

Microalgae as biological treatment for municipal wastewater – effects on the sludge handling in a treatment plant

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ABSTRACT

A mix of microalgae and bacteria was cultivated on pre-sedimented municipal wastewater in a continuous operated microalgae-activated sludge process. The excess material from the process was co-digested with primary sludge in mesophilic and thermophilic conditions in semi-continuous mode (5 L digesters). Two reference digesters (5 L digesters) fed with waste-activated sludge (WAS) and primary sludge were operated in parallel. The methane yield was slightly reduced ($\approx 10\%$) when the microalgal-bacterial substrate was used in place of the WAS in thermophilic conditions, but remained approximately similar in mesophilic conditions. The uptake of heavy metals was higher with the microalgal-bacterial substrate in comparison to the WAS, which resulted in higher levels of heavy metals in the digestates. The addition of microalgal-bacterial substrate enhanced the dewaterability in thermophilic conditions. Finally, excess heat can be recovered in both mesophilic and thermophilic conditions.

Key words | dewaterability, heat balance, heavy metals, microalgae, semi-continuous study, waste activated sludge

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ABBREVIATIONS

AD	Anaerobic digestion
ASP	Activated sludge process
CHP	Combined heat and power
CST	Capillary suction time
MAAS	Microalgal based activated sludge process
TS	Total solids
VS	Volatile solids
WAS	Waste activated sludge

INTRODUCTION

Contemporary municipal wastewater treatment plants (WWTPs) commonly utilize mechanical, biological and chemical treatment. The mechanical treatment produces a primary sludge and the biological treatment, traditionally the activated sludge process (ASP), produces waste-activated sludge (WAS) (Tchobanoglous *et al.* 2014). Aeration of the ASP accounts for a large part of the electrical consumption at a WWTP (50% according to Panepinto

et al. (2016)), and thus there is a strong incentive to make the biological treatment, and especially the aeration, more energy efficient.

One of the possible options for improving energy efficiency is the exploitation of microalgae as an alternative to bacteria in the biological treatment. Aeration via algal photosynthesis has the potential to reduce the energy demand for aeration, while retaining the reduction of pollutants from the wastewater, making this process solution attractive for municipal WWTPs. A microalgal-based biological treatment of municipal wastewater is the MAAS-process (microalgal based activated sludge process) presented by Nordlander *et al.* (2017). Here the microalgae release oxygen as a byproduct from photosynthesis. The oxygen is then utilized in the oxidation of biodegradable organic compounds and bacterial nitrification by activated sludge bacteria.

The biomass produced from microalgal biological treatment can be converted to biogas via anaerobic digestion. Co-digestion of microalgae and sewage sludge has been studied in both batch and continuous tests (Wang *et al.*

2013; Mahdy *et al.* 2015). Despite this there are insufficient comparative studies in which WAS is substituted by microalgae and co-digested with primary sludge in both thermophilic (50–57 °C) and mesophilic (30–38 °C) conditions. Such comparative studies are important when the ASP is replaced by a MAAS-process, or by any other biological treatment based on microalgae, in order to enhance the knowledge of the wastewater treatment from a system perspective.

The aim of present study was to compare, on a system level, how the anaerobic digestion and the following sludge treatment at a municipal WWTP could be influenced when the WAS was replaced by microalgal and bacterial biomass from a MAAS-process. System aspects considered included process stability, digestate quality and heat balance of the digestion. This case study can help to identify challenges when a MAAS-process is implemented as the biological treatment in a full scale municipal WWTP. Furthermore, benefits and challenges regarding the impact of digestion temperature, i.e. mesophilic and thermophilic digestion, on the mentioned aspects were evaluated. A higher digestion temperature can increase the methane yield (at unchanged hydraulic retention time (HRT)) and the degradation kinetics and consequently lower digester volumes or higher organic loading rates can be applied. However, thermophilic digestion is more sensitive to process instabilities and more importantly the heat demand of the process is increased and has to be compensated by higher methane production of the system (Yenigün & Demirel 2013).

In present study, a mix of microalgae and bacteria from a MAAS-process was co-digested with primary sludge from a full-scale municipal WWTP in a semi-continuous pilot system in both mesophilic and thermophilic conditions. The process stability and methane yield were evaluated. The dewaterability of the digestates was studied since the dewaterability of the digestate has a large impact on the running costs of a municipal WWTP. An earlier study by Wang *et al.* (2013) showed that co-digestion with microalgae can enhance the dewaterability of the digestate. Additionally, the quality of the digestates were analyzed, considering the heavy metals content, in order to evaluate how the digestates meet Swedish (SFS 1998:944) and US (40 CFR Part 503) regulatory limits for sewage sludge. Exceeding limits can hamper the applicability of the digestate as a fertilizer on arable land. A heat balance calculation comparing mesophilic and thermophilic digestion was performed from the results of the methane yield from the four digesters in order to identify optimal conditions of the process.

MATERIALS AND METHODS

Semi-continuous digestion experiment

The semi-continuous anaerobic digestion system consisted of four digesters presented in Table 1.

The active volume of the digesters in the semi-continuous experiment was 5 L. The temperature was maintained at a constant 55 °C in TherM and TherS, and at 37 °C in MesM and MesS. All four digesters were fed with substrate once a day, 6 days week⁻¹, with a double dose of substrate on Saturdays. During the 3 weeks before the experiment started, all four digesters were operated with the same substrate mixture (60% primary sludge and 40% WAS based on VS-content), the same organic loading rate (OLR) (2.4 g VS L⁻¹ d⁻¹) and the same HRT (21 days) as the full-scale digesters at Västerås WWTP in order to calibrate the methane production. The reference digesters (TherS and MesS) continued operating with the same substrate mixture throughout the study. In TherM and MesM the microalgae/bacteria material from the MAAS-pilot was substituted for the 40% WAS after 3 weeks' calibration time.

During the semi-continuous digestion experiment the HRT in all four digesters was 14 days and the OLR varied between 1–2.4 g VS L⁻¹ d⁻¹. To maintain comparable conditions, the same OLR was applied in all four digesters and was set by the amount of microalgae/bacteria that could be harvested from the MAAS-process. The study continued for 77 days, or approximately six HRTs. According to Schnürer & Jarvis (2017) semi-continuous experiments need to be maintained in the same conditions for at least three HRTs before the process is considered to be stable.

Substrates and inoculum

A microalgal-bacteria biomass was used from a MAAS-pilot with an active volume of 1 m³, a HRT of 6 days and a sludge retention time (SRT) of 20–25 days. Microscopic

Table 1 | Description of the operational details in the digesters

Digester	Operational temperature (°C)	Substrate mixture
TherM	55	Microalgal-bacterial biomass and primary sludge
TherS	55	WAS and primary sludge
MesM	37	Microalgal-bacterial biomass and primary sludge
MesS	37	WAS and primary sludge

investigation of the substrate from the MAAS-process was made by a light microscope (Optika B-353 LD2, Optika, Italy) to identify the algal strains in order to compare the active microalgal species with other studies. The incoming wastewater, used as substrate for the microalgal cultivation, was taken after the pre-sedimentation unit from a full-scale municipal WWTP in Västerås, Sweden (wastewater from 133,000 physical people). The primary sludge and the WAS used in the study was obtained from a full-scale municipal WWTP in Västerås, Sweden.

The inoculum used in the thermophilic digesters (TherM and TherS) came from a pilot digester operated with a constant loading of 60% primary sludge and 40% WAS (OLR of $2.5 \pm 0.14 \text{ g VS L}^{-1} \text{ d}^{-1}$ and HRT of 14 days) in 55°C for 60 days. The original inoculum in this digester was a combination of mesophilic digested sludge from the municipal WWTP in Västerås, Sweden (95 vol%) and sludge from a thermophilic biogas plant in Uppsala, Sweden (5 vol%) (the substrates digested in the biogas plant comprise of organic domestic waste and industrial waste). The inoculum for the mesophilic digesters (MesM and MesS) was taken from the mesophilic digester at the municipal WWTP in Västerås, Sweden.

For comparison purposes, data over the study period was collected from the full-scale digesters at the municipal WWTP in Västerås. The mixture of sewage sludge added to the full-scale mesophilic digesters contained 4–5.5% total solids (TS) and 75–80% volatile solids (VS) and was composed of 60% primary sludge and 40% WAS based on VS.

Analytical procedures

The parameters analyzed in the substrates and the methods and standards used were as follows:

1. TS: Standard technique with an oven at 105°C for 24 h (APHA 1995).
2. VS: Standard technique with an oven at 550°C for 2 h (APHA 1995).
3. Total Kjeldahl nitrogen (TKN): Foss Techator AN 300.
4. $\text{NH}_4\text{-N}$: ISO 11732/St. Methods 18th 4500B + E.
5. Lipids: SBR analysis (Schmid-Bondzynski-Ratslaff) according to standard method No. 131 from the Nordic Committee of Food Analysis (NMKL 1989).
6. Heavy metals (Pb, Cd, Cu, Cr, Ni, Zn, Cr): SS-EN ISO 11885-2:2009, SS-EN ISO 11885:2009.
7. Mercury (Hg): SS ISO 16772.

Triplicate samples were analyzed for TS and VS once a week. The theoretical methane yield for the substrates were

calculated from estimates of the contents of lipids, proteins and carbohydrates. The lipid content was determined by direct measurement on the substrates one time at the beginning of the study (HRT 1). The protein content was estimated from the TKN value (also measured at the beginning of the study) multiplied by 6.25 (conversion factor used for calculation of protein in food samples) according to Salo-Väänänen & Koivisto (1996). The carbohydrates were estimated as the remaining organic material for each substrate, calculated according to Equation (1). The inorganic content was estimated by using the standard technique for VS-measurement presented in APHA (1995).

$$\begin{aligned} \text{Carbohydrate content [W\%]} = & 100 [\text{W\%}] - \text{H}_2\text{O [W\%]} \\ & - \text{inorganic content [W\%]} - \text{lipids [W\%]} - \text{proteins [wt\%]} \end{aligned} \quad (1)$$

The methane yield was estimated from the theoretical methane yields for lipids, proteins and carbohydrates (VDI 2006). The following yields were used: $1.000 \text{ NmL gVS}^{-1}$ for lipids, $0.480 \text{ NmL gVS}^{-1}$ for proteins and $0.375 \text{ NmL gVS}^{-1}$ for carbohydrates.

The biogas produced was measured online (Ritter™ Milligascounter), and the methane content in the gas was measured continuously with a Bluescens CH_4 gas sensor (Manufacturer: BlueSens gas sensor GmbH, Herten, Germany). The biogas volume was normalized (1013.25 mbar and 273 K) according to equation presented in VDI (2006).

The digesters were equipped with a cooling system for the biogas. The gas and methane content were therefore measured on gas with a low moisture content. The methane yield was calculated weekly for each digester by adding up the measured methane production and dividing it by the incoming feed of organic matter. Statistical significance of differences between the two digesters during HRT 1–6 were evaluated by one-way analysis of variance (ANOVA) using the computer software package SPSS 22 (SPSS Inc., Chicago, IL, USA).

The VS reduction in the two digesters was calculated according to Equation (2).

$$\text{VS - reduction} = \text{VS}_{in} - \text{VS}_{out}/\text{VS}_{in}(\%) \quad (2)$$

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

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

The heavy metal content of the substrates were analyzed at the beginning (HRT 1) and at the end (HRT 6) of the

experiment to monitor the difference in the uptake of metals.

The TS and VS of the digestates were measured in the same way as for the substrates, in order to calculate the VS reduction in the four digesters. The stability of the digesters was monitored by analyzing the pH, COD, CODs, volatile fatty acids (VFA) and total alkalinity according to following the methods:

1. pH: Metrohm 744 pH meter.
2. COD, CODs: Hach Lange 214 - LCK214 - COD mercury free cuvette tube cell vial test 100–1,000 mg L⁻¹ O₂.
3. VFA: Hack Lange 365 - Volatile Acids TNTplus Vial Test (50–2,500 mg L⁻¹).
4. Total alkalinity: Method described in [Jenkins *et al.* \(1991\)](#).

All the TS, VS, COD and VFA values were measured in triplicate samples. The pH measurement was performed once a day and the other parameters were analyzed once a week.

The heavy metal content of the digestates were analyzed (with the same methods used as for the substrates) were performed on the digestates at the beginning (in HRT 1), middle (in HRT 3) and end (in HRT 6) of the semi-continuous experiment and compared with Swedish regulatory limits for sewage sludge in SFS 1998:944 and US regulatory limits for sewage sludge in 40 CFR Part 503.

The NH₄-N levels were analyzed (with the same method used as for the substrates) once a week in the digestates to monitor free NH₃-N levels. This analysis is important because high levels of free NH₃-N are known to have a toxic effect on the digestion process ([Kevbrina *et al.* 2011](#)). The NH₃-N was calculated from the NH₄⁻ N content, pH and the temperature in the digestate, according to Equation (3) ([Gallert & Winter 1997](#)).

$$NH_3 - N = NH_4 - N * 10^{pH} / \left(e^{6,344/(273+T)} + 10^{pH} \right) \quad (3)$$

NH₃ - N: Free ammonia nitrogen content (mg L⁻¹)

NH₄ - N: Ammonium nitrogen content (mg L⁻¹)

pH: pH in the digestate

T: Temperature in the digestate (°K)

Dewaterability test

A comparative dewaterability test was performed on the digestates with a capillary suction time (CST) apparatus from Triton Electronics Ltd, UK. The optimal dose of polyelectrolyte was first estimated by adding an increasing amount to 100 mL of digestate until a distinct separation

between water and sludge was seen. The slurry was then mixed and the resulting floc formation was recorded and evaluated. The type of polyelectrolyte used was the same as the one used for dewatering the digestate in the full-scale plant. The concentration of the polyelectrolyte was estimated at 0.2%. The experimental setup was the same as used by [Taylor & Elliot \(2013\)](#). To measure the stability of the floc, the digestates were stirred for 10 s, 40 s and 100 s in accordance with the instructions supplied by the equipment manufacturer. Weak flocs were identified by a steep increase in the CST after stirring. Stable flocs are important since the sludge in municipal WWTPs is most commonly dewatered by centrifugation ([Tchobanoglous *et al.* 2014](#)). The sludge samples were poured into the funnel and the CST was recorded six times for each sample.

Heat balance

A heat-balance calculation was performed to link experimental results of the methane yield from the four digesters to the energy required to heat the substrate and maintain the temperature of the full-scale digesters of the reference WWTP. In the heat requirements analysis, it was assumed that the digesters are cylindrical with a diameter of 15 m and a height of 10 m (3 m underground) and the construction details of the digesters are the same as presented in [Zupancic & Ros \(2003\)](#).

The heat energy produced (*Q_{CHP}*) from the biogas was estimated assuming the use of a combined heat and power (CHP) system manufactured by IET Energy GmbH, Austria (model IET 100 Bio) with a heat yield of 48.5%. The energy content of the biogas is assumed to be 6 kWh m⁻³ ([Deublein & Steinhauser 2008](#)). The calculations of the substrate heating assumed the use of a heat regeneration system from Lackeby Products AB. The system consists of a heat exchanger for incoming sludge and outgoing digestate with circular sludge channels surrounded by rectangular water circuits. The possible heat regeneration (*Q_{regen.}*) from the exchanger was calculated by the manufacturer in both mesophilic (37 °C) and thermophilic conditions (55 °C). The temperature of the incoming sludge for the heat regeneration was assumed to be 14 °C. The resulting temperature of the heat regenerated sludge entering the digester was calculated to be 22.1 °C and 35.4 °C in mesophilic and thermophilic conditions, respectively.

The heat consumption for heating the substrate to mesophilic and thermophilic conditions was calculated according to Equation (4) (presented in [Nordlander *et al.* \(2017\)](#)). It is assumed that heating is required for 15 m³ sewage sludge h⁻¹, with a TS content of 5.5% and VS content of 75% of

TS (data retrieved from the staff at the municipal WWTP). The temperature of the incoming substrate was based on winter (12 °C) and summer (19 °C) conditions at the reference WWTP.

$$Q_{\text{substrate}} = V_{\text{substrate}} \cdot \delta_{\text{substrate}} \cdot C_s \cdot (T - T_0) \quad (4)$$

$Q_{\text{substrate}}$: Heat required for the substrate (kWh)

$V_{\text{substrate}}$: Substrate volume (15 m³)

$\delta_{\text{substrate}}$: Substrate density (1,000 kg m⁻³)

C_s : Heat capacity of substrate (4.1855 kJ kg⁻¹ K⁻¹) (15 °C, 101.325 kPa)

T : 37 °C in mesophilic conditions and 55 °C in thermophilic conditions

T_0 : Temperature after the heat regeneration in mesophilic and thermophilic conditions

The heat losses through the digesters were calculated from Zupancic & Ros (2003). The heat loss from the sludge to the outside air, soil and groundwater were calculated according to Equation (5).

$$Q_{\text{heat losses}} = k_{\text{cout}} \cdot A_{\text{out}} \cdot (T - T_{\text{air}}) + k_{\text{cgrs}} \cdot A_{\text{gr}} \cdot (T - T_{\text{grs}}) + k_{\text{cgrw}} \cdot A_{\text{gr}} \cdot (T - T_{\text{grw}}) \quad (5)$$

$Q_{\text{heat losses}}$: Sum of all the heat losses through the digesters (kWh)

k_{cout} : Heat transfer coefficient from digestate to outside air (0.265 W m⁻² K⁻¹)

k_{cgrs} : Heat transfer coefficient from digestate to the soil (0.235 W m⁻² K⁻¹)

k_{cgrw} : Heat transfer coefficient from digestate to the groundwater (0.181 W m⁻² K⁻¹)

A_{out} : Digester surface from digestate to outside air (m²)

A_{gr} : Digester surface from digestate to the ground (m²)

T : Temperature of the digestate (37 °C or 55 °C)

T_{air} : Minimum outside air temperature (−20.3 °C in winter, +5.8 °C in summer)

T_{grs} : Standard temperature of soil (0 °C)

T_{grw} : Standard temperature of water (10 °C)

The minimum air and ground temperatures for winter and summer conditions were taken from Zupancic & Ros (2003), since the climate conditions are similar in Västerås (Sweden) and Domzale (Slovenia).

The resulting heat-balance calculation for the four digesters are presented in Equation (6).

$$Q_{\text{balance}} = Q_{\text{CHP}} + Q_{\text{regen.}} - Q_{\text{substrate}} - Q_{\text{heat losses}} \quad (6)$$

RESULTS AND DISCUSSION

Substrate analysis

Microscopic investigation of the microalgal-bacterial substrate from the MAAS-process showed a microalgae culture that was dominated by *Chlorella* sp., *Scenedesmus* sp. and filamentous *Cyanobacteria* as presented by Schwede et al. (2016). These microalgae species are considered tolerant to the conditions in municipal wastewater according to Pittman et al. (2011). The presence of these microalgae in the substrate therefore indicates that the results from the co-digestion experiment are representative for what can be expected in a possible real full-scale MAAS-process.

The composition of the primary sludge, WAS and microalgal substrate, as shown in Table 2, was compared to analyses from earlier studies in order to validate the properties of the substrates.

According to Miron et al. (2000), the composition of the organic matter for primary sludge was 9.7% lipids, 17.3% proteins and 51.3% carbohydrates, which is similar to the proportions in the present study. This indicates that the primary sludge used in the present study is a representative substrate. The WAS differs from that reported by Mahdy et al. (2015), which measured 0% lipids and 35% proteins but much higher carbohydrates content, i.e. 47%. The microalgal substrate from the MAAS-process had approximately the same composition as the microalgae in the study of Olsson et al. (2014) (3% lipids, 26% proteins and 31% carbohydrates), which was also cultivated on municipal wastewater. However, there was a significant difference

Table 2 | Substrate analysis

Parameter	Primary sludge	WAS	Microalgae
TS (%)	4.6 ± 0.6	5.1 ± 0.4	4.7 ± 1.8
VS (% of TS)	78.1 ± 2.4	74.1 ± 1.5	67.4 ± 4.2
TKN (g kg TS ⁻¹)	27.0	76.5	56.6
Lipids (% of TS)	10.3	6.3	3.7
Protein (% of TS)	16.9	47.8	35.4
Carbohydrates (% of TS)	54.1	18.1	27.9

with the composition of a *Chlorella* sp. monoculture presented by Kim & Kang (2015) (16% lipids, 67% protein and 6% carbohydrates). The monoculture was not cultivated on municipal wastewater and accordingly did not contain sludge bacteria. In addition, the higher SRT in the MAAS-process compared to algal monoculture systems results in lower amounts of storage compounds such as lipids and carbohydrates due to partial stabilization of the material as also shown by the lower VS content. The low VS content could consequently reduce the methane yield and the VS-reduction in the semi-continuous experiment making the substrate from the MAAS-process less attractive to digest.

The theoretical methane yields for primary sludge, WAS, microalgal substrate, the substrate composition in TherM and MesM and the substrate composition in TherS and MesS are presented in Table 3.

The analyses of heavy metals in the substrates are presented in Table 4. The results are compared with the results on heavy metals in the digestate presented in the section 'Digestate analysis'.

Table 3 | Theoretical methane yield

Substrate or substrate mixture	The theoretical methane yield (Nml g VS ⁻¹)
Primary sludge	475
WAS	499
Microalgal substrate	465
TherM	471
TherS	485
MesM	471
MesS	471

Table 4 | Substrate analysis: heavy metals

Parameter (mg kg TS ⁻¹)	Primary sludge		WAS		Microalgae	
	1	2	1	2	1	2
Analysis						
Zn	320	310	290	260	3,100	1,500
Cu	180	200	310	340	260	530
Ni	14	12	21	35	41	25
Pb	8.1	9	10	9.1	5.2	11
Hg	0.39	0.31	0.32	0.22	0.20	0.21
Cr	15	12	17	36	38	37
Cd	0.55	0.36	0.78	0.46	0.61	0.34

Analysis 1: beginning of the experiment, 2: end of the experiment.

The content of zinc in the microalgal-bacterial substrate was the only heavy metal that was significantly higher compared to the other substrates, both at the beginning (in HRT 1) and at the end of the experiment (in HRT 6). The probable cause seems to be leakage of zinc from the alloy on the stirrers in the MAAS-process. A comparative analysis between the reduction of zinc in the wastewater from the full-scale biological treatment and from the MAAS-process revealed a reduction of zinc in the full-scale plant of 82%, but an 11% increase of zinc in the MAAS-pilot.

Semi-continuous digestion experiment

Methane yield and VS reduction

The methane yield was higher in TherS than in TherM but the difference was only statistically significant in HRT 2 (Figure 1). The highest accumulated methane production during the whole experimental period was achieved by the thermophilic digester fed with primary sludge and WAS (TherS) with 13% higher production than the thermophilic digester fed with primary sludge and microalgae (TherM). The reason for the lower production in TherM compared to the production in the previous study by Varol & Ugurlu (2016) could be the lower VS content in the microalgal substrate in present study ($67.4 \pm 4.2\%$ compared to 82%). The possible aerobic stabilization in the MAAS-process described in the section 'Substrate analysis' could have a negative influence on the methane yield due to prior reduction of easily available organic matter. The lower VS reduction in TherM and MesM in both thermophilic and mesophilic conditions compared with the reduction in TherS and MesS support this hypothesis (see Figure 2).

When the thermophilic and mesophilic digesters were compared, the tendency was for the thermophilic digestion to give a higher accumulated methane production compared to the mesophilic digestion with both substrate compositions. The methane yield also tended to be higher in thermophilic conditions, but the only statistically significant difference was between TherS and MesS during HRT 2, 3 and 4. Caporgno *et al.* (2015) also concluded that the temperature significantly influenced biogas production when sewage sludge and microalgae were digested. It could be concluded that thermophilic digestion of both substrate mixtures with microalgae or WAS increase the methane yield making the higher temperature possibly more attractive in a system perspective since more biogas can be produced. However, the heat balance also needs to be considered

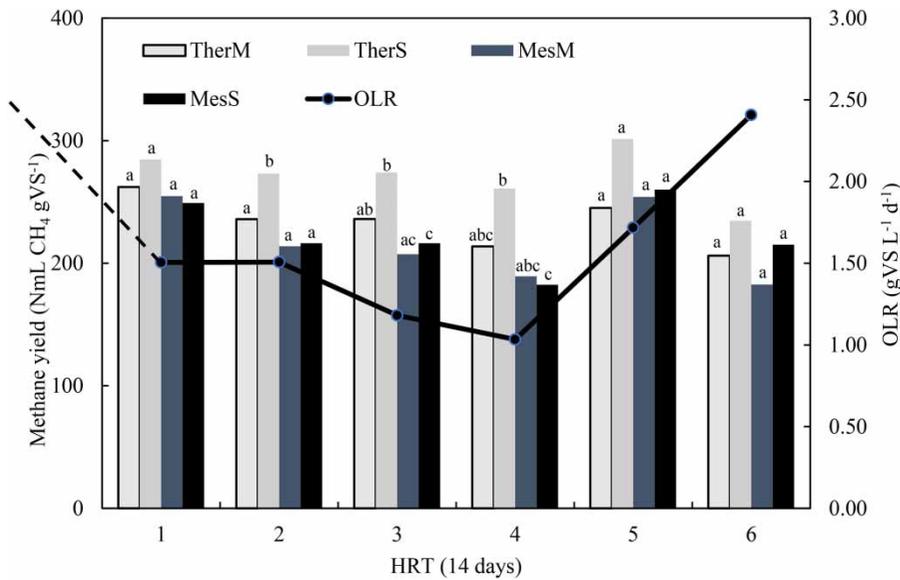


Figure 1 | Methane yield per incoming g VS for the four digesters. Statistically significant differences ($p \leq 0.05$) are indicated by different letters. Columns with no significant difference are marked with the same letter. For example, a column marked with all three letters a, b and c does not show any significant difference to other columns marked with a, b and/or c. This method was presented by Odlaire *et al.* (2011). The statistical analysis compared the four digesters with each HRT and did not compare different HRTs. (The dashed line describes the OLR in the digesters before the microalgal-bacterial substrate was applied.)

since a higher temperature requires more energy for heating up the substrate. It could also be concluded that no process imbalances related to the different temperature conditions could be seen.

The accumulated methane production in mesophilic conditions was approximately the same in both MesM and MesS, and there was no statistically significant difference between the methane yields during the entire experiment.

This contradicts results from Olsson *et al.* (2014) where a synergistic effect could be seen when 37% microalgae were added to sewage sludge and digested in mesophilic conditions. A possible reason for this may be that a more stabilized microalgal culture was digested in the present study.

Compared with the full-scale mesophilic digesters at the WWTP, the methane yields were $36 \pm 9\%$ and $34 \pm 8\%$

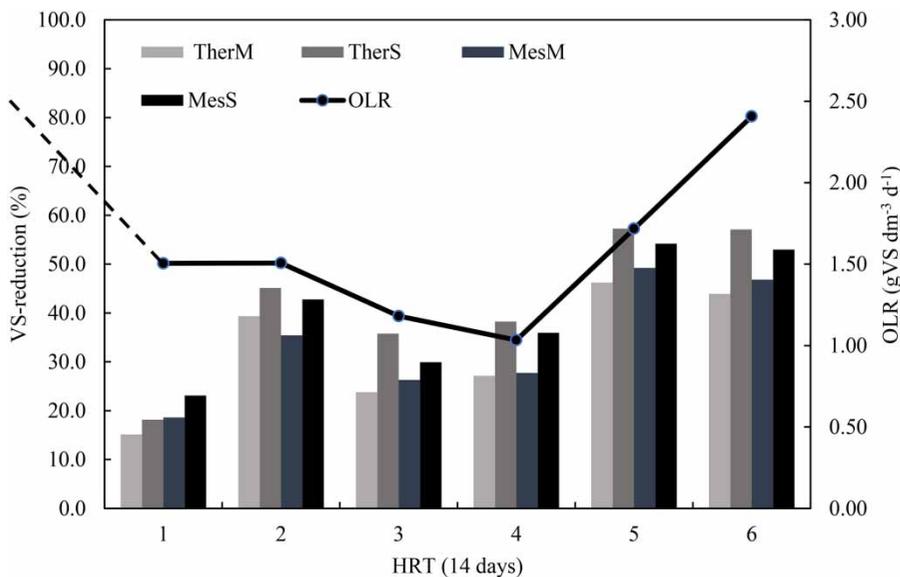


Figure 2 | VS reduction (%) for the digesters. (The dashed line describes the OLR in the digesters before the microalgal-bacterial substrate was applied.)

lower in the MesM and MesS, respectively. Possible reasons for this could be the lower HRT (6 days lower) used in the pilot digesters and that more stable continuous feeding could be applied in the full-scale digesters.

The low VS-reduction in all digesters in HRT 1–4 followed by high VS-reduction in HRT 5 and 6 indicated that steady state conditions in all digesters were only reached at the end of the experiment.

Stability analysis of the process

During the entire experiment, the pH and VFA in the digesters indicated stable conditions due to the low OLR in the digester. The previous study by Varol & Ugurlu (2016) also reported stable process conditions for co-digestion of microalgae and sewage sludge. The pH- and VFA values are presented in Table 5.

Stable conditions in anaerobic digestion can be maintained with a VFA content of 2,520 mg L⁻¹ according to Yenigun & Demirel (2013).

The total alkalinity and NH₄-N were fluctuating in all four digesters since the OLR changed but the HRT was constant during the experiment. However, the digestates with higher NH₄-N tended to have higher alkalinity. According to Labatut et al. (2014), additional alkalinity is produced due to increased protein degradation. The substrates in the

present study had a higher protein content in WAS compared with the microalgal substrate (see the section ‘Substrate analysis’), which corresponds to a higher nitrogen content in the substrate. This is in agreement with the study by Scherholz & Curtis (2013) where a compositional comparative analysis showed that the bacteria *E. coli* contained 9.6% nitrogen content by mass while the microalgae *Chlorella* sp. and *Chlamydomonas* only contained 4.6% and 5.8% nitrogen by mass, respectively. The higher protein content may explain the higher NH₄-N in TherS and MesS compared with TherM and MesM. A higher NH₄-N and total alkalinity was also observed in the thermophilic digestates, indicating increased protein degradation in thermophilic digestion compared to mesophilic digestion. Lower levels of NH₄-N in the substrate from the MAAS-process could be beneficial for the stability of the anaerobic digestion since high NH₄-N-levels could give toxic levels of NH₃-N, especially in thermophilic conditions.

Digestate analysis

The maximum levels of heavy metal content in the digestates from the four digesters are presented in Table 6, together with Swedish and US regulatory limits.

Since the digestate is a nutrient-rich product that can be used as a fertilizer, it is mandatory to maintain heavy metals below the limits established by SFS 1998:944. The only heavy metal exceeding the limits according to the Swedish regulations were Zn in TherM and MesM. This result was expected since there was much higher Zn content in the microalgal substrate (see the section ‘Substrate analysis’) (assumed to originate from the alloy on the stirrers in the MAAS-pilot). Even if no other heavy metals exceeded the limits, Cu, Ni and Cr were higher in the digestates TherM and MesM. This could have a negative effect on

Table 5 | pH and VFA in the digesters

Digester	pH	VFA (mg L ⁻¹)
TherM	7.29 ± 0.22	221 ± 82
TherS	7.47 ± 0.22	238 ± 69
MesM	7.16 ± 0.22	93 ± 22
MesS	7.21 ± 0.24	100 ± 22

Table 6 | Maximum heavy metal levels in digesters

Parameter (mg kg TS ⁻¹)	TherM	TherS	MesM	MesS	Regulation from SFS 1998:944	Regulation from 40 CFR Part 503
Zn	2,600	480	2,100	490	800	7,500
Cu	460	390	450	380	600	4,300
Ni	30	24	31	24	50	420
Pb	16	15	14	14	100	840
Hg	0.8	0.33	0.42	0.48	2.5	57
Cr	35	22	40	21	100	–
Cd	0.9	0.95	1.00	0.88	2	85

Values in bold exceed the limits of the regulations.

Table 7 | CST analysis for the digestates

Digester	CST at 10 s stirring (s)	CST at 40 s stirring (s)	CST at 100 s stirring (s)
Full-scale digester	23 ± 5	13 ± 1	24 ± 6
TherM	43 ± 12	46 ± 12	107 ± 59
TherS	155 ± 53	899 ± 93	1,362 ± 180
MesM	12 ± 1	15 ± 3	23 ± 2
MesS	12 ± 1	14 ± 1	22 ± 2

the potential to use this digestate on arable land in future, when there may be stricter limits on heavy metals.

Table 7 presents the CST analysis of the digestates from the full-scale digesters in Västerås WWTP and the four pilot-scale digesters. The dosage of polyelectrolyte in the experiment was estimated at 7.6 g kg TS⁻¹ for all digestates.

According to the manufacturer of the CST equipment, 20 s is an acceptable CST time for centrifugation of sewage sludge with a good stability of the floc. The dewaterability for the mesophilic digesters MesM and MesS was comparable with the full-scale process, with good dewaterability and stable flocs. There were no differences in the results when WAS was replaced by microalgal substrate, but it is possible that a polyelectrolyte dosage in the digestate from MesM could be reduced without affecting dewaterability, as presented in the study by Olsson *et al.* (2017). According to Wang *et al.* (2013) the dewaterability was improved when adding 4% and 11% of microalgae to sewage sludge.

Digestates from the thermophilic digesters had poorer dewaterability, with the worst result from the digestate

from TherS (Primary sludge and WAS). This is consistent with the study of Bouskova *et al.* (2006), which examined dewatering of sludge treated at operating temperatures of 33, 35, 37, 39 and 55 °C. The reduced dewaterability in thermophilic conditions was attributed to higher proportions of colloidal flocs. The better results in TherM indicate that the implementation of microalgae in thermophilic digestion can improve the dewaterability.

Heat balance

The methane yields used in the heat calculations are mean values from the six HRTs in the digesters. The biogas production in Table 8 is based on a flow of 15 m³ h⁻¹ of sewage sludge with a TS content of 5.5% and a VS content of 75%.

The heat demand for summer and winter conditions, the heat regeneration in mesophilic and thermophilic digestion and the final heat-balance calculation for each digester are presented in Table 8.

The results in Table 8 show that the heat produced from the CHP system is sufficient to provide a positive heat balance in nearly all operational conditions except for the thermophilic digestion of microalgal-bacterial substrate and primary sludge. However, when a heat regeneration system is implemented, even more heat can be used elsewhere in the WWTP. The lower methane yield in the digesters using microalgae in the substrate contributed to a lower positive heat balance both in thermophilic and mesophilic conditions, which makes the thermophilic digestion (in this setup) unfeasible (unless you have a free heat source for heating up the digester) because you get a

Table 8 | Results from the heat balance calculation

Digester	Q _{regen.} (kW)	Q _{substrate} (kW)	Q _{heat losses} (kW)	Q _{balance} without regeneration (kW)	Q _{balance} with regeneration (kW)
Winter conditions					
TherM	324	723	34	-57	+267
TherS	324	723	34	+58	+382
MesM	246	414	24	+214	+460
MesS	246	414	24	+232	+478
Summer conditions					
TherM	324	609	27	+64	+388
TherS	324	609	27	+179	+503
MesM	246	298	17	+337	+583
MesS	246	298	17	+355	+601

negative heat balance for your process. The conclusion would then be to use regeneration or a mesophilic process. As presented by Zupancic & Ros (2003), the heat losses from the digester are just a small part of the total heat requirements when heating the contents of the digester. This means that the digester size only has a minor influence on the total heat requirements.

CONCLUSIONS

This system study showed that substitution of WAS for microalgal-bacterial substrate cultivated on municipal wastewater is feasible in thermophilic and mesophilic anaerobic digestion. The methane yield decreased slightly when the microalgal-bacterial substrate were co-digested with primary sludge in thermophilic conditions, but remained approximately the same in mesophilic conditions. The uptake of heavy metals in the digester was higher with the microalgal-bacterial substrate compared with the WAS, which could decrease the value of using the sludge as fertilizer. CST measurements indicated that the microalgal-bacterial substrate enhanced the dewaterability of the digestate in thermophilic conditions. Finally, a positive heat balance was achieved in both mesophilic and thermophilic conditions with and without heat regeneration.

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