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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 treating pulp and paper industry biosludge as such or after thermal pretreatment were characterized and utilized for cultivating *Scenedesmus acuminatus*. Higher *S. acuminatus* biomass yields were obtained in thermophilic digestates (without and with thermal pretreatment prior to anaerobic digestion (AD): 10.2±2.2 and 10.8±1.2 g L^{-1}, respectively) than in pretreated mesophilic digestate (7.8±0.3 g L^{-1}), likely due to different ammonium and sulfate concentrations in the digestates. *S. acuminatus* removed over 97.4% of ammonium and 99.9% of both phosphate and sulfate from all the digestates. Furthermore, color (74–80%) and CODs (29–39%) of the digestates were partially removed. The shown differences in methane yields (18–126 L CH_4 kg^{-1} VS) from biosludge resulting from the different AD processes and different microalgal yields emphasize the importance of optimization of wood processing biorefineries and thus provide information to pulp and paper industry development.

Keywords: wastewater treatment; pulp and paper industry; digestate characteristics; microalgal growth; nutrient recovery

1 Introduction

Due to the environmental pollution and global warming, the European Council has promoted a binding EU goal of greenhouse gas emissions with at least 40% internal reduction by 2030 compared to 1990, including 27% share of renewable energy for the EU (European Council, 2014). Asia with the rapid growth and heavy dependence on fossil fuels (Lee et al., 2017) as well as other regions e.g. North America, Latin America and Africa (Tan et al., 2017) must carry out a series of policies and legislations for low-carbon and green growth. Biomass referring to all organic materials that originate from plants (algae, trees and crops) can be converted into different kinds of biofuels and energy carriers and is therefore one of the major renewable energy feedstocks (McKendry, 2002). Compared with other plants, microalgae have a high potential as a sustainable bioenergy feedstock because of several advantages e.g. higher growth rate, no requirement for arable land and potential for wastewater treatment to especially recover nutrients (Guldhe et al. 2017) but possibly also to remove toxic heavy metals (Romera et al. 2007) and remove faecal coliforms (Ansa et al. 2012). Besides, CO_2 from the exhaust gases of e.g. combustion, metallurgical, chemical and biological processes can be utilized as carbon source for microalgal cultivation (for a review, see Wang et al. 2008).

However, the existing problems, such as high demand for water and nutrients, low biomass production yields and high cost of microalgal harvesting, need to be solved before commercial utilization of microalgae to low-value products such as energy and fuels (Arenas et al. 2017). 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 wastewaters (Ansa et al., 2012; 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 the efficient nutrient removal and accumulation of high-value products (e.g. astaxanthin, carotenoids and omega-3 fatty acids) to the microalgal biomass (Polishchuk et al., 2015; Xia and Murphy, 2016). The integration of effluents of AD from pulp and paper industry biosludge and microalgal cultivation (from now on referred to as Integrated AD&MC system) has been studied to produce biomass and to 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 AD of pulp and paper industry biosludge.

Pulp and paper industry is water and energy intensive biomass refining industry typically treating its wastewaters in aerobic systems generating large amount of primary sludge and biosludge. Anaerobic digestion of the generated sludges has gained increasing attention in pulp and paper industry sludge treatment due to e.g. biomethane production as renewable energy (Kinnunen et al., 2015; Veluchamy and Kalamdhad, 2017) and possibility for nutrient recovery. More studies have focused on anaerobic digestion of biosludge than primary sludge because primary sludge from pulp and paper mill contains more wood fibres, which can be recycled to the fiber-processing system of the mill instead of being anaerobically digested (de Alda, 2008; Kamali et al., 2016). Biosludge also has a quite high content of lignocellulosic materials, which may limit its anaerobic degradability (Kinnunen et al., 2015). To enhance biomethane production, application of pre-treatment technologies have been considered. Thermal pretreatment prior to AD is one of the main approaches used to enhance the methane production of pulp and paper industry biosludge (Kinnunen et al., 2015; Kamali et al., 2016). To understand the effect of thermal pretreatment

temperatures (80 °C, 105 °C, 121 °C and 134 °C) on methane production potential from biosludge from pulp and paper industry Kinnunen et al. (2015) carried out biomethane potential batch assays at 35 °C. They reported that biomethane production was increased by 39–140% compared to untreated biosludge with the increasing pretreatment temperatures, except that biomethane production from the biosludge treated at the lowest temperature, 80°C, was lower than that obtained from the untreated one. However, although the increased pretreatment temperature increased methane production, it also increases the costs and energy consumption of the thermal pretreatment (Kinnunen et al., 2015). Because of this, Asunis (2015) further studied the anaerobic digestion of pulp and paper mil biosludge at mesophilic and thermophilic conditions since the operating temperature is a significant variable that also affects the methane yield. To our knowledge, the AD plant reported to be under planning phase is the first full-scale AD plant integrated in the pulp mill for digesting pulp mill sludges (Liikanen, 2016).

The previous studies show that biosludge with different treatments (pretreatment and AD conditions) can result in different methane production yields and digestate compositions (Kinnunen et al., 2015; Asunis, 2015). However, the microalgal cultivation in the effluents of AD operated at different conditions has not been compared. Biomethane is generated during the AD process while microalgal biomass can be produced during the cultivation by using the liquid digestates from AD. To optimize Integrated AD&MC system for maximum bioenergy (biomethane and microalgal biomass) production, it is important to study each process and thus give an overview of the Integrated AD&MC system. The aim of this work was to study *S. acuminatus* cultivation in various types of liquid digestates from AD of pulp and paper industry biosludge and provide information with practical cases to biorefinery concept in pulp and paper industries implementing AD and algal cultivation system simultaneously.

2 Materials and Methods

2.1 Microalgal strain and liquid digestates

Scenedesmus acuminatus (SAG 38.81) was obtained from the SAG Culture Collection of Algae at the University of Göttingen, Germany as a culture suspension. Stock culture was maintained in 100 mL N-8 medium in 250 mL Erlenmeyer flask on an orbital shaker (150 rpm) under fluorescent lamps (Osram L 18W/965 bio lux, Germany) at a light intensity of 40 µmol photos m^{-2} s^{-1}. The N-8 medium consisted of (g L^{-1}): KNO_3, 0.506; KH_2PO_4, 0.740; Na_2HPO_4, 0.260; $MgSO_4 \cdot 7H_2O$, 0.050; $CaCl_2 \cdot 2H_2O$, 0.018; $FeNaEDTA \cdot 3H_2O$, 0.012 and micronutrient ($ZnSO_4 \cdot 7H_2O$, 0.003; $MnCl_2 \cdot 4H_2O$, 0.013; $CuSO_4 \cdot 5H_2O$, 0.018; $Al_2(SO_4)_3 \cdot 18H_2O$, 0.007). An initial pH of 6.5 in the N-8 medium was adjusted to 8.0 by adding 5 M NaOH.

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). The 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 in this paper was anaerobically digested at 35 °C (mesophilic digestate, M) (Asunis, 2015) and utilized for 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 fibre filter (Whatman GF /A, UK). After filtration, the liquid digestates were stored at 4 °C before use.

The microalgal growth results with the mesophilic digestate (M) are not directly comparable to the other digestates in the present study as *S. acuminatus* was grown in 1.5-times diluted mesophilic digestate M in the previous study (Tao et al., 2017), whereas in this study *S. acuminatus* was cultivated in undiluted digestates. Therefore, growth yields of *S. accuminatus* in digestate M are not compared to the microalgal cultivations results obtained in this study.

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) closed with a plastic cap with two tubes going through the cap 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, ⌀ 22mm, Duran Group, Germany). The photobioreactors were continuously illuminated using white fluorescent lamps (Osram L 18W/965 de lux cool daylight, Germany) with a light intensity of 240 µmol photos m^{-2} s^{-1}) from two sides of the reactors. *S. acuminatus* was inoculated to the photobioreactors to provide an initial optical density (OD) 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 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 culture solution through a glass fibre filter (Whatman GF/A). Each filter containing the suspended solids was dried at 105 ºC overnight, weighed and then burned in a 550 ºC muffle furnace for 2 h and weighed again. VSS was determined gravimetrically as a difference of the filters after treatment at these two temperatures. The supernatant after VSS filtration was used in the analysis of digestate OD and turbidity, soluble chemical oxygen demand (CODs), soluble biochemical oxygen demand (BOD_{7s}), dissolved organic carbon (DOC) and nutrients (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 was also measured from non-filtrated samples to assess the microalgal biomass production.

CODs was determined using dichromate method according to the Finnish Standard SFS 5504. The determination of BOD_{7}s was done with a WTW OxiTop Control/ OxiTop measuring system. DOC was measured with total organic carbon analyzer (Shimadzu Model TOC-5000) with ASI-5000 autosampler. NH_4^+-N was measured with an ion selective electrode (Thermo Scientific Orion ISE meter). The potential extent of ammonium stripping was estimated by the following equation

(Emerson et al. 1975) as rate of ammonia stripping has been shown to correlate well with unionized ammonium concentration related to temperature and pH (Zimmo et al. 2003):

$$unionized\ NH_3(\%) = \frac{100}{1 + 10^{(pK_a - pH)}} \quad (1)$$

where $pK_a = 0.09018 + \frac{2729.92}{T}$ and T = temperature(ºK).

NO_3^-, NO_2^-, PO_4^{3-} and SO_4^{2-} were measured using ICS-1600 ion chromatograph (Dionex, USA) with AS-DV autosampler, Ion- Pac AS4A-SC anion exchange column, and ASRS-300 suppressor (2 mm). The eluent contained 1.9 mM Na_2CO_3 and 1.7 mM $NaHCO_3$, and the eluent flow rate was 1 mL min^{-1}.

3 Results

3.1 Characteristics of the liquid digestates

The four pulp and paper industry biosludge digestates originating from digesters operated at different temperatures treating biosludge with and without thermal pretreatment had different characteristics (Table 1). The initial pH of all the digestates was above 8.0 and the high alkalinity in the digestates provided a good buffering capacity for microalgal cultivation since *S. acuminatus* prefers slightly alkaline conditions. The color of the digestates was measured by absorbance (OD) at a wavelength of 680 nm and turbidity after removing the microalgal biomass by filtering. In terms of OD, the color of the thermophilic digestates was higher than that of the mesophilic digestates. In addition, OD value of the pretreated digestates was higher than those without pretreatment. The digestate Tp showed the darkest color (OD: 0.63±0.08, turbidity: 320 NTU) of all the digestates. However, the value of OD of the digestate T (0.59±0.06) was higher than that of

Mp (0.35±0.01) while the turbidity of the digestate T (280 NTU) was lower than that of Mp (290 NTU). Thus, there was no clear correlation between OD and turbidity.

The thermophilic digestates (T and Tp) had on an average 65 mg L^{-1} higher ammonium concentrations compared with the mesophilic digestates (M and Mp). In addition, the digestates treated at the same temperature resulted in 30–100 mg L^{-1} higher ammonium concentration with pretreatment than without pretreatment (Table 1). Ammonium was available in all the digestates as nitrogen source for microalgal growth, while nitrate and nitrite concentrations were below 1.0 mg L^{-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 the digestate M, where phosphate share was slightly higher (64.3%). In addition, the sulfate-S concentration in digestate Mp was much lower than that in the other three digestates (Table 1).

Similar phenomenon as with ammonium was observed with CODs values of the different digestates. The thermophilic digestates had higher CODs values than the mesophilic digestates and when the digestates produced at the same digestion temperature were compared, the biosludge digestates generated in a process with pretreatment resulted in higher CODs than without pretreatment (Table 1). The BOD_7s/CODs 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. This indicates that the digestate could support mainly photoautotrophic growth and that the microalgal growth in the digestates relied mainly on CO_2 as the carbon source.

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

3.2.1 Microalgal biomass production

The microalgal biomass production as indicated by VSS in the three studied digestates (T, Tp and Mp) was as shown in Fig. 1. 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 that 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. In the beginning, *S. acuminatus* in the digestate Tp grew slower than in the digestates T and Mp likely due to the higher initial ammonium concentration and poorer light penetration (due to darker color of the digestate). Before day 9, *S. acuminatus* biomass concentration in the digestate T (6.0 g L^{-1} VSS at day 9) was the highest followed by *S. acuminatus* in the digestate Mp (4.9 g L^{-1} VSS at day 9) and Tp (4.4 g L^{-1} VSS at day 9). Later, the culture concentration in the digestate Tp exceeded those in the digestates Mp and T after day 9 and day 15, respectively.

3.2.2 Nutrient removal from liquid digestates

S. acuminatus removed nutrients efficiently from the digestates (Fig. 2). The ammonium concentration decreased from 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 a little higher than the ammonium removal efficiency obtained in the mesophilic digestate (97.4%). The phosphate and sulfate were completely removed from all the liquid digestates in 7 days and 7–9 days, respectively. There was no clear difference in the phosphate and sulfate removal efficiency from the three different digestates (T, Tp and Mp).

3.2.3 CODs, DOC and color changes

CODs removal efficiency was higher from the thermophilic digestates (38% and 39%) than from the mesophilic digestate (29%) (Fig. 3a). The DOC concentration in the thermophilic digestates decreased while DOC increased in the digestate Mp during the cultivation (Fig. 3b). The OD of the digestates was measured after removing the microalgae to show the digestate color change during the cultivation (Fig. 3c). The OD values in all digestates decreased until day 9 and remained quite stable after that. In the end of the batch cultivations, the color removal efficiencies in T, Tp and Mp were 80%, 74% and 79%, respectively.

4 Discussion

This study shows that it is possible to produce a high concentration of microalgal biomass and remove nutrients (ammonium, phosphate and sulfate) efficiently from various liquid digestates of pulp and paper mill biosludge digestion without dilution. Thus, the results confirm the potential of Integrated AD&MC system where the liquid digesates originate from pulp and paper wastewater treatment plant biosludge digestion as shown in our previous study (Tao et al., 2017). It further shows that the temperature of AD and thermal pretreatment prior to AD have some but not critical impact on microalgal biomass production and nutrient, CODs and DOC removal. Thus, this study provides information for decision making on the AD system to be invested simultaneously considering the potential implementation of a microalgal system in a biorefinery concept in pulp and paper industry. An overview (treatment methods of biosludge, microalgal cultivation conditions and bioenergy production) of the studied Integrated AD&MC systems is shown in 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 was also higher both without and with pretreatment in the thermophilic digestion compared to the mesophilic process (Table 2) (Asunis, 2015) indicating that the highest biogas production and microalgal biomass yield can be obtained in the same integrated AD&MC system. However, it is clear that the influence from pulp and paper mill digestate on microalgal growth is species specific as Kinnunen and Rintala (2016) previously reported that biomass concentration less than 0.2 g L^{-1} (VSS) was obtained with a *Scenedesmu*s sp. originating from Lake Pyhäjärvi (Tampere, Finland) in an optimum mixture of 75% distilled water and 25% liquid digestate from pulp and paper industry biosludge AD. Although the biosludges used in Kinnunen and Rintala (2016) and this study were from the same pulp and paper mill, the different characteristics of the digestates (likely due to the changes of e.g. wood source used, pulp mill operation parameters and season) and microalgal strains clearly affect the obtainable biomass quantity.

The effect of sludge pretreatment before digestion on microalgal cultivation is not, however, fully clear based on this study. Asunis (2015) reported that thermal pretreatment increased the methane yield by 100% in thermophilic AD process while the increase was 460% in mesophilic AD, while the methane yield with pretreatment at thermophilic condition was still 25 L CH_4 kg^{-1} VS higher than that obtained with pretreatment at mesophilic condition (Table 2). The difference caused by the pretreatment prior to thermophilic digestion on the microalgal biomass production in the digestate was not significant. Although highest methane and microalgal biomass production was obtained at the same process (thermophilic AD with pretreatment), other factors should be considered, including e.g. costs and energy burden of the thermal pretreatment and possible

removal of residual CODs from the digestate after microalgal cultivation. However, it is impossible to strictly compare the microlagal biomass yields in the mesophilic digestates with and without thermal pretreatment in this study due to the differences in dilution used in this study and our previous study (Tao et al, 2017).

The present study also shows that it is possible to obtain a high microalgal biomass yield in the liquid digestates from pulp and paper wastewater treatment plant biosludge without dilution. This indicates e.g. that the digestate color levels in this study did not affect the microalgal growth greatly. Substances in the liquid digestates causing the color may include clay, silt, finely divided inorganic and organic matter, soluble colored organic compounds, and plankton and other microscopic organisms (Wang et al., 2010). The turbidity of liquid digestates may vary ranging e.g. from 2960 to 51400 NTU as reported in the liquid fraction of mainly manure digestates from 11 full-scale co-digestion plants (Akhiar et al., 2017). The dark color of the medium is one of the issues, which could reduce the microalgal growth due to poor light penetration (Wang et al., 2010; Marcilhac et al., 2014; Xia and Murphy, 2016). For example, in a study by Wang et al. (2010), where *Chlorella* sp. was cultivated in liquid fraction 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 (R^2 = 0.982) indicated that high turbidity can limit algal growth. However, dilution for the microalgal growth increases total wastewater treatment volume and might reduce microalgal growth owing to a reduction in nutrients and trace element concentrations. In the present study, the different AD process conditions led to color differences of the three liquid digestates (turbidity: 280–320 NTU), which however did not affect the color removal efficiency. It is not easy to compare results (e.g. microalgal biomass production and nutrients removal efficiencies) with other studies, which have similar medium turbidities as in the

present study since only few papers have mentioned the turbidity of medium during the microlalgal cultivation.

In this study, microalgal cultivation removed CODs to certain levels (29-39% removal) while DOC acted somewhat contradictory to CODs as DOC level increased in the mesophilic digestate. COD represents the demand of chemical oxidizer needed to oxidize all the oxidizable organic or inorganic materials in wastewater and DOC is used to reflect 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) and the correlation between COD and DOC in microalgal cultures is not clear based on the previous studies. For example, Marjakangas et al (2015) reported increase in both soluble COD and DOC concentrations likely due to the low pH stress after *C. vulgaris* CY5 was mixotrophically cultivated in anaerobically treated piggery wastewater. Thus, it seems that the changes in COD and DOC depend on the 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 CODs suggests that organic materials from the digestates were consumed during the cultivation and 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). The CODs in digestes in this study was probably mainly caused by lignocellulosic materials (Kinnunen et al., 2015), which are not easily biodegradable and are therefore difficult to remove with biological methods. Thus, further removal would be possible with other than biological treatments e.g. chemical oxidation, if that would be deemed necessary.

304 The results in this study demonstrate that the microalgal cultivation efficiently removed
305 ammonium, phosphorus and sulfate from all digestates from pulp and paper industry biosludge
306 AD. Phosphorus was likely removed from the digestates through adsorption on the microalgal
307 surface, intracellular uptake and precipitation (Cai et al., 2013). In the present study, it is speculated
308 that enough phosphorus ensured microalgal growth since the microalgal biomass yield did not stop
309 or slow down when phosphate was no more detected from the liquid digestates after day 7. For
310 ammonium removal, several ammonium transformations (e.g. algal uptake, ammonia evaporation,
311 bacterial growth and nitrification) can occur in algae-bacteria consortium systems (González-
312 Fernández et al., 2011). According to the average temperature (22 °C) and the observed pH range
313 (7.8–8.4), the theoretical fraction of unionized ammonia in all cultivations was 2.8%–10.3%. In
314 addition, low levels of nitrate were found in all cultivations. This data suggests that ammonium
315 stripping and nitrification may have occurred, but that the main portion of the removed ammonium
316 from the digestates was used for microbial growth.

317 Initial sulfate concentration in liquid digestates could affect the ammonium removal efficiency and
318 microalgal biomass production. This hypothesis is supported by the fact that the cultivations in the
319 digestates T and Tp having similar initial sulfate concentration (15-17 mg L^{-1}) enabled over 99.9%
320 ammonium removal and similar microalgal biomass production while the different initial sulfate
321 concentrations in the digestates T and Mp (17 vs. 3 mg L^{-1}), which had similar initial ammonium
322 concentration resulted in different ammonium removal efficiencies and algal biomass yield.
323 Biological nitrogen fixation is catalyzed during photosynthesis by nitrogenase, which contains
324 iron-sulfur clusters (Zheng and Dean, 1994) and the shortage of sulfur can therefore decrease the
325 assimilation of nitrogen (Kumaresan et al., 2017). Sulfate as primary sulfur source for microalgae
326 in aquatic environments has been proved to affect microalgal growth also in other studies (Lv et

al., 2017; Mera et al., 2016). Mera et al. (2016) reported that microalga *Chlamydomonas moewusii* growth was quite similar at sodium sulfate concentrations 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 study by Lv et al. (2017), similar *Chlorococcum* sp. growth at sulfate levels from 18-271 mg L^{-1} was obtained, but growth was much lower at 0 mg L^{-1} sulfate.

5 Conclusions

Cultivatation of *Scenedesmus acuminatus* was succesful in different undiluted digestates from pulp and paper industry biosludges treated at different AD conditions i.e. mesophilic vs. thermophilic, with and without thermal (121 °C) 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 CODs (29–39%) were partially removed. The digestate from thermophilic process with pretreatment enabled highest microalgal biomass concentration, forming a promising discovery for pulp and paper industry algae-based biorefinery applications as highest biomethane production was obtained at the same conditions.

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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 at thermophilic process (55 °C) without pretreatment (T), with pretreatment at 121 °C for 10 min (Tp) and at mesophilic process (35 °C) with pretreatment at 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 biosludges anaerobically treated at thermophilic condition (55 °C) without pretreatment (T), with pretreatment at 121 °C for 10 min (Tp) and at mesophilic condition (35 °C) with pretreatment at 121 °C for 10 min (Mp).

Fig. 3 CODs concentration and removal efficiency (a), DOC concentration (b) and OD of the cultivation medium (c), during the cultivation of *Scenedesmus acuminatus* in the digestates from the pulp and paper wastewater treatment plant biosludge anaerobically treated at thermophilic process (55 °C) without pretreatment (T), with pretreatment at 121 °C for 10 min (Tp) and at mesophilic process (35 °C) with pretreatment at 121 °C for 10 min (Mp).

Tables

Table 1 Compositions of the liquid digestates from the anaerobic digestion of the pulp and paper industry biosludge produced at thermophilic process without pretreatment (T) and with pretreatment at 121 °C for 10 min (Tp) and at mesophilic process without pretreatment (M) and with pretreatment at 121 °C for 10 min (Mp).

	T	Tp	M [a)]	Mp
pH	8.2	8.3	8.5	8.3
Alkalinity [a)] (mg L^{-1} $CaCO_3$)	2700	3100	n.a.	2600
OD	0.59 ± 0.06	0.63 ± 0.08	0.34 ± 0.01	0.35 ± 0.01
Turbidity (NTU)	280	320	n.a.	290
NH_4^+-N (mg L^{-1})	380 ± 20	480 ± 20	350 ± 50	380 ± 0
NO_3^- (mg L^{-1})	<1.0	<1.0	<1.0	<1.0
NO_2^- (mg L^{-1})	<1.0	<1.0	<1.0	<1.0
TP (mg L^{-1})	33 ± 3	27 ± 1	28 ± 1	33 ± 2
PO_4^{3-}-P (mg L^{-1})	16 ± 3	15 ± 3	18 ± 1	15 ± 1
SO_4^{2-}-S [a)] (mg L^{-1})	17 ± 1.0	15 ± 0.1	17 ± 0.9	3 ± 0.1
CODs (mg L^{-1})	1200 ± 130	2000 ± 130	910 ± 30	1170 ± 10
BOD_7s [a)] (mg L^{-1})	110 ± 5	60 ± 100	n.a.	60 ± 5
DOC (mg L^{-1})	300±4	540±110	370±40	150±0

a) The values with ± sign include standard errors

b) n.a.=data not available

458 **Table 2** Integrated processes of anaerobic digestion of pulp and paper industry biosludge and *Scenedesmus*

459 *acuminatus* cultivation in the undiluted liquid digestates from the anaerobic digestion of the biosludge

	Pretreatment	AD temperature (°C)	Microalgal cultivation duration (d)	Methane yield (L CH_4 kg^{-1} VS)	Average microalgal biomass production (g L^{-1} VSS)
M	No	35	14	18[a]	8.8[b)]
Mp	Yes	35	21	101[a]	7.8
T	No	55	21	63[a]	10.2
Tp	Yes	55	21	126[a]	10.8

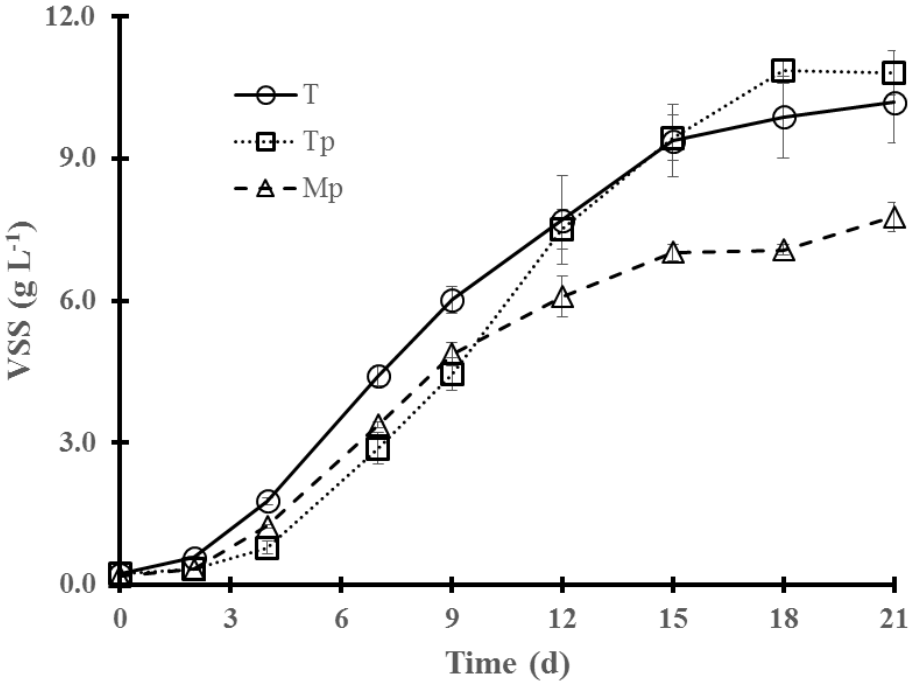
460 a) data originated from Asunis (2015)

461 b) microalgae were cultivated in 1.5-times diluted digestate (Tao et al., 2017)

462

463 **Figures**

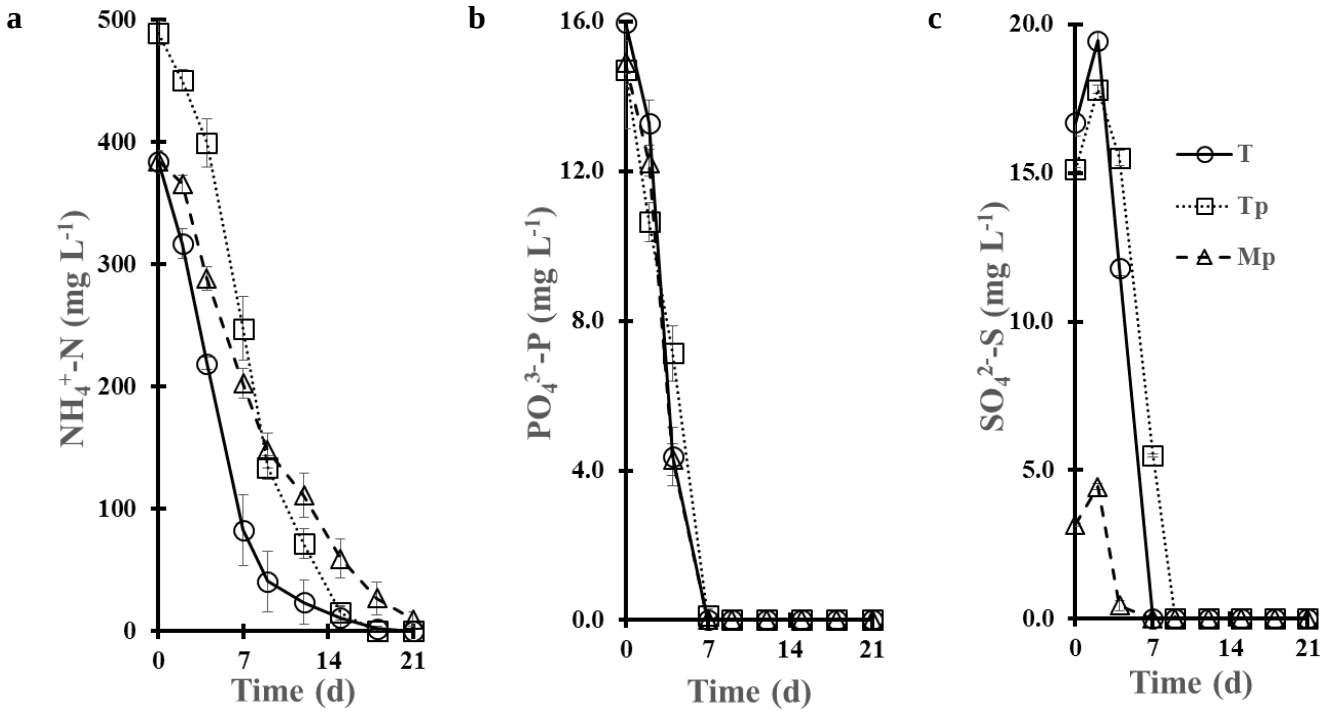
464 **Fig. 1**



465

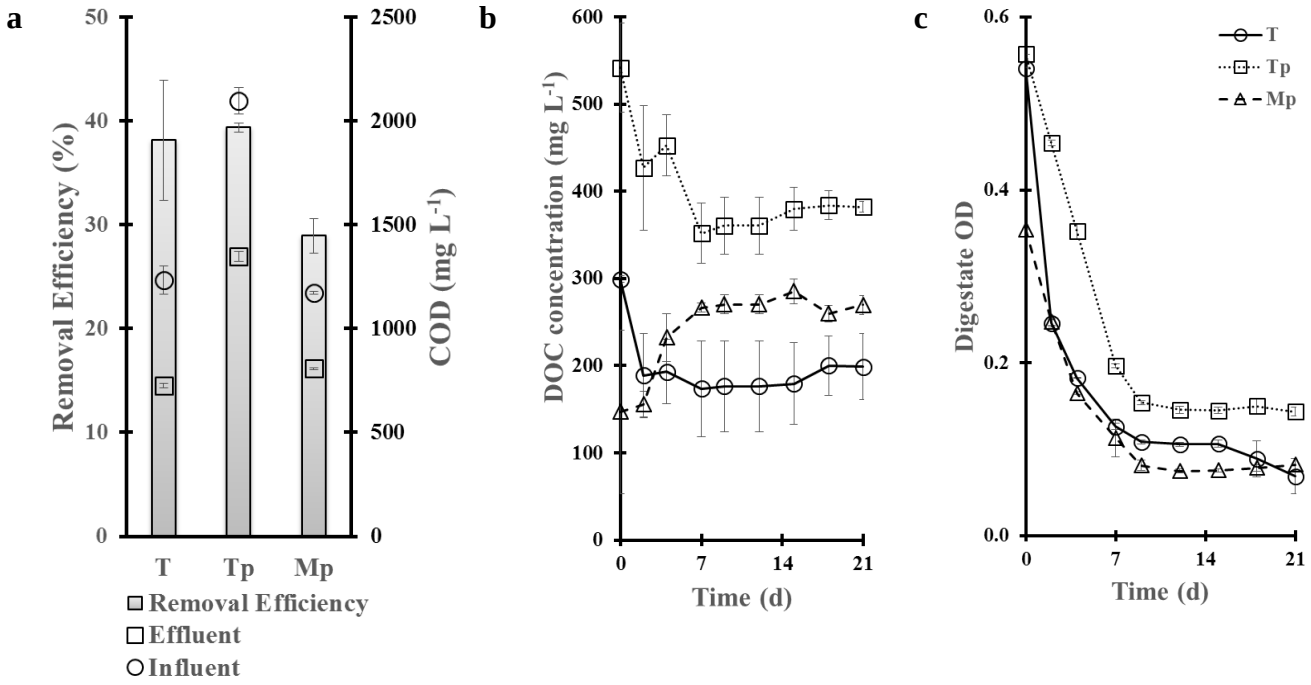
466

467 **Fig. 2**



468

469 **Fig. 3**


a
Removal Efficiency (%)
COD (mg L-1)
T
Tp
Mp
Removal Efficiency
Effluent
Influent
b
DOC concentration (mg L-1)
Time (d)
c
Digestate OD
Time (d)
T
Tp
Mp

470