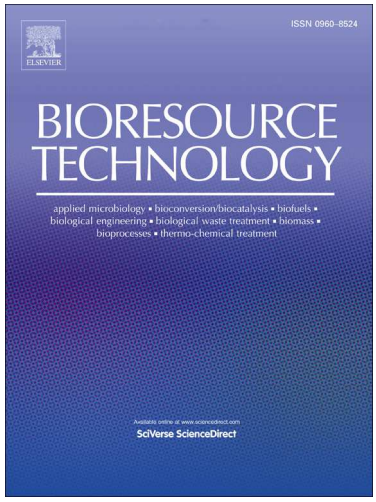
Acidogenic fermentation of Scenedesmus sp.-AMDD: comparison of volatile fatty acids yields between mesophilic and thermophilic conditions

Gruhn, Marvin; Frigon, Jean-Claude; Guiot, Serge R.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1016/j.biortech.2015.10.087
Bioresource Technology, 2015-10



Acidogenic fermentation of *Scenedesmus sp.*-AMDD: comparison of volatile fatty acids yields between mesophilic and thermophilic conditions

Marvin Gruhn, Jean-Claude Frigon, Serge R. Guiot

PII: S0960-8524(15)01490-X
DOI: http://dx.doi.org/10.1016/j.biortech.2015.10.087
Reference: BITE 15712

To appear in: *Bioresource Technology*

Received Date: 5 August 2015
Revised Date: 22 October 2015
Accepted Date: 24 October 2015

Please cite this article as: Gruhn, M., Frigon, J-C., Guiot, S.R., Acidogenic fermentation of *Scenedesmus sp.*-AMDD: comparison of volatile fatty acids yields between mesophilic and thermophilic conditions, *Bioresource Technology* (2015), doi: http://dx.doi.org/10.1016/j.biortech.2015.10.087

Acidogenic fermentation of *Scenedesmus sp*.-AMDD: comparison of volatile fatty acids yields between mesophilic and thermophilic conditions

Marvin Gruhn[a], Jean-Claude Frigon[b] and Serge R. Guiot[b,*]

[a] Department of Waste Management, Brandenburg University of Technology, 03013 Cottbus, Germany

[b] Energy, Mining and Environment, National Research Council Canada. 6100 Royalmount, Montreal, Canada, H4P 2R2

[*] corresponding author; Tel: 514-496-6181; Fax: 514-496-6265; e-mail address: serge.guiot@canada.ca

Abstract

This study compared the acidogenic fermentation of *Scenedesmus* sp.-AMDD at laboratory-scale, under mesophilic (35 °C) and thermophilic conditions (55 °C). Preliminary batch tests were performed to evaluate best conditions for volatile fatty acid (VFA) production from microalgal biomass, with respect to the inoculum, pH and nutrients. The use of bovine manure as inoculum, the operating pH of 4.5 and the addition of a nutrient mix, resulted in a high VFA production of up to 222 mg g^{-1} total volatile solid (TVS), with a butyrate share of 27%. Both digesters displayed similar hydrolytic activity with 0.38±0.02 and 0.42±0.03 g soluble chemical oxygen demand (COD) g^{-1} TVS for the digesters operated at 35 and 55 °C, respectively. Mesophilic conditions were more favourable for VFA production, which reached 171±5, compared to 88±12 mg soluble COD g^{-1} TVS added under thermophilic conditions (94% more). It was shown that in both digesters, butyrate was the predominant VFA.

Keywords:

anaerobic digestion; carboxylic acid; fatty acids; microalgae; *Scenedesmus*

Abbreviations

AD	anaerobic digestion
BMP	biochemical methane potential
COD	chemical oxygen demand
COD_{eq}	COD-equivalent
CSTR	completely stirred tank reactor
FID	flame ionization detector
GHG	greenhouse gas
HRT	hydraulic retention time
LCFA	long chain fatty acid
OLR	organic loading rate
R35	CSTR under mesophilic conditions (35 ºC)
R55	CSTR under thermophilic conditions (55 ºC)
sCOD	soluble chemical O_2 demand
TKN	total Kjeldahl nitrogen
TVS	total volatile solids
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acids
VSS	volatile suspended solids
WAS	waste activated sludge
WWTP	wastewater treatment plant

1. Introduction

Most fuels and precursor chemicals used in the chemical industry are largely based on crude oil. Considering the unsustainability of crude oil as a feedstock, considerable research efforts are underway to replace crude oil with more renewable and sustainable sources by developing biomass-based processing platforms to produce valuable bio-based chemicals and energy carriers, including versatile building blocks. An

additional platform, the carboxylate platform has been introduced recently (Agler et al., 2011; Chang et al., 2010; Holtzapple and Granda, 2009). Carboxylates refer to a mixed spectrum of dissolved short-chain volatile fatty acids (VFA), mostly acetic, propionic and butyric acids, derived from anaerobic digestion (AD). In conventional AD, VFAs are subsequently utilized by methanogens to produce methane in one or two-stage systems. Instead, AD can be limited to the acidogenic fermentation and the VFA mix separated from the culture broth can serve as a resource for the production of valuable products such as ketones, esters or alcohols (Agler et al., 2011; Bastidas-Oyanedel et al., 2015). Butyric acid is of particular importance as it has an increasing commercial value and can be transformed into butanol, a promising future fuel (Dwidar et al., 2012). A variety of large scale AD technologies are commercially available. The anaerobic acidogenic fermentation would use similar hardware to that used currently in industrial biomethanization, so bioreactor technologies are readily available for acidogenic fermentation.

Macroalgae have been used for centuries by mankind to produce food products like *nori*, a dried red alga (*Rhodophyta*) that is widely used in Japanese cuisine, or *agar-agar* (Pulz and Gross, 2004). More recently, microalgae has attracted particular attention. as a universal and sustainable "factory" for the production of precursor chemicals and thereby substitute oil-derived products. It was found that certain species of microalgae are capable of accumulating valuable substances like lipids (e.g. polyunsaturated lipids such as omega-3) and proteins. This enables several possible applications for food (Plaza et al., 2008), animal feed (Pulz and Gross, 2004), pharmaceutical (Borowitzka, 1995) and cosmetic industry (Stolz and Oberbayer, 2005). Microalgae are also a suitable feedstock for the production of energy carriers, such as biodiesel (Schenk et al., 2008)), bioethanol (Harun et al., 2010), biohydrogen (Hankamer et al., 2007) and methane (Frear et al., 2013; Golueke et al., 1957; Montingelli et al., 2015; Sialve et al., 2009). Even after maximal extraction of high value chemicals or fuels, a large portion of the algal production will still be left over. The greenhouse gas (GHG) abatement resulting from the CO_2 capture by the algal culture could be significantly compromised by GHG emissions from the algal leftover if improperly managed. This is precisely where biogas or

carboxylate platforms could be instrumental, for yet generating value from algal residues and expanding the range of products from the whole algal feedstock.

The fermentative VFA production has so far been examined for different complex substrates such as manure (Thanakoses et al., 2003), food waste (Hong and Haiyun, 2010), plant residues (Kim et al., 2013), municipal solid waste (Aiello-Mazzarri et al., 2006). However, the production of VFA-rich effluents from microalgae has received little attention (Li et al., 2013). This work focused on mixed VFA production from the microalga strain *Scenedesmus* sp.- AMDD, with special emphasis on distinctions in hydrolysis and VFA yields between mesophilic and thermophilic conditions. This work extends existing research on microalgal utilization for VFA production and attempts to intrigue more work in this domain.

2. Materials and methods

2.1. Algae feedstock

The microalga strain *Scenedesmus* sp.-AMDD was used in this study. The algae was provided by the Aquatic and Crop Resources Development division of National Research Council of Canada (NRC) located in Halifax (Nova Scotia, Canada). In preliminary tests conducted in our lab, *Scenedesmus* sp.-AMDD had shown good methane yields and was chosen as a model strain for future examinations (Frigon et al., 2013). No pre-treatments were applied and the algal biomass was kept at -20 °C before feeding. Based on the dry weight, the composition of *Scenedesmus* sp.-AMDD was as follows: 45 % carbohydrates, 44 % proteins and 4 % lipids. To maintain the desired organic loading rate (OLR), the algae paste was diluted using the same defined media as in the VFA potential assays (section 2.2). The total volatile solids (TVS) of the fed algae substrate was determined on a daily base to quantify the OLR as precisely as possible and adjust to the target value. Due to an observed hydrolysis of the algae solution over time, even when stored at 4 °C, the solution was prepared immediately prior to feeding.

2.2. Volatile fatty acid potential estimation

The VFA potential assays were based on biomethane potential (BMP) protocol that was modified so to optimize the acidogenic fermentation and the VFA yield, while preventing methane production due to acidic conditions. The modified assays were prepared as previously described (Frigon et al., 2013), using an inoculum to substrate ratio of 2:1 based on the total volatile solid (TVS) concentration, so that the bioconversion kinetics is not microbially limited. All assays were performed in triplicates. Briefly, the sample bottles were prepared anaerobically by sparging with a constant flow of a gasesous mixture containing 80% N_2 and 20% CO_2. For each experimental digestion the bottles contained: inoculum (2 g TVS), algal biomass (1 g TVS), defined media (2 mL), Na_2S-cysteine solution (0.5 mL). The final volume was adjusted to 100 mL for all bottles using boiled demineralized water. The assays were conducted for 10 days. The bottles were incubated at 35°C with an agitation of 150 rpm. One liter of defined media contained: 500 mg KH_2PO_4, 500 mg NaCl, 100 mg $CaCl_2 \cdot H_2O$, 1894 mg NH_4Cl, 100 mg $MgCl_2 \cdot 6H_2O$, 10 mg $(NH_4)6Mo_7O_{24} \cdot 4H_2O$, 0.1 mg $ZnSO_4 \cdot 7H_2O$, 0.3 mg HBO_3, 1.5 mg $F_2Cl_2 \cdot 4H_2O$, 10 mg $CoCl_2 \cdot 4H_2O$, 0.03 mg $MnCl_2 \cdot 6H_2O$, 0.03 mg $NiCl_2 \cdot 6H_2O$, 0.1 mg $AIK(SO_4)_2 \cdot 12H_2O$, 0.1 mg nicotinic acid, 0.1 mg cyanocobalamin, 0.05 mg thiamin, 0.05 mg p-aminobenzoic acid, 0.25 mg pyridoxine and 0.025 mg pantothenic acid. The assays were monitored for gas production and biogas composition along with pH and VFA at day 2, 4, 7 and 10. Parameters such as TVS, COD and ammonium were analyzed at the end of the incubation period.

The first set of assays compared the use of four different inocula: granular sludge collected from a full scale upflow anaerobic sludge blanket (UASB) digester treating apple processing wastewater (Lassonde Inc., Rougemont, Qc, Canada), bovine manure collected from a dairy farm (Ferme Larose, Verchères, Qc, Canada), concentrated waste activated sludge (WAS) from a municipal wastewater treatment plant (WWTP) (Ste-Catherine, Qc, Canada), and a blend from the three inocula (33.3% each on a TVS basis). All inocula were used as collected, except the bovine manure. The manure with initial TVS of around 140 g kg^{-1} was blended at 3200 rpm using a Retsch GM 300 knife mill (Retsch Inc., Newtown, PA, USA) to ensure a homogeneous substrate by destroying

coarse and fibrous structures. Subsequently, the blended manure was diluted with the defined media as described above, to a final concentration of *circa* 50 g TVS kg^{-1}. All inocula were starved for 48 hours prior to the start-up of the assays by incubation at 35 °C with agitation at 150 rpm with no substrate. The algae mass added to the assays varied as a function of its TVS concentration.

Bovine manure was used as the inoculum for the assays at different pH and nutrient concentrations. For the pH assays, pH was adjusted to 2, 3, 4 and 5 using H_2SO_4 6N at the initial time. Then, pH was adjusted if required at day 2, 4 and 7 in each bottle. For the nutrients assays, one set of bottles was prepared with the defined media as described above, one set of bottles was prepared without defined media, and the last set of bottles was prepared with the defined media with a nutrient content five times more concentrated.

2.3. Reactor setup

Two completely stirred tank reactors (CSTR) were operated for a period of 22 weeks under mesophilic (35 °C, R35) and thermophilic (55 °C, R55) conditions to evaluate the production of VFAs from microalgal biomass. Figure 1 shows the schematic reactor setup applied for this work. The glass CSTRs (Bellco Glass Inc., Vineland, NJ) with a working volume of 6L were equipped with a steel stirrer, temperature and pH sensors. The reactors contained three ports for effluent extraction, feeding, acid injection as well as gas sampling. Biogas was quantified by a MilliGascounter fabricated by Ritter Apparatebau GmbH & Co. KG (Bochum, Germany). Each time the reactor was to be fed, an effluent volume was first withdrawn with a peristaltic pump along with compensation in the headspace by an equivalent volume of nitrogen, and then replaced with an equal volume of algal concentrate. The withdrawn effluent was then refrigerated for further examination.

Hydraulic retention time (HRT) and OLR were monitored throughout the experiment. Variations within the batches caused some deviations in OLR. Furthermore, feeding had to be modified during week 4 and 5 due to technical difficulties, which resulted in fluctuations in OLR and HRT. This however was before stable state

conditions were achieved and hence had no impact on the data used for evaluating the performances.

Each reactor was inoculated with 6L of manure with a TVS of 49 g kg^{-1} of manure. Hence, at the beginning, the digesters contained a total of 294 g TVS. Feeding was initiated the following day. A semi-continuous feeding was applied: the reactors were manually fed once a day, five days a week. Before feeding, the substrate was heated to room temperature in order to prevent any negative effects for the microbial community.

To maintain the desired temperatures, both reactors were equipped with heat jackets and heat controllers (JSC-33A-R/M, Shinko Technos Co. LTD., Osaka, Japan). For reactor R55 an ancillary heat element was installed beneath the reactor to complement the heat jacket. To monitor the proper functioning of the temperature control, both reactors were equipped with an additional electric thermometer. The pH was controlled with an automatic injection of 6N H_2SO_4 regulated by a PHCN-410 pH controller fabricated by Omega Engineering Inc. (Stamford, CT). To avoid phototrophic algae growth during hydrolysis, the reactors were covered with aluminum foil throughout the experiment to keep light away.

2.4. Analytical methods

The pH was measured on a daily basis with a Fisher Scientific AB15 pH meter (Thermo Fisher Scientific Inc., Ashville, NC, USA) after the samples cooled down to room temperature. Prior to each measurement, the pH meter was calibrated with a two-point calibration at pH 4 and 7 using standard solutions (VWR International Inc., West Chester, PA). The TVS and COD analyses were performed according to standard methods (Eaton et al., 1995).

The gas composition (CO_2, CH_4, N_2, H_2 and O_2) was measured by injecting 300 µL of gas (model 1750 gas-tight syringe, Hamilton, Reno, NV) into an Agilent 6890 gas chromatograph (Agilent Technologies Inc., Wilmington, DE) equipped with a thermal conductivity detector and a 5 m x 2.1 mm Carboxen-1000 column (Supelco, Bellafonte, PA) with argon as the carrier gas. The column temperature was held at 60 ºC for 7 min

and increased to 225 ºC at a rate of 60 ºC min^{-1}. All gas volumes are given at standard temperature and pressure, of 273.15 K and 100 kPa.

VFAs (acetate, propionate, butyrate, iso-butyrate, valerate iso-valerate, caproate) and alcohols (methanol, ethanol, 2-propanol, tert-butanol, n-propanol, sec-butanol, and n-butanol) were measured on an Agilent 6890 gas chromatograph (Wilmington, DE) equipped with a flame ionization detector (FID) as described previously (Guiot et al., 2011). Unless explicitly mentioned, the sum of the VFA concentrations are expressed in COD equivalents (COD_{eq}), using conversion factors of 1.07, 1.51, 1.82, 1.82, 2.04, 2.04, 2.20 g COD g^{-1} for acetic, propionic, iso-butyric, butyric, iso-valeric, valeric and caproic acids, respectively (Lim et al., 2008).

For the long chain fatty acids (LCFA) extraction and analysis, one mL of liquid sample was placed into a 5 mL vial and added with 0.05 g NaCl, two drops of 50% H_2SO_4 and 2 mL of a 50:50 hexane:methyl tertiary butyl ether (MTBE) mixture. The vials were crimped with a Teflon coated septum, shaken at 200 rpm using a rotary shaker (New Brunswick, Edison, NJ) for 15 min and centrifuged at 2000 g for 5 min. The organic layer was removed and 1 µL was analyzed by gas chromatography using an Agilent 6890 chromatograph equipped with a FID at 250 ºC, an injector at 250 ºC and a 30 m x 0.53 mm Carbowax 20M capillary column (Supelco, Belafonte, PA). The helium carrier gas flow was 5 mL min^{-1} and the column temperature was held at 90 ºC for 0.5 min, then increased to 180 ºC at a rate of 20 ºC min^{-1} and held at 180 ºC for 9 min. This method quantified C8 to C22 saturated and unsaturated LCFAs with a minimum 85% extraction efficiency (Lalman and Bagley, 2000).

3. Results and discussion

3.1. Conditions conducive to significant VFA production

Microalgal biomass is a complex substrate and the efficacy of its conversion into VFAs will depend on favourable digestion conditions. A few of these conditions were verified in bottle tests prior to the digester operation, namely different inocula, working

pH and the addition of nutrients (trace metals, vitamins, nitrogen and phosphorus). The VFA production was verified for three inocula in a first set of bottles and the final values are presented in Table 1. The soluble COD (sCOD), a direct indicator of the hydrolysis of the microalgal biomass, was higher for the concentrated WAS and the manure, at 6879 and 6433 mg L^{-1}, respectively. Although the VFA concentration was slightly higher for the WAS inoculum, the highest butyrate concentration of 484 mg L^{-1} was reached with manure as inoculum. At a pH of 5 no methane was detected in the assays.

A second set of assays targeted different acidic pH using manure as the inoculum. The highest VFA concentration were measured at pH 3 and 5 (Table 1). However, the butyrate concentration was clearly higher at pH 5 compared with the other assays. Interestingly, the final butyrate concentration was very similar for this assay (476 mg L^{-1}) compared with the assay under the same conditions in the previous test (484 mg L^{-1}), validating both experiments. It has to be noted that hydrolytic activity was present at all tested pH, with soluble COD ranging from 5023 mg L^{-1} at pH 2 to 6522 mg L^{-1} at pH 5.

Finally, the impact of nutrients was verified, with manure as inoculum and at a target pH of 4. Not adding nutrients had a detrimental effect on the final sCOD and VFA concentration (Table 1). Adding five times the nutrient concentration as in defined media (Frigon et al., 2013) considerably improved the VFA concentration at the end of the digestion, and particularly the butyrate concentration, which reached 560 mg L^{-1}. These results suggest that the anaerobic digestion of microalgal biomass as a mono-substrate requires nutrients. Since the biomass itself contains an abundance of nitrogen and phosphorous, it is hypothesized that these are trace metals and vitamins in the defined media that provided the observed benefit.

These digestion assays demonstrated the hydrolytic activity of manure at pH 5 when digesting *Scenedesmus sp*.-AMDD. The VFA yield reached a maximum of 222 mg COD_{eq} g^{-1} TVS. If a ratio of 1.42 g COD g^{-1} TVS is assumed for this microalgal biomass, then approximately 15% of the biomass was converted into VFA after 10 days of incubation. This is an interesting starting point but it can be presumed that higher yield are required to draw interest in the production of carboxylic acids rather than methane.

3.2. Digesters experiment

3.2.1 Operational parameters for mesophilic and thermophilic digesters

The mesophilic (R35) and thermophilic (R55) digesters were monitored during 22 weeks of operation after which the experiment was terminated, as VFA production reached steady-state conditions. Some feeding problems were encountered with R55 during the last two weeks of operation and the results were not considered during analysis. A pH of 4.4 ± 0.1 (target 4.5) was maintained throughout the experiment for both digesters. Although a higher pH could sustain higher VFA concentration as shown by Lim et al. (2008), where with 30 – 38% improvement in VFA concentration was demonstrated at pH of 5.0 – 5.5, initial experiments performed in a 2L CSTR in our laboratory at pH 5.0 resulted in significant methane production and less VFA produced than at pH 4 (data not shown).

The feeding of the digesters was performed five times per week at an average HRT of 15 days. Although the organic concentration of the algal paste varied during the course of the experiments, the frequent TVS determination allowed for the adjustment of the recipe and a constant OLR of 2.5 ± 0.1 g TVS L^{-1} d^{-1} for both digesters. The average mass of TVS added per week was 98.1 ± 7.2 and 95.7 ± 6.2 g TVS for R35 and R55, respectively.

3.2.2 Hydrolysis performance under mesophilic and thermophilic conditions

The sCOD concentration in the effluent of the digesters was only 5.5 and 7.0 g L^{-1} for R35 and R55 after the first week of operation, but quickly reached a pseudo steady-state with 14.3 ± 0.6 and 15.6 ± 1.4 g L^{-1} during the last weeks of operation (Figure 2). To evaluate the break down of complex oligomers into soluble monomers as a crucial preliminary step for VFA production by acidogenic bacteria, two performance parameters have been defined as follows:

$$\text{Hydrolysis yield [g sCOD g}^{-1}\text{ TVS]} = \text{sCOD}_{\text{effluent}}\,[\text{g}] \cdot \text{TVS}_{\text{feed}}^{\ -1}\,[\text{g}] \quad (1)$$

$$\text{Solubilization [\%]} = 100 \cdot \left(1 - \frac{\text{VSS}_{\text{effluent}}\,[\text{g kg}^{-1}]}{\text{VSS}_{\text{feed}}\,[\text{g kg}^{-1}]}\right) \quad (2)$$

where $sCOD_{effluent}$ represents the soluble COD in the reactor discharge, TVS, the total volatile solids, and VSS, the volatile suspended solids. The hydrolysis yield followed the sCOD concentration over time and increased up to 0.38 ± 0.02 and 0.42 ± 0.03 g sCOD g^{-1} TVS at the end of the experiment for digester R35 and R55, respectively (Table 2). This indicated a similar conversion of algal biomass into soluble compounds under mesophilic and thermophilic conditions. Komemoto et al. (2009) demonstrated a clear dependency of solubilization rates of solid matter, mainly consisting of carbohydrates on the operating temperature. Considering the similar hydrolysis yield under both temperatures regimes in this study, it can be said that for microalgae like *Scenedesmus* sp.-AMDD, which is characterized by high contents of carbohydrates and proteins and only small amounts of lipids, the process temperature did not have a major impact on solid degradation. The high protein content in *Scenedesmus* sp.-AMDD may be responsible for this behaviour, since protein solubilization can decrease at temperatures > 40 °C, especially at low pH (Pelegrine and Gasparetto, 2005). A lower protein solubilization would result in a lower protein degradation. This was clearly observed with much lower ammonia concentration of 643 mg NH_4^+ L^{-1}at 55 °C compared to the concentration of 1673 mg NH_4^+ L^{-1} at 35 °C . Additional evidence of inhibition of protein degradation is suggested by the VFA data. Compared to the mesophilic digester, butyrate values in the thermophilic digester were 35% lower, but iso-butyrate was 93% lower, and no iso-valerate was produced. These two VFAs are primarily produced by amino acid fermentation (Macfarlane et al., 1992). This might counteract an improved carbohydrate solubilization at thermophilic temperatures and explain similar solubilization levels.

Although the solubilization ratio technically describes the same reality as the hydrolysis yield, it uses, however, a different approach since it refers to the VSS reduction during the fermentation rather than the increase in sCOD. Solubilization ratios of 24 ± 1 and 27 ± 3 % were obtained for R35 and R55, implying that roughly 75% of the algae cells remained in a non-hydrolyzed state and were not broken down by hydrolytic bacteria. This correlates with previous findings (Zamalloa et al., 2012), where the degradation of *Scenedesmus obliquus* was investigated. It, however, remains to be investigated if three out of ten algae cells got degraded entirely or rather only one highly

rigid component, such as the biopolymer cell wall of the *Scenedesmus* biomass remains untouched in all cells.

3.2.3 Comparative VFA production under mesophilic and thermophilic conditions

Contrary to the similar hydrolytic performance and the correlated level of sCOD during mesophilic and themophilic operation, a clear difference in acidification, defined as the VFAs in COD equivalents reported to the effluent sCOD, was observed between the two temperatures (Figure 2). Starting with initial VFA concentrations at *circa* 2 g COD L^{-1} in week 1, it increased continuously until stabilization took place at around week 12 for R35 and week 5 for R55. Average acid concentrations at steady-state were 6.46 ± 0.28 and 3.26 ± 0.42 g COD L^{-1} for reactor R35 and R55, respectively (Table 2). VFA concentration in reactor R35 was almost two folds higher than in reactor R55. VFA yields of 171 ± 5 and 88 ± 12 mg COD g^{-1} TVS^{-1} were reached for R35 and R55, respectively. This compares with the yield of 0.125 g VFA-COD/g^{-1} TVS obtained with lipid-extracted *Chlorella* sp., at pH 4 in mesophilic conditions (Li et al., 2013). The most dominant VFA was butyrate, which represented 63% and 82% of the total VFA in COD equivalents for R35 and R55. This compares with other work about acidification at low pH (Horiuchi, 2002). It has to be noted that iso-butyric concentration was significant for R35 while absent from the R55 effluent (Table 2). It appears that an accumulation of more complex soluble intermediates such as longer fatty acids (C > 14) occurred in reactor R55, explaining the similar hydrolysis level despite lower VFA concentration.

It can be expected that poor hydrolysis characteristics of *Scenedesmus* sp.-AMDD diminished potentially achievable VFA concentrations, since insoluble components are not able to be fermented. The results presented here can serve as a reference point for future work about mixed VFA production from microalgae and especially *Scenedesmus* strains.

Iso-butyric acid was found in both reactors but the concentrations were higher at mesophilic temperature (Table 2). Average concentrations of 723 ± 33 and 50 ± 14 mg L^{-1} were measured for reactor R35 and R55, respectively. Iso-butyric acid is reported to be of minor importance in well functioning anaerobic digesters. Reactors with concentrations of less than 5 mg L^{-1} can be considered as 'healthy', whereas a

concentration above 15 mg L^{-1} indicates process limiting conditions (Hill and Bolte, 1989). Lowering the pH may have triggered the accumulation of iso-butyric acid at mesophilic temperature. Usually iso-butyric acid is not produced in large amounts by any natural organism (Zhang et al., 2011). However it seems that mesophilic fermentation of algal proteins can produces branched chain VFAs, such as iso-butyrate and iso-valerate, yet in lower proportion as compared to linear VFAs (Boeckaert et al., 2008).

Longer VFAs with more than four carbon atoms (iso-valerate, valerate, caproate) were present in both reactors but only in traces for R55, compared with measurable amounts for reactor R35 (0.68 ± 0.05 g COD L^{-1}) (Table 2). Under mesophilic conditions iso-valerate, valerate and caproate was detected throughout the whole experiment, whereas iso-valerate and valerate disappeared under thermophilic conditions during week 8 and 14, respectively. Only trace amounts of caproate was detected (< 2 mg L^{-1}) in reactor R55.

Both effluents contained considerable amounts of long saturated as well as unsaturated fatty acids (C > 14), which could be an indication for incomplete degradation. Figure 3 illustrates the LCFA profiles found for mesophilic and thermophilic conditions. LCFA concentrations were considerably higher inside the reactor run at 55 °C. This underlines the previous assumption that an accumulation of longer intermediates occurred in reactor R55. The predominant LCFA found in the effluents of R35 and R55, namely palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) were always found at concentration that were below inhibition (1 g L^{-1}) as reported for thermophilic anaerobic digestion (Angelidaki and Ahring, 1992). It is however possible that synergestic effect could take place as total LCFA concentration reached 2.3 g L^{-1} in R55 (Table 2).

3.2.4 Fermentative hydrogen production under thermophilic conditions

Methane was not detected at any point during the course of the experiments, indicating a successful inhibition of acetoclastic as well as hydrogenotrophic methanogenic archaea by maintaining a low pH. Small amounts of oxygen was present in both reactors throughout the experiments. It remains unclear if this was either an effect of microleaks or phototrophic activity inside the reactor. Sánchez Hernández and Travieso

Córdoba (1993), indeed, found evidence for algae growth during anaerobic digestion of microalgae.

Although the fermentative hydrogen production has not been the primary focus of this study, the results indicate clear distinctions between mesophilic and thermophilic temperatures in terms of hydrogen production during the hydrolysis step. It is therefore worth having a closer look on these differences. The fermentative hydrogen production has been performed with a variety of substrates such as glucose, sucrose, xylose, sugary and winery wastewater. However, only few papers describe the hydrogen production through dark fermentation of microalgal biomass (Yang et al., 2010).

High hydrogen production was observed for reactor R55, whereas the hydrogen produced in reactor R35 was negligible. Under steady-state conditions, a mean hydrogen production of roughly 20 ± 3 mL g^{-1} TVS was observed for R55 with a hydrogen content of 42 ± 1.1 % in the biogas. This is comparable to that reported in literature for various complex substrates (Han and Shin, 2004; Luo et al., 2010; Zhu et al., 2008) and lipid-extracted *Scenedesmus* biomass (Yang et al., 2010). This study however is the first work on a continuous hydrogen production through dark fermentation of microalgal biomass. High hydrogen yield of 0.107 $m^3 kg^{-1}$ TVS were recently reported from the batch digestion of *Chlorella sorokiniana.* Although this was preceded by an intensive pretreatment (20% vol/vol HCl followed by autoclaving for 20 minutes) (Roy et al., 2014).

The reactor temperature is a crucial parameter as it affects the microbial ecosystem and thereby impacts fermentative product spectra. It was demonstrated that thermophilic temperatures favour a breakdown of substrate into gaseous components, namely hydrogen and carbon dioxide, whereas mesophilic conditions will lead to higher VFA concentrations and less hydrogen. It can be assumed that homoacetogenesis, i.e. the utilization of hydrogen to reduce carbon dioxide into acetate, was a more important pathway under mesophilic than thermophilic conditions. Previous studies evidenced that temperature impacted the homoacetogenic activity and especially that psychrophilic temperatures favored homoacetogenic bacteria (Kotsyurbenko et al., 2001).

4. Conclusion

At a controlled pH controlled of 4.5, acidogenic digestion of a feedstock made of *Scenedesmus* sp.-AMDD resulted in a solubilization efficiency of ~30% under mesophilic as well as thermophilic temperatures. A higher production of short chain fatty acids (acetic acid, propionic acid, butyric acid and iso-butyric acid) was obtained under mesophilic conditions, while an accumulation of long chain soluble intermediates was shown at 55 °C. It is expected that these LCFAs may negatively affect the digestion process at 55 °C. The results have shown that butyric acid can represent the dominant VFA under mesophilic as well as thermophilic temperatures when the pH is kept below 5, which is preferred if the extraction of butyric acid for e.g. biofuel production is intended.

Acknowledgements

The authors are grateful to Claudia Syed, Marie-Josée Lévesque and Punita Mehta for their help with the monitoring of methane potential assays. The authors also wish to thank Alain Corriveau for analytical assistance with the VFAs and ammonium measurement. The authors are grateful to Patrick McGinn and Crystal G. Whitney for growing and supplying the microalgal biomass. The authors also thank Abid Hussain for proofreading. This work was supported by the NRC Flagship Program on Algal Carbon Conversion. This is NRC publication no. EME-xxxx.

References

Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T. 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotech.*, **29**(2), 70-78.

Aiello-Mazzarri, C., Agbogbo, F.K., Holtzapple, M.T. 2006. Conversion of municipal solid waste to carboxylic acids using a mixed culture of mesophilic microorganisms. *Bioresour. Technol.*, **97**(1), 47–56.

Angelidaki, I., Ahring, B.K. 1992. Effects of free long-chain fatty acids on thermophilic anaerobic digestion. *Appl. Microbiol. Biotech.*, **37**, 808-812.

Bastidas-Oyanedel, J.-R., Bonk, F., Thomsen, M.H., Schmidt, J.E. 2015. Dark fermentation biorefinery in the present and future (bio)chemical industry. *Rev. Environ. Sci. Biotechnol.*, **14**(3), 473-498.

Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., Van Nespen, T., Van Straalen, W., Fievez, V. 2008. Effect of dietary starch or micro-algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows *J. Dairy Sci.*, **91**, 4714–4727.

Borowitzka, M.A. 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. *J. Appl. Phycol.*, **7**, 3–15.

Chang, H.N., Kim, N.-J., Kang, J., Jeong, C.M. 2010. Biomass-derived volatile fatty acid platform for fuels and chemicals. *Biotech. Bioprocess Eng.*, **15**(1), 1–10.

Dwidar, M., Park, J.-Y., Mitchell, R.J., Sang, B.-I. 2012. The future of butyric acid in industry. *The Scientific World Journal*, **2012**, Article No. 471417, 10 pp.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E. 1995. Standard Methods for the Examination of Water and Wastewater. 19 ed, American Public Health Association. Washington D.C.

Frear, C., Zhao, B., Ma, J., Zhao, Q. 2013. Anaerobic digestion of whole and lipid-extracted algal biomass from four industrial strains — Determination of important methane and nutrient information. *ASABE National Conference*, July 22th-24th, 2013, Kansas City, MO, USA. American Society of Agricultural and Biological Engineers, St. Joseph, Michigan. pp. Paper number 131618699.

Frigon, J.-C., Matteau-Lebrun, F., Ganda Bachir, R., McGinn, P.J., O'Leary, S.J.B., Guiot, S.R. 2013. Screening microagae strains for their productivity in methane following anaerobic digestion. *Appl. Energy*, **108**, 100-107.

Golueke, C.G., Oswald, W.J., Gotaas, H.B. 1957. Anaerobic digestion of algae. *Appl. Microbiol.*, **5**(1), 47–55.

Guiot, S.R., Cimpoia, C., Carayon, G. 2011. Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas. *Environ. Sci. Technol.*, **45**(2), 2006–2012.

Han, S.-K., Shin, H.-S. 2004. Performance of an innovative two-stage process converting food waste to hydrogen and methane. *J. Air Waste Manag. Assoc.*, **54**(2), 242–249.

Hankamer, B., Lehr, F., Rupprecht, J., Mussgnug, J.H., Posten, C., Kruse, O. 2007. Photosynthetic biomass and H_2 production by green algae: from bioengineering to bioreactor scale-up. *Physiologia Plantarum*, **131**(1), 10–21.

Harun, R., Singh, M., Forde, G.M., Danquah, M.K. 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. *Ren. Sust. Energy Reviews*, **14**(3), 1037–1047.

Hill, D.T., Bolte, J.P. 1989. Digester stress as related to iso-butyric and iso-valeric acids. *Biol. Wastes*, **28**(1), 33–37.

Holtzapple, M.T., Granda, C.B. 2009. Carboxylate platform: the MixAlco process. Part 1: comparison of three biomass conversion platforms. *Appl. Biochem. Biotech.*, **156**(1-3), 95–106.

Hong, C., Haiyun, W. 2010. Optimization of volatile fatty acid production with co-substrate of food wastes and dewatered excess sludge using response surface methodology. *Bioresour. Technol.*, **101**(14), 5487–5493.

Horiuchi, J.-I. 2002. Selective production of organic acids in anaerobic acid reactor by pH control. *Bioresour. Technol.*, **82**(3), 209-213.

Kim, N.-J., Park, G., Kang, J., Kim, Y.-C., Chang, H. 2013. Volatile fatty acid production from lignocellulosic biomass by lime pretreatment and its applications to industrial biotechnology. *Biotech. Bioprocess Eng.*, **18**(6), 1163–1168.

Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., Toda, T. 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. *Waste Manage.*, **29**(12), 2950–2955.

Kotsyurbenko, O.R., Glagolev, M.V., Nozhevnikova, A.N., Conrad, R. 2001. Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. *FEMS Microbiol. Ecol.*, **38**(2-3), 153–159.

Lalman, J.A., Bagley, D.M. 2000. Anaerobic degradation and inhibitory effects of linoleic acid. *Water Res.*, **34**(17), 4220-4228.

Li, Y., Hua, D., Zhang, J., Zhao, Y., Xu, H., Liang, X., Zhang, X. 2013. Volatile fatty acids distribution during acidogenesis of algal residues with pH control. *World J. Microbiol. Biotech.*, **29**(6), 1067-1073.

Lim, S.-J., Kim, B.J., Jeong, C.-M., Choi, J.-D.-R., Ahn, Y.H., Chang, H.N. 2008. Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. *Bioresour. Technol.*, **99**(16), 7866-7874.

Luo, G., Xie, L., Zou, Z., Zhou, Q., Wang, J.-Y. 2010. Fermentative hydrogen production from cassava stillage by mixed anaerobic microflora: Effects of temperature and pH. *Appl. Energy*, **87**(12), 3710–3717.

Macfarlane, G.T., Gibson, G.R., Beatty, E., Cummings, J.H. 1992. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. *FEMS Microbiol. Ecol.*, **101**(2), 81–88.

Montingelli, M.E., Tedesco, S., Olabi, A.G. 2015. Biogas production from algal biomass: A review. *Ren. Sust. Energy Rev.*, **43**, 961-972.

Pelegrine, D.H.G., Gasparetto, C.A. 2005. Whey proteins solubility as function of temperature and pH. *LWT-Food Science and Technology*, **38**(1), 77–80.

Plaza, M., Cifuentes, A., Ibanez, E. 2008. In the search of new functional food ingredients from algae. *Trends Food Sci. Tech.*, **19**(1), 31–39.

Pulz, O., Gross, W. 2004. Valuable products from biotechnology of microalgae. *Appl. Microbiol. Biotech.*, **65**(6), 635–648.

Roy, S., Kumar, K., Ghosh, S., Das, D. 2014. Thermophilic biohydrogen production using pre-treated algal biomass as substrate. *Biomass Bioenergy*, **61**, 157-166.

Sánchez Hernández, E.P., Travieso Córdoba, L. 1993. Anaerobic digestion of *Chlorella vulgaris* for energy production. *Resour. Conserv. Recycl.*, **9**(1-2), 127–132.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenerg. Res.*, **1**(1), 20-43.

Sialve, G., Bernet, N., Bernard, O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotech. Adv.*, **27**(4), 409-416.

Stolz, P., Oberbayer, B. 2005. Manufacturing microalgae for skin care. *Cosmet. Toiletries*, **120**(3), 99–106.

Thanakoses, P., Black, A.S., Holtzapple, M.T. 2003. Fermentation of corn stover to carboxylic acids. *Biotech. Bioeng.*, **83**(2), 191–200.

Yang, Z., Guo, R., Xu, X., Fan, X., Li, X. 2010. Enhanced hydrogen production from lipid-extracted microalgal biomass residues through pretreatment. *Int. J. Hydrogen Energy*, **35**(18), 9618-9623.

Zamalloa, C., Boon, N., Verstraete, W. 2012. Anaerobic digestibility of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophilic conditions. *Appl. Energy*, **92**, 733–738.

Zhang, K., Woodruff, A.P., Xiong, M., Zhou, J., Dhande, Y.K. 2011. A synthetic metabolic pathway for production of the platform chemical isobutyric acid. *ChemSusChem*, **4**(8), 1068–1070.

Zhu, H., Stadnyk, A., Béland, M., Seto, P. 2008. Co-production of hydrogen and methane from potato waste using a two-stage anaerobic digestion process. *Bioresour. Technol.*, **99**(11), 5078-5084.

Table 1. Final values for the biomethane potential assays under different conditions

Conditions	pH	VSS[a] ($g\ L^{-1}$)	sCOD[b] ($mg\ L^{-1}$)	C_2-C_4 VFA[c, d] ($mg\ COD_{eq}\ L^{-1}$)	Butyrate ($mg\ L^{-1}$)	NH_4^+ ($mg\ L^{-1}$)
Inocula						
Granular biomass	5.23	16.5	4624 ± 235	1491	204	251
Activated sludge	5.36	15.8	6879 ± 136	2417	326	294
Manure	5.47	22.4	6433 ± 99	2129	484	168
Blend of all inocula	5.42	19.5	5550 ± 284	2008	317	241
pH						
2	2.12	16.8 ± 2.5	5023 ± 1529	1266	298	278
3	3.00	17.4 ± 0.2	4743 ± 1030	2533	231	316
4	4.14	16.3 ± 0.0	5070 ± 422	1575	257	371
5	5.12	17.4 ± 1.2	6522 ± 919	2222	476	514
Nutrients[e]						
None	4.02	15.3 ± 1.3	2985 ± 132	507	180	26
1X	4.19	16.5 ± 0.1	3404 ± 120	1046	262	125
5X	4.01	18.8 ± 0.5	5463 ± 770	1851	561	470

[a] VSS: volatile suspended solids.
[b] sCOD: soluble chemical oxygen demand.
[c] VFA: volatile fatty acid.
[d] Values were obtained from pooled aliquots from the triplicate of bottles.
[e] 1X : nutrient concentrations as indicated in section 2.2; 5X: nutrient concentrations fivefold increased.

Table 2. Digester performance (average from weeks 18-20)

Parameters	R35	R55
Soluble organic matter in effluent (g sCOD L^{-1})	14.3 ± 0.6	15.6 ± 1.4
Hydrolysis rate (g sCOD $gTVS^{-1}$)	0.38 ± 0.02	0.42 ± 0.03
Solubilization efficiency (%)	24 ± 1	27 ± 3
Acidification (VFA-COD_{eq}:sCOD) (%)	45 ± 3	21 ± 2
VFAs (C2-C4) (g COD_{eq} L^{-1})	6.46 ± 0.28	3.26 ± 0.42
Acetate (mg L^{-1})	757 ± 105	441 ± 35
Propionate (mg L^{-1})	160 ± 4	11 ± 2
Butyrate (mg L^{-1})	2249 ± 113	1471 ± 227
Iso-butyrate (mg L^{-1})	723 ± 33	50 ± 14
VFA yield (mg COD $gTVS^{-1}$)	171 ± 5	88 ± 12
Longer VFAs (C5-C7) (g COD_{eq} L^{-1})	0.68 ± 0.05	0
Iso-Valerate (mg L^{-1})	95 ± 8	0
Valerate (mg L^{-1})	102 ± 3	0
Caproate (mg L^{-1})	128 ± 5	0
LCFA (C14-C22) (mg L^{-1})	1417	2291
Alcohols (mg L^{-1})	331 ± 16	70 ± 4
pH	4.4 ± 0.1	4.4 ± 0.1
TKN (mg L^{-1})	3240 ± 0	2948 ± 64
Total phosphates	1872 ± 45	1910 ± 5
Ortho-phosphate (mg L^{-1})	611 ± 14	622 ± 5
Ammonium (mg L^{-1})	1673 ± 83	643 ± 30

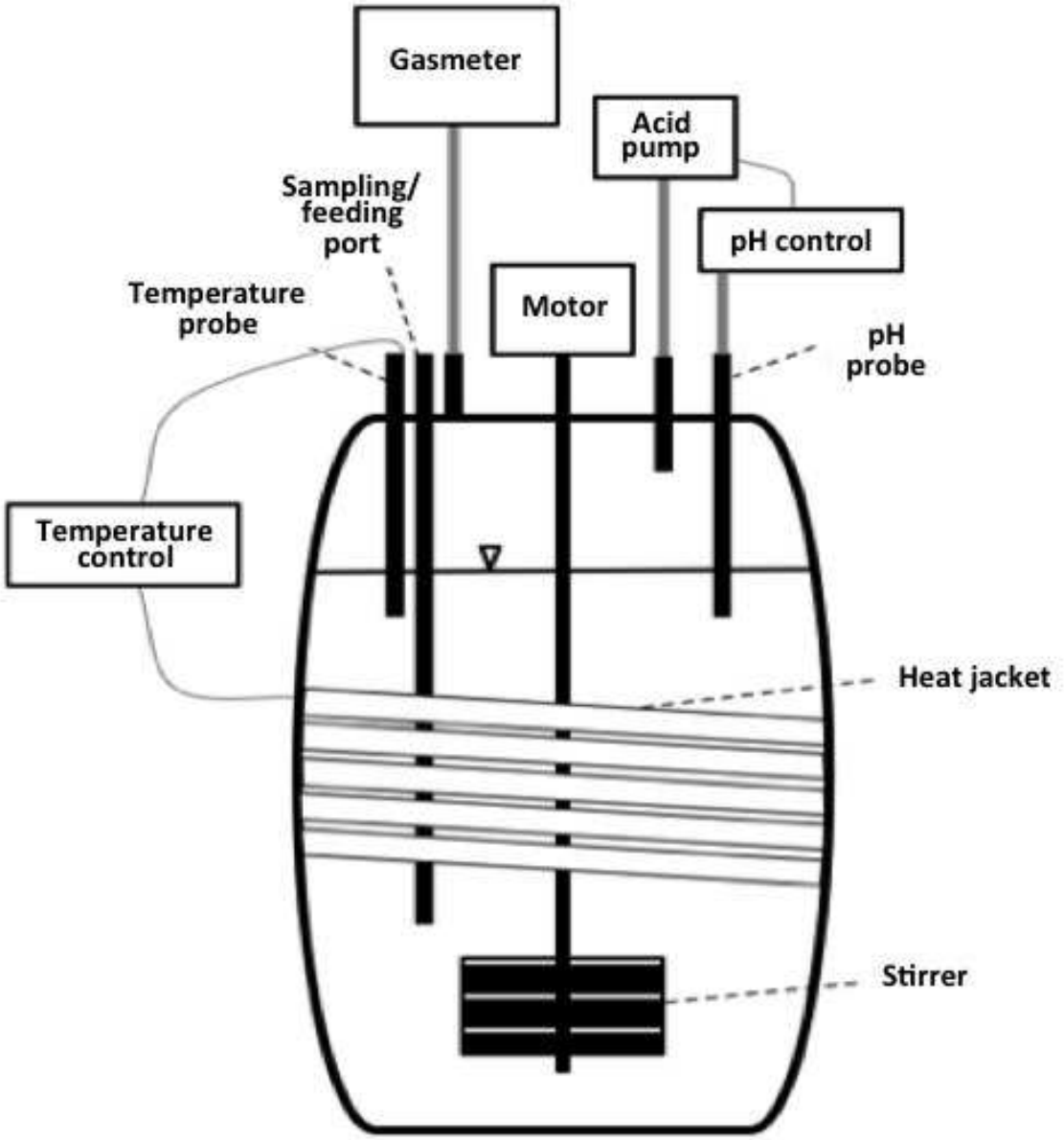
sCOD: soluble chemical O_2 demand
TKN: total Kjeldahl nitrogen
Alcohols: ethanol, methanol
Longer VFAs: caproic, valeric, iso-valeric, heptanoic acids
VFAs: acetic, propionic, butyric, iso-butyric acids
LCFA: long-chain fatty acids

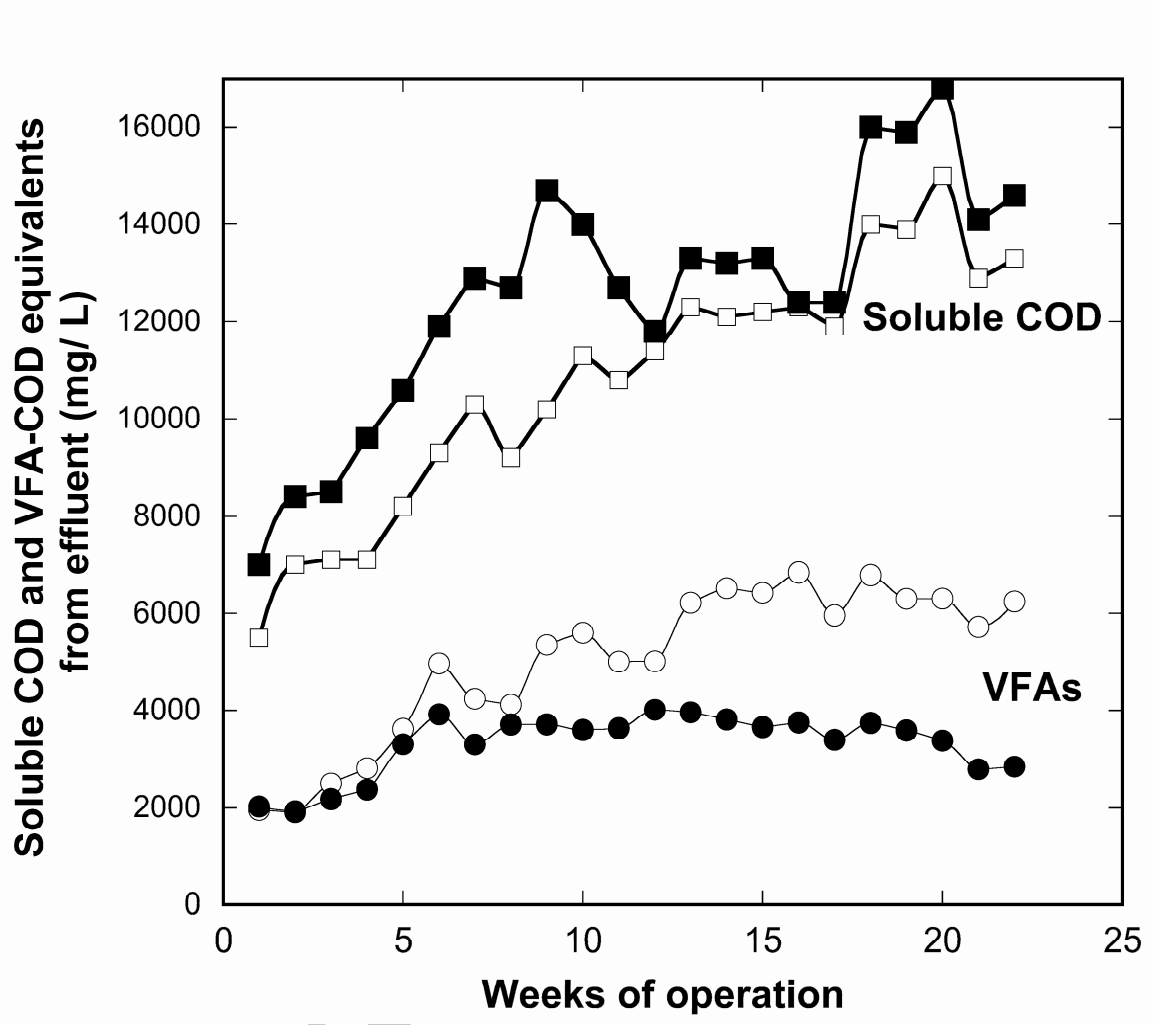
Figure captions

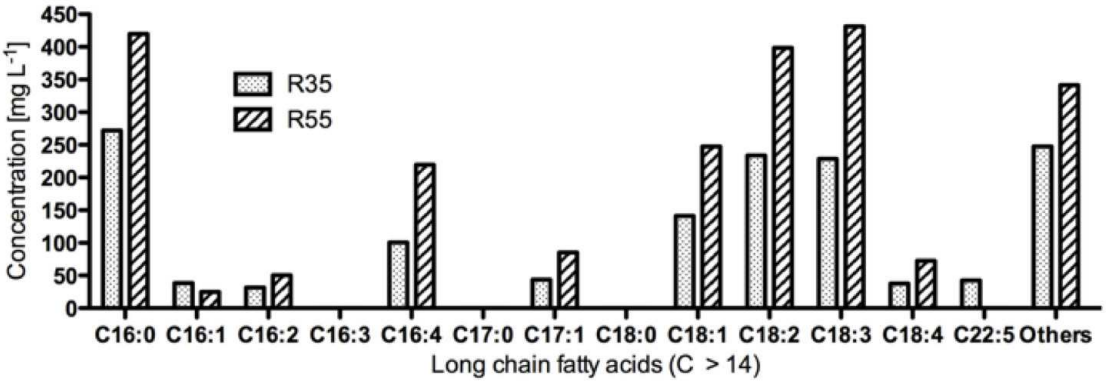
Fig. 1. Schematic reactor configuration

Fig. 2. Time-course of soluble COD (squares) and VFA (circles) concentrations in the effluent of mesophilic (blank symbols) and thermophilic (black symbols) digesters. Each data point represents an average of three values from three different days during the week.

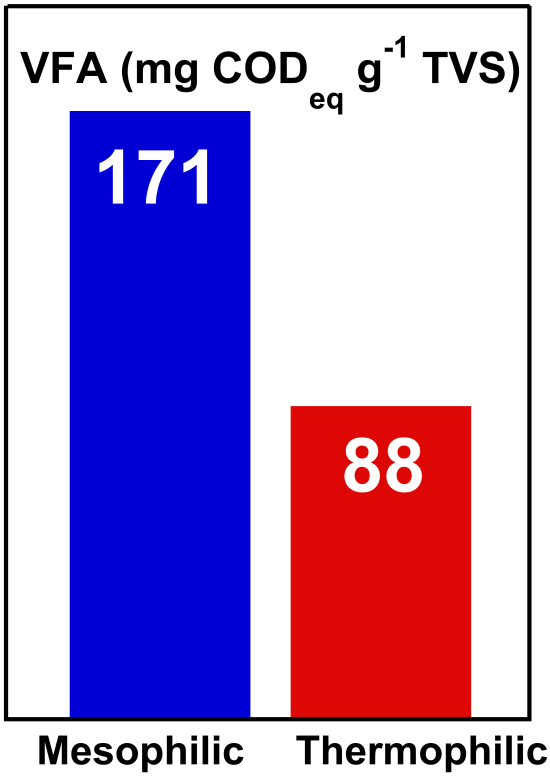
Fig. 3. Long chain fatty acids (LCFA) profile in effluent of mesophilic (R35) and thermophilic (R55) reactors.


Gasmeter
Acid pump
Sampling/ feeding port
pH control
Temperature probe
Motor
pH probe
Temperature control
Heat jacket
Stirrer





c



Highlights

- Anaerobic digestion of *Scenedesmu*s microalgal biomass into carboxylic acids.
- Similar hydrolytic activity under mesophilic and thermophilic conditions.
- Mesophilic conditions more favorable for volatile fatty acids production.
- Acid yield up to 222 mg g^{-1} total volatile solid added.