Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy

Bjorn Rusten and Ashish K. Sahu

ABSTRACT

Proof-of-concept has been demonstrated for a process that will utilize nutrients from sludge liquor, natural light, and CO_2 from biogas to grow microalgae at wastewater treatment plants. This process will reduce the impact of returning side-streams to the head of the plant. The produced algae will be fed to anaerobic digesters for increased biogas production. Dewatering of anaerobically digested sludge in centrifuges produces reject water with extremely low transmittance of light. A pre-treatment procedure was developed that improved light transmittance for reject water from the FREVAR, Norway, wastewater treatment plant from 0.1% T to 77% T (670 nm, 1 cm path). *Chlorella sp.* microalgae were found to be suitable for growth in this pre-treated reject water. Typical nitrogen removal was 80–90 g N/kg TSS of produced microalgae. The microalgae were successfully harvested by chemically assisted flocculation followed by straining through a 33 μm sieve cloth, achieving up to 99% recovery. Harvested algae were anaerobically co-digested with wastewater sludge. The specific methane gas production (mL CH_4/g VS fed) for the algae varied from less than 65% to 90% of the specific methane gas production for the wastewater sludge, depending on digester temperature, retention time and pre-treatment of the algae biomass.

Key words | Anaerobic co-digestion, *Chlorella sp.*, methane, microalgae, sludge liquor nutrients

Bjorn Rusten (corresponding author)
Ashish K. Sahu
Aquateam-Norwegian Water Technology Centre AS,
PO Box 6875 Rodeløkka,
0504 Oslo,
Norway
E-mail: *bjorn.rusten@aquateam.no*

INTRODUCTION

The work presented here is one part of a large research and development project on utilizing nutrients and CO_2 from wastewater treatment plants to grow microalgae for biogas production. The project is jointly funded by industry and the Norwegian Research Council and is carried out by two US universities, two Norwegian universities, two consulting engineering companies, one equipment supplier, and one wastewater utility company.

The overall concept is to retrofit wastewater treatment plants that currently have anaerobic sludge digesters with photobioreactors for growth of algae. The algae will utilize natural light, waste nutrients, and CO_2 from the produced biogas (either from flue gas after combustion in a CHP, or from CO_2 separated from the biogas when upgrading to fuel for vehicles). The produced algae will be harvested and fed to the existing anaerobic digesters for increased production of renewable bioenergy.

A pre-project (Rusten *et al.* 2009) showed that with the present costs for fossil fuel and CO_2 emissions the energy production from the algal biomass can not justify the costs of installing and operating photobioreactors. However, taking credit for the nutrients removed, together with an anticipated increase in energy costs and CO_2-emission costs, photobioreactors for renewable energy production may be profitable.

Algae composition will depend on species and nutrient availability. With the composition given by Grobbelaar (2000), growing of 1 kg total solids (TS) of algae will remove approximately 1.9 kg CO_2, 66 g N and 13 g P. CO_2 and light will be the limiting factors if algae are supposed to remove all the N and P at a wastewater treatment plant (WWTP). However, one attractive solution is to use algae to remove nutrients from side-streams (see Figure 1), as side-streams typically account for 20–30% of the nitrogen load at a municipal WWTP (Janus & Van der Roest 1997) and are known to often have a negative impact on the treatment processes when they are returned to the head of the plant. The most effective way to reduce the nutrient loading to the wastewater treatment plant is to treat the sludge liquor through a separate treatment procedure.

doi: 10.2166/wst.2011.722

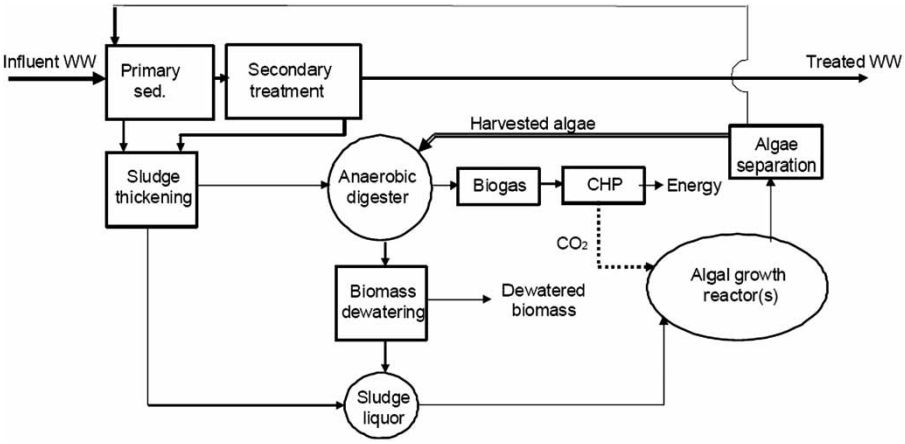

Figure 1 | Simplified flow sheet of the overall concept, utilizing nutrients in sludge liquor to grow microalgae for increased biogas production at municipal WWTPs (Rusten *et al*. 2009).

In the present study sludge liquor in the form of reject water from centrifuge dewatering of anaerobically digested sludge was pre-treated and used for growth of algae. Harvested algae were mixed with WWTP sludge and the methane potentials were measured in anaerobic laboratory digesters after different types of pre-treatment.

The intention of this paper is a proof-of-concept demonstration of the system in Figure 1, and only some of the results from laboratory and pilot scale tests will be presented.

CHALLENGES

Algae need light while dewatering of anaerobically digested sludge in centrifuges produces reject water that often is black, contains a lot of very fine solids particles, and has extremely low transmittance of light. Thus, pre-treatment of the reject water is necessary to improve transmittance and make it a suitable substrate for growth of algae.

It is also important to find a type of algae that can grow at a high rate in the highly variable and difficult conditions expected at WWTPs. Harvesting of algae in an economic and efficient manner at large scale is also a challenge.

Maximizing biogas production is very important, and anaerobic co-digestion of wastewater sludge and algal biomass may require pre-treatment of the algae.

PRETREATMENT OF REJECT WATER

Reject water from dewatering of anaerobically digested sludge in centrifuges is characterized by high concentrations of total suspended solids (TSS), organics and nutrients. Samples were collected from four wastewater treatment plants with anaerobic sludge digesters and dewatering centrifuges in the Oslo (Norway) region. The composition of the reject water is shown in Table 1.

The reject water samples were pre-treated with different combinations of processes (chemical oxidation processes, flocculation processes, particle removal processes). The optimum process must be simple, inexpensive, and able to produce reject water with a high light transmittance (T) without removing nutrients. Untreated reject water had from 0.1 to 21% T (670 nm wavelength, 1 cm path). Although the entire UV plus visible spectrum was scanned, light transmittance at 670 nm wavelength was selected for evaluation of transmission since it is close to the maximum absorption wavelength for chlorophyll a (Graham *et al*. 2008). Chemical oxidation processes were unsuccessful in improving light transmittance. Polymer addition + flocculation + anthracite (1.0–2.4 mm grain size) filtration was the best solution, with optimum results as shown in Table 2. Rapid mixing of polymer lasted for 30 s, followed by 10 min of slow mixing

Table 1 | Reject water composition from four treatment plants in the Oslo region of Norway

Treatment plant	TSS (mg/L)	% T 670 nm[a]	COD (mg/L)	Total nitrogen (mg/L)
Gardermoen	787	16	1,872	640
Nordre Follo	2,125	2	3,850	930
Soendre Follo	636	21	4,503	383
FREVAR	3,740	0.1	7,525	1,655

[a]Light transmittance at 670 nm wavelength and 1 cm light path.

Table 2 | Reject water particle concentration and light transmittance after pre-treatment, i.e. polymer addition, flocculation and filtration

Treatment plant	Polymer addition (mg/L)	Type of polymer	TSS (mg/L)	% T 670 nm
Gardermoen	50	Zetag 8125	93	85
Nordre Follo	150	Zetag 8125	750	56
Soendre Follo	25	Kemira C-496	130	65
FREVAR	250	Zetag 8125	64	77

flocculation. The optimum polymer dose was proportional with the TSS concentration in the untreated reject water, showing the importance of having a dewatering process that produces reject water with low TSS concentrations. For reject water from the FREVAR WWTP light transmittance improved from 0.1% T to 77% T.

Pretreatment of reject water for algae growth will require the WWTP to install polymer dosing equipment, flocculator basins and sand filters. The simplest will be to operate the flocculator basins in batch mode, with flocculation followed by settling, to minimize the particle load on the sand filters. Operators need to monitor the light transmittance of the treated reject water.

MICROALGAE GROWTH

Several types of wastewater that contain high concentrations of ammonia have been proposed for cultivation of algae, including livestock effluents and centrate from anaerobic digestion of sludges (Craggs *et al*. 2004; Kebede-Westhead *et al*. 2006; Mulbry *et al*. 2008).

Initial experiments at the University of Life Sciences (Ås, Norway) indicated that *Chlorella sp*., a fresh water alga, would be able to grow in sludge liquor. We obtained inoculum from the university and Figure 2 shows a result from growing *Chlorella sp*. in a mixture of 1 part pretreated reject water and 4 parts effluent from the FREVAR WWTP. The experiment was set up in 1 L Erlenmeyer flasks, with continuous air sparging and a photosynthetically active radiation of 87 μmol/m^2 s for 24 h/day. Based on the illuminated area available for algae growth a maximum biomass production rate of 13 g TSS/m^2 d was observed. Over the first 4 days, when we had the highest biomass production rates, the nutrient removal was 91.5 g NH_4-N/kg TSS grown and 3.83 g PO_4-P/kg TSS grown.

In pilot scale, inside a green house at the FREVAR WWTP, *Chlorella sp.* were grown in pretreated reject water that was undiluted, to demonstrate that reject water can be

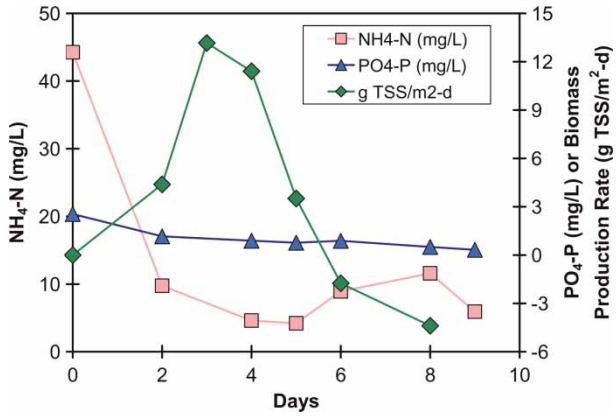

Figure 2 | Biomass production rate (g TSS/m^2 d) of microalgae in diluted reject water.

used for microalgae growth. Vertical plastic bag photo-bioreactors were operated in semi-batch mode. The photo-bioreactors were made of thin-walled polyethylene plastic tubing with a diameter of 156 mm, and had a volume to surface area ratio of 0.038 m^3/m^2. Operating water temperatures ranged from 19 to 26 °C, with pH varying from 7.9 to 8.6 and DO concentrations varying from 10 to 16 mg O_2/L. The photo-bioreactors were sparged with CO_2 for about 20 min twice a day, and the maximum biomass production rate was believed to be CO_2-limited. The reactors were aerated at a rate of 7 L of air/L reactor volume-hour. A maximum biomass production rate of about 17 g TSS/m^2 of photo-bioreactor surface area-day was observed at a liquid temperature of 25 °C, under partly cloudy skies, 15 h/day of daylight and a solar altitude at noon of 42.7°. Based on the 15 h of daylight this biomass production rate was equivalent to 1.13 g TSS/h of daylight. The concentration of the pretreated reject water feed during this period was 277 mg TSS/L, 90 mg total P/L and 616 mg total N/L. Over a 12 day growth period the nitrogen removal was 81 g NH_4-N/kg TSS produced.

MICROALGAE HARVESTING

Harvesting microalgae is an expensive and energy intensive process and presents a key challenge to the algae industry. The efficient separation, dewatering and drying of microalgae is probably the most essential factor in the economic feasibility of any microalgae production (Shelef *et al*. 1984). Harvesting costs may contribute 20–30% of the total cost of algal biomass. Harvesting method depends on the species, cell density and culture conditions (Carlsson *et al*. 2007).

There are several technologies for algae harvesting; filtration and screening, gravity sedimentation, flotation, and centrifugation (Shelef *et al*. 1984; Hoffmann 2002). Filtration is the most common method of algae harvesting but the mesh size depends on algae size and varies from species to species. The use of polymers for harvesting can achieve removal efficiencies as high as 85–95% (Shelef *et al*. 1984).

The particle size distribution of the *Chlorella sp.* microalgae biomass, grown on a mixture of reject water and WWTP effluent in our experiments, is shown in Figure 3. The dominant particle size was less than 4 μm, and 90% of the algal biomass was less than 9 μm. Without chemicals this microalgae suspension did not settle, nor did it float, and laboratory centrifugation was unsuccessful. The particles were too small for direct separation on commercial fine mesh sieves, so flocculation to create larger particles was necessary. Jar-tests were conducted using several commercial dewatering polymers and inorganic chemicals. Once the jar-test apparatus was activated, rapid mixing occurred for 10 s, followed by 10 min of flocculation.

Sedimentation

Sedimentation after flocculation was unsuccessful. Using organic polymers there was no settling. Using polyaluminum chloride (PAX-XL60 with 94 mg Al/mL, from Kemira, Norway) some settling occurred when left over night, but recovery of algae was low and unpredictable. It varied from about 15% to a maximum of 70% recovery.

Fine mesh sieve

Harvesting of flocculated algae on a fine mesh sieve was tested using a bench scale Salsnes Filter unit (Salsnes Filter AS, Norway) with vacuum attachment and sieve cloths with openings of 33, 54, 75, 90 and 213 μm. Only results for the Salsnes Filter sieve cloth with 33 μm openings and 10% open area are given here, since all sieve cloths with larger openings showed significantly poorer performance. TSS was measured before flocculation and after the sieve, to determine the recovery of algae. The algae suspension that was harvested had TSS concentrations from 810 to 980 mg/L.

Flocculation was done with addition of polymer alone, metal salts alone, and metal salts assisted with polymer. Initial screening tests indicated that the cationic polymer Zetag 7550 (BASF, Norway) was the most efficient when polymer was used alone. Polyaluminum chloride (PAX-XL60) worked well alone. However, PAX-XL60 assisted with a small amount of the anionic polymer Magnafloc 155 (MF155) (CIBA, Norway) worked very well. Figure 4 shows algae recovery after flocculation with different chemicals and straining through a 33 μm sieve cloth. All tests showed more than 90% recovery of the algal biomass. Increasing the PAX dose beyond 375 μL/L (35 mg Al/L) reduced the recovery. Almost 99% recovery was found for 375 μL/L of PAX-XL60 in combination with 2 mg/L of Magnafloc 155.

Gravity straining produced a cake with 4.3 to 6.0% dry solids for harvested algae flocculated with PAX-XL60 or PAX-XL60 assisted with a low dose of Magnafloc 155. This is a sufficiently high dry solids concentration to feed the algae directly to an anaerobic digester. Algae harvested after flocculation with the cationic polymer Zetag 7550 did gravity drain poorly, and the vacuum assisted straining was used. However, with vacuum assisted straining a cake with 11–12% dry solids was produced.

Algae harvested with PAX-XL60 contained 83 g N/kg VS and 24 g P/kg VS. PAX-XL60 is a commercial precipitation chemical for phosphorus removal. The high phosphorus

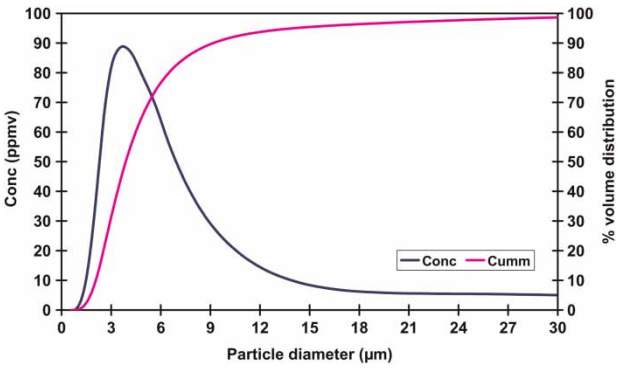

Figure 3 | Particle size distribution of the microalgae biomass, measured by a Malvern Mastersizer.

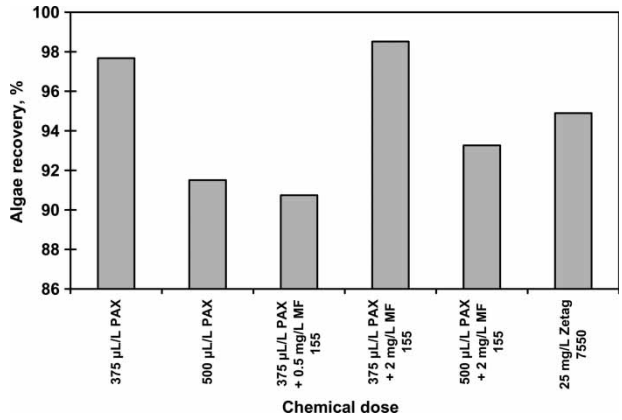

Figure 4 | Algae (*Chlorella sp.*) recovery when harvested by flocculation with different chemicals and doses, followed by straining through a 33 μm sieve cloth.

concentration in the harvested biomass was due to precipitation of the residual phosphate in the algae suspension. If the water passing through the sieve cloth is discharged, removing the residual phosphorus is a good thing.

BIOGAS PRODUCTION

Anaerobic digestion of algae to provide a source of fuel is an attractive possibility (Golueke *et al*. 1957). Co-digestion of sewage sludge with algae is an advisable practice because it can be carried out in existing digesters (Cecchi *et al*. 1996).

In our experiments a Bioprocess Control Automated Methane Potential Test System (AMPTS, Lund, Sweden) was used for measurements of methane production (Bioprocess Control 2009). The system consisted of 15 airtight bottles, each with a capacity of 500 mL and equipped with a slow rotating agitator. The bottles were immersed in a constant temperature water bath. Biogas produced in each bottle passed through an individual vial containing an alkali solution where several gas fractions such as CO_2 and H_2S were removed. The remainder gas which is methane was measured using a wet gas flow measuring device that worked according to the principle of liquid displacement and could monitor an ultra low gas flow. A digital pulse was generated when a defined volume of gas flowed through the device and a data acquisition system was used in conjunction with the flow cells in order to record, display and calculate the results. Volatile solids concentrations of inoculum, sludge and algae were measured before the batch digestion.

The tests were set up with 3 parallels for inoculum, 3 parallels for inoculum plus wastewater sludge, and 3 parallels for each combination of inoculum plus wastewater sludge plus algae. The amount of inoculum was identical in all bottles and the biogas production from the inoculum was subtracted to calculate the biogas production from wastewater sludge and algae. Standard deviations between replicates were ±2.4% at mesophilic temperatures and ±5.6% at thermophilic temperatures. The feed concentrations in the bottles were 17–18 g VS/L for the inoculum bottles, 20–21 g VS/L for the bottles with inoculum plus wastewater sludge, and 19–23 g VS/L for the bottles with inoculum plus wastewater sludge plus harvested algae.

Figure 5 shows results from anaerobic digestion over 12 days, 20 days, 30 days and 68 days at a mesophilic temperature of 37 °C. The *Chlorella sp*. algae were grown in WWTP reject water and effluent and were harvested without chemical addition using a laboratory centrifuge. The algae had been frozen and thawed, and both wastewater sludge and algae were tested with and without homogenization (rapid mixer with cutting blades) prior to digestion. The algae and wastewater sludge mixture contained 58% algae and 42% sludge as volatile solids (VS). Inoculum was from a WWTP with mesophilic digesters treating a combination of primary and biological sludge. Undigested wastewater sludge was a mixture of primary and biological solids from the same WWTP. Specific methane gas productions are shown in Figure 5 for wastewater sludge alone (tested separately) and for algae, assuming the specific methane production from wastewater sludge was the same in the algae-sludge mixture as for sludge alone. Homogenization had no effect on the wastewater sludge, and no gain in biogas production was seen by increased retention time. For the algae homogenization increased the biogas production by 19% when measured after 12 days, 14% after 20 days, 12% after 30 days and by less than 9% when measured after 68 days. Increasing the retention time from 12 to 68 days increased the biogas production by 28% without homogenization, and by 17% with homogenization. The specific methane gas production (mL CH_4/g VS fed) for frozen and thawed algae after 12 days was less than 65% of the specific methane gas production for wastewater sludge. This increased to 90% of the specific methane gas production for wastewater sludge after 68 days if the algae had been frozen, thawed and homogenized.

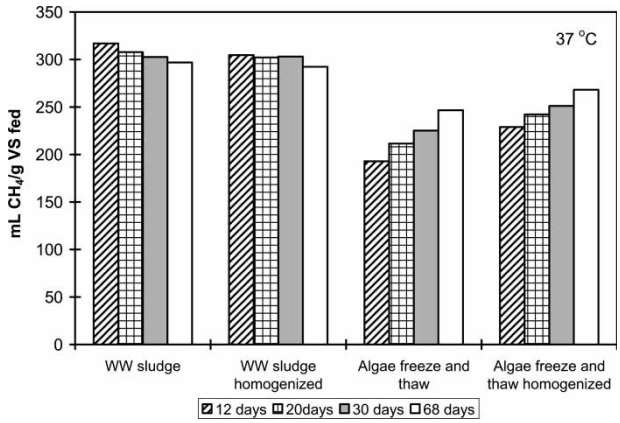

Figure 5 | Methane gas production for wastewater sludge and algae at 37 °C.

Results from anaerobic digestion over 10, 20 and 30 days at a thermophilic temperature of 55 °C are shown in Figure 6. The *Chlorella sp*. algae were grown in WWTP reject water and effluent and were flocculated with 25 mg/L Zetag 7550 polymer for harvesting. Fresh algae were used, and the algae were homogenized (rapid mixer with cutting blades) prior to digestion. The algae and wastewater

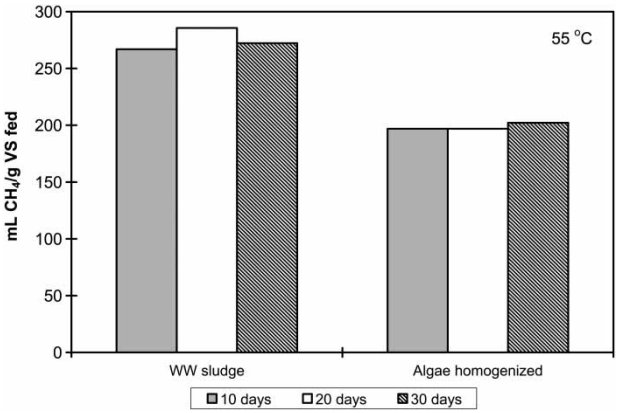

Figure 6 | Methane gas production for wastewater sludge and algae at 55 °C.

sludge mixture contained 69% algae and 31% sludge as volatile solids (VS). Inoculum was from a WWTP with thermophilic digesters treating solids from chemically enhanced primary clarifiers. Undigested wastewater sludge was enhanced primary solids from the same WWTP. Specific methane gas productions are shown in Figure 6 for wastewater sludge alone (tested separately) and for algae, assuming the specific methane production from wastewater sludge was the same in the algae-sludge mixture as for sludge alone. No significant difference in biogas production could be seen after 10, 20 or 30 days. In general the specific methane gas production (mL CH_4/g VS fed) was lower for both wastewater sludge and for algae than previously shown for the mesophilic digestion in Figure 5. The advantage of the thermophilic temperature in Figure 6 was that the maximum gas production for algae was achieved at short detention times. The specific methane gas production (mL CH_4/g VS fed) for homogenized algae after 10 days was 74% of the specific methane gas production for the wastewater sludge.

CONCLUSIONS

Proof-of-concept has been demonstrated for a process that will utilize nutrients from sludge liquor, natural light, and CO_2 from biogas to grow microalgae at wastewater treatment plants. The microalgae will remove nitrogen and phosphorus from the sludge liquor, reducing the impact of returning sidestreams to the head of the treatment plant. The produced algae will be harvested and fed to existing anaerobic digesters for co-digestion with wastewater sludge for increased production of renewable bioenergy.

Algae need light while dewatering of anaerobically digested sludge in centrifuges produces reject water that often is black, contains a lot of very fine solids particles, and has extremely low transmittance of light. Thus, pre-treatment of the reject water is necessary to improve transmittance and make it a suitable substrate for growth of algae. A pre-treatment procedure involving polymer addition + flocculation + anthracite filtration was developed, improving light transmittance for reject water from the FREVAR WWTP from 0.1% T to 77% T (670 nm wavelength, 1 cm path).

Chlorella sp. microalgae were found to be suitable for growth in pre-treated reject water that was undiluted. Typical nitrogen removal was 80–90 g N/kg TSS of produced microalgae.

The *Chlorella sp*. microalgae in our tests had a median particle diameter of less than 4 μm. Chemically assisted flocculation was necessary for harvesting. Flocculation followed by straining through a 33 μm sieve cloth was very successful. It achieved 95 to 99% recovery of the produced algae when flocculated with 25 mg/L of Zetag 7550 cationic polymer, or 35 mg Al/L (PAX-XL60), or 35 mg Al/L (PAX-XL60) + 2 mg/L of Magnafloc 155 anionic polymer. Gravity straining produced a cake with 4.3 to 6.0% dry solids for harvested algae flocculated with PAX-XL60 or PAX-XL60 + Magnafloc 155. Algae harvested after flocculation with the cationic polymer Zetag 7550 did gravity drain poorly, and vacuum assisted straining was used, producing a cake with 11–12% dry solids. These are sufficiently high dry solids concentrations to feed the harvested algae directly to an anaerobic digester.

Harvested algae were anaerobically co-digested with wastewater sludge. The specific methane gas production (mL CH_4/g VS fed) for the algae varied from less than 65% to 90% of the specific methane gas production for the wastewater sludge, depending on digester temperature, retention time and pre-treatment of the algae biomass.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Biowater Technology AS (Tønsberg, Norway) and the Norwegian Research Council for funding this project. Tina Trydal is acknowledged for contributing a part of her Masters work from the University of Stavanger. Knut Lileng from FREVAR (Fredrikstad, Norway) is acknowledged for technical and material assistance for anaerobic digestion and other facilities. We are also thankful to Meng Wang and Diane Sheppard from the University of Massachusetts for

maintaining the cultures at FREVAR during their summer internship. Ocelie Kjønnø and Arne Lundar from Aquateam (Oslo, Norway) are acknowledged for their help with the methane potential tests.

REFERENCES

Bioprocess Control 2009 *Automatic Methane Potential Test System. Operators and Maintenance Manual. 1st version with modifications*. Bioprocess Control Sweden AB, Lund, Sweden.

Carlsson, A. S., Clayton, D., Moeller, R. & van Beilen, J. B. 2007 *Micro- and macro-algae: Utility for industrial applications*. Outputs from the EPOBIO (Realising the Economic Potential of Sustainable Resources-Bioproducts from Non-food Crops) project. CPL Press, Newbury, UK.

Cecchi, F., Pavan, P. & Alvarez-Mata, J. 1996 Anaerobic codigestion of sewage sludge: application to the macroalgae from the Venice lagoon. *Resources, Conservation and Recycling* **17**, 57–66.

Craggs, R. J., Sukias, J. P., Tanner, C. T. & Davies-Colley, R. J. 2004 Advanced pond system for dairy-farm effluent treatment. *New Zealand Journal of Agriculture Research* **47**, 449–460.

Golueke, C. G., Oswald, W. J. & Gotaas, H. B. 1957 Anaerobic digestion of algae. *Applied Microbiology* **5**, 47–55.

Graham, J. E., Wilcox, L. W. & Graham, L. E. 2008 *Algae*. 2nd edition. Benjamin Cummings, San Francisco, USA.

Grobbelaar, J. U. 2000 Physiological and technological considerations for optimizing mass algal cultures. *Journal of Applied Phycology* **12**, 201–206.

Hoffmann, J. P. 2002 Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology* **34** (5), 757–763.

Janus, H. M. & van der Roest, H. F. 1997. Don't reject the idea of treating reject water. *Water Science and Technology* **35** (10), 27–34.

Kebede-Westhead, E., Pizarro, C. & Mulbry, W. 2006 Treatment of swine manure effluent using freshwater algae: production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. *Journal of Applied Phycology* **18** (1), 41–46.

Mulbry, W., Kondrad, S., Pizarro, C. & Westhead, E. K. 2008 Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot scale algal turf scrubbers. *Bioresource Technology* **99**, 8137–8142.

Rusten, B., Sahu, A. K. & Vik, E. A. 2009 *A Sustainable Process to Capture and Store CO_2 to Produce More Renewable Bio-Energy*. Pre-Project for the Bilateral Industry Oriented Research Co-operation to the Research Council of Norway. Report No. 09–001, January 22, Aquateam, Oslo, Norway.

Shelef, G., Sukenik, A. & Green, A. 1984 *Microalgae Harvesting and Processing: A Literature Review*. A subcontract report to the USDOE. US Dept of Commerce, VA.

First received 30 December 2010; accepted in revised form 3 June 2011