Cultivation of *Scenedesmus acuminatus* in different liquid digestates from anaerobic digestion of pulp and paper industry biosludge

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**Abstract:**

Different undiluted liquid digestates from mesophilic and thermophilic anaerobic digesters of pulp and paper industry biosludge with and without thermal pretreatment were characterized and utilized for cultivating *Scenedesmus acuminatus*. Higher *S. acuminatus* biomass yields were obtained in thermophilic digestates (without and with pretreatment prior to anaerobic digestion (AD): 10.2±2.2 and 10.8±1.2 g L\(^{-1}\), respectively) than in pretreated mesophilic digestates (7.8±0.3 g L\(^{-1}\)), likely due to differences in concentration of sulfate, iron, and/or other minor nutrients. *S. acuminatus* removed over 97.4% of ammonium and 99.9% of phosphate and sulfate from the digestates. Color (74–80%) and soluble COD (29–39%) of the digestates were partially removed. Different AD processes resulted in different methane yields (18–126 L CH\(_4\) kg\(^{-1}\) VS), digestate compositions, and microalgal yields. These findings emphasize the importance of optimizing each processing step in wood-based biorefineries and provide information for pulp and paper industry development for enhancing value generation.
Keywords: wastewater treatment; pulp and paper industry; digestate characteristics; microalgal growth; nutrient recovery

1 Introduction

Due to environmental pollution and climate change, the European Union has promoted a binding goal of reducing greenhouse gas emissions by at least 40% in each member country by 2030 compared to 1990, including a 27% share of renewable energy for the EU (European Council, 2014). With the rapid growth of and heavy dependence on fossil fuels in Asia (Lee et al., 2017) as well as in other regions (e.g., North America, Latin America, and Africa) (Tan et al., 2017), a series of policies and legislations to encourage a low-carbon economy and green growth should be implemented. Biomass, which refers to all organic material originating from plants (e.g., algae, trees, and crops), can be converted into biofuels and energy carriers and is therefore a major renewable energy feedstock (McKendry, 2002). Compared with terrestrial plants, microalgae have great potential as a sustainable bioenergy feedstock due to, e.g., higher growth rates, no requirements for arable land, and the potential of wastewater treatment to recover nutrients (Guldhe et al., 2017).

However, before microalgae can be commercially utilized in low-value products such as energy and fuels (Arenas et al., 2017), higher biomass yields need to be generated to make the process more economically feasible. Since wastewater can provide the water and nutrients for the microalgae, many studies have been carried out to cultivate microalgae in different kinds of wastewaters, including municipal, agricultural, and industrial wastewater (Lv et al., 2017; Guldhe et al., 2017; Kinnunen and Rintala, 2016). Microalgal cultivation
in anaerobic digestion (AD) effluents, as a specific waste stream, has shown significant potential for biorefinery applications due to efficient nutrient removal and accumulation of high-value products (e.g., astaxanthin, carotenoids, and omega-3 fatty acids) in microalgal biomass (Polishchuk et al., 2015; Xia and Murphy, 2016). The integration of AD effluents from pulp and paper industry biosludge and microalgal cultivation (hereafter referred to as integrated AD&MC system) has been studied to produce biomass and recover nutrients from wastewater (Kinnunen and Rintala, 2016; Polishchuk et al., 2015). The results of our previous study (Tao et al., 2017) indicated the possibility of high-yield microalgal biomass production and efficient nutrient removal when Scenedesmus acuminatus was cultivated in liquid digestates from the AD of pulp and paper industry biosludge.

The pulp and paper industry is a water- and energy-intensive biomass-refining industry that typically treats its wastewaters in aerobic systems, which generate a large amount of primary sludge and biosludge. The AD of the generated sludge has gained increasing attention within the pulp and paper industry due to, e.g., methane production as a renewable energy (Kinnunen et al., 2015; Veluchamy and Kalamdhad, 2017) and the possibility for nutrient recovery. Thermal pretreatment prior to AD is one of the main approaches used to enhance methane production from pulp and paper industry biosludge (Kamali et al., 2016; Kinnunen et al., 2015). To understand the effect of thermal pretreatment temperatures (80 °C, 105 °C, 121 °C, and 134 °C) on the potential for methane production from biosludge in the pulp and paper industry, Kinnunen et al. (2015) carried out methane potential batch assays at 35 °C. They reported that methane production was increased by 39–140% compared to untreated biosludge with increasing pretreatment temperatures, except for methane production from biosludge treated at the lowest temperature, 80 °C,
which was lower than that obtained from untreated biosludge. However, although increased pretreatment temperatures increased methane production, costs and energy consumption increased as well (Kinnunen et al., 2015). To our knowledge, the first full-scale AD plant integrated with a pulp mill for digesting pulp mill sludge is currently being planned in Finland (Liikanen, 2016).

Previous studies have shown that biosludge with different treatments (pretreatment and AD) can result in different methane production yields and digestate compositions (Asunis, 2015; Kinnunen et al., 2015). To optimize an integrated AD&MC system for maximum bioenergy (methane and microalgal biomass) production, it is important to study each component and thus provide an overview of the AD&MC system itself. The aim of this work was to study *S. acuminatus* cultivation in various types of liquid digestates from the AD of pulp and paper industry biosludge, which to our knowledge has not been studied before. The objective was to provide scientifically and practically relevant information to pulp and paper industry biorefineries that consider implementing AD of biosludge and microalgal cultivation in the resulting liquid digestate. The following research questions were addressed: (1) How do different AD conditions change the composition of the digestates and in turn affect the growth of *S. acuminatus*? (2) Can *S. acuminatus* grow in and simultaneously remove nutrients from undiluted digestates from pulp and paper mill biosludge? The microalga *S. acuminatus* was chosen due to its high growth rate and ability to grow in various types of waste streams (Adamsson, 2000; Tao et al., 2017).

2 Materials and Methods
2.1 Microalgal strain and liquid digestates

Scenedesmus acuminatus (SAG 38.81) was obtained as a culture suspension from the SAG Culture Collection of Algae at the University of Göttingen, Germany. The stock culture was maintained in 100 mL of modified N-8 medium (Praveenkumar et al., 2014) in a 250-mL Erlenmeyer flask on an orbital shaker (150 rpm) and continuously illuminated using fluorescent lamps (Osram L 18W/965 Biolux, Germany) at a light intensity of 40 µmol photos m\(^{-2}\) s\(^{-1}\). Since there was no growth of S. acuminatus in the modified N-8 medium with an initial pH of 6.5, the pH was adjusted to 8.0 by adding 5 M NaOH. Based on a previous study by Xu et al. (2015), 8.0 is an optimal initial pH for the cultivation of Scenedesmus sp.

Four types of digestates characterized in this study were collected from anaerobic, semi-continuously fed, completely stirred tank reactors (5 L liquid volume) treating biosludge from a pulp and paper industry wastewater treatment plant (Asunis, 2015). Three different pulp and paper mill biosludge digestates used in the microalgal cultivation experiments of the present study were anaerobically digested at 55 °C (thermophilic digestate, T), anaerobically digested at 55 °C after thermal pretreatment at 121 °C for 10 min (pre-treated thermophilic digestate, Tp), and anaerobically digested at 35 °C after thermal pretreatment at 121 °C for 10 min (pre-treated mesophilic digestate, Mp). The fourth pulp and paper mill biosludge digestate referred to in this paper was anaerobically digested at 35 °C (mesophilic digestate, M) (Asunis, 2015) and utilized for the cultivation of S. acuminatus in our previous study (Tao et al., 2017). The digestates were centrifuged at 5200 rpm for 4 min, and the supernatant was filtered through a glass fiber filter (Whatman GF/A, UK).
After filtration, the liquid digestates (Fig. S1 in the Supplementary Material) were stored at 4 °C before being used.

The microalgal growth results with the mesophilic digestate (M) are not directly comparable to the three digestates used for microalgal cultivation in the present study because, in our previous study, *S. acuminatus* was grown in 1.5-times diluted mesophilic digestate M (Tao et al., 2017), whereas in this study *S. acuminatus* was cultivated in undiluted digestates. Therefore, growth yields of *S. acuminatus* in digestate M were not compared to the microalgal cultivation results obtained in this study. However, the composition of the digestate M was provided in order to show more clearly how the digestate characteristics change depending on the AD temperature and presence or absence of a pretreatment step.

### 2.2 Photobioreactors

*S. acuminatus* was grown separately in the three different digestates (digestate refers to liquid, filtered digestate) for 21 days in photobioreactors (four replicates with each digestate), which consisted of a 1-L glass bottle (Pyrex) sealed with a plastic cap, with two tubes penetrating the cap serving as the gas inlet and outlet. Air with 5% CO$_2$ (v/v) at a flow rate of 0.105 L min$^{-1}$ was sparged from the bottom by a glass distribution tube (porosity 0, $\varnothing$ 22 mm, Duran Group, Germany). The photobioreactors were continuously illuminated using white fluorescent lamps (Osram L 18W/965 De Luxe Cool Daylight, Germany) with a light intensity of 240 µmol photos m$^{-2}$ s$^{-1}$ (Xu et al., 2015) from two sides of the reactors. *S. acuminatus* was inoculated to the photobioreactors to provide an initial optical density (OD$_{680}$) of 0.2. The initial total culture volume in the reactors was 600 mL.
The temperature of the reactors was maintained at 22±2 °C. Water evaporated during the cultivation due to the constant sparging, and therefore distilled water was added to compensate for the evaporated water volume (marked with lines on the photobioreactors) each time before taking samples for analyses.

2.3 Analytical methods

The culture pH was measured using a WTW 330 pH meter (WTW, Germany) with a Slimtrode electrode (Hamilton, Germany). The light intensity was controlled by measuring the average value of six sites on two sides of the photobioreactors’ outer surface by a MQ-200 quantum meter (Apogee, USA).

Volatile suspended solids (VSS) were measured by filtering 10–15 mL of culture solution through a glass fiber filter (Whatman GF/A) to assess microalgal biomass production. Each filter containing the suspended solids was dried at 105 °C overnight, then weighed and burned in a 550 °C muffle furnace for 2 h before being weighed again. VSS was determined gravimetrically as the difference between the filters after treatment at these two temperatures. The supernatant after VSS filtration was used in the analysis of digestate color (OD\textsubscript{680}) and turbidity, soluble chemical oxygen demand (soluble COD), soluble biochemical oxygen demand (BOD\textsubscript{7d}), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and nutrient (N, P, S) concentrations. The OD was measured at a wavelength of 680 nm using a Shimadzu UV-1700 Pharmaspec spectrophotometer after proper dilution with distilled water to give absorbance values between 0.2–0.7. Turbidity was measured with a TN-100/T-100 turbidimeter. OD\textsubscript{680} was also measured from non-filtrated samples to assess microalgal biomass production (ODm\textsubscript{680}).
The growth rates were calculated using the following equation:

\[ \mu = \frac{\ln(X_t/X_0)}{t - t_0} \]  

where \( X_0 \) is the concentration of biomass measured as VSS (g L\(^{-1}\)) at initial time \((t_0)\) and \( X_t \) is the concentration of biomass at a specific time \((t)\).

Soluble COD was determined using a dichromate method according to the Finnish Standard SFS 5504. The determination of BOD\(_7\)s was achieved with a WTW OxiTop Control/OxiTop measuring system. DOC and DIC were measured with a total organic carbon analyzer (Shimadzu Model TOC-5000) with an ASI-5000 autosampler. \( \text{NH}_4^+ \)-N was measured with an ion-selective electrode (Thermo Scientific Orion ISE meter). The nutrients’ (ammonium, phosphate, and sulfate) removal rate was calculated as \( \text{NRR} = (C_0 - C_t) \ t^{-1} \), where \( C_0 \) is the nutrient concentration on day 0, and \( C_t \) is the nutrient concentration after decreasing to below 0.1 mg L\(^{-1}\), which represents > 99.9% nutrient removal. \( \text{NO}_3^- \), \( \text{NO}_2^- \), \( \text{PO}_4^{3-} \), and \( \text{SO}_4^{2-} \) were measured using an ICS-1600 ion chromatograph (Dionex, USA) with an AS-DV autosampler, Ion-Pac AS4A-SC anion exchange column, and ASRS-300 suppressor (2 mm). The system was operated in isocratic mode using an eluent containing 1.9 mM \( \text{Na}_2\text{CO}_3 \) and 1.7 mM \( \text{NaHCO}_3 \), and an eluent flow rate of 1 mL min\(^{-1}\).

3 Results and Discussion

3.1 Characteristics of the liquid digestates

The four pulp and paper industry biosludge digestates originating from digesters operating at different temperatures to treat biosludge with and without thermal pretreatment had
different characteristics (Table 1). The initial pH of all the digestates was above 8.0, and the buffering capacity was good because the pH remained relatively stable in all cultivations despite efficient ammonium utilization, the uptake of which usually decreases culture pH, as shown by, e.g., Goldman and Brewer (1980). The OD_{680} of the thermophilic digestates were higher than those of the mesophilic digestates. In addition, the OD_{680} of the digestates indicated that pretreatment leads to increased color, as their OD_{680} were slightly higher than those without pretreatment. Digestate Tp showed the darkest color (OD_{680}: 0.63±0.08; turbidity: 320 NTU) of all the digestates. However, the OD_{680} of digestate T (0.59±0.06) was higher than that of Mp (0.35±0.01), while its turbidity (280 NTU) was lower than that of Mp (290 NTU). The correlation between OD_{680} and turbidity is unclear, likely due to the different wavelengths used in the two measurements. Substances in the liquid digestates responsible for their color may include clay, silt, finely divided inorganic and organic matter, soluble-colored organic compounds, plankton, and other microscopic organisms (Wang et al., 2010). The turbidity of liquid digestates may vary, ranging from, e.g., 2960 to 51400 NTU in the liquid fraction of mainly manure digestates from 11 full-scale co-digestion plants (Akhiar et al., 2017). The turbidities of our samples were much lower than those in Akhiar et al. (2017), likely due to different sampling methods. In the study of Akhiar et al. (2017) the liquid fractions of the digestates were separated from the solids either by screw press, centrifugation or vibrating screen, whereas in this study digestates were centrifuged and then filtered through glass fiber filters with a nominal pore size of 1.6 µm. The dark color of the medium, which results in poor light penetration, is one of the issues that could reduce microalgal growth (Wang et al., 2010; Xia and Murphy, 2016). For example, in a study by Wang et al. (2010) where Chlorella sp. were cultivated in
a liquid fraction (filtered through glass microfiber filters with pore size of 1.5 µm) of anaerobically digested dairy manure (turbidity: 1800–1900 NTU) with different dilutions (10-, 15-, 20-, and 25-times) for 21 days, the inverse correlation between turbidity and specific algal growth rates (R² = 0.982) indicated that high turbidity may limit algal growth. However, dilution for the benefit of microalgal growth increases total wastewater treatment volume and might actually reduce microalgal growth due to a reduction in nutrients and trace element concentrations.

The thermophilic digestates (T and Tp) had on average 65 mg L⁻¹ higher ammonium concentrations compared with the mesophilic digestates (M and Mp). In addition, the pretreatment also led to increased ammonium concentration in the digestate especially in the case of thermophilic digestion. The digestate Tp had on average 100 mg L⁻¹ higher ammonium concentration than digestate T (Table 1). Ammonium was available in all the digestates as a nitrogen source for microalgal growth, while nitrate and nitrite concentrations were below 1.0 mg L⁻¹. The sulfate-S concentration in digestate Mp was much lower than corresponding concentrations in the other three digestates (Table 1). The total phosphorus content was similar (27–30 mg L⁻¹) in all the digestates, and approximately 50% of the phosphorus existed in the form of phosphate — except in digestate M, where the phosphate share was slightly higher (64.3%). Xin et al. (2010) have reported an optimal N/P ratio (mass per mass) for Scenedesmus sp. LX1 growth to range between 5 and 8, while Scenedesmus sp. in the study of Rhee (1978) required an N/P ratio of approximately 13.5 to grow without limitations by either nutrient. The optimal ratio is also species-specific. The N/P ratios of the digestates in this study ranged from 12 to 18 (Table 1) and were thus somewhat higher than the reported values. However, no extra
phosphate was added to the digestates since it did not help with microalgal biomass production or ammonium removal in the digestates of sewage sludge in our previous study (Tao et al., 2017).

A phenomenon similar to that with ammonium was observed with soluble COD values of the different digestates. The thermophilic digestates had higher soluble COD values than the mesophilic digestates; and when the digestates produced at the same digestion temperature were compared, those generated with pretreatment resulted in higher soluble COD values than those without pretreatment (Table 1). The BOD/soluble COD ratios were lower than 1:20 in the measured digestates (T, Tp, and Mp), which means that most of the organic material left in the liquid digestates after anaerobic digestion was not easily biodegradable. The DIC concentration (520–690 mg L⁻¹) of each digestate was higher than the corresponding DOC concentration (150–540 mg L⁻¹).

3.2 Cultivation of *S. acuminatus* in the liquid digestates

3.2.1 Microalgal biomass production

Microalgal biomass production as indicated by VSS in the three studied digestates (T, Tp, and Mp) was as shown in Fig. 1. The OD₆₈₀ and VSS had a positive correlate in each digestate (T: R² = 0.96; Tp: R² = 0.96; Mp: R² = 0.97). The final microalgal biomass concentration after 21 days of batch cultivation was higher with both thermophilic digestates (T, Tp: 10.2±2.2–10.8±1.2 g L⁻¹) than the concentration obtained with the mesophilic digestate (Mp: 7.8±0.3 g L⁻¹). Despite the relatively high initial ammonium concentrations (380–480 mg L⁻¹) in all cultures, no clear lag phase was observed in microalgal growth. The biomass concentration started to stabilize on day 15–18. *S.*
*acuminatus* in digestate Tp initially grew more slowly than in digestates T and Mp, likely due to its higher initial ammonium concentration potentially inhibiting or slowing down photosynthesis (Abeliovich and Azov, 1976) as well as poorer light penetration (due to the darker color of the digestate). Before day 9, the *S. acuminatus* biomass concentration in digestate T (6.0 g-VSS L\(^{-1}\) at day 9) was the highest, followed by *S. acuminatus* in digestate Mp (4.9 g-VSS L\(^{-1}\) at day 9) and Tp (4.4 g-VSS L\(^{-1}\) at day 9). After day 9 and day 15, the VSS concentration in digestate Tp exceeded that in digestates Mp and T, respectively. The highest specific growth rates for all digestates were obtained during different periods (Table 2). These values are relatively high, as previous studies have reported growth rates ranging from 0.41 to 1.06 day\(^{-1}\) (Diniz et al., 2017; Wang et al., 2010).

The results of this study show that liquid digestates from pulp and paper wastewater treatment plant biosludge digestion can support high microalgal biomass yields and thus confirm the results of our previous study (Tao et al., 2017). In addition, in this study high microalgal biomass concentrations were obtained in the liquid digestates without dilution. To our knowledge, this has not been reported before. The light path in this study was not optimized, but it was shown that the color of the digestates was not a problem in the simple cultivation systems used. Thus, the microalgae should also grow well in more optimized short-path photobioreactors without dilution of the digestate. Bacteria were observed in the cultures, which was expected since the digestates were not sterilized in this study. Thus, the measured VSS values did include some bacteria associated with the microalgae. However, majority of the biomass was likely microalgae. For example, Hulatt and Thomas (2010) found an increased number of bacteria during 30-day microalgal cultivation, but reported that less than 1% of carbon of the total biomass comprised of bacteria.
The influence from pulp and paper mill digestates on microalgal growth is also species-specific. For example, Kinnunen and Rintala (2016) previously reported that the highest biomass concentration (less than 0.2 g-VSS L$^{-1}$) was obtained with *Scenedesmus* sp. originating from Lake Pyhäjärvi (Tampere, Finland) in 4-times diluted liquid digestate from pulp and paper industry biosludge AD after optimizing the dilution. Although the biosludge used in Kinnunen and Rintala (2016) and in this study were from the same pulp and paper mill, the different characteristics of the digestates (likely due to changes in, e.g., wood source, pulp mill operation parameters, and seasons) and microalgal strains clearly affected the obtainable biomass quantity.

### 3.2.2 Nutrient removal from liquid digestates

*S. acuminatus* removed nutrients efficiently from the digestates (Fig. 2). The ammonium concentration decreased from an initial 380–480 mg L$^{-1}$ to less than 0.2–10 mg L$^{-1}$. The ammonium removal efficiency in the thermophilic digestates was over 99.9%, which was slightly higher than that obtained in the mesophilic digestate (97.4%). The pH fluctuated between 7.8 and 8.4 (Fig. S2 in Supplementary Material) and showed a decreasing trend likely due to ammonium uptake, which is known to reduce pH (Goldman and Brewer, 1980). The overall ammonium removal rates during the 21-day cultivation period were similar in all cultures (T: 18.3 mg L$^{-1}$ day$^{-1}$; Tp: 23.3 mg L$^{-1}$ day$^{-1}$; and Mp: 17.8 mg L$^{-1}$ day$^{-1}$). However, a clear change in the ammonium removal rate was seen in all digestates after day 7, likely due to exhaustion of phosphate and sulfate (Fig. 2). Ammonium removal rates before and after day 7 were 43.1 and 5.9 mg L$^{-1}$ day$^{-1}$, 34.5 and 17.7 mg L$^{-1}$ day$^{-1}$, and 26.0 and 13.8 mg L$^{-1}$ day$^{-1}$ for digestate T, Tp, and Mp, respectively. This finding indicates that the exhaustion of phosphate and sulfate from the cultures could slow
ammonium uptake as previously shown also by Xin et al. (2010). Several ammonium transformations (e.g., algal uptake, ammonia evaporation, bacterial growth, and nitrification) can occur in algae–bacteria consortium systems (González-Fernández et al., 2011). According to the average temperature (22 °C) and observed pH range (7.8–8.4), the theoretical fraction of unionized ammonia in all cultivations was 2.8%–10.3% (the equation used for calculation shown in Tao et al., 2017). In addition, only low levels of nitrate and nitrite (< 3 mg L⁻¹) were found in all cultivations. These data suggest that ammonium stripping and nitrification may have occurred, but that the main portion of the removed ammonium from the digestates was used for microbial growth.

Sulfate concentration increased in all cultures from day 0 to day 2 (Fig. 2c). The resulting sulfate likely originated from other sulfur compounds present in the digestates. During anaerobic digestion, sulfate can be converted to sulfide by sulfate-reducing bacteria, and result in the presence of H₂S and HS⁻ in the liquid phase (Cirne et al., 2008). H₂S and HS⁻ could be converted into sulfate during cultivation via chemical and biological reactions in the cultures supplied with air (Chen and Morris, 1972). Additionally, microalgae are capable of releasing enzymes that can split inorganic sulfur from organic compounds and make the sulfur available for algal growth (Giordano and Raven, 2014; Kertesz, 2000). After the initial increase, however, sulfate was completely removed by day 7-9. Phosphate removal, on the other hand, started immediately and phosphate was completely removed by day 7 in all cultures. The overall phosphate and sulfate removal rates were 2.28 and 2.39 mg L⁻¹ day⁻¹, 1.63 and 1.68 mg L⁻¹ day⁻¹, and 2.13 and 0.45 mg L⁻¹ day⁻¹ for digestates T, Tp, and Mp, respectively. The removal rates of both phosphate and sulfate in digestate T were the highest among all digestates. Phosphorus was likely removed from the digestates
through adsorption on the microalgal surface, intracellular uptake, and precipitation (Cai et al., 2013). In the present study, VSS continued to increase even though phosphate was no longer detected from the liquid digestates after day 7, which indicates that initial phosphorus level in the digestates was high enough to support microalgal growth.

Based on the results of this study, initial sulfate concentrations in liquid digestates could affect ammonium removal efficiency and microalgal biomass production. This hypothesis is supported by the fact that the cultivations in digestates T and Tp had similar initial sulfate concentrations (15–17 mg L\(^{-1}\)) that enabled over 99.9% ammonium removal and similar microalgal biomass production, while the different initial sulfate concentrations in digestates T and Mp (17 vs. 3 mg L\(^{-1}\)), which had similar initial ammonium concentrations, resulted in different ammonium removal efficiencies and algal biomass yield. Biological nitrogen (N) uptake is catalyzed during photosynthesis by nitrogenase, which contains iron–sulfur clusters (Zheng and Dean, 1994). A shortage of either sulfur or iron can, thus, decrease the microalgal growth rate (Kumaresan et al., 2017; Liu et al., 2008). Sulfate is a primary sulfur source for microalgae in aquatic environments, but the effect of sulfate concentration on microalgal growth has not been widely studied. Mera et al. (2016) reported that the growth of microalga *Chlamydomonas moewusii* was quite similar at sodium sulfate concentrations of 0.1–3 mM (SO\(_4^{2-}\)-S: 3.2–96 mg L\(^{-1}\)), but microalgal biomass yields were lower at higher and lower sodium sulfate concentrations. In a study by Lv et al. (2017), similar *Chlorococcum* sp. growth at SO\(_4^{2-}\)-S levels from 6–90.3 mg L\(^{-1}\) was obtained, but was much lower at 0 mg L\(^{-1}\) sulfate. Due to the small number of related studies, the effect of sulfate and combined effect of iron and sulfate on microalgal growth should be further studied in the future. However, it should be also noted that other
micronutrients and trace elements that were not measured in this study could have caused some differences in microalgal growth.

### 3.2.3 Soluble COD, DOC, DIC, and color changes

In this study, microalgal cultivation removed soluble COD and DIC to a certain extent; 29–39% removal and 47–57% removal, respectively (Fig. 3a, d). DOC acted somewhat contradictory to soluble COD, as the DOC level increased in the mesophilic digestate (Fig. 3c). Soluble COD removal efficiency from the thermophilic digestates (38% and 39%) was higher than that from the mesophilic digestate (29%). The total removed dissolved carbon (<1 g L\(^{-1}\)) from the digestates was lower than the total carbon present in the biomass (3.9–5.4 g L\(^{-1}\)), when assuming that approximately 50% of the total produced biomass is carbon (Chisti, 2008). Hence, the cultivation was mixotrophic as both organic and inorganic carbon was utilized, but mainly photoautotrophic as CO\(_2\) was the main carbon source used for microalgal growth.

COD represents the concentration of chemical oxidizer needed to oxidize all the oxidizable organic or inorganic materials in wastewater, and DOC is used to reflect the dissolved organic carbon content of a sample. In most microalgal studies, either DOC or COD has been measured during microalgal cultivation (Eloka-Eboka et al., 2017; Guldhe et al., 2017; Wang et al., 2010), yet the correlation between COD and DOC in microalgal cultures remains unclear. For example, Marjakangas et al. (2015) reported an increase in both soluble COD and DOC concentrations, likely due to a stress caused by an initial pH decrease after *C. vulgaris* CY5 was mixotrophically cultivated in anaerobically treated piggery wastewater. Thus, it seems that changes in COD and DOC depend on growth
conditions. In our study, organic carbon release from photosynthetic microalgal cells might explain the observed increase in DOC during the cultivations in mesophilic digestate. The decrease in soluble COD suggests that organic materials from the digestates were consumed during cultivation and that the amount of consumed materials was higher than the organic carbon released by the microalgae during normal photosynthetic growth. Some studies have reported relatively high COD removal efficiencies (75–80%) from liquid digestates integrated with microalgal cultivation (Yan and Zheng, 2014; Yang et al., 2015). Soluble COD in this study was not easily biodegradable and was not therefore fully removed. Further removal of soluble COD would be possible with non-biological treatments, e.g., chemical oxidation, if deemed necessary. However, further soluble COD removal would probably not be needed as the COD load (both low flow and COD concentration) from algae treatment reject waters would be minimal compared to the effluent COD load from the activated sludge plant the sludges originates, which may be up to tens of tons COD per day (e.g., Regional State Administrative Agency of Eastern Finland, 2016). Furthermore, in practice the effluent from algae treatment could be circulated to the beginning of the activated sludge process, as is typically done with dewatering reject waters after AD in municipal wastewater treatment plants.

The OD$_{680}$ of the digestates were measured after removing the microalgae to demonstrate their color change during cultivation (Fig. 3b). The OD$_{680}$ values in all digestates decreased until day 9 but remained stable afterward. At the end of the batch cultivations, the color removal efficiencies in T, Tp, and Mp were 80%, 74%, and 79%, respectively. The mechanism of color removal is not clear based on the results of this study. However, Graham and Wilcox (2000) suggested that lignin (one cause of color) could be converted
into other non-colored materials by microalgal metabolism. Tarlan et al. (2002) also reported that the main mechanism of color removal from pulping effluents with a mixed culture of microalgae was metabolic conversion of colored molecules to non-colored molecules rather than adsorption. Thus, the possible reason for the lower removal efficiency of COD (29–39%) than color (74–80%) in this study was that the colored organic molecules were converted into non-colored organic molecules.

3.2.4 Integration of methane production and microalgal cultivation in the digestate

To evaluate the different integrated AD&MC systems, the performance of each processing step is shown in an overview (treatment methods of biosludge, microalgal cultivation conditions, and bioenergy production) (Table 2). During the 21-day cultivation, approximately 35% more microalgal biomass (as VSS) was obtained in the thermophilic digestates than in the mesophilic digestate. This is a promising discovery, as methane production in thermophilic digestion with pretreatment was higher than that obtained in the corresponding mesophilic process; likewise, methane production in thermophilic digestion without pretreatment was also higher than that obtained in mesophilic digestion without pretreatment (Table 2). This finding indicates that the highest methane production and microalgal biomass yields can be obtained in the same integrated AD&MC system.

The effect of sludge pretreatment before digestion on microalgal cultivation is not, however, fully clear based on the results of this study. Asunis (2015) reported that thermal pretreatment increased the methane yield by 100% in thermophilic AD, while the increase was 460% in mesophilic AD. The difference caused by pretreatment prior to thermophilic digestion on microalgal biomass production in the digestate was not significant. Although
maximum methane and microalgal biomass production were obtained with the same process (thermophilic AD with pretreatment), other factors should be considered, including the cost and energy burden of thermal pretreatment.

4 Conclusions

The cultivation of *Scenedesmus acuminatus* was successful in different undiluted digestates from pulp and paper industry biosludge treated at different AD conditions (mesophilic vs. thermophilic, with and without thermal pretreatment). *S. acuminatus* grew well (7.8–10.8 g L\(^{-1}\)) and removed nutrients efficiently (over 97%) from all the digestates. Color (74–80%) and soluble COD (29–39%) were partially removed. The digestates from the thermophilic process with pretreatment generated the highest microalgal biomass concentrations, which is a promising discovery for pulp and paper industry algae-based biorefinery applications as maximum methane production was also obtained at the same conditions.

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Appendix A. Supplementary data

Figure S1. The photos of liquid digestates from the pulp and paper wastewater treatment plant biosludge, anaerobically treated under thermophilic conditions (55 °C) without pretreatment (T), with pretreatment (121 °C) for 10 min (Tp), and under mesophilic conditions (35 °C) with pretreatment (121 °C) for 10 min (Mp) before (day 0) and after cultivation (day 21).

Figure S2. pH evolution during the cultivation of *Scenedesmus acuminatus* in the liquid digestates from the pulp and paper wastewater treatment plant biosludge, anaerobically treated under thermophilic conditions (55 °C) without pretreatment (T), with pretreatment (121 °C) for 10 min (Tp), and under mesophilic conditions (35 °C) with pretreatment (121 °C) for 10 min (Mp).
References


Figure Captions

Fig. 1. Biomass concentration as volatile suspended solids (VSS) during the cultivation of *Scenedesmus acuminatus* in the liquid digestates from the pulp and paper wastewater treatment plant biosludge, anaerobically treated under thermophilic conditions (55 °C) without pretreatment (T), with pretreatment (121 °C) for 10 min (Tp), and under mesophilic conditions (35 °C) with pretreatment (121 °C) for 10 min (Mp).

Fig. 2. The soluble ammonium-N (a), phosphate-P (b), and sulfate-S concentrations (c) during the cultivation of *Scenedesmus acuminatus* in the digestates from the pulp and paper wastewater treatment plant biosludge, anaerobically treated under thermophilic conditions (55 °C) without pretreatment (T), with pretreatment (121 °C) for 10 min (Tp), and under mesophilic conditions (35 °C) with pretreatment (121 °C) for 10 min (Mp). The nitrate and nitrite concentrations are not shown since they remained below 3 mg L$^{-1}$ in all cultures.

Fig. 3. Soluble COD concentration and removal efficiency (a), OD$_{680}$ of the cultivation medium (b), DOC concentration (c), and DIC concentration (d) during the cultivation of *Scenedesmus acuminatus* in the digestates from the pulp and paper wastewater treatment plant biosludge, anaerobically treated under thermophilic conditions (55 °C) without pretreatment (T), with pretreatment (121 °C) for 10 min (Tp), and under mesophilic conditions (35 °C) with pretreatment (121 °C) for 10 min (Mp).
Table 1 Composition of the liquid digestates from the anaerobic digestion of the pulp and paper industry biosludge produced under thermophilic conditions without pretreatment (T) and with pretreatment (121 °C) for 10 min (Tp) and under mesophilic conditions without pretreatment (M) and with pretreatment (121 °C) for 10 min (Mp).

<table>
<thead>
<tr>
<th></th>
<th>T</th>
<th>Tp</th>
<th>M a)</th>
<th>Mp</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.2</td>
<td>8.3</td>
<td>8.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Alkalinity (mg L(^{-1}) CaCO(_3))</td>
<td>2700</td>
<td>3100</td>
<td>n.a. b)</td>
<td>2600</td>
</tr>
<tr>
<td>OD(_{680})</td>
<td>0.59 ± 0.06</td>
<td>0.63 ± 0.08</td>
<td>0.34 ± 0.01</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>280</td>
<td>320</td>
<td>n.a.</td>
<td>290</td>
</tr>
<tr>
<td>NH(_4^+)-N (mg L(^{-1}))</td>
<td>380 ± 20</td>
<td>480 ± 20</td>
<td>350 ± 50</td>
<td>380 ± 15</td>
</tr>
<tr>
<td>NO(_3^-) (mg L(^{-1}))</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>NO(_2^-) (mg L(^{-1}))</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>TP a) (mg L(^{-1}))</td>
<td>33 ± 3</td>
<td>27 ± 1</td>
<td>28 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>PO(_4^{3-})-P (mg L(^{-1}))</td>
<td>16 ± 3</td>
<td>15 ± 3</td>
<td>18 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>N:P c)</td>
<td>12.1 ± 2.3</td>
<td>17.6 ± 1.5</td>
<td>12.5 ± 2.0</td>
<td>11.6 ± 1.0</td>
</tr>
<tr>
<td>SO(_4^{2-}) S a) (mg L(^{-1}))</td>
<td>17 ± 1.0</td>
<td>15 ± 0.1</td>
<td>17 ± 0.9</td>
<td>3 ± 0.1</td>
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<tr>
<td>Soluble COD (mg L(^{-1}))</td>
<td>1200 ± 130</td>
<td>2000 ± 130</td>
<td>910 ± 30</td>
<td>1170 ± 10</td>
</tr>
<tr>
<td>BOD(_5) a) (mg L(^{-1}))</td>
<td>110 ± 5</td>
<td>60 ± 100</td>
<td>n.a.</td>
<td>60 ± 5</td>
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<tr>
<td>BOD(_5)/soluble COD a)</td>
<td>0.09 ± 0.04</td>
<td>0.03 ± 0.77</td>
<td>n.a.</td>
<td>0.05 ± 0.50</td>
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<tr>
<td>DOC (mg L(^{-1}))</td>
<td>300 ± 4</td>
<td>540 ± 110</td>
<td>370 ± 40</td>
<td>150 ± 0</td>
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<tr>
<td>DIC (mg L(^{-1}))</td>
<td>570 ± 10</td>
<td>690 ± 46</td>
<td>520 ± 5</td>
<td>680 ± 47 a)</td>
</tr>
</tbody>
</table>

a) The values with ± sign include standard errors (n = 2)
b) n.a. = data not available
c) N:P (mass per mass): N refers to NH\(_4^+\)-N and P refers to TP
Table 2 Integrated processes of anaerobic digestion of pulp and paper industry biosludge and *Scenedesmus acuminatus* cultivation in the undiluted liquid digestates from the anaerobic digestion of the biosludge

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>AD temperature (°C)</th>
<th>Cultivation duration (d)</th>
<th>Methane yield (L CH₄ kg⁻¹ VS)</th>
<th>Highest obtained biomass concentration (g-VSS L⁻¹)</th>
<th>Highest specific growth rate (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>No</td>
<td>35</td>
<td>14</td>
<td>18&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>8.8 ± 0.8&lt;sup&gt;b)&lt;/sup&gt; (day 4–7)</td>
</tr>
<tr>
<td>Mp</td>
<td>Yes</td>
<td>35</td>
<td>21</td>
<td>101&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>T</td>
<td>No</td>
<td>55</td>
<td>21</td>
<td>63&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>10.2 ± 2.2</td>
</tr>
<tr>
<td>Tp</td>
<td>Yes</td>
<td>55</td>
<td>21</td>
<td>126&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>10.8 ± 1.2</td>
</tr>
</tbody>
</table>

<sup>a</sup) data originated from Asunis (2015)

<sup>b</sup) microalgae were cultivated in 1.5-times diluted digestate (Tao et al., 2017)
Figures

Fig. 1

![Graph showing VSS (g L⁻¹) over time (d) for different conditions T, Tp, and Mp.](image-url)
Fig. 2

(a) NH$_4^+$-N (mg L$^{-1}$) vs. Time (d)

(b) PO$_4^{3-}$-P (mg L$^{-1}$) vs. Time (d)

(c) SO$_4^{2-}$-S (mg L$^{-1}$) vs. Time (d)

- **I**
- **Tp**
- **Mp**
Fig. 3

(a) Graph showing removal efficiency and soluble COD levels for T, Tp, and Mp.

(b) Graph showing digestate OD_{500} decrease over time (d) for T, Tp, and Mp.

(c) Graph showing DOC concentration over time (d) for influent and effluent.

(d) Graph showing IC concentration over time (d) for T, Tp, andMp.