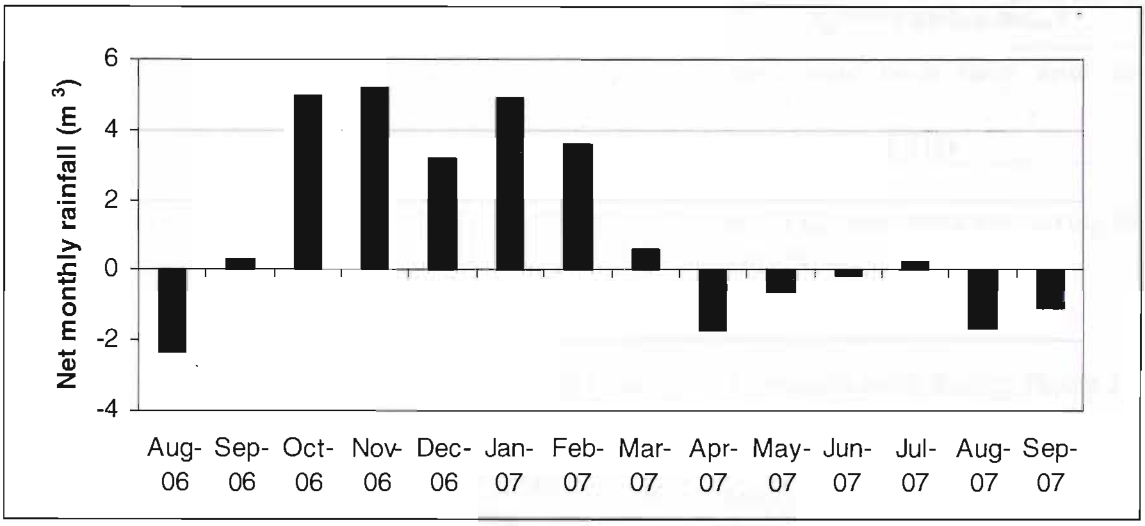


Figure 7.17 Incoming net monthly rainfall to the pond system recorded at Lockerley

During the filling period in Phase 1 a water balance was calculated for Pond 1 using inflow and net precipitation values. The results showed good agreement with volumes calculated from pond depth measurements ($R^2 = 0.991$).

7.3.3 Phase 1

7.3.3.1 COD and BOD_5 removal

Filtered and unfiltered BOD_5 and COD concentrations for Phase 1 are shown in Figure 7.18 and monthly average and range values for unfiltered BOD_5 and COD in Table 7.2. Unfiltered COD doubled from 300 mg l^{-1} at the start of August to 600 mg l^{-1} at the beginning of September coinciding with the filling period. Filtered values did not show a sudden drop once the pond stopped receiving, probably due to the addition of organic matter from algal growth as indicated by high levels of chlorophyll-a observed in the ponds during this time. Unfiltered COD remained high for longer than filtered COD due to the presence of the algal bloom. Both COD and BOD_5 concentrations fell to low levels throughout the winter period but then increased in the spring warm-up period with the onset of a new algal bloom.

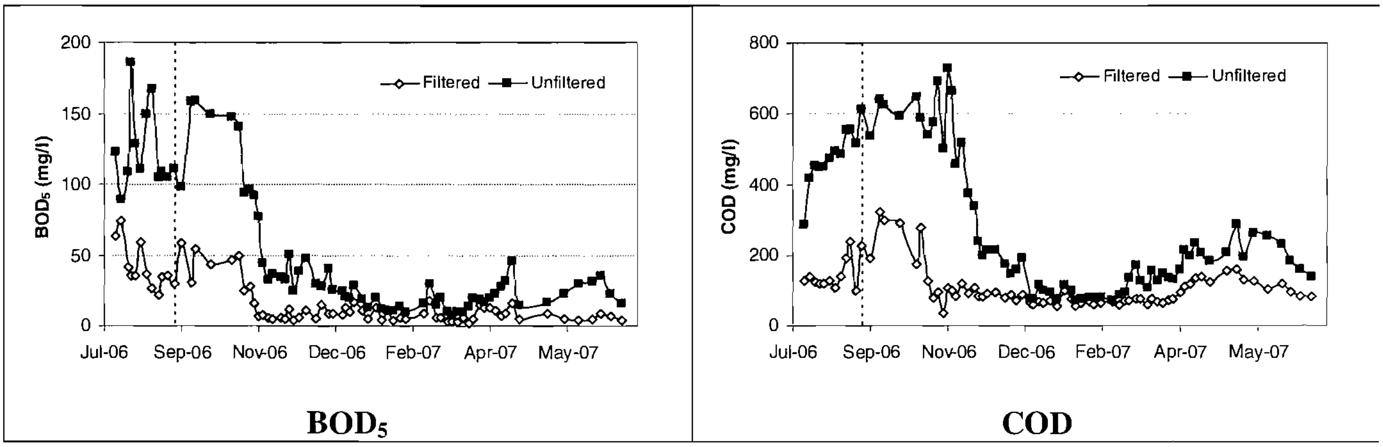


Figure 7.18 Monthly average filtered and unfiltered BOD_5 and COD concentration during Phase 1 (---) represents end of filling period

Table 7.2 Mean monthly unfiltered BOD_5 and COD concentration during Phase 1

Month	Average BOD_5 concentration (mg l^{-1})	Range (mg l^{-1})	n	Average COD concentration (mg l^{-1})	Range (mg l^{-1})	n
Aug-06	130	89-187	9	452	288-552	9
Sep-06	106	98-111	4	581	516-640	6
Oct-06	120	94-147	4	607	543-694	6
Nov-06	47	25-92	10	423	198-727	10
Dec-06	32	21-47	6	149	74-215	7
Jan-07	17	11-29	8	94	73-117	8
Feb-07	17	10-30	5	82	72-96	6
Mar-07	14	7-20	9	118	72-170	9
Apr-07	27	15-45	6	200	161-233	6
May-07	23	17-30	3	241	194-288	5
Jun-07	26	15-35	6	178	141-229	4

Apparent removal efficiencies were calculated using concentration values, which include the effects of net rainfall dilution and wastewater removal; these are therefore higher than the actual efficiencies due to biologically-mediated removal. Apparent monthly removal efficiencies for filtered BOD_5 and COD increased rapidly from 6% and 24% in

September-October to 79% and 49% in October-November respectively. The pond was monitored from late July 2006 until late June 2007. The results shown in Figure 7.17 indicated that from the viewpoint of COD concentrations, the best time for discharge of treated wastewater in this period would have been in February 2007. Apparent overall removal efficiencies were therefore calculated using average August values at the start of the experiment and average values in February. Apparent removal efficiencies for filtered and unfiltered BOD_5 were on average 80 and 87% and for COD 57 and 82% respectively.

In order to calculated the loss of COD from the pond system as a result of diluting out by rainwater, the COD and BOD removal rates were calculated by a mass balance approach, taking into account net rainfall and the removal of excess rainwater in winter. The total filtered COD added to the pond during the filling period was 6.38 kg. A volume of 13.77 m^3 of excess rainfall was removed from the pond was between 5 December 2006 - 9 March 2007: at the end of this the total filtered COD in the pond was 2.22 kg. The quantity of filtered COD removed with the excess rainfall was calculated in 2 ways: first by taking the average unfiltered COD concentration between in this period and multiplying this by the volume of water removed which gave a total loss of 0.98 kg and secondly by a more accurate mass balance approach using the values of 23 COD samples and rainfall measurements taken approximately twice a week in the period; this latter method gave a value of 1.03 kg of filtered COD removed. The actual removal efficiency in this period was therefore 49% compared with an apparent efficiency of 65% due to the dilute-out effect of rainfall.

Overall performance of the pond was good; typical effluent values from a facultative pond range from 50-80 mg BOD_5 l^{-1}, 120-200 mg COD l^{-1} with removal efficiencies of 75-85% and 65-80% respectively (von Sperling and Chernicharo, 2005). On the basis of BOD_5 and COD levels, filtered BOD_5 was low enough to meet EU effluent quality of $\leq$ 25 mg l^{-1} and filtered COD less than 125 mg l^{-1} from the beginning of November. If the '20/30 Royal Commission standard' was used by the EA for the River Dun then discharge to the river would only be possible in February-March but BOD_5 and COD levels would meet discharge to land requirements throughout the monitoring period.

7.3.3.2 Chlorophyll-a

Figure 7.19 shows chlorophyll-a concentrations during Phase 1. Levels rose as high as 10 mg l^{-1} during October, indicating an algal bloom that was also reflected in the difference between filtered and unfiltered COD and BOD_5. Mara *et al.* (1992) reported chlorophyll-a concentrations of 1-1.5 mg l^{-1} in effluent from UK facultative ponds during the summer. Chlorophyll-a concentration in the Lockerley pond reached seven times this value. This may have been due to a number of factors, including optimum water temperature, high light intensities and an influent with high concentrations of ammonia and phosphate. The sampling method may also have contributed to high values, as the grab samples taken could have included surface algal foam. Chlorophyll-a concentrations such as those found in this study have however been reported in similar ecosystems; Kyando and Nkotagu (2003) reported chlorophyll-a values of between 13 and 24 mg l^{-1} in an enclosed shallow lake in Tanzania; Mara (2006) commented on results from facultative ponds in Brazil where effluent chlorophyll-a concentrations of over 10 mg l^{-1} were observed. pH values steadily rose from around 7.8 to as high as 9.8 to coincide with increased chlorophyll-a content. The pH of the influent was usually around 7.6, so it is likely that algal photosynthesis was the cause of this increase.

Chlorophyll-a levels remained below 0.3 mg l^{-1} from mid-January until the end of February. As noted in Chapter 6, Pearson (1996) suggested that the performance of facultative ponds becomes less reliable if the algal biomass drops below 0.3 mg l^{-1} because of the potential for negative net oxygen production. While this guideline may not be applicable to intermittently-loaded ponds, Pond 1 experienced failure according to this criterion from the start of January for a period of less than two months. With such conditions discharge to land may be an option at this time as concentrations of all measured parameters were very low and likely to meet discharge requirements. Figure 7.18 (b) highlights the rapid rise in chlorophyll-a concentration at the start of March which reflected the spring algal bloom and the recovery from winter conditions. Chlorophyll-a fluctuated between 0.4 mg l^{-1} and 0.9 mg l^{-1} during the spring reaching a maximum of 1.3 mg l^{-1} on 20/03/07. Concentrations began to decline at the start of April and stabilised at approximately 0.4 mg l^{-1}. There then appeared to be a second smaller bloom at the start of May with the algal population rising and then gradually falling to 0.15 mg l^{-1} by the end of June. This gradual decline in chlorophyll-a concentration correlated well with the fall in orthophosphate ($R^2 = 0.96$; $p < 0.001$) and also, but less strongly, with ammonia levels ($R^2 = 0.66$; $p = 0.05$) suggesting the pond became nutrient limited following the spring bloom.

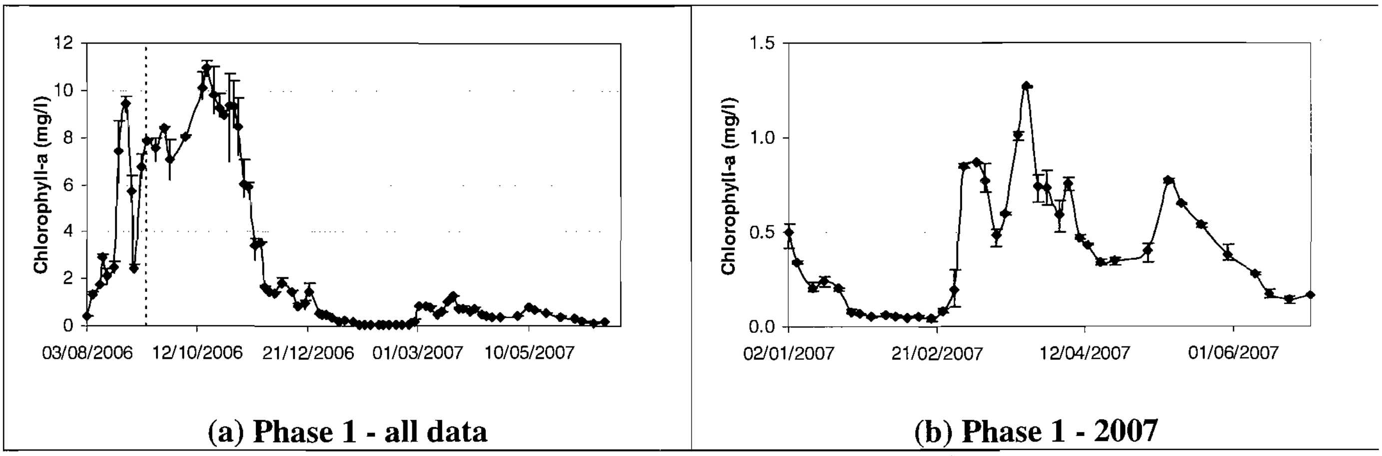


Figure 7.19 Chlorophyll-a concentration during Phase 1 (2007 graph shows spring algal bloom) (NB. different y-axis to highlight changes in chlorophyll-a concentration) (---) represents end of filling period

After the filling period during Phase 1, unfiltered BOD_5 and COD correlated strongly with chlorophyll-a (R^2 = 0.76, 0.91; $p < 0.001$). When all data points were used the relationship was not as close with variation dropping to 56% and 81% for BOD_5 and COD respectively due to the presence of influent organic matter. The increase in organic matter towards the end of the filling period was due to an increase in algae. As algae died and settled to the bottom in the autumn, organic matter concentrations decreased proportionately. The highest monthly removal of BOD_5 and COD occurred in November as temperatures dropped and algal settlement took place which would suggest a potential opportunity to discharge.

7.3.3.3 Suspended solids and pH

Suspended solids concentration and the relationship between suspended solids and chlorophyll-a during Phase 1 are shown in Figure 7.20. Regression analysis revealed a highly significant relationship between the two variables when data points after the filling period were used ($R^2 = 0.94$; $p < 0.001$). The relationship is given in equation *7.1*:

$$\text{SS (effluent)} = 25.08 + 38.23 \text{ Chl-a} \qquad (7.1)$$

The result suggests that effluent suspended solids concentrations in excess of 25 mg l^{-1} were most likely due to the contribution of algal solids. When all data points were used the correlation weakened ($R^2 = 0.80$; $p < 0.001$) giving the relationship in equation *7.2*:

SS (effluent) = 44.30 + 37.96 Chl-a *(7.2)*

The result suggests when all data is used effluent suspended solids concentrations in excess of 44 mg l^{-1} were most likely due to the input of algal solids which accounts for the solids from the influent. Abis (2002) reported a value of 33 mg l^{-1} for ponds in Yorkshire, UK treating 50% trade and 50% domestic waste, which was mid range of values obtained in this study.

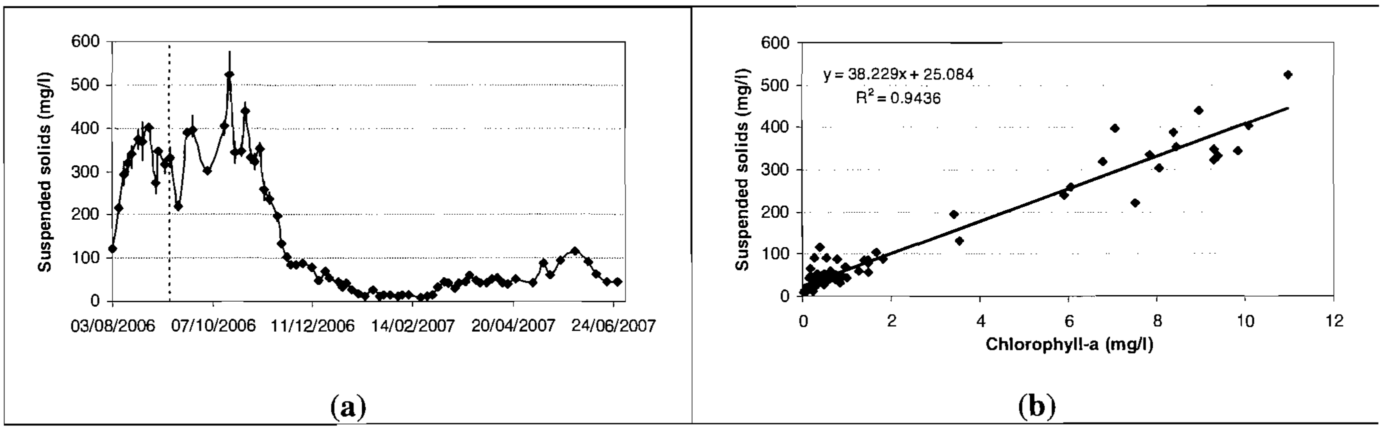


Figure 7.20 (a) Suspended solids and (b) relationship between chlorophyll-a and suspended solids concentration during Phase 1 (Filling period not included)
(---) represents end of filling period

According to Mara (1996), the suspended solids from facultative ponds are about 60 to 90% algae and each 1 mg of algae generates a BOD_5 around 0.45 mg. von Sperling and Chernicharo (2005) stated that 1 mg l^{-1} of suspended solids in the effluent would therefore be capable of generating a BOD_5 in the range 0.3 to 0.4 mg l^{-1} and a COD of 1.0 to 1.5 mg l^{-1}. When suspended solids concentration was plotted against both BOD_5 and COD concentration from Phase 1 the relationship between the parameters was a good fit with that proposed by von Sperling and Chernicharo (2005) (Figure 7.21). Relationships were significant with p-values < 0.001 and the variation accounted for was 75% and 91% for BOD_5 and COD respectively. The results imply that 1 mg l^{-1} of suspended solids in the effluent generated a BOD_5 of 0.30 mg l^{-1} and a COD of 1.32 mg l^{-1}. Although the system was not typical of continuous-fed WSP, the results demonstrate relationships similar to those in fed systems. In terms of discharges, suspended solids in the effluent are predominantly algae that or may not exert oxygen demand in the receiving water body; it is therefore important to have an estimate of the effluent suspended solids concentration. If the '20/30 Royal Commission standard' was in place then in terms of suspended solids concentration, discharge to river would only be possible from mid-January to the end of February without further treatment; but if the wastewater is discharged to land this can offer a minor benefit as soil conditioners and sources of nutrients in irrigated agriculture. The less stringent EU UWWTD standard (CEC, 1991) for suspended solids in the wastewater is ≤ 150 mg l^{-1} which implies that effluent could potentially be discharged to river from the end of November onwards.

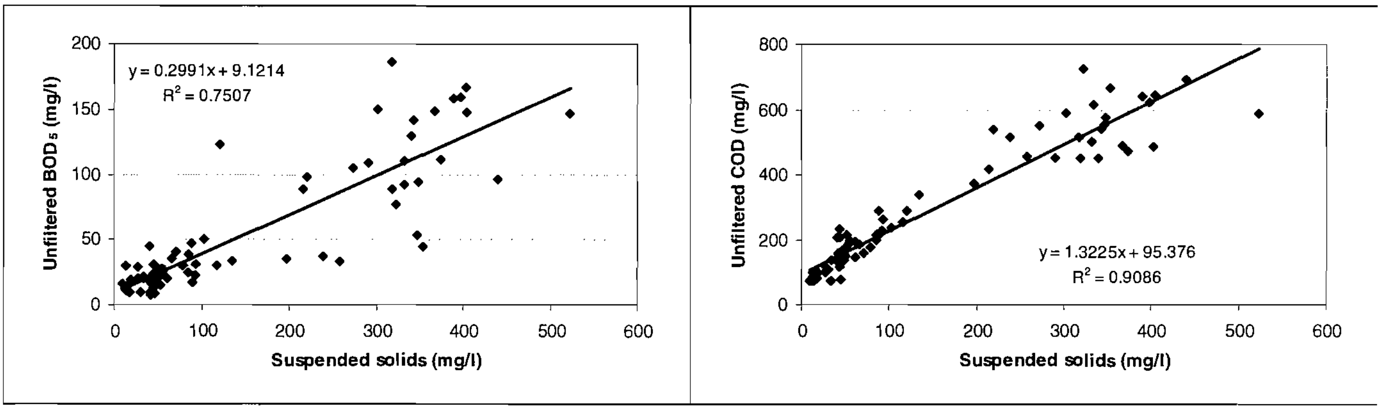
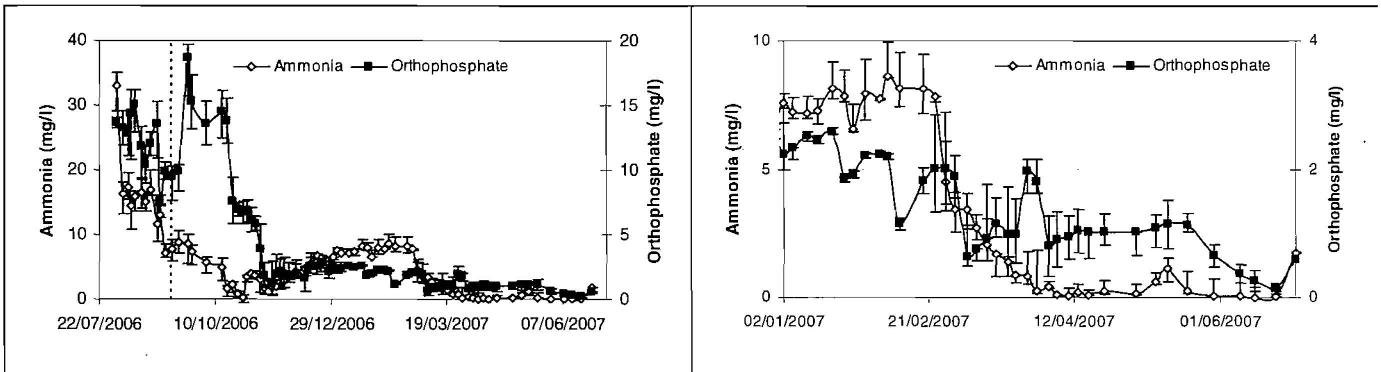


Figure 7.21 Relationship between suspended solids and BOD_5/COD concentration during Phase 1 (NB. different unit of measurement)

7.3.3.4 Nutrients

Ammonia and orthophosphate concentrations in Pond 1 during Phase 1 are shown in Figure 7.22. Ammonia levels fell from 33 mg l^{-1} to approximately 16 mg l^{-1} in four days at the beginning of August then showed a continued downward trend before reaching less than 1 mg l^{-1} at the end of October. Over the winter period ammonia levels began to rise. Welch (1992) stated of dimictic lakes that a large nutrient supply exists because there is little or no growth to remove nutrients. Chlorophyll-a levels remained low through winter thus supporting this suggestion. Ammonia then fell from 7.8 mg l^{-1} to less than 0.2 mg l^{-1} by the beginning of April coinciding with the rise in chlorophyll-a levels. Nitrate concentrations remained very low fluctuating between less than 0.1 and 0.7 mg l^{-1} indicating that there was no ammonia removal through nitrification. Algal uptake has been suggested as the major pathway for ammonia removal by Lai and Lam (1997) and Ferrara and Avci (1982). Ammonia is the preferred source of nitrogen for algae and has been shown to be taken up in preference to nitrate which has to be reduced to ammonia before it can be assimilated (Konig *et al.*, 1987; Mainer *et al.*, 2000).

Abis (2002) found strong correlations between ammonia removal and pH and surface temperature. In this study using all data after the filling period, ammonia concentration correlated strongly with pH ($p < 0.001$) with 55% of the variation accounted for, the same value as reported by Abis (2002); and with surface water temperature ($R^2 = 0.52$; $p < 0.001$). Both parameters showed inverse relationships with ammonia concentration. High pH is important in the stripping of ammonia but by the time pH levels had risen above 9.0, ammonia concentration had decreased by 78%. Higher pH is also associated with the presence of algae which have optimum growth at warmer temperatures. Therefore although chlorophyll-a did not directly correlate with ammonia concentration, the results suggest that algal uptake would have been the primary mechanism of removal.



(a) Phase 1 - all data | **(b) Phase 1 - 2007**

Figure 7.22 Ammonia and orthophosphate concentration during Phase 1
(NB. different y-axis to highlight changes in nutrients in spring) (---) represents end of filling period

Orthophosphate declined less quickly than ammonia perhaps because there are fewer mechanisms of removal: for example, the main form of routes for phosphate removal in WSP are via algae and bacteria and through precipitation of phosphate under high pH conditions. pH values rose above 9.0 from the beginning of September until the end of October which is an essential requirement for significant precipitation to take place, though there was no significant relationship between orthophosphate concentration and pH or surface water temperature ($p > 0.05$). Figure 7.23 shows a strong relationship existed between chlorophyll-a concentration and the level of orthophosphate in the pond during Phase 1 ($R^2 = 0.75$; $p < 0.001$), suggesting phosphate was a controlling factor for algal growth and that algal uptake was the primary mechanism for phosphate removal. High phosphate levels around the time of the algal bloom also suggest that the high chlorophyll-a concentration measured during the bloom may have been correct and representative. As orthophosphate levels declined mid-October, chlorophyll-a levels subsequently fell suggesting phosphate was the limiting nutrient for algae to maintain growth. The corresponding dataset from mid-October to end of November showed a significant relationship between the two parameters ($R^2 = 0.73$; $p < 0.001$). Using the same time frame for ammonia, the correlation between ammonia and chlorophyll-a in contrast was very weak ($R^2 = 0.01$; $p = 0.73$). In spring, orthophosphate levels began to increase perhaps as a result of a spring turnover although there is no corresponding rise in ammonia to confirm this suggestion (Figure 7.22 (b)). Phosphate levels immediately fell in association with increased chlorophyll-a levels and then stabilised. Nutrient levels were very low by the end of February suggesting a potential time to discharge. Ammonia and orthophosphate levels were less than 2 mg l^{-1} for a period of 20 days in November which in terms of nutrient levels would also have been an optional time to discharge.

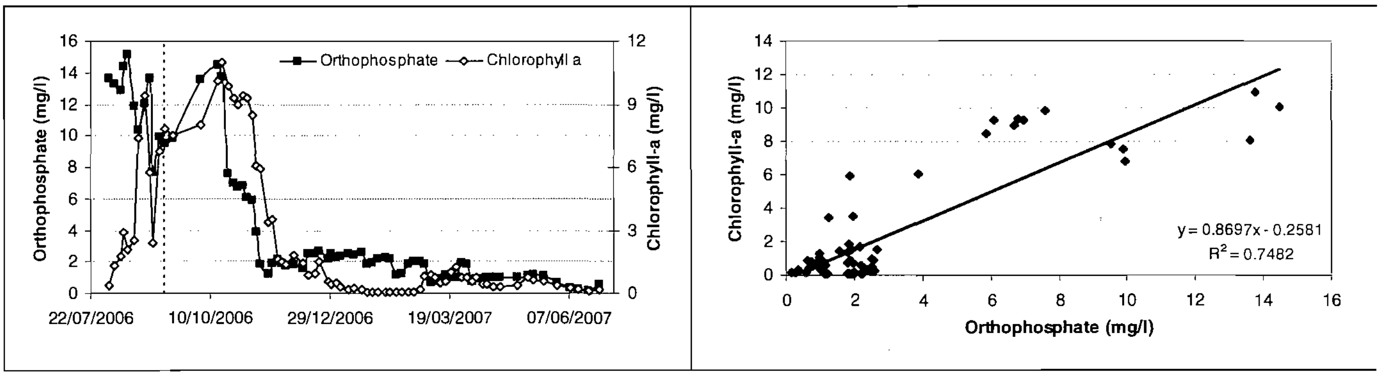


Figure 7.23 Relationship between chlorophyll-a concentration and orthophosphate during Phase 1

7.3.3.5 Dissolved oxygen

Figure 7.24 shows the weekly average and maximum dissolved oxygen concentrations during Phase 1. From the end of August to the beginning of October, corresponding to the filling period, there were very low levels of dissolved oxygen in the system. Although chlorophyll-a concentrations were high during this time, the oxygen demand on the pond was also high. Oxygen consumption usually equalled or exceeded production on a 24-hour basis. Abis (2002) found that higher loadings of 107 kg BOD ha^{-1} d^{-1} led to more stable chlorophyll-a concentrations during the summer, but these were not associated with high dissolved oxygen concentrations. The reason suggested was that the chlorophyll-a concentration did not reveal stratification effects: under these conditions, the algae trapped on the surface could contribute > 500 μg l^{-1} to the chlorophyll-a, but hardly anything to the dissolved oxygen concentration. Abis (2002) also stated that at the lower loading of 63 kg BOD ha^{-1} d^{-1}, the relationship between dissolved oxygen and chlorophyll-a was closer, suggesting that at this reduced loading, the oxygen supply and demand of the system was more equally balanced. In the current study when the loading on the pond stopped from the middle of September onwards maximum dissolved oxygen values increased. It was not until the beginning of November that mean oxygen levels rose from 1 mg l^{-1} to nearly 9 mg l^{-1}.

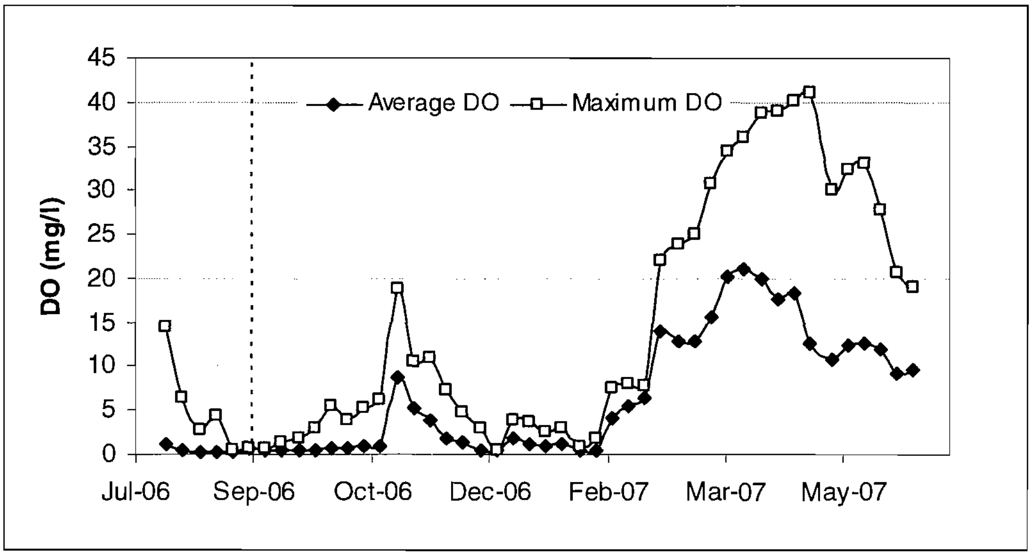


Figure 7.24 Weekly average and maximum dissolved oxygen levels during Phase 1
(---) represents end of filling period

As Figure 7.25 shows, the relationship between chlorophyll-a and average dissolved oxygen became much closer from the beginning of November. Dissolved oxygen levels dropped as chlorophyll-a levels declined, fluctuating between 0.5 and 2.75 mg l^{-1} until the end of January. At this time chlorophyll-a levels were on average below 0.3 mg l^{-1} and pH values below 7. From 09/02/07 mean dissolved oxygen began to increase rapidly reaching saturated conditions within three weeks. At the beginning of April, average oxygen concentrations were above 20 mg l^{-1}. Higher oxygen concentrations were usually associated with high pH values. Although only spot pH measurements were taken values were often over pH 10 when average dissolved oxygen levels were above 20 mg l^{-1}. By the beginning of May, maximum oxygen levels were higher than 40 mg l^{-1} by mid-afternoon (Figure 7.25). As chlorophyll-a concentration began to fall during mid-May, oxygen levels dropped accordingly, although the pond reached saturation on a daily basis until the end of Phase 1.

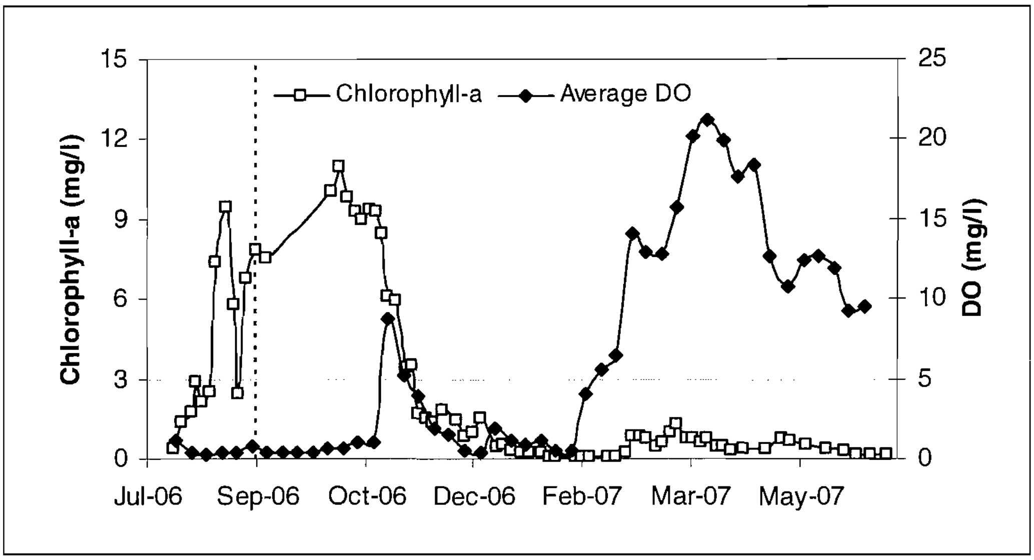


Figure 7.25 Average chlorophyll-a concentration and dissolved oxygen levels during Phase 1

The occurrence of a spring turnover and its associated changes in nutrient concentration was not crucial to the onset of a bloom as there was little evidence of a turnover apart from a small increase in orthophosphate levels. The rise in water temperature during the period of most rapid growth was less than 1°C indicating that temperature change was not a major factor in the rise in chlorophyll-a levels. Changes in light levels at the start of spring therefore appeared to be responsible for the rise in algal population as indicated by chlorophyll-a concentration. Welch (1992) stated that spring blooms usually occur when the light intensity reaches a level so that gross production exceeds respiration. Figure 7.26 (a) shows light intensity and dissolved oxygen levels over a few days at the end of February. From the end of February to the end of April supersaturated levels were maintained in the pond overnight, presumably due to low levels of microbiological activity associated with low temperatures and low BOD. As nutrients became limiting by the end of April and chlorophyll-a levels started to decline, solar radiation became the limiting factor for photosynthetic oxygen production as shown in Figure 7.26 (b). Average oxygen levels were dictated by light availability and although peak light intensities were much higher in May than February there was not enough surplus oxygen to maintain saturated conditions in the dark in contrast to the overnight saturated conditions shown in Figure 7.26 (a). In terms of discharge times, oxygen levels and chlorophyll-a content in the pond during February would be more beneficial to receiving waters as respiration by algae during the night was minimal and would not cause any undue demand on the system. In May however, chlorophyll-a levels were higher and oxygen levels dropped rapidly to less than 2 mg l^{-1} during the night; with the additional increase in BOD, discharge at this time may lead to deoxygenation of the receiving water. This would only be an issue if discharging into a water body but as land discharge is more likely at this site, oxygen levels are of less importance.

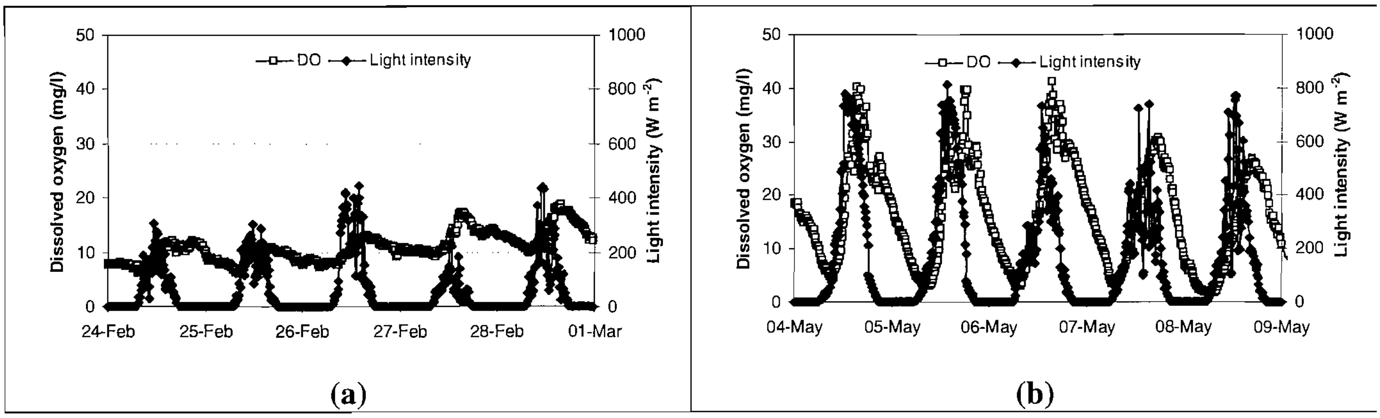
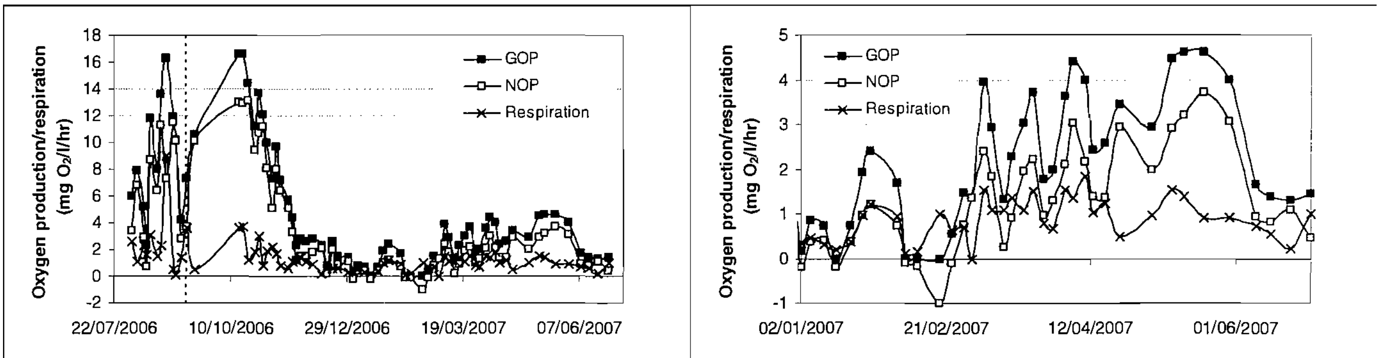


Figure 7.26 Change in relationship between average dissolved oxygen levels and light intensity in (a) February and (b) May during Phase 1

7.3.3.6 Oxygen production

The results of gross and net oxygen production measurements are shown in Figure 7.27. Both gross and net production were highest at the end of the filling period and lowest in winter. Gross production ranged from 16.6 mg O_2 l^{-1} h^{-1} in mid-October to zero in January. Net oxygen production was 13 mg O_2 l^{-1} h^{-1} in mid-October and showed small negative values on five occasions in January and February. Negative values in January coincided with low dissolved oxygen and chlorophyll-a concentrations and colder temperatures, and were therefore probably due to minor measurement errors. As noted in the literature review, dissolved oxygen concentrations exceeding about 120% have been known to inhibit photosynthesis (Sanchez Miron *et al.*, 2000; Acien Fernandez *et al.*, 2001); when there is an accumulation of oxygen in a closed system inhibition of photosynthesis will lead to an underestimation of the photosynthetic rate. In the latter half of February, oxygen levels varied from 100-120% saturation and therefore net oxygen production may have been adversely affected, leading to the apparent negative values. Average deviations from the mean of duplicate values were small, ± 0.39 mg O_2 l^{-1} h^{-1} for gross production and ± 0.45 mg O_2 l^{-1} h^{-1} for net production.

Figure 7.27 shows that as expected the average respiration rate was higher in periods when the organic load was at its highest and levels of dissolved oxygen were low, due to increased bacterial activity associated with biodegradation of the waste. The respiration rate was very low from December to February when oxygen production was also low and the oxygen demand on the system was minimal. Both oxygen production and respiration increased in the spring with the rise in chlorophyll-a levels.



(a) Phase 1 - all data **(b) Phase 1 - 2007**

Figure 7.27 Average gross and net oxygen production and respiration rate during Phase 1 (NB. different y-axis to highlight changes in oxygen production) (---) end of filling period

Table 7.3 shows values for gross and net oxygen production and respiration rate divided into three seasonal periods: August to October including the filling period and into autumn; November to February representing the colder winter months; March to June representing the spring warm-up period. The results clearly show that the highest levels of oxygen production occurred during the summer/autumn months with an average of 10.52 mg O_2 l^{-1} h^{-1} for gross production and 8.22 mg O_2 l^{-1} h^{-1} for net production. The respiration rate was also highest during these months with an average of 2.30 mg O_2 l^{-1} h^{-1}. Values for production and consumption were quite similar from November to June although the period from November to February included some values from November which still saw relatively high production levels and therefore increased the average.

Table 7.3 Average gross and net oxygen production and respiration rate during different months of the monitoring period

Months	GOP (mg O_2 l^{-1} h^{-1})	Range	NOP (mg O_2 l^{-1} h^{-1})	Range	Respiration (mg O_2 l^{-1} h^{-1})	Range
Aug-Oct	10.52	2.34-16.63	8.22	0.70-13.12	2.30	0.12-8.97
Nov-Feb	2.65	0.00-9.98	1.84	-1.00-8.08	0.81	0.11-2.25
Mar-Jun	2.88	1.31-4.62	1.85	0.24-3.71	1.04	0.00-1.85
Average	4.83	-	3.55	-	1.28	-

Using all data points a close relationship existed between gross and net oxygen production and chlorophyll-a concentration ($R^2 = 0.76$ and 0.71 respectively, $p < 0.001$). Figure 7.28 shows the relationship between gross oxygen production and chlorophyll-a using data points separated into three different sections of the monitoring period as in Table 7.3. The strongest relationship was apparent for data points from November to March ($R^2 = 0.96$). During August-October the relationship was still significant ($p < 0.001$) but accounted for only 49% of the variation in gross oxygen production: the high organic loading during the filling period is likely to have affected the oxygen demand irrespective of chlorophyll-a levels. There was a very poor correlation between chlorophyll-a and oxygen production from March-June, possibly due to the frequent conditions of oxygen saturation. Low chlorophyll-a concentrations in some samples also demonstrated notable oxygen production rates providing an interesting contrast with the suggestion by Pearson (1996) that a threshold of 0.3 mg l^{-1} of chlorophyll-a is generally required to maintain positive net oxygen production in continuously-fed facultative ponds. In this study positive net oxygen production was apparent when chlorophyll-a levels were less than 0.1 mg l^{-1}, due to an absence of loading on the pond from autumn onwards and therefore a lower oxygen demand on the system. A poor correlation existed between dissolved oxygen concentration and oxygen production ($p > 0.05$). In WSP, measurable oxygen largely represents the photosynthetic rate minus the consumption by decomposition and respiration and loss by diffusion after supersaturation is reached (Neel *et al.*, 1961). Measurable oxygen therefore represents a momentary surplus and concentration is not always indicative of maximum photosynthetic rate.

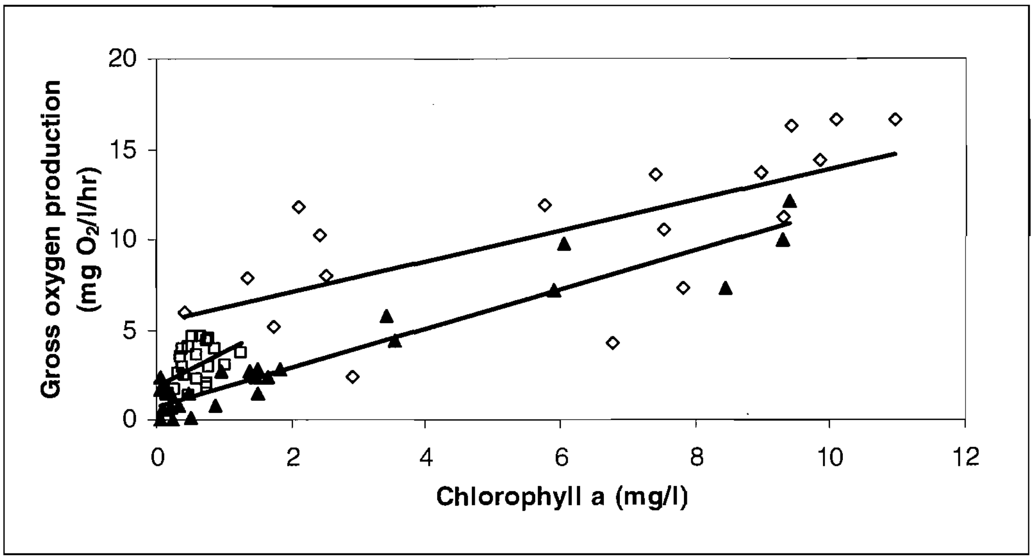


Figure 7.28 Relationship between chlorophyll-a and gross oxygen production during Phase 1 (◊) August-October ($R^2 = 0.49$); (▲) November-February ($R^2 = 0.91$); (□) March- June ($R^2 = 0.22$)

Gross and net oxygen production values were found to correlate strongly with both unfiltered BOD_5 and COD concentration ($p < 0.001$). The closest fit was using gross production values following the filling period with 81% and 82% of the variation accounted for in BOD_5 and COD respectively. When all data points were used R^2 values dropped to 76% and 75% for BOD_5 and COD respectively. In contrast, using net production measurements this reduced to 69% and 73% for BOD_5 and COD respectively. The results suggest that measurements of oxygen exchange can provide an indication of pond performance and more quickly than current methods of assessment.

7.3.3.7 Faecal coliforms

Samples were tested for faecal coliform (FC) numbers from February to April during Phase 1. No FC were found in any samples, suggesting that the long detention time and absence of influent over the winter period were adequate preventative measures in FC survival making this an appropriate time for possible discharge.

7.3.3.8 Phase 1 - Conclusions

Results from Phase 1 have demonstrated excellent performance at this scale. In terms of EC discharge requirements to water, the most appropriate period appears to be anytime between November through to spring for all parameters except nutrients, which would limit discharge to November and February. Alternatively some form of tertiary treatment could be added to reduce nutrient concentrations. If the more stringent '20/30 Royal

Commission' standard was imposed, then February might be a possible time to discharge when BOD_5 levels and suspended solids were still low prior to the spring bloom. According to World Health Organisation guidelines (WHO, 2006), BOD_5 and nutrient levels were low enough for discharge to land throughout the monitoring period.

7.3.4 Phase 2

7.3.4.1 COD and BOD_5 removal

In Phase 2, the flow rate into and therefore the loading on Pond 1 was nine times higher than during Phase 1, but BOD_5 and COD concentrations recorded in Pond 1 throughout August were lower. This was due to dilution of the incoming wastewater in Phase 2 by the treated wastewater remaining in the pond from Phase 1. Average filtered and unfiltered BOD_5 and COD concentrations for Phase 2 are shown in Figure 7.29. Sampling of Pond 2 did not start until 16/08/07 when the pond was completely full but satisfactory removal was achieved as values in Pond 2 were immediately lower than in Pond 1. Reflective of the feeding period, concentrations in Pond 1 rose from 190 mg unfiltered COD l^{-1} at the end of July to 500 mg COD l^{-1} by the beginning of September while filtered COD rose from 100 to over 230 mg l^{-1}. Unfiltered BOD_5 levels rose from an average of 50 to 150 mg l^{-1} and filtered BOD_5 rose from 30 to 90 mg l^{-1}. After 06/09/07, concentrations of filtered and unfiltered BOD_5 and COD began to stabilise as there was no further influent to Pond 1. Monitored filtered BOD_5 and COD concentrations in Pond 2 may have satisfied effluent quality standards of ≤ 25 mg l^{-1} and ≤ 125 mg l^{-1} except for samples taken during the last week. Small improvements to the system could produce effluent of suitable quality for discharge throughout the summer.

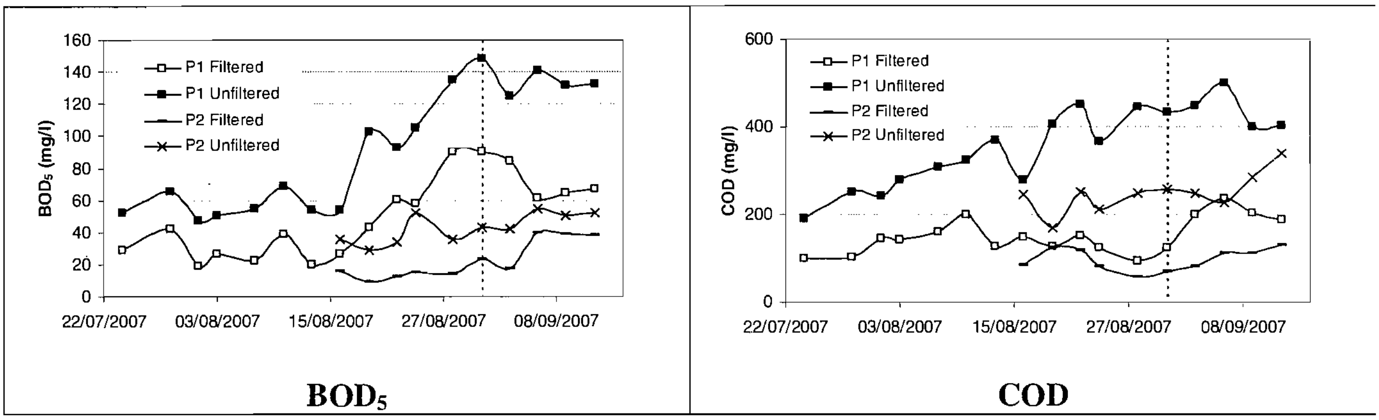


Figure 7.29 Average filtered and unfiltered BOD_5 and COD concentration in both ponds during Phase 2 (using both composite and grab samples) (---) represents end of filling period

Figure 7.30 shows removal efficiencies for BOD_5 and COD calculated for Pond 1. Negative removal efficiencies following the filling period were probably due to increasing levels of chlorophyll-a.

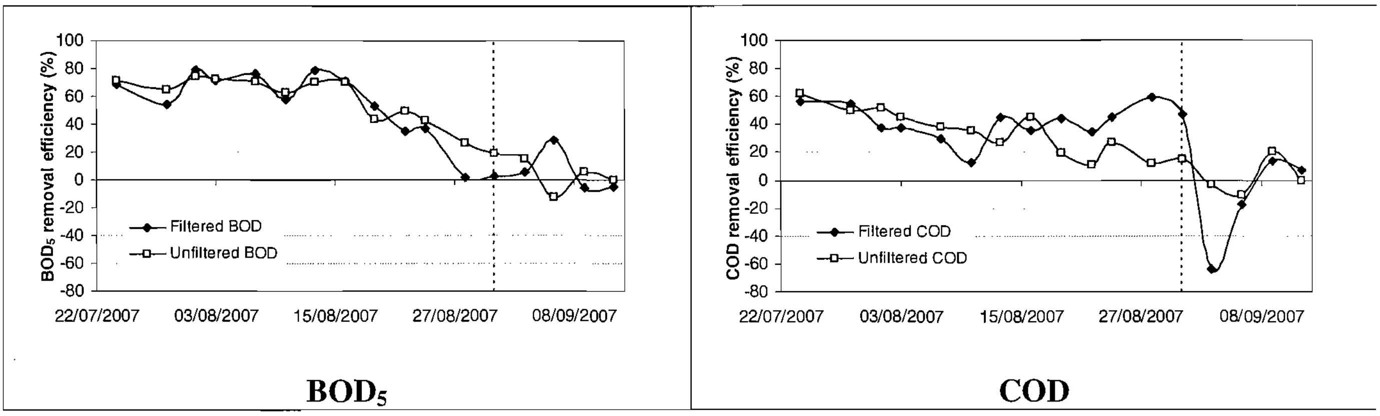


Figure 7.30 Average filtered and unfiltered BOD_5 and COD removal efficiencies in Pond 1 during Phase 2

Figure 7.31 shows removal efficiencies for BOD_5 and COD in Pond 2 using average values from Pond 1 and 2 to assess performance in a two-pond system. Removal efficiencies were calculated using equation *3.6* where C_o = influent concentration from Pond 1 (mg l^{-1}) and C_e = effluent concentration from Pond 2 (mg l^{-1}). BOD_5 removal efficiencies until 03/09/07 when Pond 1 stopped receiving influent were excellent in the whole system exhibiting a range of 74 to 84% and in accordance with those obtained from previous studies (Soniassy and Lemon, 1986; Mara *et al.*, 1998). Removal efficiency from Pond 1 to Pond 2 then fell to 35% before showing a slight increase. Similar to Pond 1, COD removal was lower with an initial drop to just 2% and then a continual rise reaching a removal efficiency of 59% on the 03/09/07. There was then a steady decline to 30% on the final day of sampling reflecting the increasing COD concentration due to the rise in chlorophyll-a levels. In Phase 1, BOD_5 and COD levels did not fall immediately following the end of the filling period but a subsequent drop to very low levels was observed in November; it is assumed that pollutant levels in Phase 2 will behave similarly and fall to levels again suitable for discharge.

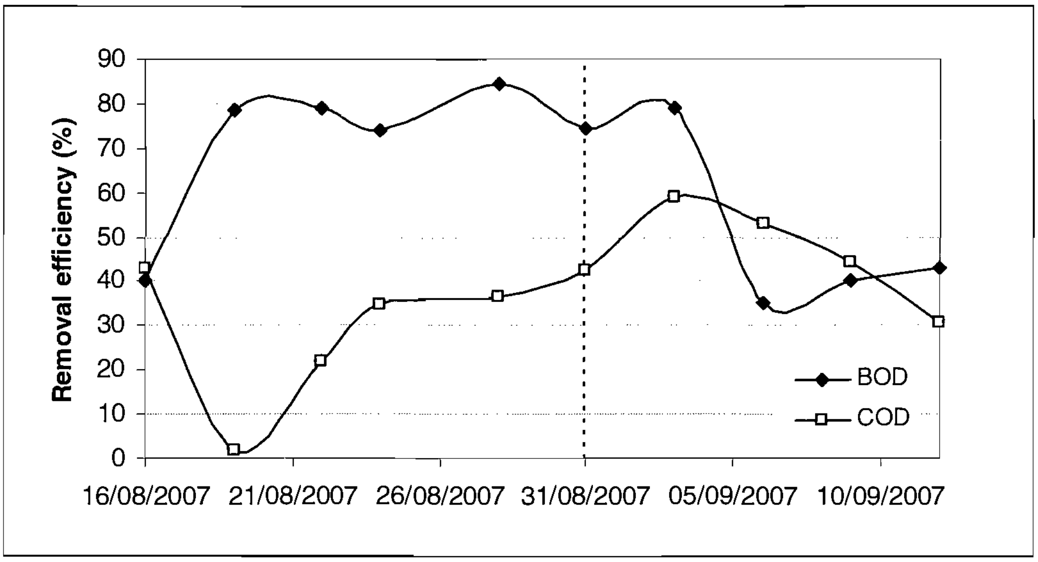


Figure 7.31 Average filtered BOD_5 and COD removal efficiencies in Pond 2 during Phase 2

7.3.4.2 Chlorophyll-a

Figure 7.32 shows chlorophyll-a concentrations in Pond 1 and 2 during Phase 2. Levels in Pond 1 rose to 0.9 mg l^{-1} by the beginning of August. In this period, there was a decrease in chlorophyll-a levels (sampled 01/08/07) that coincided with a duckweed infestation. This would have shaded out the light and caused some algae to die. The duckweed was removed and chlorophyll-a levels subsequently recovered. Algal growth rose steadily in both ponds represented by maximum chlorophyll-a concentration recorded on the final day of sampling in Pond 1 as 2 mg l^{-1}. During the end of the period when the ponds were still operating as a typical facultative pond system i.e. continuous feed, chlorophyll-a concentration was typical of values found in effluent from UK facultative ponds during the summer according to Mara *et al.* (1992) who reported concentrations of 1.0-1.5 mg l^{-1}. The increase in chlorophyll-a levels appears similar to that obtained from an exponential growth model. The growth rate to achieve the closest fit was 0.12 d^{-1} in Pond 1 and 0.16 d^{-1} in Pond 2 giving R^2 values of 0.80 and 0.88 respectively. At this growth rate, the algae should need a HRT of a little over 8 and 6 days in the ponds otherwise cell washout may occur. As a conservative measure, the retention time in Pond 1 which was approximately 10 days may therefore need to be increased. Typical growth rates for algae in UK ponds are 0.05-0.7 d^{-1} (Arceivala, 1999) which suggests that these values were fairly low in comparison with results from other studies, possibly as a result of lower than average summer temperatures (Met Office, 2008a).

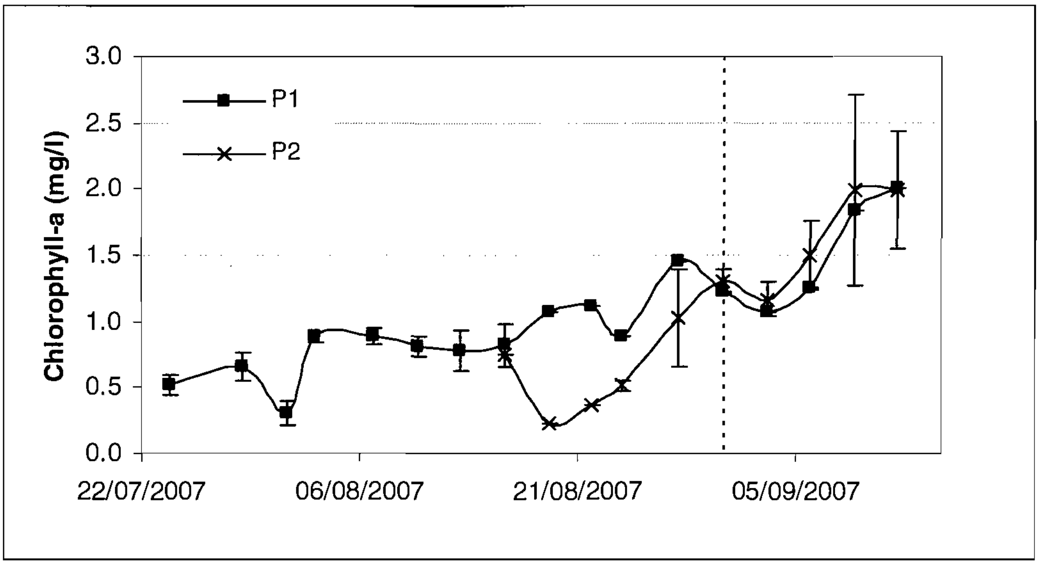


Figure 7.32 Chlorophyll-a concentration in both ponds during Phase 2

During Phase 2, unfiltered BOD_5 and COD correlated well with chlorophyll-a in Pond 2 ($R^2 = 0.50, 0.61$; $p \leq 0.02$) suggesting that the presence of an algal bloom following the period of incoming wastewater may have been responsible for reducing the removal efficiency. BOD_5 levels in Pond 1 correlated most strongly with chlorophyll-a ($R^2 = 0.63$; $p < 0.001$) which saw a steady increase in organic matter corresponding to both incoming wastewater and the growth of algae. The relationship with chlorophyll-a was not as strong in Pond 2 perhaps because COD and especially BOD_5 was much lower in Pond 2.

7.3.4.3 Suspended solids and pH

Suspended solids concentrations for both ponds during Phase 2 are shown in Figure 7.33. Levels rose more gradually than in Phase 1 with an average of 85 mg l^{-1} in Pond 1 at the end of July, reaching a maximum of 200 mg l^{-1} at the beginning of September; perhaps due to not stirring up the bottom sediments as much as filling into a full rather than an empty pond. Levels in Pond 2 were always 50-100 mg l^{-1} lower than in Pond 1 until the beginning of September when the difference between the two was on average 30 mg l^{-1}. The higher concentrations of suspended solids in Pond 1 reflect the incoming load, possible sludge feedback from Phase 1 and algal biomass. The wastewater fed into Pond 2 contained fewer solids due to settling out in Pond 1 and therefore levels in Pond 2 represent predominantly algal content. This can also be seen in Figure 7.32 where chlorophyll-a levels were higher in Pond 2 than Pond 1 from the end of August but this was not reflected in the suspended solids concentration. Therefore at the end of the filling period Pond 1 had stopped receiving wastewater and the suspended solids concentration subsequently stabilised with levels at 190 mg l^{-1} and 160 mg l^{-1} in Pond 2. Suspended solids in Pond 2 would have been able to meet discharge requirements of $\leq$ 150 mg l^{-1} for most of the monitoring period except where levels rose above this in mid-September due to rising chlorophyll-a concentration. At no stage would levels have met the '20/30 Royal Commission' standard for discharge to river.

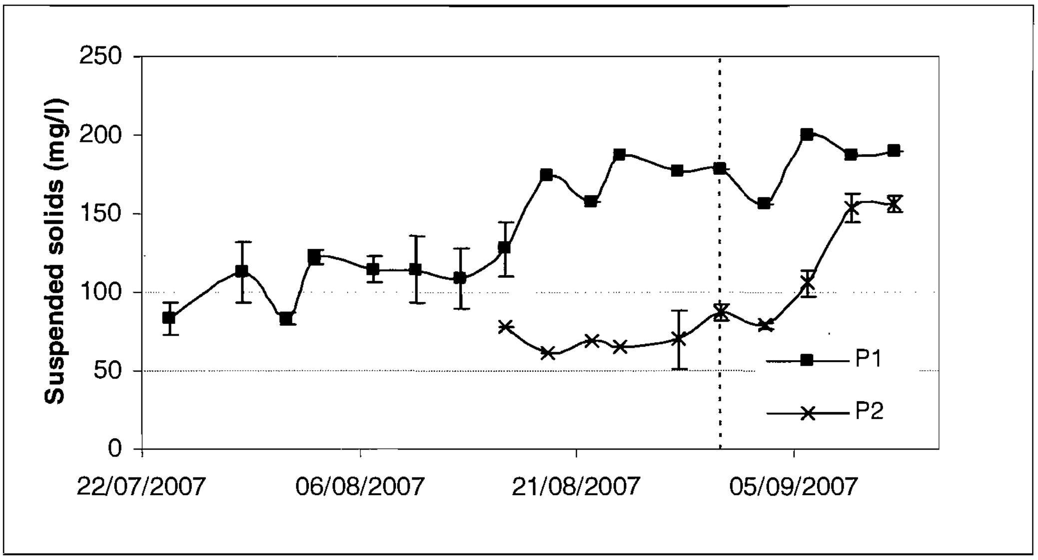


Figure 7.33 Suspended solids concentration in both ponds during Phase 2

Figure 7.34 shows the relationship between suspended solids concentration and chlorophyll-a in both ponds during Phase 2. A strong relationship existed between the two parameters for both ponds ($p < 0.001$) although the variation accounted for was 78% in Pond 1 compared with 90% in Pond 2. The relationships are given in equations *7.3* and *7.4*, which suggest that effluent suspended solids concentrations in excess of 68 mg l^{-1} and 47 mg l^{-1} in Pond 1 and Pond 2 respectively are most likely due to the contribution of algal solids.

SS (P1 effluent) = 67.74 + 70.83 Chl-a *(7.3)*

SS (P2 effluent) = 47.03 + 38.05 Chl-a *(7.4)*

The value for both quantity of algae contributing to suspended solids concentration and the relationship between chlorophyll-a and suspended solids for Pond 2 showed a similar result to the relationship obtained in Phase 1 suggesting this is a 'real' value. The higher residual suspended solids value of 71 mg l^{-1} in Pond 1 was similar to the value reported by Abis (2002) who found 1 mg l^{-1} of chlorophyll-a to be equal to 68 mg l^{-1} of suspended solids. The similarity between the result in Pond 1 in Phase 2 and the result reported by Abis (2002) is perhaps due to Phase 2 results predominantly consisting of values when the pond was being fed. The lower values for residual solids observed in Phase 1 are likely to be a consequence of the number of results included when the pond was not fed and equally for Pond 2 in Phase 2, as the pond itself was not directly fed. It is therefore reasonable to suggest that more of the suspended solids were accountable to chlorophyll-a concentration when the ponds were not continuously fed.

pH values during Phase 2 in Pond 1 gradually increased from 6.5 at the end of July to pH 8 by the start of September coinciding with the rise in chlorophyll-a levels. The two parameters were only weakly related ($R^2 = 0.28$; $p = 0.028$) possibly due to the use of spot measurement of pH value. pH in Pond 2 ranged from 7.4 to 8.5 and was always higher than in Pond 1 reflecting the lower loading on the system and higher chlorophyll-a levels.

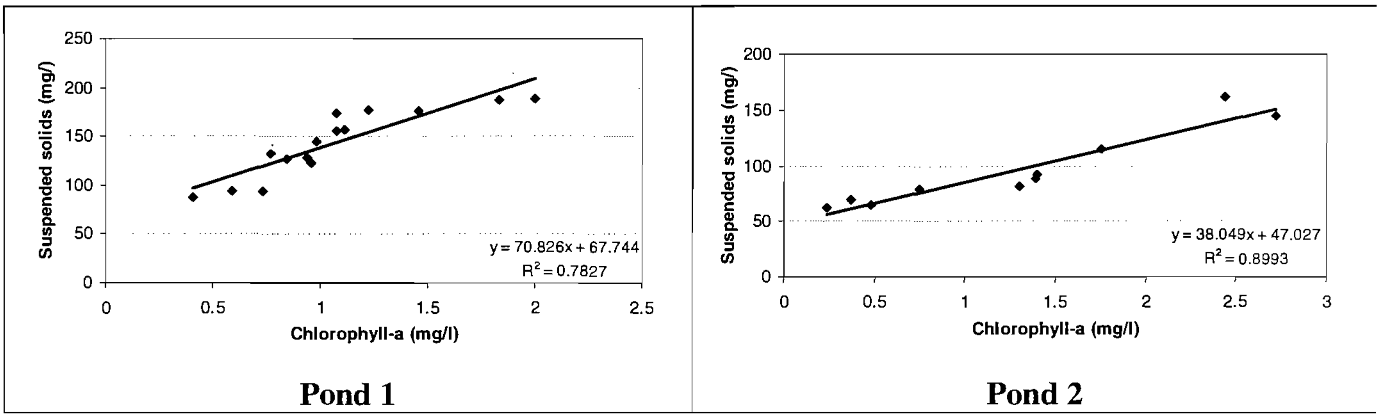


Figure 7.34 Relationship between chlorophyll-a and suspended solids concentration in both ponds during Phase 2 (using both composite and grab samples)

7.3.4.4 Nutrients

The change in ammonia and orthophosphate concentration during Phase 2 is shown in Figure 7.35. Ammonia concentration was related to chlorophyll-a in Pond 2 but not in Pond 1 ($R^2 = 0.75$; $p = 0.001$). In contrast, orthophosphate was related to chlorophyll-a in Pond 1 and not in Pond 2, although the relationship was weaker ($R^2 = 0.38$; $p = 0.009$). Nutrient levels did not correlate well with pH except orthophosphate concentration in Pond 2 ($R^2 = 0.50$; $p = 0.022$). Orthophosphate removal was good in Pond 2 with a reduction of up to 30% from the values recorded in Pond 1 at the end of August but levels remained too high to allow for discharge. Chlorophyll-a levels had increased rapidly at this time which suggests removal through the mechanism of algal uptake; supported by the correlation with pH. The system would benefit from a longer retention time to reduce both ammonia and orthophosphate concentration to adequate levels.

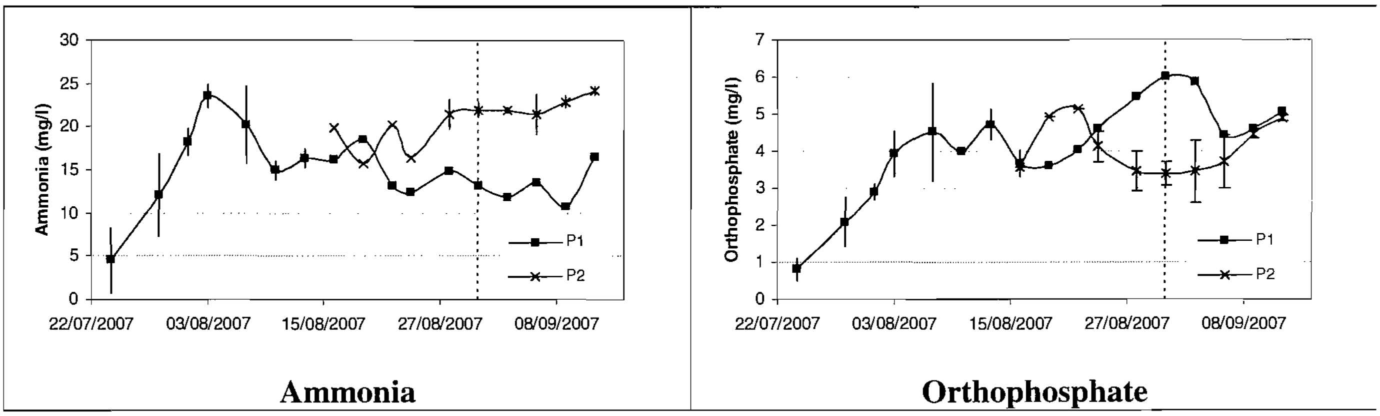


Figure 7.35 Ammonia and orthophosphate concentration in each pond during Phase 2 (NB. different y-axis)

7.3.4.5 Dissolved oxygen

Figure 7.36 shows daily average dissolved oxygen levels in Pond 1 during Phase 2. The effect of loading is clearly visible with average oxygen levels in Pond 1 only rising to above 1 mg l^{-1} following the end of the filling period; maximum levels reached were 1.4 mg l^{-1}. A second probe positioned 0.3 m from the bottom averaged 0.20 mg l^{-1} and minimum values of 0.13 mg l^{-1} (reaching the probes lower limit of sensitivity) which indicated that conditions remained anoxic, as might be expected at this depth in a facultative pond.

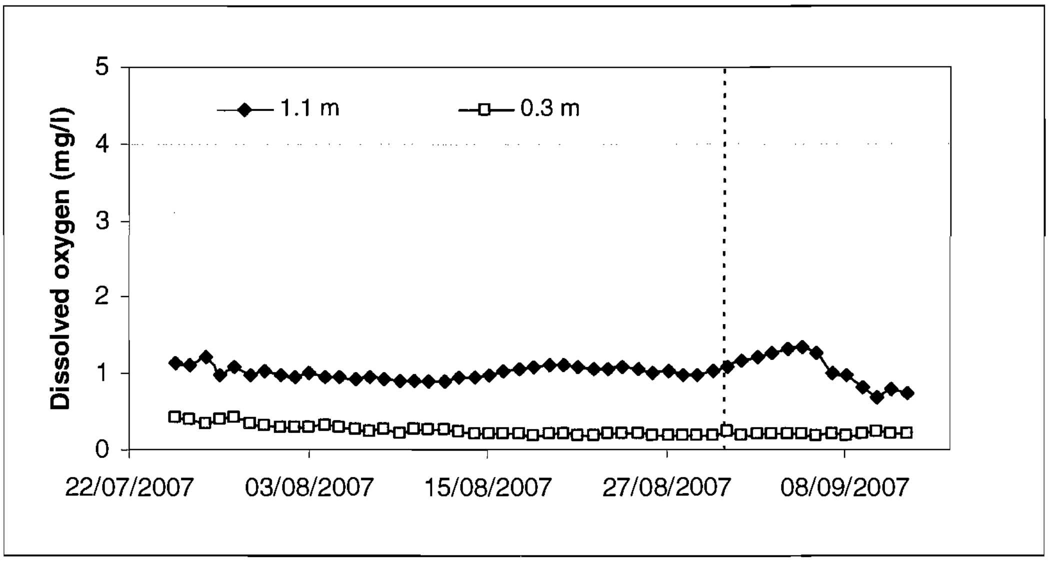


Figure 7.36 Daily average dissolved oxygen levels in Pond 1 during Phase 2

Oxygen levels in Pond 2 fluctuated between a maximum of 0.5 and 18 mg l^{-1} and an average of 0.3 and 7 mg l^{-1} (Figure 7.37), reflecting the reduced loading compared with Pond 1.

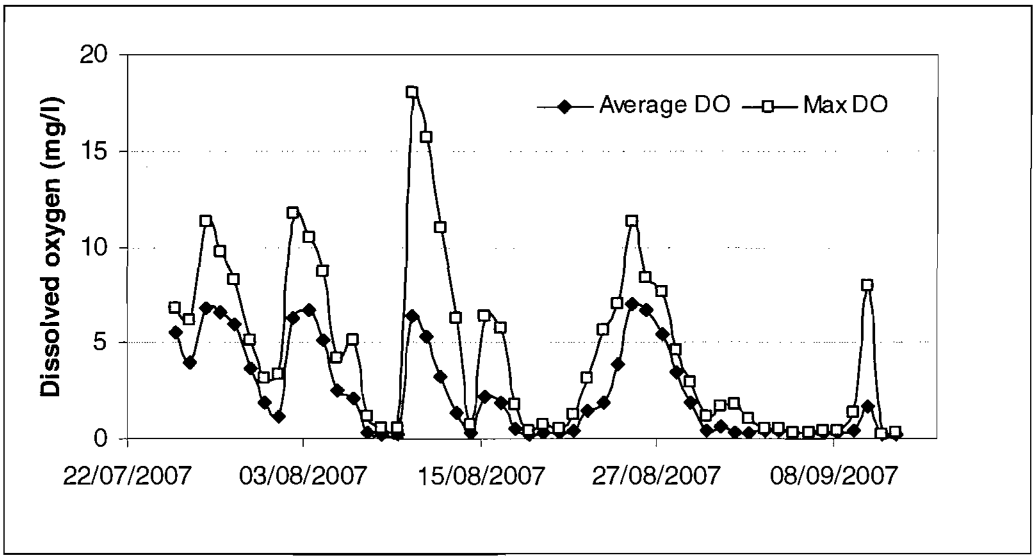


Figure 7.37 Daily average and maximum dissolved oxygen levels (1.1 m) in Pond 2 during Phase 2

Measurable oxygen in Pond 2 was highly variable and this may have been a consequence of light variability (Figure 7.38). Average dissolved oxygen levels were significantly related to light intensity ($p < 0.001$) though the correlation coefficient was poor ($R^2 = 0.22$). This value increased to 0.44 when values after 11/08/07 were used. From this date onwards there appeared to be a light threshold required to maintain photosynthetic activity. Daily average light intensity was less than 70 W m^{-2} over a period of four days during mid-August and dissolved oxygen dropped to an average of 0.25 mg l^{-1}. When light intensity increased above 100 W m^{-2}, oxygen levels also increased. A further fall in light intensity occurred from 240 to 100 W m^{-2} over four days at the end of August, leading to a drop in oxygen levels from an average of 7 to 0.45 mg l^{-1} over the same period. The pond then remained anoxic for the rest of the monitoring period apart from a rise to 1.70 mg l^{-1} on 10/09/07. Daily average light intensity during this period was below 180 W m^{-2}. The suggestion of light intensity thresholds exerting an effect on pond performance has been reported in previous studies but these were continuous-feed primary facultative ponds. Neel *et al.* (1961) suggested a minimum monthly average of 75 W m^{-2} would be needed to maintain oxygen in ponds loaded up to 112 kg ha^{-1} d^{-1}. Thirumurthi (1974) noted that standard environmental conditions required for a load of 67 kg ha^{-1} d^{-1} are 44 W m^{-2}. In the study by Abis (2002), pond failure and recovery threshold values were used to estimate required monthly solar radiation levels for loadings of 80 kg ha^{-1} d^{-1} or less. Recovery and failure were defined by the presence or absence of dissolved oxygen at the surface of the ponds. It was suggested that 57 W m^{-2} was required for recovery and 20 W m^{-2} for failure to take place. From the results in this study, it is difficult to estimate a threshold light intensity required to maintain dissolved oxygen levels as light intensities much higher than those quoted by previous authors appeared to reduce dissolved oxygen levels to a minimum. It is possible that in the slowly mixing pond and with increasingly larger populations of algae as exhibited in rising chlorophyll-a concentration that individual algae may have come into contact with optimal sunlight less and less frequently. The effective loading into Pond 2 had also increased as BOD concentration in Pond 1 increased from mid-August and as previous studies have shown an increased load on the pond requires an increase in light availability to maintain aerobic conditions.

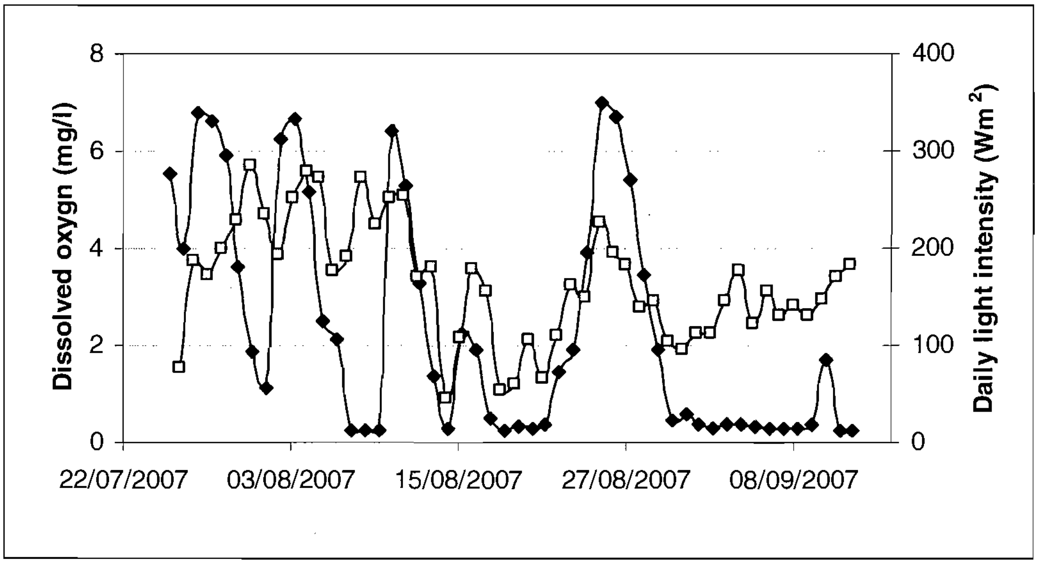


Figure 7.38 Relationship between light intensity and dissolved oxygen levels in Pond 2 (♦) daily average dissolved oxygen; (□) daily average light intensity

Figure 7.39 shows two examples where dissolved oxygen levels were controlled by changing light intensities. Both examples showed significant relationships ($p < 0.001$) but the correlation coefficient was low for both data sets ($R^2 = 0.07$ (a); 0.22 (b)). Although only two days separated the data sets there was a significant difference ($p < 0.001$) in light levels. Total measurable oxygen was far higher in (a) at 2773 mg l^{-1} as the pond remained aerobic overnight compared with a total of 192 mg l^{-1} measured in the data set in (b). There was no notable difference in COD and nutrient levels between examples. Surface temperature did not correlate with oxygen levels in (a) ($R^2 = 0.00$; $p > 0.05$) but showed a more significant relationship with (b) ($R^2 = 0.08$; $p < 0.001$) when light levels were lower. The average temperature for each example was 18.6°C and 17.0°C respectively. The results support the argument that a light threshold existed in the pond as a significant drop in light intensity caused a substantial drop in oxygen levels. When light levels fell, temperature was also perhaps more important in supporting pond oxygenation through its role in speeding up metabolic reactions.

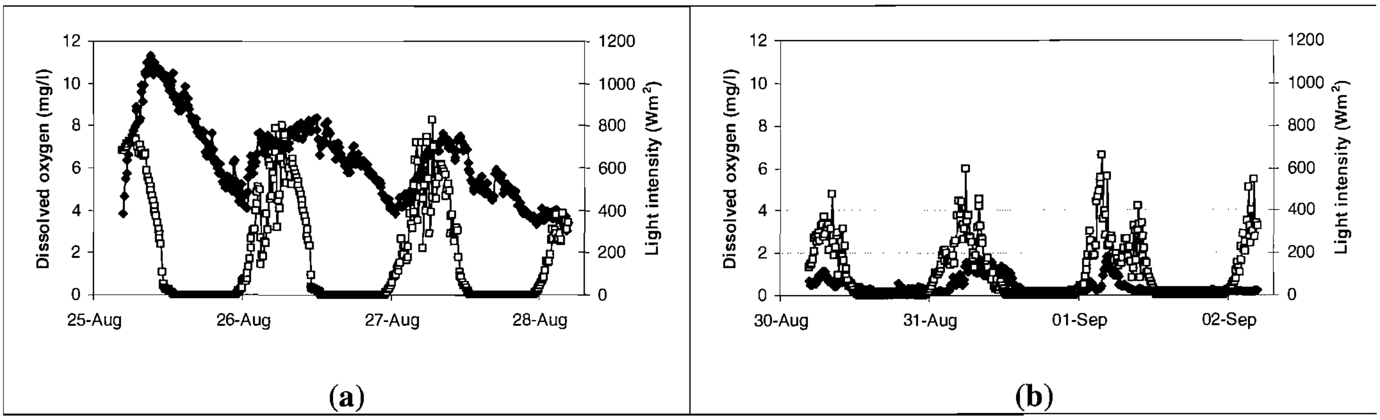


Figure 7.39 Relationship between light intensity and dissolved oxygen levels in Pond 2 (♦) dissolved oxygen; (□) light intensity

7.3.4.6 Oxygen production

Figure 7.40 shows gross and net oxygen production in both ponds during Phase 2. The effect of the untreated influent in Pond 1 was evident as oxygen consumption rates from late July onwards always exceeded gross production until the pond stopped receiving wastewater in early September. Concurrently net oxygen production became positive with values increasing from -6.5 mg O_2 l^{-1} h^{-1} to 5.7 mg O_2 l^{-1} h^{-1}. Maximum gross production levels of 8.7 mg O_2 l^{-1} h^{-1} were measured at the same time. In contrast, in Pond 2, gross oxygen production always exceeded consumption, giving a positive value of net production. All three measurements gradually increased over time reaching values of 11.4 mg O_2 l^{-1} h^{-1} for gross production, 8.7 mg O_2 l^{-1} h^{-1} for net production and 2.7 mg O_2 l^{-1} h^{-1} for the respiration rate at the end of the monitoring period. Average deviations from the mean for duplicate samples were ± 0.26 mg O_2 l^{-1} h^{-1} and ± 0.44 mg O_2 l^{-1} h^{-1} for gross and net production respectively.

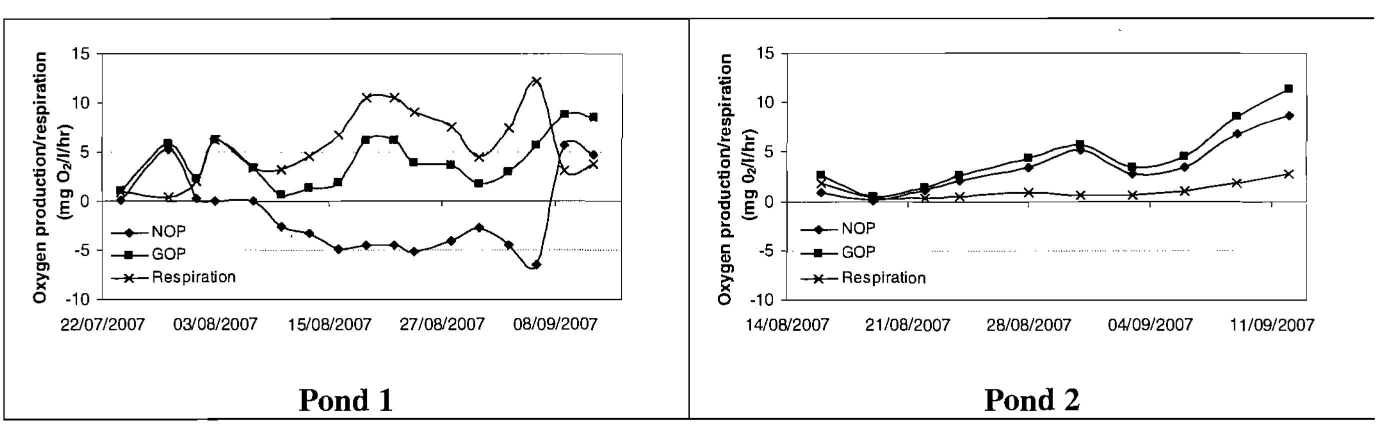


Figure 7.40 Average gross / net oxygen production and respiration rate in both ponds during Phase 2

The increase in production and consumption in Pond 2 was probably due to the gradual increase in algal population as indicated by chlorophyll-a levels and unfiltered COD. Figure 7.41 shows a strong relationship between gross oxygen production and chlorophyll-a was apparent in both ponds. By the time sampling began in Pond 2 in mid-August gross oxygen production had begun to increase in Pond 1 and resemble the trend in Pond 2. Unfiltered COD and gross oxygen production were closely related in Pond 2 with a p-value of < 0.001 and an R^2 of 0.82. In comparison, the correlation was very poor in Pond 1 with an R^2 of 0.06. It is possible that Pond 1 was slightly overloaded as odour was present during the filling period and this is confirmed by higher respiration rates compared with gross oxygen production. Pond 2 on the hand performed well throughout the monitoring period with no odour and this too is reflected in the results for oxygen production. These findings further support light-dark experiments as a possible alternative to the BOD test.

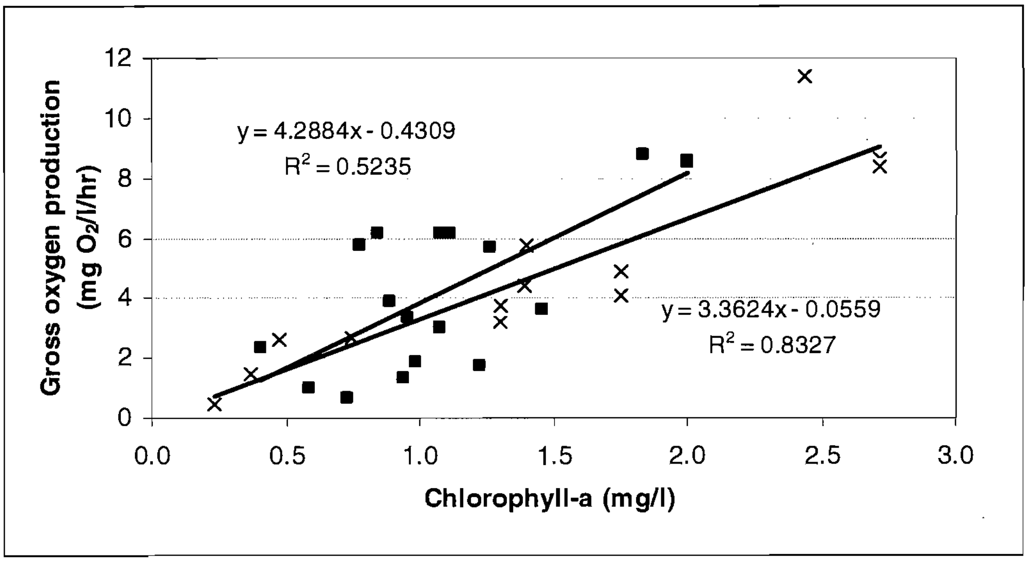


Figure 7.41 Relationship between chlorophyll-a and gross oxygen production during Phase 2 (■) Pond 1; (×) Pond 2

7.3.4.7 Phase 2 - Conclusions

Results from Phase 2 demonstrated that discharge to land from Pond 2 would be possible throughout the monitoring period in terms of BOD_5 and nutrient concentration meeting recommended WHO guidelines (WHO, 2006). Nutrient levels remained high over the summer and therefore could present a problem for river discharge consents but if the wastewater was to be used for crop irrigation then nutrients may be beneficial. BOD_5 concentrations began to rise in September with the increase in chlorophyll-a concentration but based on results from Phase 1 it is likely that a fall in nutrient levels and a subsequent drop in algae could lead to further potential discharge opportunities in the autumn.

7.4 Discussion

7.4.1 BOD_5/COD removal

Based on BOD_5 and COD removal, performance of Pond 1 during Phase 1 was excellent. Overall removal efficiencies for BOD_5 and COD compared favourably with previous reported values of 75-85% and 65-80% respectively (von Sperling and Chernicharo, 2005). While the pond was operated in containment mode during the summer filling period, this performance probably reflects the low loading rate of 14 kg BOD ha^{-1} d^{-1} and the long nominal retention time of about 93 days. In Phase 2, the system was only monitored until the end of the filling period and started with Pond 1 full of treated wastewater from the previous year. Results for Pond 1 alone suggest that a loading of approximately 130 kg BOD ha^{-1} d^{-1} and a retention time of just over 10 days there was some loss in performance, as BOD_5 and COD removal efficiencies showed a tendency to decline over the monitoring period, averaging just 40% and 30% respectively. BOD_5 removal quoted by other authors has been in the range 50-90% (Middlebrooks *et al.*, 1983; Mara *et al.*, 1998; Gray, 2002) and COD removal in the range 55-70% (Mara et al., 1998) with the wide variations reflecting differences in design and climatic conditions. The fact that during Phase 2 removal efficiencies in Pond 1 were below the published range suggests that performance in Pond 1 was relatively poor, probably due to the short retention time and high loading rate. The existence of Pond 1 to act as an initial buffer to Pond 2 and the addition of a second pond contributed to removal efficiencies for BOD_5 in the whole system being very good, with COD removal increasing over time. The increase in load by a factor of 9 in Phase 2 did not reduce overall performance levels due to the two-pond system, which gave a loading rate on the whole system of 46.5 kg BOD ha^{-1} d^{-1} and a total retention time of 35 days. The operating protocol in the second phase accommodated approximately half of the campsite's wastewater and demonstrated effective removal efficiencies. It is likely that Pond 1 acting as a dilutant enhanced pond performance in the early stages as although removal efficiencies in Pond 1 were comparatively low, the pond did not become anaerobic at any stage. Alternative arrangements include providing ponds of more equal size or using Pond 2 as the first pond in the series to give a reduced surface loading rate and increased retention time.

Pond 1 was filled over the summer in Phase 1, making use of the treatment period and then storing the wastewater over winter. The treated wastewater could be discharged once it meets consent standards, or kept for dilution purposes the following year, possibly enabling discharge earlier in the summer. During Phase 2, BOD removal efficiency in Pond 2 averaged 80% without dilution; however COD removal efficiency remained below 40% until the end of the filling period, suggesting that pre-dilution by treated wastewater or rainwater stored in the second pond might be of use in this case. One operating mode would thus be to keep both ponds full at the end of the camping season for the purpose of dilution the following summer, although the above average rainfall observed during the winter of 2006 and the potential for similar weather in the future means a requirement for larger storage volumes in this mode. Leaving the second pond free to fill with rainwater over the winter could provide an alternative. Unfortunately it was not possible to try these modes during the current study as the second pond was only built prior to the start of Phase 2.

Pond 1 had a higher surface area-to-volume ratio of 1.33 to 1 compared with Pond 2 (0.99 to 1) because of its shape although this included some side surface area. During filling, both ponds also had high top surface area-to-volume area ratio, as would also be the case in a full-scale pond. The consequence is that wastewater would be exposed to more than the normal quantity of sunlight per unit volume during the filling period. It is possible that this had an effect on the rate of pollutant removal, and this could be a useful side-effect of filling the pond from empty rather than diluting into an existing volume of water.

7.4.2 Nutrients

Abis (2002) found that ammonia removal in temperate climate ponds was better in summer than winter. It was suggested that if the pond becomes anoxic or devoid of algae over winter then ammonia removal is likely to drop to zero; while there was no inflow of wastewater during winter, both of these conditions occurred in the ponds in the current study. Both ammonia and orthophosphate peaked at the end of February following the winter and were quickly reduced corresponding to the rise in chlorophyll-a levels. Although ammonia correlated poorly with chlorophyll-a concentration it was shown to correlate strongly with pH. High pH levels were found to occur with the highest levels of dissolved oxygen suggesting that although chlorophyll-a did not directly correlate with ammonia concentration, algal uptake was the likely mechanism of removal.

Orthophosphate declined less quickly than ammonia but was shown to correlate strongly with chlorophyll-a levels suggesting algal uptake was also the key mechanism for orthophosphate removal. Nutrient levels did not fall to below discharge requirements during the summer but were less than 2 mg l^{-1} for a period of 20 days in November which would have been an optional time to discharge and from the beginning of March onwards.

In Phase 2, Pond 1 ammonia and orthophosphate concentration rose coinciding with the filling period and then fluctuated with an overall tendency for ammonia values in particular to drop and orthophosphate concentration to continue to rise. Ammonia concentration did not correlate closely with chlorophyll-a in Pond 1 even though ammonia removal occurred whilst chlorophyll-a levels were rising. In contrast, orthophosphate showed a negative correlation with chlorophyll-a in Pond 1. Algal uptake was probably the primary form of ammonia removal as pH levels were not high enough to suggest ammonia volatilisation was responsible. As pH was based on spot values rather than 24 hour monitoring it was not possible to determine whether levels rose above pH 9.0 but as dissolved oxygen levels remained low in Pond 1, it is likely that this was not the case. Orthophosphate removal was good in Pond 2 with removals of up to 30% from the values recorded in Pond 1. Ammonia removal from Pond 1 to Pond 2 was very poor with ammonia concentrations in Pond 2 exceeding those in Pond 1. The effect of the increased loading is most apparent when comparing ammonia concentrations in Phase 2 with those in the same period in Phase 1. Ammonia concentration was over twice as high by the end of the filling period in Phase 2. This may be a consequence of not only the increased load on Pond 1 but also higher chlorophyll-a levels to aid removal were observed in Phase 1 compared with those in Phase 2. Phase 2 would also have developed an established sludge layer in Pond 1 which may have fed back ammonia into the pond water. Nutrient levels stayed high throughout the monitoring period and could prevent discharge at this time but it is possible that with the holding period through the autumn and winter ammonia levels would reduce to < 2 mg l^{-1}.

7.4.3 Chlorophyll-a and dissolved oxygen

Chlorophyll-a levels were higher during Phase 1. Following stabilisation of the BOD at the beginning of November, oxygen concentrations began to rise with maximum levels in the supersaturated range. During the winter months, non-algal sources of dissolved oxygen and low uptake rates combined with the higher solubility of oxygen at lower temperatures were not always able to maintain oxygen concentrations of > 1 mg l^{-1} at the surface. Dissolved oxygen levels dropped as chlorophyll-a levels declined reaching anoxic levels by mid-December. The fall in algal population was concurrent with the drop in surface water temperature which fell to 5°C by December. No observed odour problems were associated with the transition from aerobic to anoxic conditions due to the very low levels of organic matter in the pond at the time. Unfortunately light intensity was not monitored at this time and therefore its importance in the decline in algal growth cannot be evaluated. From mid-February dissolved oxygen levels increased and reached saturated conditions by the beginning of March coinciding with the rise in chlorophyll-a levels. There was little evidence of a spring turnover though nutrients were present in sufficient quantities in the pond at the timing of the spring bloom. Water temperature changes which were insignificant during the timing of increased algal growth appeared to be less important. By this stage, light intensity was being measured and its effect on dissolved oxygen levels in the pond could be assessed. In spring, maximum photosynthetic activity was in response to a high level of solar radiation and when nutrients became limiting by the end of April and chlorophyll-a levels started to decline, solar radiation became the determining factor for photosynthesis to take place with oxygen levels corresponding to available light intensity. It is apparent from these observations that solar radiation was one of several factors exercising control over photosynthetic oxygen production although results suggest light intensity appeared to be most important. When the loading on the pond was high during the summer months, available light was able to support measurable oxygen production. During the spring warm-up period when the load on the pond was low the effects of light intensity were supported by available algal nutrients.

Chlorophyll-a concentration in Phase 2 took longer to rise to levels higher than 1 mg l^{-1} in Pond 1 compared with results from the previous year. This was probably a consequence of the higher loading but also of the weather conditions which were not as conducive to algal growth as the conditions observed in the previous year and estimated growth rates suggest that washout at the imposed retention time may have been responsible. A duckweed infestation that caused a decline in chlorophyll-a levels due to light shading in Pond 1 in early August was also a factor in Phase 2 that was not observed during Phase 1. Chlorophyll-a levels were higher in Pond 2 than Pond 1 which suggested that the reduced loading on Pond 2 was more beneficial to algal growth. Although Pond 1 received the entire influent load this was effectively diluted and prevented the pond from becoming anaerobic. Both ponds maintained healthy chlorophyll-a levels which were able to cope with the oxygen demand on the system.

The effect of loading on measurable oxygen was evident during Phase 2 with oxygen levels averaging 1 mg l^{-1} in Pond 1 and fluctuating between 0.5 and 18 mg l^{-1} in Pond 2. As a consequence of the higher loading in Pond 1 there was some odour but this was not commented on by the campers and was therefore presumably not a nuisance outside of the immediate pond area. The odour was less apparent once the incoming wastewater had ceased. The variability in oxygen levels in Pond 2 appeared to coincide with changes in light intensity. When average light intensity was quite low, for example less than 70 W m^{-2}, photosynthetic activity was adversely affected and dissolved oxygen levels dropped below 1 mg l^{-1}. When light intensity increased above 100 W m^{-2} oxygen levels also increased. There appeared to be a light threshold required to maintain photosynthetic activity although this varied being higher towards the end of the monitoring period. It was suggested that this change in threshold was caused by increased chlorophyll-a concentration reducing light availability thereby creating conditions of self-shading and a reduction in production potential. Alternatively it was the result of increased loading from higher pollutant concentrations flowing from Pond 1; therefore a higher oxygen demand on the system and a requirement for higher light intensities to satisfy the oxygen consumption imposed by the heavier load.

In terms of possible discharge from Pond 2, it is important that oxygen levels in the wastewater are above the minimum allowable; 5 mg l^{-1} is generally accepted to be the minimum level which should be maintained in the water body. Although experiments determining oxygen dynamics revealed that production outweighed consumption, dissolved oxygen levels in Pond 2 fluctuated widely throughout the summer falling as low as 0.20 mg l^{-1}. The trend in oxygen levels appeared to follow the pattern of changing light intensity. The lack of reliably consistent oxygen levels above minimum allowable levels could prevent summer discharge. The results from Phase 2 suggest that the volume of Pond 1 should be increased to lengthen the retention time and prevent algal wash-out which may improve oxygen relations in the whole system.

7.4.4 Oxygen production

Although chlorophyll-a levels were fairly high during the filling period in both phases, oxygen consumption usually equalled or exceeded its production on a 24-hour basis in Pond 1 with low or zero values throughout August and into September.

Measurements on oxygen dynamics in the pond revealed that the highest levels of oxygen production occurred during the summer/autumn months in Phase 1. The respiration rate was also highest during these months coinciding with the oxygen demand imposed during the filling period. Irving and Dromgoole (1986a) monitored the oxygen balance in a facultative pond over a 7-month period. Maximum gross daily oxygen production was 33 mg O_2 l^{-1} d^{-1} determined from hourly rates obtained in the field. The maximum value obtained in the current study was more than ten times higher than that obtained by Irving and Dromgoole (1986a). Although chlorophyll-a concentrations were not given by Irving and Dromgoole (1986a) it is likely that chlorophyll-a levels were not as high as those measured during the summer/autumn of Phase 1. It is suggested that the likely cause of such high levels of gross oxygen production was due to the high levels of chlorophyll-a in the sample.

In Phase 2, the effect of loading resulted in a notable difference in oxygen production between ponds. The effect of receiving the untreated influent wastewater in Pond 1 was evident as oxygen consumption rates always exceeded gross production until the pond stopped receiving the wastewater in early September suggesting that the pond was overloaded or the HRT was too low. In contrast, oxygen production always exceeded consumption and net production remained positive in Pond 2. A close relationship existed between gross and net oxygen production and chlorophyll-a concentration in Phase 1 and 2. Gross and net oxygen production values were found to correlate strongly with both unfiltered BOD_5 and COD concentration in Phase 1. The relationship between unfiltered COD and gross oxygen production in Phase 2 was closely correlated in Pond 2 but showed a poor relationship in Pond 1. These results were similar to Phase 1 in that the closest fit between the two parameters was using values after the filling period. This is probably because the unfiltered COD and BOD_5 levels in Pond 2 largely represented chlorophyll-a levels. Equally the values in Phase 1 with the absence of incoming wastewater were predominantly of algal content.

The results suggest that measurements of oxygen exchange can provide an indication of pond performance and more quickly than current methods of assessment. In discussing the relevance of the results to performance in the field it should be noted that natural temperature variation and the possible variable effect of temperature changes on metabolism of the different species have not been taken into account. The results of these laboratory studies which were conducted at 20°C can therefore only specifically relate to the reference temperature.

7.4.5 Overall operational performance

The operating protocols trialled at Lockerley proved reasonably successful. Table 7.4 shows possible discharge times to river or land when pollutant concentrations met typical discharge requirements.

Table 7.4 Possible discharge periods for the main pollutants to river or land according to the requirements of different discharge standards

Parameter	Source of guidelines	Discharge standard (mg l^{-1})	Phase 1	Phase 2
		Discharge to river		
COD (f)	UWWTD	125	Nov-May	Aug
BOD (f)	UWWTD	25	Nov-May	Aug
BOD (f)	Royal Commission	20	Feb-Mar	Aug
Chlorophyll-a	n/a	n/a	Jan-May*[1]	Aug
SS	UWWTD	150	Nov-May	Aug
SS	Royal Commission	30	Jan-Feb	-
Ammonia*[3]	UWWTD	2	Nov; April-May	-
Phosphorus	UWWTD	1	Nov; April-May	-
		Discharge to land		
BOD (uf)	WHO	< 400	Aug-May	Jul-Sept
Chlorophyll-a	n/a	n/a	Jan-Feb; April-May*[2]	Aug
SS	WHO	< 100	Dec-May	Aug
Ammonia	WHO	< 30	Aug-May	Jul-Sept
Phosphorus	WHO	< 20	Aug-May	Jul-Sept

*[1] There is no standard for chlorophyll-a, although high concentrations indicate large algal populations which may cause problems of eutrophication in receiving waters. Dates are for periods when concentrations are below 1.35 mg l^{-1} equivalent to 150 mg l^{-1} SS (assuming SS in WSP effluent 60% algae).
*[2] Dates are for periods when chlorophyll-a concentrations are below 0.90 mg l^{-1} equivalent to 100 mg l^{-1} SS.
*[3] UWWTD sets standards of 10-15 mg l^{-1} for total N. Both total N and P are requirements for discharge in sensitive water bodies only.

In Phase 1, it would have been possible to discharge to river in accordance with the 20/30 Royal Commission standard in February but with nutrients potentially being the limiting factor, which might be addressed by tertiary treatment for nutrient removal. According to the UWWTD standard, discharge to river could have been possible from November to

May, although nutrient levels were only low enough for discharge in April/May. Nutrient limits are only a requirement when discharging to sensitive water bodies; although the River Dun is not classified as sensitive, it is a tributary of the River Test which has been classified as such. The main problem of the protocol trialled in Phase 1 is the need to provide additional storage capacity for rainfall, the majority of which occurs in autumn and the first half of winter in the UK climate. Phase 2 attempted to address this issue by trialling a continuous discharge system over the summer. This failed to meet nutrient standards without some form of tertiary treatment although discharge to land at Lockerley could have been an option.

UWWTD standards are applicable for discharges to water while standards applying to land discharge are found in the World Health Organisation's 'Guidelines for the safe use of wastewater, excreta and greywater' (2006) whose primary objective is to maximise the public health benefits of wastewater use in agriculture. In terms of BOD_5, discharge to land would have been possible throughout the filling and monitoring period in Phase 1 and 2 as unfiltered levels were lower than 400 mg l^{-1}. Concentrations of organic matter higher than this have been found to clog soil pores (WHO, 2006). Hence, wastewater with suspended solids levels above 100 mg l^{-1} are not recommended for irrigation and therefore limit the possibility for discharge to land from December onwards. Chlorophyll-a concentration may pose a problem during the spring bloom and in March; Phase 1, levels exceeded 0.9 mg l^{-1}, equivalent to approximately 100 mg l^{-1} of suspended solids. In Phase 2, continuous summer discharge to land was limited by the level of suspended solids. Nutrient levels met WHO Guidelines at all times; nutrients are generally beneficial to soils and for crop productivity; however, nitrogen and phosphorus levels higher than recommended values can have adverse affects on crop quality.

The research carried out was primarily concerned with water quality parameters relevant to discharge into rivers. The work did not look in detail at microbiological performance, which is the key parameter for reuse in irrigation/land application, although faecal coliforms were not detected in tests during spring in Phase 1. Where irrigated crops are not intended for direct human consumption and the site is isolated with restricted public access such as at Lockerley, standards are less stringent as there is less risk of pathogen transfer: in practice, precautions might be necessary to ensure either that appropriate microbiological standards are met or that children on the campsite could not gain access to any irrigated areas.

7.5 Conclusions

The performance of the systems monitored suggests that WSP are a feasible and practical option for sites where there is a short-term intermittent generation of wastewater. Pollutant removal was generally very good but tertiary treatment for further nutrient removal before discharge to the receiving water would be required or land application is another option. The application of an intermittent discharge system at Lockerley may have site specific limitations due particularly to the locality of the River Dun.

8 GENERAL DISCUSSION

The research focused on the operation of WSP in variable climates, in particular the effect of dilution on pond performance during the spring warm-up period. Experiments conducted in three different locations, at different scales and under varying environmental conditions addressed a number of key themes as summarised in the sections below. Despite differences in experimental conditions, common trends were apparent in the results.

8.1 The effect of dilution on treatment efficiency

The use of dilution to alter the strength of wastewater had a significant effect on the relative BOD_5 and COD removal rates, with negative correlations between initial organic load and removal rate in batch systems and in the unsteady state period in fed systems. The latter systems based in Kazakhstan also showed noticeable improvements to removal efficiencies where the pollutant load was reduced. Despite the wide range of COD removal rates under different experimental conditions, the response of removal rate to changing concentrations was fairly similar. Comparable results were obtained in bottle and flask experiments due to high light penetration in small vessels whilst depth effects were a feature of the buckets and tanks (Chapter 5). The change in overall removal rates with COD concentration was 0.00008 g g^{-1} d^{-1} per mg l^{-1} for buckets and 0.0001 g g^{-1} d^{-1} per mg l^{-1} for tanks. Increased light penetration is likely to account for the difference between overall removal rates obtained in the bucket and tank experiments and that obtained for the bottle experiment which was 4-5 times higher at 0.0004 g g^{-1} d^{-1} per mg l^{-1}. The overall filtered COD removal rate for tanks which out of the batch-fed experiments were the closest replica of a WSP, suggested that an approximate four-fold reduction in the initial COD could increase the removal rate by three-fold. Overall filtered BOD_5 removal rate for tanks, demonstrated a three-fold increase in overall removal rate from a three-fold reduction in BOD_5 concentration. In practical terms the latter finding might require a 2% increase in the pond volume, but which is offset by a 33-day reduction in retention time and the likelihood of no accompanying odours. This is an oversimplification, as removal rates did not remain constant throughout the experiment, and the result is based on batch experiments while WSP are generally fed systems with mixing.

Whether dilution is worthwhile depends on the cost and on water quality requirements early in spring. If the most important factor in pond performance is maintenance of or early recovery from anaerobic to aerobic (i.e. facultative) conditions, then a reduction in the winter load is necessary. The results of the work are therefore likely to have an application in arid climates where water is needed early in the year for irrigation, and in areas where the reduction or prevention of odours is a priority. Prince *et al.* (1995a) reviewed the findings of a survey carried out to assess public concern with respect to wastewater treatment in Alberta, Canada finding that spring-time odours were the most common complaints.

Dilution studies conducted in 25-l units in Almaty, Kazakhstan, starting before and after the winter freeze demonstrated some important differences between the behaviour of systems experiencing a winter period followed by the non-steady state in spring. BOD, COD and nutrient removal in units with higher initial substrate concentrations were affected by the addition of a winter period with delays of up to 24 days in the increase of removal efficiencies at the start of spring. Steady-state values for BOD_7/COD concentration were reached immediately in the most diluted units, however, with removal efficiencies operating at 86%. With no period of ice cover and an absence of a non-steady state period, pollutant concentration and thus removal efficiencies were found to be similar throughout the monitoring period.

Results of varying retention times/loading rates in 780-l tank experiments conducted in Kazakhstan demonstrated the capacity to reduce high COD and nutrient concentrations in the spring, followed by sustained low levels throughout the start of the summer period. The recovery from winter conditions is due to the increase in light and temperature necessary to promote the rapid development of a spring bloom, followed by the hot summer typical of a continental climate. Shorter retention times however, caused a delay in the tanks reaching steady-state values (Section 6.3.6.1). The 20-d HRT delayed the reduction in COD concentration to steady state values by one month and the 15-day HRT by approximately 42 days. COD removal rates calculated during the steady-state phase in winter and summer were similar to predicted values from the US EPA (1983) where rates increase with increasing load. The results demonstrate that the tank experiments were consistent with typical performance in full-scale plants. For the unsteady-state phase, a mass balance for the duration of the unsteady state period at each HRT was used to obtain spring COD removal rates. These showed that at shorter HRT the transition to low COD concentration took slightly longer as well as occurring at later date. The amount of

COD removed per kg available for removal was similar between HRT, probably reflecting that for the 15-d HRT, this occurred much later in the season, when higher temperatures and total daily light input were present. At the time of transition, therefore treatment capacity was similar in all ponds.

In Chapter 6, the effect of loading on COD removal rates were discussed with the view that there needs to be enough oxygen generation capacity to deal with the load by more efficient aerobic metabolic routes. This capacity can be reduced by a number of factors e.g. higher washout of organisms due to shorter HRT, slower growth of algae or bacteria due to lower temperature, and lower oxygen production due to lack of light, etc. The reason for the delay in falling COD concentration demonstrated by the shorter retention times could therefore be due to several of these factors. Results from Chapter 5 suggest that light is highly important, although these results only provide evidence and are not conclusive. The capacity of the system to degrade the waste is most important, and modelling of the factors that affect the capacity for treatment is required for more quantitative results.

The trials based at Lockerley provided the opportunity to test two distinct modes of operation: intermittent once-per-year discharge mode utilising one facultative pond, and a two-pond system effectively in continuous summer discharge mode with a 10-day HRT incorporating the concept of dilution into its design. Although single pond systems are still common, much better effluent quality can of course be obtained with more than one pond in series and/or with extended storage. Both modes of operation proved reasonably successful because the once-per-year discharge operation was run with prolonged storage time. When operating under continuous discharge in Year 2, the availability of treated water buffered the effects of a low retention time imposed on the primary pond. At 10 days HRT in Pond 1 and a loading of approximately 130 kg BOD ha^{-1} d^{-1}, the system essentially coped but the presence of odours for a short period suggested that increasing the retention time might improve performance: Abis (2005) reported that a 20-day HRT was adequate for a UK climate. According to UWWTD standards, performance in Phase 1 allowed for possible river discharge from November through to spring. The more stringent 20/30 Royal Commission standard would limit this to just February, however nutrients would be the limiting factor which were only low enough in November or from April-May. The increase in load by a factor of 9 in Phase 2 and the addition of a two-pond system meant BOD_5/COD levels were low enough for possible discharge to river in August during the filling period. Nutrient levels however, did not meet typical requirements at any stage, implying that tertiary treatment is required to meet these standards. In terms of BOD_5 and nutrient concentration, discharge to land was possible throughout the entire monitoring period of Phase 1 and 2. Due to high chlorophyll-a levels in the summer of Phase 1, suspended solids were above the 100 mg l^{-1} recommended value (WHO, 2006) until December. In Phase 2, chlorophyll-a levels steadily rose during the summer, as a result, suspended solids exceeded the recommended level by the beginning of September.

8.2 Environmental effects

Light is the ultimate energy source for primary producers, and temperature an important regulator of metabolic processes. Both factors are subject to strong seasonal variation, especially at mid and high latitudes. Controlled experiments to assess the effect of temperature on pond performance in spring were not conducted during this study but relationships were found between the change in temperature and COD concentration and its effect on the timing of an algal bloom during simulated UK batch-fed experiments. Results demonstrated that the temperature required to initiate a rise in chlorophyll-a concentration increased with the increase in initial load: for example, algal growth was observed at 10°C for an approximate starting load of 100 mg l^{-1} compared with 20°C for a load of 400 mg l^{-1} (Section 5.6.6.4). It has been found that higher light intensities are required to saturate growth at suboptimum growth temperatures (Bartosh, 2004). This suggests that higher temperatures may be required for the formation of algal blooms to counteract the effects of reduced light penetration when the load is increased.

Light intensity as a variable affecting pond performance was assessed in the laboratory and its role during the spring warm-up period at Lockerley. Light intensity was monitored during each of the experiments in the UK but was only controlled during the flask and tank experiments. It was possible to comment on the difference in COD removal rates between experiments but the variability from using different light sources, vessels and temperatures prevented quantitative conclusions. Flask experiments involving different total daily light inputs revealed that although no noticeable difference was observed between daylengths 12:12h and 24:0h, by dramatically reducing light energy to a daylength of 6:18h, the process of COD removal did slow down to some extent (Section 5.5.1). Overall, lowest removal rates were observed in the tank experiments and then the buckets. It was expected that removal rates would be lowest in the tanks as these received least light; the bucket experiment however was conducted under the highest light intensity of 705 μmol m^{-2} s^{-1} and was therefore expected to perform more efficiently. Both vessels incorporated depth effects, however, and therefore the surface area exposed to light was limited to the top surface which although corresponds to normal pond conditions did reduce the surface area to volume ratio compared with the smaller more transparent flask and bottles. Consequently, highest removal rates were observed in those experiments where the surface area to volume ratio encouraged algal growth and oxygen production.

High light intensities observed during the bucket experiment, similar to those expected in the summer in Almaty, resulted in the lowest overall change in removal rate with COD concentration, at 0.00008 g g^{-1} d^{-1} per mg l^{-1} (Figure 5.12). This may indicate that any effect of dilution was lessened with increasing light intensity, with just a 2-fold increase in removal rate for a 7-fold reduction in filtered COD. Dilution as a potential operational option may therefore be questionable at latitudes that experience high light intensities early in the spring and could be better suited to more northerly climates where light availability is limited.

Low initial substrate concentrations created less oxygen demand on the system with the oxygen balance reaching equilibrium sooner. This may have been aided by the fact that dilution reduced not only the suspended solids concentration but also apparent colour (as measured by absorbance at 440 nm), with potentially important consequences on the amount of light energy reaching pond microbial communities. An example of the effect of light input on the appearance of free oxygen is shown in the tank and bucket experiments. No significant rise in oxygen levels was recorded for the tanks with the highest load (431 mg filtered COD l^{-1}), but a change from negative to positive net oxygen production and bubbles on the surface was observed in the buckets that received double the initial substrate concentration (872 mg filtered COD l^{-1}). Temperature showed no significant changes to dissolved oxygen levels at an initial load above 400 mg filtered COD l^{-1} in the tank experiments even when water temperature rose above 20°C (Section 5.6.6.4). The increase in temperature over the course of the experiment also had no apparent effect on removal rate of organic matter. Regression analysis of COD concentration rather than removal rate with temperature resulted in only 11 and 24% of the variability in COD concentration explained by temperature for the two highest loads. This poor correlation was suggested to be a consequence of the length of time the experiments ran when initial loads of 417 and 431 mg l^{-1} were used; temperature remained at 20+ 2°C for half the duration which would have affected the correlation coefficient. The difference in results for system performance is therefore likely to be a result of light intensity which was much greater during the bucket than the tank experiment.

Low dissolved oxygen levels were present even in the dark variants at low loadings, as light was still adequate to support detectable oxygen production (Figure 5.10 and Figure 5.34). The rate of oxygen consumption from higher initial loads was too great to be satisfied by oxygen production under low light intensities. A similar phenomenon was reported by Neel *et al.*, (1961) who found that higher light intensity became more important to the photosynthetic process when the levels of organic matter present were high. Conversely, high light intensities appeared to be less important to system performance when loadings were reduced and algal growth appeared to be controlled by the availability of nutrients. Work by Thirumurthi (1974) supports the idea that the adverse effect on removal rate of higher loading is greater when exposed to inadequate light energy, as lower loaded systems performed well at all light intensities.

Monitoring the effects of light intensity on dissolved oxygen during the spring at Lockerley revealed maximum dissolved oxygen levels in the ponds was closely linked to a high level of solar radiation. With the depletion of nutrients, in particular ammonia, by the end of April and the decline in chlorophyll-a levels, solar radiation became the limiting factor for photosynthesis to take place. During Phase 2, the variability in oxygen levels in the secondary pond coincided with changes in light intensity. There appeared to be a threshold on the light required to maintain net oxygen production, although this varied being higher towards the end of the monitoring period. It was suggested that this change in threshold was caused by increased chlorophyll-a concentrations corresponding to a higher algal population, leading to reduced light availability due to conditions of self-shading and a reduction in oxygen production potential. Alternatively it was the result of increased loading from greater pollutant concentrations flowing from Pond 1; thus requiring higher light intensities to satisfy the oxygen consumption imposed by the increased load.

8.3 Algal growth

A further trend common to all experiments was an increase in the lag phase of algal growth as a result of increased loading, and subsequent delays to conditions of net oxygen production. For example, in the UK batch-fed tank experiments, there was a lag phase of 21 days in the highest strength wastewater compared with just 5 days for the lowest initial concentration. Reducing the pollutant load through dilution consequently improved system performance promoting oxygenated conditions and a healthy algal population.

In Kazakhstan, findings from the 25-l unit experiments concurred with those from the 780-l systems and from the UK experiments. Both lower initial pollutant concentrations and reduced loading rates resulted in the earlier onset of algal growth following a period of dormancy under the ice. In contrast, less diluted units showed a clear lag phase following the winter period with peak levels of chlorophyll-a delayed by up to 10 days.

8.4 Oxygen production

In addition to the traditional approach of BOD measurement, measurement of oxygen production potential was used to determine how a pond system behaved under different conditions, e.g. of organic loading. The approach was used to obtain an estimate of the microbial activity occurring and the overall oxygen balance in the pond at that time. Primary production was measured in the laboratory under standard conditions by the light and dark bottle method. This method, in common with all other methods for primary production determination, is not devoid of errors. Sources of error include an increase in algal population in the light bottles during the experiment. Most errors however result from long exposure time. Since the presence of non-photosynthetic organisms in pond samples affects net production, the photosynthetic oxygen production profile is not the same as that determined for the mixed community. In practical terms, the data produced are of value because they give an indication of oxygen production which is in excess of immediate requirements and which is available to the community, and the method appeared able to provide an accurate assessment of pond performance in a short amount of time.

8.5 Proposed operating protocols

The performance of pond systems varies significantly with season and temperature and therefore potential improvements to pond operating protocols in variable climates were considered, including those with intermittent loading. On the premise that the ideal operating regime for variable climates is to create enough storage in the maturation pond to hold wastewater entering in the period when it cannot be discharged, the initial aim was to establish when the accumulated water would be clean enough to discharge.

Discharge could then continue through the spring and summer when the water is needed. The difference from the existing North American (cold region) design of operating WSP is a reduced storage time, with discharge over a longer period and not necessarily equal to the entire volume of the pond.

Figure 8.1 shows an example of a potential operational protocol over one year in a cold or continental climate region. The design allows for the possibility of building a smaller pond as suggested in Heaven *et al.*, (2007) or retaining some treated water in the pond at the end of the summer for dilution of the incoming winter load as investigated in the current work.

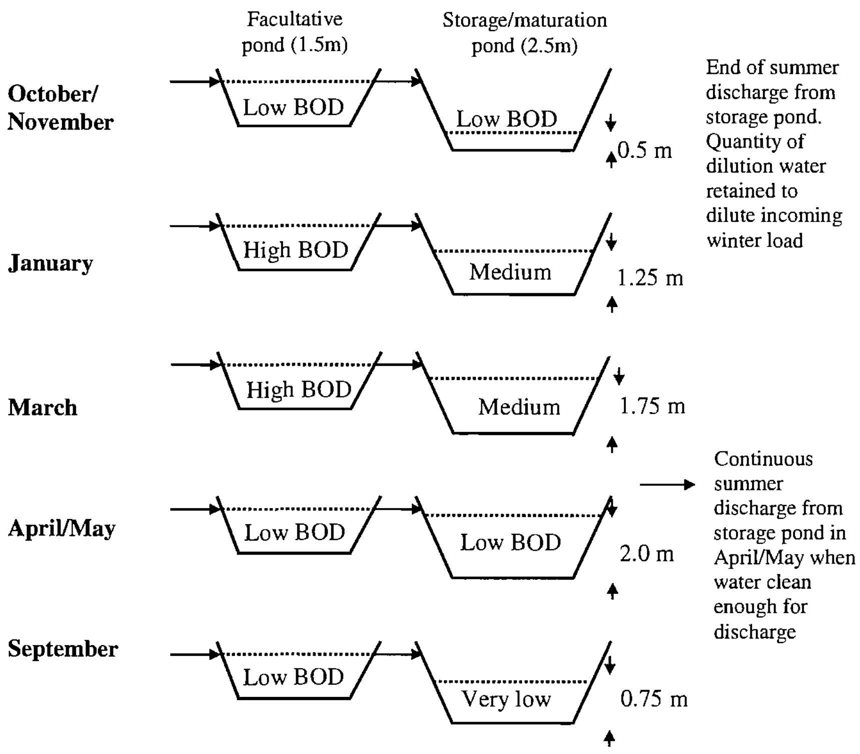


Figure 8.1 Schematic of the proposed operational design for WSP in cold regions (adapted from Heaven and Banks (2005))

Testing the proposed protocol in Kazakhstan of reducing the winter load through the retention of treated wastewater from the previous year produced good results and an effluent of suitable quality for discharge as early as mid-March. Typically, treated effluent is discharged into rivers, however excessive nutrients may cause eutrophication and impact ecosystem health. At the same time, soil erosion and increased agriculture are removing nutrients from the soil, causing a depletion of fertility. Water reclamation and reuse can return nutrients and water to the land and bring life back to aquatic ecosystems. However, a major issue and risk with sewage effluent reuse is the potential of sewage-borne pathogens. Faecal coliform numbers were only monitored in the spring at Lockerley, where none were found. Clearly this observation is only applicable to ponds receiving no wastewater over the winter period as there is evidence indicating the potential for extended survival of pathogens in cold conditions in WSP (Torrella *et al.*, 2003). Unfortunately there is little guidance on appropriate design parameters and therefore further research is required to assess the extent to which pathogens are depleted following the winter period and during the spring warm-up. In continental climates with their typically low precipitation, however, the possibility of wastewater reuse should be an important factor in choice of technology and design.

The results of the Kazakhstan 25-1 experiments showed a notable difference in improvements to removal efficiencies for experiments conducted before and after the winter freeze. These suggest another operating protocol where, instead of holding clean effluent at the end of summer and adding untreated wastewater over the autumn and winter, the two are mixed at the start of winter without further addition of wastewater until the following spring. At suitable dilution ratios (e.g. 75:25%) this approach appears to allow feeding from the start of spring with no increase in COD and BOD levels. The disadvantage in this protocol is the need for a batch component which does not deal with incoming wastewater in the winter period, but it may have specific applications for example in sites with existing infrastructure where there is a need for clean water early in spring.

Trials at Lockerley showed potential for both operating protocols, but site specific limitations were evident. The locality of the River Dun may dictate future restrictions on discharge to river, particularly the requirement to comply with nutrient standards. Reasonably successful BOD removal suggests that in general, both protocols are capable of being used at other sites warranting an intermittent discharge system.

8.6 Summary

The research has demonstrated that dilution offers the potential to improve WSP operation in variable climates. Previous work on removal kinetics has typically not included information from ponds experiencing winter conditions and ice cover. During the spring warm-up period, both removal rates and the time to reach maximum removal efficiency were found to increase as the initial load decreased. This contrasts with typical first-order reactions where the rate is proportional to concentration of substrate. Performance during the spring warm-up period improves when the loading is reduced suggesting existing design models may therefore not be applicable to ponds in variable climates. Altering the initial load through dilution is a potential mode of operation that could reduce the winter load and bring forward the date at which treated wastewater reaches discharge standards as well as preventing occurrence of odours in spring.

9 CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

9.1 Conclusions

Batch-fed experiments

1) Substrate concentration has an effect on pollutant removal rate. In small-scale (bottle and flask) batch tests under natural and simulated light, this appeared to be on the order of 0.0004 g g^{-1} d^{-1} per mg l^{-1}.

2) In larger-scale tests simulating depth effects, with light penetration at the top surface only, the effect is smaller; overall removal rates with changing COD concentration were 0.00008 to 0.0001 g g^{-1} d^{-1} per mg l^{-1}.

3) Some removal was obtained even at very low light levels. Bottle experiments (5-15 μmol m^{-2} s^{-1}) at the lowest initial concentration of 68 mg COD l^{-1} achieved overall removal rates of 0.12 g g^{-1} d^{-1} compared to 0.27 g g^{-1} d^{-1} for bottles in full light of 147 μmol m^{-2} s^{-1}. Flask experiments under a light intensity of 1 μmol m^{-2} s^{-1} also achieved quite high removals (54-78% after 8 days). While some of this removal was due to physical mechanisms of sedimentation, the presence of measurable levels of dissolved oxygen indicated that biological removal was also occurring.

4) High light intensities of 705 μmol m^{-2} s^{-1} significantly reduced the effects of dilution in UK bucket experiments with the overall removal rate changing with COD concentration at 0.00008 g g^{-1} d^{-1} per mg l^{-1}, suggesting that an operating protocol incorporating dilution would be more applicable in northerly climates where light is limited.

5) Substrate concentration prolonged the lag phase of an algal bloom. A lag of 3-4 days occurred in buckets with an initial load of approximately 868 mg COD l^{-1}; an 8-fold decrease in dilution in tank experiments increased the delay in an algal bloom from 5 to 21 days. In some instances when the light intensity or total daily light input was reduced, e.g. in flask experiments at a 6:18h light regime, the onset of a bloom was completely inhibited at higher loads.

Semi-continuous fed experiments

6) Results based on experimental data from five 780-l tanks operating as facultative ponds showed that increasing the surface loading from 33 to 133 kg BOD ha^{-1} d^{-1} with a corresponding reduction in retention time from 60 to 15 days had no adverse effect on COD and nutrient removal under steady state conditions in early summer. During the spring warm-up period, however, the change in loading and retention time led to a delay of up to 6 weeks in achieving steady state values for both parameters.

7) There was a good correlation between loading rate and the number of days from ice thaw to the first day of steady-state COD values. The result corresponded to approximately 1.9 days longer per day of HRT.

8) At the time of complete ice-thaw, chlorophyll-a levels rose rapidly in all tanks due primarily to incoming solar radiation and in part to minor changes in water temperature. The onset of an algal bloom in the 780-l tanks was delayed by 10 days when the HRT was reduced to 15-20 days.

9) No notable difference was observed in performance between dilutions in 25-l units started after the winter period, with minimum/steady-state values reached on the same day for all dilutions. Removal rates on day 4 ranged from 0.178 to 0.185 g g^{-1} d^{-1} compared to 0.11 and 0.14 g g^{-1} d^{-1} after 5 days for the UK buckets. Higher light intensity in Almaty compared with the UK may have been a factor in the increased removal rates and a factor in the minor difference between removal rates at different loadings.

10) Performance in the 25-l units was affected by the addition of a winter period; BOD_7 removal in dilution 20:80% took 24 days from the start of the monitoring period in spring to reach removal efficiencies above 80% and dilution 50:50% took 17 days, compared with immediate steady-state values in the more diluted units. The findings concurred with those for ammonia removal. A delay of 12 days in the onset of an algal bloom was observed in the least diluted units compared with the highest diluted systems. In Year 1, without an over-wintering period, no difference in the start date of an algal bloom was observed between dilutions.

11) In Year 2, gross and net oxygen production reflected the loading on each 25-l system with maximum gross oxygen production at 3.95 mg O_2 l^{-1} h^{-1} for 80:20% dilution compared with 2.90 mg O_2 l^{-1} h^{-1} for 50:50% dilution. Maximum net oxygen production

was 6.53 mg O_2 l^{-1} h^{-1} for 80:20% dilution compared with 3.00 mg O_2 l^{-1} h^{-1} for 50:50% dilution. Oxygen production in the units containing 20:80% dilution fluctuated primarily below 1.20 mg O_2 l^{-1} h^{-1}.

12) Results indicate that there may be considerable potential for modified operating protocols that could offer improved performance in variable climate pond systems. With the proposed operational design using dilution achieved by the carry-over of treated wastewater, it would be possible to maintain normal loadings over the winter months but have the option of a reduced pollutant load in the pond system for when the critical spring warm-up period occurs.

Experimental ponds at Lockerley

14) Two trial modes of operation in pilot-scale ponds at Lockerley, UK proved reasonably successful. Phase 1 demonstrated that a storage time of six months was adequate to produce an effluent of quality likely to be suitable for river discharge in February. In Phase 2, results of a continuous discharge system through the summer proved it was possible to meet UWWTD standards of 25 mg l^{-1} filtered BOD l^{-1} and 150 mg l^{-1} suspended solids. It is likely that the availability of treated water from Phase 1 initially buffered the impact of a 10-day retention time in Pond 1 but discharge to river may be limited by insufficient nutrient removal during the summer.

15) Results from the light and dark bottle method found strong correlations between oxygen production and both chlorophyll-a concentration and pollutant levels, suggesting the method could be useful in providing a more rapid indication of pond performance.

9.2 Recommendations for further work

In response to the findings obtained from this study, further work is suggested as follows:

1) Further research is needed at full-scale to establish optimum dilution factors for incoming wastewater, optimum organic loadings and hydraulic retention times, as results of the current study lacked the actual hydraulic regime of a pond system and had the problem of artificial conditions present in laboratory scale experiments.

2) Modelling of the factors affecting the capacity for pond treatment at different loadings is required for more quantitative results, i.e. oxygen generation, algal and bacterial biomass, light intensity and temperature during the spring warm-up period.

3) A matrix study using mixed populations of microorganisms normally found in ponds of the effects of light intensity, temperature and dilution would be appropriate to identify the maximum rate of oxidation at the temperature/light observed in spring. This may determine at what temperature/light intensity level, organic loading could become significant to engineering solutions.

4) Sharply continental climates are characterised by cold winters, hot summers and very low precipitation, making water a precious resource. The potential for wastewater reclamation and reuse depends on water quality parameters. Further research into pathogen survival is critical to the proposed operating protocol if the wastewater is to be discharged in spring for the purpose of crop irrigation.

5) An assessment of the economic implications and cost-effectiveness of implementing an operating protocol incorporating dilution into the design is essential for future adoption of the approach.

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