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Washing of wheat straw to improve its combustion properties with energy recovery by anaerobic digestion of the washwater

by

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ABSTRACT

FACULTY OF ENGINEERING AND THE ENVIRONMENT
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WASHING OF WHEAT STRAW TO IMPROVE ITS COMBUSTION PROPERTIES WITH ENERGY RECOVERY BY ANAEROBIC DIGESTION OF THE WASHWATER

Syazwani Idrus

Wheat straw is a major potential source of waste biomass for renewable energy production, but its high salt content causes problems in combustion. Work was undertaken to evaluate straw washing as a means of reducing the alkali index of the straw by washing out light metal cations, primarily potassium. In addition to loss of salts, organic matter is also washed out of the straw and this is a potential source of energy through anaerobic biodegradation to produce methane as a fuel gas. The rate of washout of both potassium and organic carbon was dependent on the temperature of the washwater, although cold water washing could reduce the alkali index to a suitable level, after a long retention time. Using this technique an organically dilute washwater was produced with a chemical oxygen demand of around 2.0 g l^{-1}, suitable for a short hydraulic retention time immobilised cell digester. An upflow anaerobic sludge blanket (UASB) was chosen for initial trials, but this was later compared with an anaerobic filter. As a control throughout the experiments digesters were also maintained on a synthetic wastewater which gave a performance baseline against which the activity and methane production potential of the UASB digesters could be judged. Initial trials showed an accumulation of potassium in the granular sludge bed and an initial apparent drop in the specific methane production and COD removal efficiency. This could be recovered and the potassium washed out of the bed by switching the feed from wheat straw washwater (WSW) to synthetic sewage. Repetitive cycling between these two substrates did not damage or disrupt the digestion process. When allowed to stabilise on WSW alone the COD removal was around 83% and the specific methane production was 0.216 l g^{-1} $\mathrm{COD}_{\mathrm{added}}$ under pseudo steady state conditions. The accumulation of potassium also stabilised at around 11 mg g^{-1} granule wet weight. Under these conditions the organic loading rate could be increased to ~3 g COD l^{-1} day^{-1} without adversely affecting digester performance. Whilst operating on wheat straw washwater the conversion of COD to methane compared to the stoichiometric potential was less than that seen for the synthetic wastewater in the same digester with the same granular biomass. It is hypothesised that a proportion of the carbon converted is used in maintaining the osmotic integrity of the cells by a metabolically-linked potassium transport system. Evidence to show intracellular accumulation of potassium was provided by transmission electron microscopy coupled to EDX analysis of granular sections. Complementary studies were carried out to determine the resistance to salt toxicity of two different types of anaerobic inoculum, taken from a mesophilic municipal wastewater biosolids digester and a saline estuarine mud, for comparison with the UASB granules. Both of these inoculums had a higher tolerance to both Na and K than the granular material, and the wastewater plant digestate was used to further acclimate a

dispersed growth inoculum to seed an anaerobic filter. In the acclimatisation, which was carried out in semi-continuous fed stirred tank digesters, the digestate successfully acclimated to 10 g l^{-1} of KCl, NaCl and a mixture of the two salts. When operated at the same loading on either synthetic wastewater or WSW there was no difference between performance of the UASB and anaerobic filter in either COD removal efficiency or specific methane production.

A simple energy balance was conducted taking into account only the energy required for heating washwater to reduce the washing time necessary to meet the alkali index for the straw. This would, however, consume most of the energy produced by anaerobic digestion of the washwater even when other energy consuming activities were not considered.

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Declaration of Authorship

I, Syazwani Idrus declare that the thesis entitled **Washing of wheat straw to improve its combustion properties with energy recovery by anaerobic digestion of the washwater** and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
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- Parts of this work have been presented and published as conference and journal paper:

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Definitions and Abbreviations

AD	Anaerobic Digestion
AF	Anaerobic Filter
AFFR	Anaerobic fixed film reactor
ADP	Adenosine Disphosphate
ATP	Adenosine Triphosphate
CSTR	Continuously Stirred Tank Reactor
CWW	Cold Water Wash
DEFRA	Department of Farming and Rural Affairs
DFAFBR	Down flow anaerobic fixed bed reactor
EDX	Energy Dispersed X-Ray
EPS	Extracellular Polymeric Substances
EU	European Union
HHV	Higher Heating Value
HRT	Hydraulic Retention Time
LHW	Liquid Hot Water
MBR	Membrane Bioreactor
OLR	Organic Loading Rate
COD	Chemical Oxygen Demand
SEM	Scanning Electron Microscope]
SMP	Specific Methane Production
SW	Synthetic Sewage
TAN	Total Ammonia Nitrogen
TDS	Total Dissolved solid
TS	Total Solids
UAF	Upflow anerobic filter
UASB	Upflow Anaerobic Sludge Blanket
UASBF	Upflow sludge bed filter
VFA	Volatile Fatty Acid
VS	Volatile Solids
WSW	Wheat straw washwater

1. Introduction

1.1 Background

The use of biomass resources for energy production is a rapidly developing area due to increasing awareness of both the limited extent of fossil fuel reserves and the emission of CO_2 during fossil fuel combustion (Cardu and Baica, 2003). Primary biomass residues comprise agricultural and forestry wastes and by-products from downstream processing, and include materials such as wood, straw, husks, seed cases, non-edible vegetative material, pulps, liquors and food residues. A classification of biomass fuels is presented in Table 1.1.

Table 1.1 Classification of biomass fuels (Source: Khan *et al.,* 2009)

Classification	Source
Primary residues	By-products of food crops and forest products (wood, straw, cereals, maize etc.).
Secondary residues	By-products of biomass processing for production of food products or biomass materials (saw and paper mills, food and beverage industries, apricot seed etc.)
Tertiary residues	By-products of used biomass derived commodities (waste and demolition wood etc.).
Energy crops	Specifically cultivated for energy purposes

Straw from cereal production has attracted attention for many years as a potential biomass fuel source, and there is interest in using it as a combustible fuel in both large and small-scale plant for heat and power production. The abundance of straw, its short rotation period and intensive culture systems have led to considerable interest from researchers in exploring efficient methods for its utilization as an energy source (He *et al.,* 2008).

A study conducted by Bloomberg New Energy Finance (2010) estimated the potential of different biomass residues that are available for energy conversion in the EU. For crop residue the main potential is from wheat straw which comprised 74 million tonnes year^{-1} out of 180 million tonnes year^{-1}. Barley straw and rye residues comprised 26 and 6 million tonnes year^{-1} respectively. The combined value for wheat, barley and rye residues is thus equivalent to 60% of the total crop residues potential.

In the UK, according to Defra Statistics: UK Cereal Production Survey (accessed 18 May 2012), of 12.2 million tonnes of straw (wheat, barley, oats and oil seed rape), more than half is from wheat which comprised 7.7 million tonnes. Based on the findings from Biomass Futures project, it is estimated that in the UK more than 5 million tonnes of straw (dry matter), equivalent to approximately 15 million tonnes wet weight, will be available by year 2020 (Elbersen *et al.*, 2012). One straw-burning power station in East Anglia, UK uses 210,000 tonnes of straw per year to produce 38 MW, which can supply power to 80,000 homes (Stoddart and Watts, 2012). In addition to being a source of renewable energy, the use of straw is a means of recycling carbon and thus reducing Greenhouse Gas (GHG) emissions. Straw bales (as shown in Figure 1.1) produced from 1 ha are reported to replace 2.4 tonnes of coal and save over 5,000 kg carbon dioxide (CO_2) (Stoddart and Watts, 2012).

Figure 1.1 Straw Bales in a field near Dunnotar Castle, Aberdeenshire (photo by S Idrus)

The major problem associated with the use of straw in thermal energy production is the low fusion temperature ash which can cause fouling during the combustion process. The high salt content, particularly K and Cl, can also give rise to acidic gases with associated boiler corrosion problems. Cl plays a shuttle role in the formation of alkali sulphates and alkali silicates which results in increased corrosion and deposit formation in the combustion furnace. Furthermore, Jensen *et al.* (1997) concluded that higher gas temperature can result in a higher amount of direct alkaline condensation on the probe and lead to the increase of hard deposits.

Jensen *et al.* (2001) reported that the inorganic components in wheat straw comprised Potassium (K) (0.5-1.0%) with smaller amounts of magnesium (Mg), Calcium (Ca), Sodium (Na), Sulphur (S) and Phosphorus (P). Nutalapati *et al.* (2007) reported that Ca and Na were present at 0.43% and 0.05% respectively on average on a dry weight basis.

Pre-treatment techniques such as washing can improve the quality of the straw for thermal processing by reducing the concentrations of the salts K and Na as well as Cl (Jensen *et al.* 1997). Sander (1997) reported that the leaching process allows rapid removal of K because it is not associated with the structural components of plants. Jenkins *et al.* (1998) also showed that large fractions of K, Cl and P in straw can be removed by water, and that most of the divalent and monovalent cations are water soluble or ion exchangeable. Capablo *et al.* (2009) found that 40% of K was removed from rice straw after 15 minutes submergence. Dayton *et al.* (1999) and Knudsen *et al.* (2004) demonstrated straw washing with water as pre-treatment technique. Results showed 90% of K can be removed from straw by applying this method.

Although salts can be removed from straw in a relatively simple process, the saline wheat straw washwater (WSW) obtained requires treatment before disposal as in addition to the leached salts it is also rich in organic matter. Anaerobic digestion (AD) has been identified as an option for treatment of this washwater, as it has a low operating cost, low sludge production, and the methane produced could be used as an energy source in a fully integrated process of washing, drying, pelletisation and combustion.

Several studies have looked at the application of anaerobic treatment for the removal of organic pollutants in highly saline wastewaters, including the treatment of tannery wastewater, seafood processing effluent, fishery and fish farm wastewater effluent. The presence of excessive amount of salts, particularly Sodium, has been revealed as inhibitory for anaerobic wastewater treatment at concentrations of more than 10 g L^{-1} due to the effect of osmotic shock (Lefebvre and Moletta, 2006a).

The toxic effect of high Na concentrations has been reported by many researchers, but little attention appears to have been given to the effects of high concentrations of K, which is the salt most likely to contribute to salinity in straw washings. Kugelman and McCarty (1965) and Mouneimne *et al.* (2003) investigated the toxicity of K in an acetate batch assay test, while Fernandez and Foster (1994) demonstrated the threshold of K inhibition using glucose feed substrate in batch study.

1.2 Aim and objectives

The overall aim of the work was to determine the effectiveness of washing for removal of salt from wheat straw as a pre-treatment to its thermal conversion, with evaluation of the possibility of using anaerobic digestion to recover energy from the washwater produced.

The work was divided into a number of experiments with specific objectives which developed sequentially throughout the course of the research. These objectives were:

- To determine the mass balance for light metal cations and anions in raw wheat straw, washed wheat straw and wheat straw washwater, and the dependency of the partitioning of these on the duration and temperature of the wash.
- To determine the biodegradability and methane potential of wheat straw washwater (WSW) using UASB digesters. To ensure the reliability of the experimental approach by using control digesters run on a tried-and-tested

synthetic wastewater the characteristics of which were known in terms of its biodegradability and specific methane potential.

- To assess the effect of increasing the strength of the WSW on the performance of the UASB digesters in terms of methane production, COD removal and K accumulation.
- To test the performance of a UASB digester under longer-term continuous exposure to WSW and determine if the accumulation of K leads to digestion failure or whether a longer-term steady state condition could be achieved.
- To assess the effect of increasing the concentration of K in synthetic wastewater fed to a UASB digester in order to determine whether the adverse effects seen on feeding with WSW were due to the Potassium content rather than any other potentially inhibitory components in the WSW.
- To use Energy Dispersed X-ray (EDX) spectra analysis and electron microscopy to identify surface accumulation of light metal cations and also to examine the inside of granules by taking cross sections of them.
- To show the kinetic for sorption of the Potassium contained in WSW onto anaerobic granular sludge and to quantify the sorption capacity of the granular sludge.
- To assess whether anaerobic sludge taken from a variety of sources could be acclimated to relatively high concentrations of Sodium and Potassium and whether a dispersed growth sludge acclimated in this way would be a suitable inoculum for an anaerobic filter.
- To compare a UASB digester with an anaerobic filter for the treatment of WSW at high loading rates and to establish the highest methane production that could be achieved in either of these types of digester.
- To evaluate the results to obtain a preliminary carbon and energy balance based on the energy input into washing of the wheat straw and the energy output from the two products, WSW and wheat straw fibre

2. Literature Review

A literature review was carried out to identify the current state of the art and the key issues relevant to the research. The following topics are discussed, in 12 main sections.

In section 2.1 the review briefly summarises the main problems related to ash deposition and fouling, which lead to the requirement to remove salts from wheat straw. Issues considered in section 2.2 include relevant properties of biomass and the indices used to determine its suitability for combustion, as a basis for determining the required performance for the washing techniques to be developed. Previous reports of leaching techniques and their performance are also considered, in section 2.3.

After a brief overview of the anaerobic digestion process in section 2.4, the next section of the review considers the main concepts and operating parameters associated with upflow anaerobic sludge blanket reactors, as the selected technology (Section 2.5). A considerable amount of attention is then given to the mechanisms of granulation and the effect of cations on formation and maintenance of granules in section 2.6, as this is potentially a key issue for digestion of saline washwaters.

The review then considers past experience in anaerobic digestion of saline wastewaters (section 2.7), including specific studies on potassium inhibition as this ion appears likely to be abundant in the washwater.

This is followed by a more general section on osmoregulation in micro-organisms that provides a background to the potential causes of instability and loss of digestion performance (section 2.8). Section 2.9 considers the speciation and interaction of salts, with respect to possible mechanisms for the alleviation of toxicity. This leads on to section 2.10 on the theory of sorption which is also of potential relevance to the accumulation of salts in UASB digesters.

A separate section considers the use of microscopic techniques as a means of understanding the changes occurring in UASB granular sludge when exposed to saline environments (section 2.11).

The review ends with a summary of the main conclusions and their relevance to the proposed research, in section 2.12.

2.1 Problems of ash deposition

Ash is defined as the inorganic incombustible part of fuel which is left after complete combustion, and usually contains K, Mg, Ca, Na, S and P. Ash deposition in the furnace chamber from combustion of low quality biomass can lead to loss of efficiency of in heat transfer and to unscheduled plant shut-downs Baxter (1993). The mechanisms of ash deposition are divided into condensation and chemical reactions such as that of S or Cl with fly ash; and particle impaction and diffusion as in the case of deposits on water walls (Miles *et al.*, 1996). All types of biomass fuels tend to cause fouling, but the rates depend on ash content. The problem with straw as compared to wood; at the same fuel firing rate, is that there is more ash produced than during combustion of wood due to the unfavourable ash composition of straw (Jenkins *et al.*, 1998). The forms of ash deposition are summarised in Table 2.1

Table 2.1 Form of ash deposition and its effect on the combustion process (Sources: Baxter 1993; Jenkins *et al.*, 1998; Baxter *et al.*, 1998; Obernberger and Thek, 2004; and Khan *et al.*, 2009).

Form of deterioration	Factor influenced	Effect on combustion process
Alkali silicates	Produced by reaction of K and Silicon (Si)	Developed heavily sintered and fused glassy deposits on super heater surface thus lower the melting point.
Slagging on the furnace wall	Radiated heat transfer is dominant	Disrupt fuel feeding and ash removal process. Can cause bridging across tube bundles and partial melting of the ash.
Fouling	Behaviour of component during gas cooling down	Retardation during heat transfer as a result of low thermal conductivity and high reflectivity. Sticky ash particles on heat exchanger tubes.
Corrosion	Salts from the tube wall react with a component from an ash deposit or flue gas	Gradual destruction of the boiler tubes and other surfaces.
Erosion	The impact of hard particles on tube surfaces, and is aggravated by partial blockage due to fouling deposits (occurs in convective part due to high velocity)	Development of bridge across tube surfaces and increase in the combustion side pressure drop. Can reduce flow through the convection pass.

Lopez *et al.* (2004) described the steps of slagging and fouling occurrence which are initiated by the release of volatile alkali salts: formation of aerosols and fly ash; transport of ash species from bulk gas to heat transfer surfaces; adhesion of ash species to heat transfer surfaces; and finally, build-up, consolidation, and shedding of deposits as illustrated in Figure 2.1.

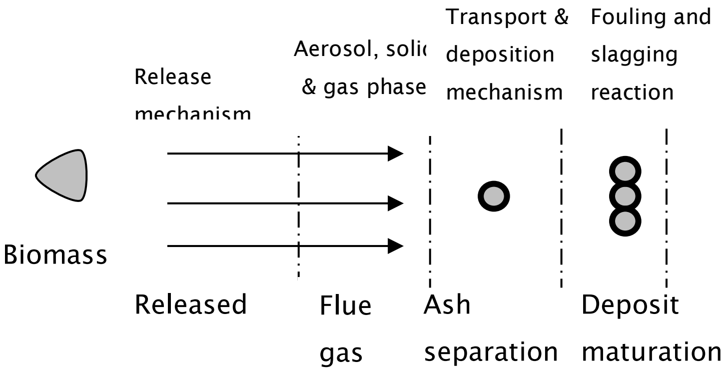


Figure 2.1 Ash Formation and Deposition Phenomena (Lopez *et al.,* 2004)

Several studies have reported on the problems associated with combustion of wheat straw in existing power plants. Excessive quantities of K, Cl and S may cause formation of deposits (Baxter, 1993; Baxter *et al.*, 1998 and Jensen *et al.*, 1997), corrosion of superheaters (Nielsen, 2000, Nutalapati *et al.*, 2007 and Nielsen *et al.*, 1995) and fouling and agglomeration (Khan *et al.*, 2009), low ash fusion temperature in the combustion chamber which vulnerable to slagging due to accumulation of clinker around the heat transfer pipes and leads to the corrosion of furnace components (Jensen *et al.*,2000).

2.1.1 Behaviour of alkali and chlorine

There are two major sources of the above mentioned problems which include (1) The reaction of alkali (Potassium) with silica to form alkali silicates at low temperature (2) The reaction of alkali (Sodium) with sulphur to form alkali sulphates; where K is the dominant source of alkali in most biomass fuels. The formation of alkali silicates and sulphates at low temperature (can be below 700°C) provides a sticky surface which can expedite ash deposition. The combination of alkaline earth salts like Ca with K reacts with silicates deposited as fly ash to form molten glassy phases leading to hard sintered structures (Obernberger *et al*, 2004).

At high temperature K acts as a glue gas which makes Si, Mg and Ca stick on the furnace probe and super heater coils. In addition, part of these compounds can also condense on the fly ash thus affecting the heat exchanger. This phenomenon is explained by the fact that organic compounds have a larger impact and stick on the furnace probe and superheater coil at high temperature (Baxter, 1993).

According to Miles *et al.* (1996) the reaction between alkali and alkaline earth metals, particularly K and Na, with S and Si is facilitated by Cl which is thus responsible for many undesirable reactions in combustion furnace and power boilers. Cl appears to play a shuttle role, facilitating the transport of alkali from fuel to surface to form sulphates or silicates where it can increase the volatility (or mobility) of K and Na and release them as KCl and NaCl. Furthermore, Cl has the ability to dictate the amount of alkali vaporized during combustion and this may have a stronger influence than the alkali concentration itself. In some cases Cl can combine with K to form KCl which is a high-temperature stable, gas-phase, alkali-containing species. In the absence of chlorine, alkali hydroxides are the major stable gas-phase species in moist and oxidising environments (Jenkins *et al.*, 1998). Therefore, the removal of Cl from fuels is particularly attractive due to its role as a facilitator in alkali deposition, as a contributor to corrosion, and as an agent of toxic emission formation during thermal combustion (Bjorkman and Stromberg, 1997).

Khan *et al.* (2009) investigated the behaviour of alkali and Cl during the combustion process and found that particles collected at 900 °C contained K and S while those collected at 560 °C contained only Cl, implying that Potassium Sulphate (K_2SO_4) nucleation occurs at higher temperature and KCl condenses at lower temperatures. This phenomenon also described the competitive behaviour of C and S in detaining K vapours at the exit of a combustion chamber. In this process S acts first, since the formation of K_2SO_4 occurs at higher temperatures, followed by Cl which condenses as KCl.

Besides their adverse effects on the combustion system, a further impact of alkali salts is that they are observed to produce pollutant emissions (Jenkins *et al.*, 1998). It is therefore clear that the present research has a valuable role in reducing these to enhance the value of the biomass for thermal processing.

2.1.2 Ash deposition control indices

Easterly and Burnham (1996) suggested the use of a biomass type ratio [(Na_2O+K_2O/SiO_2] to as a measure of the risk of ash deposition associated with a given biomass fuel. Values above 2 require special precautions to avoid fouling problems; below 0.2, erosion may occur.

An alternative measure which has become popular is known as alkali index .The alkali index is a threshold indicator (as shown in Table 2.2) for fouling and slagging and expresses the quantity of alkali oxide in fuels in the unit of kg alkali GJ^{-1}. The alkali index can be calculated as follows:

$$\text{Alkali index (kg alkali } GJ^{-1}) = (1/HHV) \times [\text{ash } (K_2O\text{ash} + Na_2O\text{ash})] \qquad \text{Equation 1}$$

Where HHV = higher heating value – value obtained from bomb calorimeter based on volume of dry basis (Jenkins *et al.*, 1998)

Table 2.2 Threshold of alkali index (Source: Miles *et al.*,1996)

Alkali Index (kg Alkali GJ^{-1})	Severity
> 0.17	Fouling is probable
> 0.34	Fouling is virtually certain to occur

2.2 Combustion properties of biomass

2.2.1 Energy value of biomass

Demirbas *et al.* (2004) described how biomass can be categorised by breaking it down into structural components (known as proximate analysis) or into chemical elements (known as ultimate analysis). Examples of these two approaches are shown in Table 2.3 for some typical biomass fuels including wheat straw and for coal.

Table 2.3 Properties of biomass and coal (Source: Khan *et al.*, 2009)

	Proximate Analysis			**Ultimate Analysis**						
	Moisture %	Volatile %	Ash %	C %	H %	O %	N %	K_2O %	S %	Cl %
Wheat straw[a]	13.9	77.9	6.8	56.6	6.7	48.8	1.0	30.0	0.2	N.R
Wood pellets	4.9	80.4	0.2	45.5	6.6	47.7	BDL	16.8	BDL	BDL
Sunflower pellets	11.2	65.2	8.2	44.1	5.17	34.6	0.5	22.8	0.1	0.1
Bituminous coal[b]	4.9	32.3	14.7	65.7	5.6	7.7	1.2	2.3	0.5	BDL

BDL – below detection limit

NR – Not Reported

Units - % dry fuel

[a b] Ashing at 815° C

In earlier work Demirbas (1997) suggested a formula for estimating the higher heating value (HHV) using ultimate analysis data. The HHV (MJ kg^{-1}) can be calculated from:

$$HHV = \{33.5 [C]+142.3[H] -15.4[O]-14.5[N]\} \times 10^{-2} \qquad \text{Equation 2}$$

Equation (2) was derived by considering the oxidation heats of C and H and the reduction heat of O, assuming the effect of the N content of a biomass fuel on HHV is negative. Therefore, the HHV for wheat straw, wood pallets, sunflower pallets and bituminous coal are 20.83, 17.29, 16.73 and 28.61 MJ kg^{-1} respectively.

From the viewpoint of heating value, as indicated by proximate and ultimate parameters, wheat straw and other biomass show lower heating values as compared to coal. This is due to the higher percentage of carbon in coal, which increases the heating value, while oxygen decreases it. The moisture content in biomass tends to cause ignition issues and reduce combustion temperature, requiring high fuel usage and generating large amounts of flue gas.

The heating value of biomass can be correlated with ash concentration, where each 1% increase in ash translates roughly into a decrease of 0.2 MJ kg^{-1}. The heating value can also be correlated with carbon concentration with each 1% increase in carbon elevating the heating value by approximately 0.39 MJ kg^{-1} (Bridgwater, 2003).

2.2.2 Salt content

High concentrations of K and Cl in wheat straw have been reported by several researchers as summarised in Table 2.4.

Table 2.4 Potassium and chlorine in wheat straw

Potassium (% of dry weight matter)	Chlorine (% of dry weight matter)	Source
0.5-2	0.2-1.0	Sander *et al.* (1997)
0.2-1.9	0.58-1.76	Hernandez Allica *et al.* (2001)
0.5-1.3	0.2-0.7	Jensen *et al.* (1997)
0.91	0.3	Nutalapati *et al.* (2007)
20 $^{\text{(ash)}}$	3.6 $^{\text{(ash)}}$	Demirbas (2004)
21.7$^{\text{(ash)}}$	1.8$^{\text{(ash)}}$	Miles *et al. (*1996)
1.2	0.41	Zolin *et al. (*2001)
1.7	0.25	Baxter *et al.* (1998)
15000 (mg kg$^{-1\ \text{dry}}$)	0.31	Ronald *et al.* (2008)
25.6$^{\text{(ash)}}$	0.23	Jenkins *et al.* (1998)

According to Miles *et al.* (1996), wheat straw typically contains 1.66 kg alkali GJ^{-1} while Ronald *et al.* (2008) and Jenkins *et al. (*1998) reported at 0.9 kg alkali GJ^{-1} and 1.07 kg alkali GJ^{-1} respectively. Sander (1997) concluded that there were correlations between the K and Cl contents in straw and the soil type, K fertiliser dose and geographical location. In particular for wheat farms there was a strong correlation between the chlorine content in the straw and the supply of chlorine in fertiliser. If the straw is left lying in the field after harvesting, however, rain may leach significant amounts of K and Cl from it (Sander, 1997).

Ash can be defined as inorganic incombustible part of fuel which is left after complete combustion and can be inherent in the fuel or added to the fuel during processing steps. Table 2.5 shows the typical elemental composition of the straw in ash form, which indicates the presence of SiO_2 and K_2O are among the highest as compared with other components. These two components typically result in low ash melting temperature while MgO and CaO have been identified as increasing the ash melting temperature. Melting temperature is as essential characteristic to avoid sintering or slagging (resulting in deposits on boiler tubes) (Khan *et al*, 2008).

Table 2.5 Ash analysis of wheat straw

Dry Basis (wt %)	Zolin *et al.* (2001)	Miles *et al.* (1996)	Khan *et al.* (2009)	Jenkins *et al.* (1998)
K_2O	21.0	21.7	30.0	25.60
CaO	7.8	4.91	17.7	6.14
SiO_2	57.0	37.06	53.1	55.32
SO_3	2.70	4.44	N.R	4.40
Fe_2O_3	0.10	0.84	1.2	0.73
Na_2O	0.11	9.74	4.5	1.71
MgO	1.20	2.55	3.0	1.06

N.R : Not Reported

2.3 Leaching techniques

Leaching is one of the most promising techniques to reduce the concentrations of alkali salts and improve the ash fusion temperature which is the main influence for fouling by biomass fuels. Several methods can be used for washing wheat straw, including hand spraying treatment over a straw bed, pouring water through the wheat straw and submerging the wheat straw in water for certain residence time. Jenkins *et al.* (1998) demonstrated three leaching techniques on rice and wheat straw which were conducted at room temperature (20-25ºC). The study revealed, however, that hand spraying techniques are not likely to be effective due to the limited quantity of water used. For the leaching of wheat straw, only 3.5% of the ash content was reduced on 1 minute of hand spraying. Flushing and submerging in tap water were found to be more effective which removed about 4% and 6 % of ash respectively.

Baxter *et al.* (1998) stated that K concentrations are higher in plant materials where it occurs primarily as a facilitator of osmotic processes. They also discussed K modes of occurrence in wheat straw and suggested that 80-90% of K is water soluble or ion exchangeable.

Precipitation can also be considered as a natural washing process. This phenomenon has been investigated by several researchers, and it has been noted that rainfall intensity seems to be correlated with relative decrease in Potassium content. Sander (1997) examined the effect of rainfall on a range of biofuels lying in the field, including barley, rye, rape and wheat straw, and discovered rain after harvest leached significant amounts of K and Cl as these elements are not associated with the structural components of plants. In contrast, the amount of Ca in wheat straw was not affected by rainfall. Sander (1997) also found that 100 mm of accumulated rainfall was sufficient to remove approximately 80% of the initial K content of barley straw lying on the field; whereas Hernandez Allica *et al.* (2001) discovered 150 mm of accumulated precipitation was able to decrease the K in wheat straw by about 60% - 68% and 82% of K removed in barley straw at 163mm of accumulated rainfall. This study revealed a correlation between the electrical conductivity K and Cl after wheat straw and barley straw were exposed to the rainfall. The analysis of the ash content indicated reduction in minerals (as % of dry matter) after washing, with an initial value of 11% and a final ash content of 6.27%. In addition to reducing the K, Cl and ash content, washing of wheat straw was found to improve the heating value from 17.83 to 18.57 MJ kg^{-1}.

While the above studies provide useful information, it appears that little work has been carried out to determine mass balances for light metal cations and anions or their partitioning in raw wheat straw, washed wheat straw and wheat straw washwater depending on the duration and temperature of the washing process.

2.4 Anaerobic digestion

2.4.1 Brief overview of the anaerobic digestion process

Anaerobic digestion (AD) can be defined as a series of processes that involve microorganisms breaking down biodegradable material in the absence of oxygen to produce methane and carbon dioxide (biogas). Biogas production from anaerobic digestion is widely used as renewable energy source to replace fossil fuel, and the nutrient-rich solids left after digestion can be used as a fertiliser.

Demirel and Scherer (2008) and Gerardi (2003) explained anaerobic digestion as the degradation of particulate organic material (carbohydrates, proteins, lipids) which are first hydrolysed to organic monomers (the hydrolysis process). This reaction produces sugars, amino acids (utilised by fermentative organisms) and fatty acids (utilised by anaerobic oxidisers) which are converted during the acidogenesis process into hydrogen or intermediate products such as propionate and acetate, as shown in Figure 2.2. Acetogenic bacteria play roles in converting the products of acidogenesis to acetate and hydrogen (acetogenesis). The final step is methanogenesis which may include (1) acetoclastic based on the use of acetate (2) hydogenotropic through the use of hydrogen and carbon dioxide and (3) methyltropic through the use of methanol. Degradation rates in all stages can provide an indicator of an efficient digestion process.

Five metabolic groups represent the anaerobic digestion process: the hydrolytic fermentative bacteria, proton-reducing acetogenic bacteria, hydrogenotrophic methanogens, and acetoclastic methanogens (Gerardi, 2003).

In evaluating the performance of anaerobic digestion, Demirel and Yenigun (2006) compared the application of one phase (conventional) and two phase systems. In a one phase system, acid and methane formation take place in a single reactor. This may create an imbalanced environment due to differences in the physiology, nutritional needs and growth kinetics of different groups, leading to the accumulation of volatile fatty acids (Mata Alverez *et al.*, 2000).

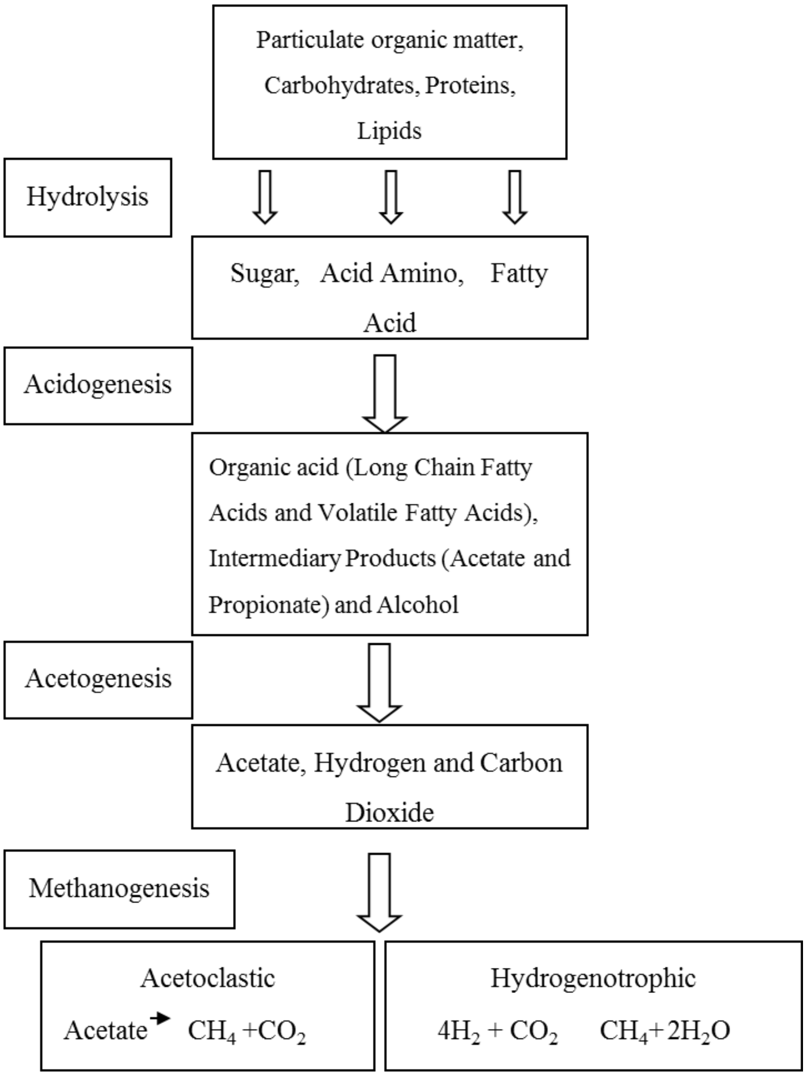


Figure 2.2 Anaerobic conversion of biomass to methane (Source: Demirel and Scherer, 2008 ; Gerardi, 2003)

2.5 UASB reactors

2.5.1 Main concept and operational parameters of UASB reactors

During the operation of UASB, wastewater (feedstock) flows through a sludge bed in the bottom of reactor, where physical and biochemical process play a role in retaining and degrading organic substances which are acidified and then converted into methane and other biogas components. The sludge bed also acts as filter to suspended solids which can extend the residence time and thus achieve high rate Chemical Oxygen Demand (COD) removal (Ruiz *et al.*,1998).

The sludge bed (known as granular sludge) is originally formed by accumulation of incoming suspended solids and bacterial growth. It is very important to retain a good settling velocity in order to enable good treatment performance, as the treatment capacity of UASB reactors depends on the amount of active biomass retained (Seghezzo *et al.*, 1998). Previous studies have shown how operational parameters such as temperature, hydraulic retention time, organic volumetric loading rate can affect methane production and COD removal efficiency (Sánchez *et al.*, 2005)

The concept of the upflow anaerobic sludge blanket (UASB) reactor was developed in the 1970s and is based on retaining the active biomass in contact with wastewater for an efficient treatment (Lettinga *et al.*, 1980). UASB reactors have several advantages over conventional continuously-stirred tank reactors, including their compactness, low sludge production and low operating costs. The application of UASB has been highly successful for easily hydrolysed substrates, including domestic wastewater, sugar industry wastes, distillery wastes, and brewery wastes (Blonskaja *et al.*, 2003, Fang *et al.*, 1995, Lettinga and Pol, 1991).On the other hand substrates which include phenols, and effluents from the food industry including milk and slaughterhouse wastewaters have been identified as difficult to treat. The disadvantages of UASB systems have been described as long start-up time and require for post treatment at certain circumstances, as well as poor gas production, susceptibility to shock loading, inability to form self-immobilized bacterial granules in some cases (Blonskaja *et al.*, 2003).

2.5.2 Operational requirements and conditions for UASB reactors

UASB granules can be defined as concentric layers of near-spherical biofilm retaining different bacterial population groups or trophic groups that produce biomass and extracellular polymeric substances in their vicinity, thus allowing other trophic groups to attach and gain nutrition (Henze and Harremoes, 1983). According to Lettinga *et al.* (1993); Schmidt and Ahring (1996) and Show *et al.* (2004) granules may range in size from 0.1 to 2 mm and are distinguished from flocculated sludge by their higher shear strength. Granulation has a significant role in determining the performance of a UASB reactor. The improved settling properties of granular sludge allow higher hydraulic loading rates, while granulation also reduces the limitations on inter-species mass transfer between syntrophic groups. Furthermore, microbial aggregates or granules are more resistance to sudden physical changes or process inhibitors due to their ability to withstand high gas and liquid shear stresses without disintegrating into dispersed microorganisms.

Nutrient requirements for the UASB process have been discussed by Ahn *et al.* (2006); Chen *et al.* (2008) and Veeresh *et al.* (2005). Operational conditions have also been investigated including the effects of pH and alkalinity, and strength and composition of influent. The pH value can represent the current state of reactor and can be the indicator for formation of volatile fatty acid. In order to obtain a good quality of granular sludge, a stable pH is required between 6.3-7.8.

Henze and Harremoes (1983) reported that operational temperature can affect the methanogens more than the acidogens in fixed film processes. From the point of view of start up time, mesophilic granules have a shorter start up time and stabilise more quickly as compared to thermophilic granules.

Shear due to upflow velocities > 1 m h^{-1} can lead to granules disintegration and washout of microbial aggregate from the reactor. Too much gas production at high organic loading rate may also results in shearing off of cells from the granule surface contributing to granule erosion (Syutsubo *et al.*, 1997) .

Hollow granules with a grey and white colour have been reported to appear in effluent (Kosaric *et al.*, 1990). This phenomenon is caused by poor settling velocity. The hollow core section becomes filled with gas produced by the anaerobic microbial culture and separates from the granular mass. Effluent recirculation can prevent the granules from maintaining this hollow core and consequently from floating out of the reactor. The hollowing of the granule can also be correlated with the size of granules. Since the feed can only penetrate through a diffusion process, when the size of the granule exceeds a certain limit the amount of feed reaching the middle of the granule is small. As a result, the microbial population at this location suffers starvation and gas may be entrapped thus decreasing the density of granules which as a consequence may float (Kosaric *et al.*, 1990).

2.6 Mechanisms of sludge granulation

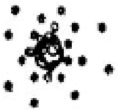
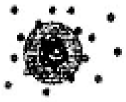
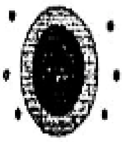
2.6.1 Overview of granulation in UASB reactor

The implementation of UASB treatment requires proper consideration of the aspect of granulation. Previous studies have attempted to explain the theory of granulation process. Three main mechanisms involved during granulation process were reversible adsorption by physico-chemical forces, irreversible adhesion of cells by microbial polymer, multiplication of cells and finally development of granules(Schmidt and Ahring, 1996, Tiwari *et al.*, 2006 and Hulshoff Pol *et al.* 2004b).

The adhesion of cell to cell was reported to be the mechanism promoting the production of an external layer of extracellular polymers which are excreted or exposed under certain physiological conditions. Extracellular polymeric substances (EPS) are high molecular weight compounds produced by microorganisms which originate from different sources such as biological synthesis and excretion. EPS can also develop from lysis of bacterial cells and the adsorption of organic matter from the incoming wastewater, e.g. cellulose and humic acids (Epstein, 2003 and Morgan *et al.*, 1991). The charge surface layer formed by EPS promotes the interaction between cells where it allows adjacent bacteria to aggregate by bridging cell surfaces electrostatically and physically.

Ahn *et al.* (2006) suggested a five-step model for granule biofilm formation as shown in Table 2.6.

Table 2.6 Ahn's proposed model for the anaerobic sludge granulation (Ahn *et al.*, 2006)

Appearance	**Stage**	**Diameter Approximate**
	(A) growth of filamentous (acetoclastic) methanogens and other microorganisms in low hydrogen partial pressure conditions.	Filament
	(B) bridging and rolling effects on the growth of filamentous methanogens. Transport of cells to the surface of an uncolonised inert material or other cells (substratum).	< 100 μm
	(C) growth of a small conglomerate as a loose core; crowded syntrophic acetogens around the surface of the core.	< 1 mm
	(D) growth of a small granule with a dense core; crowded syntrophic hydrogenotrophs and acidogens around the surface of a small granule. Irreversible adhesion of the cells to the substratum by microbial appendages and/or polymers.	1-2 mm
	(E) growth of a large granule with multi-layered structure, due to accumulation of extracellular polymers by hydrogenotrophs. Multiplication of the cells and development of the granules.	2-3 mm

2.6.2 Role of cations in granule formation and maintenance

The cell walls of bacteria are negatively charged and thus cations play a role in binding to the EPS Liu *et al.* (2003a). Carboxyl and amino groups in protein have been identified as the binding groups that link the salts on the surface of bacteria.

Besides linking the EPS, multivalent cations condense the diffuse double layers and enhance flocculation due to Van der Waals forces. The presence of inert solids in the granules can also allow the development of abiotic surfaces to interact with the salts ions and thus enhance the toxicity resistances of biogranules.

Numerous studies have reported on the positive effect of Ca addition in enhancing granulation. Hulshoff Pol *et al. (*1998) discovered that granule formation was stimulated by calcium at concentrations of 100±200 mg l^{-1} and noted adverse effects on granulation at Ca concentrations above 500 mg l^{-1}. Kugelman and McCarty (1965) stated the minimum concentration of Ca in the granules was 200 mg l^{-1} and the upper limit was from 2000 - 5000 mg l^{-1}. Yu *et al.* (2001a) suggested an upper limit for granulation of 1250-6700 mg l^{-1} and an optimal value between 150-300 mg l^{-1} at an influent Chemical Oxygen Demand (COD) of 4000 mg l^{-1}. Influent COD was also found to have a substantial effect on the optimum Ca concentration.

At higher concentrations, however, Ca has been found to have a detrimental effect where biomass concentrations decreased and granules became loose and expanded easily. Excess amounts of calcium can lead to precipitation of calcium carbonate ($CaCO_3$), which can block the intragranular pores and cause cementation of the bed and severe mass transfer limitation (Yu *et al.*, 2001a,b) . $CaCO_3$ precipitation may also result in reduced buffer capacity, scaling of reactor walls and effluent pipework, decreasing efficiency due to sludge washout and occupation of void space by inorganic precipitates, and loss of specific methanogenic activity (Van Langerak *et al.*, 1998).

Besides addition of Ca, Yu *et al.* (2001b) reported improved biomass settlability from the addition of Aluminium Chloride ($AlCl_3$), a leading to lower effluent Volatile Suspended Solid (VSS) concentrations. This phenomenon implies that the presence of $AlCl_3$ enhanced granulation process

2.7 Anaerobic digestion of saline wastewater

Salinity in wastewater is known to lead to low COD removals in anaerobic digestion due to the loss of activity of organisms (Kargi and Dincer, 1996). Concentrations of salt more than 1% were reported to produce inhibitory or toxic effects on bacteria not adapted to high salinity; high salt concentrations (>1%) have been shown to cause plasmolysis and/or loss of activity of cells. Some studies have reported a detrimental effect on COD removal efficiency immediately after addition of salt, due to leading to changes in surface charge leading to alterations in the community (Reid *et al.*, 2006). This finding is consistent with Kincannon and Gaudy Jr, 1968, Kargi and Dincer, 1996 and Campos *et al.*, 2002 who reported that shocking with salt caused a decrease in COD removal.

Several studies have looked at the application of anaerobic treatment by ordinary (i.e. non-halophilic) methanogenic consortia for the removal of organic pollutants in highly saline wastewaters. Habert *et al.* (1997) demonstrated the treatment of inuline effluent using UASB at 10 g l^{-1} of salt at OLR of 23-32 kg COD m^{-3} day^{-1} and achieved 65% - 85% of COD removal rate. Other research which employed non halophilic methanogens was conducted by Rovirosa *et al.* (2004) using downflow anaerobic fixed bed reactors for the treatment of piggery effluent. The study revealed 90% of COD removal at an OLR of 0.5 kg COD m^{-3} day^{-1} at a salt concentration of 15 g l^{-1}. The used of non halophilic groups in an upflow anaerobic filter has been demonstrated by Guerrero *et al.* (1997) during the treatment of seafood processing effluent at 15 g l^{-1} of salts and revealed 83% of COD removal at COD influent of 34 g l^{-1} and OLR of 2.8 kg COD m^{-3} day^{-1}. Furthermore, Boardman *et al.* (1995) also reported on the treatment of seafood processing wastewater using UASB and discovered 77% of COD removal at 7.7 to 26.3 g l^{-1} of salt. The OLR applied in the study was at 13.6 kg COD m^{-3} day^{-1} and COD influent of 1.7 g l^{-1}. Omil *et al.* (1995) investigated the performance of an anaerobic contact system in treating seafood processing at 13.7 – 33.7 g l^{-1} of salts and discovered 70% - 90% of COD removal at COD influent of 20-60 g l^{-1}.

Anaerobic digestion has not worked well in all such cases, however, and there are reports of saline wastewaters being inhibitory in AD systems when employing non-halophilic methanogens. Gebauer (2004) investigated the recycling of fish farm wastewater with an influent COD of 70.1 g l^{-1} and salt concentration at 35 g l^{-1} using a Completely Stirred Reactor (CSTR). This study found only 50% of COD removal rate at OLR of 2.5 kg COD m^{-3} day^{-1}. Feijoo *et al.* (1995) demonstrated the effect of antagonism and the adaptation process in treating seafood processing wastewater in an AD system. This experiment was conducted in a continuous digester by introducing three different anaerobic digestates including; (1) sludge from an AF system after 2 years treating mussel wastewater, (2) suspended biomass from a central digester treating a mixture of seafood processing wastewater and (3) sludge from UASB which previously treated potato processing wastewater. This study found 50% of inhibition at concentration of 3 - 16 g Na l^{-1} but reported adaptation of methanogens to the saline environment, with increased Na tolerance and a shorter lag phase before the onset of methane production.

Mosquera Corral *et al.* (2001) investigated the efficiency of a Hybrid Anaerobic Sludge Bed Filter in treating seafood processing effluent which contained 1-1.5 g l^{-1} of influent COD concentration at OLR of 1.5-2 kg COD m^{-3} day^{-1}. The findings showed about 70%-90% of COD removal at HRT of 18 hrs. Application of an Upflow Anaerobic Sludge blanket at OLR of 0.5 kg COD m^{-3} day^{-1} has also been reported by Lefebvre *et al.* (2006b) during the treatment of tannery wastewater at 71 g l^{-1} of salts and found 78% of COD removal at COD influent of 2.3 g l^{-1}. Vidal *et al.* (1997) investigated the performance of anaerobic filters in treating seafood processing wastewater at salt concentration of 30 g $l^{-1.}$ This study revealed the COD removal efficiencies remained at 70% with an Organic Loading Rate (OLR) ranging from 1 to 15 kg COD m^{-3} day^{-1}. In contrast, a study on the treatment of fishery effluent in CSTR reported only 50% of COD removal at salt concentration of 40 g l^{-1}. This experiment was conducted at OLR of 2 kg COD m^{-3} day^{-1} and COD influent of 6 g l^{-1} (Aspé *et al.*, 1997).

Microorganisms requiring salt for growth are designated halophilic and halotolerant, and have developed more acidic enzymes that require the ion K for optimal activity. Therefore, they can maintain an osmotic balance of their cytoplasm with the external medium by accumulating high concentrations of various organic osmotic solutes (Lai and Gunsalus, 1992). This suggests the potential of using salt tolerant microorganisms in biological saline wastewater treatment. Furthermore, halophilic and halotolerant microorganisms require a high salt concentration, particularly of K, for most biochemical reactions and lower concentrations may lead to cell disintegration (Wood, 2006).

Studies on the treatment of saline wastewater indicate that salt concentration is not the main indicator in determining the level of inhibition. Apart from pre adapted sludge and population of the bacteria, the presence of other cations and OLR also need to be considered. These statements are supported by Feijoo *et al.* (1995) who noted that performance in treating saline wastewater in AD system depends on nutrient in the feedstock, previous adaptation of the sludge, antagonistic or synergistic effect (due to the presence of other cations) and lower substrates to biomass ratio used.

2.7.1 Studies on K inhibition

Numerous studies on saline wastewater have highlighted the role of Na in contributing to inhibition in AD systems. The effect of other salts, particularly K, has been given relatively little attention. Fernandez and Foster (1994) investigated the threshold of K inhibition using glucose feed substrate (batch study) and observed that low concentrations of K (less than 400 mg l^{-1}) facilitated performance in both the thermophilic and mesophilic conditions, while at higher concentrations (greater than 2500 mg l^{-1}) toxicity of K was significant. Mouneimne *et al.* (2003) investigated the toxicity of K in an acetate batch assay test and found the toxicity threshold was 0.43 mol l^{-1}. Kugelman and McCarty (1965) reported that 5.87 g l^{-1} of K caused 50% inhibition of acetate utilizing methanogen. Most of the K toxicity thresholds which have been reported were conducted in batch studies, which implied that further investigation is required in continuous system.

None of the previous research has focused on the potential for anaerobic digestion of washwaters from pre-treatment of straw for thermal combustion, or investigated to what extent this is a reliable source of energy. The salt content in wheat straw washwaters may cause problems during the biodegradation process and this issue will be assessed in the current study. Recent studies have shown that Na can cause inhibition in AD systems. Only a few studies have been conducted to identify the threshold of K inhibition, and most of these were carried out in batch systems rather than in continuous digesters which are more relevant in practice. The limitations of wheat straw washwaters in terms of their low organic carbon and nutrient content, as compared to common highly biodegradable feedstocks, also require further investigation.

2.8 Salt regulation in micro-organisms

2.8.1 Osmoregulation theory

In non halophilic or halotolerant bacteria, intracellular K+ is relatively high (>0.5 M), which implies that under normal conditions these cells exist with a high turgor pressure. Turgor pressure is the pressure by water inside the cell against the cell wall. For instance, when a cell is exposed to a hyperosmotic environment, the influx of water into the cell requires a large turgor pressure to maintain the cell wall and to prevent it swelling or bursting (Brabban *et al.*, 1999 and Roberts, 2004).

In general, accumulation of internal monovalent cations can inhibit various enzymes. Cells in bacteria react by adjusting the flow of solutes across the cytoplasmic membrane. The solutes comprise K+ and organic osmolytes that are also protein stabilisers (amino acid derivatives such as glycine betaine). When extracellular osmotic pressure rises, however, transmembrane water fluxes concentrate the cytoplasmic membrane thus disrupting the cell structure and function. Furthermore, the presence of excessive salt alters many cellular properties which include cell volume (dehydration of bacterial cell), turgor pressure, ionic strength, and crowding of macromolecules in the cytoplasmic membrane (Wood, 2006). Alteration of the concentration of intracellular metabolites can lead to inhibition of various cellular processes. This phenomenon is known as cell lysis (dehydration under high-osmolarity growth conditions) (Kraegeloh *et al.*, 2005)

Microorganisms accumulate cations, including ionic K, and/or low-molecular-weight organic compounds known as compatible solutes from the surrounding medium to achieve an osmotic equilibrium. Most cells also maintain [K+(in)] >> [K+(out)] (Schönheit *et al.*,1984; Sprott *et al.*, 1985). Inhibition or dehydration occurs when K+ in the extracellular solute concentration exceeds K+ in the cell cytoplasm (Lai and Gunsalus, 1992).

2.8.2 Physiological roles and intracellular concentration of Potassium and Sodium in methanogens

Osmoregulation theory describes the function of K+ in the metabolic process in order to regulate intracellular osmolarity and promote proton motive force for adenosine triphosphate (ATP) synthesis. K+ is accumulated by the cells electrogenically as a response to the membrane potential which is created by electrogenic proton extrusion (Bakker and Mangerich, 1981).

Takashima *et al.* (1990) discussed the role of K+ for the growth of bacteria. K+ limitation can lead to restriction of nucleic acid synthesis in the cell wall and thus reduced permeability of its membrane structure. This study also noted that the Na+ and K+ content of the cells consisted of 0.3 to 4.0% and 0.13 to 5.4% respectively.

A comparison of K+ and Na+ showed that the inhibitory activities of these ions were equivalent, whether chloride or acetate salts were used. In vitro nitrogenase enzyme assays showed that at low concentration (100 mol l^{-1}) Potassium glutamate enhanced activity but at higher concentrations this compound inhibited activity; 50% at concentration of approximately 400 mol l^{-1} (Brabban *et al.*, 1999, Mulkidjanian *et al.*, 2008).

There are two energies which can correlate K+ and Na+ in uptake system which consist of:

(i) The transport system between K+ and Na+ for methanogenic bacteria is dependent on each other for optimal activity (Perski *et al.*, 1981).

K+ is accumulated in the cytoplasm of methanogens while Na+ is extruded. A Na+/K+ pump is responsible for moving these ions in opposite directions at a rate of three Na+ out of the cell for every two K+ pumped in. The role of this pump is to maintain the volume of the cell in equilibrium condition. Inside the cytoplasm, organic compounds that are negatively charged are surrounded by positive ions .If the Na+/K+ pump is working well, it should be able to prevent water influx into the cell during hyper osmotic shock, and thus prevent as cell lysis (Mitchell, 1973 and Kashket, 1985).

(ii) K+ transport is coupled directly to ATP hydrolysis

Chemi-osmotic theory says that both Na+ and K+ play important roles for ATP synthesis and nutrient transport. ATP synthesis produces energy for the cell, to be consumed through the synthesis of adenosine triphosphate (ATP). The ATP releases energy by the combination of adenosine diphosphate (ADP) and inorganic phosphate (P_i) (Schonheit *et al.*, 1984).

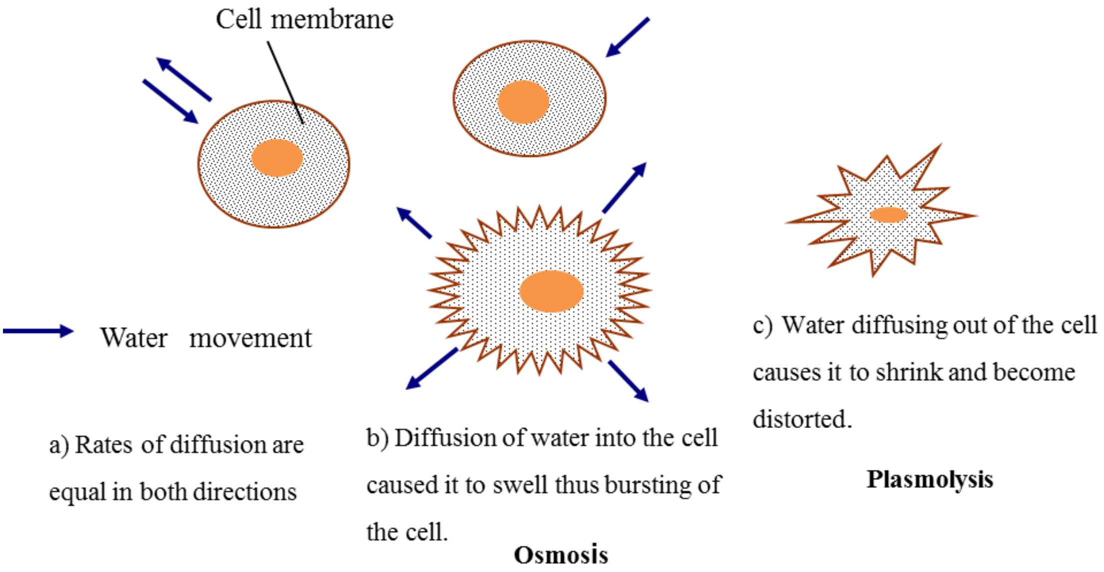
2.8.3 Proton motive force

Inhibitor studies suggest a role for the proton motive force in providing the energy required for a reaction early in methanogenesis. The proton motive force is generated by an electron transport change during oxidation-reduction reactions from an electron carrier to transport hydrogen ion (H+) across the membrane. As the H+ concentration increases, an electrochemical gradient called proton motive force develops. The distribution of protons provides the energy for the synthesis of ATP from ADP and phosphate (Gober and Kashket, 1986).

2.8.4 Changes in cell during exposure to external Sodium chloride

Figure 2.3 shows the effects of hyperosmotic shock after sudden exposure to a high concentration of NaCl which causes water to efflux from the cell. After certain period, the external NaCl concentration decreased allowing an influx of water into the cell, and leading to cell lysis. Diffusion of water into the cell causes it to swell and burst if there is no mechanism to remove the water (Woolverton et al, 2008).

Inhibition due to osmotic shock induces K+(in) to efflux from the cell. In this condition [K+(in)] << [K+(out)] thus promoting a decline in the proton motive force. This alters the concentrations of intracellular K+ and can lead to inhibition of cellular processes (Sprott *et al.*, 1985).



a) Rates of diffusion are equal in both directions

b) Diffusion of water into the cell caused it to swell thus bursting of the cell.

Osmosis

c) Water diffusing out of the cell causes it to shrink and become distorted.

Plasmolysis

Figure 2.3 Changes in cell during exposure to Sodium Chloride (NaCl) (Woolverton *et al*, 2008)

2.8.5 Adaptation to change in Sodium chloride after osmotic shock

From the viewpoint of microbial ecology, a non halophilic or halotolerant inoculum requires adaptation to high salt concentrations. The adaptation of bacteria during the treatment of saline wastewater can occur in two different ways, due to internal changes in the predominant species of methanogens or to a shift in the methanogenic population. If either of these states is achieved during the treatment of saline wastewater, the microorganisms can accommodate gradual increments of salt content which can far exceed the normal limit of inhibitory concentration (Chen *et al.*, 2008). Analyses of *Methanococcus thermolithotrophicus* showed cells respond to changes in external NaCl the adaptation process is indicated in Figure 2.4. After hyperosmotic shock, water effluxes from the cell followed by K+ re-entering the cell and an influx of water into the cell. On a longer period, there is a cycle of influx and efflux of water and K+ from the cell with water moving in the opposites direction to K+, until the intracellular K+ decreases to a new steady-state above that observed for cells grown at a lower NaCl concentration. Therefore the cells begin to readjust to the 'adapted' levels and grow (Roberts, 2004).

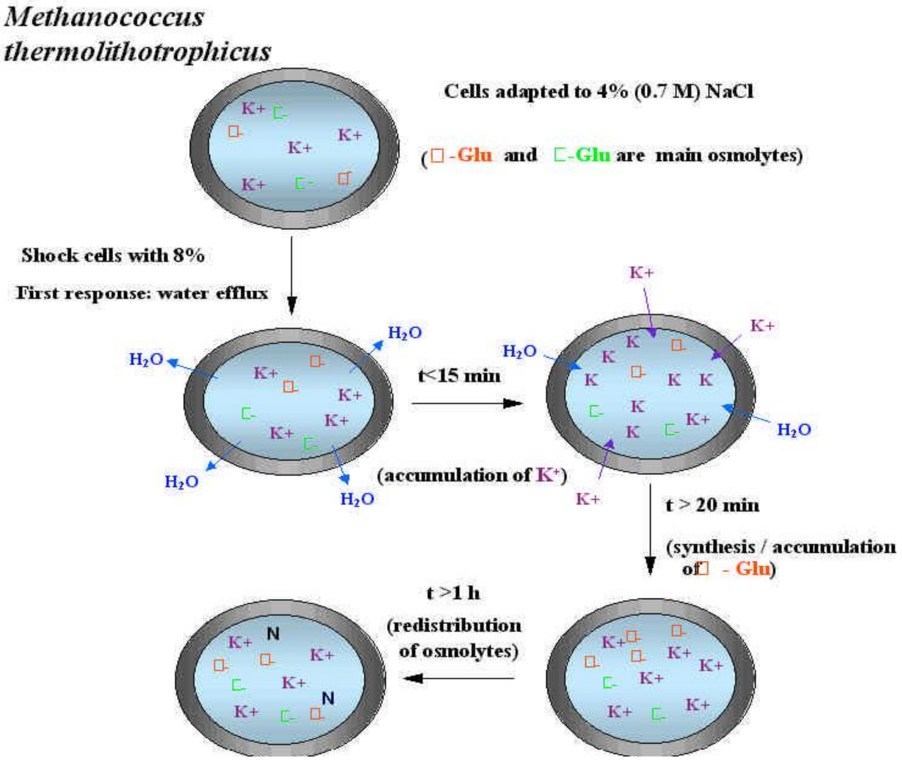


Figure 2.4 Response to changes in external NaCl (Roberts, 2004) .

2.9 Salt speciation and interactions

2.9.1 Salt antagonism

The toxicity effect of K can be eliminated by addition of pair cations as described by the rule of antagonism which can be defined as the ability of a cation to alleviate the toxicity produced by another cation. Kugelman and McCarty (1965) reported that toxicity of Potassium varied with the presence of other cations. They also reported that toxicity of K can be mitigated by a certain ratio of Na to K and Ammonium (NH_4^+) to K. This finding was supported by Muller *et al.*, (1986) who discovered that Na at 4.3 mol l^{-1} largely suppressed the inhibition of K at 50 mol l^{-1}. Fernandez and Foster (1994) also demonstrated the effect of K inhibition in the thermophilic and mesophilic digestion of coffee waste and confirmed the addition of Calcium Phosphate ($Ca_3(PO_4)_2$ increased the COD removal rate from 60% to 80%.

McCarty and McKinney (1961) showed that toxicity of cations in anaerobic systems on a molar basis increases according to the sequence Na, NH_4^+, K, Ca and Mg. A study by Kugelman and McCarty (1965) found the primary antagonists of K, with Na most effective in isolation. Ca and Na in combination were reported to improve 15% of performance as secondary antagonists of K as compared to Na alone. Na was reported as a dominant antagonist to all other cations but produced a significant antagonism effect on K, Ca and NH_{4+} (Kugelman and McCarty, 1965). The role of Na in mitigating K inhibition has been discussed by Jarrell *et al.* (1987) who reported that the ATP ion pump which brings the cation into the microbial cell through the hydrolysis/synthesis process can be stimulated by the presence of Na. Feijoo *et al.* (1995) demonstrated the effect of antagonism and adaptation process in treating seafood processing wastewater in AD system. This toxicity assay was conducted in a batch study where the seawater used was characterized for cations including K, Na, Ca and Mg. The effect on methane production of addition of seawater or NaCl (at the same concentration of Na in seawater) to the same digestate were examined. The findings indicated that at a minimum concentration of Na at 1 g l^{-1}, methane production was stimulated after addition of seawater and increased further up to Na at 9 g l^{-1}. On the other hand, addition of NaCl (instead of seawater) to the same sludge and the same Na concentration did not give any significant improvement. This phenomenon again revealed the effect of other cations in reducing or eliminating the toxicity effect of Na.

2.9.2 Speciation, accumulation and precipitation

The addition of salts into anaerobic systems has received attention due to the stimulatory and toxic effects on the microbial activity. Salts speciation is significant in understanding the rational dosage of salts to prevent disturbances in reactor performance.

The fate (bioavailability and retention) of salts is influenced by the interactions between the liquid and solid phases in the bioreactor. Transport of free salts ion by microorganisms occurs across the cell membrane. During the process, salts actually reach the biomass present in a biofilm or sludge granule and processes such as precipitation occur (e.g., as sulphides) with the formation of inorganic and organic complexes. This process can significantly reduce the free salts concentration in solution to extremely low values.

Precipitation of salts such as sulphides, phosphates or carbonates may also affect the bioavailability of salts in granular sludge. The interaction between the salts and EPS is known as biosorption. During salts speciation, three interactions occur consisting of the salts - microbe interaction, salts - liquid phase interaction and salts interaction with carbonates and sulphides. Salts accumulation is governed by the nature of the mineral and organic constituents, the nature of the salts, the composition of the influent and its pH (Zandvoort *et al.*, 2006). The accumulation of salts within granular sludge is useful for synergistic effects and metabolic requirements during reactor operation but may also cause toxicity at excess concentrations.

Accumulation of salts in granular sludge can be demonstrated by batch equilibrium techniques and the sorption data described by isotherms. The results obtained provide information on the retention capacity and the strength by which the sorbate is retained onto the sludge. Nevertheless, sorption isotherm studies are limited due to cross linkage of the interaction of salts and bacteria. This research investigated the mechanisms involved in accumulation of salts through both adsorption capacity and binding.

The processes involved in salts speciation are shown in Figure 2.5. Precipitation of salts as carbonates and sulphides is one of the factors in accumulation of salts in the sludge. Salts can be retained in the sludge due to precipitation processes. However, salts are no longer available after sulphide precipitates for a long period of exposure due to transformation of salts from amorphous form to crystalline forms. Interactions with granules are summarised in Table 2.7 – 2.9.

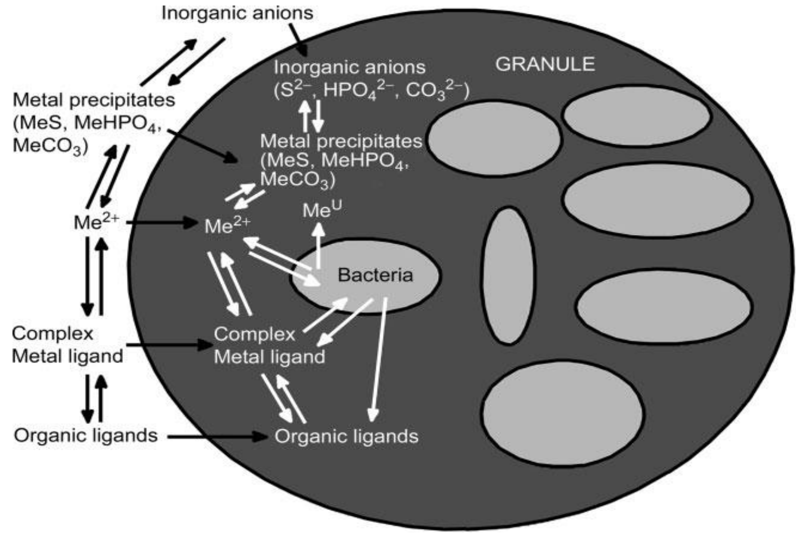


Figure 2.5 Salts speciation in UASB granular sludge (Zandvoort *et al.*, 2006)

Table 2.7 Salts–microbe interaction

Elements involved in interaction	Description
Role of salts in the enzymatic system	Salts act as inhibitors by blocking enzyme functions due to the effect of osmotic pressure (Liu *et al.*, 2003b).
Salts–biofilm interaction	Salts sorption by anaerobic granules has been studied by many researchers (Gonzalez-Gil *et al.*, 2001; Gould and Genetelli, 1978 and Zandvoort *et al.*, 2006). Generally, these studies revealed a strong sorption of salts ions in granules due to precipitation, co-precipitation, adsorption and binding by EPS and bacterial cells. EPS are known to be the major components of granular matrix, and up to 90% of the dry biomass is EPS material, which bind salts. Furthermore, translocation process between the bacterial cell and the salts also contribute to the accumulation.

Table 2.8 Salts–liquid phase interaction

Elements involved in interaction	Description
Particulate salts binding	Apart from precipitation, co precipitation and adsorption are also important phenomena in a mixture of salts ions and are highly influenced by kinetic factors such as the capacity of adsorption onto and desorption from granular sludge.
Liquid phase speciation	Precipitation can be categorised into 5 stages which comprise nucleation, growth of nuclei, aggregation, formation of irreversible aggregates and formation of larger crystals. The final stages determine transformation of salts from amorphous precipitates to more stable crystalline forms (Zandvoort *et al.*, 2006).

Table 2.9 Salts accumulation by inorganic components

Elements involved in interaction	Description
Salts accumulation by carbonates	During the anaerobic process of methanogenesis from acetate to methane and carbon dioxide, bicarbonate ions are produced and precipitate with calcium to form calcium carbonate.
Salts accumulation by sulphides	Salts accumulated in the sludge are mainly released from the organic matter/ sulphides fraction (van Hullebusch *et al.*, 2004), in which they accumulated as sulphides minerals either by adsorption and precipitation or lattice exchange.

2.10 Sorption

Salts are known to favour specific binding sites. The attachment of mercury to cell surfaces in activated sludge, binding of cadmium to the extracellular polymers and attachment of nickel on cell walls have been reported by Buzzini and Pires (2007). Furthermore, Buzzini *et al.* (2006) reported the results of a series of trials in which salts (zinc, copper, nickel and lead) were added to a starch-based substrate.

The binding of salts to activated sludge has been widely studied and is known to be linked to the ion exchange capabilities of the surface polymers in the sludge. Salts removal in activated sludge is also well documented where they can exist as inorganic precipitates (sulphides, carbonates) or attached to organic ligands (Gould and Genetelli, 1978). Most of the studies to date have documented interaction between activated sludge and salts. In addition, inhibition of methanogenic activity has been reported at different salt concentrations. This can promote error due to the fact that total salts were measured in the liquid phase, ignoring the salts speciation.

Van Hullebusch *et al.*, (2004) reported that the main mechanisms involved in salts accumulation within biofilms are complex formation which includes chelation of salts, ion exchange, adsorption, inorganic micro precipitation and translocation of salts into the cell. All these mechanisms, apart from translocation, are metabolism independent, and may also occur in dead microbial cells.

Besides EPS, polysaccharides and amino acids constituting the basic structure of the cell wall also contain polarizable groups, such as phosphate, carboxyl, hydroxyl and amino groups, which are capable of interaction with cation ligands (salts) (Gould and Genetelli, 1978). In physical processes, equilibrium conditions between adsorbed salts ions in solid phase and free ions remaining in solution are rapidly attained for granular sludge as described by van Hullebusch *et al.*, (2004). The Langmuir and Freundlich equations are the most widely applied in order to study the equilibrium for biosorption

2.10.1 Mechanism of sorption

Adsorption is the process of accumulating substances which are in solution. The adsorbate is the substance removed from the liquid phase during interface; the adsorbent is the solid, liquid or gas phase onto which the adsorbate accumulates. Adsorption occurs on the outer surface of the adsorbent or inside the pores. There are three major groups of adsorption which comprise physical, ion exchange and chemical adsorption. Physical adsorption results from the action of Van der Waal forces and is relatively easy to desorb into water phase. Ion exchange involves electrostatic attachment of ionic species. Chemical adsorption is a result of the interaction between adsorbate and adsorbent in the change of chemical form of adsorbate.

Biosorption is a physico-chemical process, independent of metabolic processes (Hulshoff Pol *et al.*, 2004a). Hulshoff Pol *et al.*, (2004) and Yu *et al.*, (2001a) reported that precipitation in granular sludge systems occurs as a result of metabolic activities and physico-chemical reactions, and precipitates are accumulated either inside or on the surface of granules.

2.10.2 Equilibrium parameters

Adsorption equilibrium state between granules and salts can be referred as the dynamic adsorptive behaviour of any substance from fluid to liquid phase. Metcalf and Eddy (2003) defined the adsorption isotherm as the amount of material adsorbed as the function of the concentration in the solution at constant temperature. The two common isotherm models which have been widely applied are the Langmuir and Freundlich Models.

The Langmuir isotherm was developed based on the assumption that (1) adsorption occurs at a fixed number of definite site; (2) the surface is homogeneous; (3) monolayer adsorption occurs on the surface of the adsorbent; (4) there is no lateral interaction between adsorbate molecules. The Langmuir sorption isotherm is often used to describe sorption of a solute from a liquid and its value depends on the experimental conditions, particularly pH, which remains a major factor in biosorption. None of the studied salts ions has significant side reactions at the pH values (van Hullebusch *et al.*, 2004).

The Freundlich equation assumes the salts concentration on the adsorbent will increase as long as there is an increase in the salts concentration in the liquid. The Freundlich isotherm is most frequently used to describe the adsorption of organic components in solution. This empirical isotherm can be used for non-ideal sorption that involves heterogeneous sorption.

2.10.3 Adsorption and desorption efficiency.

Adsorption capacity:

$$q_e = \frac{V(C_o - C_e)}{m} \qquad \text{Equation 3}$$

Where C_o is the initial salts concentration (mg l^{-1}), C_e is the final or equilibrium concentration (mg l^{-1}) after adsorption. V is volume of liquid in conical flask (L) and q_e is known as adsorbent phase concentration after equilibrium (mg adsorbate/ g adsorbent) and m is mass of adsorbent (g).

In this study, the desorption efficiency was calculated based on the following equation

$$Desorption\ capacity = \frac{\mathrm{C_{ed} - C_0}}{\mathrm{q_e m}}\ \mathrm{V_{dvbv}} \qquad \text{Equation 4}$$

Where C_{ed} is the equilibrium concentration (mg L^{-1}) after desortion, C_o is the initial concentration of salts in the eluent (mg L^{-1}) which was 0 in this experiment, V_d is the volume of the eluent, q_e is the adsorption capacity achieved in the upstream adsorption experiment and m is mass of adsorbent (g).

2.10.4 Langmuir isotherm

The Langmuir isotherm equation was developed with two assumptions: 1) a fixed number of accessible sites are available on the adsorbent surface and have the same energy (2) adsorption is reversible. Equilibrium is reached when the rate of adsorption of molecules onto the surface is the same as the rate of desorption of molecules from the surface. Langmuir isotherm was used to explain the uptakes adsorbates occurring on homogenous.

$$\frac{C_e}{q_e} = \frac{1}{Q_{ob}} + \frac{C_e}{Q_o}$$ Equation 5

Where C_e is the final or equilibrium concentration (mg l^{-1}) after adsorption, q_e is the amount of salts adsorbed at equilibrium (mg g^{-1}).Q_e and b are Langmuir constants related to the adsorption capacity and energy adsorption.

2.10.5 Freundlich isotherm

The Freundlich isotherm is the most commonly used to describe the adsorption characteristics of a sorbent. Freundlich (1912) derived the isotherm which is given as:

$$log q_e = log K_f + n^{-1}\, log C_e$$ Equation 6

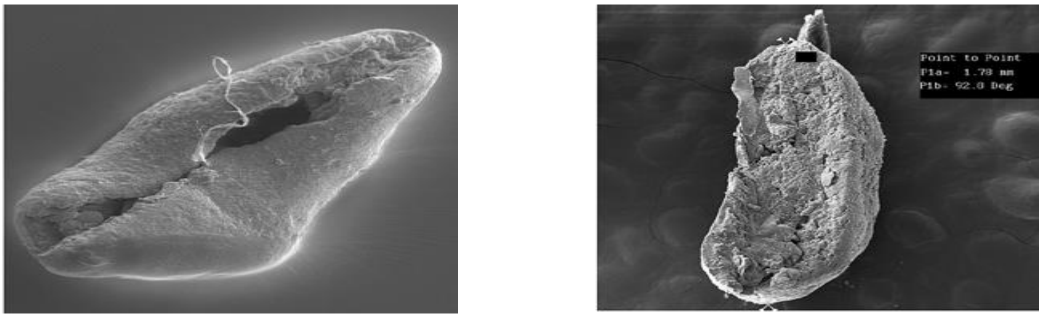
Where q_e is the amount of salts adsorbed at equilibrium (mg g^{-1}), C_e is the final or equilibrium concentration (mg l^{-1}) after adsorption, and K_f and n^{-1} are Freundlich constant incorporating all factors affecting the adsorption process such as capacity and intensity.

2.10.6 Kinetic parameters of biosorption

van Hullebusch *et al.* (2004) discovered that metals sorption on anaerobic granular sludges was very efficient in the first minutes of the process due to the availability of active sites around or inside the sludge. This was especially so for Cadmium, for which equilibrium was reached in about two hours. In contrast Cobalt required almost 10 days to reach equilibrium. pH was reported to influence the adsorption of salts directly, by affecting speciation, or indirectly by affecting the biological surface. These findings indicated that both mass transfer and pore diffusion are important in determining the adsorption rates. The study also showed that the Langmuir equation gave the best isotherm results for the interaction of the metals with anaerobic granular sludge. Salts accumulation was governed by the nature of the mineral and organic constituents, the nature of the salts, the composition of the influent and its pH (van Hullebusch *et al.*, 2004). Removal of salts from anaerobic granular sludge was also straightforward, with >90% removal efficiencies.

2.11 Microscopic and spectroscopic observation

Microscopic evidence for hollowing of large granular aggregates is shown in Figure 2.6a and b, and was attributed to substrate insufficiency (Gonzalez-Gil *et al.*, 2001). Hollow granules are reported to be more susceptible to shear stresses thus facilitating the disintegration of granules within the reactor. From the point of view of microscopic observation, there are significant differences in granules where the granule prior to acclimation formed a bed with a clear supernatant and without fluffy conglomerates as compared to the granule after acclimation.



(a) cross section of floated granule (74x) after 180 days of operation

b) cross section of floated granule (120x) after 180 days

Figure 2.6 Photomicrographs from a SEM analyses of granules (Buzzini *et al.*, 2006).

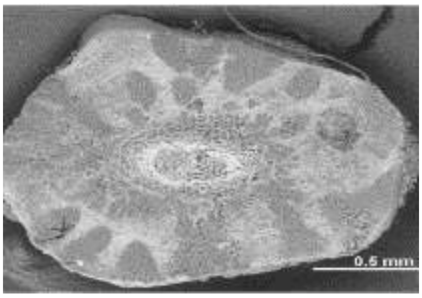


Figure 2.7 Photomicrographs from SEM showing cross section of granules (Gonzalez-Gil *et al.*, 2001)

The effect of salts accumulation in a granule can be seen on the cross section (Figure 2.7). Energy-dispersed X-ray analysis and back-scattered electron microscopy showed spherical aggregates in which dark areas indicate regions with higher biomass concentrations (low atomic mass such as carbon) whereas light areas indicate regions with high salts content (high atomic mass) (Gonzalez-Gil *et al.*, 2001). Figure 2.8 shows the exact mapping location of iron, sulphur, calcium and phosphorus. The accumulation of salts is observed as a whitish cluster which indicates the presence of inorganic precipitates such as calcium carbonate or calcium phosphate. Energy Dispersed X-ray (EDX) analysis of UASB granules confirmed the existence of salts precipitation.

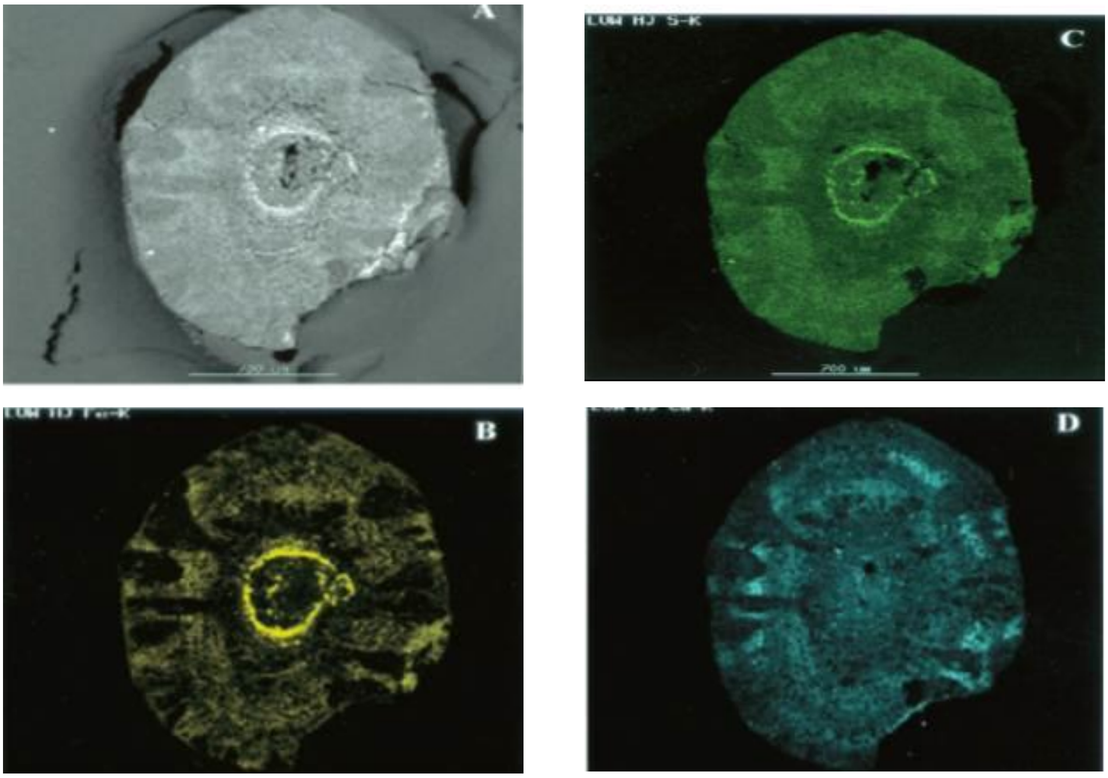


Figure 2.8 EDX analysis of a cross-sectioned aggregate. Back-scattered electron image from which mappings of (A) iron (B) sulphur (C)calcium (D) phosphorus . Note that the deposition of salts corresponds to the light areas observed in the back-scattered electron image. (Gonzalez-Gil *et al.*, 2001)

Furthermore, microbial aggregates were found to have homogeneous or heterogeneous structures. The heterogeneous structure observed was mainly related to the presence of concentric biomass layers. Granules exposed to salts were reported to be more spherical with crystal particles developed on the surface (Van Langerak *et al.,* 1998a). This finding is consistent with Fang *et al.*, (1995) who reported an excessive amount of salts can accelerate agglomeration. This agglomeration process is known as crystal ripening (van Langerak *et al.*, 1998b)

The SEM analyses in Figure 2.9 indicated that the cross-section of a granule revealed a large amount of material with crystal-like structures that suggest the presence of extra cellular polymer. Large amounts of these materials are commonly formed when the growth of microbial cultures is affected by a nutritional imbalance as described by (Buzzini *et al.*, 2006).

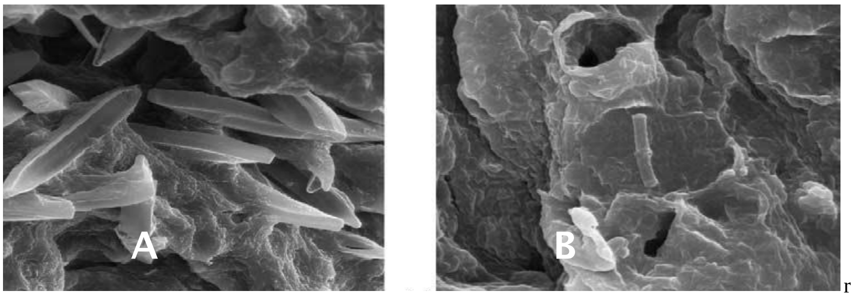


Figure 2.9 Photomicrographs from a SEM analysis of cross section of granules

(a) cross-section of the granule, showing the presence of crystal-like structures (x 1000); (b) cross-section of the granule, showing material that may be extracellular or polymeric (x 5000). (Buzzini *et al.*, 2006)

Van Langerak *et al.* (1998a) founds cementation of sludge (in the form of spherical particles) in a reactor fed with synthetic sewage at a high calcium concentration. The spherical particles consisted of a dense crystal structure with a small amount of biomass. Agglomeration of crystals was found to be due to high density, low shear forces and the availability of free crystal surface due to poor overgrowth. This phenomenon can cause severe operational problems.

In contrast, granulation proceeded better in reactors with a moderate concentration of calcium which had a dense core of calcium carbonate. The surface of most granules was covered with a dense biofilm and little amount of crystal layer (Figure 2.10).

Furthermore, specific methanogenic activity was not affected by scaling of the biomass. This can be explained by the type of precipitation, where biofilm precipitation is not the dominant process. Nevertheless, the bulk precipitation that took place allows attachment of biomass on the formed crystal surfaces, as confirmed by microscopy.

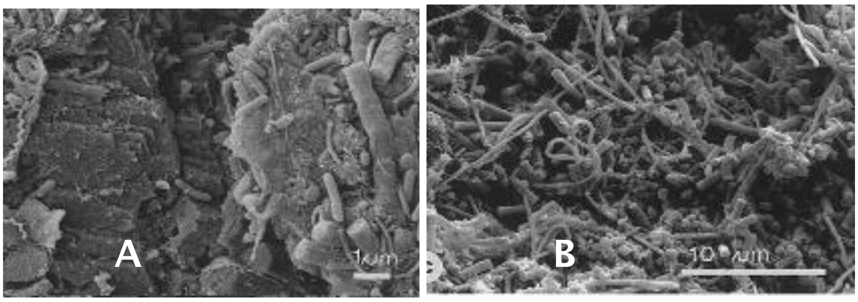


Figure 2.10 Scanning electron microscope photographs of crystal surface and EPS

(A) details of the surface of a spherical particle showing a dense crystal surface with some attached bacteria; (B) granule from the bottom of reactor at the end of the experiment, showing a dense biofilm on the surface (Van Langerak *et al.*, 1998a)

2.12 Conclusions from literature review

The following main conclusions can be drawn from the literature review:

Little work has been carried out on mass balances for light metal cations and anions or their partitioning in wheat straw processing streams with respect to the temperature and duration of the washing process, although this is likely to be crucial in terms of achieving the appropriate alkali index for combustion of the wheat straw, and an optimum overall process energy balance.

Similarly there appear to have been no studies to determine the methane potential and biodegradability of wheat straw washwater. This represents a major gap in our knowledge of all aspects of this potential technology for upgrading of straw as a renewable energy source through combined combustion and anaerobic digestion.

The difficulties associated with digestion of saline wastewaters are well known, and there is a considerable body of knowledge about probable causes of these and some potential alleviating factors. There has, however, been relatively little work on the threshold for K inhibition, which is known to be the major salt product from washing or leaching of straws.

No information is available on the effects of longer-term continuous exposure of anaerobic systems to wheat straw leachate, and in particular whether salt accumulation could lead to digestion failure or whether stable steady state conditions can be achieved

Mechanisms relating to osmoregulation and K accumulation are complex and there is as yet insufficient understanding of these to allow prediction of how anaerobic systems will react to a specific feedstock. The literature review has revealed that a combination of biological and chemical processes are involved, some favourable to digestion and some not, and many of these may interact in an anaerobic system. As a result of this, a sensible approach is to carry out experimental studies on the anaerobic treatment process, rather than considering more complex multi-stage or pre-treatment options in the first instance.

In a complex substrate such as wheat straw washwater, which contains a variety of antagonistic ions, it is likely to be difficult to identify whether differences in performance are due to a single factor such as the concentration of potassium, or are affected by other potentially inhibitory compounds. Use of a synthetic wastewater in which the concentration of individual cations can be controlled and varied is thus likely to be a good experimental strategy, coupled with long-term experiments with WSW that will allow the effects of acclimatisation to be taken into account.

While UASB digesters appear to be a promising choice for recovery of energy from wheat straw washwater, there are potential problems associated with the role of cations in formation and maintenance of granulation. It would thus be useful to know whether anaerobic biomass from other sources could be acclimated to wheat straw leachate for use as inoculum in anaerobic filters, and also to have information on the comparative performance of these two systems. To date, however, the scarcity of work on this substrate means that no comparison between the performance of UASB digesters and anaerobic filters on this substrate has been undertaken.

Electron microscopy and Energy Dispersed X-ray spectra analysis appear to be promising techniques for identifying and localising the accumulation of light metal cations in granular sludge, and thus potentially elucidating the mechanisms underlying any accumulation. Well-established isotherm techniques are also available for determining the key characteristics and kinetic parameters of sorbents. Use of these to quantify the sorption capacity of anaerobic granular sludge for potassium could thus add to our knowledge of this complex system.

The literature review has thus identified some key gaps in knowledge, and indicated a number of concepts and tools that may be useful in exploring these. In the absence of some of the critical information and operating parameters identified above, detailed evaluation of the potential practical applications of this technology is likely to be problematic. The research will therefore focus on filling some of these gaps in knowledge through a programme of experimental work, and use the results to develop a preliminary carbon and energy balance for the process based on the raw energy inputs and outputs from the wheat straw fibre and washwater components.

3. Materials and Methods

3.1 General

Ultra pure deionised water was obtained from a Barnstead Nanopure ultrapure water purification system (Thermo Scientific, UK)

Before use, all glassware was washed with detergent and rinsed with tap water then deionised water.

All glassware and apparatus used in the analysis of trace concentrations of metals was washed with detergent, rinsed twice with tap water and twice with deionised water, then soaked in an acid bath of 10% hydrochloric acid for at least twelve hours. Deionized water was used in the acid bath, subsequent rinsing and all wet chemical analyses.

Chemicals used were from Fisher Scientific (Loughborough UK) except where otherwise specified. All acids used in metal analysis were trace analysis grade. All reagents, standards and VFA samples were prepared using deionised water.

3.2 Analytical methods

3.2.1 Light metal cations by Flame Atomic Absorption Spectrometry

Light metal cations were measured on a Flame Atomic Absorption Spectrometry (Spectr AA-200, Varian, Australia) operated according to manufacturer's instructions using a hollow cathode lamp. The conditions used are shown in Table 3.1, including interference suppressors and check standard concentrations. Calibration solutions were prepared from a stock solution of K, Mg, Na and Ca (Fisher Scientific Standard solution, 1000 mg l^{-1} in HNO_3 for atomic spectroscopy) by dilution to the required concentration range using 12.5% nitric acid (HNO_3).

Samples of wheat straw were first ashed in the furnace for 2 hours at 550ºC and 0.5 g of the ash was added to a digestion tube followed by the addition of 3 ml concentrated HNO_3 and 9 ml of Hydrochloric acid (HCl) for acid digestion. Anti-bumping granules were added to the tube and the mixture was left for 24 hours prior to the digestion, which was carried out using a Gerhardt Kheldatherm heating block and triple condensers. The heating process commenced at 50 ºC for 10 mins before the temperature was raised to 80ºC for 10 mins and then to 120ºC for 10 mins before reaching the final temperature of 180 ºC which was maintained for 2.5 hours. After cooling the mixture was filtered (Filter paper Whatman No 1 - 11cm) and the residue was leached out from the digestion tube using warm 12.5% HNO_3 and made up to 100 ml in a volumetric flask. Digested wheat straw was then analysed for K, Na, Mg and Ca as described above with additional dilution by 12.5% nitric acid (HNO_3) if required

Table 3.1 Parameters for each of elements involves

Element	Wave length (nm) /slit setting (nm)	Fuel/ support	Interference elimination
K	766.5/ 1.0	Acetylene/ Air	Cesium Chloride (CsCl)
Na	589/0.5	Acetylene/ Air	Potassium Chloride (KCl)
Mg	285.2/0.5	Acetylene/ Air	Lanthanum Chloride (LtCl)
Ca	422.7/0.5	Nitrous Oxide/ Acetylene	LtCl

3.2.2 Measurement of anions using ion chromatography

Chloride (Cl), Sulphate (SO_4) and Phosphate (PO_4) were measured by ion chromatography using a Dionex 500 Ion chromatography system (Dionex Corporation, Sunnyvale, USA) fitted with an electrochemical detector and Ion Pac (4x250mm) column (AS14A). The eluent (mobile phase) used was 8 mmol l^{-1} Sodium carbonate (Na_2CO_3) and 1 mmol l^{-1} Sodium bicarbonate ($NaHCO_3$) prepared as shown in Table 3.2, filtered (Nylon 66 membrane -0.45 µm x 47mm). 1000 mg l^{-1} standards were prepared using chemicals as shown in Table 3.3 using analytical grade reagents. Wheat straw and wash straw samples were prepared as explained in section 3.2.1. Samples were filtered (cellulose nitrate membrane filter, 0.45µm pore size x 25mm) followed by dilution to the correct concentration range with the eluent. All reagents were made up to the required volume using high quality deionised water (18.2 M Ω - cm resistivity, Thermo Barnstead)

Table 3.2 Eluent prepared for mobile phase

Chemical (Pre-dried at 105° C for 3 hours)	Concentration (g l^{-1})
Sodium carbonate (Na_2CO_3)	0.848
Sodium bicarbonate ($NaHCO_3$)	0.084

Table 3.3 Standard calibration solution for Anion measurement

Chemical / Element to be measured	Weight (g) in 100ml of eluent
Na Cl / (Cl)	0.1649
KH_2PO_4 /(PO_4)	0.9999
Na_2SO_4 / (SO_4)	0.4394

3.2.3 Chemical Oxygen Demand (COD)

COD was measured by the closed tube reflux method with titrometric determination of the end point (Environment Agency, 2007). If the sample COD was more than 400 mg l^{-1} pre-dilution was carried out. 2 ml of sample (or 2ml deionised water for blanks) was placed into the reflux tubes followed by the addition of 3.8 ml of FICODOX-plus reagent (Fisher Scientific Ltd, UK) the composition of which is shown in Table 3.4. The tube was sealed with a PTFE screw cap and the mixture refluxed at 150°C for 2 hours. After cooling, a few drops of ferroin indicator (Table 3.5) were added (Fisher Scientific Ltd, UK) and titrated with acidified (2% Sulphuric acid) 0.025N ferrous ammonium sulphate solution, the normality of which was calculated using equation 7. The end point was a colour change from blue to red. The COD value of the sample was calculated using equation 8.

Dilutions of a standard solution containing 3.8 g l^{-1} of Potassium hydrogen phthalate with a COD of 4 g COD l^{-1} were used as a check against calculated values of COD.

$$Normality_{FAS} = \frac{0.12884}{TitrantVolume_{FASstd}}$$ Equation 7

$$COD(mgO_2 / l) = \frac{8000.(TitrantVolume_{Blank} - TitrantVolume_{Sample}).Normality_{FAS}}{2.Dilution}$$

Equation 8

Table 3.4 FICODOX-plus Composition

Chemical	**Concentration**
Potassium di-chromate	1.7 g l^{-1}
Silver sulphate	8.1 g l^{-1}
Sulphuric acid	81.1%

Table 3.5 Ferroin Indicator Composition

Chemical	**Concentration**
1,10-phenantroline monohydrate	14.85 g l^{-1}
Iron (II) sulphate heptahydrate	6.95 g l^{-1}

3.2.4 Total Organic Carbon

Total Organic Carbon was measured by Dohrmann TOC (DC-190) following the manufacturer's manual and method which is based on Standard Method 5310 (APHA, 2005). In all analytical runs the linearity of the instrument was checked using standard solutions of 50 mg l^{-1}, 500 mg l^{-1} and 1000 mg l^{-1} of Potassium Hydrogen Phthalate (KHP).

3.2.5 Conductivity

This was measured with a conductivity meter (WTW LF330) using units of µS cm^{-1}.

3.2.6 Solids analysis

3.2.6.1 Total Solids (TS) and Volatile Solids (VS)

After thorough agitation, approximately 10 g of sample was transferred into a weighed crucible by pipetting (digestate samples) or spatula (substrate samples). Samples were weighed to an accuracy of 10±0.001 g (Sartorius LC6215 balance, Sartorius AG, Gottingen Germany) and placed in an oven (Vulcan laboratory oven, LTE Scientific Ltd., Oldham UK) for drying overnight at 105^{o}C ± 2 ^{o}C. After drying the samples were transferred to a desiccator to cool for at least 40 minutes. Samples were then weighed again with the same balance, transferred to a muffle furnace (Carbolite Furnace 201, Carbolite UK, Hope Valley UK) and heated to 550 ^{o}C ± 10 ^{o}C for two hours. After this ashing step, samples were again cooled in a desiccator for at least one hour before weighing a third time.

After all analyses, crucibles were washed with detergent, rinsed with deionised water, and stored in an oven until required for the next analysis. Crucibles were transferred from the oven to a desiccator for cooling to room temperature before each analysis. Total and volatile solids were calculated according to the following formulae:

$$\%TS = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \qquad \text{Equation 9}$$

$$\%VS(based \cdot on \cdot total \cdot weight) = \frac{W_3 - W_4}{W_2 - W_1} \times 100 \qquad \text{Equation 10}$$

$$\%VS(based \cdot on \cdot total \cdot solids) = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \qquad \text{Equation 11}$$

Where

W_1 is the weight of the empty crucible;

W_2 is the weight of the crucible containing fresh sample;

W_3 is the weight of the crucible and sample after drying at 105^oC;

W_4 is the weight of the crucible and sample after heating to 550^oC.

3.2.6.2 Suspended Solids

Suspended solids content was measured by passing a sample of known volume through a glass fibre filter paper (Whatman, UK, pore size 0.4µm) of known dry weight. After drying at 105^oC the paper was again weighed and the difference determined.

3.2.7 Calorific value

Calorific value was quantified using a bomb calorimeter (CAL2k EC – Digital Data System Ltd, South Africa). Wheat straw was cut using scissors to give mean length of 0.2 to 0.5 mm and prior to pre dried process in the oven for overnight 105 ºC. Then, 0.150 g (with an accuracy of 0.1 mg) of wheat straw was placed into stainless steel crucible in the filling station. A cotton thread was attached to the ignition wire and crucible. The bomb vessel was then filled with oxygen until the pressure reached 3 MPa. Then, the vessel was moved into static polystyrene jacket. As the sample started to burn by electrical energy, the temperature of bomb vessel rise thus allow calorific content of sample to be calculated. A blank is made to account for the electrical energy input and the energy released in burning the fuse. The standard used was benzoic acid (around 1 g with an accuracy of 0.1 mg) which gives the HHV of 26.454 kJ g^{-1} TS. The same procedure was applied when measuring the samples and blank. For each runs including the standard, the vessel can only be used when it cool to room temperature and the crucible was cleaned using deionised water.

3.2.8 Gas volume

Gas was collected in gas impermeable Tedlar bags (SKC Ltd, Blandford Forum). The sample bag volumes were measured using a weight-type water displacement gasometer (Walker *et al.*, 2009). The initial height was recorded before the sample bag was connected to the gasometer. After the gas bag was empty, the final height and weight of water was recorded. All gas volumes are reported at a standard temperature and pressure (STP) of 101.325 kPa and 0 ^{o}C.

3.2.9 Biogas composition

Biogas composition was quantified using a Varian Star 3400 CX gas chromatograph (GC), (Varian Ltd, Oxford, UK). The device was fitted with a Hayesep C column with argon as the carrier gas at a flow of 50 ml min^{-1} and a thermal conductivity detector. The biogas composition was compared with a standard gas sample containing 65.12% (v/v) CH_4 and 34.88% (v/v) CO_2 for calibration. A sample of 5 ml was taken from a Tedlar bag used for sample collection and was injected into a gas sampling loop.

3.2.10 pH

pH was measured using a Jenway 3010 pH meter (Jenway, London, UK) with a combination glass electrode calibrated in buffers at pH 4, 7 and 9 (Fisher Scientific, UK). The calibration solutions were prepared weekly and stored in sealed jars. Deionised water was used to clean the probe. When measuring sample pH this was done immediately after the sample was taken, to avoid loss of dissolved carbon dioxide resulting from changes in temperature and equilibrium conditions. During measurements, the sample was stirred to be homogeneous. In addition, the pH probe was rinsed with deionised water in between measurements and placed into a mild acid solution to avoid cross-contamination.

3.2.11 Ammonia

The total ammonia nitrogen (TAN) analysis was performed using the Standard Method 4500-NH3 B and C (APHA, 2005). Approximately 100 ml effluent (from UASB and AF) measured by mass for accuracy using My Weigh i201 balance (capacity 200 g ± 0.1 g, My Weigh Europe, Huckelhoven Germany), was poured into the digestion tubes. For CSTR, 3- ml of digestate was measured by mass as mentioned above. 50 ml of DI water were added to the digestate and poured into digestion tubes. Furthermore, both blank (50 ml DI water) and standard samples (containing 10 ml of 1000 mg l-1 NH_4Cl with 40 ml DI water) were also prepared. 5 ml of 10 M Sodium hydroxide (NaOH) was added to the samples to raise the pH greater than 9.5 to increase the volatilisation of ammonia. Total ammonia nitrogen was measured using a Kjeltec System 1002 distillation unit (Foss Tecator AB, Hoganas, Sweden) which distils off the ammonia into a boric acid solution which is then titrated with 0.25N H_2SO_4 until a colour change from green to pale lavender. Digestate samples were measured immediately after sampling to avoid any ammonia losses.

3.2.12 Alkalinity

The alkalinity of liquid samples was measured by titration with a 0.25N solution of sulphuric acid and is based on the Standard Method 2320B for Alkalinity (APHA, 2005). End points of pH 5.7, 4.3 and 4.0 were measured in order to calculate partial, intermediate and total alkalinity (PA, IA and TA) as described by Ripley *et al. (*1986). IA indicates the measure of volatiles fatty acids (VFA) buffering while the PA showed the measure of carbonate buffering. The alkalinity ratio can be described as the ratio partial to IA which implies the level of stability in AD process. Alkalinity is presented in equivalent concentration of calcium carbonate and is given by Equation 12.

$$Alkalinity\,(mgCaCO_3 l^{-1}) = \frac{Volume_{Acid}.Normality_{acid}.50000}{Volume_{sample}} \qquad \text{Equation 12}$$

The probe was calibrated before each alkalinity titration using the buffer solutions noted above for pH measurement. Cross-contamination was avoided by thorough rinsing of the probe between each measurement, and storage of the probe in a mild acid solution between uses. Where necessary samples were first diluted with deionised water.

3.2.13 Volatile Fatty Acids (VFA)

The method used was based on SCA (1979): Determination of Volatile Fatty Acids in Sewage sludge (1979). Samples were prepared for analysis by centrifugation at 14,000 g (micro-centrifuge, various manufacturers) for 15 minutes. 0.9 ml of the supernatant was transferred by pipette to vials with 0.1 ml formic acid to give a final concentration of 10% formic acid. Where dilution was necessary, deionised water was used and formic acid was added to give a concentration of 10% of the total volume for analysis. VFA concentrations were quantified in a Shimazdu 2010 gas chromatograph using a flame ionisation detector and a capillary column type SGE BP 21 with helium as the carrier gas at a flow of 190.8 ml min^{-1}, with a split ratio of 100 giving a flow rate of 1.86 ml min^{-1} in the column and a 3.0 ml min^{-1} purge. The GC oven temperature was programmed to increase from 60 to 210 ^{o}C in 15 min, with a final hold time of 3 min. The temperatures of injector and detector were 200 and 250 ^{o}C, respectively. Samples were acidified using formic acid. Three standard solutions containing 50, 250 and 500 mg l^{-1} of acetic, proprionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids were used for VFA calibration.

3.3 Substrates

3.3.1 Wheat straw

3.3.1.1 Source

Wheat straw was collected at the time of harvest from a farm at Marwell in Hampshire, UK and was supplied as cut and baled with a sample size of approximately 100 kg. The straw was then stored under cover and kept dry to prevent deterioration.

3.3.1.2 Preparation

Wheat straw was preselected in order to remove particulate component which includes small stone. Then the wheat straw was cut using scissors into small pieces to give a mean length of about 10 cm and stored inside boxes at room temperature before pre treatment process started.

3.3.2 Straw washing methods

Figure 3.1 shows the pre-treatment washing process used for wheat straw. The cold (20^oC) and hot (50°C) water washes were carried out in a glass reactor maintained in a water bath (Figures 3.2 – 3.4). The hot water wash at 100°C used a stainless vessel with a lid; the vessel was heated directly using a hot-plate. The steam treatment used a steam basket maintained over water at 100°C in a stainless vessel stainless vessel with a lid, and the vessel was heated directly using a hot-plate. The hot water wash at 120°C used a pressure cooker with direct heat from a hot-plate. Washes at 60°C were also carried out in a reciprocating action washing machine with a perforated drum with a 1 mm mesh size as shown in Figure 3.5 and 3.6.

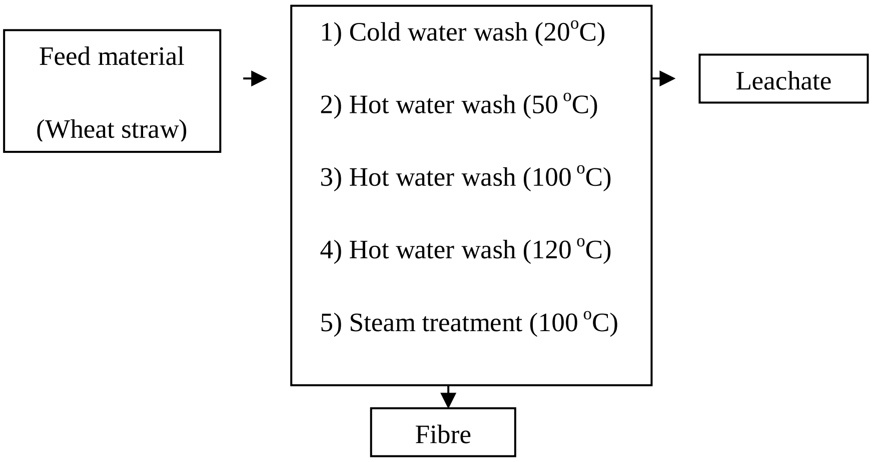


Figure 3.1 Pre treatment washing process for wheat straw

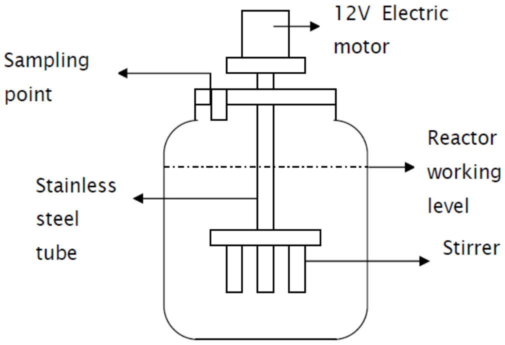


Figure 3.2 Sketch of pre-treatment glass reactor (6 litres)

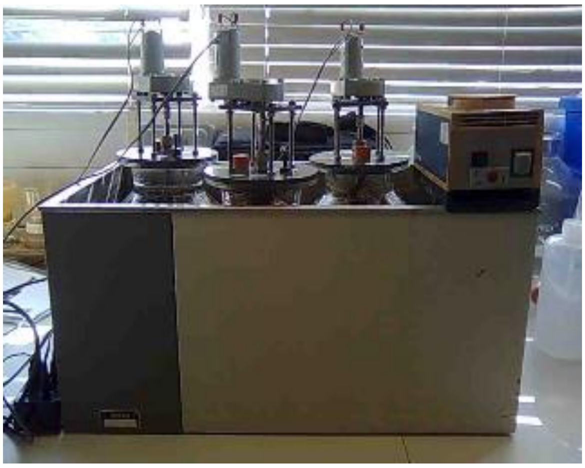


Figure 3.3 Cold water washing/ liquid - hot water in water bath system

Figure 3.4 Straw washing in glass reactor

Figure 3.5 Washing machine during construction

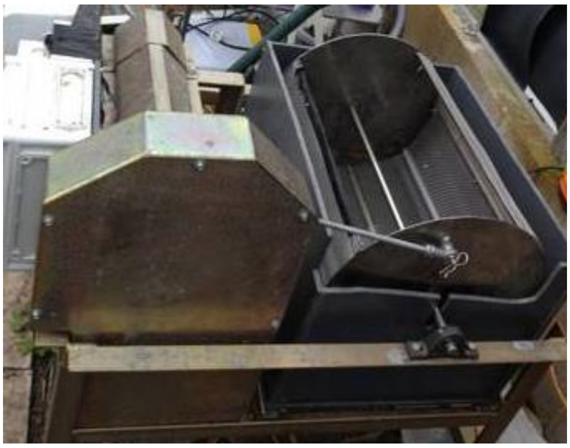


Figure 3.6 Washing machine with perforated drum fitted

3.4 Synthetic wastewater

Synthetic sewage was chosen as a surrogate material for certain experiments as it allowed the use of a complex chemical feedstock of high biodegradability without the risk of exposure to pathogens present in real sewage. The material also allowed experiments to be performed with repeatable results against an already established baseline of data. The composition of the synthetic wastewater concentrate (before dilution to working strength) is given in Table 3.6. Table 3.7 gives the composition of the trace element (TE) solution added to the synthetic sewage during operation of the CSTR.

Table 3.6 Composition of synthetic sewage concentrate (diluted 1:100 to give a working solution)

Component	Quantity	Unit
Trace element solution (mg l^{-1} : Fe 10; Co, Mn, Ni, and Zn 1; Cu, B, Al, Mo, and Se 0.1)	1	ml
Yeast (block bakers form)	23	g
Urea	2.14	g
Full cream milk (UHT sterilised)	144	ml
Sugar (granulated white)	11.5	g
Dried blood	5.75	g
Ammonium phosphate $(NH_4)_2HPO_4$	3.4	g
Tap water	Make up volume to 1 l	

Table 3.7 Composition of additional TE solution as a working and stock solution.

Trace element as cation	Compound used	Element concentration in the working condition (after diluted by 1000 times), mg l^{-1}	Compound concentration in stock solution, g l^{-1}
Aluminium (Al)	$AlCl_3\cdot 6H_2O$	0.1	0.895
Boron (B)	H_3BO_3	0.1	0.572
Cobalt (Co)	$CoCl_2\cdot 6H_2O$	1	4.038
Copper (Cu)	$CuCl_2\cdot 2H_2O$	0.1	0.268
Iron (Fe)	$FeCl_2\cdot 4H_2O$	5	17.80
Manganese (Mn)	$MnCl_2\cdot 4H_2O$	1	3.602
Nickel (Ni)	$NiCl_2\cdot 6H_2O$	1	4.050
Zinc (Zn)	$ZnCl_2$	0.2	0.41
Concentration of oxyanion elements in stock solution (make up to the volume of 500 ml volumetric flask)			
Trace element as oxyanion	Compound used	Element concentration in the working condition (after diluted by 1000 times), mg l^{-1}	Compound concentration in stock solution, g l^{-1}
Molybdenum (Mo)	$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	0.2	0.368
Selenium (Se)	Na_2SeO_3	0.2	0.438
Tungsten (W)	$Na_2WO_4\cdot 2H_2O$	0.2	0.359

3.5 Anaerobic digesters

3.5.1 Inoculum

Granular sludge was obtained from a UASB reactor treating paper mill effluent (Smurfit Kappa Ltd, Kent, UK). The granular sludge was stored in the laboratory at ambient temperature until required.

Digestate was obtained from an anaerobic digester at Millbrook wastewater treatment plant, Southampton. This digestion plant is operated by Southern Water PLC and receives an influent substrate of mixed primary and secondary sludge from municipal wastewater treatment. Digestate was collected as needed, maintained at 37oC and used as inoculum within 48 hours from the time of collection.

Salt-acclimated digestate was taken from laboratory-scale continuously-stirred tank reactor (CSTR) digesters which had run for 7 months on synthetic sewage with the addition of KCl and NaCl.

3.5.1 Upflow Anaerobic Sludge Blanket (UASB) reactors

Two designs of UASB digesters were used in the experiments

3.5.1.1 Early design UASB digesters

Four 1-litre working volume UASB digesters were used as shown in Figure 3.7. These were maintained at 37±2°C using external heating coils connected to a thermostatically controlled water circulator. Influent was fed into each digester by peristaltic pump (Model 505S, Watson Marlow, UK), and effluent and any gas produced was collected in a sealed receptacle connected to a gas sampling bag. Biogas production was determined as the volume of gas in the sampling bag, minus that displaced by incoming effluent (established by weight). The digesters were seeded with 450 g of granular sludge which was initially resuscitated by raising it to the digester operating temperature and feeding with a synthetic wastewater (Section 3.3) until all of the digesters were achieving a specific methane production of 0.32 l CH_4 g^{-1} COD added.

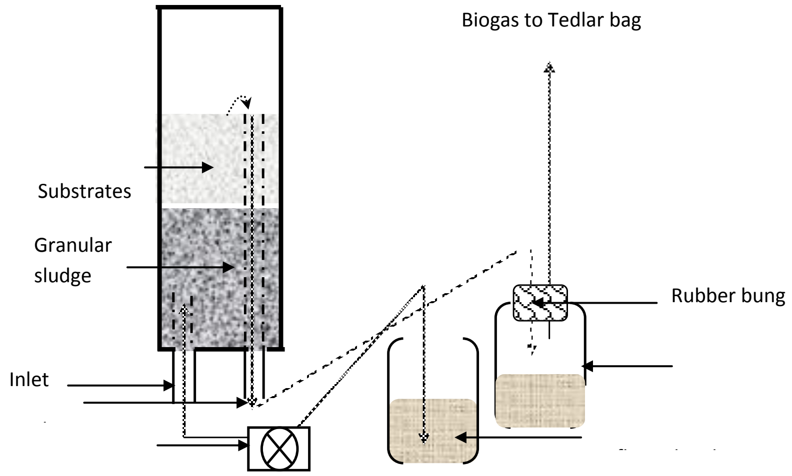


Figure 3.7 Upflow Anaerobic Sludge Blanket digester

3.5.1.2 Later design UASB digesters

These were designed to have a working capacity of 1.5 litres and were operated without a sealed effluent container, as the design incorporated a gas liquid separator at the top of the digester. Since later design of UASB (Figure 3.8 and 3.10) in a bigger working volume, liquid gas separator was introduced to ensure that biogas and effluent efficiently separated and biogas was not flow out with the effluent which can lead to the waste of biogas (see Figure 3.9. The digesters were seeded with 1 kg of granular sludge.

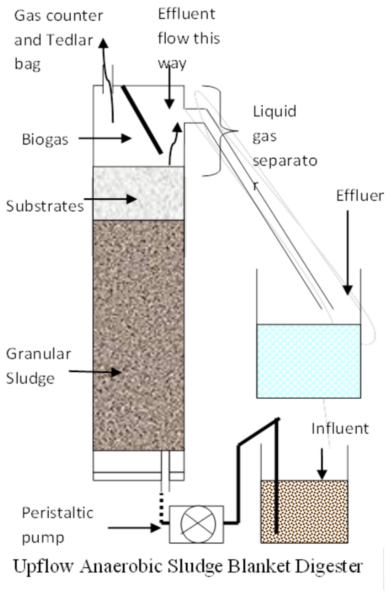
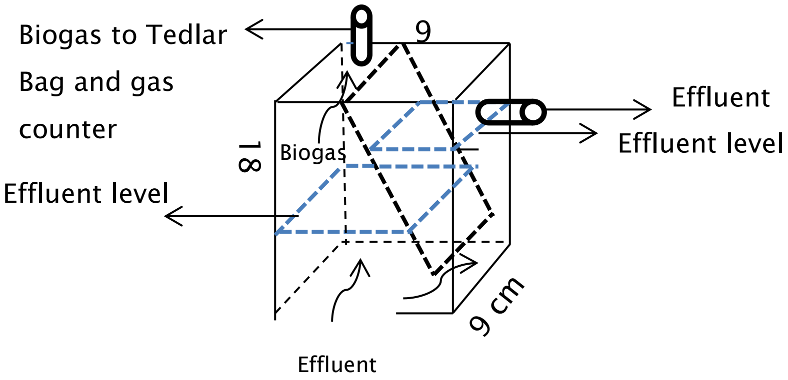


Figure 3.8 Anaerobic sludge blanket digester



Flow of effluent + biogas from digester

Figure 3.9 Gas liquid separator

3.5.2 Anaerobic filters

These were of the same design as the second set of UASB digesters. The AF digesters (Figure 3.10 and 3.11) were filled with 288g of biological filter media named as Biocell with the specific surface area of 500 m^2 m^{-3} and dimension of 10 mm X 7 mm (width X depth). Salt-acclimated digestate was recirculated for a week using a peristaltic pump (Watson Marlow 550S) to allow biomass to attach to the filter media.

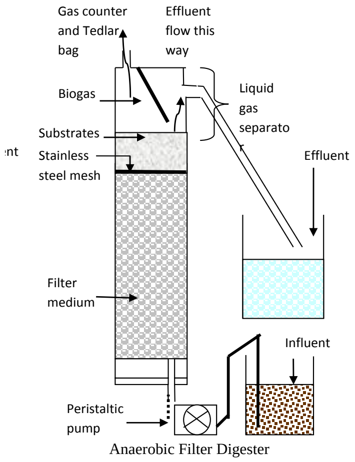


Figure 3.10 Anaerobic filter digester

Figure 3.11 Anaerobic filter and UASB digester

3.5.3 Continuously Stirred Tank Reactors

The continuously stirred tank reactors (CSTR) each had a 5-L capacity and a 4-L working volume. They were constructed of PVC tube with gas-tight top and bottom plates. The top plate was fitted with a gas outlet, a feed port sealed with a rubber bung, and a draught tube liquid seal through which an asymmetric bar stirrer was inserted with a 40 rpm motor mounted directly on the top plate (Fig.3.12). Temperature was controlled at 35 ^{o}C by circulating water from a thermostatically-controlled bath through a heating coil around the digesters. Biogas was measured using tipping bucket gas counters with continuous datalogging (Walker *et al.*, 2009) and all gas volumes reported are corrected to standard temperature and pressure (STP) of 0 ^{o}C and 101.325 kPa. Semi-continuous operation was achieved by removing digestate through an outlet port in the base before adding feed via the feed port. During this process a small amount atmospheric air enters the headspace but in insufficient quantities to affect the redox conditions in the digester: any nitrogen detected in the gas composition is corrected for, as this is not normally produced as a result of the digestion process. Feed was added on a daily basis with regular checks to ensure a constant level was maintained in the digesters. The CSTR digesters were initially seeded with digestate from a digester treating municipal wastewater biosolids at Millbrook Wastewater Treatment plant, Southampton, UK and then acclimated to synthetic wastewater.until they achieved stable specific methane production of 0.32 l CH_4 g^{-1} COD $_{added.}$

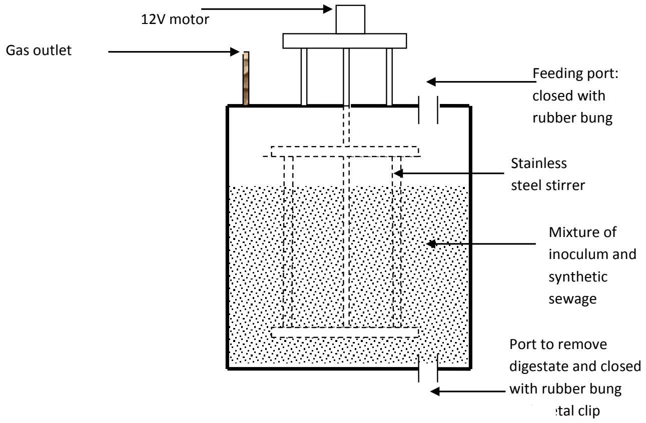


Figure 3.12 Continuously stirred tank reactors

3.5.4 Serum bottle batch assay

This experiment was conducted in 124 mL serum bottles, with septum sealed. Twelve (duplicate each) serum bottles were used. Each of the serum bottles containing 25 mL of inoculumn (marine mud or Millbrooke digestate) and 1.1 mL of saline synthetic sewage concentration at 1 g COD l^{-1}. Rock salt (as NaCl) was added into synthetic sewage to represent the saline environment at 0%, 1% and 3%. The headspace of the serum bottle was flushed with N_2/CO_2 (80:20) (BOC, UK) and the bottle was sealed using a crimp cap with a PTFE coated septum. Inoculum-only controls were also prepared. The test and control bottles were incubated at 37°C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). The headspace pressure of each serum bottle was measured at regular intervals using a digital absolute pressure meter (Digitron 2025P, Digitron Instrumentation Ltd, Cambridge, UK).

Pressure readings were converted to gas volume and corrected to STP conditions using the ideal gas law: $PV = nRT$ where P is the pressure of the gas, V is the gas volume (which in this case is the fixed headspace volume), n is the amount of gas (in moles), T is the gas temperature and R is the ideal gas constant ($8.314\ \mathrm{J{\cdot}K^{-1}{\cdot}mol^{-1}}$). The gas volume was also corrected to take water vapour into account using the Goff-Gratch equation:

$$\mathrm{Log}_{10}\, e_w = -7.90298\,(373.16/T-1) + 5.02808\,\mathrm{Log}_{10}(373.16/T) - 1.3816\ 10^{-7}\,(10^{11.344\,(1-T/373.16)} - 1) + 8.1328\ 10^{-3}\,(10^{-3.49149\,(373.16/T-1)} - 1) + \mathrm{Log}_{10}(1013.246) \qquad \text{Equation 13}$$

where e_w is the saturation water vapour pressure (in hPa) and T is the air temperature (in K).

3.6 Sorption of salts on anaerobic granular sludge: kinetics and isotherm studies

The objective of this study was to investigate the kinetics and sorption capacity of K that contained in wheat straw washwater onto anaerobic granular sludge.

3.6.1 Sorption study

Batch adsorption test are required to investigate the effect of adsorption kinetics and adsorption isotherm. The results were interpreted in term of adsorption efficiency and adsorption isotherm model. Six conical flasks seeded with 1.6 g wet weight of granular sludge were used at initial volume of WSW at 100 ml. The initial concentration of Potassium in WSW was 111.11, 88.41, 65.99 mg l^{-1}. The conical flasks were incubated at 37^{o}C with agitation at ~100 rpm (Hybrid Maxi 14, Thermo Scientific, UK). Readings were taken within the interval of 30 minutes for the first 3 hours followed by hourly sampling for the following 7 hours and once in every 24 hours until 144hours. Final concentration of K was determined using the AAS after sample were filtered (Filter paper Whatman No 1- 11cm) followed by dilution into 12.5% of Nitric acid (HNO_3).

3.6.2 Isotherms experiments

Ten conical flasks seeded with 1.6 g wet weight of granular sludge were used at initial volume of WSW at 100 ml. The conical flasks were incubated at 37^{o}C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). An isotherm experiment was carried out at 37C° with initial wheat strawwashwater concentrations of 111.11, 88.41, 65.99, 43.57 and 18.52 mg l^{-1} with 1.6 g wet weight of granular sludge as the adsorbents. Final concentration of K was determined using the AAS after sample were filtered (Filter paper Whatman No 1- 11cm) followed by dilution into 12.5% of Nitric acid (HNO_3).

Isotherm study was investigated at 37Cº with different initial concentration of wheat strawwashwater ranging from 111.11 to 65.99 mg l^{-1}. Langmuir and Freundlich isotherm were used to interpret the sorption capacity of K onto granules.

3.6.3 Desorption study

6 conical flasks seeded with 1.6 g wet weight of granular sludge were used at initial volume of synthetic sewage at volume of 100 ml at concentration of 1 g COD l^{-1}. The conical flasks were incubated at 37°C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). Readings were taken within the interval of 24 hours until saturation point. Final concentration of K was determined using the AAS after sample were filtered (Filter paper Whatman No 1- 11cm) followed by dilution into 12.5% of Nitric acid (HNO_3).

3.7 Characterisation of granular sludge

3.7.1 Light metal cation composition

Samples of digester granules were ashed in the muffle furnace for 4 hours at 550 Cº. The ash obtained was acid digested using the method of Langerak *et al.*, (1997). This involved taking 0.5 g of the ash and boiling it with 3 ml of concentrated HNO_3 and 9 ml of HCl in a reflux apparatus (Gerhart Kheldaterm). Three temperature/heating time combinations were used: 1hr at 80 Cº, 1hr at 120 Cº and 2 hr at 180 Cº. After cooling, the mixture was filtered (Filter paper Whatman No 1- 11cm) and any residue was rinsed out of the digestion tube using warm 12.5% HNO_3. The volume was made up to 100 ml in a volumetric flask with 12.5%HNO_3. The acid extract was then analysed for K, Na, Mg and Ca using the Varian AAS as described in section 3.1.1.

3.7.2 Energy Dispersed X-ray (EDX) analysis

Chemical composition analysis was carried out using the Energy Dispersed X-ray (EDX) microanalysis technique. The analysis was carried out using a JSM 6500F, JEOL 15kV electron microscope and the EDX spectra allowed the composition of salts that were present on the surface or within the granules to be determined. It also allowed observations to be made of the crystalline structure of the granules.

3.7.2.1 EDX of granule surface

During the first phase, analysis was carried out on after 120 days of operation. Two or three clusters were samples using a stainless tube from control digester, feeding on synthetic sewage (R1&R3) and experimental digester, feeding on wheat strawwashwater and switching with synthetic sewage (R2&R4). For second phase by using the same method, two or three cluster were selected from both reactor (R2&R4 - feeding on wheat straw washwater, R3&R1 – feeding on synthetic sewage in addition of KCl) after 530 days of operation (after 130 days feeding on wheat strawwashwater without switching to synthetic sewage). For both phases, 0.05 g of the sampled granules was air dried for 2 days. Dried material was then mounted on a specimen stub using electrically-conductive double-sided adhesive tape, and sputter coated with gold before examination in the microscope (JSM 6500F, JEOL) 15kV.

3.7.2.2 EDX of granule cross section

EDX analysis were conducted on granules which were samples using the same method in measuring surface morphology. Samples were air dried for 2 days. The granules were fixed in 3% glutaraldehyde and 4% formaldehyde for 24 hours. They were then rinsed twice for 10 minutes with Sodium cacodylate buffer and dehydrated using a series of ethanol solutions at 30, 50, 70, 95% and then two further steps using absolute ethanol. Each dehydration step in the series took 10 minutes. The dehydrated granules were then critical-point dried with CO_2 using a Critical Point dryer (Balzers).

In order to obtain a uniform cross section the granules were further stabilised by fixation in acetonitrile over two 10-minute exposures. The granules were then left overnight in acetonitrile and resin before being embedded into resin for 6 hours followed by further embedding in fresh resin and polymerisation at 60 Cº for 24 hours**.** Clip point blade was used to cut the granule which successfully embedded into resin. After gold sputter coating using SEM sputter coating unit (ES100 Polaron), the aggregates were examined on SEM (Quanta 200) (20 kV).

4. Results

4.1 Experiment 1: Characterisation of wheat straw, wheat straw washwater, and washed fibre.

The objective of Experiment 1 was to determine the mass balance for light metal cations and anions in raw wheat straw, washed wheat straw and wheat straw washwater, and the dependency of the partitioning of these on the duration and temperature of the wash.

To achieve this, experiments were carried out in which the wheat straw was washed under different conditions (section 3.3.2). Light metal cations were measured using flame atomic absorption spectrometry (section 3.2.1), and anions using ion chromatography (section 3.2.2). Some method development was also undertaken to establish the relationship between cation concentration and electrical conductivity to allow more rapid estimation of this for future experiments.

The experimental results are considered with respect to the necessary characteristics of washed straw for good combustion in terms of the agglomeration and fouling indicators reported for wheat straw and the quality of the washed fibre is also expressed in terms of the alkali index (See Table 2.2)

4.1.1 Cold water washing (20°C)

50 g of wheat straw was washed in 5.2 L of de-ionised water for 144 hours. The mass of anions and cations measured in the unwashed sample, the washed sample and the washwater is shown in Figure 4.1a and b. On a dry weight basis Potassium (15.0 mg g^{-1}) was the most abundant light metal cations in the straw followed by Calcium (10.10 mg g^{-1}). Chloride (10.77 mg g^{-1}) was the most abundant anion followed by Phosphate (3.49 mg g^{-1}). The other measured ions Sodium, Magnesium and Sulphate had concentrations of 0.50, 3.82 and 1.67 mg g^{-1} respectively.

Elements were found to be leached from the wheat straw with the following % efficiencies (weight of element in washwater / weight of element in raw straw); Mg (90), Na (89), K (87), Ca (86), SO_4 (82), Cl (82) and PO_4 (79).

Although the trends of removal are consistent among elements in terms of the mass balance, the total weight in the washwater and washed straw is slightly lower than the weight in the raw straw. The difference is believed to be due to the elemental analysis procedure in which some fine material is lost during ashing and some during the digestion process, as well as losses in sample preparation prior to measurement. The above results are consistent with those in the literature. Jenkins *et al. (*2001) had previously confirmed that Potassium was the major light metal cation species. Sander (1997) reported that water leaching allows rapid losses of K and concluded that this was because it was not associated with the structural components of plants. Based on the data obtained, the very high removal of Cl and K in from the straw is likely to give a washed straw with much better combustion properties.

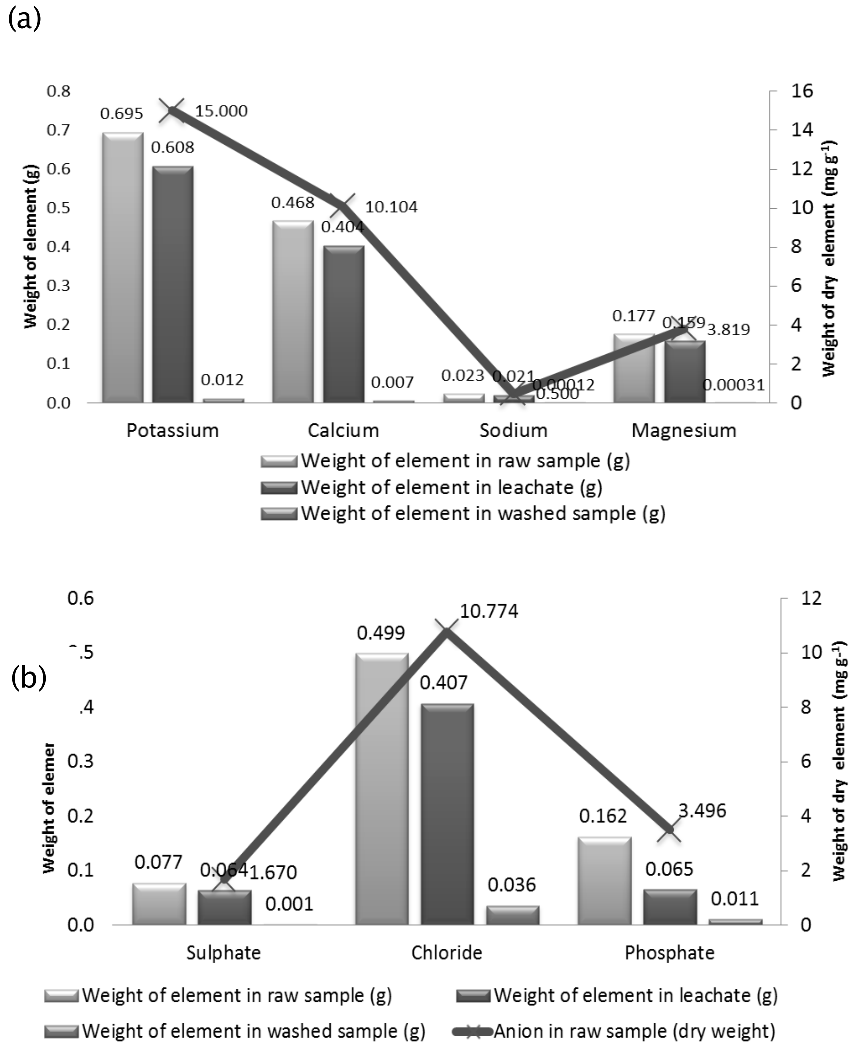


Figure 4.1 Partitioning of selected elements between wheat straw, washed fibre and washwater following a CWW

4.1.2 Relationship between electrical conductivity (EC) and Potassium and Sodium concentrations in washwater

Figures 4.2 and 4.3 show the relationship between conductivity (EC) and concentration of K and Na. There is a strong correlation between Potassium (K) and EC when extracted using three different water volumes (5.2, 4.2 and 3.2 litres) for 50 g of wheat straw over a period of 144 hours. On the other hand Sodium (Na) showed only a very weak correlation with EC (Figure 4.2 and 4.3). This therefore indicated that the presence of K is likely to be the major contributor to the EC in wheat straw washwater and the Na content does not greatly influence the result. This is confirmed by the relative abundance of K and Na in the washwater, with very low Na concentrations.

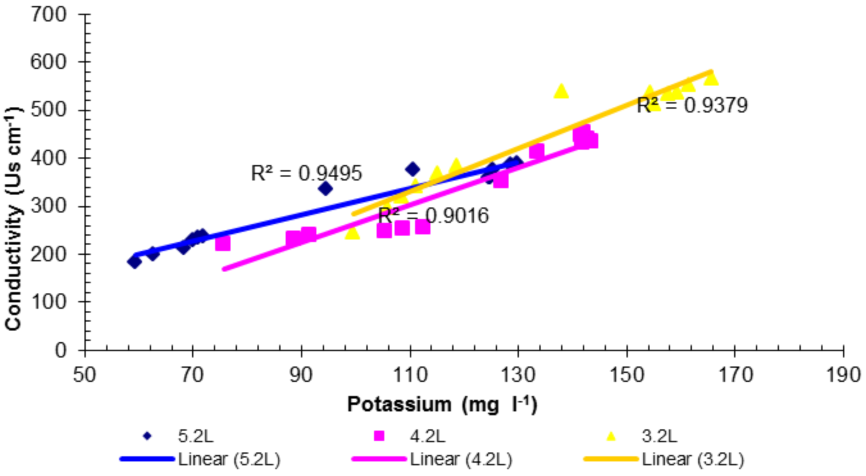


Figure 4.2 Influence of Potassium on conductivity in wheat straw washwater and correlation to the wheat straw : washwater mass ratio

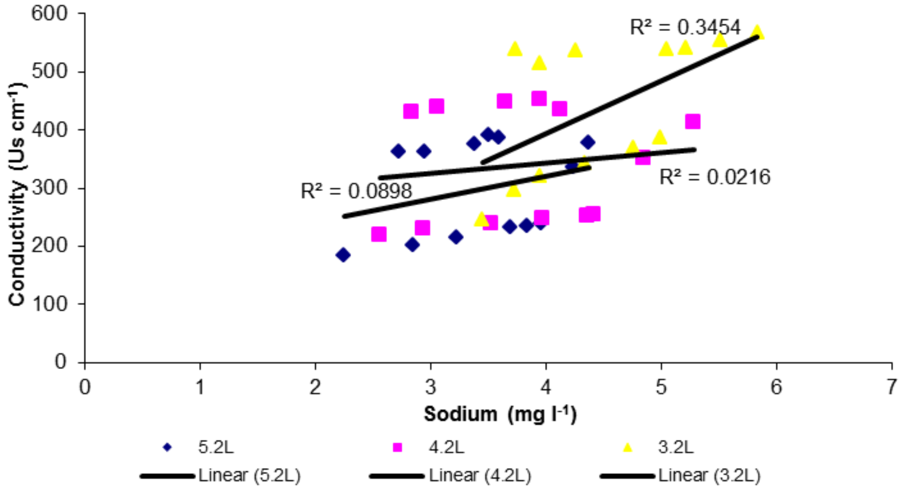


Figure 4.3 Influence of Sodium on conductivity in wheat straw washwater and the correlation to the wheat straw : washwater mass ratio

4.1.3 Changes in electrical conductivity in cold water washing over time

In the second part of the experiment 50g of wheat straw was leached with 5.2L of de-ionised water over a period of 170 hours. During this time measurements were made of the electrical conductivity and the concentrations of Sodium and Potassium. The results are shown in Figure 4.4 (NB: timescale not linear). During the first 6 hours of treatment, the EC of the washwater increased to 237 µS cm^{-1} which was equivalent to 71.73 mg L^{-1} of K. The maximum removal of EC and Potassium corresponded to an EC of 390 µS cm^{-1} which was achieved after 96 hours. It was concluded that even with a cold water wash (CWW) 60% and 55% respectively of conductivity and K could be removed within 24 hours, and 100% after 48 hours.

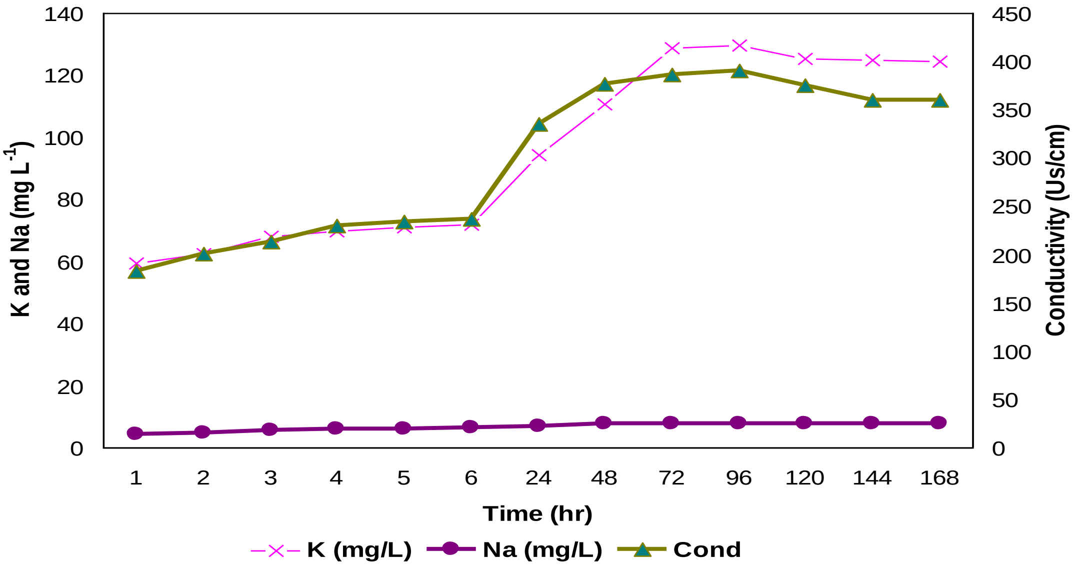


Figure 4.4 Change in conductivity of washwater over time and its relationship to Potassium and Sodium concentrations during the wheat straw washing process

4.1.4 Comparison of washing at different temperatures and mass ratios

The removal of salt, measured as an increase in the EC of the washwater, was determined at different ratios of wheat straw to water and at different temperatures. Because of the close correlation between TDS, Potassium concentration, and EC the latter was chosen as a simple but reliable method to estimate the salt content in the washwater. Salt removal (g) per wheat straw (g) can then be calculated using equation 14.

$$\frac{(g)\ TDS/L}{(g)\ Straw/L} = \frac{Salt\ removal\ (g)}{Straw\ (g)} \qquad \text{Equation 14}$$

Expressing the results in this way allows comparisons to be made independent of the water volume used in the extraction.

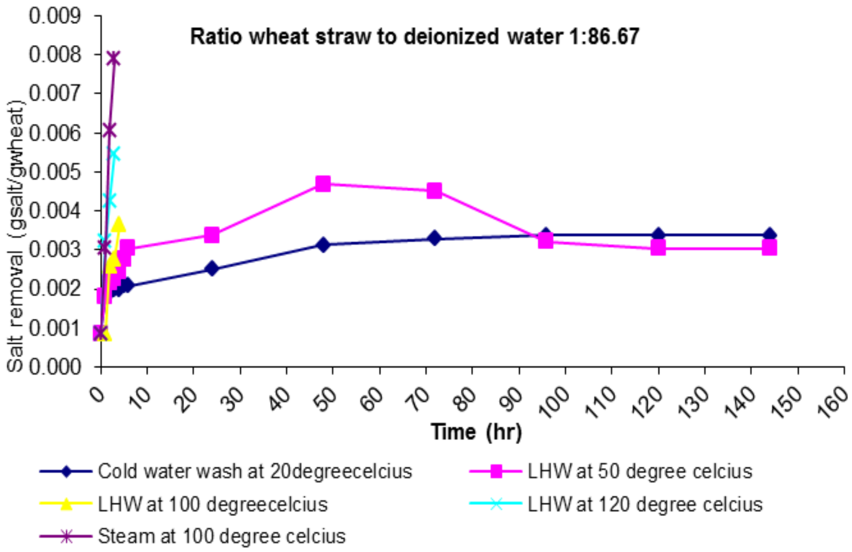


Figure 4.5 Salt removed from wheat straw during washing at different temperatures at a wheat straw: deionised water ratio of 1:86.67

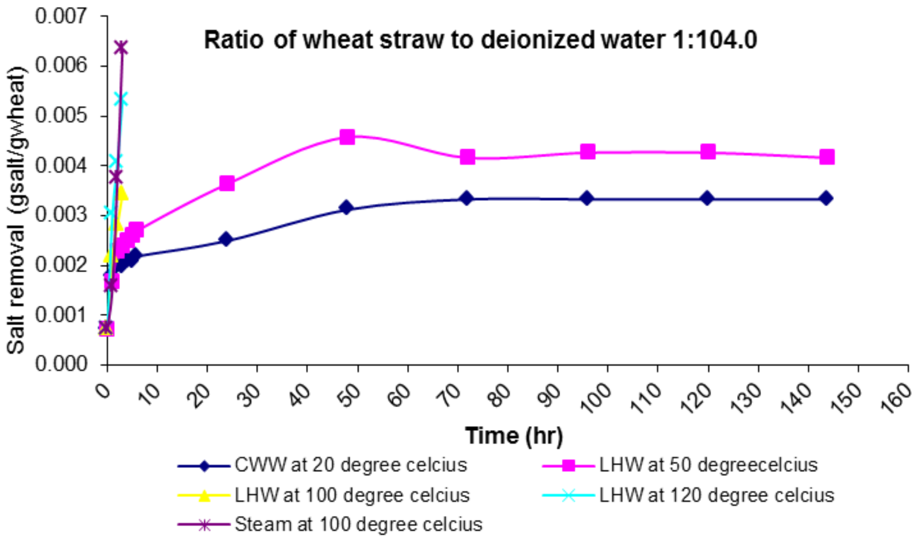


Figure 4.6 Salt removed from wheat straw during washing at different temperatures at a wheat straw: deionised water ratio of 1:104

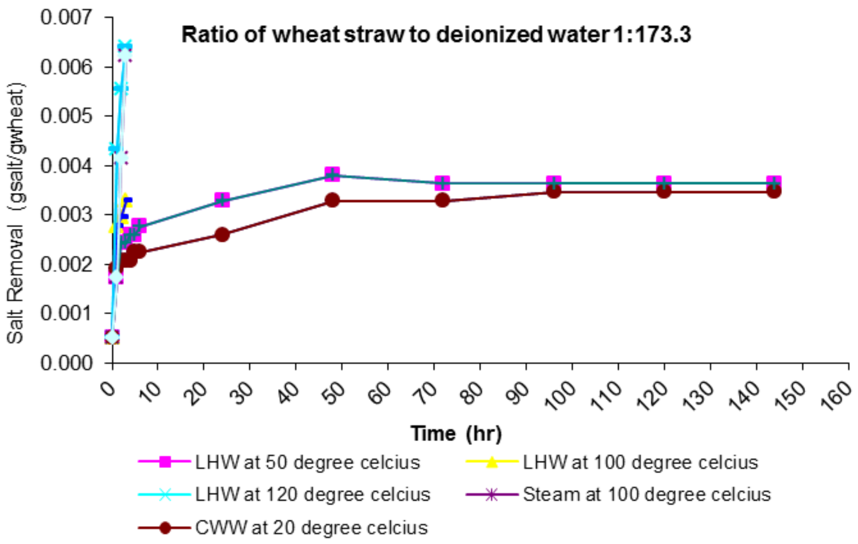


Figure 4.7 Salt removed from wheat straw during washing at different temperatures at a wheat straw: deionised water ratio of 1:173.3

The results are shown graphically in Figures 4.5 – 4.7 and clearly demonstrate that there is an initial rapid release of salt irrespective of the temperature, but the rate of release then slows and is temperature dependent. The magnitude of the release is also temperature dependent and was greater at the highest temperatures. In cold water washing, there was less than 35% removal of salt compared to the value achieved at 120 °C. Likewise using liquid hot water (LHW) at 50°C resulted in 48.3%, 48.8% and 41.7% salt removal compared to 120°C for the wheat straw/water ratios of 1:86.67, 1:104.0 and 1:173.3 respectively. At 120^{o}C after one hour of treatment the washwater had an EC of 60.2 µS cm^{-1} (25 mg TDS L^{-1}), equivalent to 0.004 salt g^{-1} wheat treated. The highest removal achieved was at a treatment temperature of 120 ^{o}C over a 3-hour period where the measured washwater EC was 222 µS cm^{-1} (91 mg TDS L^{-1}), equivalent to 0.0079 g salt g^{-1}wheat treated.

From the perspective of reaction time, both CWW and LHW achieved maximum removal at 48 to 96 hours and started to reach the saturated condition by 144 hours. Experiments using temperatures of 100 ^{o}C and above did not continue past 3 hours and at this point there was still no flattening of the extraction curve.

Removing ash components as salts from the straw by leaching will also increase the higher heat value of the biomass. In this study, it was found that unleached wheat straw had a value of 13.52 MJ kg^{-1} while straw leached at 120°C showed a value of 19.81 MJ kg^{-1}, an increase of 31%. The hot water wash at 50°C increased the calorific value to 14.14 MJ kg^{-1}, only a 3.1% rise. The results reported here are in line with the findings of others. Sander (1997) stated that washing had in all cases increased the melting temperature of the ash and reduced the ash content, thus improving the calorific value of biomass. Bridgwater (2003) estimated that a 1% increase in ash content roughly decreased the calorific value by 0.2 MJ kg^{-1}. Cuiping *et al.* (2004) reported the heating value of biomass is in the range of 16–20 MJ kg^{-1}, which is similar to the range of values found here for washed and un-washed straw biomass.

4.1.5 Potential effect of straw and washed straw on combustion processes

Table 4.1 compares results from the characterisation analysis of the wheat straw and the washed wheat straw with the agglomeration and fouling indicators which have been reported for combustion with wheat straw.

Table 4.1 Comparison of calculated values for Agglomeration and Fouling Indicators with those of Ronald *et al.* (2008).

		This study		Ronald's study	
	Unit	**Raw wheat straw**	**Washed straw**	Raw Straw	Lime treated
Fluidized bed combustion					
agglomeration indicators					
1) Sinter induced					
limit value (Na+K)/(2S+Cl) > 1	(g/kg)	**1.10**	**0.31**	2.70	0.80
2) Smelt induced					
limit value (K + Na) >1	(g/kg)	**15.50**	**0.96**	15.00	5.00
Fluidized bed combustion					
fouling indicators					
Limit value Alkali Index <0.17	(kg alkali index/GJ)	**0.90**	**0.05**	0.90	0.30

The fluidised bed agglomeration indicators show a reduction to below the limit values in all cases for the washed straw produced in this study compared to the raw unwashed straw. Results for the sinter induced indicator showed a value of 0.31 compared to the threshold value of 1. Sinter induced, smelt induced and alkali index reduced after washing to about 71%, 94% and 94% respectively.

The fouling indicator as assessed by the alkali index showed a reduction from 0.9 to 0.05 kg GJ^{-1} which was below the threshold of 0.17 kg GJ^{-1}. The results show that water washing gave better reductions than those found by Ronald *et al. (*2008) using an alkaline treatment. This result indicates that cold water washing might be an effective pre-treatment as the removal of Potassium is very high; it may however be limited where there is a high chlorine content.

Figure 4.8 shows the reduction in alkali index for a cold water wash of 50g of straw using three different water volumes. The results show that the washwater volume has a significant effect on the alkali index, and this can be explained by the quantity (gram) of salts removed per gram of wheat straw.

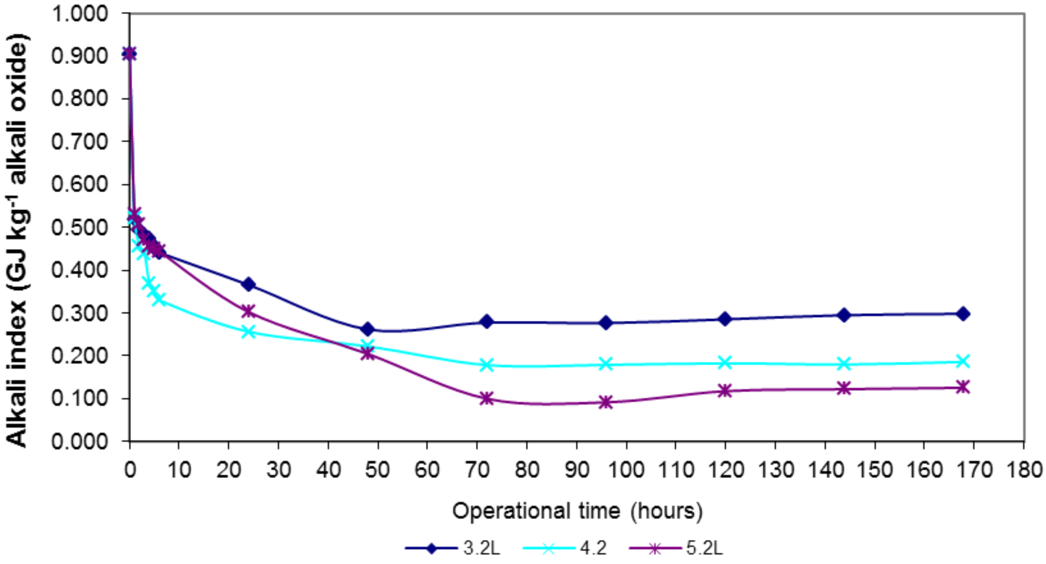


Figure 4.8 Change in Alkali Index over time for 50 g samples of wheat straw washed in 3 different volumes of deionised water (3.2, 4.2 and 5.2 L) at ambient temperature.

Miles *et al.* (1996) stated that an alkali index of 0.17 was the threshold limit necessary for complete avoidance of fouling problems which can be caused by high ash deposition rates. An upper limit of 0.34 was also given as an indicator that fouling will probably occur above this point. Results from the most concentrated washwater (Figure 4.8) showed the alkali index to be around 0.26 kg GJ^{-1} after 48 hours of washing using 3.2 litres of water for 50 g of wheat straw. Although not guaranteed to be safe from fouling problems, the value is well inside the upper limit. 4.2 L of water gave an alkali index of 0.18 kg GJ^{-1}, and 5.2 L a value of 0.09 kg GJ^{-1}. In general, all treatment required a minimum of 48 hours to remove salt to its lowest concentration and after 72 hours the alkali index tended to stable.

4.2 Experiment 2: Potential of wheat straw washwater for anaerobic digestion

The objective of Experiment 2 was to determine the biodegradability and methane potential of wheat straw washwater (WSW) using UASB digesters. To ensure the reliability of the experimental approach control digesters were run on a tried-and-tested synthetic wastewater, the characteristics of which were known in terms of its biodegradability and specific methane potential.

The experiment was carried out in the early design digesters labelled R1 to R4, which were set up as described in section 3.4.2.1. The digesters were fed on synthetic wastewater (SW) (section 3.3) or on WSW obtained by washing 50 g of wheat straw in 5.2 litres of deionised water at ambient temperature for 144 hours (Section 3.2.2). In order to maintain a supply of WSW throughout the experiment the washing procedure had to be carried out a number of times, and the variability between the batches prepared is shown in Table 4.2.

Table 4.2 Characteristics and variability of wheat straw washwater used as feedstock and prepared in different batches.

Period (days)	49-77	104 – 140	181- 209	233 - 258	276 - 297	321 – 339
COD (mg L^{-1})	1200.0	1021.0	1021.0	1005.0	1005.0	1037.0
K (mg L^{-1})	149.7	124.0	124.0	111.7	111.7	138.9
Na (mg L^{-1})	4.7	4.0	4.0	5.6	5.6	4.7
Mg (mg L^{-1})	37.8	32.0	32.0	35.2	35.2	45.1
Ca (mg L^{-1})	82.2	82.3	82.3	74.4	74.4	65.3
Zn (mg L^{-1})	3.4	3.2	3.2	5.9	5.9	6.1
Cl^- (mg L^{-1})	56.9	83.0	83.0	77.7	77.7	74.3
SO_4^{2-} (mg L^{-1})	119.9	113.0	113.0	125.4	125.4	124.4
PO_4^- (mg L^{-1})	13.3	13.0	13.0	14.6	14.6	15.3
Conductivity (µS cm^{-1})	548.0	437.0	437.0	532.3	532.3	521.9

Two of the UASB digesters were used as a control and fed only on the synthetic wastewater to ensure that biogas production could be maintained under the conditions used (temperature, OLR, HRT). A hydraulic retention time (HRT) of 24 hours was used coupled to an organic loading rate (OLR) of 1 g COD l^{-1} day^{-1}; these values have previously been shown to be suitable for this type of digester and were well within safe operational limits (section 2.7.1). The other two UASB digesters were fed on the washwater and synthetic wastewater in a cyclic manner, but at the same OLR and HRT. The duration of each cycle was based on observations of changes in biogas production and loss in performance. The extended period of continuous sequential exposure to the WSW was to assess the potential for longer-term acclimatisation. As a result of this cyclic feeding pattern the accumulation of Potassium in the digesters during WSW feeding was noted (section 4.2.2) and its subsequent loss on switching the feed to synthetic sewage. These changes were quantified and a mass balance applied. The results were then used to assess the effect of Potassium accumulation on specific methane production as this was thought to be an energy-consuming cellular activity in maintaining osmotic gradients. The exact feeding pattern followed is shown in Table 4.3 and extended over a period of 366 days.

Table 4.3 Feed regime for the UASB digesters

Cycle	**From** day	**To** day	**Days** no.	**Feed**	**Target OLR** g COD l^{-1} day^{-1}
R1 and R3					
-	1	208	207		1
-	209	221	12		2
R2 and R4					
0	1	48	47	SW	1
1	49	77	28	WSW	1
1	78	103	25	SW	1
1	104	104	0	No feed for one day	1
2	105	140	35	WSW	1
2	141	145	4	Tap water	1
2	146	180	34	SW	1
3	181	209	28	WSW	1
3	210	232	22	SW	1
4	233	258	25	WSW	1
4	259	275	16	SW	1
5	276	297	21	WSW	1
5	298	320	22	SW	1
6	321	339	18	WSW	1
6	340	366	26	SW	1

4.2.1 Methane production and COD removal

4.2.1.1 Control digesters R1&R3

Digesters R1 and R3 were controls and were fed on synthetic wastewater (SW) at an organic loading rate (OLR) of 1 g COD l^{-1} day^{-1} from day 1 to day 208. Stable gas production was achieved by day ~45 (Figure 4.9). Average specific biogas production (SBP) between day 46 and day 208 was 0.558 and 0.559 l g^{-1} COD_{added}, with an average specific methane production (SMP) of 0.309 and 0.306 1 CH_4 g^{-1} COD_{added}, for R1 and R3 respectively. COD removal rates were also stable, and averaged 95.0% for both digesters. The specific methane production in terms of COD removal was 0.325 and 0.323 l CH_4 g^{-1} $COD_{removed}$ for R1 and R3 respectively, corresponding to 92.9% and 92.2% of the theoretical value based on stoichiometric calculation.

The OLR on R1 and R3 was increased to 2 g COD l^{-1} day^{-1} on day 209 by doubling the strength (i.e. halving the dilution used when preparing it from the concentrated SW), while maintaining the same HRT. A slight reduction in SMP and COD removal rates was seen in the following 3-4 days, followed by a recovery to previous values.

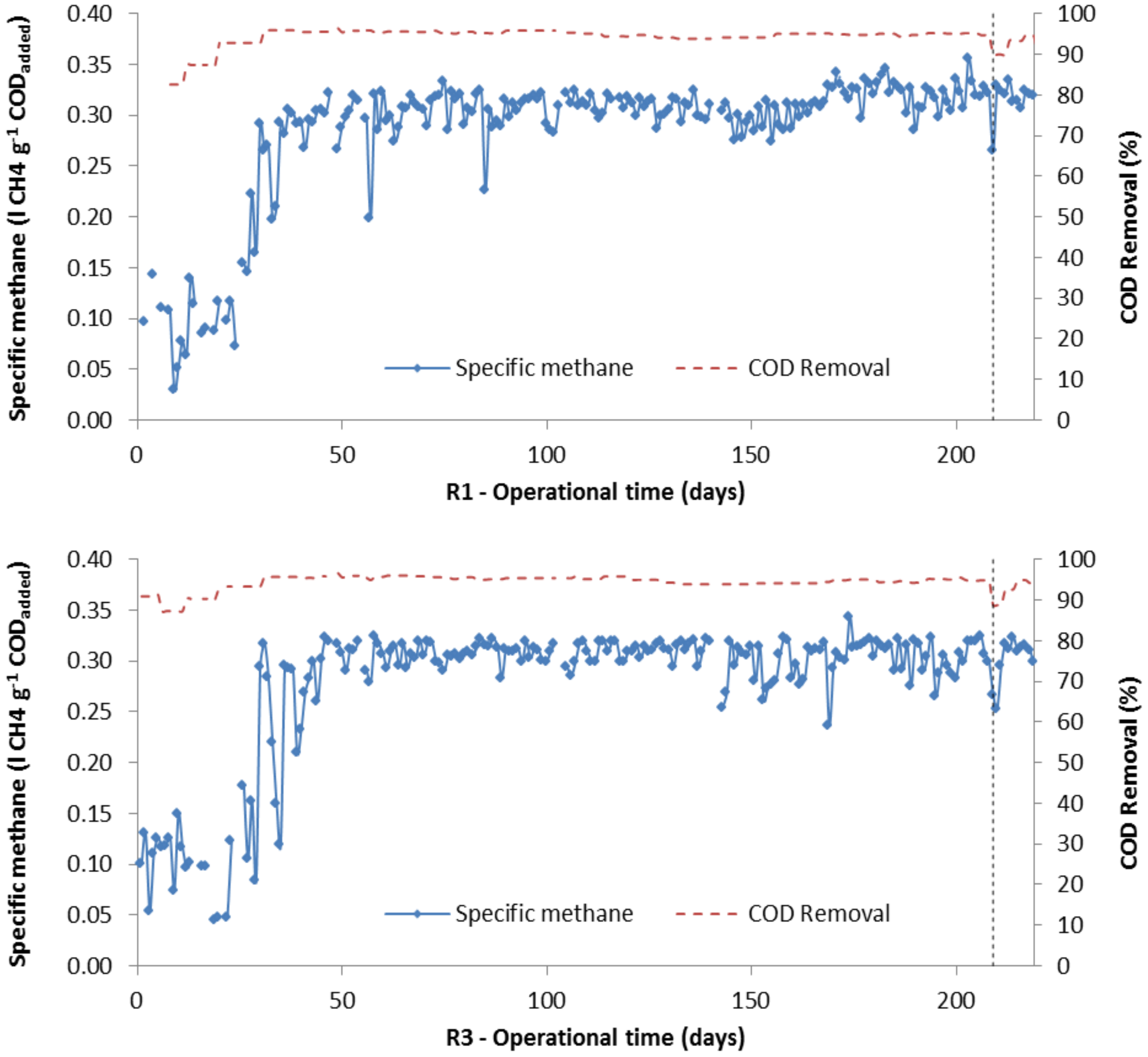


Figure 4.9 Daily specific methane production and average COD removal (based on twice-weekly sample) in control UASB digesters R1 and R3 fed on synthetic wastewater from day 1-209. The vertical line at day 209 represents a change in OLR from 2 to 4 g COD l^{-1} day^{-1}.

4.2.1.2 Experimental digesters R2 & R4

Trends in SMP and in the % COD removal in the two experimental reactors showed good agreement throughout the period (Figure 4.10).

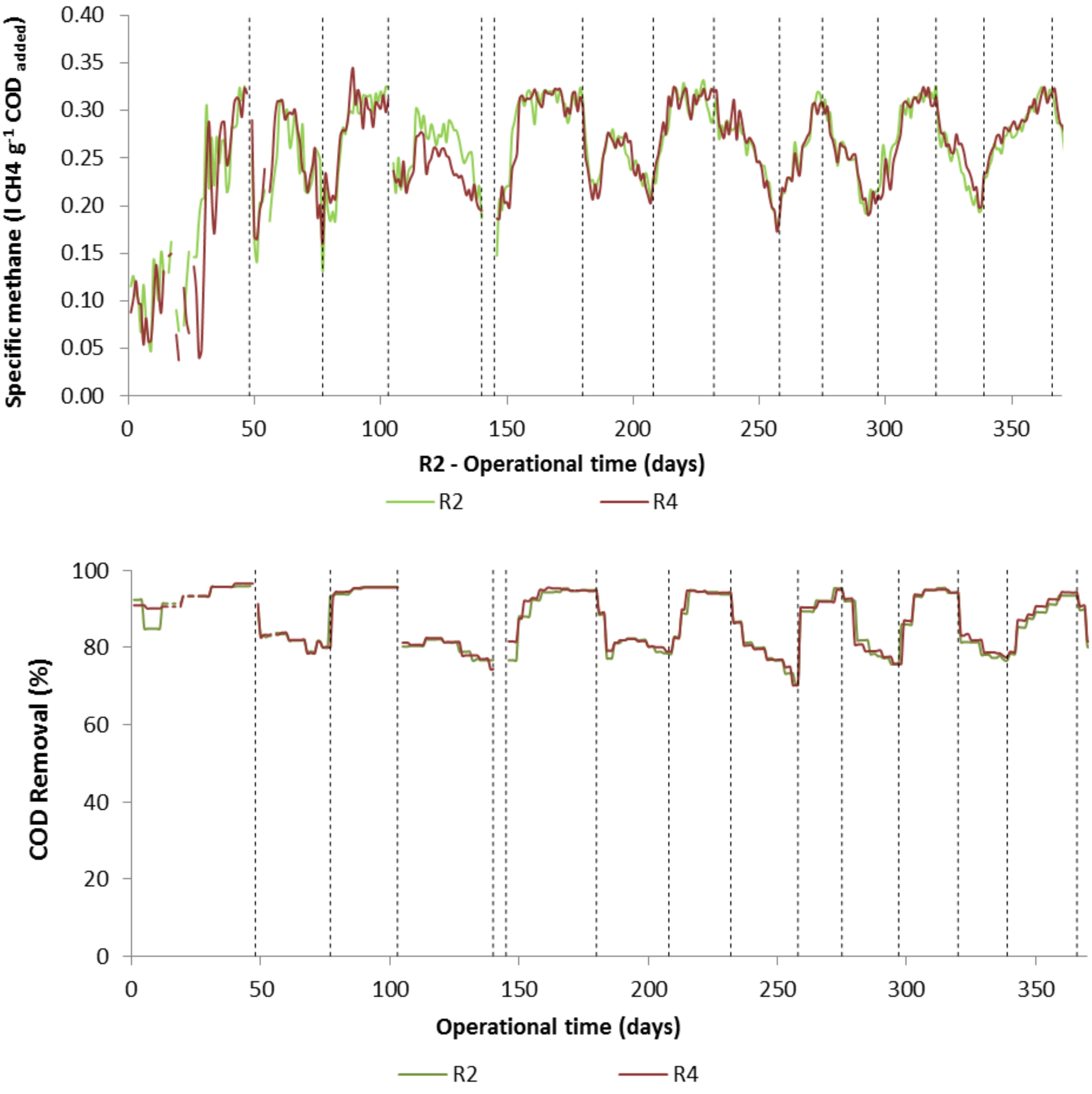


Figure 4.10 Specific methane production and %COD removed in experimental UASB digesters R2 and R4 fed with a cyclic feed pattern of synthetic wastewater and WSW. Vertical lines indicate a change in feed.

From day 1 to day 48 digesters R2 and R4 were fed on SW at an organic loading rate (OLR) of 1 g COD l^{-1} day^{-1}, and showed very similar behaviour to the control digesters R1 and R3 in terms of both gas production and COD removal (Figure 4.11).

On day 49 the feed to both digesters was changed to wheat straw washwater (WSW) at the same OLR of 1 g COD l^{-1} day^{-1}. In the next 3 days the SMP dropped significantly to 0.20 and 0.19 l CH_4 g^{-1} COD $_{added}$ for R2 and R4 respectively, reflecting the change in feed (Figure 4.12). The SMP then recovered in the period up to day ~60 in both digesters to a value of around 0.30 l CH_4 g^{-1} COD $_{added}$, which was maintained for around 6-10 days. Both digesters then showed a gradual decline in SMP to below 0.2 l CH_4 g^{-1} COD_{added} by day 77. When the feed was changed to WSW there was also an initial reduction in COD removal rate to 83% in both digesters, and this further declined by day 77 to around 80%. At this point the feed was switched back to SW, and by day 90 SMP and COD removal rates had recovered to the previous values for this substrate of around 0.31 l CH_4 g^{-1} COD_{added} and 95%, indicating that the inhibition seen was reversible. Stable operation on SW continued until day 103.

On day 105 the feed was switched to WSW at the same OLR of 1 g COD l^{-1} day^{-1}. This was again accompanied by an immediate fall in both SMP and COD removal, but the fall in SMP was not as severe as observed on the previous exposure to WSW. Recovery again occurred after about 10 days leading to fairly stable gas production for a further ~10 days, before starting once again to decline. The maximum SMP achieved with the WSW, however, was lower than that previously observed. By day 140 the SMP had again fallen to below 0.21 l CH_4 g^{-1} COD_{added}, with COD removal rates of 77% or below.

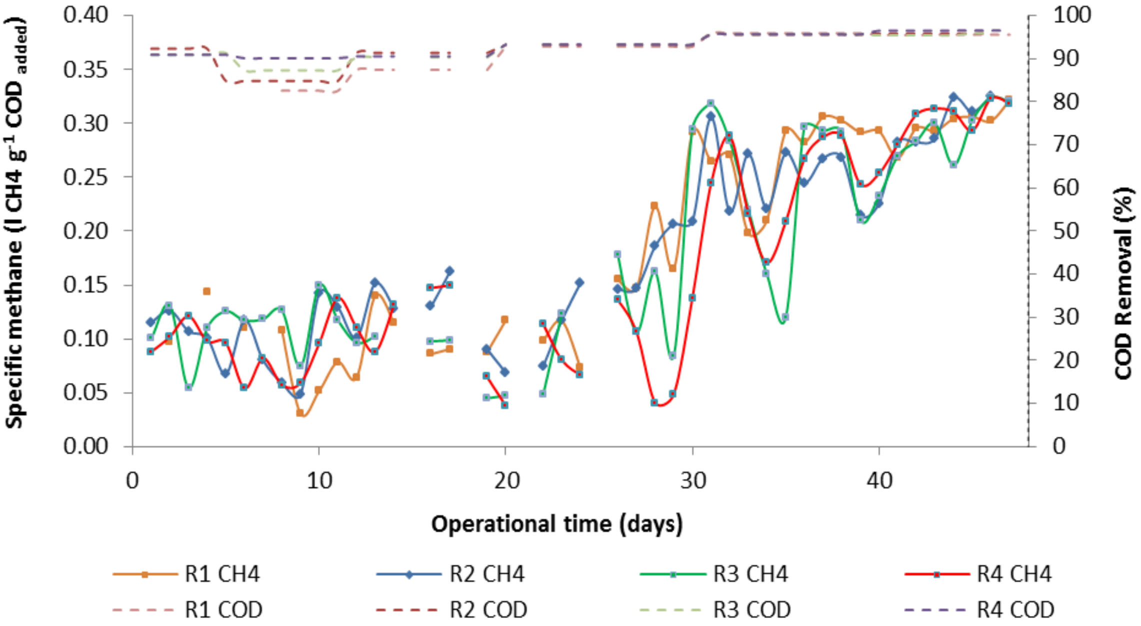


Figure 4.11 Specific methane production and % COD removal in experimental UASB digesters R2 and R4 compared to the control digesters R1 and R3 over the first 48 days of operation. All digesters were fed on synthetic wastewater.

During these two cycles it was observed that when the feed was WSW the concentration of Potassium in the effluent was lower than in the influent, indicating that Potassium was accumulating in the digesters. When the feed was switched to SW, the effluent concentration increased, indicating washout of Potassium. In response to this, after the second period of feeding with WSW ended on day 140, R4 was fed with tap water only for 5 days (day 141-145) and R2 was left without feed for 2 days then fed with tap water only for 3 days, to allow further observations on Potassium removal (see section 4.2.2). On day 146 the feed was switched to SW until day 180 to confirm that SMP and COD removal rates were stable and at similar values to those in the control reactors (Figure 4.12).

From day 180 – day 366 a further four cycles of alternating feed with WSW and SW were performed (cycles 3-6, Table 4.4, Figure 4.13) to determine whether there was any further acclimatisation to the WSW feed, both in terms of specific methane production and of COD removal. Cycle 3 showed a similar pattern to the first two cycles, with a rapid initial fall in SMP to 0.21 l CH_4 g^{-1} COD_{added} followed by recovery over the next ~10 days to 0.27 l CH_4 g^{-1} COD_{added}, then a decline as before. In cycle 4 (day 233-275) the initial fall in SMP was less sharp, possibly suggesting some acclimatisation, but the recovery was less pronounced and the decline started earlier, at around day 240. When the feed was switched back to SW in the second half of the cycle the rate of recovery was slower. In the fifth and sixth cycles this trend continued with an almost continuous decline in SMP after the introduction of WSW and a slower recovery once the feed was changed back to SW.

The pattern for SMP per g COD removed was broadly similar to that for COD added (Figure 4.14) but the peak values for WSW were higher, indicating that the lower SMP of the WSW was in part due to the presence of a non-anaerobically degradable fraction.

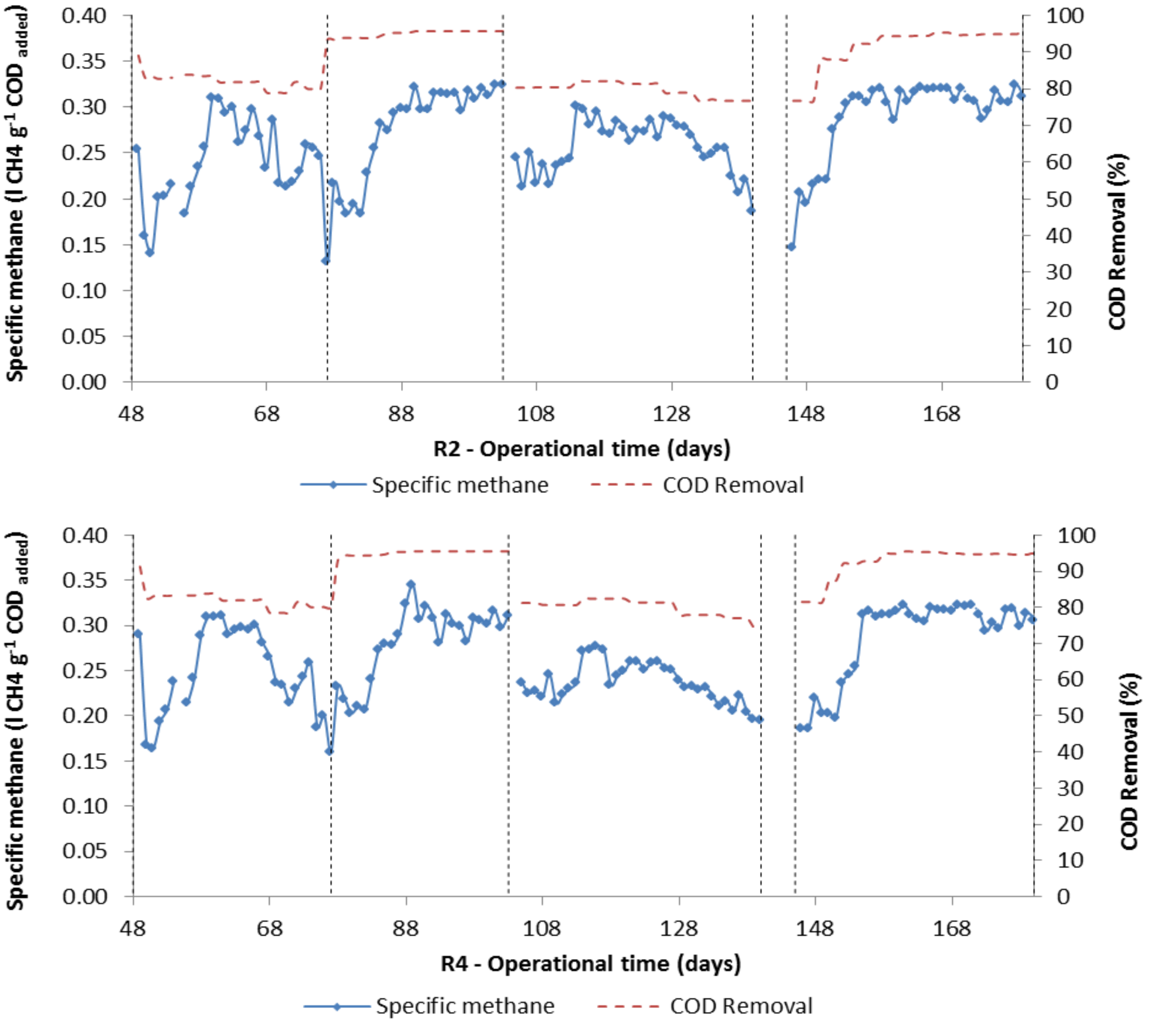


Figure 4.12. SMP and % COD removal in experimental UASB digesters R2 and R4 over the first 180 days of cyclic feeding (Table 4.3). Tap water (no line) was fed from day 141 to day 145. Vertical lines indicate a change in feed.

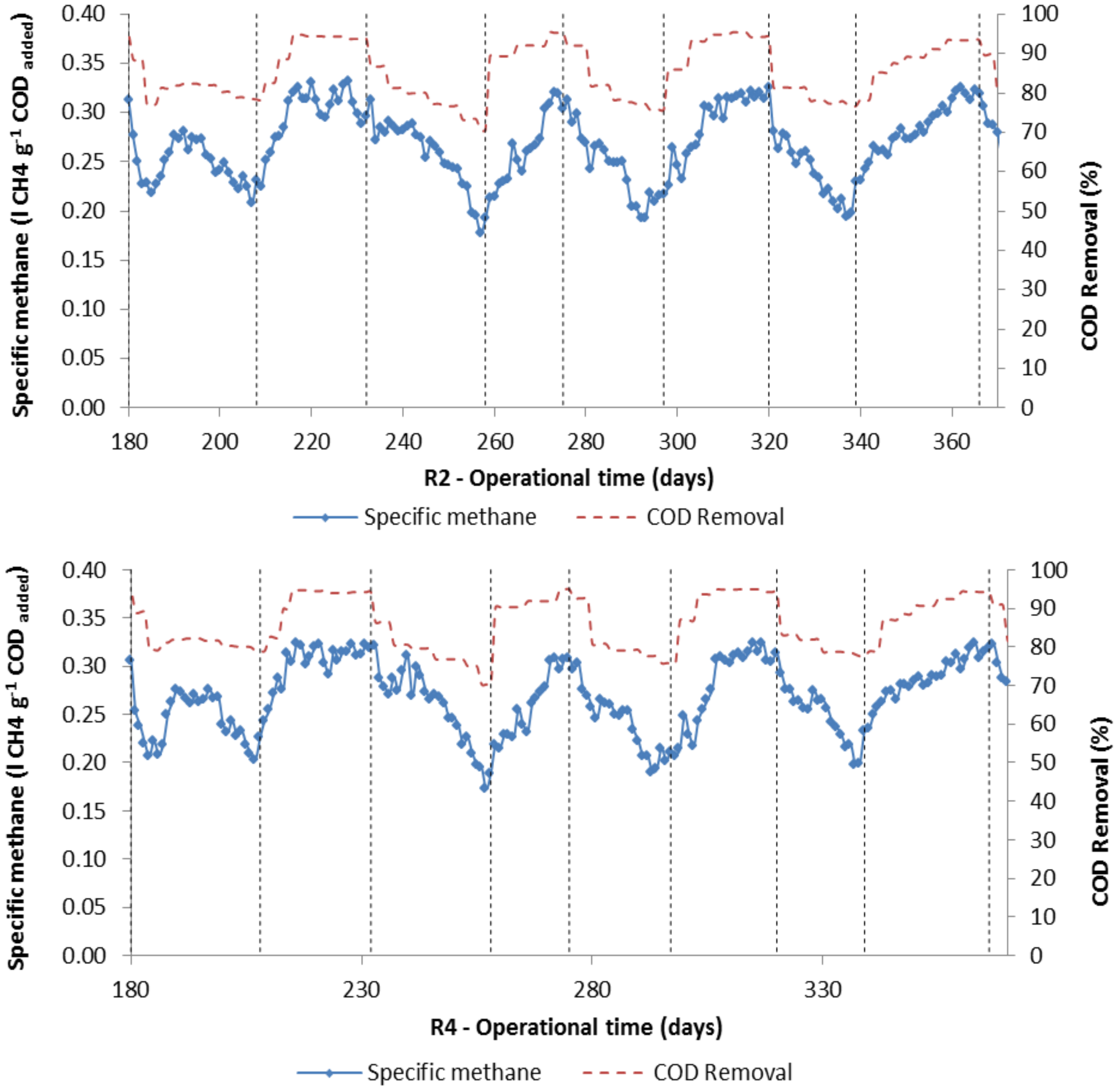


Figure 4.13. SMP and % COD removal in experimental UASB digesters R2 and R4 between day 181 and 366 when subjected to cyclic feeding (Table 4.3). Vertical lines indicate a change in feed.

4.2.2 Potassium accumulation

As noted above, changes were observed in the UASB effluent Potassium concentrations when switching feeds between WSW and SW, indicating selective removal of Potassium from WSW. The amount accumulating in the digesters was calculated by mass balance, based on the difference in influent and effluent concentration, the daily feed volumes and the digester capacity. The results are shown in Figure 4.14 together with the SMP.

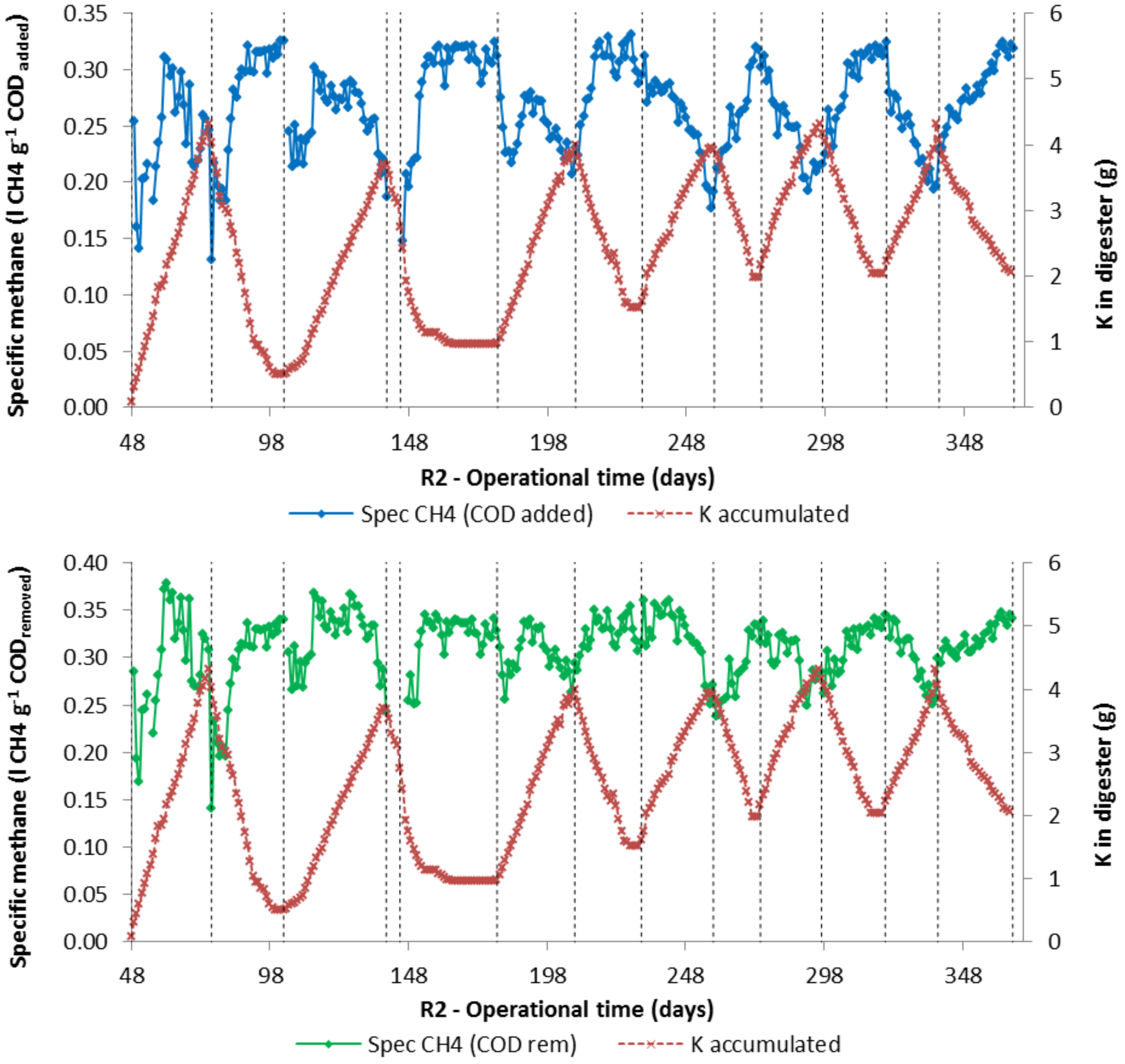


Figure 4.14. SMP and accumulation and washout of Potassium over time in experimental UASB digesters R2 and R4 in relation to changes in feed between WSW and synthetic wastewater for cycles 1-6.

In the first cycle an additional 4.32 [4.21] g K accumulated in R2 [values for R4 shown in brackets], equivalent to around 9.5 mg K g^{-1} wet weight of the original granular sludge inoculum. On feeding with SW this quantity rapidly fell to around 1.0 [0.98] g K, at which point the rate of washout decreased. The net accumulation at the end of cycle 1 was 0.50 [0.45] g K from the initial value before any WSW addition. In the second cycle the initial loss of K due to water washing followed by SW was rapid, but again decreased, leaving a final accumulation of 0.96 [0.94] g K. Over the next four cycles this pattern was repeated with incremental increases in the quantity of K accumulated in the digester (Figure 4.14 and Table 4.4).

Table 4.4 Maximum and minimum accumulation of K in digester R2 and R4 during each cycle and the average daily rate of accumulation.

Cycle	**Feed**	**K accumulated** (mg K g^{-1} WW)*		**Rate of k accumulation** (g K g^{-1} WW day^{-1})[a]			
		R2	*R4*	*average between days*		*R2*	*R4*
1.1	WSW	9.6	9.4	49	77	0.33	0.32
1.1	SW	1.1	1.0	78	92	-0.40	-0.49
1.2	WSW	8.2	8.2	107	140	0.22	0.23
1.2	SW	2.1	2.1	145	149	- [b]	- [b]
1.3	WSW	8.9	8.9	181	209	0.25	0.24
1.3	SW	3.4	3.2	210	225	-0.26	-0.27
1.4	WSW	8.8	9.6	233	258	0.20	0.22
1.4	SW	4.4	4.0	259	275	-0.29	-0.33
1.5	WSW	9.6	9.1	276	297	0.23	0.20
1.5	SW	4.5	4.3	298	315	-0.27	-0.26
1.6	WSW	9.6	9.1	321	339	0.23	0.23
1.6	SW	4.6	4.4	340	366	-0.16	-0.16

[a] WW = wet weight of original inoculum

[b] no stable slope due to water washing

At the end of the WSW feed in cycle 2 R2 and R4 were fed with tap water from day 141 to day 145. The quantity of K accumulated in R2 in this period fell from 3.68 to 3.11 g [equivalent values for R4: 3.37 g to 2.63 g], corresponding to a rate of -0.41 [-0.32] mg K g^{-1} WW day^{-1}. These results indicated that the rate of washout with water was similar to that with SW, despite the fact that the SW contained approximately 16 mg K l^{-1}.

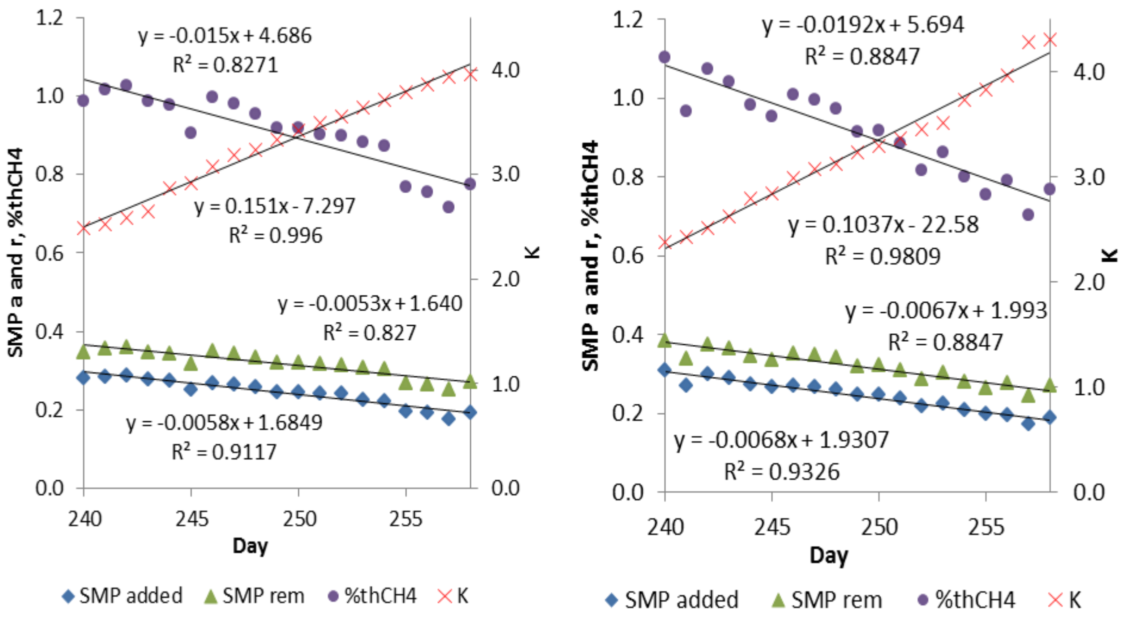
Table 4.5 shows the SMP per g COD removed as a percentage of the theoretical value based on stoichiometry, on the last day before each change of feed from WSW to SW and vice versa. Immediately before feeding with WSW is discontinued, when the K accumulation in the digesters is highest, the percentage of removed COD converted to methane is at its lowest; while at the end of the cycle when the removable portion of the accumulated K has been washed out the COD conversion is close to 100% of the theoretical value. This result may indicate that when Potassium accumulation occurs, a proportion of the energy available from COD conversion is not being channelled into methane production. This is consistent with the theory that methanogenic organisms operate a Potassium pump to maintain ionic equilibrium, and as this is an energy-driven process it will therefore reduce the overall methane yield. The results are not fully consistent as the periods of feeding with each substrate differed slightly in each cycle and also as there were minor day to day variations in biogas production while influent and effluent COD were only measured weekly. It was therefore decided that feeding with WSW should continue for an extended period in a subsequent experiment to allow more accurate assessment of the differences in SMP as a proportion of the theoretical methane yield per g COD applied or removed.

Table 4.5 SMP per g COD removed at end of each cycle of feeding on either synthetic sewage or WSW. COD converted to methane is expressed as a % of that expected based on stoichiometry.

Cycle	Feed	Day	SMP per g COD removed as % of theoretical value*	
			R2	R4
1.1	WSW	77	40	57
1.1	SW	103	97	93
1.2	WSW	140	70	75
1.2	SW	180	98	95
1.3	WSW	209	82	88
1.3	SW	232	90	97
1.4	WSW	258	78	77
1.4	SW	275	91	93
1.5	WSW	297	81	80
1.5	SW	320	98	95
1.6	WSW	339	85	86
1.6	SW	366	97	97

* based on 0.35 l CH_4 per g COD converted at 101.325 kPa and 0 ^{o}C

Rates of change in the SMP per g COD added, SMP per g COD removed, COD removal rate, and SMP as a proportion of the theoretical yield were calculated within each cycle over periods when they were approximately uniform (see Figure 4.15 and Table 4.6). No apparent relationships were found between these parameters and either the rate of K accumulation or the quantity of K accumulated. As all of the batches of WSW were of similar strength, however, the range of rates of K accumulation was relatively small. In a following experiment the strength of the WSW was increased.



R2 cycle 4

R4 cycle 1

Figure 4.15 Example of method used for determination of rates of change in the SMP per g COD added, SMP per g COD removed, COD removal rate, and SMP as a proportion of the theoretical yield, calculated over a uniform period within one cycle, for experimental UASB reactors R2 and R4.

Table 4.6 Rate of change in K accumulation, SMP per g COD added, COD removal rate, SMP per g COD removed and SMP removed as a % of theoretical value calculated over uniform periods within each cycle. Results for R4 (R2 results were similar).

Cycle	Feed	Average between days		Rate of change				
				K	SMPa	COD	SMPr	$thCH_4$
				mg l^{-1} day^{-1}	l CH_4 g^{-1} COD added day^{-1}	g COD day^{-1}	l CH_4 g^{-1} COD removed day^{-1}	ratio day^{-1}
1.1	WSW	63	77	0.15	-0.009	-0.131	-0.010	-0.029
1.1	SW	77	89	-0.23	0.013	0.616	0.012	0.034
1.2	WSW	121	140	0.10	-0.004	-0.365	-0.003	-0.009
1.2	SW	146	155	-0.12	0.011	1.566	0.009	0.025
1.3	WSW	197	207	0.11	-0.007	-0.227	-0.008	-0.022
1.3	SW	208	214	-0.13	0.013	1.928	0.008	0.023
1.4	WSW	240	258	0.10	-0.007	-0.529	-0.007	-0.019
1.4	SW	258	271	-0.14	0.007	0.735	0.005	0.015
1.5	WSW	288	293	0.05	-0.012	-0.395	-0.014	-0.039
1.5	SW	302	307	-0.14	0.016	1.137	0.014	0.039
1.6	WSW	328	337	0.11	-0.008	-0.358	-0.009	-0.025
1.6	SW	339	344	-0.12	0.008	2.048	0.003	0.007

SMPa = SMP per g COD added, SMPr = SMP per g COD removed, $thCH_4$ = theoretical CH_4 yield based on COD removed

4.3 Experiment 3: Use of higher strength wash water feed

The objective of Experiment 3 was to assess the effect of increasing the strength of the WSW on the performance of the UASB digesters in terms of methane production, COD removal and K accumulation.

Experiment 3 was carried out in digesters R1 and R3, which were set up as described in section 3.4.2.1 and had served as control reactors fed on SW only for the preceding 220 days (section 4.2.1.1). The feed used was WSW prepared by washing 50 g of wheat straw in 2.6 litres of water at ambient temperature for 144 hours. This gave a feed COD concentration strength about double that used in Experiment 2. The HRT remained the same and therefore the OLR and the concentration of K were also about double. The feeding pattern was cyclic alternating the WSW with synthetic wastewater as shown in Table 4.7.

The WSW had the following characteristics (mg l^{-1}): COD 2000; K 248.75; Na 7.83; Mg 92.34; Ca 104.50; Zn 12.36; Cl^- 123.00; SO_4^{2-} 169.61; PO_4^- 23.45, conductivity 995.43 µS cm^{-1}.

Table 4.7 Feed regime for digesters R1 and R3 in Experiment.

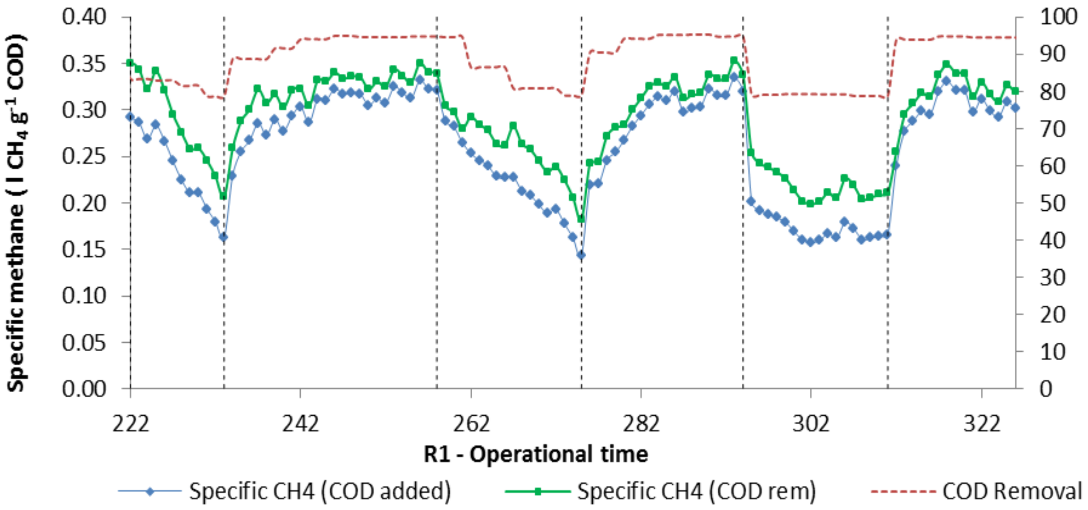
Cycle	from day	to day	days	R1 and R3
2.1	222	233	11	WSW
2.1	234	258	24	SW
2.2	259	275	16	WSW
2.2	276	294	18	SW
2.3	295	311	16	WSW
2.3	312	326	14	SW

4.3.1 Methane production and COD removal

When feeding with WSW commenced on day 222 the SMP in both digesters fell slightly over the next 2-3 days and then more sharply (Figure 4.16), reaching 0.163 and 0.165 l CH_4 g^{-1} COD_{added} (0.207 and 0.208 l CH_4 g^{-1} $COD_{removed}$) by day 233 in R1 and

R3 respectively, equivalent to COD removal rates of 78.7 and 79.2. The rate of fall of SMP was around -0.015 l CH_4 g^{-1} COD_{added} day^{-1} in both cases.

On day 234 the feed was switched to SW synthetic in in the following 4-5 days the SMP recovered rapidly, then climbed more slowly back to a value of around 0.32 l CH_4 g^{-1} COD_{added}, equivalent to or slightly better than the performance of the digesters before the first addition of WSW. A second cycle of feeding with WSW began on day 259 and led to a steady fall in SMP, at a rate of around -0.008 l CH_4 g^{-1} COD_{added} day^{-1} in both digesters. This was again recovered by switching the feed to SW. In the third cycle starting on day 295, however, the SMP showed an immediate fall in both digesters then stabilised over the next few days at around 0.17 and 0.19 l CH_4 g^{-1} COD_{added} in R1 and R3 respectively. The SMP per gram of COD removed also appeared to be lower in R1 than R3, with respective values of 0.209 and 0.245 l CH_4 g^{-1} $COD_{removed}$, corresponding to 60% and 70% of the theoretical value based on stoichiometric considerations. The COD removal rate was the same in both digesters, at 79.0%.



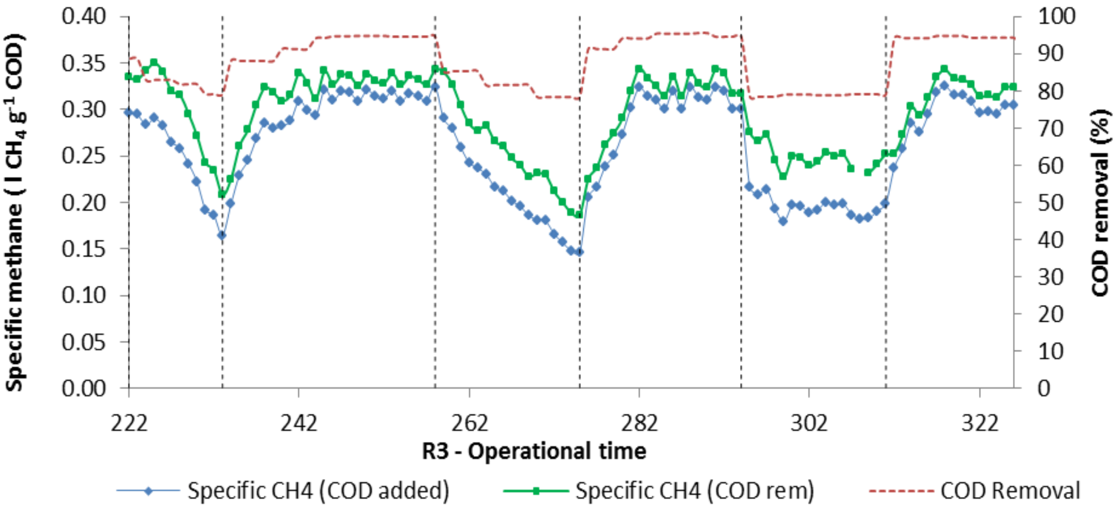
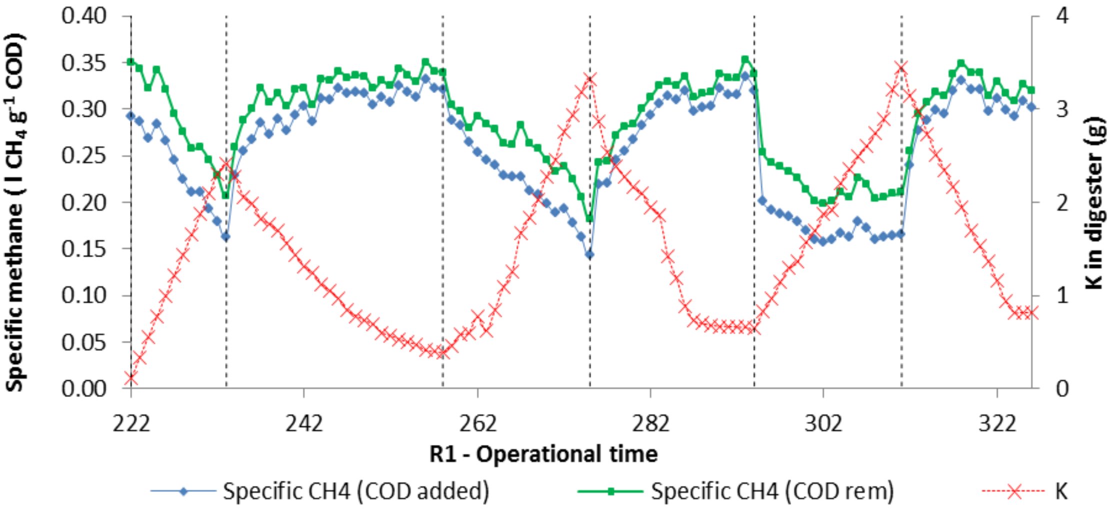


Figure 4.16 SMP and % COD removal in UASB digesters R1 and R3 when subjected to cyclic feeding with higher strength washwater over 3 cycles (Table 4.7). Vertical lines indicate change of feed.

4.3.2 K accumulation

Figure 4.17 shows the accumulation of Potassium in R1 and R3 during the three cycles. The pattern was similar to that seen in the previous experiment, with accumulation on the WSW feed followed by incomplete washout on SW. Final values and average rates of accumulation taken over periods when these were relatively steady are shown in Table 4.8.



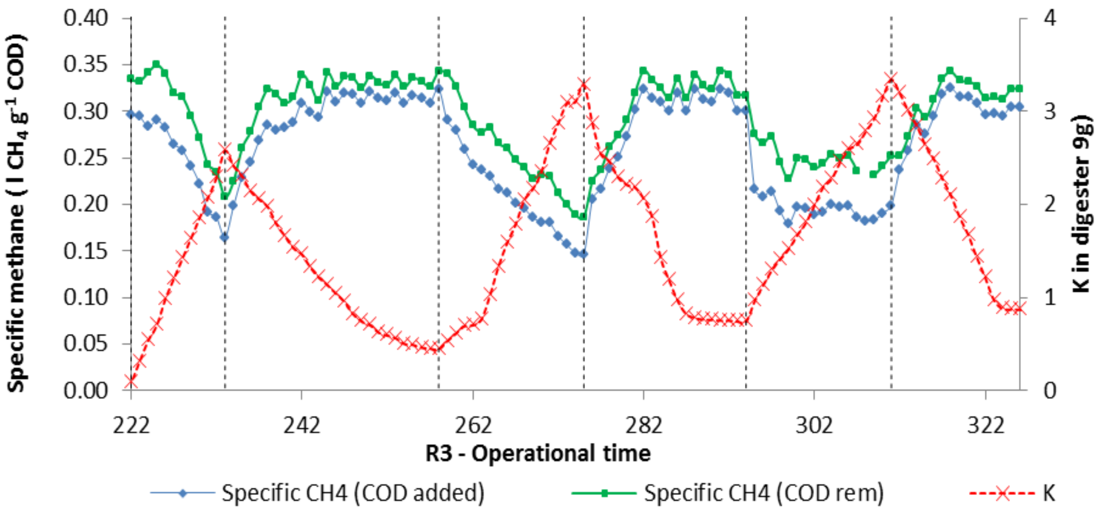


Figure 4.17 SMP and accumulation and washout of Potassium over time in UASB digesters R1 and R3 when subjected to cyclic feeding with higher strength washwater over 3 cycles. Vertical lines indicate changes in feed.

Table 4.8 Maximum and minimum accumulation of K in digester R1 and R3 during each cycle and the average daily rate of accumulation

Cycle	Feed	K accumulated (mg K g^{-1} WW inoculum)		Rate of K accumulation (mg K g^{-1} WW inoculum day^{-1})			
		R1	R3	average between days		R1	R3
2.1	WSW	5.4	5.7	222	233	0.477	0.495
2.1	SW	0.9	1.0	234	247	-0.234	-0.255
2.2	WSW	7.4	7.3	259	275	0.431	0.426
2.2	SW	1.4	1.6	276	287	-0.405	-0.398
2.3	WSW	7.7	7.4	295	311	0.347	0.319
2.3	SW	1.8	1.9	312	323	-0.443	-0.445

WW = wet weight

The rate of K accumulation during feeding with WSW fell during successive cycles, while the rate of washout when fed with SW increased. The final value of K accumulated and the rate of K accumulation was in all cases higher than the values in the previous experiment, reflecting the higher concentration of K in the WSW.

Figure 4.18 shows rate of K accumulation plotted against g K accumulated at the start of the respective period: as the amount of K remaining in the digester changes with time, while the rate of accumulation or loss remains relatively consistent (figure 4.18), the difference in initial concentration cannot fully account for the different rates of accumulation. The rate of washout of K by SW falls appears to fall more sharply with inoculum K concentration than does the rate of K accumulation with WSW as the feed, however, and this may reflect the operation of a K+ pump to regulate concentration in the cells.

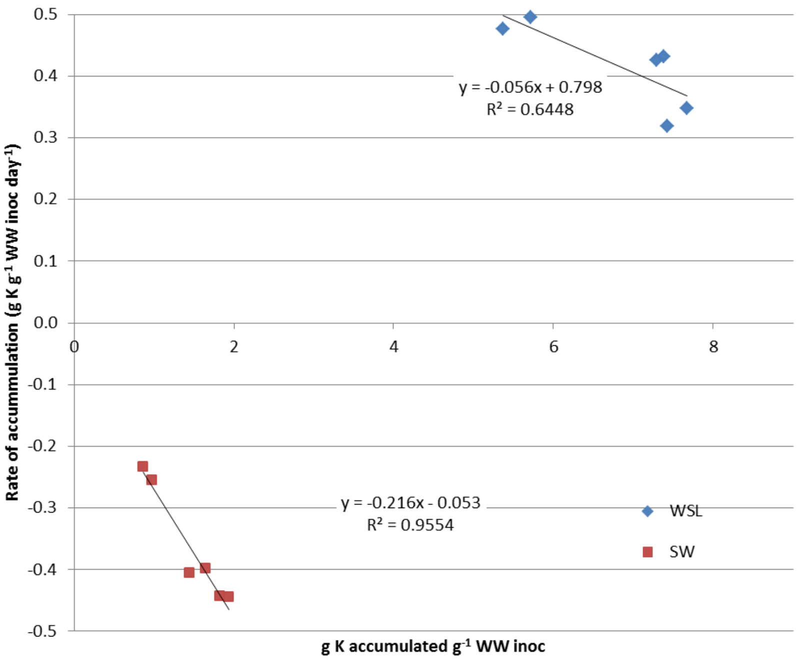


Figure 4.18 Rate of K accumulation in each cycle in digesters R1 and R3 versus initial K accumulation during cyclic feeding with higher strength washwater.

4.4 Experiment 4: Long-term acclimatisation to WSW

The objective of Experiment 4 was to test the performance of a UASB digester under longer-term continuous exposure to WSW and determine whether the accumulation of K leads to digestion failure or whether a longer-term steady state condition can be achieved.

The previous experiments (section 4.2.1.2) had shown the response to cyclical feeding with WSW, with a drop in specific methane yield associated with an accumulation of K and then recovery and a loss of K when the feed was switched to SW. In this experiment one pair of digesters was maintained on WSW for 320 days. Digesters R2 and R4 were fed only on WSW after day 367, initially at an OLR of 1 g COD l^{-1} day^{-1} and a hydraulic retention time of 24 hours. Feeding at this loading continued until day 577 after which the OLR was successively increased by increasing the strength of the WSW, by adding less dilution water. The characteristics of the WSW over the experimental period are shown in Table 4.9.

Table 4.9 Characteristics of WSW prepared to allow increases in OLR at a constant HRT of 24 hours by decreasing the amount of dilution water added

Operational time (day)	367 - 577	578 - 587	588 - 605	606 - 633	634 - 645	646-678
OLR (g COD l^{-1} day^{-1})	1.00	1.81	2.12	2.49	3.67	3.17
K (mg l^{-1})	138.9	200.1	248.8	278.1	325.6	384.6
Na (mg l^{-1})	4.7	6.5	7.8	9.1	12.4	15.4
Mg (mg l^{-1})	45.1	75.6	92.3	125.7	151.9	189.1
Ca (mg l^{-1})	65.3	92.3	104.5	134.0	158.7	189.4
Zn (mg l^{-1})	6.1	9.8	12.4	15.7	18.9	23.5
Cl^- (mg l^{-1})	74.3	99.5	123.0	146.3	174.9	221.5
SO_4^{2-} (mg l^{-1})	124.4	151.4	169.6	19.6	221.3	256.8
PO_4^- (mg l^{-1})	15.3	19.8	23.5	29.8	37.9	49.8
Conductivity (µS cm^{-1})	521.9	783.2	995.4	1231.6	1679.0	1996.9

4.4.1 Specific methane production

After switching the feed to WSW the SMP fell, as had been observed in previous cycles, and after 19 days (day 385) was around 0.20 l CH_4 g^{-1} COD_{added} in both digesters, equivalent to 76% COD removal. The rate of decline then reduced, and the SMP reached a minimum value of 0.168 and 0.157 l CH_4 g^{-1} COD_{added} in R2 and R4 respectively around day 400. After this there was an improvement in SMP in both reactors, reaching ~0.26 l CH_4 g^{-1} COD_{added} by day 430 and continuing at this for a short period before declining to a value that fluctuated around 0.22 l CH_4 g^{-1} COD_{added} between days 460 and 577. This steady state value corresponded to a COD removal of around 80% as shown in Figure 4.19.

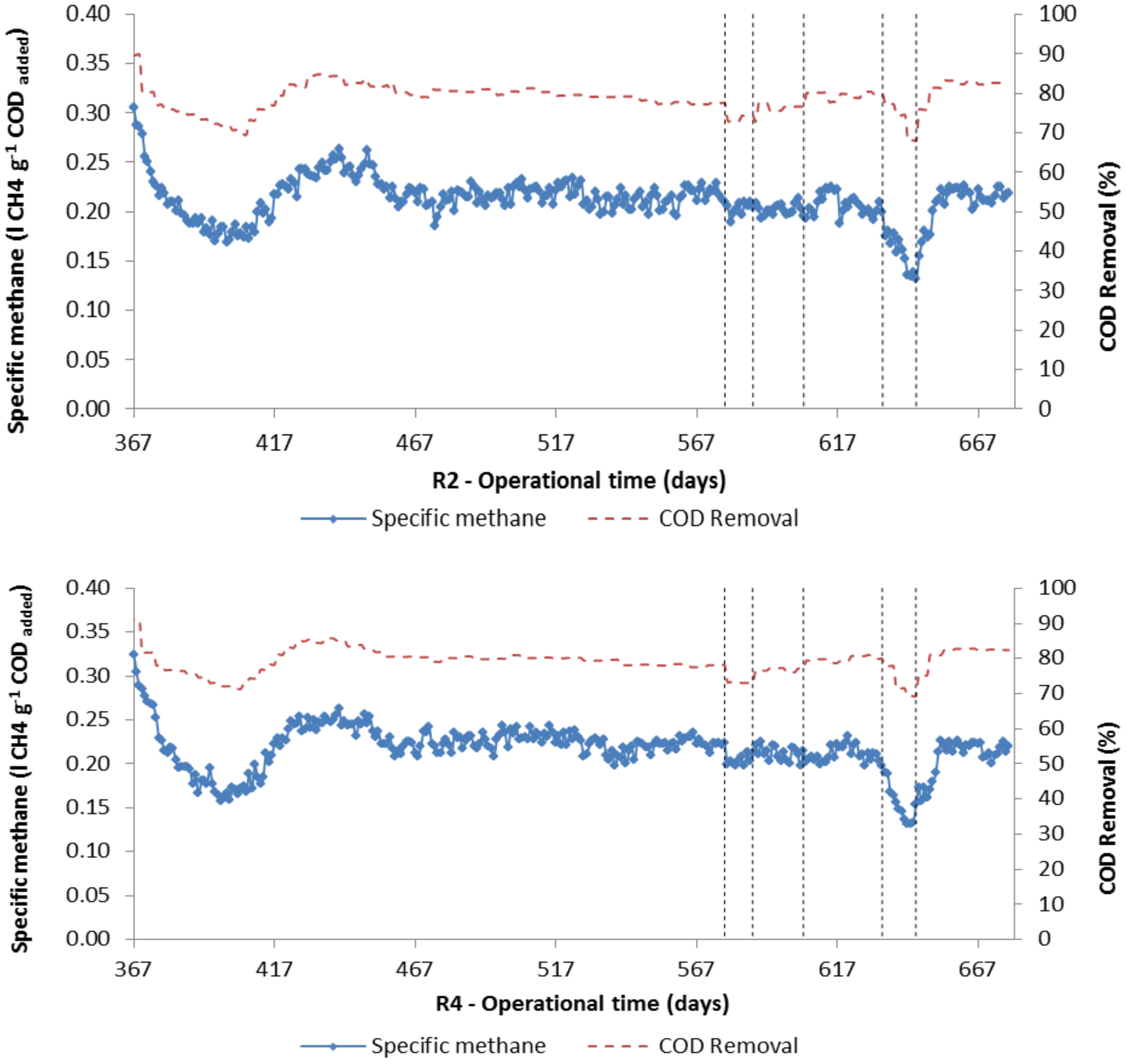


Figure 4.19 Specific methane production and %COD removal in digesters R2 and R4 fed continuously on WSW. Vertical lines indicate changes in OLR as shown in Table 4.9

It was clear that the digesters had acclimated to the WSW at this low loading and on day 578 the OLR was increased to 1.8 g COD l^{-1} day^{-1} in both digesters. The SMP fell to around 0.20 l CH_4 g^{-1} COD $_{added}$ in response to this sudden increase but gradually recovered over the next 21 days, at which point the loading was again increased to 2.1 g COD l^{-1} day^{-1} and then to 2.5 g COD l^{-1} day^{-1} on day 606. These sharp increases in loading did not appear to adversely affect the digesters, and the SMP in both R2 and R4 remained between 0.20 and 0.21 l CH_4 g^{-1} COD_{added} while COD removal recovered to 80%. On increasing the OLR to 3.7 g COD l^{-1} day^{-1} there was a sharp drop in SMP to 0.16 and 0.13 l CH_4 g^{-1} COD $_{added}$ in R2 and R4 respectively, and a corresponding fall in COD removal to around 73%. The OLR was therefore reduced to 3.2 g COD l^{-1} day^{-1} on day 646, and within 7 days COD removal had recovered to over 80%. Operation remained stable until the end of the experiment on day 678, with average SMP and COD removals of 0.216 l CH_4 g^{-1} COD_{added} and 82.6% respectively in both digesters for the last 20 days of the run (Table 4.10).

It can be concluded from this experiment that it was possible to acclimate the methanogenic consortium to the WSW, although not all of the COD in the WSW was anaerobically biodegradable under the conditions used. It is likely that the non-biodegradable fraction was humic materials released from lignin as these give a characteristic yellow/brown colour (Flaig, 1964), which was observed in thewashwater and the effluent. The SMP value expressed in terms of COD removed was 0.262 l CH_4 g^{-1} $COD_{removed}$, lower than the theoretical stoichiometric productivity. This phenomena can be explained by the reaction of microorganism when the transport system (K+ pump) inside the cell failed to function for optimal activity. Under these circumstances, some of COD were not converted into methane but were consumed to maintain the cell for proton motive force development which provides the energy for the synthesis of adenosine triphosphate (ATP) for reaction in methanogenesis.

Table 4.10. SMP as a % of the theoretical stoichiometric conversion

Day	to day	Average for OLR 1.0 g COD l^{-1} day^{-1}		R2	R4
460	577	Ave SMP for COD added	l CH_4 g^{-1} COD_{added}	0.215	0.223
		Ave COD removal	%	79.3	79.4
		Ave SMP per g COD removed	l CH_4 g^{-1} $COD_{removed}$	0.271	0.280
		% of stoichiometric yield	%	77.4	80.1
		Average for OLR 3.17 g COD l^{-1} day^{-1} (last 20 days)			
659	678	Final SMP for COD added	l CH_4 g^{-1} COD_{added}	0.216	0.216
		Final COD removal	%	82.6	82.6
		Ave SMP per g COD removed	l CH_4 g^{-1} $COD_{removed}$	0.262	0.262
		% of stoichiometric yield	%	74.9	74.8

4.4.2 Accumulation of K

As was observed previously, on switching the feed to WSW there was an accumulation of K in the UASB (Figure 4.20) and this continued until around day 400 when the total K accumulated in the bed had reached about 5 g, equivalent to around 11.0 mg K g^{-1} wet weight of original inoculum. After this no significant change in the quantity of K accumulated took place irrespective of the loading applied. Rate of accumulation for this period are shown in Table 4.11.

The maximum value of K accumulated at any time in the experimental run was 5.21 g in R2 [5.18 g in R4] equivalent to 11.58 [11.51] mg K g^{-1} wet weight of original inoculum.

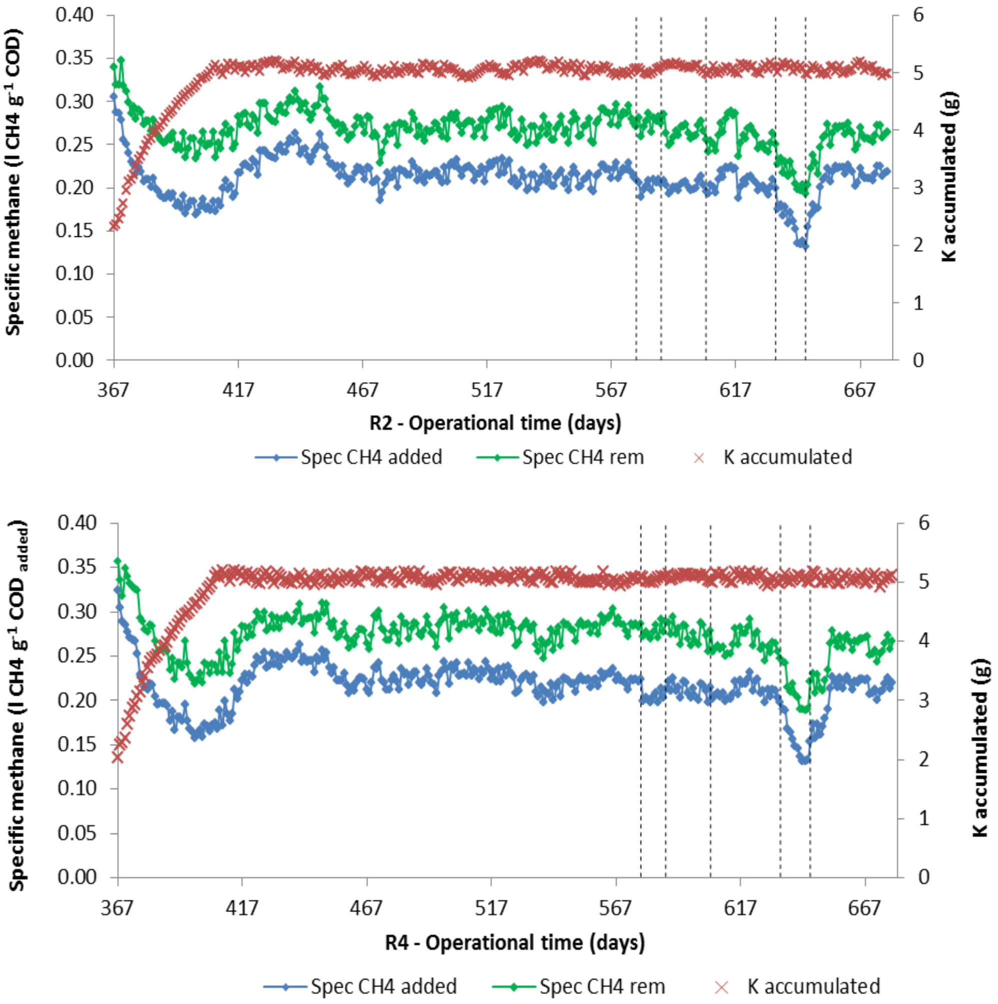


Figure 4.20 SMP and accumulation and washout of Potassium over time in UASB digesters R2 and R4 when fed on WSW. Vertical lines indicate changes in OLR.

Table 4.11 K accumulated during the first 40 days of WSW feed and the daily rate of accumulation

Day	K accumulated (mg K g^{-1} WW inoculum)		Rate of K accumulation (mg K g^{-1} WW inoculum day^{-1})			
	R2	R4	average between days		R2	R4
367	5.2	4.5	370	378	0.264	0.270
405	11.0	11.0	381	405	0.108	0.119

4.5 Experiment 5: Acclimatisation of UASB sludge to KCl augmented SW

The objective of Experiment 5 was to assess the effect of increasing the concentration of K in synthetic wastewater fed to a UASB digester, in order to determine whether the adverse effects seen on feeding with WSW were due to the Potassium content rather than any other potentially inhibitory components in the WSW.

Experiment 5 used two UASB reactors (R1 and R3) that had been exposed to cycles of SW and WSW at an OLR of 2 g COD l^{-1} day^{-1}. From day 328 feeding was continued at a constant OLR of 2 g COD l^{-1} day^{-1} but using a synthetic wastewater made up to a feed concentration of 2 g COD l^{-1} to which had been added in the form of Potassium Chloride (KCl) at 0.67 g K l^{-1}. This concentration was increased to 0.9 g K l^{-1} on day 430, then to 1.5 g K l^{-1} on day 470 and finally to 1.8 g K l^{-1} on day 534. The digester HRT was maintained at 24 hours

4.5.1 Specific methane production

Figure 4.21 shows that there was an initial drop in SMP in response to the addition of KCl to the synthetic wastewater feed. This decline was slow but continued for around 60 days in each reactor with the lowest SMP being close to 0.20 l CH_4 g^{-1} COD_{added}, a fall of 33% from the SMP obtained with SW alone. This was accompanied by a fall in COD removal from 93 to 82%. SMP showed signs of improvement from day 405 onwards and had returned to around 0.27 l CH_4 g^{-1} COD_{added} by day 425, at which point the recovery appeared complete. The salt concentration was increased to 0.9 g K l^{-1} on day 430, then to 1.5 g K l^{-1} on day 470 and finally to 1.8 g K l^{-1} on day 534. After each increase in concentration there was some disturbance and partial loss in performance, but in each case the reactors recovered and gave an average SMP of 0.255 l CH_4 g^{-1} COD_{added} and 92.7% COD removal for the last 30 days of operation (Table 4.12).

The COD removal by the UASB with addition of KCl to the synthetic wastewater was slightly lower than the value without addition (95.0%, section 4.2.1.1), and the corresponding SMP was around 0.05 l CH_4 g^{-1} COD_{added} lower when KCl was present. The introduction of potassium as KCl in synthetic sewage has caused a 'shock phenomena'. This phenomenon can be described by the reaction of microorganism when the transport system (K+ pump) inside the cell was upset to function for .optimal activity. Under these circumstances, some of COD were not converted into methane but were consumed to maintain the cell for proton motive force development which provides the energy for the synthesis of adenosine triphosphate (ATP) for reaction in methanogenesis (metabolic balance) (Mitchell, 1973; Kashket,1985; Gober and Kashket, 1986). Based on the COD removal, the threshold inhibition level for K is around 1.8 g K l^{-1}, but this critical concentration was not determined accurately as concentrations of K in the WSW would not exceed this value.

Table 4.12 SMP COD removal and proportion of stoichiometric conversion

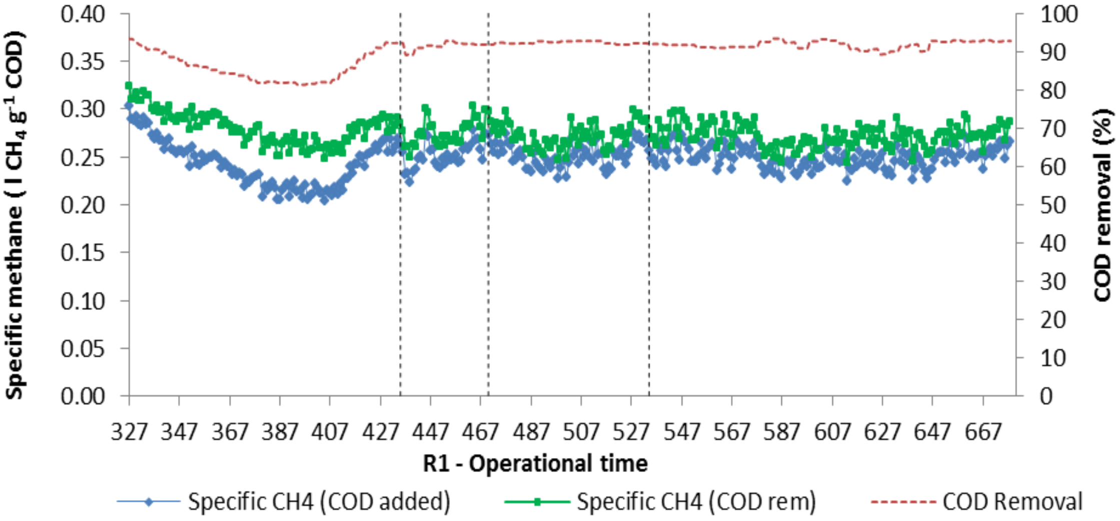
From day 649 to day 678	Unit	R1	R3
Ave SMP added	l CH_4 g^{-1} COD_{added}	0.255	0.255
Ave COD removal	%	92.7	92.7
Ave SMP rem	l CH_4 g^{-1} $COD_{removed}$	0.276	0.275
% of stoichiometric yield	proportion	0.787	0.787

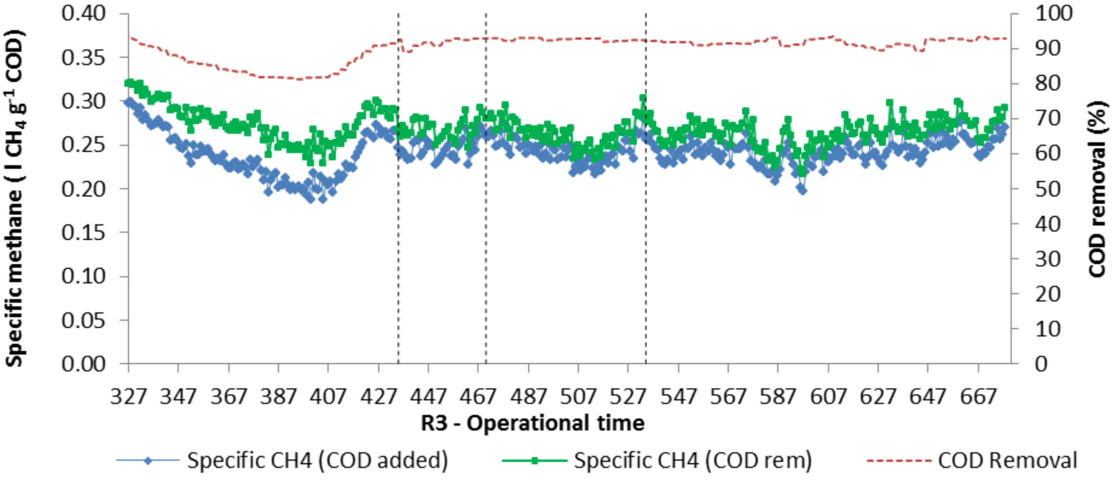
4.5.2 Accumulation of K

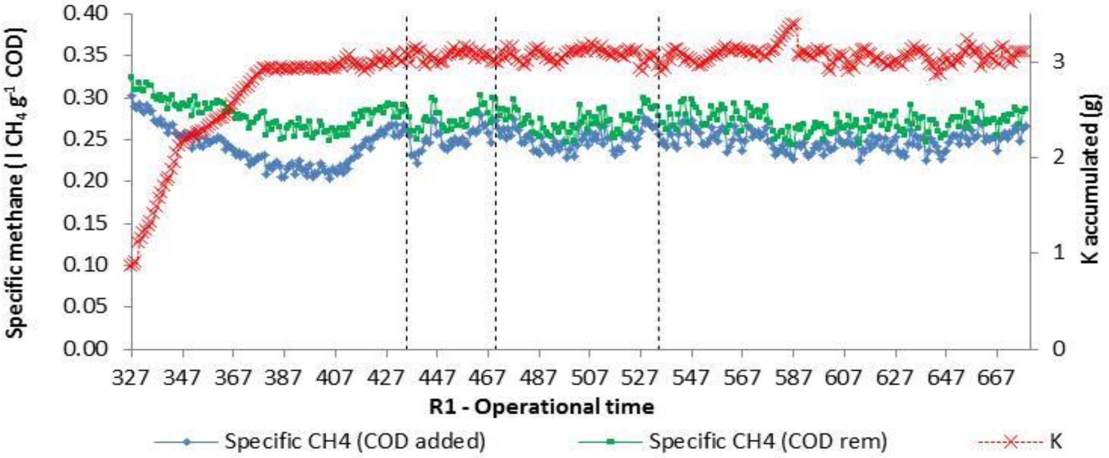
The accumulation of K in the UASB reactors was also determined, as shown in Table 4.13. Rates of accumulation in the period from the first day of KCl supplemented SW (day 328) until the lowest SMP was recorded (around day 380-385) were much lower than had been observed when feeding on WSW, despite the fact that the K concentration was much higher in SW+KCl than in WSW.

There was no further accumulation of K inside the digesters as the KCl concentration was further increased and the accumulated K load was around 3.0 g in each reactor, equivalent to 6.5 mg K g^{-1} wet weight of granular sludge. The accumulation of potassium in digesters fed on WSW was higher, at around 11.0 mg K g^{-1} wet weight of granular sludge (section 4.4.2). This may be explained by the reaction of the cell in response to the effect of potassium pumps for ATP synthesis and nutrient transport. The additional nutrient in SW is used to reduce the effect of ‘cell damaging’ and thus reduce the accumulation of potassium (used for cell repair) inside the digester fed on KCl supplemented SW.

Where K accumulation had occurred it was found that it was strongly bound to the sludge granules. The binding of salts to granular sludge is attributed to the ion exchange capabilities of the surface polymers in the sludge. Metal removal by granular sludge has been reported to exist as inorganic precipitates (sulphides, carbonates) or attached to organic ligands. The ability of micro-organisms to take up cations and/or low molecular-weight organic compounds as compatible solutes to achieve an osmotic equilibrium is known as osmo-regulation (Roberts, 2004) . In the case of K this is taken up transiently from the surrounding medium and allows the micro-organism to function normally until a point when the solute is no longer in equilibrium (i.e. where the concentration of ion K in the extracellular solute exceeds that of ion K in the cell cytoplasm). The different degrees and rates of accumulation of K between the KCl supplemented SW and the WSW could be related to the relative osmotic strength of the two different media.


Specific methane (l CH$_4$ g^{-1} COD)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
COD removal (%)
100
90
80
70
60
50
40
30
20
10
0
327 347 367 387 407 427 447 467 487 507 527 547 567 587 607 627 647 667
R1 - Operational time
Specific CH4 (COD added)
Specific CH4 (COD rem)
COD Removal


Specific methane (l CH$_4$ g^{-1} COD)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
COD removal (%)
100
90
80
70
60
50
40
30
20
10
0
327 347 367 387 407 427 447 467 487 507 527 547 567 587 607 627 647 667
R3 - Operational time
Specific CH4 (COD added)
Specific CH4 (COD rem)
COD Removal


Specific methane (l CH$_4$ g^{-1} COD)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
K accumulated (g)
3
2
1
0
327 347 367 387 407 427 447 467 487 507 527 547 567 587 607 627 647 667
R1 - Operational time
Specific CH4 (COD added)
Specific CH4 (COD rem)
K

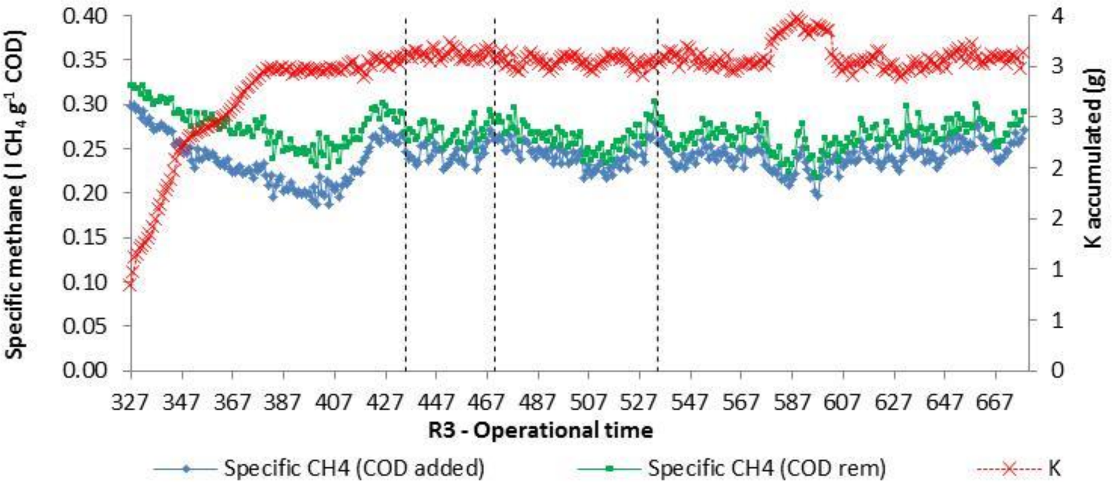


Figure 4.21 Methane production, % COD removal and K accumulated in R1 and R3 when fed on synthetic wastewater + KCl. Vertical lines indicate changes in K concentration in feed as follows. From day 328 0.67 g K l^{-1}, increased to 0.9 g K l^{-1} on day 430, to 1.5 g K l^{-1} on day 470 and finally to 1.8 g K l^{-1} on day 534.

Table 4.13 K accumulated and the daily rate of accumulation between days 328 – 379

Day	K accumulated (mg K g^{-1} WW inoculum)		Rate of K accumulation (mg K g^{-1} WW inoculum day^{-1})			
	R1	R3	average between days		R1	R3
328	2.0	2.2	330	344	0.136	0.133
344	4.3	4.4	347	362	0.037	0.036
379	6.5	6.6	364	377	0.076	0.068

Table 4.14 shows the results for COD removal and SMP based on COD added and removed for SW, WSW and SW + KCl obtained in experiments 3, 4 and 5. It can be seen that the SW+ KCl feed achieved 79% of the theoretical stoichiometric yield based on COD removed, slightly higher than the value of 75% obtained in experiment 4 with WSWbut still below the 93% achieved with SW. The 'missing' CH$_4$ for SW + KCl was slightly higher than for WSW, however, and this may be due to the higher concentration of K.

Table 4.14 Comparison of the SMP for SW, WSW and SW + KCl for different experiments

	COD removal	SMP added	SMP rem	$thCH_4$ rem [d]	% $thCH_4$	'Missing' CH_4 [e]
Unit	%	l CH_4 g^{-1} COD_{added}	l CH_4 g^{-1} $COD_{removed}$	l CH_4 g^{-1} $COD_{removed}$	-	l CH_4 g^{-1} $COD_{removed}$
SW [a]	95.0	0.308	0.324	0.333	0.92	0.009
WSW[b]	82.6	0.216	0.262	0.289	0.75	0.027
SW+KCl [c]	92.7	0.255	0.276	0.325	0.79	0.049

[a] from Experiment 2

[b] from Experiment 4

[c] From Experiment 5

[d] calculated assuming 1 g COD = 0.35 l CH_4

[e] calculated by the difference between theoretical stochiometric yield and the actual yield per g COD removed

4.6 Experiment 6: EDX spectra analysis to identify regions of metal accumulation in and on granules

The specific objective of Experiment 6, which used Energy Dispersed X-ray (EDX) spectra analysis and electron microscopy, was to identify surface accumulation of light metal cations (section 3.7.1) and also to examine the inside of granules by taking cross sections of them (section 3.7.2) .

The work was carried out using the methods described in Section 3.7 and its subsections.

4.6.1 Accumulation of salts on the surface of granules

Micrographs at low magnification of sludge granules both before and after exposure to wheat straw washwater and SW in the first feed cycle (after 120 days operation) are shown in Figure 4.22a and b. These revealed differences in surface morphology of the granules: those that had been exposed to wheat straw washwater were less uniform. At higher magnifications (Figure 4.22c and d crystal-like structures could be observed on the surface of the WSW exposed granules. EDX analyses (Figure 4.23) showed that these crystals and agglomerates were primarily composed of salts of Sodium and calcium, and only low levels of potassium were found. The EDX analysis also confirmed that there was no salt accumulation on the surface of the granules grown on SW.

Fang *et al.* (1995) and Van Langerak *et al.* (1998a) have also reported that exposure of granules to metals can lead to crystal development on the surface. This is as a result of the high density, low shear forces between granules and the availability of a crystal-free surface due to poor overgrowth; this process of crystal formation is known as ripening (van Langerak *et al.*, 1998b). Both these studies and the current research suggest that salt accumulation is in a crystalline rather than an amorphous form. The fact that there was no evidence of K accumulation on the granule surface suggested that this ion had been taken inside the granular structure. This further supports the view that there is a differential translocation of salts in granular sludge by mechanisms of adsorption, precipitation and possibly absorption across membranes as a result of selective active transport mechanisms.

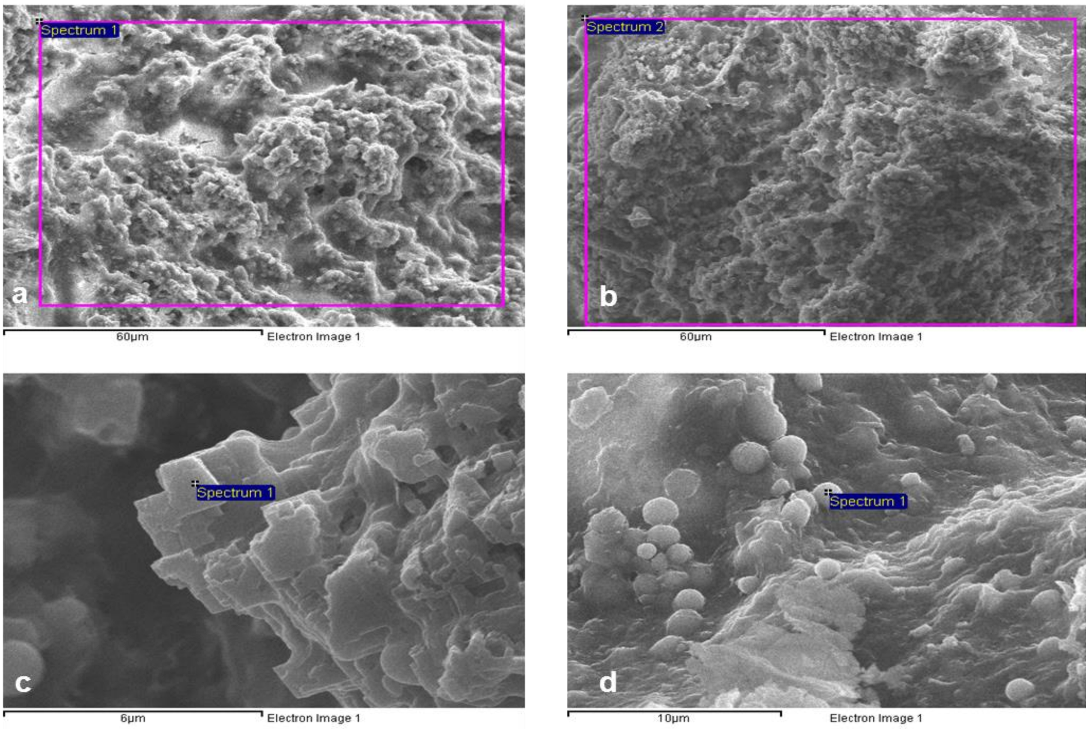


Figure 4.22 Scanning electron micrographs showing the granule morphology after 120 days of operation (a) Surface of granules fed with synthetic sewage (x 1000); (b) Surface of granules fed with wheat straw washwater (x 1000); (c) Surface of granules fed with washwater showing the presence of crystal-like structures (x 5000); (d) Surface of granules fed with washwater showing agglomeration of salts (x 5000)

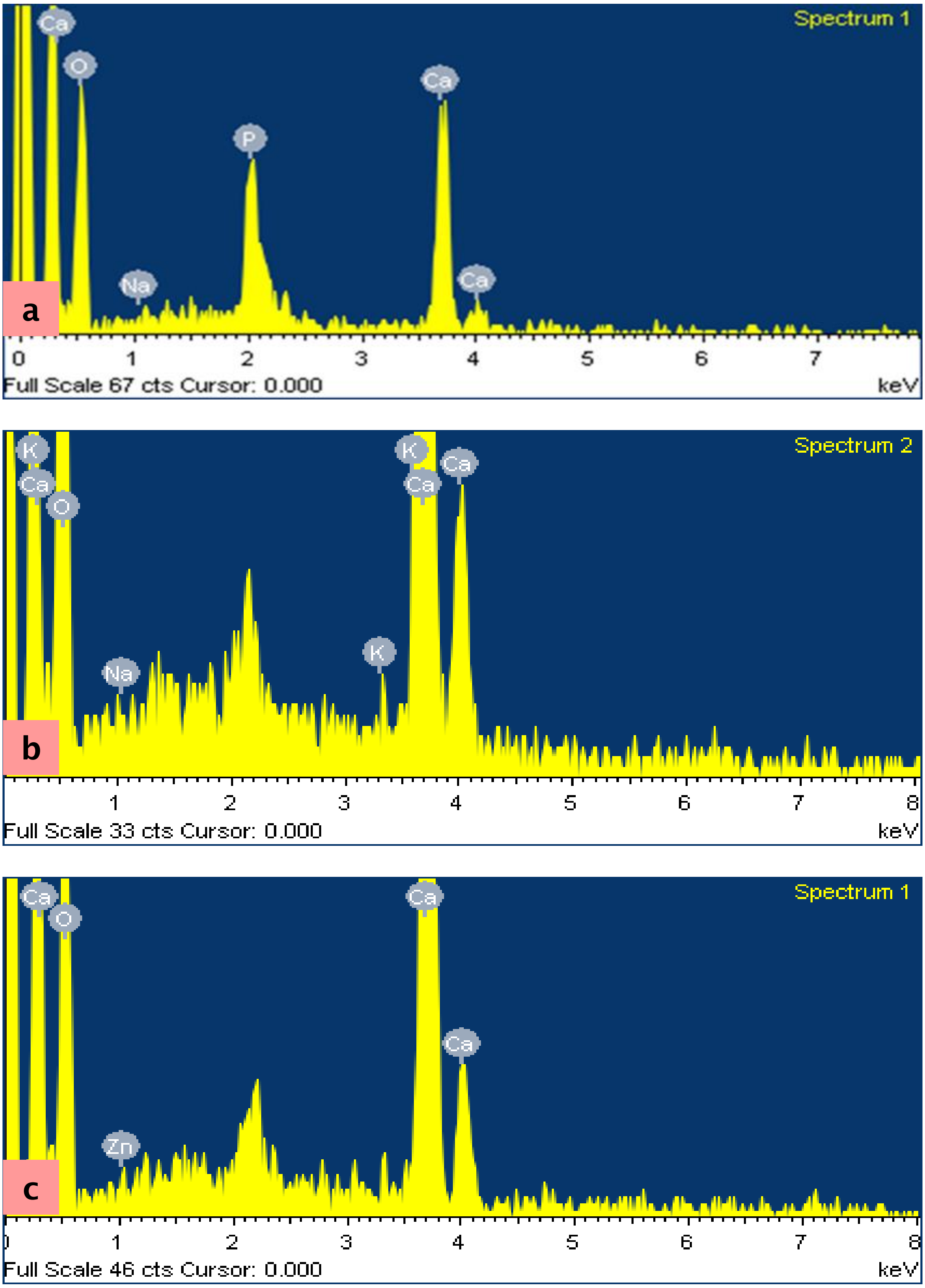


Figure 4.23 EDX spectra associated with different areas on the granule surface. (a) granule surface after feeding on synthetic sewage; (b) EDX Spectra for granule surface after feeding on WSW at 60 µm (surface covered by pink line in Figure 4.22). (c) EDX Spectra for granule surface fed on WSW at 6 µm (as marked on Figure 4.22).

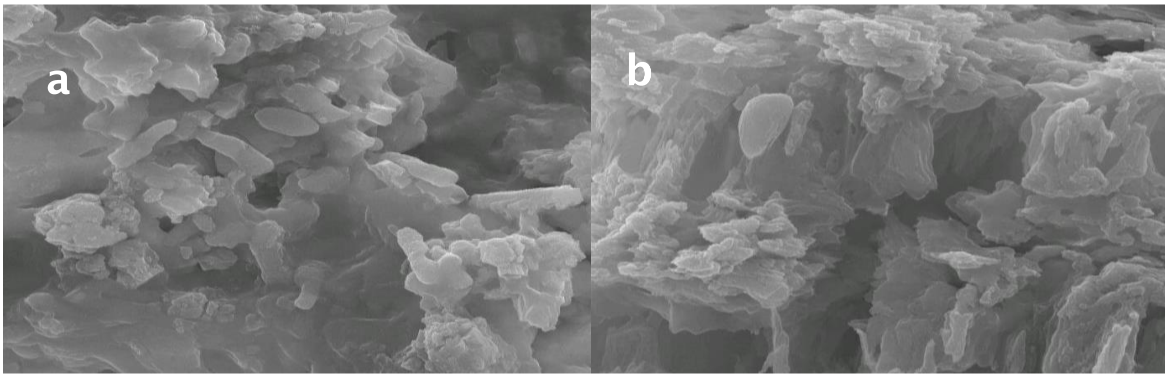


Figure 4.24 Scanning electron micrographs of granule morphology (2^{nd} cycle - after 140 days operation) (a) Surface of granules fed with washwater showing agglomeration of salts (x 5000) (b) Surface of granules fed with washwater showing the presence of crystal-like structures (x 7000)

Observation of the granule morphology after the second cycle of feeding on wheat straw washwater showed further agglomeration and more apparent salt crystal like structure (Figure 4.24a and b). This supports the observation that the accumulation of salt increases after prolonged exposure and appears to be in the form of crystals.

4.6.2 Surface of granule after 530 days operation

Figure 4.25 showed the mineral content on the surface of the granule after 530 days of operation (after 130 days feeding on wheat straw washwater without switching to synthetic sewage). K was found at both points which again indicate the accumulation of K on the surface of granules. EDX result associated with the SEMs are shown in Figure 4.26

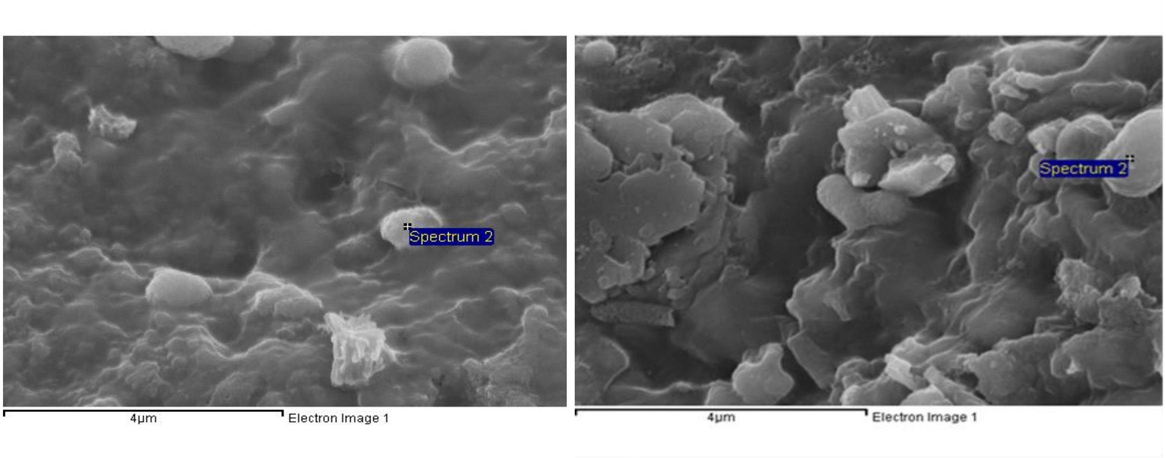


Figure 4.25 Scanning electron micrographs of: (a) Surface of granules fed on WSW showing potassium agglomeration (x 15000) after 530 days operation. (b) Surface of granules fed on WSW showing potassium agglomeration (x 15000) after 530 days operation

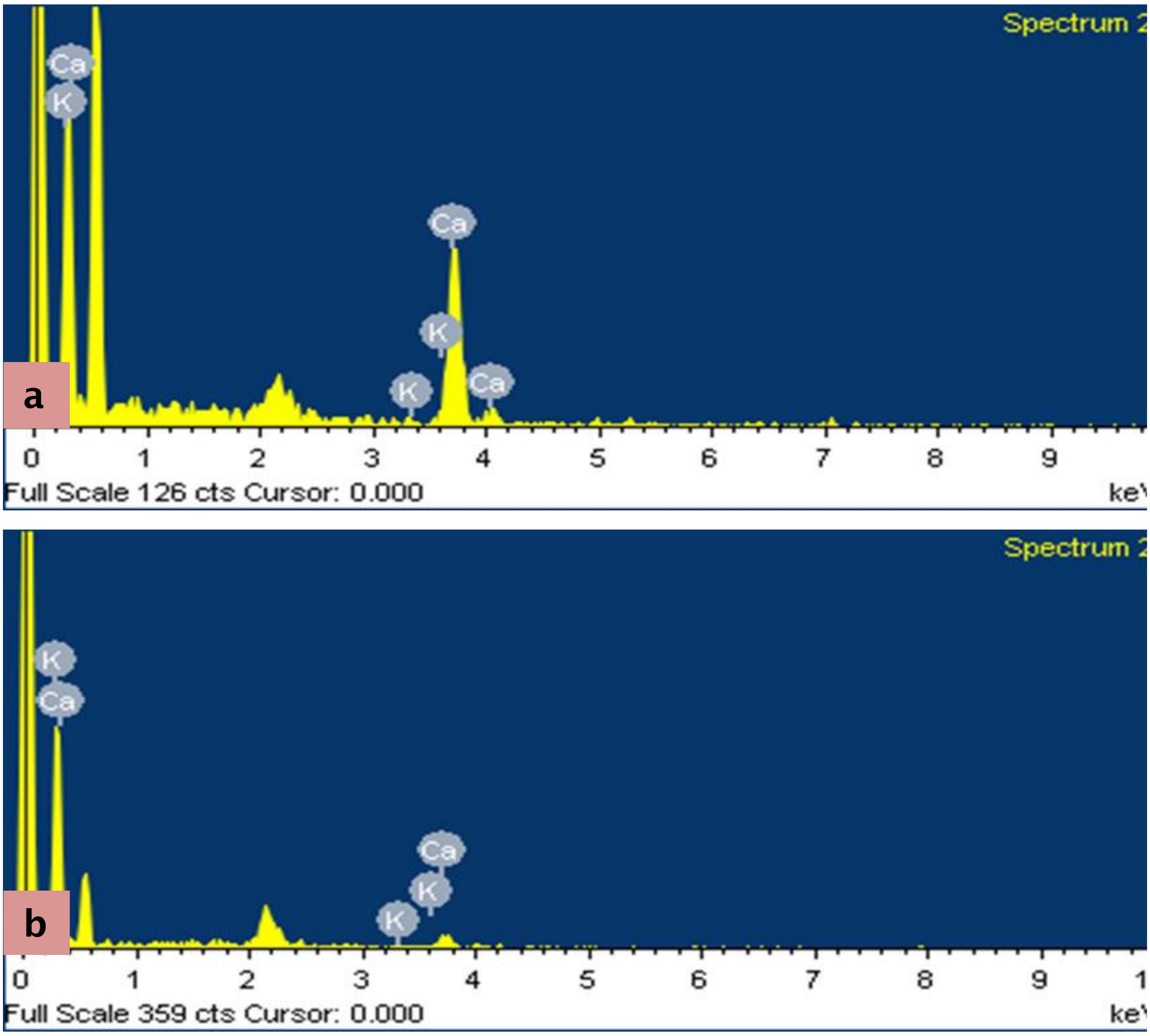


Figure 4.26 EDX spectra of the granule surface 530 days operation. (a) Spectra at point indicated in Figure 4.25a (b) Spectra at point indicated in Figure 4.25b. The presence of K can be clearly seen after feeding on WSW.

4.6.3 Accumulation of salts inside granules after 140 days operation

The SEMs shown in 4.27 c, d, e, and f show a dense crystal surface with some attached bacteria on the granule cross-section, which suggests the presence of extracellular or polymeric matrix.

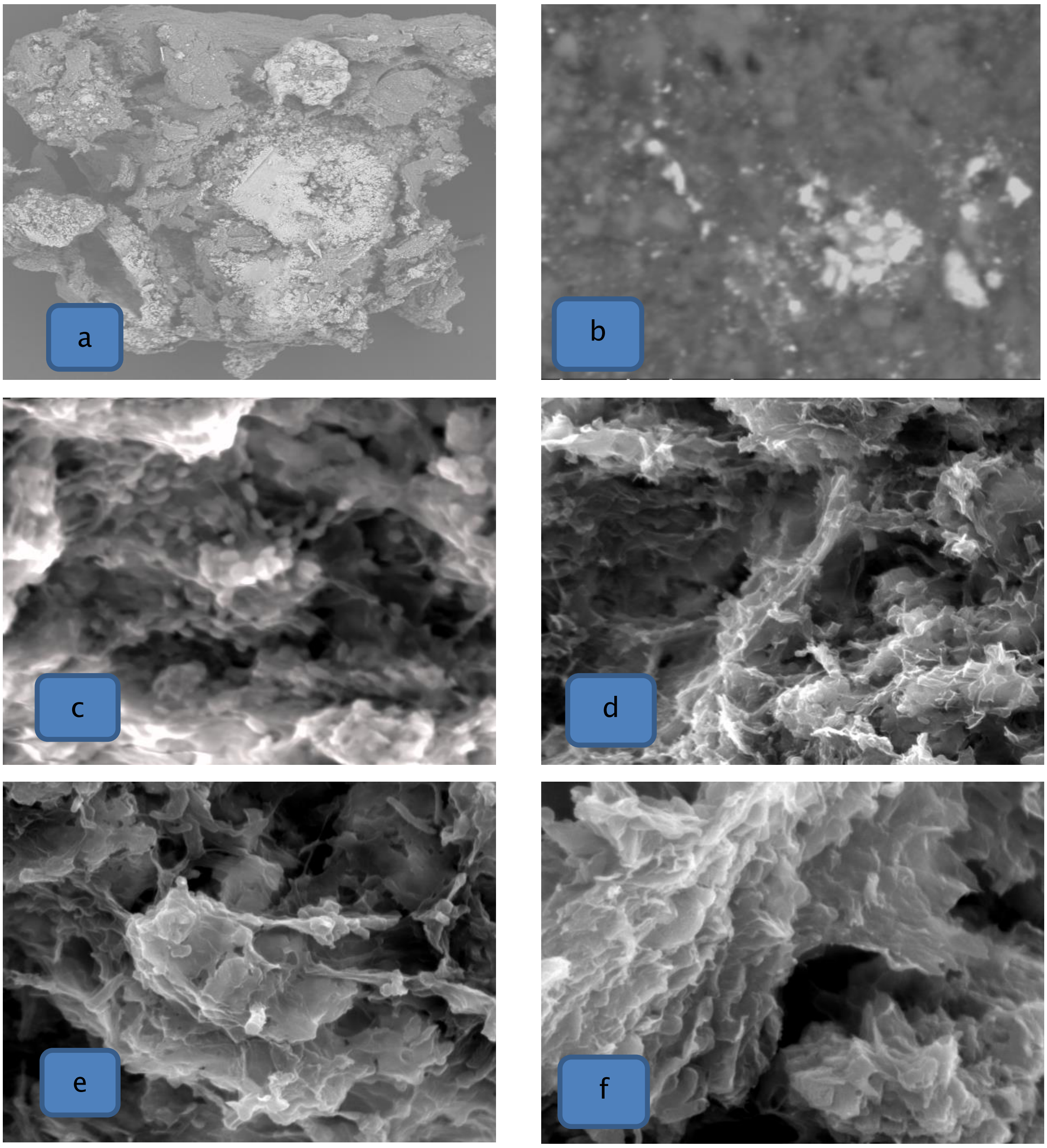


Figure 4.27 Scanning electron micrographs of granule cross section after 140 days (cycle 2) operation a) cross-section of the granule, showing metals accumulation on the whitish cluster (x100) b) cross-section of the granule, showing metals accumulation on the whitish cluster (x6000) c) cross-section of the granule, showing bacteria (cocci) attached (x12000) d) details of the cross section showing extracellular or polymeric material with some attached bacteria (*methanosaeta like genus*) (x12000) e) and f) cross section of granules showing crystal like structure and agglomeration of salts (x16000)

The back scattered electron microscopy images (Figure 4.28) show different zones within the granule to have different chemical compositions which can be identified. The dark areas indicate regions with high concentrations of elements of low atomic mass (for example, carbon), whereas the brighter areas indicate regions rich in elements of high atomic mass (metals). EDX spectra analysis (Figure 4.29) showed Ca, Na, P and K found in the cross section of granules suggesting that K accumulating both inside and outside the granules. Lower intensity of K as compared to Ca showed that K could be lost in the preparation of the granule section due to the long exposure of in a series of ethanol washes and acetonitrile over a period of more than 24 hours.

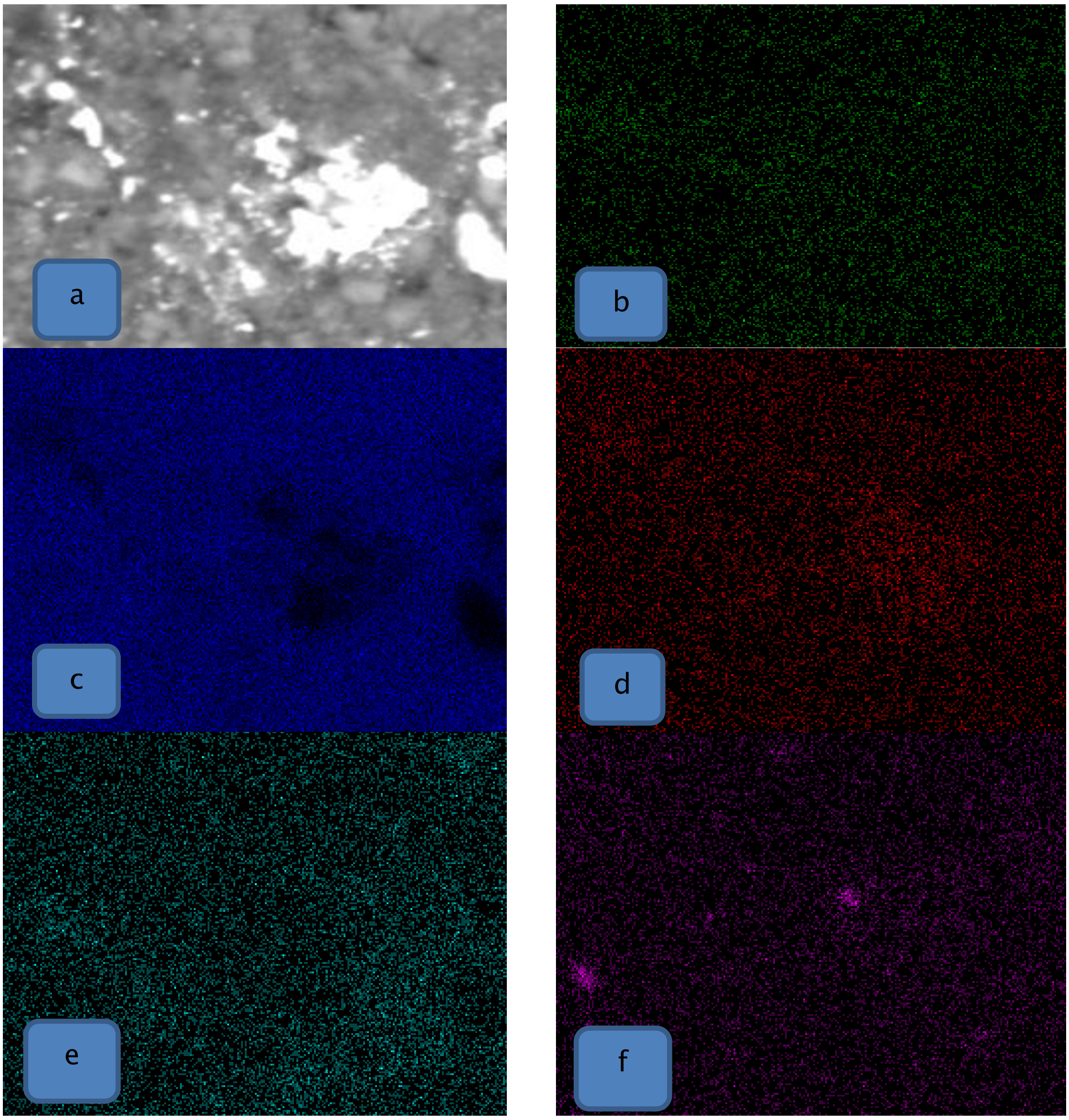


Figure 4.28. Back-scattered electron image from a granule cross section. The minerals content, are represented at different intensity and colour. (a) showing presence of (b) Potassium (c) Calcium (d) Chlorine (e) Phosphorus (f) Silica

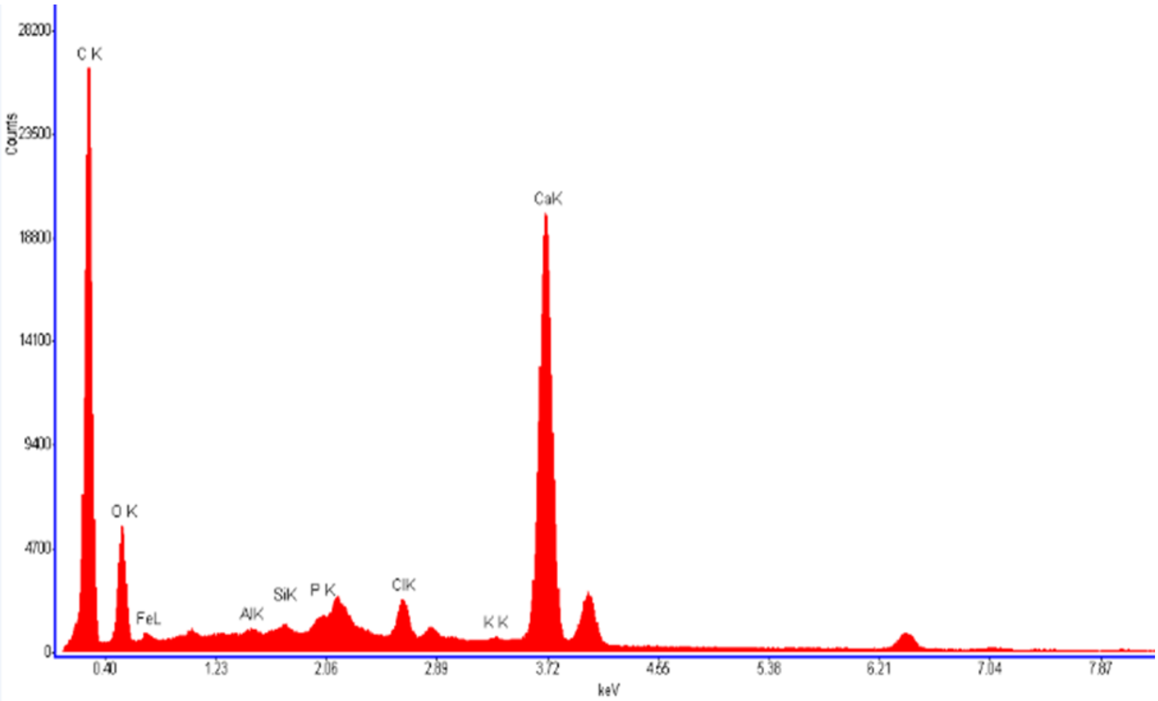


Figure 4.29. EDX analysis of a cross-sectioned aggregate after 140 days of operation (2nd cycle) showing the presence of K, Cl, P and Si inside the granule after feeding on WSW. Ca was present in the granule before feeding on WSW as a result of Calcium Carbonate precipitation. (data not shown)

4.7 Experiment 7: Sorption and desorption of K onto granular sludge

The objectives of Experiment 7 were to show the kinetic for sorption of the potassium contained in WSW onto anaerobic granular sludge, and also to quantify the sorption capacity of the granular sludge.

The rate of sorption of K from solution was determined using the methods detailed in section 3.5. These used a simple experimental set up in which the sorption was determined by exposing a predetermined quantity of granules to a fixed concentration of K ions in solution. An isotherm approach was also used in which a fixed quantity of granules was exposed to differing concentrations of K and the adsorption data fitted to both the Langmuir (section 2.1.8.4) and Freundlich (2.1.8.5) equations to determine the type of adsorption taking place as well as the capacity of the granules for K accumulation. The ability of synthetic sewage to desorb potassium was also tested by exposure of the granules to this medium.

4.7.1 Batch sorption test

Six conical flasks seeded with 1.6 g wet weight of granular sludge were used at initial volume of WSW at 100 ml. The conical flasks were incubated at 37 ^{o}C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). At an initial concentration of 111.1 mg K l^{-1}, it was found that 20% of available K was taken up over the first several hours as shown in Figure 4.30. The rate of sorption then slowed down, but continued up to 50 hours at which time saturation of the granules had occurred with 4.07 mg of K removed per gram of wet granules. The sorption capacity was determined by calculating the difference between the initial concentration and final concentration after reaching saturation point. The value was then multiplied by the initial volume and the result divided by the wet weight of granules to give the sorption capacity of 4.12 mg of K per gram of wet granules.

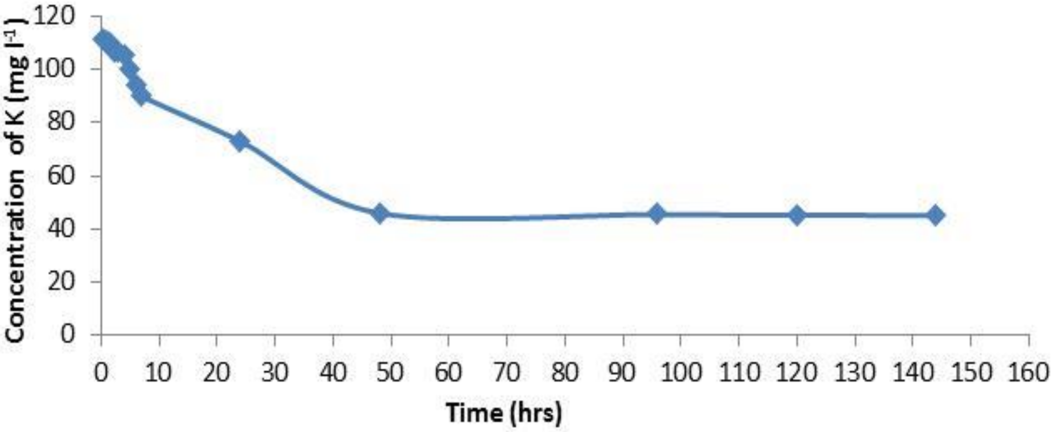


Figure 4.30 The reduction of K concentration in wheat straw washwater (at initial volume of 100 ml) when exposed to 1.6g of granules.

4.7.2 Isotherm experiments

Ten conical flasks seeded with 1.6 g wet weight of granular sludge were used at an initial volume of WSWat 100 ml. The conical flasks were incubated at 37 ^{o}C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). An isotherm experiment was carried out at 37 °C with initial WSW concentrations of 111.11, 88.41, 65.99, 43.57 and 18.52 mg l^{-1} with 1.6 g wet weight of granular sludge as the sorbent.

Plots of the data according to the Langmuir and Freundlich isotherms are shown in Figure 4.31 and 4.32 with a linear regression fit to the data in both cases. From the Langmuir plot the values for Q_{max} and b were calculated from the slope and intercept and from the Freundlich plot the values of the two coefficients were estimated (Table 4.15).

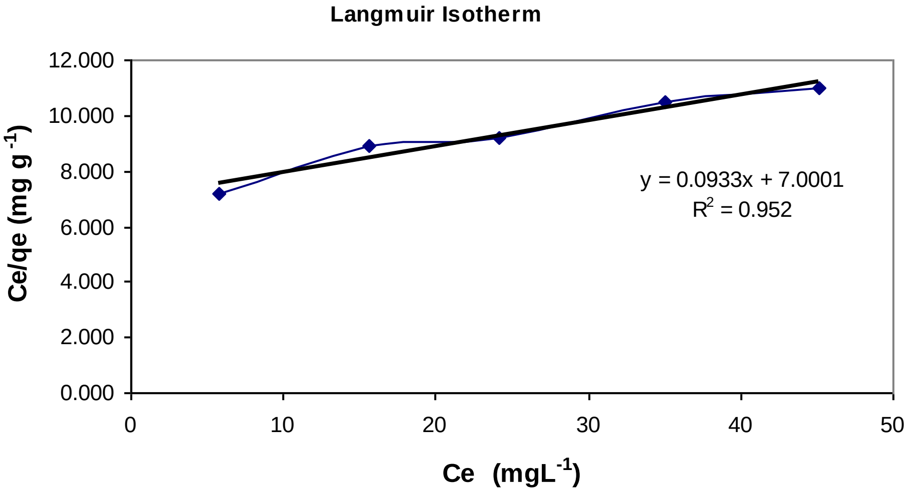


Figure 4.31 Langmuir Isotherm for K sorption to granular anaerobic sludge

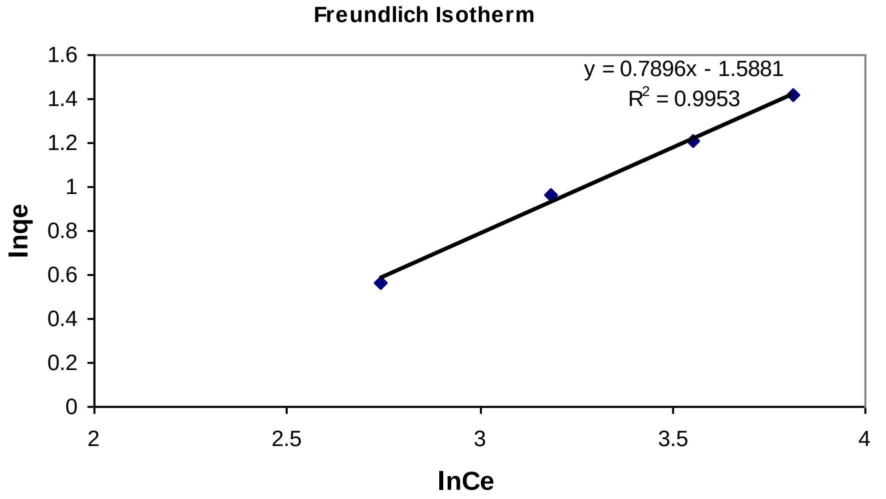


Figure 4.32 Freundlich Isotherm for K sorption to granular anaerobic sludge

Table 4.15 Parameters associated with the Langmuir and Freundlich isotherms

Langmuir constant			
Adsorbent	Qo (mg g^{-1})	b (lmg^{-1}	R^2
Granular sludge	4.120	0.083	0.9520
Freundlich constant			
Adsorbent	Kf	1/n	R^2
Granular sludge	7.519	1.219	0.9953

4.7.3 Desorption study

Six conical flasks seeded with 1.6 g wet weight of granular sludge were used at an initial volume of synthetic sewage of 100 ml. The conical flasks were incubated at 37 °C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). A sorption experiment was previously carried out at 37 ºC with initial WSW concentrations of 111.11, 88.40 and 65.99 mg l^{-1} with 1.6 g wet weight of granular sludge as the adsorbents (Table 4.16). Synthetic sewage at 1 g l^{-1} of COD was used as the eluent over a period of six days.

Table 4.16 Mass balance derived from the adsorption and desorption study

Eluent for sorption	K initial inwashwater (Prior to sorption)	K sorbed into granules (mg)	K desorbed from granules (mg)	Percentage of recovery (desorbed) (%)
SW	111.113	6.52	5.60	85.77
SW	88.405	5.03	4.33	86.06
SW	65.989	3.96	3.09	78.00

Figure 4.33 shows that the quantity of K leached from the granules was greatest in those granules that had been grown in the most concentrated K solutions and had sorbed the most K in the sorption phase. The removal rate was, however, similar in all cases when SW was used as the desorption medium, and the desorption rate using SW was also much faster than when using tap water, suggesting that the energy input from the feed played a role in desorption. A recovery rate of 86% (equivalent to a concentration of ~55 mg l^{-1} in the SW eluent) was found for the granules with the highest initial K load.

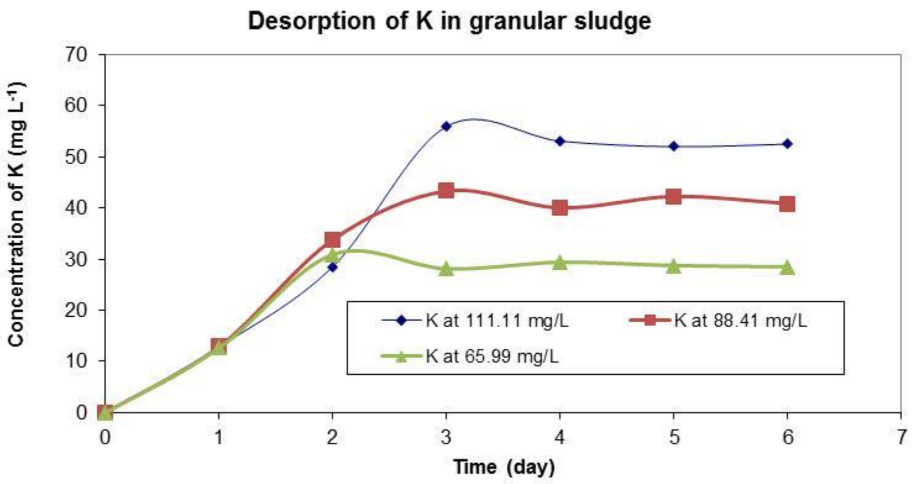


Figure 4.33. Desorption of K from 1.6g of granular sludge (at initial volume of 100 ml).

4.7.4 Second cycle sorption of K following SW desorption.

Two conical flasks seeded with 1.6 g wet weight of granular sludge were used at an initial volume of WSWat 100 ml. The conical flasks were incubated at 37^{o}C with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). The granules which were desorbed in SW were used a second time for sorption to determine the sorption capacity. 1.6 g of granules were exposed to 100 ml of WSWith a K concentration of 111.13 mg l^{-1} as shown in Figure 4.34.

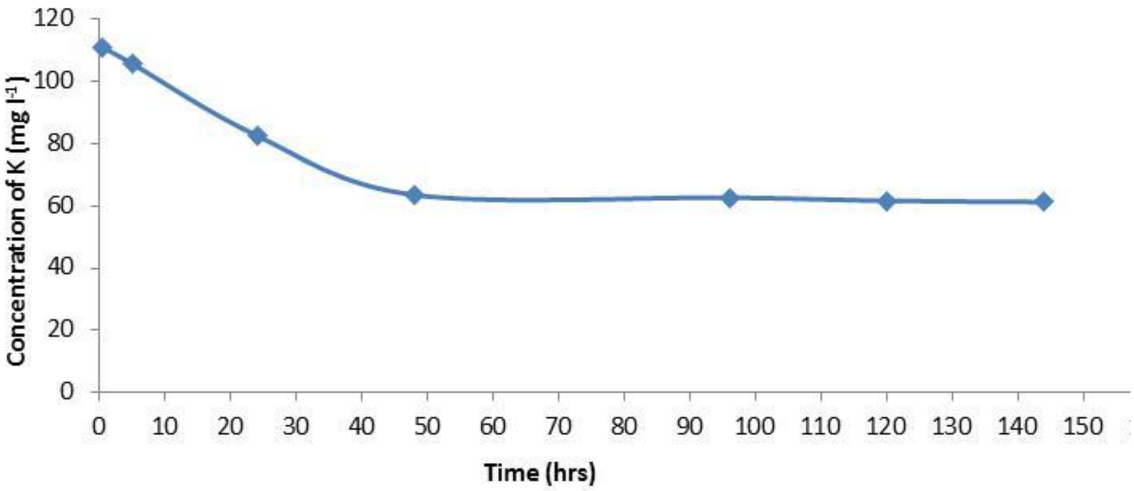


Figure 4.34 Sorption of K from WSW onto granular sludge which had been previously desorbed using synthetic sewage.

A similar trend was observed as in the first study where less than 10% of K was sorbed within the first 10 hours, as shown in Figure 4.30. The removal rate showed a significant increment after 24 hours where it reached more than 25% followed by 43% in the following 48 hours. Equilibrium was reached after 48 hours which is similar to the first sorption cycle. Sorption capacity for this cycle was 3.11 mg of K per gram of wet granules. The 44% removal was, however, lower than the 59% obtained in the first sorption cycle. Nevertheless, as only 86% was desorbed in the previous stage, however, 14% was left on the granules giving a total of 44 + 14 = 58% which is close to the previous value of 59%.

4.8 Experiment 8: Continuously stirred tank reactor trial

The objective of Experiment 8 was to assess whether anaerobic sludge taken from a variety of sources could be acclimated to relatively high concentrations of Sodium and Potassium and whether a dispersed growth sludge acclimated in this way would be a suitable inoculum for an anaerobic filter. If this was the case a further objective would be use this type of biofilm digester for the treatment of WSW in a subsequent experiment.

In the previous experiments using UASB digesters (Experiments 2 - 6) it was shown that the granular sludge could be acclimatised to K concentrations greater than those found in the WSW. From the literature review it was considered likely that the accumulation of K seen, and its possible role in the osmotic balancing the granular sludge to a slightly saline feedstock, would also apply to a dispersed growth anaerobic consortia typically found in CSTR digesters or to the biofilms typical of anaerobic filters. It was therefore decided to carry out an experiment using CSTR digesters fed on synthetic wastewater in which the ionic strength was altered by addition of the light metal cations K and Na and of a mixture of these, as chloride salts.

For this purpose three sources of inoculum were tested. One was a sample of digestate taken from the mesophilic anaerobic digester treating municipal wastewater biosolids at

Millbrook WWTW, Southampton, UK. The other was a sample of approximately 0.025 kg of saline mud taken from the foreshore of the west bank of the Solent estuary near the Redbridge flyover, (50^o 55.127' N, 1^o 28.752' W). Half of this sample was taken from the rear of the foreshore and the other half from a waterlogged area of the salt marsh. At the time of sampling it was noted that there was a strong smell of H_2S associated with the marsh mud but not with the foreshore mud, although both appeared to be anaerobic based on their black colour. The third was the granular sludge obtained from a UASB reactor treating paper mill effluent (Smurfit Kappa Ltd, Kent, UK). The granular sludge had been stored in the laboratory at ambient temperature prior to use.

4.8.1 Initial batch testing of inoculums (saline mud and Millbrook digestate)

This experiment was conducted in 124 mL serum bottles with a septum seal. Each of the serum bottles contained 25 mL of inoculum (saline mud, Millbrook digestate or granular sludge) and synthetic sewage or sodium acetate as the feed. The serum bottles received additions of either KCl or NaCl (as rock salt) to give NaCl concentrations of 0%, 1% and 3% (w/v). Details of the experimental set-up are shown in Table 4.17. All experiments were carried out in duplicate unless noted.

The headspace of each serum bottle was flushed with N_2/CO_2 (80:20) (BOC, UK) and the bottle was sealed with a crimp cap with a PTFE coated septum. Inoculum-only controls were also prepared. The test and control bottles were incubated at 37^oC with agitation at ~100 rpm (Hybaid Maxi 14, Thermo Scientific, UK). The headspace pressure of each serum bottle was measured at regular intervals using a digital absolute pressure meter (Digitron 2025P, Digitron Instrumentation Ltd, Cambridge, UK).

Table 4.17 Batch experimental conditions for the serum bottle tests

Test reference	Feed	Salt addition)
Saline mud		
B1	1.1 ml SW at 1 g COD l^{-1}	0%
B2	"	1% NaCl
B3	"	3% NaCl
B4	"	1% KCl
B5	"	3% KCl
Granular sludge		
B6	1.1 ml SW at 1 g COD l^{-1}	0%
B7	"	1% NaCl
B8	"	3% NaCl
B9	"	1% KCl
B10	"	3% KCl
Millbrook digestate		
B11	1.1 ml SW at 1 g COD l^{-1}	0%
B12	"	1% NaCl
B13	"	3% NaCl
B14	1.1 ml SW at 48 g COD l^{-1}	0%
B15	"	1% KCl
B16	"	3% KCl
B17	0.064 g of sodium acetate to give 0.05 g of COD	0%
B18	"	1% NaCl
B19	"	3% NaCl

Table 4.18 shows the methane produced in the control bottles at 0% of salt addition after three weeks of incubation. This was greater than the calculated theoretical methane for the substrates applied, due to residual methane production potential present in the inoculum at the start of the experiment. This can be seen from the results for Millbrook digestate, which produced on average 3.96 ml of methane from the original inoculum (Table 4.18). The result indicated that the inoculums used could produce significant amounts of methane under the test conditions and were suitable for use in the experiment.

Table 4.18 Methane production from control serum bottles compared to the theoretical calculated quantity.

Inoculum and Feed (control at 0% of salt)	Loading added (mg of COD)	Obtained methane (ml) [a]	Theoretical methane (ml) [b]	Methane in original inoculum (ml) [c]
B1 - Saline Mud +SW	1.10	1.95	0.39	1.56
B6 - Granular Sludge +SW	1.10	7.98	0.39	7.60
B11 - Millbrook + SW	1.10	4.19	0.39	3.81
B14 - Millbrook + SW	52.80	21.90	18.48	3.42
B17 - Millbrook + Acetate	50.00	22.16	17.50	4.66

[c] difference between [a] (cumulative methane) and [b]

Tables 4.19-4.21 show the experimental results for saline mud, granular sludge and Millbrook digestate, while Figure 4.35 shows the cumulative methane production (average values shown, duplicates showed reasonable agreement in all cases).

Methane production from the saline mud inoculum showed no apparent inhibition from 1% KCl addition and only a slight reduction from 1% NaCl (Figure 4.35a). At 3% salt addition the 21-day methane yields were reduced by more than one third compared to the control, with NaCl clearly causing the greatest inhibition in the early stages, although by day 21 the rate of methane production had increased for both KCl and NaCl. In the granular sludge sample salt addition at all concentrations caused a major reduction in methane yield (Figure 4.35b), to < 10% of the 21-day value for the control. For this inoculum, however, NaCl appeared to be less inhibitory than KCl, with 3% NaCl addition equivalent to 1% KCl (Figure 4.35c) and 3% KCl addition allowing only negligible methane production. The Millbrook digestate showed a clear effect from NaCl addition (Figure 4.35d), with 21-day methane yields 53% and 23% of the control value at 1% and 3% addition, respectively. Methane production rates in the granular sludge and Millbrook digestate samples appeared to be fairly steady, apart from in the Millbrook digestate sample with 1% NaCl addition. The saline mud sample appeared to have a longer initial lag, however, possibly reflecting the fact that this is not a conventional inoculum acclimated to regular feeding.

Table 4.19 Methane production using saline mud in tests with NaCl and KCl at 0%, 1% and 3% concentrations (average values for duplicates).

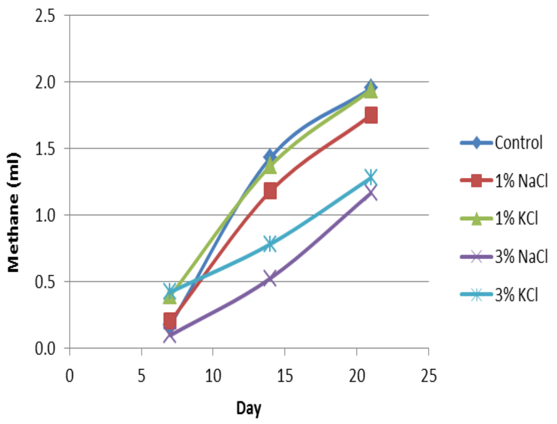
	Biogas (ml)		Methane %		Methane (ml)	
Additions	SW+NaCl	SW+KCl	SW+NaCl	SW+KCl	SW+NaCl	SW+KCl
mg COD added (SW)	1.1	1.1	1.1	1.1	1.1	1.1
0% salt	*B1*	-	*B1*	-	*B1*	-
Week 1	13	-	1.34%	-	0.17	-
Week 2	12	-	10.48%	-	1.26	-
Week 3	6	-	8.64%	-	0.52	-
Total					1.95	
1% salt	*B2*	*B4*	*B2*	*B4*	*B2*	*B4*
Week 1	16	18	1.26%	2.18%	0.20	0.39
Week 2	11	11	8.89%	8.89%	0.98	0.98
Week 3	7	7	8.13%	8.13%	0.57	0.57
Total					1.75	1.94
3% salt	*B3*	*B5*	*B3*	*B5*	*B3*	*B18*
Week 1	10	13	0.97%	3.25%	0.10	0.42
Week 2	8	8	5.36%	4.51%	0.43	0.36
Week 3	6	5	10.64%	9.92%	0.64	0.50
Total					1.16	1.28

Table 4.20 Methane production using granular sludge in tests with NaCl and KCl at 0%, 1% and 3% concentrations (average values for duplicates).

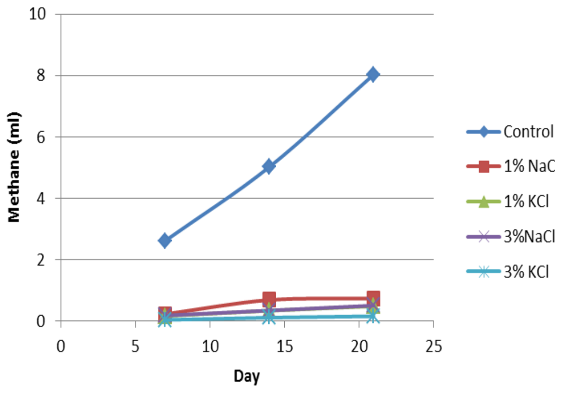
	Biogas (ml)		Methane %		Methane (ml)	
Additions	SW+NaCl	SW+KCl	SW+NaCl	SW+KCl	SW+NaCl	SW+KCl
mg COD added (SW)	1.1	1.1	1.1	1.1	1.1	1.1
0% salt	*B6*		*B6*		*B6*	
Week 1	49.5	-	5.28%	-	2.61	-
Week 2	70	-	3.44%	-	2.41	-
Week 3	58	-	5.20%	-	3.01	-
Total					8.04	
1% salt	*B7*	*B9*	*B7*	*B9*	*B7*	*B9*
Week 1	9.5	18	2.38%	0.93%	0.23	0.17
Week 2	29	8.5	1.59%	2.04%	0.46	0.17
Week 3	12.5	10	0.39%	1.46%	0.05	0.15
Total					0.74	0.49
3% salt	*B8*	*B10*	*B8*	*B10*	*B8*	*B10*
Week 1	9	6.5	2.01%	0.61%	0.18	0.04
Week 2	13	6.5	1.26%	1.13%	0.16	0.07
Week 3	5.5	7	2.95%	0.66%	0.16	0.05
Total					0.51	0.16

Table 4.21 Methane production using Millbrook digestate in tests with NaCl at 0%, 1% and 3% concentrations and 1 mg COD feed as SW (average values for duplicates).

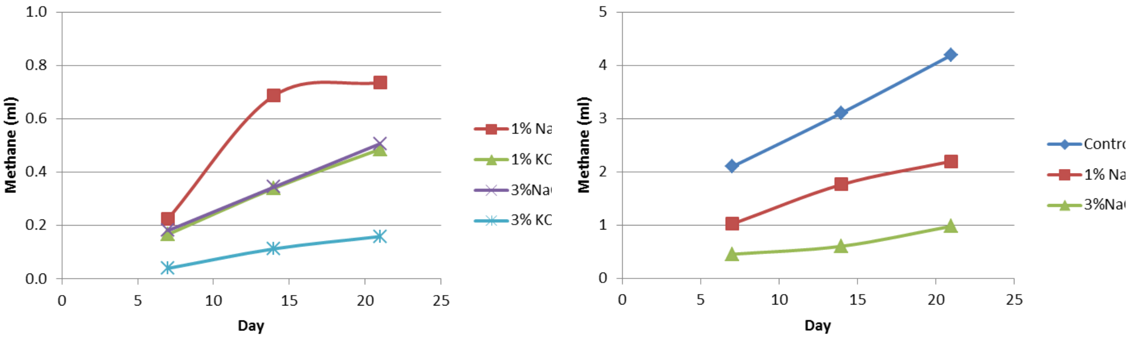
	Biogas (ml)	Methane %	Methane (ml)
Additions	SW+NaCl	SW+NaCl	SW+NaCl
mg COD added	1.1	1.1	1.1
0% salt	*B11*	*B11*	*B11*
Week 1	35	6.00%	2.10
Week 2	21	4.81%	1.01
Week 3	14	7.73%	1.08
Total			4.19
1% salt	*B12*	*B12*	*B12*
Week 1	17	6.02%	1.02
Week 2	15	4.94%	0.74
Week 3	8	5.44%	0.43
Total			2.20
3% salt	*B13*	*B13*	*B13*
Week 1	15	3.06%	0.46
Week 2	11	1.36%	0.15
Week 3	8.2	4.57%	0.37
Total			0.98



a) Saline mud



b) Granular sludge (control shown)



c) Granular sludge (control not shown)

d) Millbrook

Figure 4.35 Cumulative methane production from different inoculums fed with 1 mg of COD as SW at different concentrations of NaCl and KCl.

For the tests run at a higher COD input (B14-18), the controls on SW and acetate were in good agreement (Figure 4.36). Addition of either NaCl or KCl at 1% caused a reduction in methane yield of between 5-10%, but at 3% the addition of NaCl in combination with acetate had a greater effect than KCl with SW.

Table 4.22 Methane production using Millbrook digestate in tests with NaCl and KCl at 0%, 1% and 3% concentrations.and and 50 mg COD feed as SW or Acetate (average values for duplicates).

	Biogas (ml)		Methane %		Methane (ml)	
Additions	SW+KCl	Acetate+ NaCl	SW+KCl	Acetate+ NaCl	SW+KCl	Acetate+ NaCl
mg COD added	52.8	50	52.8	50	52.8	50
0% salt	*B14*	*B17*	*B14*	*B17*	*B14*	*B17*
Week 1	84	57	15.28%	22.09%	12.83	12.59
Week 2	19	20	23.47%	25.64%	4.46	5.13
Week 3	18	16	25.56%	27.86%	4.60	4.46
Total					21.89	22.18
1% salt	*B15*	*B18*	*B15*	*B18*	*B15*	*B18*
Week 1	54.5	42.5	16.65%	23.16%	9.08	9.84
Week 2	21	19	24.80%	26.02%	5.21	4.94
Week 3	19	20.5	28.08%	29.43%	5.34	6.03
Total					19.62	20.82
3% salt	*B16*	*B19*	*B16*	*B19*	*B16*	*B19*
Week 1	36.5	15.5	4.30%	3.61%	1.57	0.56
Week 2	17.5	10.5	16.28%	4.88%	2.85	0.51
Week 3	16.5	5	19.03%	7.03%	3.14	0.35
Total					7.56	1.42

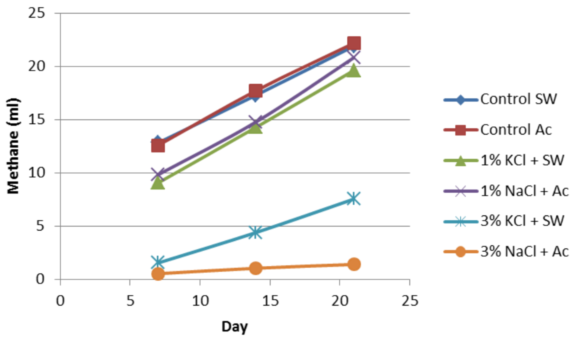


Figure 4.36 Cumulative methane production from Millbrook digestate fed with approx 50 mg of COD as SW or acetate at different concentrations of NaCl and KCl.

Using the above data an attempt was made to estimate the concentration at which 50% inhibition of the 21-day methane yield occurred in each case. Figure 4.37 shows the concentration plotted against 21-day cumulative methane yield, while Table 4.23 presents the estimated concentration for 50% inhibition based on linear regression. It can be seen that the estimated value lies within the range tested for the Millbrook and granular inoculum: the assumption of a linear relationship is weak but Figure 4.37 suggests about 2.2 and 2.7% for NaCl and KCl respectively for Millbrook digestate; and around 0.5% in both cases for the granular sludge. For the saline mud, the assumption of a linear relationship appears more valid but the estimated inhibitory concentrations of around 3.7 and 4.7% for NaCl and KCl respectively are outside the range tested.

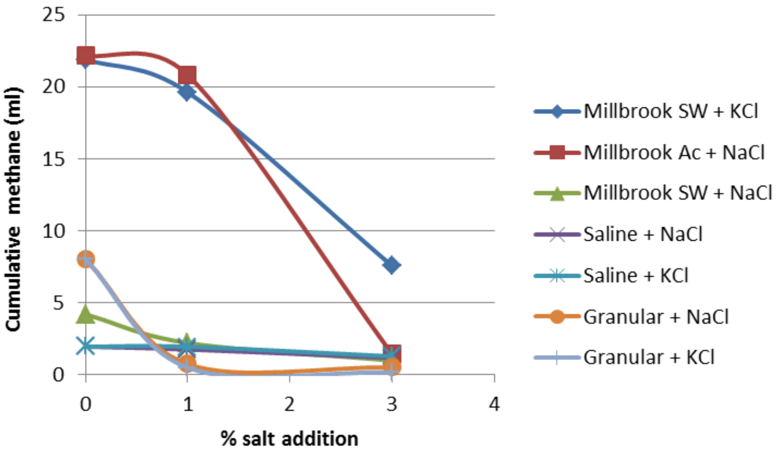


Figure 4.37 Salt addition versus 21-day cumulative methane yield for batch tests with different inoculum types and addition of NaCl and KCl at 0%, 1% and 3% concentrations.

Table 4.23 Estimation of additional salt concentration to produce 50% reduction in 21-day methane yield, assuming a linear relationship between salt addition and methane yield

	50 mg COD addition		1 mg COD addition				
21-day methane	Millbrook SW + KCl	Millbrook Ac + NaCl	Millbrook SW + NaCl	Saline + NaCl	Saline + KCl	Granular + NaCl	Granular + KCl
Salt addition	ml CH_4	ml CH_4	ml CH_4	ml CH_4	ml CH_4	ml CH_4	ml CH_4
0%	21.9	22.2	4.2	1.95	1.95	8.04	8.04
1%	19.6	20.8	2.2	1.75	1.94	0.74	0.49
3%	7.6	1.4	1.0	1.16	1.28	-[a]	-[a]
Linear regression for % salt versus 21-day cumulative methane							
Slope	-4.96	-7.31	-1.00	-0.27	-0.24	-7.30	-7.55
Intercept	23.0	24.6	3.8	2.0	2.0	8.0	8.0
R^2	0.97	0.93	0.90	0.99	0.90	(1.00)	(1.00)
Estimated % salt for 50% inhibition	2.42	1.84	1.69	3.76	4.46	0.55	0.53

[a] value omitted from regression calculation

The results of the batch tests suggested that the saline mud showed least inhibition from either NaCl or KCl addition, indicating some adaptation to saline conditions. The granular sludge showed the worst response to salt addition. The methanogenic population of the saline mud was likely to be much lower than in Millbrook digestate, however, meaning that a long time would be required to build up a large enough inoculum for experimental digesters. As the Millbrook digestate also showed reasonably good performance with respect to inhibition, and was readily available, it was therefore decided to use it for further study for the effect of salt.

4.8.2 CSTR trial

In this part of Experiment 8 four CSTR digesters (C1-4) were used to test the tolerance of Millbrook sludge to both Na and K both in combination and alone under semi-continuous feeding conditions. C1 was the control digester and fed only on SW. C2 was fed on SW supplemented with NaCl, C3 on SW with KCl and NaCl in a 1:1 ratio by weight and C4 on SW and KCl. The SW was used at a concentration of 48 g COD l^{-1} and the applied organic loading rate was increased during the experiment as shown in Figure 4.38. From day 11 salt was added to the SW to give a concentration of 10 g TS l^{-1} of either the individual salt or the mixed NaCl and KCl. No salt additions were made to the inoculum and the increase in salt concentration in the digester therefore occurred by dilution into the reactor. From day 65, additional trace element supplementation was carried out by adding of 0.132 ml of oxyanion and cation solutions twice a week for 3 weeks, as in section 3.3.

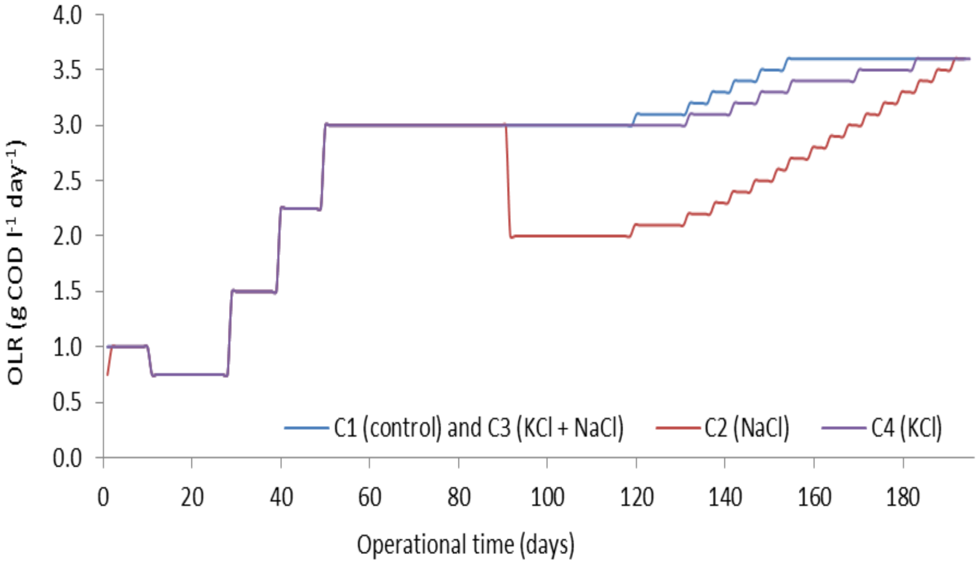


Figure 4.38. OLR applied to digesters C1-4 during CSTR trial. The control and digester C3 fed on KCl and NaCl received the same OLR. Digester C2 was inhibited by NaCl and required a temporary reduction in loading. Digester C4 with KCl addition also had a slightly reduced load compared to the control.

The additional salt concentration in each digester was calculated by mass balance and is shown in Figure 4.39. The conductivity in the unfiltered digestate was measured from day 40 until values appeared stable. Additional salt concentrations of 9.5 g l^{-1} were achieved by day 65 and by the end of the experimental period the concentration in each of the digesters C2-4 was 10 g l^{-1}.

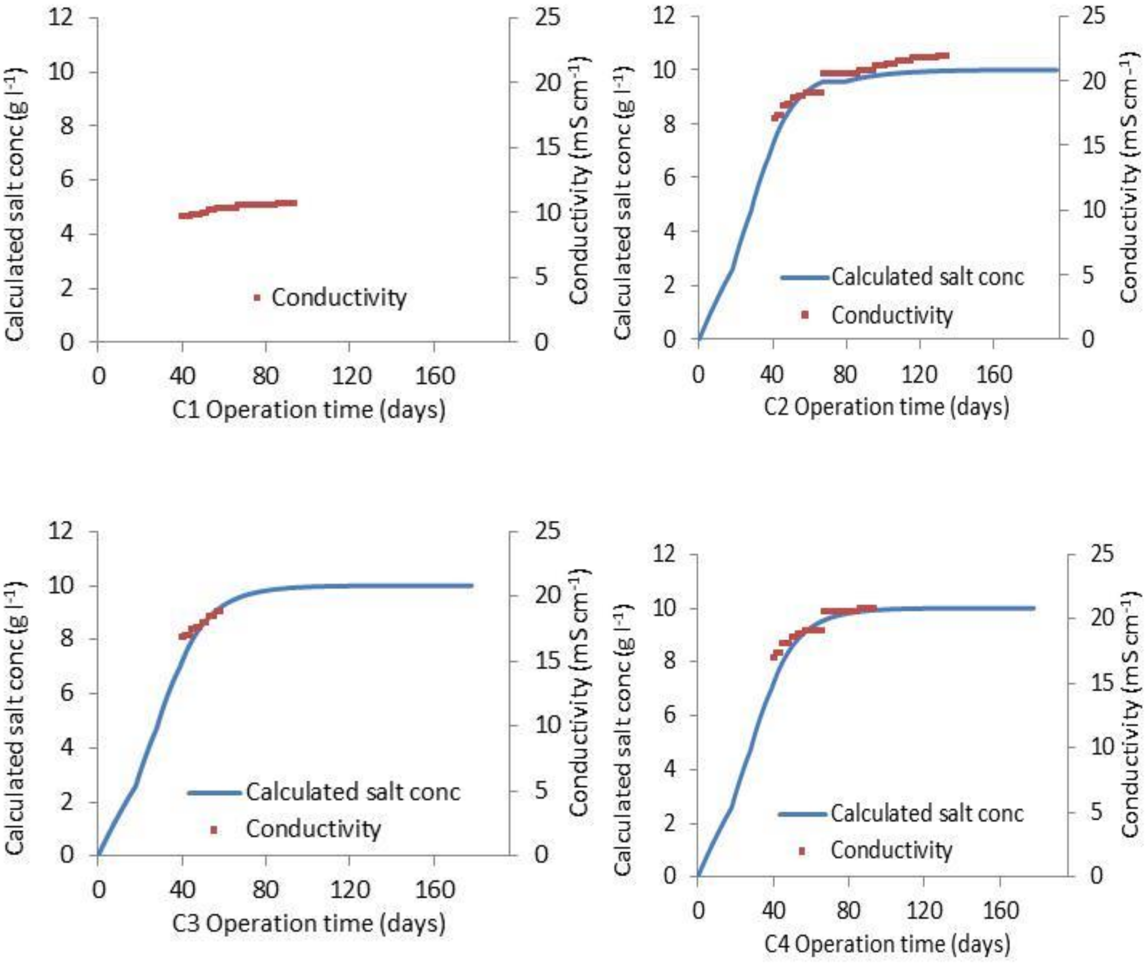


Figure 4.39. Measured conductivity in digesters C1-4 and the calculated amount required to reach a steady state.

On starting to feed the digesters with SW there were some initial fluctuations in specific biogas production (SBP) and SMP, probably due to the change in feedstock and the gradual depletion of old substrate. By day 25 the SMP had reached a typical value for the SW of around 0.32 l CH_4 g^{-1}, similar to that achieved at steady state in the UASB trials. Subsequently on each occasion when the OLR was increased there was a fall in SBP and SMP followed by recovery over the next 5-10 days (Figure 4.40). Until day 45 the behaviour of all four digesters was similar in terms of gas production: by this point the salt concentration had increased to around 6.5 g l^{-1}, a concentration already higher than that in the UASB trials. From this time, however, the digesters receiving the salt-supplemented feedstock began to show some signs of stress, with a reduction in SBP and SMP relative to the control.

Signs of stress were also seen in the pH, which fell first and most sharply in C2 (receiving NaCl), then less sharply in C4 (KCl) and C3 (KCl + NaCl). The drop in pH was associated with a rise in the alkalinity ratio and in the total VFA concentration (Figure 4.41). This increase in VFA also occurred in the control digester, and has previously been observed in the laboratory at Southampton following a change of substrate (Zhang *et al.*, 2012, and unpublished data). The change in VFA concentration could therefore not be attributed to salt addition alone: however the increase in concentration started earlier, lasted longer and gave a higher peak in the following order: C2 (NaCl) > C4 (KCl) > C3 (KCl + NaCl) > C1 (control).

VFA profiles for each digester are shown in Figure 4.42. In the control digester C1 the VFA peak was mainly composed of acetic acid, which peaked around day 72. A smaller peak in propionic acid was seen between days ~60 to 90. In C2 (NaCl) propionic acid rose much more rapidly than acetic, reaching a peak around day 72 that declined only slowly in the next 20 days, before falling rapidly. In C3 (KCl + NaCl) acetic and propionic acid increased at similar rates and the propionic peak was both higher and more prolonged than the acetic, but fell rapidly from day 78 onwards. In C4 (KCl) the acetic acid concentration increased first, with a very sharp rise in propionic after day 61 by which time the salt concentration had reached around 9.3 g l^{-1}. The appearance of propionic acid and of other longer-chain VFA (Figure 4.42) thus also suggested that the digesters adapted more easily to KCl + NaCl or KCl alone than to NaCl. Addition of TE twice a week for 3 weeks starting from day 65 may also have helped to reduce the VFA concentration and thus improve biogas production particularly in the digester fed on SW+NaCl alone.

Ammonia concentrations in the digesters showed good agreement throughout the experimental period (Figure 4.41), and in general remained between 2.5 - 3.0 g N l^{-1}, a level likely to provide some buffering without a strong risk of inhibition.

Because of the particularly high alkalinity ratio and VFA concentration in C2, the OLR on this digester was reduced from day 92 to promote recovery. This lower loading was maintained until day 120, by which time the alkalinity ratio in all digesters was less than 0.3 and the total VFA concentration was below 1000 mg l^{-1}. SBP and SMP values were converging and remained stable from day 140 onwards, during which time the OLR on all digesters was increased to the final value of 3.6 g COD l^{-1} day^{-1}. During, the last 30 days of operation (Table 4.24), all digesters achieved stable operation with methane production of 0.30 l CH_4 g^{-1} COD_{added} and VFA of 100 mg l^{-1}.

Average performance and operating parameters for last 30 days of run are shown in table 4.24. From these results it appears that the inoculum adapted more readily to the mixture of KCl + NaCl than to KCl alone, and to KCl alone better than to NaCl. This circumstance can be explained by the presence of excessive salts alters many cellular properties which include cell volume (dehydration of bacterial cell), turgor pressure and the ionic strength in the cytoplasmic membrane. The alteration of intracellular metabolites concentration can lead to the inhibition of a variety of cellular processes. The phenomenon is known as cell lysis (dehydration under high-osmolarity growth conditions) (Kraegeloh *et al.*, 2005). Despite this difference, in all cases the digesters were able to adapt successfully to the added salt concentrations applied.

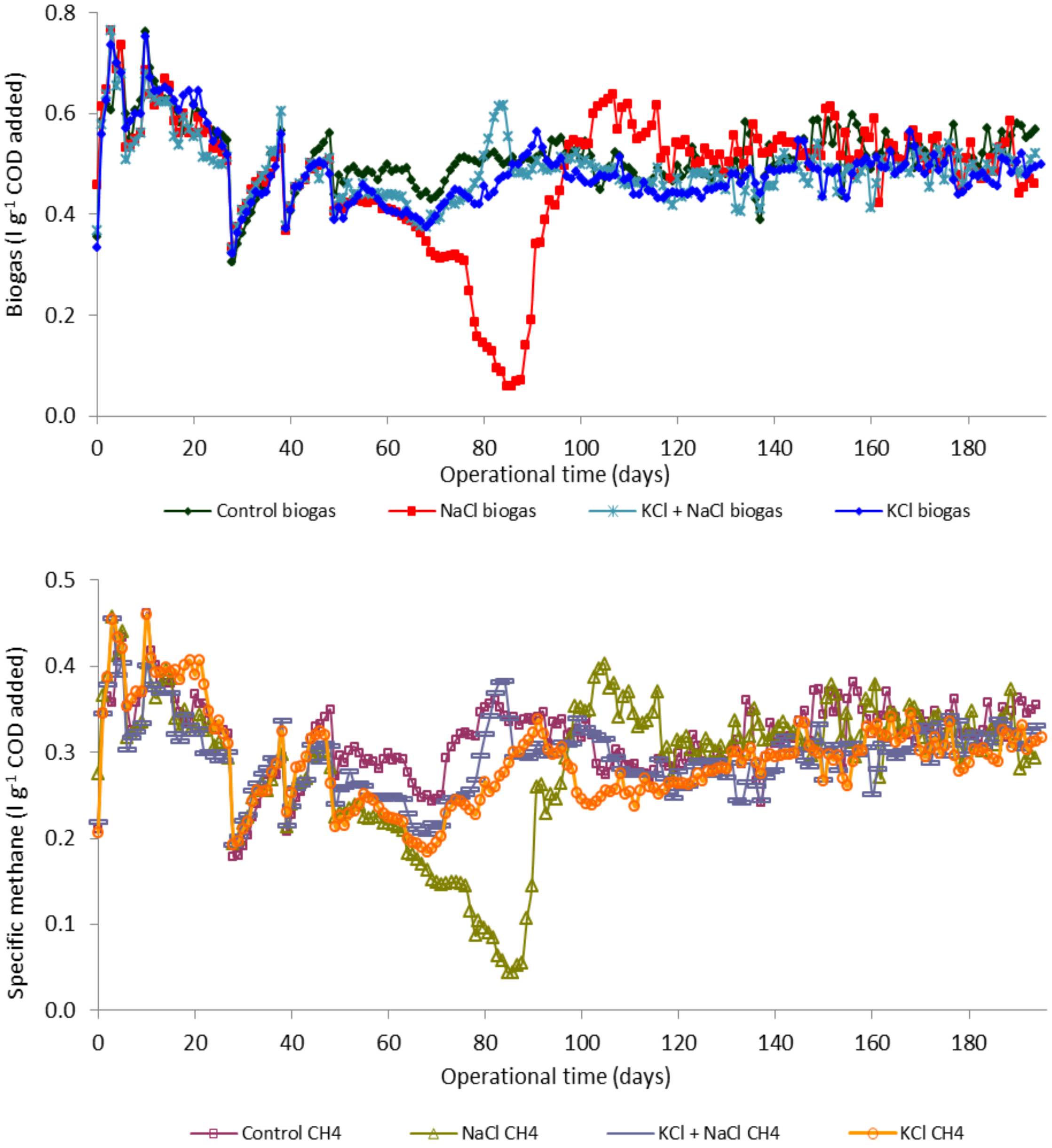


Figure 4.40. Specific biogas and methane productions for digesters C1-4 during CSTR testing of salt addition

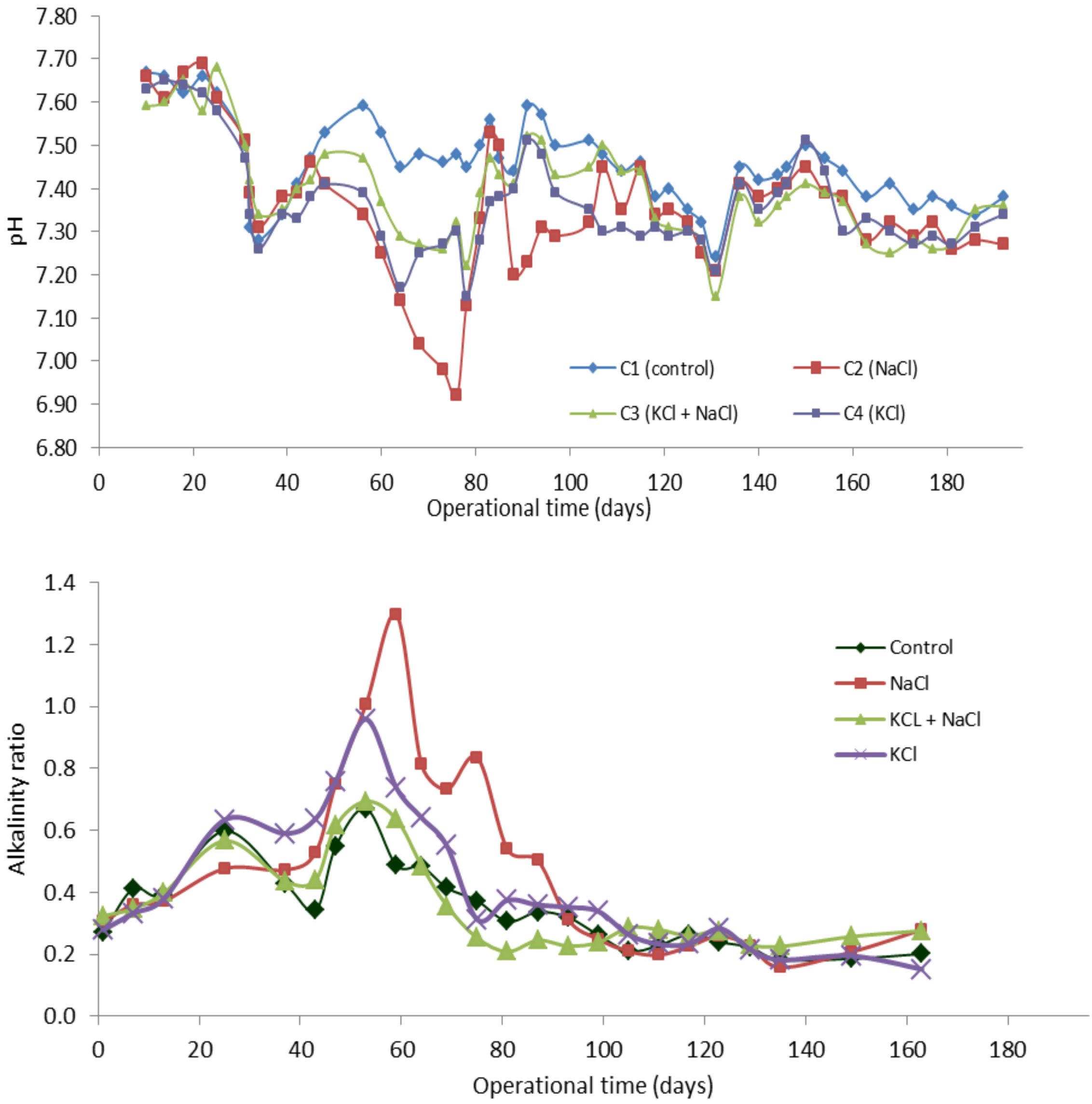


Figure 4.41 pH and alkalinity in digesters C1-4 during CSTR testing of salt addition

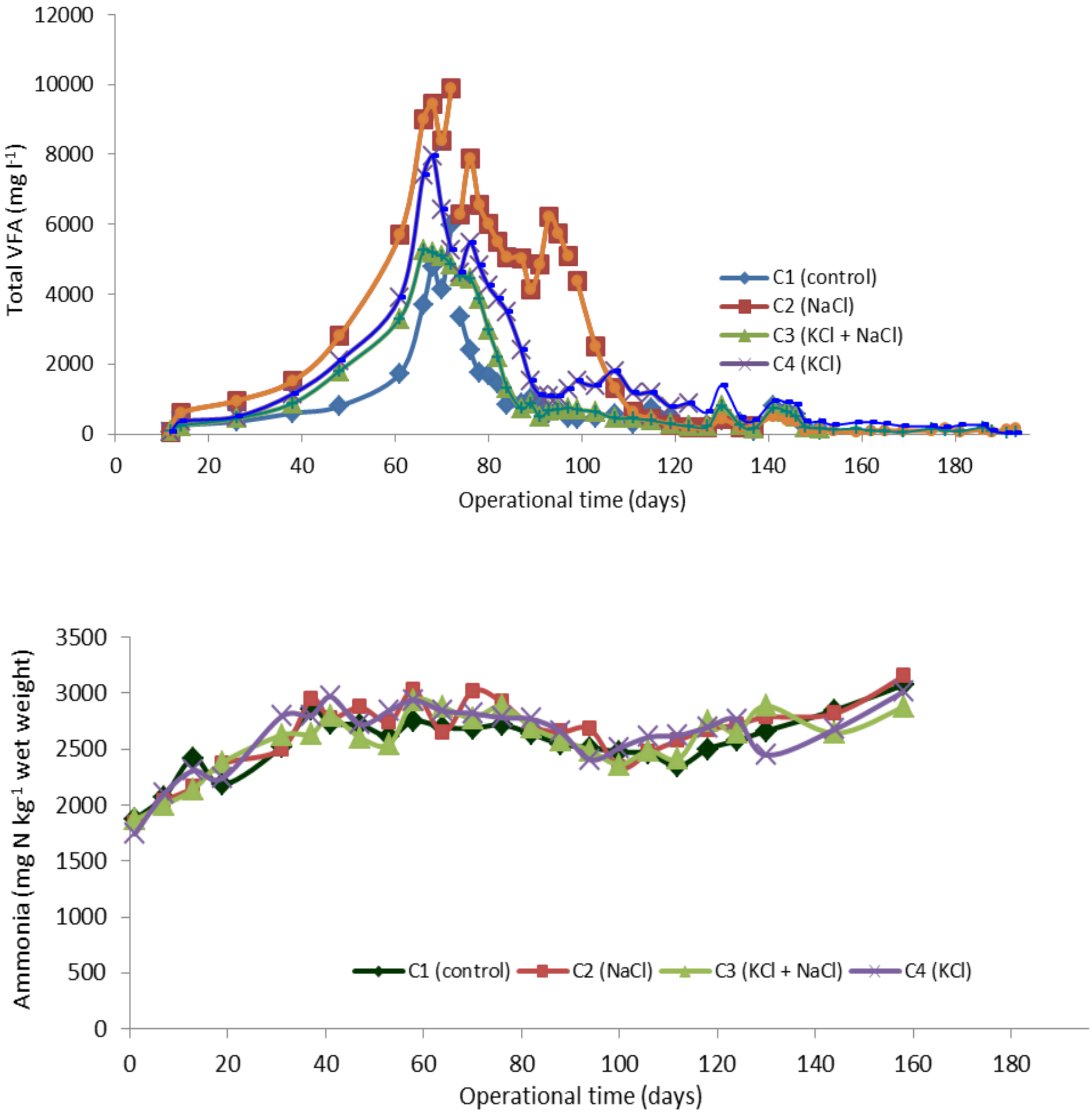
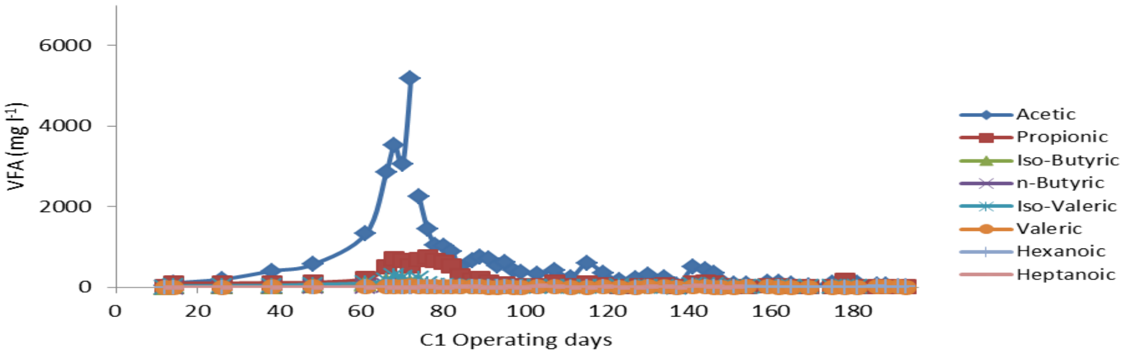


Figure 4.41 VFA and ammonia concentrations in digesters C1-4 during CSTR testing of salt addition



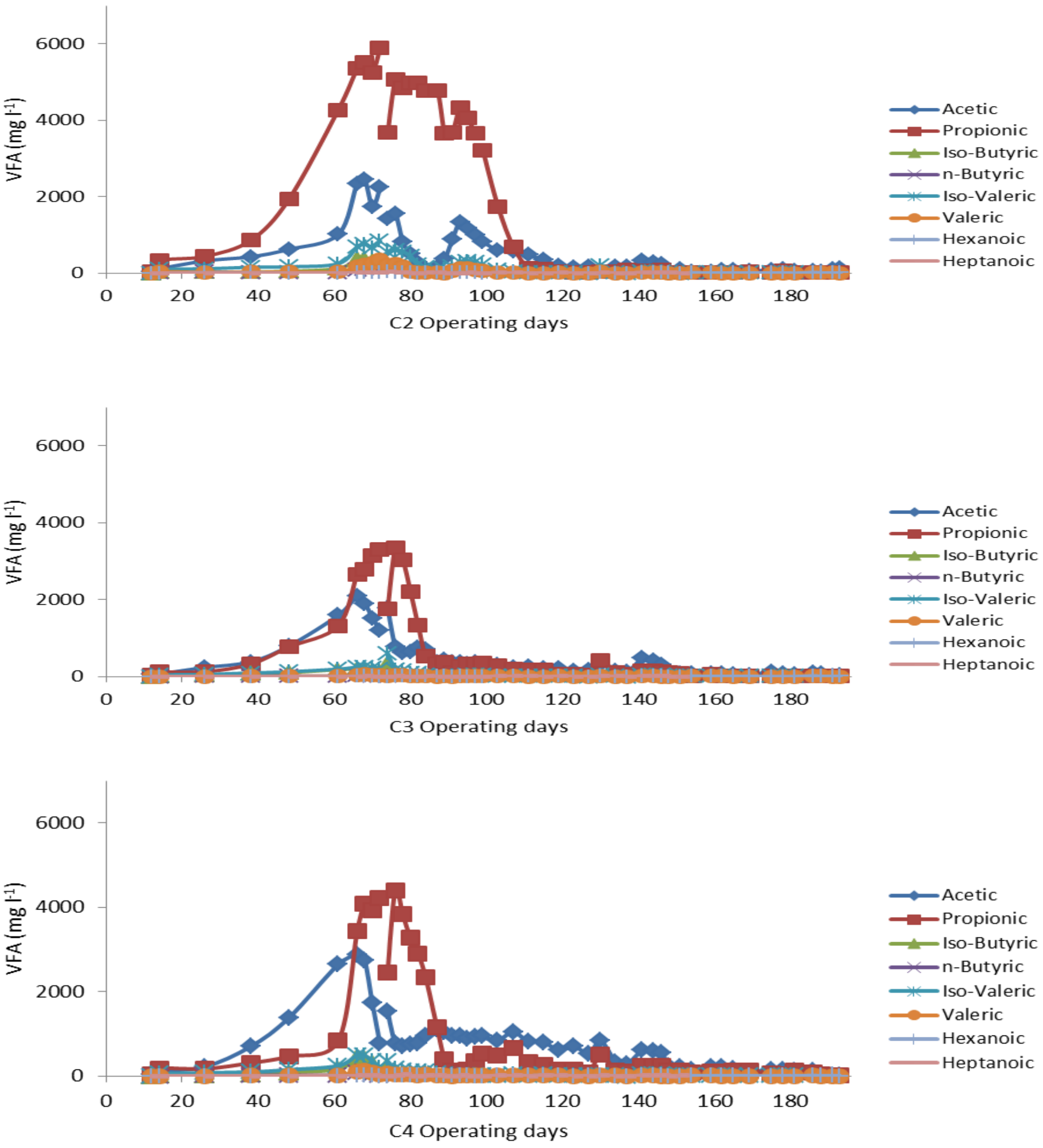


Figure 4.42 VFA profiles in digesters C1-4 during CSTR testing of salt addition

Table 4.24 Average performance over the last 30 days of the experimental run*

	Unit	C1 Control	C2 NaCl	C3 KCl + NaCl	C4 KCl
SBP	l biogas g^{-1} COD $_{added}$	0.531	0.509	0.498	0.490
SMP	l CH_4 g^{-1} COD $_{added}$	0.335	0.323	0.316	0.308
pH	-	7.37	7.29	7.29	7.30
VFA	mg l^{-1}	117	107	91	169

* NB Only C1 control and C4 KCl are at full steady state conditions at this point

4.9 Experiment 9: A comparison of an upflow anaerobic sludge blanket and anaerobic filter for treating WSW

The objective of Experiment 9 was to compare a UASB digester with an anaerobic filter for the treatment of WSW at high loading rates and to establish the highest methane production that could be achieved in either of these types of digester.

The WSW for this experiment was prepared at a high strength by washing the wheat straw in a washing machine (as described section 3.2.2), at temperature of 60 ºC for 3 hours. Two batches of WSW were prepared in this way and had the following characteristics (mg l^{-1}): batch 1- COD 4500; K 637.83; Na 28.13; Mg 351.84; Ca 367.32; Zn 42.70; Cl^- 432.17; SO_4^{2-} 483.77; PO_4^- 86.13, conductivity 3553.57 µS cm^{-1} ; batch 2- COD 2250; K 316.87; Na 8.47; Mg 112.31 ; Ca 122.57; Zn 14.02; Cl^- 135.78; SO_4^{2-} 182.15 ; PO_4^- 26.17, conductivity 1113.54 µS cm^{-1}

The experimental work used two anaerobic filters (AF1 and 2) as described in section 3.4.3 and two UASB reactors (U1 and 2) of the later design as described in section 3.4.2.2. Both sets of digesters had a 1.5-litre working volume. The anaerobic filters were inoculated using salt-acclimated digestate from the previous CSTR study (section 4.8.2). For this purpose, 8 litres of digestate from digesters C3 (KCl + NaCl) and C4 (KCl) were thoroughly mixed and divided evenly between AF1 and AF2. The digestate was then recirculated within each reactor by peristaltic pump for 7 days without feeding. The temperature of the AFs was maintained at 37 ºC. The inoculum for each of the UASB reactors was 1 kg wet weight of granular sludge: this was left inside the digesters at 37 ºC without feeding for 7 days but with recirculation.

All four digesters were then fed on SW for 48 days to allow acclimatisation. During this period the OLR was gradually increased to around 6 g COD l^{-1} day^{-1}, as shown in Figure 4.43 this was achieved by changing the degree of dilution of the SW using tap water, to maintain a HRT of between 18 - 19 hours. Minor fluctuations in OLR which can be seen were due to slight variations in pumping rates; the very low values for AF2 on day 27 and U2 on day 32 were due to pumping failures.

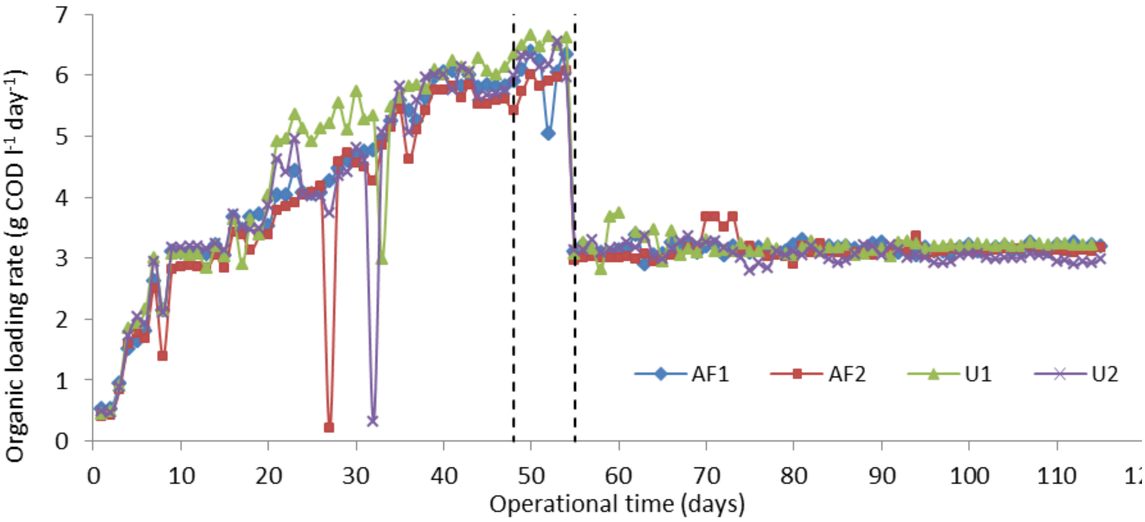


Figure 4.43 OLR applied to the UASB and AF digesters.

4.9.1 Specific methane potential

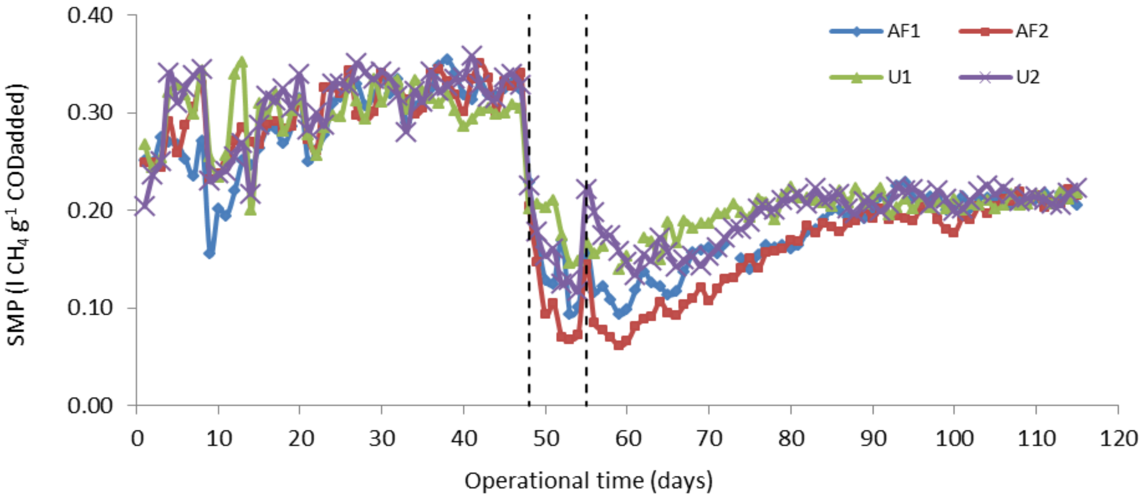
The SMP in the AF was initially slightly lower than in the UASB reactors, indicating the need for additional time to acclimate, but by day 25 all digesters had achieved SMP values of around 0.32 l CH_4 g^{-1} COD_{added} and 95% COD removal, similar to previous performance with SW. All four digesters responded well to the rapid increase in OLR up to around 6 g COD l^{-1} day^{-1}, although U1 which received a marginally higher loading showed a marginally lower SMP. Between day 25-40 the pH in U1 and 2 dropped below 7, but recovered to around 7.4 by day 48 (Figure 4.44).

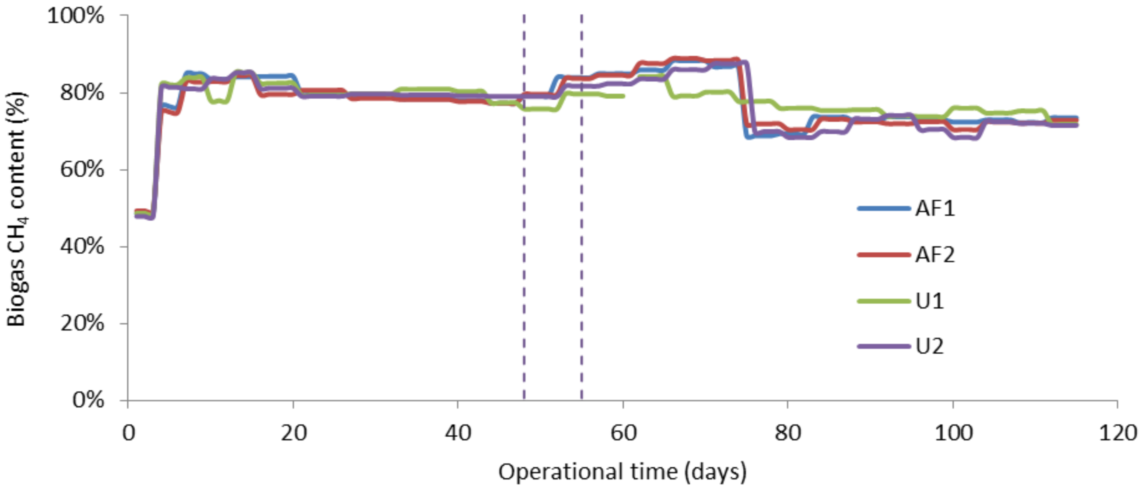
On day 55 the feed was switched to WSW at an OLR of 6.0 g COD l^{-1} day^{-1}. As the organic strength of the WSW was greater than that of the SW, this was achieved by reducing the volume added each day. At this point the SMP fell sharply in all reactors (Figure 4.44), with the AF affected more than the UASB, and U2 more than U1. On day 55 the OLR was reduced to 3.0 g COD l^{-1} day^{-1} by diluting the WSW with tap water to give an influent concentration of around 2.5 g COD l^{-1} and a HRT of around 26 hours: these values were similar to the final values for the SW feed. The SMP in both UASB reactors improved over the next 25 days, with U2 showing an immediate peak in gas production in response to the reduced load. The AF digesters responded more slowly, but by day 90 the SMP in all digesters had recovered to around 0.21 l CH_4 g^{-1} COD_{added} with a methane concentration of around 73%, fairly typical of the other WSW feedstocks tested.

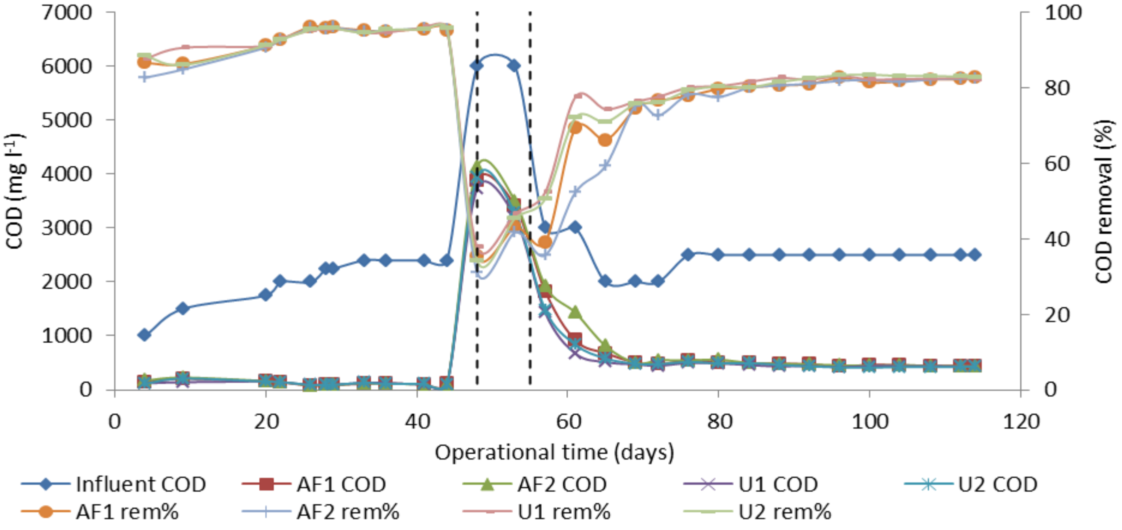
4.9.2 Other performance and stability criteria

Influent and effluent COD concentrations and removal rates are shown in Figure 4.44. On switching to WSW the effluent COD concentration rose sharply to around 4 g COD l^{-1} in all digesters, corresponding to removal rates of around 33%. These values showed some signs of recovery even before the OLR was reduced. By day 69 COD removal was close to 75% in all digesters, and by the end of the run this had stabilised at around 83% removal. Effluent VFA concentrations (not shown) remained extremely low throughout the experimental run (measured values < 10 mg l^{-1}), indicating that the COD peaks consisted of unhydrolysed material and/or other intermediate products. Suspended solids concentrations also showed little variation throughout the experiment (Figure 4.44). In terms of performance parameters (SMP, COD and suspended solids removal), the two systems thus performed equally well. During the last 10 days of operation (Table 4.24), the SMP stable at 0.20 l CH_4 g^{-1} COD $_{added}$ and COD removal rate of 82% for all digesters.

During the period of feeding on WSW, the pH in the AF was slightly higher and more stable than in the UASB, which operated at just below 7 for much of the time. The ammonia concentration in the effluent of all four digesters stabilised at around 0.1 g N l^{-1} indicating little buffering was available from this source.


SMP (l CH$_4$ g^{-1} CODadded)
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
50
60
70
80
90
100
110
120
Operational time (days)
AF1
AF2
U1
U2


Biogas CH$_4$ content (%)
100%
80%
60%
40%
20%
0%
0
20
40
60
80
100
120
Operational time (days)
AF1
AF2
U1
U2


COD (mg l^{-1})
7000
6000
5000
4000
3000
2000
1000
0
100
80
60
40
20
0
COD removal (%)
0
20
40
60
80
100
120
Operational time (days)
Influent COD
AF1 COD
AF2 COD
U1 COD
U2 COD
AF1 rem%
AF2 rem%
U1 rem%
U2 rem%

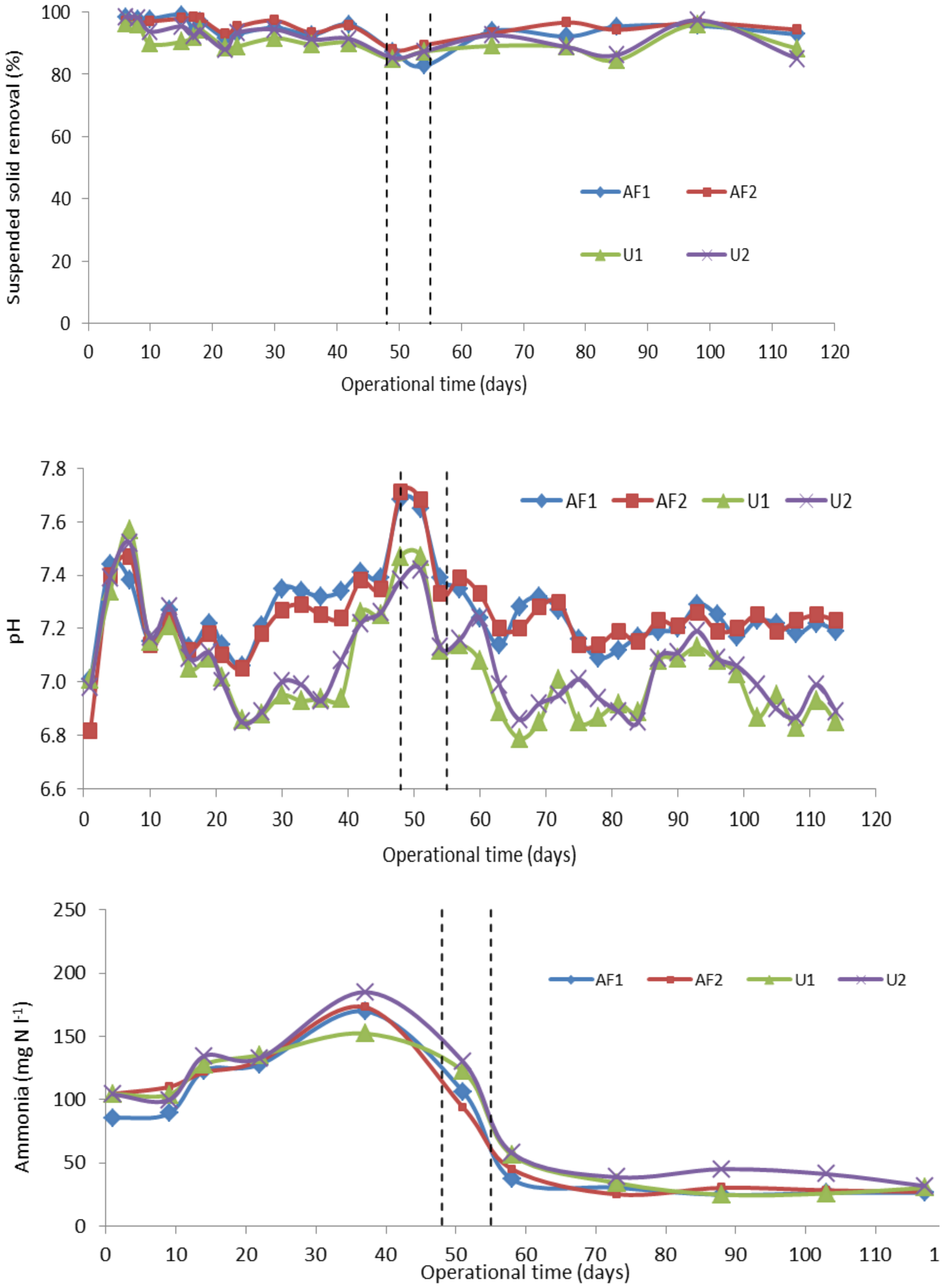


Figure 4.44 Digester performance and monitoring parameters: a) SMP; b) SBP; c) COD removal; d) suspended solids removal; e) pH; f) ammonia, during experimental period. The first vertical line indicates the change from SW to WSW; the second the reduction of OLR to 3 g COD l^{-1} day^{-1}.

Table 4.25 Average values for performance parameters in last 10 days of the experimental run

Parameter	Unit	AF1	AF2	U1	U2
SBP added	l biogas g^{-1} COD added	0.290	0.292	0.288	0.297
SMP added	l CH_4 g^{-1} COD added	0.212	0.212	0.212	0.214
SMP rem	l CH_4 g^{-1} COD removed	0.256	0.257	0.257	0.260
COD removal	%	82.5	82.2	82.4	82.2

4.9.3 Accumulation of K in the digesters

Figure 4.45 and Table 4.26 show the accumulation of K in the digesters. At an OLR of 6 g COD l^{-1} day^{-1} rates of accumulation were around 0.3 g K day^{-1}. When the OLR was reduced by diluting the WSW, this rate fell dramatically to 0.033 and 0.034 g K day^{-1}.in U1 and U2. Accumulation in the AF showed some variation with time, but in the last 35 days of operation the rate was 0.024 and 0.031 in AF1 and 2 respectively. The rates in the two reactor types were surprisingly similar, and showed no obvious sign of falling by the end of the run. It should be noted, however, that the quantity of granular sludge inoculum in U1 and U2 was larger than in R1-4 in the earlier experiments. The final values for K accumulation equated to a concentration of 4.5 mg K g^{-1} wet weight of granular sludge inoculum, less than the steady state totals of 11.0 mg K g^{-1} wet weight accumulated in R2 and R4 in Experiment 4 and of 6.6 mg K g^{-1} wet weight accumulated in R1 and R3 when fed on SW + KCl in Experiment 5 (see sections 4.2.3 and 4.2.4).

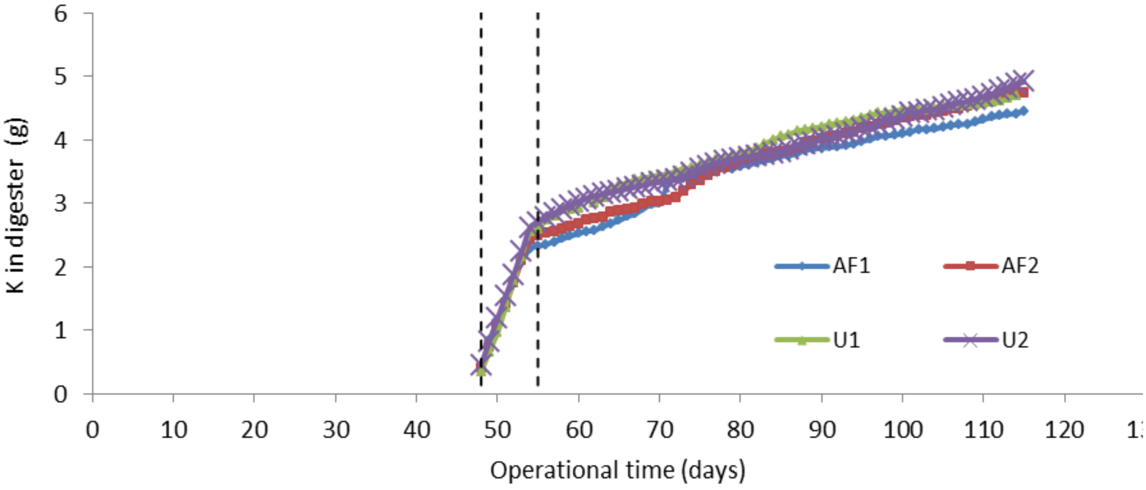


Figure 4.45 Accumulation of K in the digesters. R2 and R4 in Experiment 4.

Table 4.26 Accumulation of K in digesters

	AF1	AF2	U1	U2
K accumulated by day 56 (g)	2.35	2.52	2.73	2.78
K accumulated by day 115 (g)	4.46	4.74	4.74	4.92
K accumulated by day 115 (mg g^{-1} WW of original inoculum)	4.46	4.74	4.74	4.92
Rate of accumulation at OLR 6 (g K day^{-1})	0.281	0.314	0.349	0.336
Rate of accumulation at OLR 3 (g K day^{-1})	0.024*	0.031*	0.033	0.034

* Average for last 35 days

4.9.4 Stoichiometric relationship between COD and SMP

Table 4.27 shows the results for COD removal and SMP based on COD added and removed for SW, WSW and SW + KCl obtained in experiments 3, 4, 5 and 9. It can be seen that the results for Experiment 9 are closely similar to those for WSW in Experiment 4. ‘Missing’ methane can be explained by the small proportion of carbon which is being converted to other intermediate products that are not metabolised to CH_4.

Table 4.27 SMP for SW, WSW and SW + KCl compared (based on average values for duplicate reactors)

	COD rem	SMP added	SMP rem	$thCH_4$ rem [e]	% $thCH_4$	'Missing' CH_4 [f]	K accumulated (mg g^{-1} WW of original inoculum)	CO_2
Unit	%	l CH_4 g^{-1} COD_{added}	l CH_4 g^{-1} COD_{rem}	l CH_4 g^{-1} COD_{rem}	-	l CH_4 g^{-1} COD_{rem}		%
SW[a]	95.0	0.308	0.324	0.333	0.92	0.009	NA	42
WSW[b]	82.6	0.216	0.262	0.289	0.75	0.027	11.0	41
SW+ KCl[c]	92.7	0.255	0.276	0.325	0.79	0.049	6.56	39
WSW[d]	82.3	0.213	0.259	0.288	0.74	0.029	4.71 [g]	27

[a] from Experiment 2, OLR 1.00

[b] from Experiment 4, OLR 3.17

[c] From Experiment 5, OLR 2.00

[d] From Experiment 9, OLR 3.00

[e] calculated assuming 1 g COD = 0.35 l CH_4

[f] calculated from difference between theoretical stoichiometric yield and actual yield per g COD removed

[g] K accumulation has not reached final value

[h] NA: Not Available

rem : removed

4.10 Carbon and energy balance

The objective of this part of the study was to evaluate the results obtained and apply them in an outline carbon and energy balance based on the energy input into washing of the wheat straw and the energy output from the two products, WSW and wheat straw fibre.

Earlier results (section 4.1) established the washing requirements for wheat straw to remove salts (particularly K) which can combine with sulphate and chloride to form alkali salts with corrosive properties and will promote fouling, and thus reduce the energy output from the combustion process. A simple calculation was carried out to compare the energy used in washing and the energy produced from the AD process. A carbon balance was also conducted to determine the recovery of carbon utilized for biogas production and in the effluent.

4.10.1 Process for energy balance

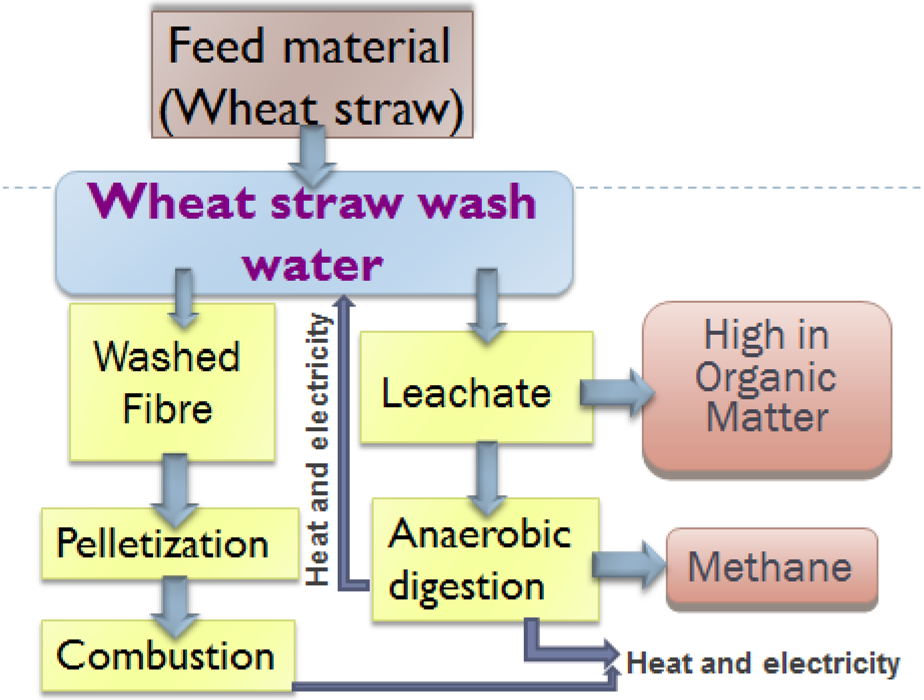


Figure 4.46 Basic stages in energy production from wheat straw fractions

Figure 4.46 outlines the basic process steps in conversion of wheat straw to energy through washing followed by combustion of the solid fraction and AD of the washwater. In this analysis, the potential energy production and the heat requirement for washing were calculated. Two types of washing technique were compared, namely cold water washing (CWW) at an ambient temperature of 20 ºC and liquid hot water washing (LHW) at 60 ºC. The potential energy produced is tabulated in Table 4.28. All data used were obtained from experimental results in earlier parts of this work.

Table 4.28 Comparison of energy consumed and produced for cold and hot water washing

Parameter	CWW at ambient temperature in 4.2 litres of tap water	Liquid hot water washing in 4.2 litres of tap water
Sample	50 g for 144 hours	50 g for 3 hours
Total Carbon in washwater (mg l^{-1})	652.6	963.0
g C g^{-1} straw	0.055	0.081
g C as CH_4 g^{-1} straw [a]	0.033	0.049
l CH_4 g^{-1} straw [b]	0.061	0.091
m^3 CH_4 $tonne^{-1}$ straw	61.40	90.60
Calorific value of CH_4 (MJ $tonne^{-1}$) [b]	2199.2	3245.3
Cal Value of CH_4 (kWh $tonne^{-1}$)	611.0	901.0
Energy required to heat water to washing temperature (MJ $tonne^{-1}$) [d]	0.0	14065.0
Energy required (kWh kg^{-1})	0.0	3.91
Energy required to heat water to digestion temperature (37ºC) (kWh kg^{-1})	1.66	0.0
ΔT (C°)	6.3	9.2
Min temperature (C°)	13.7	50.8
Measured energy in fibre (kWh kg^{-1})	5.16	4.18
Potential energy output (kWh kg^{-1}) [e]	5.77	5.09
Alkali Index (kg alkali oxide GJ^{-1})	0.116	0.094
Net energy output	4.11	1.18

[a] Assuming 60% of C is converted to CH_4. [b] Assuming 22.4 litres mol^{-1} at STP

[c] Assuming calorific value of CH_4 at STP = 35.82 MJ m^{-3}

[d] Assuming specific heat capacity of water = 4.186 J g^{-1} K^{-1}, and required washwater 4.2 litres/50g = 84 m^3 $tonne^{-1}$ straw

[e] Sum of potential energy yield from thermal and AD - not including heat demand for washwater etc

The results of the calculation indicated that the overall energy output produced for CWW and LHW are 5.77 and 5.09 kW h kg^{-1} respectively. LHW treatment gave a higher carbon content in the washwater of about 963 mg C l^{-1}; however, the energy required to reach this washing temperature is a significant fraction of the total potential energy output from combustion plus AD. This energy balance could possibly be improved by reducing the volume of washwater and by recycling heat in the process (e.g. waste low-grade heat from combustion), but CWW gives the higher potential energy value. Furthermore, the calculated alkali index revealed that excessive salts were removed during LHW, to 0.094 kg alkali oxide GJ^{-1}, where the value required was only 0.17 kg alkali oxide GJ^{-1}. The findings imply that the application of CWW for wheat straw washing is more effective in terms of the input energy required, the energy output and in washing straw to 0.12 kg alkali oxide GJ^{-1}; a level just below the threshold alkali index. From the point of view of net energy output, the use of CWW as pre treatment method offered a higher net energy output of about 4.11 kWh kg^{-1} as compared to LHW which about 1.18 kWh kg^{-1}.

4.10.2 Carbon balance

A carbon balance was calculated for the laboratory-scale UASB reactors used in Experiment 4, as shown in Table 4.29 and 4.30. Carbon in the substrate was converted into methane and carbon dioxide in the biogas, TOC in the effluent and carbon used for biomass growth. The total feed carbon was calculated from the carbon fraction of WSW fed per day into the UASB as OLR, whereas the carbon released daily in the biogas and effluent TOC were tabulated in Table 12.2a and b for both R2 and R4. Carbon in the feedstock (influent) was not wholly accounted for by the carbon in the effluent and carbon converted into methane and carbon dioxide. At OLR of 1, 1.81, 2.12, 2.49 and 3.17 g COD l^{-1} day^{-1}, carbon recovery for R2 [R4] was 88% [87%], 76% [81%], 73% [72%], 73% [72%] and 86% [85%] respectively. The loss of carbon was expected as some will be accumulated in the digester for biomass growth and a small quantity may be washed out in solid form in the daily effluent.

4.10.3 Considerations for practical application of the technology

As the net energy output for LHW was lower than for the CWW, it appears that it will be more efficient to employ CWW for further development. Based on the ratio of water to wheat straw used, however, water consumption is clearly a major issue: for example the 5 litres of water for 50 g of wheat straw used in the laboratory trial would be equivalent to 21 million m^3 for a single straw-burning power station with 210,000 tonnes $year^{-1}$ of feedstock (Stoddart and Watts, 2012). At the ratio used the washwater was also too dilute, leading to the need for larger reactor volumes at a giving retention time and a greater energy requirement to heat it to the digester temperature, as well as adding to the problem of storage and land area required. These factors may make CWW less efficient in terms of cost.

There are several alternative washing techniques which could be applied at a larger scale. Possible methods include the use of leach beds, which could be operated in series to increase the strength of the washwater in each stage while minimising water input. Another alternative is the use of a reciprocating drum system with a counterflow washwater stream (a system similar to the washing machine used, but with many units in series). The application of this system allows gradual concentration of the washwater stream, so that the clean washwater is in contact with the almost-washed material and the dirty washwater with the unwashed material. Recycling of all or part of the effluent to be used for straw washing would also reduce water demand. This may require bleeding of a proportion of the flow and replacement with fresh water to maintain the salt concentration below critical operating level, or further treatment to remove the minerals content. In the former case, further studies would be needed to determine the critical concentration, which could depend on the salt tolerance of the anaerobic treatment system or the concentration needed to achieve the required alkali index. In the latter case, options include the application of reverse osmosis or membrane technology which will also have energy and cost implications. Potassium salts or other minerals could potentially be collected and returned to the soil as nutrient in order to make a closed-loop system.

From the net energy balance for LHW, it can be seen that employing this system gives an increased energy demand. In practice, however, this could be reduced by process integration. The calculation in Table 4.28 already takes into account the fact that no further energy input will be required to raise the temperature of the washwater to that of the digester, but in practice the heat from the washwater could also be recycled to heat the following batch of washwater and wheat straw for LHW treatment using a heat exchanger. The implementation of these systems would reduce the overall energy demand. Operation at a higher wash temperature would also reduce the reaction time and required storage volume.

Further energy savings could be potentially obtained by operating the digestion system at a lower temperature: recent work has shown that UASB can be acclimated to operate successfully at temperatures of 20 ^{o}C or less (McKeown *et al.*, 2012). It should also be considered, however, that in most power generation stations heat is available as a waste product and if the straw washing and digestion is to be co-located on the same site further process integration to make effective use of this waste heat may be possible.

For practical application of the proposed technology reductions in both water use and energy consumption are essential. The current work did not consider options such as low temperature digestion or the effects of water recycling, and it is therefore not possible to make a full assessment of the whole system energy balance. The work clearly shows, however, that washing of wheat straw as a pre-treatment for salt removal before thermal conversion, with anaerobic digestion to recover energy from the washwater produced, can provide a net energy benefit when considered at the simplest level. Based on these results there is considerable scope for optimisation and integration of the process to produce a practical technology for renewable energy production through combined combustion and anaerobic digestion.

Table 4.29 Carbon balance for digester R2 fed on WSW

OLR	Average between days		Biogas	CH_4	CO_2 %	Influent TC	Effluent TC	g C as CH_4	g C as CO_2	Total C recovered	% C recovered
g COD day^{-1}	From	To	litres STP	%/100	%/100	g C l^{-1}	g C l^{-1}	g C	g C	g C	
1	460	577	0.39	0.58	0.42	0.55	0.235	0.12	0.13	0.49	88%
1.81	578	587	0.62	0.60	0.40	0.77	0.263	0.20	0.13	0.59	76%
2.12	588	605	0.72	0.59	0.41	1.13	0.469	0.23	0.13	0.83	73%
2.49	606	633	0.87	0.59	0.41	1.31	0.557	0.28	0.13	0.96	73%
3.67	-	-	-	-	-						NR [a]
3.17	654	678	1.11	0.62	0.38	1.48	0.783	0.37	0.13	1.28	86%

Table 4.30 Carbon balance for digester R4 fed on WSW

OLR	Average between days		Biogas	CH_4	CO_2 %	Influent TC	Effluent TC	g C as CH_4	g C as CO_2	Total C recovered	% C recovered
g COD day^{-1}	From	To	litres STP	%/100	%/100	g C l^{-1}	g C l^{-1}	g C	g C	g C	
1	460	577	0.40	0.59	0.41	0.55	0.227	0.13	0.13	0.48	87%
1.81	578	587	0.63	0.59	0.41	0.77	0.297	0.20	0.13	0.63	81%
2.12	588	605	0.74	0.59	0.41	1.13	0.447	0.23	0.13	0.81	72%
2.49	606	633	0.87	0.60	0.40	1.31	0.544	0.28	0.13	0.95	72%
3.67	-	-	-	-	-						NR [a]
3.17	654	678	1.16	0.61	0.39	1.48	0.758	0.38	0.13	1.26	85%

[a] Not Reported

5. Discussion

The overall aim of the research was to determine the effectiveness of washing for removal of salt from wheat straw as a pre-treatment to its thermal conversion, and to evaluate the possibility of using anaerobic digestion to recover energy from the washwater produced. The research carried out has expanded knowledge in this field and presents new data. It had previously been reported that washing could remove salts, in particular Potassium, thus improving the combustion characteristics of straw. The first part of the research investigated this further by considering the conditions required and comparing these to the results of previously reported work. Jenkins *et al.* (1998) showed that hand spraying with water could remove 3.5% of salt content, while flushing and submerging in tap water removed about 4% and 6% of ash respectively. Hernandez Allica *et al.* (2001) investigated natural washing by rain falling on straw left lying in the field, and reported that between 60-68% of K was removed after 150 mm of cumulative rainfall; and also confirmed that Potassium is the dominant salt in wheat straw. Baxter *et al.* (1998) also reported that K is the major mineral and suggested that 80-90% of K is water soluble or ion exchangeable. The results of Baxter *et al.* (1998) are consistent with those of this study in terms of K removal potential. The previous work also showed that the effectiveness of removal depends on the method used, and in particular on the ratio of water to wheat straw, as also shown in this study. No previous studies have looked at how the leaching of K is affected by temperature and whether rates of removal could be increased in this way. The correlation between K and conductivity, which proved very useful in the current work, was also found by Hernandez *et al.* (2001).

No consideration was given in the current work to more complex pre-treatments, of which previous examples include lime treatment (Ronald *et al.*, 2008) to reduce the salt content to an alkali index of 0.3 kg GJ^{-1}. Using the techniques proposed as a result of the current research, a value of between 0.12 and 0.18 kg alkali index GJ^{-1} could be achieved by either CWW or LHW, suggesting that chemical pre-treatments are not necessary to achieve a satisfactory value.

Although there have been many previous studies on wastewater treatment using high rate anaerobic processes such as UASB, these have mainly focused on industrial applications such as sugar industry wastes, distillery wastes, and brewery wastes (Blonskaja *et al.*, 2003, Fang *et al.*, 1995 and Lettinga and Pol, 1991). No previous study has been carried out on the use of an AD system specifically for energy recovery from wheat straw washwater, making the current research unique in this respect. The results indicated that methane recoveries for synthetic wastewater were in line with the expected values based on stoichiometric COD removal, giving confidence in the reliability of the experimental data and the equipment and methods used.

It is apparent that even with the CWW technique the washwater had an appreciable chemical oxygen demand, of between 1-2 g l^{-1}. The COD washed out under these conditions was also readily convertible to biogas, with around 83% removal in steady state conditions. A limited number of studies have been carried out in which different types of straw have been used as feedstock in two-phase leach bed digesters (Zhang and Zhang, 1999; Schönberg and Linke, 2012). In a different context, straw hydrolysates or stillages have also been fed to second-stage methanogenic digesters (Kaparaju et al., 2009, 2010). The results are not directly comparable with those from the current work, however, especially where the materials have been pre-treated to optimise the extraction of organic matter, using methods such as the mechanical, thermal and chemical treatments applied to rice straw by Zhang and Zhang (1999). In other circumstances the straw may be used only as a carrier medium in digesters (Andersson and Björnsson, 2002).

One effect of high salt concentrations is to cause bacterial cells to dehydrate due to high osmotic pressures (de Baere *et al.*, 1984; Yerkes *et al.*, 1997). The toxicity of salts, however, is predominantly determined by the cation (McCarty and McKinney, 1961). Light metal ions including Sodium, Potassium, Calcium, and Magnesium are present in many substrates and are thus often present in the feed to anaerobic digesters. They are also sometimes added for pH adjustment. They are required for microbial growth and moderate concentrations stimulate this, whilst excessive amounts slow down growth and even higher concentrations can cause severe inhibition or toxicity (Soto *et al.*, 1993a). Much of the work in the current research has concentrated on Potassium as this was found to be the major cation leached from straw; and the effect of Potassium on the

UASB digesters was also demonstrated by its addition to synthetic sewage over a range of concentrations up to 1.8 g K l^{-1}. A study conducted by Fernandez and Forster (1994) shown that low concentrations of Potassium (less than 400 mg l^{-1}) can result in enhancement of performance in both the thermophilic and mesophilic ranges. Limited information is available on the toxic range, although Potassium is thought to be more toxic at thermophilic temperatures (Fernandez and Forster, 1993, 1994; Shi and Forster, 1994). There are also conflicting views on which part of the microbial consortium suffers the greatest impact: Fernandez and Forster (1994) found the acidogenic phase to be most sensitive, while Mouneimne *et al.* (2003) showed greater sensitivity in acetate utilizers. The threshold values of toxicity reported are wide, ranging from 2.5 g l^{-1} (Fernandez and Foster, 1994) to 5.87 g l^{-1} reported by Kugelman and McCarty (1965) to cause 50% inhibition of acetate-utilizing methanogens.

The results of this study supported those from previous research in showing that Potassium had no detrimental effect on performance when present at a low concentration, which in the case of the lowest strength WSW was only 150 mg l^{-1}. The highest concentration of K extracted from wheat straw and used in this study was 700 mg l^{-1} (Experiment 9), and this is much lower than the range reported above to be toxic. The major difference from previous studies is that this research looked at continuous exposure over long periods, whereas previous work has only assessed the effect in batch studies. The work reported here provides clear evidence that K concentrations of around 700 mg l^{-1} in the feed can have an impact on the overall methane production. In the supplementation study where KCl was added to synthetic sewage, the starting concentration of 0.67 g K l^{-1} produced an initial 33% decrease in SMP. There was then a longer-term recovery and by gradual adaptation the digesters were able to operate successfully with ~93% COD removal at 1.8 g l^{-1} K in the feed. This was clearly an energy-consuming process, however: evidence for this came from the stoichiometric conversion of COD to methane, which was found to be lower in cultures with a higher K content. This has not previously been reported, and is a significant finding which may help in understanding the mechanism by which osmotic balances are maintained in microbial cultures. The research did not investigate this further, but proposed that there may be a requirement for additional cell maintenance energy in order to maintain an osmotic gradient, and that the active transport of K across the cell membrane would allow this by its accumulation in the cells. Evidence for this was found from the EDX studies which showed intracellular K in cell sections. There was also evidence of crystalline salt deposits forming on the granule surface, similar to previous observations made by Fang *et al.* (1995) and Van Langerak *et al.* (1998a)

In Experiment 8 anaerobic sludge was taken from a variety of sources to assess whether it could be acclimated to relatively high concentrations of Sodium and Potassium. In the first part of this experiment a batch approach was used to assess levels of inhibition. Sodium and potassium were used in the tests at concentrations of up to 30 g l^{-1}, well above the toxicity thresholds suggested by Kugelman and McCarty (1965) and McCarty and Fernandez and Foster (1994). A full range of intermediate concentrations were not tested, but the results for both Na and K suggested relatively high values for the 50% inhibition threshold for inoculums taken from saline mud and from a municipal wastewater treatment plant. The results therefore show a tolerance greater than that found in the studies of Fernandez and Foster (1994) and Kugelman and McCarty (1965). It was apparent, however, that the degree of tolerance in a batch test was dependent on the source of the original inoculum and that the anaerobic granular sludge showed a relatively low inhibition threshold.

Anaerobic digestate taken from the Millbrook wastewater treatment plant was used in CSTR digestion trials with KCl and NaCl applied both individually and in combination. It appeared that the inoculum adapted more readily to the mixture of KCl + NaCl than to KCl alone, and to KCl alone better than to NaCl. This could be due to differences in the ability of the cell to transport different ions across the cell membrane in order to maintain an osmotic balance, which is necessary to prevent dehydration and maintain cell turgor pressure and ionic strength in the cytoplasmic membrane (Brabban *et al.*, 1999 and Roberts, 2004). An imbalance or alteration of intracellular metabolites concentration can lead to inhibition of a variety of cellular processes (Kraegeloh *et al.*, 2005). As the population was able to adapt successfully to all of the added salt combinations in all of the concentrations applied, it was concluded that the differences were transient and most likely due to adjustment of temporary imbalances which could be achieved more easily and rapidly when a mixture of cations was available rather than single species. The mixture of cations and anions present can greatly influence the concentration causing 50% inhibition and it been has found that the sodium concentration can be increased more than 4 fold when sea water is used in the tests instead of NaCl (Feijoo *et al*., 1995).

Despite differences in the rate of acclimatisation, all the CSTR digesters were able to adapt successfully to the added salt applied and concentrations of 10 g l^{-1} were achieved in all cases. A considerable number of studies have been carried out on the adaptation of wastewater treatment plants to saline conditions: the most recent review of these was by Lefebvre and Moletta (2006a). As most treatment systems in use are aerobic there is greater knowledge on these than on anaerobic systems. The concentration of NaCl added in synthetic wastewater and applied to digestate acclimated from sewage sludge was 10 g l^{-1}: this is equal to the concentration reported by Kugelman and McCarty (1965) as strongly inhibitory to methanogenesis. This reported concentration was from tests carried out in batch mode, which are now known to overestimate the sensitivity of the microorganisms to saline conditions: this may be attributable to the sudden change in levels of cations that microorganisms are exposed to, allowing them little time to adapt. In continuous testing, sodium concentrations are often increased gradually, giving sufficient time for adaptation (Feijoo *et al.*, 1995). Since these earlier studies the IC50 for sodium inhibition has been reported to be between 5.6–53 g l^{-1}, depending on the adaptation period, antagonistic/synergistic effects, substrate, and reactor configuration (Patel and Roth, 1977; Rinzema *et al.*, 1988; Liu and Boone, 1991; Soto *et al.*, 1993b; Feijoo *et al.*, 1995; Omil *et al.*, 1995a,b; Aspé *et al.*, 1997; Kim *et al.*, 2000; Vallero *et al.*, 2002; Chen *et al.*, 2003; Vallero *et al.*, 2003a,b). The salt concentration achieved in the current study is low compared to some of these values, but these are 50% inhibition concentrations and in the current work the methane production was not suppressed. Compared to the relatively limited number of anaerobic digestion studies on saline wastewaters, the performance obtained was equal to or better than those previously reported (Lefebvre and Moletta, 2006). There have been no other studies on the continuous adaptation or acclimatisation of anaerobic digestion to a high concentration of KCl in isolation of other cations, and in this respect the current study is unique.

The comparison of a UASB digester with an anaerobic filter for the treatment of WSW showed that there was no difference in the COD removal or the specific methane production for these two process types. Comparisons of this type have been carried out in the past, but not using wheat straw washwater. In the study by Hutnan *et al.* (1999) a synthetic wastewater was used containing trace elements and with a COD of 6 g l^{-1}. The loading was successfully increased to 15 g COD l^{-1} day^{-1} and comparable COD removal was seen between the reactors used, which included a UASB reactor and an anaerobic filter. This is higher than the loading applied in this study, but the strength of the wastewater in Hutnan *et al.* (1999) was higher and the study was thus not affected by the reduction in hydraulic retention time which may become critical when dealing with lower strength wastewaters.

A loading of 6 g COD l^{-1} day^{-1} could not be maintained with WSW, and the highest steady loading achieved was 3 g COD l^{-1} day^{-1} at an influent concentration of 2.5 g COD l^{-1}, giving a HRT of around 26 hours. The loading was low compared to that normally associated with high rate methanogenic processes, despite the relatively long hydraulic retention time. As expected when fed on WSW the digesters accumulated K: the rate of accumulation and final amount accumulated in both the UASB and AF were very similar and had reached a concentration of between 4.5 – 4.9 mg k g^{-1} wet weight of granular sludge.

A positive energy balance was seen when using both hot and cold water washing to remove the salts for wheat straw. The energy balance was shown to be more favourable for the cold water wash, as energy was not required for heating the water. It must be emphasised however that this was very provisional estimate, based on a very limited data set for washing. Using the higher temperature wash more salt was removed than is required for trouble free combustion, and more of the carbon was transferred from the solid phase to the liquid phase which will also reduce the calorific value of the straw. The experiments carried out did not determine the optimum temperature to reach the required alkali index value. Although this was clearly achieved with a cold water wash, the washing time required with the technique employed was very long, with a period of 144 hours used in the energy balance calculation. This in itself has energy implications if the embedded energy in the tanks is taken into consideration, as would be the case in a full life cycle assessment (LCA). Likewise if the washing process involves mechanical agitation then the motor power to achieve this would also need to be taken into consideration.

The calculation is also limited in that it assumes that the two products formed from the washing process have no further energy input requirements: this is of course unrealistic, as the wheat straw itself is wet after the washing process and requires drying before combustion. The final lower heat value, and thus energy return, will depend upon the moisture content of the straw and the effectiveness of any drying process, which in turn may depend on the energy input for both heat and mechanical turning. The energy balance calculation also assumed that the effluent from the anaerobic treatment process is either fit for discharge without any further treatment or could be directly used for further extraction, neither of which is likely to be true. It would therefore be necessary to take into account the energy requirement for further treatment, which may involve an aerobic polishing process. Even then the effluent would be slightly saline and may not be acceptable for discharge. In the case of water reuse a desalination process might be required and these are generally very high energy usage processes. No attempt was made to assess the potential for washwater reuse in the current work. The energy balance calculation was carried out simply to show that the anaerobic digestion process could convert sufficient of the washed-out carbon into a fuel to allow a hot water wash, if this is required to remove the alkali metals to an acceptable concentration.

Other factors that would need to be considered in a full energy balance would be any transport requirements to move the straw from one location to another; and the embedded energy costs in infrastructure plant and equipment for the washing, straw drying and effluent treatment facilities. These would also need to be weighed against the positive benefits of having a fuel with a reduced alkali index, which would reduce maintenance on the straw combustion or co-firing power plant; and a reduced ash output from the power plant with a lower salt content.

It was never intended to carry out a full LCA for this process in the current work, as this is a specialised area of knowledge: but the research has provided quantitative data that could contribute towards such an assessment if the overall process can be shown to be attractive in terms of energy yield and economic viability.

6. Conclusions

The following conclusions can be drawn from the results obtained:

1) Leaching was shown to be a highly effective technique for removal of alkali salts and chlorine from wheat straw. Observations in this study confirmed the capability of this technique to remove up to 90% of the problematic compounds.

2) Leachate Potassium concentration and conductivity were shown to have a strong correlation, as Potassium represents the major salts in wheat straw.

3) The optimum pre-treatment conditions will be highly dependent on the maximum salt removal required, the energy consumption and recovery, reaction time and ratio of wheat straw to water. When comparing analytical data for pre-treatment and salt removal rate to find the best pre-treatment, it is clear that while steam pre-treatment at 100°C achieved the highest salt removal from the fibre (to about 0.789 mg salt g^{-1} wheat straw), this option is unlikely to be optimal due to the target of low energy consumption. CWW treatment at 20°C can achieve about 3.47 mg salt g^{-1} wheat straw at reaction times of about 3 days.

4) Based on the potential energy output from the two streams (washed wheat straw and washwater), CWW was identified as a more efficient technique for wheat straw washing than LHW with a net potential energy yield of 4.11 kWh kg^{-1}, compared to only 1.18 kWh kg^{-1} for LHW (taking into account energy used in heating water to washing temperature and washwater to 37 ^{o}C for digestion).

5) In terms of alkali index, CWW was able to wash straw for combustion to 0.12 kg alkali oxide GJ^{-1}, just below the threshold index at 0.17 kg alkali oxide GJ^{-1}. Washing salts out to the level of 0.09 kg alkali oxide GJ^{-1} in LHW represents a waste of energy.
6) The study confirmed that the organics washed from wheat straw were to a large extent biodegradable in UASB digesters, with a specific methane yield of around 0.21-0.22 l CH_4 g^{-1} COD $_{added}$ and 82-83% of COD removal.

7) During the first cycle of feeding on WSW, K accumulated in the UASB to a concentration of around 9.5 mg K g^{-1} WW inoculum. On switching to SW, the accumulated K dropped to about 1 mg K g^{-1} WW. Similar patterns were repeated over 4 successive cycles of alternating WSW and SW feed, with incremental increases in the quantity of K accumulated when feeding on WSW.

8) Long term acclimatisation to higher strength WSW showed an initial reduction in SMP to 0.20 l CH_4 g^{-1} COD $_{added}$ at COD removal of 76%. Further exposure to WSW gave a steady state value of SMP of 0.216 l CH_4 g^{-1} COD $_{added}$ and a COD removal of 82.6%. At the point when the SMP achieved a steady state value, the maximum value of K accumulation also occurred. This was equivalent to ~11.5 mg K g^{-1} WW of inoculum.

9) Comparison of SMP and COD removal rates during accumulation and washing out of K indicate that when accumulation occurs, a proportion of the energy available from COD conversion is not being channelled into methane production. This may be consistent with the theory that methanogenic organisms operate a Potassium pump to maintain ionic equilibrium, and as this is an energy-driven process it will therefore reduce the overall methane yield.

10) UASB reactors fed on SW achieved a stable SMP of around 0.31 l CH_4 g^{-1} COD $_{added}$ with a COD removal of 95%. The specific methane production in terms of COD removal corresponded to around 92.5% of the theoretical value based on stoichiometric calculation. Increasing the KCl concentration to 1.8 g K l^{-1} gave an average SMP of 0.26 l CH_4 g^{-1} COD $_{added}$ and COD removal of 93%.

11) The rate of washout of K when fed on SW appeared to fall more sharply with inoculum K concentration than did the rate of K accumulation with WSWas the feed, and this may possibly reflect the operation of a K+ pump to regulate concentration in the cells.

12) Adsorption of Potassium by granular sludge reached equilibrium within 72 hours at concentration of 59 mg l^{-1} with a sorption capacity of 4.21 mg g^{-1}. The Freundlich model could adequately represent the distribution of salts between the solution and the granular sludge.

13) The desorption study showed that 1.5 mg l^{-1} of synthetic sewage was an effective eluent to release K from granules as it achieved 90% desorption efficiency at 3.8 days at the highest K concentration of 111.13 mg l^{-1}. Desorption of K in tap water revealed the lowest removal rate of 65% (equilibrium) after 10 days and showed that tap water is unsuitable for desorption of K from granules. These findings indicate that nutrient available in synthetic sewage is required to release the K accumulated particularly inside the granules, suggesting that the dominant process was translocation.

14) The re-sorption after desorption study showed a reduction (44% of K removal) of sorption capacity from 4.12 mg g^{-1} to 3.11 mg g^{-1} . Nevertheless, by considering the amount of K left in the granules, which about 14% (as only 86% was desorbed), the total sorption became (44%+14%) 58% which is close to the previous value of 59%. This findings revealed the sorption capacity of K was not reduced in the following cycle by considering the remaining K inside the granules.

15) A CSTR digestion study revealed that that digesters seeded with an inoculum from a conventional mesophilic digester treating municipal wastewater biosolids and fed on SW plus salts were able to acclimate successfully to a final salt concentration of 10 g l^{-1}. This was considerably higher than the concentration likely to occur in WSW, and indicated that this source of inoculum would be suitable for start-up of a large-scale CSTR or anaerobic filter plant. The digesters showed some disturbance during the acclimatisation period as indicated by reductions in SBP, SMP and pH and increases in IA/PA ratio and VFA concentration: the order of severity of disturbance was NaCl > KCl > KCl + NaCl. Average values for SBP and SMP after stabilisation were below those

in the control digester, at 0.335 (control), 0.323 (NaCl), 0.316 (KCl + NaCl) and 0.308 l CH_4 g^{-1} COD $_{added}$ (KCl).

16) A change in feedstock from SW to WSWat an OLR of 6 g COD l^{-1} day^{-1} caused a sudden reduction in SMP. Reduction of the OLR to 2.5 g COD l^{-1} day^{-1} allowed adaptation to WSW, and both the UASB and the AF reactors achieved an SMP of 0.21 l CH_4 g^{-1} COD_{added} at 82% of COD removal. The results for this experiment are closely similar to those found previously for WSWin acclimated UASB. The final values for K accumulation equated to a concentration of 4.5 mg K g^{-1} wet weight of granular sludge inoculum, less than the steady state totals of 11.0 mg K g^{-1} wet weight accumulated in the previous experiments, indicating that steady state K accumulation had not yet been achieved.

17) Scanning electron micrographs showed a change in surface structure of the granules and it was confirmed by EDX analysis that there was some light metal crystallisation and salt agglomeration on the sludge granule surface and in the cross section of the granules.

7. Further work

The laboratory experiments performed as part of this work were restricted due to time limitations, and it was therefore not possible to further investigate the effect of higher loading rate for WSW, the threshold inhibition of Potassium as KCl in R1 and R3, final K accumulation in UASB and AF digesters, and the effect on SMP and COD removal of running digesters at a lower operating temperature (20°C). Extension of this work could provide further understanding on the potential for wheat straw washwater in AD systems. Specific suggestions of how the work could be extended are given below.

Confirmation of maximum OLR for WSW (Experiment 4)

The operating time at the maximum OLR of 3.67 g COD l^{-1} day^{-1}(day 634 to day 645) applied to UASB was insufficient to confirm whether the reduction of SMP and COD removal were due to the effect of shock loading, or 3.5 g COD l^{-1} day^{-1} was the allowable limit for digestion of WSW. After two weeks the system seemed to be recovering from the shock increase in loading. It would be of interest to determine the operational characteristics at a steady state condition at this loading rate by providing a longer operational time and adaptation period for the digesters. Another interesting study in anaerobic digestion of WSW is to fix the same operational time for every cycle when feeding WSW and SW. This would allow more detailed analysis on the trend of K accumulation and the role of organic matter in SW to wash out accumulated K.

Inhibition threshold for Potassium as KCl in R1 and R3 (Experiment 5)

As noted in the literature review, Fernandez and Foster (1994) reported the threshold for Potassium inhibition was at 400 mg l^{-1} and toxicity of K was significant at 2500 mg l^{-1}; while Kugelman and McCarty (1965) reported that 5.87 g l^{-1} of K caused 50% inhibition of acetate-utilising methanogens. In this study, the highest concentration of K applied in continuous mode was 1.8 g K l^{-1}. The findings showed an SMP of 0.26 l CH_4 g^{-1} COD_{added} and COD removal of 93% which implied that the 50% inhibition threshold is at a still higher concentration. Further investigation is required particularly in continuous systems as most of the K toxicity thresholds which have been reported were conducted in batch studies.

Final K accumulation in UASB and AF digester (Experiment 9)

Accumulation of K in UASB and AF digesters had not reached a final value as K was still increasing on the last feeding the digesters. It would be interesting to provide a longer operational time, which would allow the accumulated K to reach a stable value. The findings of this experiment can be used to compare the accumulation rate with that found in the first UASB study in which the digesters reached a steady state with respect to K accumulation.

Effect on SMP and COD removal of running digesters at lower temperature

Net energy production from CWW treatment was higher than from LHW, even allowing for the need to heat the washwater to the digester operating temperature. It is possible that the digesters could be run at a lower temperature, thus reducing the overall energy demand. Recent research at Southampton and elsewhere is looking at the adaptation of UASB digesters to temperatures of 20^{o}C and below and this would be a valuable extension to the current work which might make the overall process favourable in energetic and economic terms.

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Appendices

Appendix 1

1. **Gasometer Governing Equations** (Walker *et al.*, 2009)

Assumptions

- Biogas acts as a perfect gas. $\frac{p_1 V_1}{T_1} = \frac{p_2 V_2}{T_2} = \frac{p_{stp} V_{stp}}{T_{stp}}$
- The biogas once leaving the anaerobic digester quickly cools to ambient temperature. $T_1 = T_2 = T_{atm}$
- The cross-sectional area in the columns is constant.

2. **Weight Gasometer**

Volume of gas introduced into the column at standard conditions

$V_{stp} = V_{2stp} - V_{1stp}$ Eq.1

Perfect Gas Law $\frac{p_a V_a}{T_a} = \frac{p_b V_b}{T_b}$ Eq.2

$$V_{stp} = \frac{T_{stp} A}{T_{atm} p_{stp}} \left[\left(\left(p_{atm} - p_{h2o}(T_{atm}) - \rho_b g \left(H - h_1 - \frac{m_w}{A \rho_b} \right) \right) . \left(h_1 + \frac{m_w}{A \rho_b} \right) \right) - \left(p_{atm} - p_{h2o}(T_{atm}) - \rho_b g (H - h_1) \right) h_1 \right]$$

Eq.3

3. **Height Gasometer**

Equations 1 and 2 apply to this setup.

Volume of gas in column $V_1 = A h_{c,1}$ Eq.4

$$V_{stp} = \frac{T_{stp} A}{T_{atm} p_{stp}} \left(\left(p_{atm} - p_{h2o}(T_{atm}) - \rho_b g (h_{t2} - h_{c2}) \right) h_{c2} - \left(p_{atm} - p_{h2o}(T_{atm}) - \rho_b g (h_{t1} - h_{c1}) \right) h_{c1} \right)$$

Eq. 5

Where,

V	Volume (m^3)
P	Pressure (Pa)
T	Temperature (K)
H	Total height of column (m)
h	Distance to liquid surface from a datum (m)
A	X-section of gasometer (m^2)
m_b	Mass of barrier solution (kg) b m
P_{H_2O}	Density (kg m^{-3})
g	Gravitational acceleration (m s^{-2})