Cultivation and anaerobic digestion of *Scenedesmus* spp. grown in a pilot-scale open raceway

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Abstract

Digestibility of a micro-algal mixture was evaluated by mesophilic anaerobic digestion in continuously-stirred tank reactors. The culture consisted primarily of *Scenedesmus* spp. continuously cultivated over a 6-month period in a 100 m^2 raceway reactor instrumented to record pH, dissolved oxygen and temperature. The raceway received supplementary carbon in the form of flue gas from a diesel boiler (10% CO_2) injected into a 1-m deep sump to control pH in the range 7.8-8.0. Dilution was optimised to biomass productivity and gave values of 10-15 and 20-25 g total suspended solids (TSS) m^{-2} day^{-1} in winter (December - February) and Spring (April - May), respectively. The culture for the anaerobic digestion trial was harvested in February by centrifugation to give an algal paste containing 4.3% volatile solids (VS). Semi-continuous digestion at organic loading rates of 2.00, 2.75 and 3.50 g VS l^{-1} day^{-1} gave volumetric biogas productions of ~0.66, ~0.83 and ~0.99 l l^{-1} day^{-1}, respectively. Specific methane yield ranged from 0.13-0.14 l CH_4 g^{-1} VS_{added} with biogas methane content ~62%. Overall the digestion process was stable, but only ~30% VS destruction was achieved indicating low biodegradability, due to the short retention times and the recalcitrant nature of this type of biomass.

Keywords

Micro-algae; *Scenedesmus* spp.; raceway; flue gas; anaerobic digestion; biodegradability

1 Introduction

The finite nature of fossil fuel supplies combined with an increasing global demand for energy has led to substantial interest in developing renewable biologically-produced carbon neutral fuels sources [1]. In the search for renewable biofuels, micro-algae have been considered an attractive source of bioenergy for several reasons: they have a rapid growth rate and high biomass production, do not compete with terrestrial crops for arable land, have minimal needs for pesticides or herbicides, and offer the potential for cultivation using wastewater nutrients [2-5].

The major disadvantages of micro-algal biomass are the cost and energy requirements for growth and harvest, which currently make it uncompetitive with other biomass types [6]. Reduced costs of production are thus essential to the success of this technology. Open raceways, suggested more than fifty years ago by Oswald and Golueke [7, 8], are still the only realistic large-scale engineered method available for micro-algal biomass production for biofuels: this is because of the lower initial investment compared to alternative systems, due to lower construction costs, and the low operating costs [9-11]. The biomass productivity reported for this type of reactor is, however, lower than required to achieve commercial bioenergy production [6].

Micro-algae can be utilised to produce different types of biofuels, and amongst these bio-methane appears increasingly attractive. The main reason is that most of the macromolecules (i.e. protein, lipid, carbohydrate) in algal biomass can theoretically be converted to biogas through the anaerobic digestion process [12]. Algal residues after biodiesel production can also be utilised to produce biogas [13].

Although the idea of using micro-algae as feedstock for biogas production is not new [14], this research area has seen an upsurge of interest in the past few years. Literature on anaerobic digestion of micro-algae is still limited, however, in part due to the limited availability of sufficiently large quantities of feedstock material. This paper reports on a study of the semi-continuous digestion of a mixed micro-algal culture grown in an open raceway.

2 Materials and methods

2.1 Algal cultivation

2.1.1 Bioreactors

The algae were grown in experimental reactors at the Estación Experimental Las Palmerillas of Fundación CAJAMAR in El Ejido (Almería), Spain. The inoculum for the raceway was grown in a closed tubular fence-type photobioreactor constructed of 0.09 m diameter acrylic tubes with a total length of 400 m and stacked vertically to a height of 2.2 m. The unobstructed space on either side of the reactor was 1.4 m, and the reactor was located in a greenhouse with a polythene covering to diffuse direct sunlight (Figure 1a). The volume to surface ratio was 75 l m^{-2}, and oxygen was stripped from the culture using coarse air bubbles in a vertical tower 3.5 m high and 0.4 m diameter, with a heat exchanger inside to control the reactor temperature. Pure CO_2 was injected directly into the reactor to meet the growth needs of the algae, with the addition based on pH control.

The open channel raceway reactor (Figure 1b) used for long-term cultivation consisted of two

48 m channels, each 1 m wide and connected by 180º bends at both ends to give a total surface area of 100 m^2. The raceway was constructed out of 3 mm white fibreglass and the culture was circulated and mixed by a marine plywood paddlewheel with a diameter of 1.2 m driven by a 0.37 kW geared electric motor (Ebarba and WEG Iberica, Barcelona, Spain). In order to supply CO_2 and enhance the mass transfer capacity of the system, flue gas from a diesel boiler was bubbled on demand through three membrane plate diffusers positioned at the bottom of a sump (0.65 m long × 0.90 m wide × 1 m deep) located 1.5 m downstream of the paddlewheel. The raceway was equipped with pH-T and dissolved oxygen (DO) probes (5083T and 5120, Crison, Barcelona, Spain) calibrated and maintained in accordance with the manufacturers' instructions and connected to transmitters (MM44, Crison, Barcelona, Spain). Information was collected by a data acquisition card and software (LabJack U12 and Daqfactory Azeotech Arizona USA, respectively). Solar radiation was measured on-site by means of a light sensor (Li-Cor, Pyranometer, PY 61654, USA). A full hydraulic characterisation of the raceway and studies of O_2 and CO_2 mass transfer are reported elsewhere [15-17].

The total suspended solids content (TSS) of the raceway culture was measured by vacuum filtration of a 250 ml sample through a pre-weighed glass fibre filter (MN 85/90, Macherey-Nagel, Spain) and subsequent drying of the filter at 80 ^oC for 24 hours. Total solids (TS) content of centrifuged algal material was measured according to Standard Method 2540 G [18].

2.1.2 Culture medium

The culture medium used in both tubular photobioreactor and raceway reactor was prepared

in fresh water using commercial agricultural fertilisers to give ionic concentrations additional to those naturally present in the fresh water source as follows (mmol l^{-1}): NO^-_3 9.49, Na^+ 8.20, NH^+_4 0.59, Cl^- 11.10, $H_2PO^-_4$ 1.00, Fe^{3+} 2.00, K^+ 1.60, Mn^{2+} 0.84, Ca^{2+} 5.00, Zn^{2+} 0.56.

2.1.3 Seed culture, inoculation and raceway operation

A seed culture of *Scenedesmus* spp. was grown in the tubular photobioreactor in batch mode to a concentration of 2 g TSS l^{-1}. 2000 l of the seed culture was inoculated into the raceway, which had previously been filled with 8 m^3 of fresh culture medium. The final operating depth was 0.1 m, giving a total culture volume of 10 m^3, and the paddlewheel rotational speed was set to give a liquid velocity in the channel of 0.27 m s^{-1}. The raceway was initially operated in batch mode for 11 days, until a biomass concentration of 0.9 g TSS l^{-1} was achieved. The culture was then switched from batch to semi-continuous mode, in which part of the culture was removed on each weekday by gravity drainage and replaced by fresh culture medium to restore the raceway to its 0.1 m working depth. The difference between the volume harvested and the added medium was taken as the loss due to evaporation. The pH in the raceway was controlled between set points of 7.8 and 8.1 by bubbling exhaust gases (10 % CO_2, 18.1 ppm CO, 38.3 ppm NO_x, and 0.0 ppm SO_x) from a diesel oil-fired boiler (Tradesa, MOD SF 20, RA-GTI, TRADE, Italy) at 6 m^3 $hour^{-1}$ (flow meter FR4500, Key Instruments, USA). Before injection into the raceway, the flue gas was cooled to air temperature and stored in a 1.5 m^3 reservoir at a constant pressure of 2 bar (BUSCH, Mink MM 1104 BP, model C-1000 compressor, Barcelona). Flue gas was only injected during daylight hours (solar radiation >50 W m^{-2}) as no CO_2 demand was exerted by the algae during the night.

Biomass concentration was measured daily, and average daily production (g TSS m^{-2} day^{-1}) was calculated based on this concentration and the harvested volume, taken over weekly periods. The culture was maintained from December until May to provide information on algal growth during winter and spring periods. The micro-algal culture used in the AD experiment was harvested in batches from the raceway between 19 January - 3 February by continuous centrifugation (Alfa Laval Clara 15, LAPX 404 SGP-31G/TGP-61G). The batches of algal paste had TS contents ranging from 7-12 %, and were frozen immediately on harvesting then shipped under refrigeration from Almeria (Spain) to Southampton (United Kingdom). On arrival the paste was thawed and the batches mixed to give a homogeneous mass that was distributed into small containers and re-frozen immediately, then thawed as required before use.

2.2 Anaerobic digestion

Six digesters each with a working volume of 1.5 litres were operated for a period of 136 days. These 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. Temperature was maintained at 35±0.5 ^{o}C by water circulating through an external heating coil. During semi-continuous operation digestate was removed through an outlet port in the base plate, and feed added via the top plate. Gas production was measured using tipping-bucket gas counters with continuous datalogging. Calibration of gas counters was checked weekly by collecting the gas from the outlet of the gas counter in a Tedlar bag (SKC Ltd, Blandford Forum, UK); the volume was then measured accurately in a water

displacement weight-type gasometer [19]. All gas volumes reported are corrected to standard temperature and pressure of 0 ^{o}C, 101.325 kPa.

The inoculum was taken from laboratory-scale digesters that had been operating with a feedstock of freeze-dried *Scenedesmus spp.*; its characteristics were: pH 7.5, TS content 6.17%, VS content 4.71%, total ammonia nitrogen (TAN) 2070 mg N l^{-1}, and total alkalinity 13.3 g $CaCO_3$ l^{-1}. The digestion feedstock composition is shown in Table 1. On day 1 of the digestion experiment, three pairs of digesters were fed with the thawed micro-algal mixture at an organic loading rate (OLR) of 2 g VS l^{-1} day^{-1}. After 75 days, the OLR of one pair was kept unchanged, and the OLRs of the other two pairs were increased to 2.75 and 3.5 g VS l^{-1} day^{-1} respectively. All six digesters were then operated for at least three hydraulic retention times at these OLR. System performance was monitored by weekly measurement of digestate pH, TS and VS, TAN, total alkalinity, and volatile fatty acids (VFA).

pH was determined using a Mettler Toledo FE20/EL20 pH meter with a combination glass electrode, calibrated in buffers at pH 4, 7 and 9.2 (Fisher Scientific, UK). TS and VS were measured according to Standard Method 2540 G [18]. Alkalinity was measured by titration using 0.25 N H_2SO_4 to endpoints of 5.7, 4.3 and 4.0 [20]. Total Kjeldahl Nitrogen (TKN) and ammonia were determined using a Kjeltech block digestion and steam distillation unit according to the manufacturer's instructions (Foss Ltd, UK). VFA concentrations were quantified in a Shimazdu 2010 gas chromatograph (Shimadzu, UK). Calorific values (CV) were determined using a bomb calorimeter (CAL2k-ECO, South Africa). Biogas composition was measured three times a week using a Varian CP-3400 gas chromatograph (Varian, UK). Elemental composition (C, H, N) content was determined using a FlashEA 1112 Elemental

Analyzer, (Thermo Finnigan, Italy) based on the manufacturer's instructions using methionine, L-cystine and sulphanilamide as standards.

Theoretical CV was calculated using the Dulong equation according to the method in Combustion File 24 [21]. Theoretical biogas composition was calculated based on the Buswell equation [22]. The calorific value of methane was taken as 39.84 MJ m^{-3} at STP.

3 Results and discussion

3.1 Raceway cultivation

The method of carbonation of the culture medium in the raceway was found to be adequate to maintain high micro-algal productivity in the raceway. The CO_2-rich flue gas injected at the bottom of the sump gave turbulence in this zone and allowed almost complete dissolution of the CO_2 into the aqueous phase with only around 10% of CO_2 entering the sump leaving in the exhaust gas, which had a CO_2 content of < 0.5 % (Figure 3a) (de Godos et al., 2014). By supplying flue gas 'on demand' it was possible to control the raceway pH in the desired range of 7.8-8.1 even during peak carbon demand in the mid-day period when solar radiation was at its highest (Figure 3b).

During the batch start-up the average water temperature was 13.6 ^{o}C, with a maximum daytime temperature of 18.4 ^{o}C, and was therefore lower than the optimal growth temperatures reported for the most common species [23, 24]. The temperature increased in the transition from winter to spring conditions and this had a pronounced effect on algal growth and on the evaporation rate. In December the average air temperature was around 15

205 ^{o}C with an evaporation rate of around 200 l day^{-1}; while by the end of the experimental period
206 in May the temperature had risen to 25 ^{o}C with an evaporation rate of 700 l day^{-1} (Figure 4a).
207 The evaporation rate was therefore between 2 % and 7 % of total reactor volume and a
208 significant fraction of the total daily volume removed for harvesting: this loss was taken into
209 account when calculating the biomass yield.
210
211 Figure 4b shows the total suspended solids (TSS) concentration in the raceway culture, which
212 remained around 0.75 g l^{-1} for the first 6 weeks of operation when the average water
213 temperature was fairly constant. Once the temperature started to rise there was a
214 corresponding increase in culture density, reaching ~1.4 g TSS l^{-1} by early April. Further
215 increases in temperature did not increase culture density, which in fact declined slightly as
216 temperatures rose further in May. Figure 4b also shows the solar efficiency, which was
217 between 0.6 and 0.7 g E^{-1}, in the range for cultures that are photosynthetically active [25].
218 Small variances in this parameter could be explained by changes in the average irradiance
219 due mainly to the variation in weather conditions from winter to spring; some variations,
220 however, may also be as a result of changes in the cell size and concentration or pigment
221 content [26]. The higher temperatures seen at the end of the study period resulted in a
222 reduction in solar efficiency and TSS concentration as a result of photoinhibition in part of
223 the culture. Turbidity and absorbance at 680 nm (Figure 4c) were related to the culture TSS
224 concentration (R^2 = 0.613, n = 111 and R^2 = 0.775, n = 108, respectively; $p < 0.001$ in both
225 cases) showing that, as expected, absorption of light in the culture increased with the culture
226 density.
227
228 To determine the optimum dilution rate a trial-and-error approach was adopted, hence the
229 dilution rate was modified weekly. Figure 4d shows the dilution rate expressed as the amount

of fresh medium added as a percentage of the total reactor volume; and the productivity as the mass of algae produced per unit area per day. The results indicate that 43% of the variation in productivity can be attributed to the dilution rate ($R^2 = 0.43$, $n = 23$, $p < 0.001$), and also show how productivity increased during the spring period from late February through to the end of May, reflecting the higher temperatures and longer days with more solar radiation. When averaged over weekly periods, estimated productivities of 10-15 g TSS m^{-2} day^{-1} were achieved in winter (December - February) and of 20-25 g TSS m^{-2} day^{-1} during spring (April-May) (Figure 4d). Maximum biomass productivity was comparable to that reported in the literature, with values of 19 g TSS m^{-2} day^{-1} obtained by Ketheesan and Nirmalakhandan [10] in an airlift-driven raceway, and by Vonshak and Guy [27] in outdoor cultivation with natural sunlight and a culture depth of 10 cm. Similar results were obtained by Boussiba et al. [28] with CO_2 addition and pH in the range 7.0-7.5. Yoo et al. [29] achieved 21.8 g m^{-2} day^{-1} for *Scenedesmus* spp. cultivated for CO_2 mitigation. These productivities have been suggested as indicating a good potential for fuel production [30]. The highest productivity was obtained in Spring at a dilution rate of 20-25 %. The increment in productivity in Spring is due to the increasing intensity of irradiance and the duration of daylight periods. The raceway design may also have affected productivity during the winter period as the angle of inclination of the sun during these months is low, and the narrow width of the raceway resulted in more than half of the culture surface being in the shadow of the freeboard of the channel at mid-day; this area was reduced as the sun's angle of inclination increased throughout the experimental period.

Figure 5 shows continuous monitoring data for selected periods of operation corresponding to batch start-up, first week of semi-continuous operation, period of maximum productivity and the onset of reduced productivity in early summer conditions (Figure 5a, b, c and d

255 respectively). During the batch start-up period (Figure 5a) peak daily solar radiation was
256 between 300-550 W m^{-2} and both water temperature and DO concentrations showed diurnal
257 fluctuation, with day and night temperatures varying by up to 10 ^{o}C. A similar pattern was
258 reflected in the first week of continuous operation (Figure 5b), and during December DO
259 concentrations never rose above 200% saturation. During the entire study period pH
260 remained within narrow limits, with only small increases during the dark period which were
261 more pronounced in winter than spring. The control of raceway pH by the addition of flue gas
262 CO_2 addition thus proved very effective, and the single carbonisation sump was adequate for
263 its purpose. In the maximum productivity period (Figure 5c), peak solar radiation was
264 between 800-900 W m^{-2}, and water temperature peaked between 25-30 ^{o}C falling to around
265 15 ^{o}C at night. DO concentrations during the day could reach 350 % saturation. DO
266 concentrations as high as 45 mg l^{-1} have been reported in raceways [31, 32], causing
267 inhibition of photosynthesis and growth. By May water temperature had increased to over 30
268 ^{o}C in the middle of the day. This was associated with reduced productivity and lower peak
269 DO concentrations of 200-250 % saturation (Figure 5d). In all cases temperature and DO
270 peaks lagged behind peak solar radiation.
271
272 The accumulation of photosynthetic oxygen in raceways during operation at high irradiancies
273 was one of the main potential problems observed, as this can lead to a loss of productivity
274 due to inhibition. Accumulation was minimised by operating the gas exchange sump with
275 flue gas rather than pure CO_2 as this resulted in a greater gas volume passing through the
276 sump, which in turn stripped more oxygen out of the culture medium [16]. The sump proved
277 to be one of the main zones in the raceway where oxygen could effectively be removed. The
278 other major problem was the increase in temperature between spring and summer. As air
279 temperatures increased in late May the water temperature in the raceways rose above 35 ^{o}C

resulting in very marked effects on productivity as a result of thermal inhibition. This was apparent as the dissolved oxygen concentration in the raceway was reduced during the mid-day period along with a reduced demand for CO_2, both strongly indicating inhibition or even death of the culture. As the summer progressed it became increasingly difficult to maintain the raceways in a stable condition.

3.2 Anaerobic digestion trials

pH. The pH in all six digesters fluctuated between 7.07 and 7.52 (Figure 6a). From day 1 to day 75 when all digesters were operating under the same conditions, the daily pH values were almost identical: small day-to-day differences are therefore believed to reflect slightly different intervals between sampling and pH measurement. From day 75 onward, there was some variation between digesters at different OLRs, with the lower-loaded A1 and A2 operating at a slightly higher pH than A3 - A6. Nevertheless, pH remained in the optimum range for the growth of methanogens in all cases.

TS and VS. As seen in Figure 6b, in the first 28 days the digestate TS increased gradually from 6% and stabilised at around 9.5%. This increase reflected the high proportion of inorganic matter (VS/TS = 0.40), which may have been in part due to a combination of wind-blown dust entering the raceway and high salinity due to surface evaporation. The digestate VS content decreased gradually from an initial value of 4.7% and stabilised at 3% by the end of experiment. Based on the feedstock VS concentration of ~4.3 %, the apparent VS destruction was low, with only ~30% of VS degraded.

304 *Alkalinity, ammonia and VFA.* Similar to the trend in digestate TS, in the first 28 days there
305 was an increase in total alkalinity, starting at 13 g $CaCO_3$ l^{-1} and then stabilising at around 35
306 g $CaCO_3$ l^{-1} until the end of experiment. The TAN concentration in all digesters rose slightly
307 to around 2.4 g N l^{-1} in the first 14 days of operation then declined during the experimental
308 run and stabilised at around 1.8 g N l^{-1} in all cases. The digestate TKN concentration at the
309 end of the run was around 4.1 g N l^{-1} indicating a breakdown rate for organic nitrogen-
310 containing compounds of around 44%, slightly above the VS destruction rate but still quite
311 low. Total VFA concentrations in the digestate were initially between 100-250 mg l^{-1} and
312 after day 75 remained consistently below 120 mg l^{-1}. Despite the low VFA concentration, the
313 IA/PA ratio rose during the first 28 days then fluctuated between 1-4, a range often
314 considered to indicate potential instability [20]. The reason for this high value for the IA/PA
315 ratio under apparently stable conditions is unknown.
316
317 *Biogas production and methane yield.* Volumetric biogas production (VBP) was low, with
318 average values over the last 30 days of the experiment of 0.66, 0.87 and 1.06 l l^{-1} day^{-1} for
319 OLR of 2.0, 2.75 and 3.5 g VS l^{-1} day^{-1} respectively (Figure 6c). The specific methane
320 production (SMP) was almost unaffected by the applied OLR and in the same period
321 averaged around 0.139, 0.133 and 0.131 l CH_4 g^{-1} VS_{added} at OLR 2.0, 2.75 and 3.5 g VS l^{-1}
322 day^{-1}, respectively (Figure 6d). The average biogas methane content for all digesters in this
323 period was around 62%, slightly above the value of 59% predicted by the Buswell equation
324 [22]. This SMP was considerably below the specific methane yield of 0.220 l CH_4 g^{-1} VS_{added}
325 found in a 90-day batch test on the same feedstock (results not shown), but relatively similar
326 to the yield of around 0.15 l CH_4 g^{-1} VS_{added} in the first 1.6 days of the batch test,
327 corresponding to a change of slope in the cumulative gas production curve. This indicates
328 that only the readily degradable proportion of the biomass is being digested effectively in the

329 semi-continuous trials.

330

331 The estimated calorific value based on the elemental composition was 21.95 MJ kg^{-1} VS, in
332 reasonably good agreement with the measured value of 21.36 MJ kg^{-1} VS. The energy
333 recovered as methane therefore corresponded to ~25% of the measured calorific value in the
334 semi-continuous trials at OLR 2.0, 2.75 and 3.5 g VS l^{-1} day^{-1}, respectively. This compares to
335 ~41% recovery in the BMP test, again confirming the poor degradability of the material. At
336 the time of harvesting the average algal biomass productivity was 7.3 g TSS m^{-2} day^{-1},
337 equating to an energy output as raw methane of 161 MJ ha^{-1} day^{-1} at the maximum SMP in
338 semi-continuous digestion of 0.139 l CH_4 g^{-1} VS_{added}. Using an average productivity of 10.3
339 g TSS m^{-2} day^{-1} over the winter to spring period and the BMP value for methane yield, this
340 increases to a 362 MJ ha^{-1} day^{-1} or 66 GJ ha^{-1} for the half-year period.

341

342 The SMP in this study was similar to that found by Yen and Brune [33], but lower than
343 reported by some others [7, 34, 35]. When a mixed algal sludge consisting mainly of
344 *Scenedesmus* and *Chlorella spp.* was digested at 10 days HRT and OLR of 2.0, 4.0 and 6.0 g
345 VS l^{-1} day^{-1}, Yen and Brune [33] reported methane yields of 0.09 - 0.14 l CH_4 g^{-1} VS_{added}.
346 Oswald and Golueke [7] reported a methane yield of 0.26 l CH_4 g^{-1} VS_{added} from digestion of
347 mixed algal cultures at 30 days HRT. Ras et al. [35] obtained a methane yield of 0.15 l CH_4 g^{-1}
348 VS_{added} for 16 days HRT and 0.24 l CH_4 g^{-1} VS_{added} from digestion of *Chlorella vulgaris*
349 grown in a laboratory photobioreactor at 28 days HRT. There is considerable debate on the
350 digestibility of micro-algal biomass, with a wide range of reported values [36]. The value
351 reported here for a mixed algal culture primarily consisting of *Scenedesmus* spp. is lower than
352 the BMP value of 0.261 l CH_4 g^{-1} VS_{added} for a laboratory culture of *Scenedesmus* spp., and
353 lower than that of 0.331 l CH_4 g^{-1} VS_{added} for laboratory-grown *C. vulgaris* run at the same

time under the same test conditions [37]. The lower values found in the semi-continuous digestion compared to the BMP suggest that longer retention times could give slightly higher methane production, but pre-treatment of this type of substrate may also be required to improve methane yield. For example, thermal treatment of micro-algae collected from a high-rate sewage stabilisation pond at 100 ^{o}C increased methane fermentation up to 33 % [38]. Similarly, a 2.2-fold increase in methane production in comparison to untreated substrate was achieved when *Scenedesmus* biomass grown in a laboratory bioreactor was pretreated at 90 ^{o}C [39].

4 Conclusions

The culture conditions in an open pond photobioreactor were strongly related to the climatic conditions. Therefore, the correct choice of location for this kind of reactors is essential if satisfactory productivity is to be achieved across the seasons. The biggest problem encountered in operation of the raceways was the daytime accumulation of photosynthetic oxygen in the culture: even during the period of relatively low light intensity and temperature DO concentrations of 200% saturation occurred. At higher light intensities, DO concentrations of 350% saturation could have caused some reduction in productivity due to inhibition of photosynthesis, and this problem was compounded by water temperatures of up to 35 ^{o}C by late May.

Anaerobic digestion of the harvested raceway culture was possible at a VS content of 4.3% and HRT as low as 12.4 days, as indicated by stable volumetric and specific methane production and low VFA concentrations. The methane yield and biodegradability of this mixture was low, however, with a VS destruction of only about 30%. Possible reasons could

be the recalcitrant nature of this type of biomass, and the short retention times used due to the high moisture content. It is suggested that either longer retention times or pre-treatment techniques may be required in order to improve biodegradability and methane yield, if this mixture is to be grown and harvested at large scale as a feedstock for biomethane production.

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493 **List of figures and tables**

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495

496 **Figure 1.** Algal cultivation facilities. (a) Tubular fence-type photobioreactor used to grow the
497 inoculum of Scenedesmus sp. (F.G. Acién Fernández et al., 2013) [40]. (b) Raceway reactor
498 in which the main algal culture was grown.

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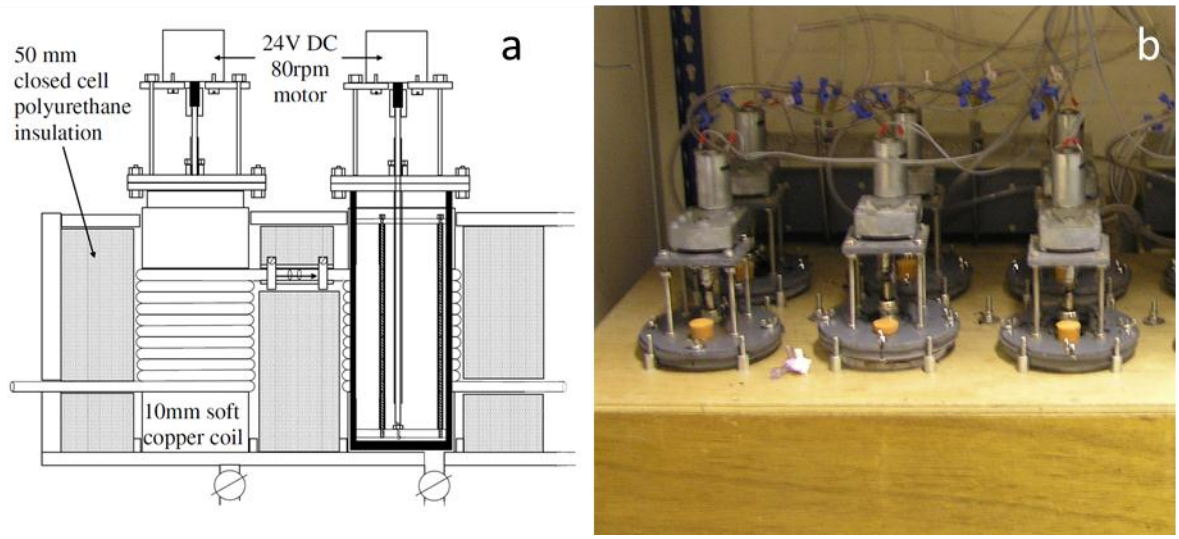

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501 **Figure 2.** Anaerobic digesters: (a) schematic cross-section; (b) photograph

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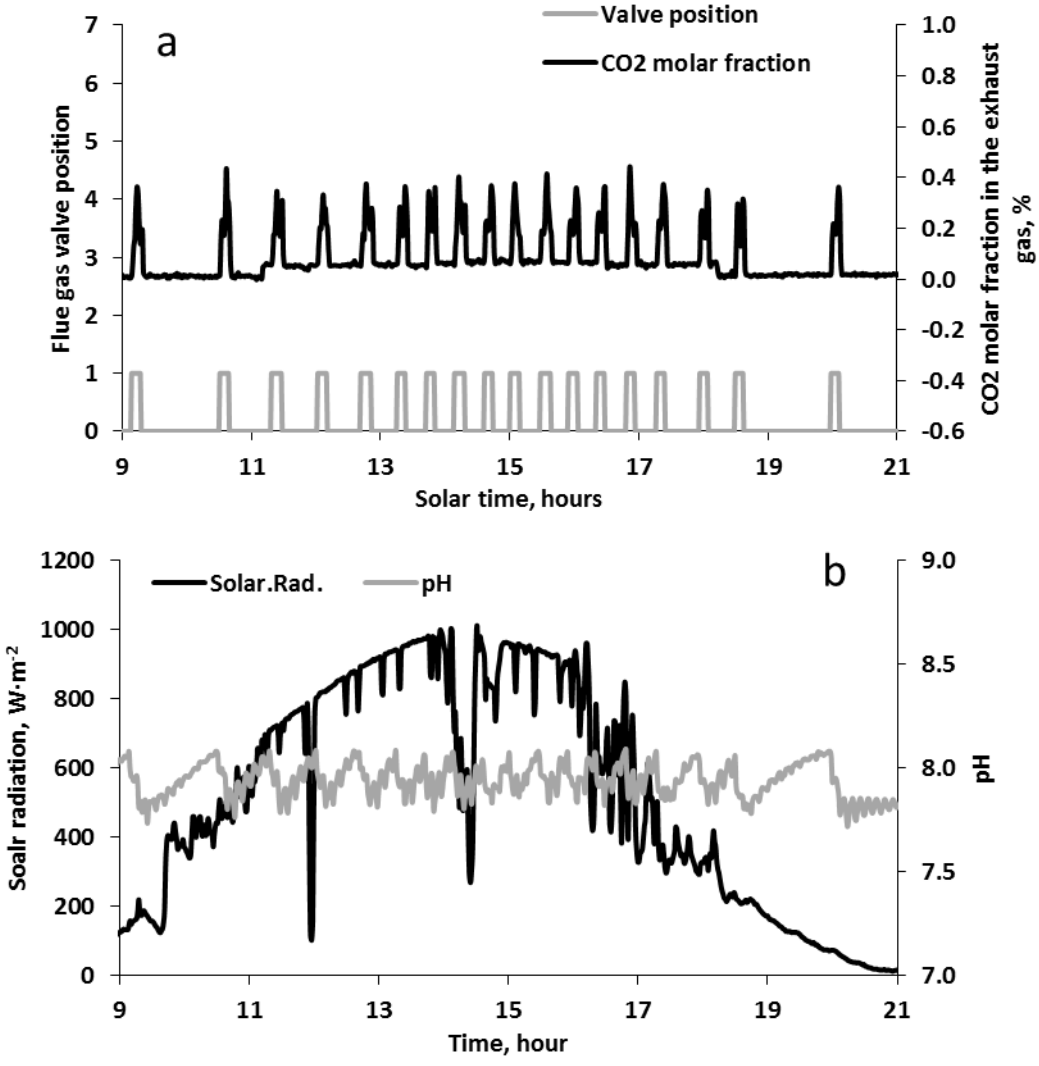

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Figure 3. CO_2 demand on the sump during a daylight period: (a) pH control hysteresis in the range 7.8-8.1 as maintained by flue gas injection (0 is flue gas valve closed and 1 is flue gas valve opened). (b) Timing of flue gas injections and % of CO_2 in gas exhausted to atmosphere.

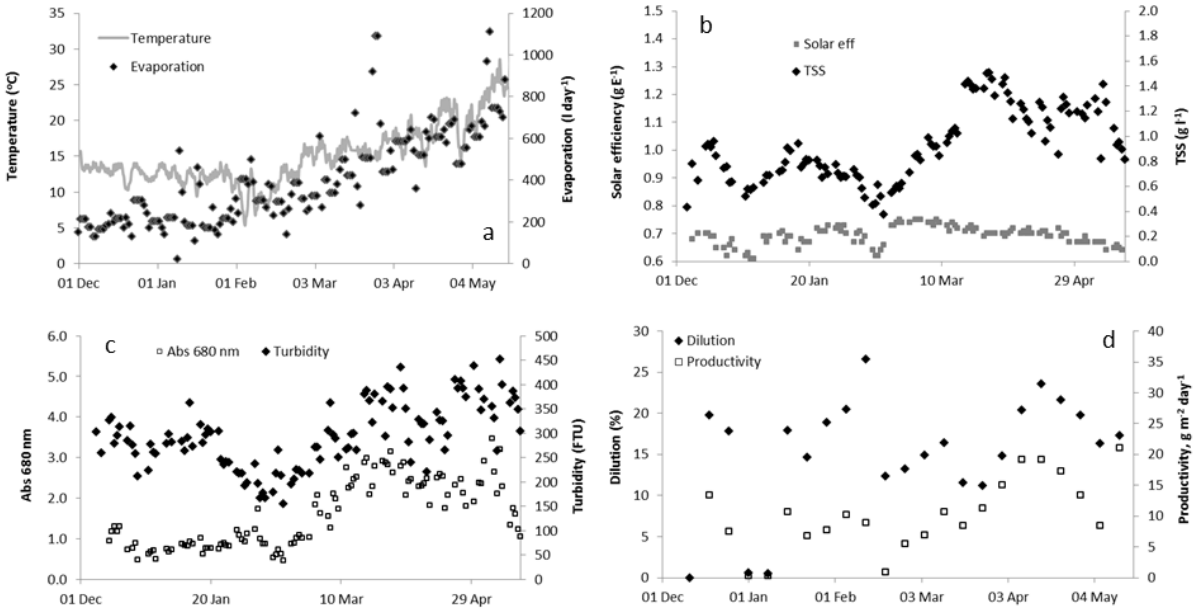

Figure 4. Parameters studied during the culture period. (a) Online culture temperature registered and daily evaporation in the raceway. (b) Daily analysis of total suspended solids and quantum yield. (c) Daily analysis of absorbance at 680 nm and turbidity. (d) Weekly average dilution and productivity in raceway algal culture.

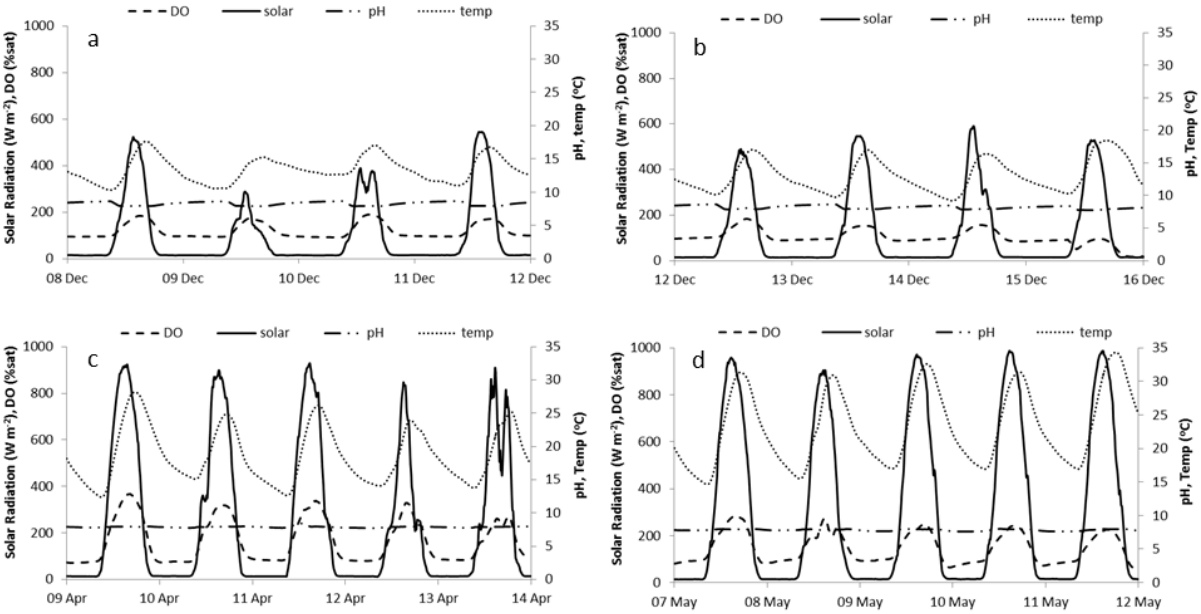

Figure 5. Selected results from continuous monitoring of raceway culture during the experimental period: (a) Batch start-up, (b) First week semi-continuous, (c) Maximum productivity, (d) Reduced productivity.

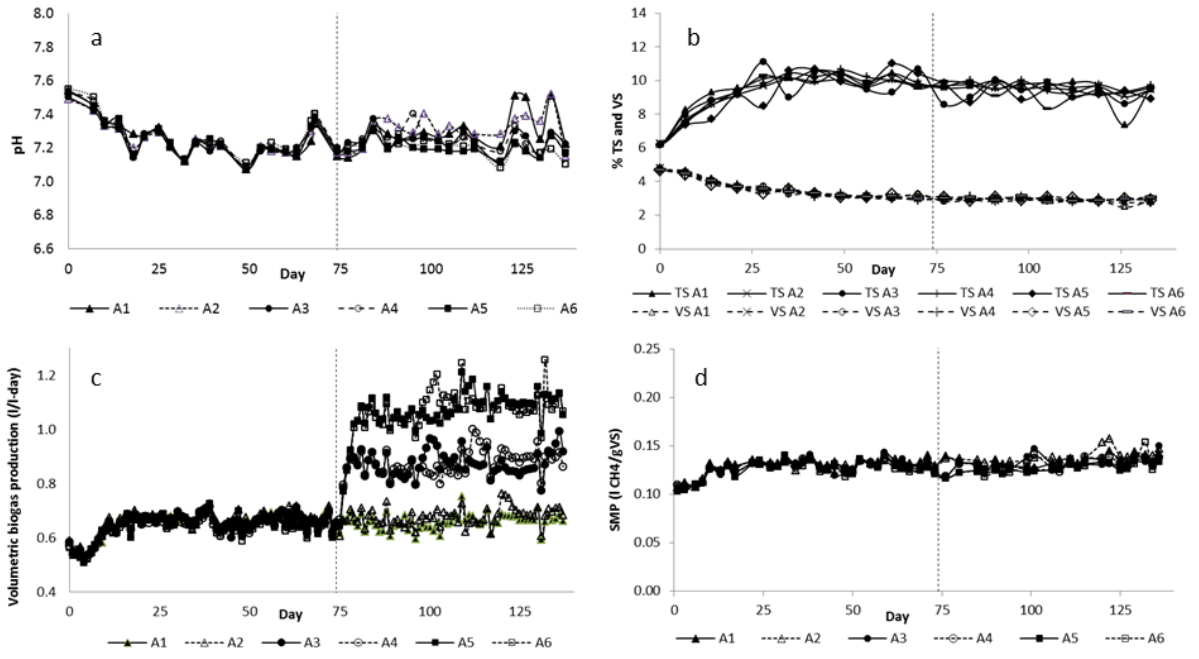

Figure 6. Results from anaerobic digestion trial with raceway culture of *Scendesmus* sp. (a) Digestate pH. (b) TS and VS of digestate. (c) Volumetric biogas production. (d) Specific

methane production. Vertical dotted line indicates change in organic loading rates on day 75.

Table 1. Characteristics of the algal mixture after centrifugation

Parameter	Unit	Algal mixture
TS	g kg^{-1} WW	108.5[b]
VS	g kg^{-1} WW	43.31[b]
TKN	g kg^{-1} WW	3.77[a]
C	% (on a VS basis)	45.6
H	% (on a VS basis)	9.0
O	% (on a VS basis)	35.7
N	% (on a VS basis)	8.7
S	% (on a VS basis)	1.0
C/N	-	5.24/1
Calorific Value	MJ kg^{-1} VS	21.36

[a] measured as dry matter

[b] measured as wet matter