Anaerobic co-digestion of sludge and microalgae grown in municipal wastewater – a feasibility study

J. Olsson, T. Forkman, F. G. Gentili, J. Zambrano, S. Schwede, E. Thorin and E. Nehrenheim

ABSTRACT

In this study a natural mix of microalgae grown in wastewater of municipal character was co-digested with sewage sludge in mesophilic conditions, in both batch and semi-continuous modes. The semi-continuous experiment was divided into two periods with OLR_1 (organic loading rate) of 2.4 kg volatile solids (VS) m^{-3} d^{-1} and HRT_1 (hydraulic retention time) of 15 days, and OLR_2 of 3.5 kg VS m^{-3} d^{-1} and HRT_2 of 10 days, respectively. Results showed stable conditions during both periods. The methane yield was reduced when adding microalgae (from 200 ± 25 NmL CH_4 g VS_{in}^{-1}, to 168 ± 22 NmL CH_4 g VS_{in}^{-1}) but VS reduction was also decreased by 51%. This low digestibility was confirmed in the anaerobic batch test. However, adding microalgae improved the dewaterability of the digested sludge. The high heavy metals content in the microalgae resulted in a high heavy metals content in the digestate, making it more difficult to reuse the digestate as fertilizer on arable land. The heavy metals are thought to originate from the flue gas used as a CO_2 source during the microalgae cultivation. Therefore the implementation of CO_2 mitigation via algal cultivation requires careful consideration regarding the source of the CO_2-rich gas.

Key words | biogas, dewaterability, Gompertz model, mesophilic, semi-continuous study, waste activated sludge

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INTRODUCTION

Municipal wastewater treatment plants (WWTPs) are commonly based on: 1. mechanical treatment with screens, grit chamber and primary settling; 2. biological treatment for removal of dissolved nutrients and organic material in an activated sludge process (ASP); and 3. a chemical polishing step where precipitation chemicals reduce the phosphorus and suspended solids in the outgoing water. In municipal WWTPs, the ASP is a large consumer of electricity due to the need for mechanical aeration. This process mainly supports the bacterial oxidation of organic material and the nitrification of nitrogen compounds to nitrate. The nitrate can be further denitrified in anoxic conditions to nitrogen gas (Tchobanoglous *et al.* 2014).

A complementary process to the ASP is the utilization of autotrophic microalgae for the addition of oxygen and the removal of dissolved nutrients in the wastewater (Selvaratnam *et al.* 2015). The oxygen produced by photosynthesis can then be used as an electron acceptor for the oxidation of biodegradable organic compounds by the heterotrophic activated sludge bacteria. Microalgae enhance nitrogen removal from the municipal wastewater by assimilating it into the microalgal biomass instead of releasing it to the atmosphere (Posadas *et al.* 2017). Moreover, microalgae utilize CO_2 and convert it to biomass. The use of microalgal photobioreactors as a CO_2 mitigation system has been suggested as a practical approach to reduce CO_2 emissions from waste gas from anthropogenic sources (Chiu *et al.* 2008). At a municipal WWTP, the biogas produced from anaerobic sludge stabilization can be combusted in a combined heat and power (CHP) system or upgraded to vehicle gas. The CO_2 from these systems can then be utilized by microalgae by bubbling the gas through the microalgal plant as described by Rusten & Sahu (2011).

The microalgae harvested from the biological step can be used as a co-substrate together with primary sludge and waste activated sludge (WAS) in anaerobic digestion as presented by Rusten & Sahu (2011). The methane potential of microalgae depends on the species and the cultivation conditions.

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According to Thorin *et al.* (2017), a majority of studies where microalgae are co-digested with sewage sludge in anaerobic conditions use batch tests in mesophilic conditions.

Wang *et al.* (2013) reported improved dewaterability of the digestate when microalgae are co-digested with sewage sludge. This improvement has an impact on the costs of running a full-scale WWTP since disposal of digested sludge is a large expense for a municipal WWTP. Microalgae can also affect heavy metal content in the digestate. According to Inthorn (2001), microalgae have high removal capacity for Hg, Pb and Cd in wastewater. It is therefore important to compare heavy metal levels in a digestate containing a microalgal substrate with regulatory limits for sewage sludge. The utilization of microalgae as a co-substrate with sewage sludge in anaerobic digestion should not obstruct the further use of the digestate as fertilizer on arable land.

In the present study, microalgae grown autotrophically on locally produced municipal wastewater were co-digested in mesophilic conditions together with WAS and primary sludge in both anaerobic batch and semi-continuous tests. Flue gas from a CHP plant was recycled as the source of CO_2 for the microalgae plant. The overall aim of the study was to investigate how the biomass generated in the microalgae process influenced the process stability, methane yield and digestibility in the anaerobic digestion. The dewatering properties of the digestate and changes in the heavy metals content were also investigated.

This case study will help to identify challenges and aid understanding of the full scale implementation of microalgal biomass in future municipal WWTPs.

MATERIALS AND METHODS

Microalgae were cultivated in a pilot-scale pond and used as a substrate together with primary sludge and WAS in an anaerobic batch experiment and a semi-continuous digestion experiment. The modified Gompertz model was used to obtain kinetic parameters for the anaerobic degradation of the sewage sludge and the microalgae. Capillary suction time (CST) analysis was used to investigate the dewatering properties of the digestate. The experimental conditions are summarized in Figure 1.

Substrates

The microalgae used in the study were cultivated in locally produced wastewater in a pilot-scale algae pond located near a power plant in Umeå, Sweden. The locally produced wastewater had a total nitrogen concentration of 21.4 ± 5.4 mg L^{-1} and a total phosphorus concentration of 2.5 ± 0.7 mg L^{-1} (mean ± sd of 59 samples collected over the duration of the cultivation period and colorimetrically analysed as previously reported (Gentili 2014)). The pilot plant is described in Zhu *et al.* (2015). The pond was one meter deep and had a total volume of 20 m^3. The microalgae were cultivated without any mixing, hence without any additional energy input aside from the incident sunlight. Cultivation took place from May to the middle of November. At the end of the growing period (end of October), algae settled on the bottom of the pond; the clear water was then drained from the pond using a siphon (without any energy demand) to a water level of 20 cm. After two weeks the remaining water was pumped out using a submersible pump. The concentrated algae slurry left on the bottom of the pond was collected in plastic containers using a wide shovel and immediately frozen at −20 °C to prevent microbial degradation. The algae were cultivated autotrophically without addition of CO_2 for four months, and with addition of flue gases from the local CHP plant (Umeå Energi, Umeå, Sweden) for the last two months. The flue gases had a CO_2 concentration of approximately 10%, and were added such that the pH was maintained at

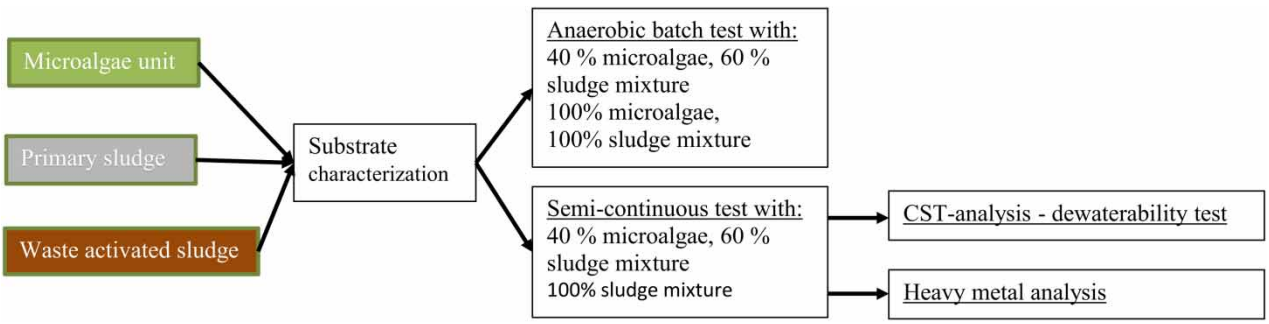

Figure 1 | Experimental conditions.

Table 1 | Parameters and methods used to analyze the substrates

Parameter	Method or standard
TS (Total Solids)	Standard technique with an oven at 105 °C for 24 h (APHA 1995)
VS (Volatile Solids)	Standard technique with an oven at 550 °C for 2 h (APHA 1995)
COD, CODs	Hach Lange 214 - LCK214 - COD mercury free cuvette tube cell vial test 100–1,000 mg L^{-1} O_2
VFA (Volatile Fatty Acid)	HPLC equipped with refractive detector and ion exchange 28 Rezer ROA. Separation was conducted at 60 °C and flow of 0.6 mL min^{-1} with 5 mM sulfuric acid as eluent
N-total	SS-ISO 13878
TKN (total Kjeldahl nitrogen)	Foss Techator AN 300
NH_4-N	ISO 11732/St. Methods 18th 4500B + E
C-total	SS 02 83 11
P-total	SS 02 83 11
Heavy metals (Pb, Cd, Cu, Cr, Ni Zn, Cr)	SS-EN ISO 11885-2:2009, SS-EN ISO 11885:2009
Hg	SS ISO 16772
Lipids	SBR analysis (Schmid-Bondzynski-Ratslaff) according to standard method No. 131 from the Nordic Committee of Food Analysis (NMKL 1989)

8.3. A light microscope (Optika B-353 LD2, Optika, Italy) was used to identify the algal strains in accordance with Bellinger & Sigee (2010).

The microalgal population was intended to represent wild algal strains grown under natural conditions in wastewater rather than laboratory-grown monocultures which are often used in similar studies (Wang *et al.* 2013; Ficara *et al.* 2014).

The co-substrate was a representative mixture of sewage sludge from a full-scale municipal WWTP in Västerås, Sweden. In order to create a representative mixture, defined ratios of primary sludge from pre-sedimentation and WAS from the biological ASP were mixed together (see Table 1). The ratios were based on the full-scale conditions at the WWTP. Primary sludge was taken from the bottom of the gravimetric thickener and WAS samples were taken after the mechanical thickener. Fresh sludge samples were collected once a week and kept refrigerated at +2 °C to prevent biological degradation. The sludge mixture was used in the semi-continuous digestion experiment (see below) together with the microalgae and in the anaerobic batch experiment to determine the biochemical methane potential (BMP).

Inoculum for anaerobic digestion

The inoculum in both the anaerobic batch experiment and the semi-continuous digestion experiment was mesophilic digested sludge from the municipal WWTP in Västerås, Sweden. The two full-scale digesters have a total volume of 3,400 m^3 and operate with a representative mixture of primary sludge and WAS. The mixture is composed of 60% primary sludge and 40% WAS based on volatile solids (VS). Before the mixture is fed into the digesters, it is thickened from 2% to 4–5% TS by gravimetric and mechanical thickening (Mälarenergi 2013).

In order to ensure degradation of the remaining easily degradable organic matter and to remove dissolved methane, the inocula were stored with an anaerobic headspace for 10 days at 37 °C prior to the start of the anaerobic batch experiment according to the method described by Angelidaki *et al.* (2009).

Cellulose with a known theoretical methane potential was used as a reference material in the anaerobic batch experiment to evaluate the activity of the inoculum. The theoretical biogas potential of the cellulose was 740–750 NmL g TS^{-1}. In order to validate the activity and suitability of the inoculum the measured biogas potential must reach 80% of the substrate's theoretical potential (VDI 4630 2006).

Anaerobic batch experiment

Anaerobic batch experiments were conducted to monitor the methane yield of the microalgae culture, the representative mix of primary sludge (60%) and WAS (40%), and a mixture of all three substrates. The proportions of the mixture of algae and sewage sludge were chosen based on the mixture that showed the highest methane potential (37% microalgae and 63% sewage sludge based on g VS content)

in Olsson *et al.* (2014). The results presented in this earlier study give an indication of how the implementation of algae affects various parameters during digestion and digestate handling. The experiment was performed according to the protocol described in Olsson *et al.* (2014) and was conducted in mesophilic conditions (35 °C) in 1 L conical bottles. Each bottle contained substrate equivalent to 3 g VS (corresponding to 4.3 g L^{-1}) and inoculum equivalent to 6 g VS (corresponding to 8.6 g VS L^{-1}) (total active volume 700 mL). All substrate mixtures and blanks (inoculum only) were run in triplicate and incubated for 50 days. The bottles were mixed manually every time gas samples were taken.

Biogas production was determined by measuring the overpressure in the bottles with a pressure gauge (model: GMH 3111) equipped with a pressure sensor (GMSD 2BR, −1,000 to 2,000 mbar). The volume of gas produced was calculated from the overpressure according to Equation (1). The calculated volume was normalized (1013.25 mbar and 273 K) according to Equation (2) (VDI 4630 2006).

$$V = \frac{(p_a + p_m) \cdot V_h}{p_a} - V_h \quad (1)$$

V: Calculated gas volume (mL)
p_a: Ambient pressure (mbar)
p_m: Measured pressure (mbar)
V_h: Headspace volume (mL)

$$V_0 = V \cdot \frac{(p_a - p_w) \cdot T_0}{p_0 \cdot T_a} \quad (2)$$

V_0: Normalized gas volume (NmL)
V: Calculated gas volume (mL)
p_w: Vapour pressure of the water as a function of the temperature of the ambient space (VDI 4630 2006) (mbar)
T_0: Normalized temperature; 273.15 K
p_0: Normalized pressure; 1013 mbar
T_a: Ambient temperature (K)

Each time the pressure was released from the bottles, a gas sample was taken for methane content analysis by gas chromatography (PerkinElmer Arnel Clarus 500; column: 7″ HayeSep N 60/80, 1/8″ SF; FID Detector 250 °C, carrier gas: helium, flow 31 mL/min, injector temperature: 60 °C; injection using Headspace sampler Turbo Matrix 110). The methane content was then multiplied by the biogas production to obtain the methane produced in each bottle. In order to determine the amount of methane from each substrate mixture the production in the inoculum bottles was subtracted from the production in the bottles containing substrates. The methane yield was obtained by dividing the production by the amount of VS substrate added to each bottle under standard conditions (NmL gVS^{-1}). Standard deviation was calculated for each set of triplicates.

Model of data fit

The modified Gompertz model has previously been used to obtain kinetic parameters in anaerobic degradability tests, see for example Olsson *et al.* (2014). This model was also applied in the present study, and can be expressed as:

$$B(t) = BMP \exp\left\{-\exp\left[\frac{R_m \cdot e}{BMP}(\lambda - t) + 1\right]\right\}, \quad (3)$$

$B(t)$: Cumulative methane yield (NmL CH_4 g VS^{-1}),
BMP: Ultimate methane yield (NmL CH_4 g VS^{-1} added)
R_m: Maximum methane production rate (NmL CH_4 g VS^{-1} day^{-1})
λ: Lag phase time (day)
t: Digestion time(day)
e: Euler's number ($e = 2.7182$)

The R^2 coefficient was calculated to evaluate the fit of the Gompertz equation to the experimental data.

The experimental data were fitted to Equation (3) using *fmincon*, a function in Matlab which finds the minimum value of a function with several variables and with linear and nonlinear constraints. In this case, the constraints included the minimum difference between the model and the experimental data, and that the parameters must be positive.

Semi-continuous digestion experiment

The system used for the semi-continuous digestion experiment consisted of two reactors for wet digestion (3–4% TS) with an active volume of 5 L (Figure 2). The reactors were stirred continuously at 200 rpm and had a temperature control system to maintain the digestate at 37 °C.

The system was fed manually with substrate once daily. To ensure that stable conditions were reached, constant organic loading rate (OLR) and hydraulic retention time (HRT) were applied for three retention times in each period. The substrate composition was also constant for both periods. To ensure stable conditions before commencing the study, the two digesters were fed with the same type and amount of substrate as the full-scale process and

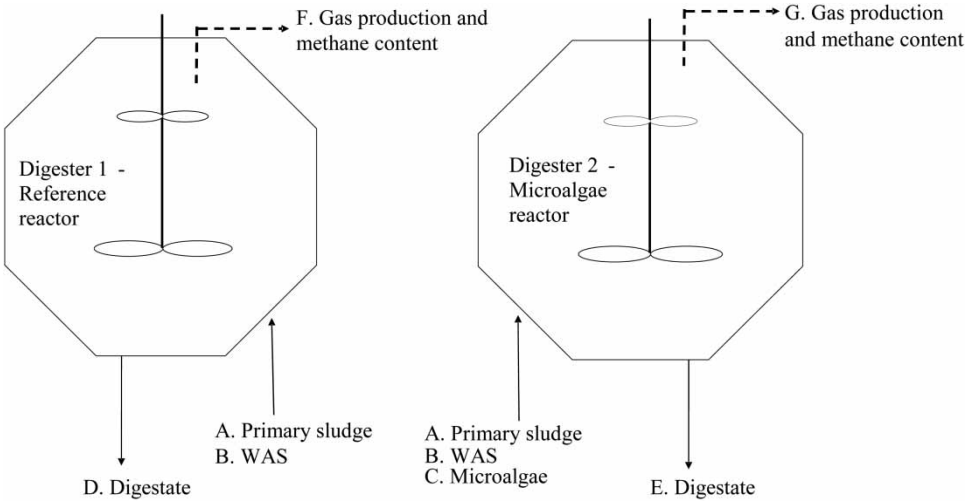

Figure 2 | Semi-continuous pilot digesters.

the methane production and the VFA content in the digestate was monitored. The system allowed online measurement of biogas production with a volumetric gas flow measurement device using a column and the water displacement method. Two inductive sensors were placed on the water column; the top sensor provided a signal to a three-way valve to release the gas, while the bottom sensor transmitted the signal to close the outlet. When sufficient gas was produced, the valve was activated to release the gas and allow the water column to settle. One cycle corresponded to 30 mL of gas produced. The methane content in the gas was measured continuously with a BlueSens CH_4 gas sensor (Manufacturer: BlueSens gas sensor GmbH), which contains an IR light source, a detector and the evaluation electronics. The reactor system was connected to the DOLLY© fermentation process control program, based on Wonderware® FactorySuite™ (Manufacturer: Belach Bioteknik AB), to regulate the process and store information from the reactors.

In this study, digester 1 was the reference reactor and digester 2 was the experimental reactor, see Figure 2. The proportions of the mixture of algae and sewage sludge were the same as used in the anaerobic batch experiment.

The experiment was divided into two separate periods, each with a duration of three retention times. In period 1, HRT = 15 days and OLR = 2.4 g VS L^{-1} d^{-1}, and in period 2 HRT = 10 days and OLR = 3.5 kg VS L^{-1} d^{-1}. The aim of increasing the loading in period 2 was to investigate the possibility of stressing the system. To ensure stable conditions before the second period started, methane production was monitored and VFA content was measured. Biogas production was normalized according to Equation (2) and methane content was normalized according to Equation (4) (VDI 4630 2006).

$$CH_{4_0}^{st} = CH_4 * \frac{p_a}{p_a - p_w} \quad (4)$$

$CH_{4_0}^{st}$: Normalized methane content (%)
CH_4: Measured methane content (%)

The VS removal in the two digesters was calculated according to Equation (5).

$$VS\ reduction = \frac{VS_{in} - VS_{out}}{VS_{in}}(\%) \quad (5)$$

VS_{in}: Incoming organic matter to the digesters (g d^{-1})
VS_{out}: Outgoing organic matter from the digesters (g d^{-1})

The methane yield was calculated by monitoring the methane production in the two digesters and the incoming VS feed.

Statistical significance of differences between the two digesters during HRT 1-6 were evaluated by one-way ANOVA using the computer software package SPSS 22 (SPSS Inc., Chicago, IL, USA).

Dewaterability study with CST analysis

According to Tchobanoglous *et al.* (2014), the polyelectrolyte required for conditioning of sewage sludge can be determined by tests that measure the index of the filterability of the digestates produced by the two digesters. This was done at the end of the semi-continuous study using a CST apparatus (Triton Electronics Ltd, UK) using a 1.8 cm diameter cylinder and Whatman No. 17 filter paper.

The digestates were treated with a cationic polyelectrolyte Zetag 8127 (BASF), which is also used in the full-scale WWTP for dewatering of digested sludge. The optimal dose of polyelectrolyte was first estimated by adding known amounts of polyelectrolyte to 100 mL of sludge from the full-scale plant, mixing, and evaluating the resulting floc formation.

The CST test was performed as described by Taylor & Elliot (2012). In order to measure the stability of the floc, the CST was measured after 10 s, 40 s, and 100 s of vigorous stirring of the sludge. Weak flocs were identified by a steep increase in the CST after stirring.

Analytical procedures

Substrate analysis

The microalgae, primary sludge and WAS were analysed for the parameters listed in Table 1.

The primary sludge and WAS were analysed for TS and VS every week when new material was taken from the sludge thickeners. TS and VS contents of the microalgal substrate were measured once at the beginning of the semi-continuous experiment. The microalgae were taken from one batch, and it was assumed that there was no biological degradation (since it was frozen) and therefore no change in TS and VS.

All the TS, VS, COD and VFA values in the results were average values of triplicate measurements.

The substrates were analysed for lipids, protein and carbohydrates in order to estimate the theoretical methane potential of the microalgae and the sewage sludge. Protein content was determined by the Kjeldahl method for organic nitrogen analysis, according to Salo-Väänänen & Koivistoinen (1996). The nitrogen content was multiplied by 6.25 (NH_4^+ deducted), which is the conversion factor used for calculation of protein in food samples (Salo-Väänänen & Koivistoinen 1996). Carbohydrates were estimated as the remaining portion of organic material for each substrate, calculated according to Equation (6).

$$\begin{aligned}&\text{Carbohydrate content } [W\%] \\ &= 100\ [W\%] - H_2O\ [W\%] - \text{inorganic content } [W\%] \\ &\quad - \text{lipids } [W\%] - \text{proteins } [W\%] \end{aligned} \tag{6}$$

In the present study, the following yields have been used based on the German standard VDI 4630 (2006): 1.000 NmL g VS^{-1} for lipids, 0.480 NmL g VS^{-1} for proteins and 0.375 NmL g VS^{-1} for carbohydrates.

Digestate analysis

The pH and electrical conductivity of the digestate (sampling points D-E in Figure 2) were measured daily in order to monitor the process stability. Weekly analyses of the parameters presented in Table 2 were performed on the digestate. Heavy metals analysis and micronutrients analysis were performed on the digestates on two occasions during the experiment.

All the TS, VS, COD and VFA values in the results are average values of triplicates.

The amount of free NH_3-N in the digestate was calculated from the NH_4-N content, pH and the temperature of the digester, according to Equation (7) (Gallert & Winter 1997).

$$NH_3 - N = \frac{NH_4^+ - N \cdot 10^{pH}}{e^{\left(\frac{6344}{273+T}\right)} + 10^{pH}} \tag{7}$$

$NH_3 - N$: Concentration of free ammonia (mg L^{-1})
$NH_4^+ - N$: Concentration of ammonium (mg L^{-1})
pH: pH-value
T: Temperature (°C)

RESULTS AND DISCUSSION

Substrate

Microalgae composition

Microscopic analysis of the microalgae revealed a diversified population as shown in Figure 3. The identified

Table 2 | Parameters and methods used to analyze the digestates

Parameter	Method or standard
pH	Metrohm 744 pH meter
TS	The content of total solids (TS) was determined using standard technique with an oven at 105°C for 24 h (APHA 1995)
VS	The content of volatile solids (VS) were determined using standard technique with an oven at 550 °C for 2 h (APHA 1995)
COD, CODs	Hach Lange 214 LCK214 - COD mercury free cuvette tube cell vial test 100–1,000 mg L^{-1} O_2
VFA	Hack Lange 365 Volatile Acids TNT plus Vial Test (50–2,500 mg L^{-1})
Total Alkalinity	Method described in Jenkins et al. (1991)
NH_4-N	Foss Techator AN 300

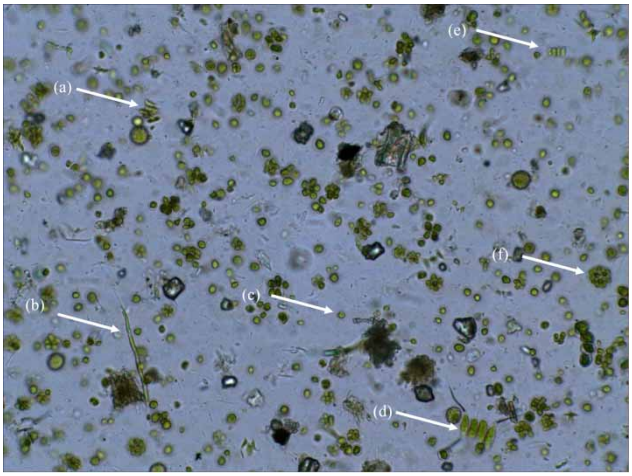

Figure 3 | Microalgae present in the substrate. (a) *Scenedesmus* sp., (b) *Ankistrodesmus* sp., (c) *Chlorella* sp., (d) *Scenedesmus opoliensis*, (e) *Scenedesmus quadricauda*, (f) *Coelastrum* sp., magnification: ×200. Illustration: F. Gentilli.

species included *Ankistrodesmus, Chlorella, Coelastrum, Scenedesmus opoliensis, Scenedesmus quadricauda* and *Scenedesmus* sp. Other species were also present but could not be identified.

Substrate analysis

The composition of the substrates is presented in Table 3.

The results in Table 3 indicate that the microalgal substrate was more stabilized (higher content of inorganics), with a lower VS/TS ratio than the primary sludge and WAS. The VS/TS ratio can influence the kinetics of the anaerobic batch experiment and the methane production as well as the VS removal in the semi-continuous experiment. The low VS-content in the microalgal substrate may be a result of the long SRT (sludge retention time) for the microalgae in the pond. A common method in municipal WWTPs is aerobic stabilization of excess biological material. The biomass is aerated for approximately 15 days creating a long SRT for the material in an oxygen rich condition. This reduces the VS-content in the biomass and the overall biosolids generated from the plant (Tonkovic 1999). A long SRT time for the microalgae can also cause the same stabilization with a lower VS-content in the substrate as a result.

The VS in the microalgal substrate may also be affected by the absence of light during settling and storage. In these conditions algae can start to consume stored organic molecules, thereby reducing the VS content in the substrate.

The theoretical methane potential of the substrates based on the carbohydrate, protein and lipid content were

Table 3 | Analysis of the three different substrates used in the semi-continuous experiment

Parameter	Primary sludge	WAS	Microalgae
TS (%)	5.4 ± 0.6	5.3 ± 0.3	8.4 ± 0.3
VS (% of TS)	76.7 ± 3.9	73.1 ± 3.6	59.2 ± 0.9
COD (mg L^{-1})	45,300 ± 3,700	48,800 ± 2,700	26,100 ± 4,500
CODs (mg L^{-1})	6,700 ± 480	4,500 ± 600	13,600 ± 3,500
Acetate (mg L^{-1})	330	220	210
Propionate (mg L^{-1})	170	n.d.	280
i-Butyrate (mg L^{-1})	n.d.	n.d.	n.d.
Butyrate (mg L^{-1})	n.d.	n.d.	n.d.
i-Valerate (mg L^{-1})	n.d.	n.d.	n.d.
Valerate (mg L^{-1})	n.d.	n.d.	n.d.
i-Capronate (mg L^{-1})	n.d.	n.d.	n.d.
Capronate (mg L^{-1})	n.d.	n.d.	n.d.
Lipids (% of TS)	8.91	5.54	3.02
TKN (g kg TS^{-1})	29.1	71.0	53.1
Protein [% of TS]	18.2	44.4	33.2
Carbohydrates [% of TS]	45.3	19.0	34.9
N-total [g kg TS^{-1}]	30.6	77.2	59.5
C-total [g kg TS^{-1}]	382	362	377
P-total [g kg TS^{-1}]	9.2	19.9	4.6
Zn [mg kg TS^{-1}]	260	240	1,700
Cu [mg kg TS^{-1}]	150	250	330
Ni [mg kg TS^{-1}]	12	16	40
Pb [mg kg TS^{-1}]	8.4	9.1	15
Hg [mg kg TS^{-1}]	0.26	0.18	0.76
Cd [mg kg TS^{-1}]	0.35	0.61	15

n.d. = not detected.

477 NmL g VS^{-1}, 488 NmL g VS^{-1} and 446 NmL gVS^{-1} for primary sludge, WAS and microalgae, respectively. These methane potentials were similar for all the substrates; however, availability and degradability may have varied during the digestion. This was also indicated by the CODs/COD ratio, which was significantly higher in the microalgal substrate (52%) compared to primary sludge (15%) and WAS (9%). This may increase the availability of a proportion of the organic matter in the microalgal substrate, thereby influencing the methane production in the

semi-continuous experiment. This high CODs/COD ratio may be due to the freezing of the microalgal substrate, which is known to increase the solubilization of the substrate, according to Samson & LeDuy (1983).

The calculated C/N ratios for the primary sludge, WAS and microalgae were 12.5, 4.5 and 6.4, respectively. Previous studies have indicated variable optimum C/N ratios in substrate mixtures for anaerobic digestion. For example, Mshandete *et al.* (2004) suggested a C/N ratio between 12 and 16 as an optimum ratio. A low C/N ratio can lead to high ammonia levels and can lead to inhibition of methane production, especially in thermophilic conditions (Caporgno *et al.* 2015). In the semi-continuous experiment, presented above, the substrate compositions in both digesters (with and without microalgae) had C/N ratios below the reported optimum ratio (digester 1: 9.38 and digester 2: 8.49).

The heavy metals content in the microalgae was much higher than in the other substrates. For example, the Cd^{2+} level was 42 times higher in the algae than in the primary sludge, and the Hg^{2+} level was three times higher in the algae than in the WAS. This may be due to uptake of heavy metals by the algae from the treated flue gases from the local CHP plant (Umeå Energi, Umeå). The metals could also have originated from the locally produced wastewater used in the cultivation, but this is unlikely since the wastewater was of municipal character. The heavy metal content in the digested sludge from the municipal WWTP in the same area as the wastewater used for the cultivation of the microalgae was much lower than in the microalgal substrate (UMEVA 2014). For example, the Cd^{2+} level in the digested sewage sludge was only 1.2 mg kg TS^{-1}.

According to Yin *et al.* (2008), the most volatile metal contained in biomass fuels is Hg^{2+}, which is completely vaporized in the flue gas or condensed on the surfaces of aerosols and fly ash particles. Cd^{2+} is also volatile and transferred to fly ash. Terry & Stone (2002) demonstrated efficient uptake of Cd^{2+} by *Scenedesmus* (one of the microalgae in the substrate). The mechanism of the effective removal of heavy metals from wastewater by microalgae has been suggested to be by adsorption onto the microalgal surface (Inthorn 2001).

Anaerobic batch experiment

The results from the anaerobic batch experiments showed that the methane potential of the microalgae was only 118.2 NmL CH_4 g VS^{-1} (Figure 4(a)), which is only 27% of the theoretical methane yield. A similar low methane potential was reported using dried microalgae substrate from the same pilot plant (Olsson *et al.* 2014). This methane potential was much lower than in the study by Frigon *et al.* (2013), where the methane potential of pure strains of different types of *Scenedesmus* and *C. vulgaris* ranged from 258 ± 7 to 410 ± 6 NmL CH_4 g VS^{-1}, and from 263 ± 3 to 361 ± 11 CH_4 g VS^{-1}, respectively at 37 °C.

Previously, co-digestion of microalgae and sewage sludge has improved the BMP of undigested sewage sludge significantly, particularly in mesophilic conditions (Olsson *et al.* 2014). This synergetic effect was not seen in the present study. The calculated BMP from the mono-digestion of the microalgal substrate and the sewage sludge was 232.2 ± 2.4 CH_4 g VS^{-1}, which is similar to the measured BMP when the two substrates were co-digested (237.1 CH_4 g VS^{-1}). Similar results were seen in the study by Caporgno *et al.* (2015), where the freshwater microalgae *Selenastrum capricornutum* was co-digested in mesophilic and thermophilic conditions with sewage sludge. The results showed that there was no synergetic effect between microalgae and sewage sludge. The low yields were attributed to microalgae species characteristics.

The methane potential of the sewage sludge in the present study was 319.0 CH_4 g VS^{-1}, which is comparable to the results presented for sewage sludge in Olsson *et al.* (2014).

When all three BMP curves are plotted (Figure 4(a)–4(c)), it is clear that there are two distinct exponential phases during which methane production increases. This is called a 'diauxie', and was described by Monod (1965).

A single modified Gompertz equation did not fit the experimental data well for the three BMP curves (100% microalgae: $R^2 = 0.85$, 100% sewage sludge: $R^2 = 0.81$, 58% sewage sludge and 42% microalgae: $R^2 = 0.81$). However, when two modified Gompertz equations were added together, they resulted in a function that fitted the experimental data much better, i.e. one with a higher R^2 coefficient. This function is presented in Equation (8). This methodology presents a new approach to describe diauxic degradation patterns in anaerobic digestion experiments.

$$B(t) = f_1 + f_2 = BMP_1 \exp\left\{-\exp\left[\frac{R_{m1} \cdot e}{BMP_1}(\lambda_1 - t) + 1\right]\right\} + BMP_2 \exp\left\{-\exp\left[\frac{R_{m2} \cdot e}{BMP_2}(\lambda_2 - t) + 1\right]\right\} \quad (8)$$

The first part of Equation (8) (i.e. f_1) represents the easily degradable organic material, and has a short lag phase and rapid degradation; the second part (i.e. f_2) represents the

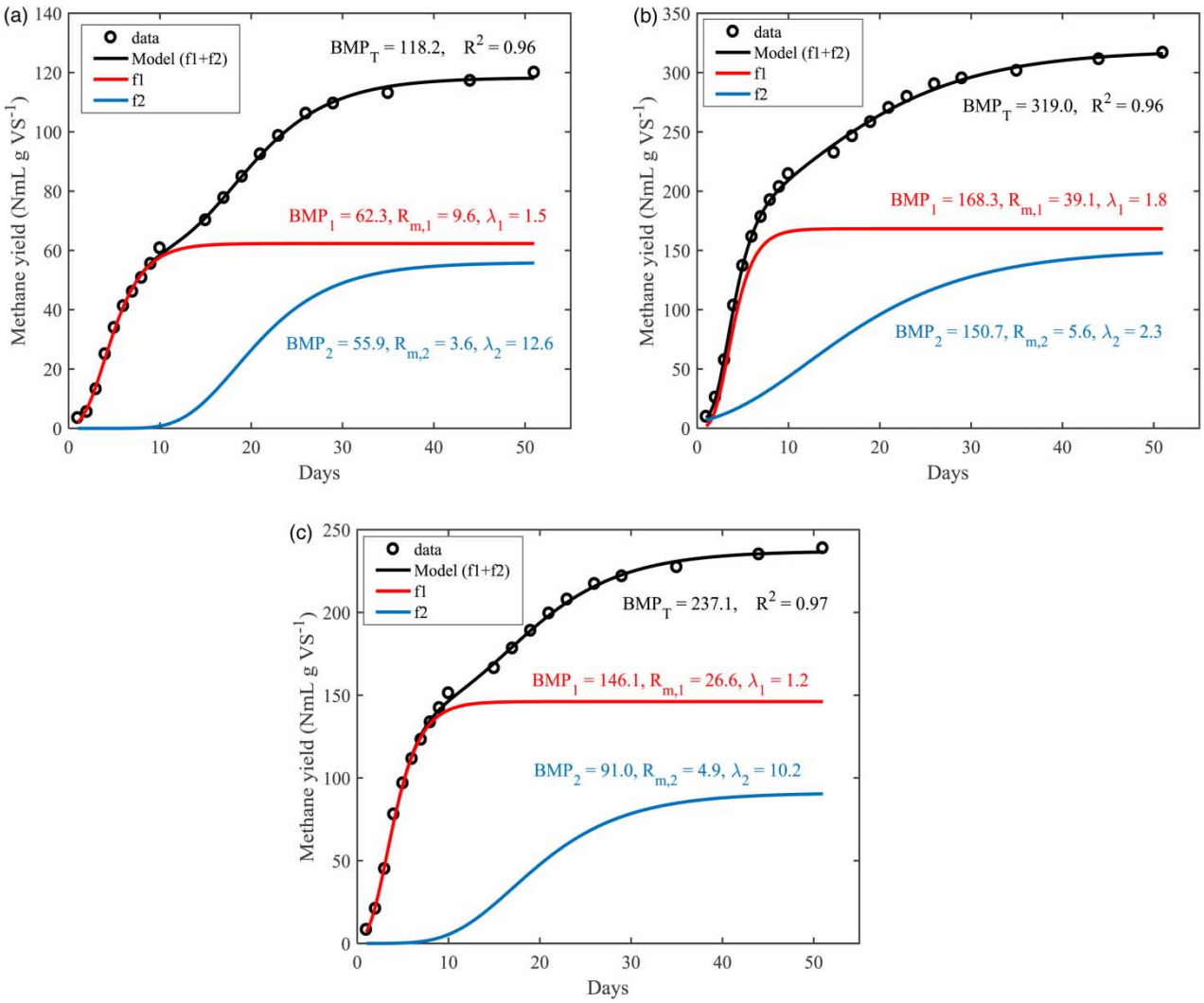

Figure 4 | Kinetic model (cf. Equation (8), black line) and experimental data (circles) for (a) 100% microalgae, (b) 100% sewage sludge, and (c) 58% sewage sludge and 42% microalgae. Parameters (BMP_1, R_{m1}, λ_1) refer to the function f1 (red line), parameters (BMP_2, R_{m2}, λ_2) refer to the function f2 (blue line). $BMP_T = BMP_1 + BMP_2$. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2017.583.

less accessible organic material. Figure 4(a) (100% microalgae) shows that BMP_1 (maximum methane yield for the easy available organic matter) was 53% of BMP_T (Total maximum methane yield). This was approximately the same as the proportion of CODs in the microalgal substrate (see substrate analysis, above). BMP_1 in Figure 4(b) (100% sewage sludge) was also approximately 53% of the BMP_T. The CODs for sludge were 15% and 9% for primary sludge and WAS, respectively. It can therefore be concluded that the sewage sludge had more particulate organic matter that was easily degradable by anaerobic digestion than the microalgal substrate. When the two substrates were co-digested (see Figure 4(c)), the BMP_1 increased to 62% of the BMP_T. It is therefore possible that co-digestion of microalgae and sewage sludge makes some of the hard degradable organic matter more easier to degrade.

The R_m values in Figure 4(a) and 4(b) indicate that the degradation rate was higher for the digestion of sewage sludge than for the digestion of microalgae. This may be due to a difference between the substrates in the availability of the organic matter. According to Schwede *et al.* (2013) the low availability of the microalgal substrate could be due to the robust cell wall structure of the microalgal cell.

Since λ_2 was larger when the microalgae were digested compared with the sewage sludge, it is possible that the microorganisms in the digestion needed an enzymatic adaption to degrade the organic matter in the co-digestion experiment, as in the case presented by Monod (1965).

Semi-continuous digestion experiment

Biogas production and composition of the biogas

During the first 3 HRTs with an OLR of 2.4 g VS L^{-1} d^{-1}, the normalized methane yields were 199.8 ± 24.7 NmL CH_4 g VS_{in}^{-1} and 168.2 ± 21.6 NmL CH_4 g VS_{in}^{-1} in digesters 1 and 2, respectively. During the second period (HRT 4-6) with an OLR of 3.5 g VS L^{-1} d^{-1}, the normalized methane yield decreased to 170.3 ± 17.2 NmL CH_4 g VS_{in}^{-1} and 157.5 ± 14.3 NmL CH_4 g VS_{in}^{-1} in digesters 1 and 2, respectively. The only statistically significant difference in the methane yield between the digesters was in HRT 6 (see Figure 5), but the tendency was towards a higher methane yield in digester 1. The full-scale digesters in Västerås WWTP have a methane yield of approximately 250 ml CH_4 g VS^{-1} (HRT of approximately 20 days).

The VS reduction was 50.8% in digester 1 and 25.1% in digester 2 during HRT 3 (stationary period) in period 1. In HRT 6 (stationary phase in period 2) the VS reduction was 44.3% in digester 1 and 31.1% in digester 2. The methane content in the biogas was consistently higher in digester 1 than in digester 2. During HRTs 1–3 the methane content was 63.7 ± 2.0% in digester 1 and 59.7 ± 1.2% in digester 2. During HRTs 4–6 the methane content was 63.1 ± 0.7% in digester 1 and 61.6 ± 0.8% in digester 2. The higher alkalinity (Table 4) in digester 1 indicates that more CO_2 was dissolved in this digester, which could cause the higher methane content.

The lower methane yields in digester 2 in both periods may be due to the smaller reduction of organic matter in digester 2, since the organic matter was more stabilized (see substrate analysis, above). The lower methane content in digester 2 differed from other studies where co-digestion of microalgae and sewage sludge has resulted in higher methane content in the biogas (Sialve *et al.* 2009). According to Mussgnug *et al.* (2010), different microalgae species give different results in both biogas production and methane content in the gas. Therefore, this difference may be accounted for by differences in the composition of the microalgal populations.

It can also be argued that the lower methane yield in digester 2 can originate from higher heavy metal content in the microalgae substrate making toxic conditions for the methane production in the anaerobic digestion. This is unlikely since it would influence the development of the methane potential of the microalgae in the anaerobic batch experiments (see Figure 4(a)). There would be a longer lag-phase if the heavy metals created toxic conditions for the anaerobic digestion. Moreover, the introduction of additional amounts of Ni^{2+}- and Cd^{2+}- ions in the study of Rosinska & Dabrowska (2014) did not disturb the proceeding of sewage sludge mesophilic digestion even if the levels of these two heavy metals were a lot higher in the digestate compared to the present study (see Table 5).

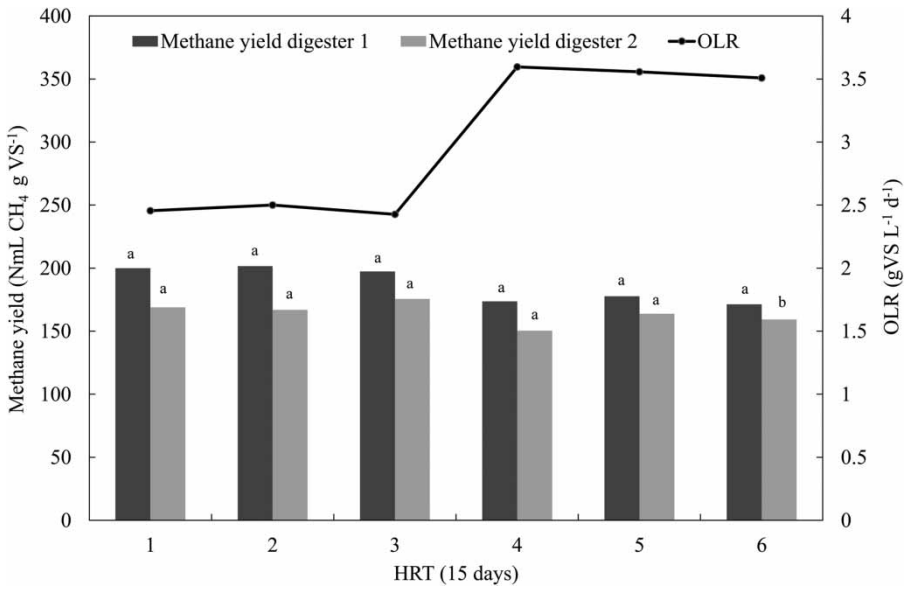

Figure 5 | Methane yield per incoming g VS for digester 1 (Reference digester) and digester 2 (Experimental digester). Statistically significant differences ($p = 0.05$) are indicated by different letters. The statistical analysis only compared the two digesters for each HRT and did not address differences between HRTs.

Table 4 | Stability analysis of the two reactors

	Period 1 (HRT 1-3)		Period 2 (HRT 4-6)	
Parameter	**Digester 1**	**Digester 2**	**Digester 1**	**Digester 2**
pH	7.51 ± 0.09	7.51 ± 0.06	7.14 ± 0.22	7.04 ± 0.27
VFA [mg L^{-1}]	190 ± 70	140 ± 30	150 ± 30	120 ± 10
NH_4-N [mg L^{-1}]	760 ± 60	690 ± 40	790 ± 130	730 ± 40
NH_3-N [mg L^{-1}]	37 ± 10	30 ± 6	13 ± 4	13 ± 7
Total alkalinity [mg $CaCO_3$ L^{-1}]	4,263 ± 147	3,921 ± 181	4,478 ± 320	3,718 ± 357

Stability analysis of the process

The evaluation of the stability of the process in the two reactors is presented in Table 4 for period 1 and period 2 (HRT 1-3 and HRT 4-6, respectively).

During both periods, the pH values were neutral in digesters 1 and 2, indicating stable conditions, even when the OLR was increased to 3.5 g VS L^{-1} d^{-1} and HRT of 10 days. A pH value below 6.8 can inhibit methanogens, leading to accumulation of VFA and lower methane production (Tchobanoglous *et al.* 2014).

According to Tchobanoglous *et al.* (2014), the total alkalinity should not be below 2,000 mg HCO_3^- L^{-1} in order to maintain a stable process. In both digesters and during both periods the total alkalinity was much higher than 2,000 mg HCO_3^- L^{-1}, indicating stable conditions.

The VFA content was low in both digesters during both periods. Earlier studies have shown that stable conditions in anaerobic digestion can be maintained with a VFA content of 2,520 mg L^{-1} (Yenigün & Demirel 2013). The low VFA content indicates that acidogenesis, acetogenesis and methanogenesis were balanced during both OLR periods. Further, the results indicate that a higher OLR could have been applied in both digesters.

Previous studies have indicated that high levels of NH_3-N (>100 mg 1^{-1}) may have an inhibitory effect on digestion (Yenigün & Demirel 2013). In the present study, the ammonia levels were much lower than 100 mg L^{-1}, as presented in Table 4.

A previous study by Samson & LeDuy (1983) also reported stable process conditions for semi-continuous co-digestion of microalgae and sewage sludge. Other earlier studies also indicate synergetic effects. However, these studies were performed under different operation conditions with respect to process configuration, loading rate, retention time and/or proportions of algae and sludge.

Digestate analysis

The heavy metals contents in the digestates from the two reactors are presented in Table 5 alongside Swedish regulatory limits for sewage sludge in SFS 1998:944 and US regulatory limits for sewage sludge in 40 CFR Part 503. If these limits are exceeded, the sewage sludge cannot be used as fertilizer on arable land.

Since stabilized sludge is a nutrient rich product that can be used as a fertilizer, it is desirable to maintain heavy metals below the limits in SFS 1998:944. Zn, Pb and Cd levels in digester 2 were above the limits in the Swedish regulations (indicated in bold in Table 5). These results were expected since the microalgae substrate had a much higher heavy metals content (see substrate analysis, above) (assumed to originate from the flue gas) than the sewage sludge. A reduction of the metal content in the microalgae could be achieved by using a different CO_2 source for the growth of the microalgae. One suggestion would be to use the CO_2 in the exhaust gas from a CHP system at a municipal WWTP as described by Sahu *et al.* (2013). This gas should have a much lower heavy metals content since the CO_2 comes from the anaerobic digestion of sewage sludge, which does not usually contain high levels of heavy metals as it is derived from municipal wastewater (Table 3).

Table 5 | Digestate analysis – heavy metals. values in bold exceed limits in the regulations

Parameter	**Digester 1**	**Digester 2**	**Regulation from SFS 1998:944**	**Regulation from 40 CFR Part 503**
Zn [mg kg TS^{-1}]	420	**1,350**	800	7,500
Cu [mg kg TS^{-1}]	310	345	600	4,300
Ni [mg kg TS^{-1}]	20	33	50	420
Pb [mg kg TS^{-1}]	15	**140**	100	840
Hg [mg kg TS^{-1}]	0.33	0.70	2.5	57
Cr [mg kg TS^{-1}]	22	40	100	–
Cd [mg kg TS^{-1}]	0.92	**10.3**	2	85

Dewaterability study with CST analysis

Table 6 presents the CST analysis of the sludge from both digesters. The dosage of polyelectrolyte was estimated at 12.5 g kg TS^{-1} in the first experiment. A second experiment was conducted with a lower polyelectrolyte dosage (6.6 g kg TS^{-1}) for the digestate in digester 2. This was done because the optimal dose seemed to be much lower than for the reference reactor (digester 1).

The results indicate that the first polyelectrolyte dose was sufficient to create a stable floc able to withstand rough treatment in a sludge dewatering unit. Similar results were also seen with the lower polyelectrolyte dosage for the digestate from digester 2, indicating that the dewaterability was improved by adding microalgae to the sewage sludge. Similar improvements in dewaterability were demonstrated by Wang *et al.* (2013) when adding 4% and 11% of algae (weight% by VS). However, Wang *et al.* (2013) found that when 41% microalgae was added the dewaterability was worse due to the increase in dissolved material in the digestate, whereas in the present study adding 42% microalgae improved the dewaterability. Polyelectrolyte dosage for dewatering of sewage sludge in dewatering units in full-scale applications varies depending on the molecular weight, ionic strength and activity level of the polyelectrolyte used (Tchobanoglous *et al.* 2014).

According to Novak *et al.* (2003), a release of biopolymers (proteins and polysaccharide) from sewage sludge in anaerobic conditions negatively influences the dewatering rate, the resistance to filtration and the amount of polyelectrolyte required. It is possible that adding the microalgal substrate to digester 2 in the semi-continuous experiment (see above) reduced the release of biopolymer. A possible explanation could be that a large proportion of soluble CODs in the microalgae was degraded during the digestion, while only a small proportion of the particulate COD was released as soluble biopolymers.

CONCLUSIONS

The study indicates that microalgae, cultivated in locally produced wastewater, could be implemented as a substrate in the anaerobic stabilization in a municipal WWTP and for biogas production. The semi-continuous digestion experiment showed stable conditions even when a higher OLR was applied. A smaller decrease in VS was observed in the digester containing microalgae, primary sludge and WAS because the microalgae was more stabilized. This is likely to have contributed to the lower methane yield in this digester. CST measurements indicated that the addition of microalgae enhanced the dewaterability of the digested sludge and lowered the demand for polyelectrolyte significantly. In the anaerobic batch experiments, no synergetic effects could be seen between the microalgae and the sewage sludge, but the kinetic model indicates that it is possible that co-digestion of microalgae and sewage sludge makes it easier to degrade some of the organic matter in the substrate. However, the high heavy metals content in the microalgae substrate increased the heavy metals content in the digestate, making it more difficult to reuse on arable land. A possible source of the metals could be the flue gas from power plants that was used as a CO_2 source. Thus, the implementation of CO_2 mitigation via algal cultivation requires careful consideration regarding the source of the CO_2-rich gas.

Table 6 | CST analysis in digesters 1 and 2

Parameter	Dosage (g kg TS^{-1})	CST at 10 s stirring (s)	CST at 40 s stirring (s)	CST at 100 s stirring (s)
Digester 1	12.5	238.6 ± 18.7	12.1 ± 0.5	19.9 ± 0.5
Digester 2	12.5	32.1 ± 7.4	11.9 ± 1.0	12.0 ± 1.8
Digester 2	6.6	67.1 ± 45.3	19.3 ± 2.3	16.1 ± 2.2

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REFERENCES

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P. & van Lier, J. B. 2009 Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* **59**, 927–934.

APHA 1995 *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, New York.

Bellinger, E. G. & Sigee, D. C. 2010 *Freshwater Algae: Identification and Use as Bioindicators.* Wiley-Blackwell, Oxford, UK. p. 271.

Caporgno, M. P., Trobajo, R., Caiola, N., Ibáñez, C., Fabregat, A. & Bengoa, C. 2015 Biogas production from sewage sludge and microalgae co-digestion under mesophilic and thermophilic conditions. *Renew. Ener.* **75**, 374–380.

Chiu, S.-Y., Kao, C.-Y., Chen, C.-H., Kuan, T.-C., Ong, S.-C. & Lin, C.-S. 2008 Reduction of CO_2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor. *Biores. Technol.* **99** (9), 3389–3396.

Ficara, E., Uslenghi, A., Basilico, D. & Mezzanotte, V. 2014 Growth of microalgal biomass on supernatant from biosolid dewatering. *Water Sci. Technol.* **69**, 896–902.

Frigon, J.-C., Matteau-Lebrun, F., Hamani Abdou, R., McGinn, P. J., O'Leary, S. J. B. & Guiot, S. R. 2013 Screening microalgae strains for their productivity in methane following anaerobic digestion. *Appl. Ener.* **108** (0), 100–107.

Gallert, C. & Winter, J. 1997 Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: effect of ammonia on glucose degradation and methane production. *Appl. Microbiol. Biotechnol.* **48** (3), 405–410.

Gentili, F. G. 2014 Microalgal biomass and lipid production in mixed municipal, dairy, pulp and paper wastewater together with added flue gases. *Biores. Technol.* **169**, 27–32.

Inthorn, D. 2001 *Removal of Heavy Metal by Using Microalgae.* Springer-Verlag, Tokyo, Tokyo.

Jenkins, S. R., Morgan, J. M. & Zhang, X. 1991 Measuring the usable carbonate alkalinity of operating anaerobic digesters. *Res. J. WPCF* **63** (1) 28–29.

Mälarenergi, A. B. 2013 *Environmental Report, Kungsängsverket 2013* Mälarenergi AB [in Swedish].

Monod, J. 1965 From enzymatic adaption to allosteric transitions. *Nobel lecture*, December 11.

Mshandete, A., Kivaisi, A., Rubindamayugi, M. & Mattiasson, B. 2004 Anaerobic batch co digestion of sisal pulp and fish wastes. *Bioresour. Technol.* **95**, 18–24.

Mussgnug, J. H., Klassen, V., Schlüter, A. & Kruse, O. 2010 Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* **150**(1), 51–56.

NMKL 1989 *Fat. Determination According to SBR in Meat and Meat Products,* Vol. 131, Nordic Committee on Food Analysis, Lyngby, Denmark.

Novak, J. T., Sadler, M. E. & Murthy, S. N. 2003 Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Wat. Res.* **37**, 3136–3144.

Olsson, J., Feng, X. M., Ascue, J., Gentili, F. G., Shabiimam, M. A., Nehrenheim, E. & Thorin, E. 2014 Co-digestion of cultivated microalgae and sewage sludge from municipal waste water treatment. *Biores. Technol.* **171** (0) 203–210.

Posadas, E., Muñoz, R. & Guieysse, B. 2017 Integrating nutrient removal and solid management restricts the feasibility of algal biofuel generation via wastewater treatment. *Algal Res.* **22**, 39–46.

Rosinska, A. & Dabrowska, L. 2014 Sewage sludge digestion at increased micropollutant content. *Chem. Eng. Res. Design* **92** (4), 752–757.

Rusten, B. & Sahu, A. 2011 Microalgae growth for nutrient recovery from sludge liquor and production of renewable bioenergy. *Water Sci. Technol.* **64** (6), 1195–1201.

Sahu, A. K., Siljudalen, J., Trydal, T. & Rusten, B. 2013 Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion. *J. Environ. Manag.* **122** (0), 113–120.

Salo-Väänänen, P. P. & Koivistoinen, P. E. 1996 Determination of protein in foods: comparison of net protein and crude protein (N× 6.25) values. *Food Chem.* **57**, 27–31.

Samson, R. & LeDuy, A. 1983 Improved performance of anaerobic digestion of *Spirulina maxima* algal biomass by addition of carbon-rich wastes. *Biotechnol. Lett.* **5**, 677–682.

Schwede, S., Kowalczyk, A., Gerber, M. & Span, R. 2013 Anaerobic co-digestion of the marine microalga *Nannochloropsis salina* with energy crops. *Biores. Technol.* **148** (0), 428–435.

Selvaratnam, T., Pegallapati, A., Montelya, F., Rodriguez, G., Nirmalakhandan, N., Lammers, P. J. & van Voorhies, W. 2015 Feasibility of algal systems for sustainable wastewater treatment. *Renewable Energy* **82**, 71–6.

Sialve, B., Bernet, N. & Bernard, O. 2009 Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol. Adv.* **27**, 409–416.

Taylor, M. & Elliot, H. A. 2012 Influence of water treatment residuals in dewaterability of wastewater biosolids. *Water Sci. Technol.* **67** (1) 180–185.

Tchobanoglous, G., Burton, F. L. & Stensel, H. D. 2014 *Wastewater Engineering: Treatment and Resource Recovery,* Volume 1, 5th edn. McGraw-Hill, Boston.

Terry, P. A. & Stone, W. 2002 Biosorption of cadmium and copper contaminated water by *Scenedesmus abundans*. *Chemosphere* **47**, 249–255.

Thorin, E., Olsson, J., Schwede, S. & Nehrenheim, E. 2017 Co-digestion of sewage sludge and microalgae – biogas production investigations. *Appl. Ener.* http://dx.doi.org/10.1016/j.apenergy.2017.08.085.

Tonkovic, Z. 1999 Aerobic stabilisation criteria for BNR biosolids. *Water Sci. Technol.* **39** (6), 167–174.

UMEVA. 2014 *Environmental Report, Öns avloppsreningsverk 2014*, Umeå Vatten och Avfall AB, [in Swedish].

VDI 4630 2006 *Fermentation of Organic Materials e Characterization of the Substrate, Sampling, Collection of Material Data, Fermentation Tests.* The Association of German Engineers, Düsseldorf, Germany.

Wang, M., Sahu, K. A., Björn, R. & Chul, P. 2013 Anaerobic co-digestion of microalgae *Chlorella* sp. and waste activated sludge. *Biores. Technol.* **142**, 585–590.

Yenigün, O. & Demirel, B. 2013 Ammonia inhibition in anaerobic digestion: a review. *Process Biochem.* **48** (5–6), 901–911.

Yin, C., Rosendahl, L. & AKær, S. K. 2008 Grate-firing of biomass for heat and power production. *Prog. in Energy and Combust. Sci.* **34** (6), 725–754.

Zhu, Y., Piotrowska, P., Van Eyk, P. J., Boström, D., Kwong, C. W., Wang, D., Cole, A. J., de Nys, R., Gentili, F. G. & Ashman, P. J. 2015 Co-gasification of Australian brown coal with algae in a fluidized bed reactor. *Energy and Fuels* **29**, 1686–1700.

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