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Impact of organic solvents on lipid-extracted microalgae residues and wastewater sludge co-digestion

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ABSTRACT

This study investigates the combined production of lipids and biogas via anaerobic mono- and co-digestion of microalgae biomass grown in municipal untreated wastewater. In the co-digestion assays, extracted microalgae and wastewater sludge were mixed at different ratios: 25–75, 50–50, and 75–25% total volatile solids (VS) of each substrate, respectively. The neutral and polar fatty acid methyl esters constituted approximately 13 and 1.5% of the total lipids, respectively. Mono-digestion of lipid-extracted microalgae had a lower biochemical methane potential (BMP) than of non-extracted microalgae. Organic solvents were shown to negatively impact both mono- and co-digestion of extracted microalgae. Co-digestion experiments showed synergy between sludge and microalgae residues, increasing the BMP from 91.4 Normalized mL (NmL) CH_4/g VS in the mono-digestion of evaporated extracted microalgae up to 228.6 NmL CH_4/g VS in the co-digestion of the mixture with 25% VS of microalgae biomass with 75% VS of sludge.

1. Introduction

Microalgae are considered one of the most sustainable bioenergy feedstocks (Lage et al., 2018). Advantages of algae include (i) ten times higher growth rates and corresponding CO_2 fixation rates and relatively high-lipid contents than conventional forest and agricultural crops (Chisti, 2007) (ii) tolerance to harsh environments, such as non-potable industrial, urban wastewater, and arid land areas, thus, algae cultivation does not compete with human and animal food production (Pittman et al., 2011). Nevertheless, with the current technology, the production of biodiesel from microalgae biomass faces several challenges in becoming a mainstream industry able to produce the quantity of biofuel required at competitive prices (Lakaniemi et al., 2013; Rashid et al., 2013). Additionally, a larger portion of the biomass ends up as a by-product residue that will require disposal (Lardon et al., 2009; Ward et al., 2014).

An alternative use for the microalgae residual biomass is the production of biogas via anaerobic digestion, considering that it does not require highly concentrated biomass and anaerobes can use the three biomass macromolecules (proteins, carbohydrates, and lipids) for methane production (Mussgnug et al., 2010; Olsson et al., 2014). However, as a limiting step, microalgae anaerobic digestion has been reported to have low production yields due to the biological inaccessibility to microalgae cells with intact membranes and inhibitory conditions (e.g., low C/N ratios and high salinity) and agents (e.g., ammonia and long-chain fatty acids) experienced during digestion (Frigon et al., 2013; Sialve et al., 2009). Several types of pre-treatment of microalgae biomass have been proposed to improve the methane production yield (Passos et al., 2013). However, pre-treatment is an energy-consuming process, which is equal to or higher than the energy gained with the methane production (Passos et al., 2014). Alternatively, the co-digestion of microalgae biomass with other substrates has been shown to circumvent the inhibitory conditions (Olsson et al., 2014; Schwede et al., 2013). Co-digestion can also assure macro and micro-nutrient equilibrium, balance the moisture content, optimize the organic loading rate, and dilute possible inhibitory compounds released during the anaerobic digestion process (Herrmann et al., 2016; Schwede et al., 2013).

Previously, a significant decrease in methane production due to residual organic solvents from lipid extraction has been documented

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(Ehimen et al., 2009; Neves et al., 2016; Yun et al., 2014; Zhao et al., 2014). For instance, chloroform, which is commonly used as an organic solvent to extract lipids from microalgae (Palmquist and Jenkins, 2003), is toxic to microbial cells because it disrupts the microbial cell membrane and compromises cell viability, resulting in the inactivation of essential membrane function and denaturation of essential enzymes (Inoue and Horikoshi, 1991; Sardessai and Bhosle, 2004). However, it was shown that co-digestion of lipid extracted microalgae biomass with food waste leachate could reduce the chloroform inhibition on biogas production (Yun et al., 2016b). Even n-hexane could inhibit the metabolic pathway of methanogens in *Chlorella vulgaris* residues after lipid extraction (Yun et al., 2016a).

The purpose of this work was to investigate the combination of lipid extraction from biomass of microalgae cultivated in untreated municipal wastewater with the biogas production of the lipid-extracted microalgae using hexane and isopropanol as organic solvents. The methane potentials of (i) anaerobic mono-digestion of non-extracted microalgae and lipid-extracted microalgae and (ii) co-digestion of lipid-extracted microalgae with municipal wastewater sludge at different mixture ratios were evaluated. Additionally, the effect of the removal of solvents from the lipid-extracted microalgae biomass on the methane potentials was determined.

2. Materials and methods

2.1. Substrates

A local microalgae consortium constituted by mainly green algae of the genera *Scenedesmus* and *Desmodesmus* was cultivated on a raceway pond during the 2016 summer season. The pond was 10 m long, 2 m wide, and approximately 0.3 m deep with a surface area of 19.14 m^2 and a volume of about 6 m^3, and it was equipped with paddle wheels with six blades (Lage et al., 2021). The pond was located inside a greenhouse at algae pilot facilities of the Umeå Energi combined heat and power plant (CHP-plant) in Umeå, Northern Sweden (63°87 N, 20°80E). The microalgae consortium was grown in municipal untreated wastewater collected at the local wastewater treatment plant (Vakin, Umeå). The microalgae culture was bubbled with flue gases containing approximately 10% CO_2 (*v*/v) from the CHP-plant at Umeå Energi, which incinerates both municipal and industrial solid wastes. The flue gas addition was regulated by pH to maintain a pH value of 8 during the cultivation. The microalgae biomass was harvested three times a week by sedimentation for approximately one day in 1 m^3 plastic containers. A portion of the pre-concentrated microalgae biomass was kept in the freezer until anaerobic mono-digestion. The other portion was centrifuged at ca. 5000 rpm (US Filtermaxx, Jacksonville, Florida, USA) and frozen until lipid extraction.

The wastewater treatment plant (WWTP) sludge was sampled at a conventional municipal WWTP in Vasa Vatten (Vaasa, Finland). The WWTP sludge sample was taken in May 2017, and the sampling point was after thickening but before the addition of a dewatering polymer and centrifugation (in this particular WWTP, the sludge is dewatered and transported to an anaerobic digestion plant). The sludge was stored frozen before the digestion. The inoculum was taken from a food waste anaerobic digester facility at Stormossen (Korsholm, Finland), more precisely at the outflow of one thermophilic reactor. The inoculum was incubated at 55 °C for 5 days before the biogas trials to minimize indigenous gas production.

2.2. Lipids extraction and fatty acid methyl esters (FAMEs) characterization

2.2.1. Lipids extraction

The lipids extraction was performed according to a modified Hara and Radin (1978) method using a mixture of hexane:isopropanol (3:2) and with a solvent: microalgae ratio of 75:1 based on the dry weight (DW) of the microalgae biomass (Fig. S1). The hexane (≥ 99%) and isopropanol (≥ 95%) was of GPR Rectapur quality (Avantor, Pennsylvania, USA). Two independent experiments were performed with 1000 g wet weight (equivalent to 134.0 g DW) of microalgae biomass, 6030 mL of hexane, and 4020 mL isopropanol in the first experiment; and 1100 g wet weight (equivalent to 147.4 g DW) of microalgae biomass, 6630 mL of hexane, and 4420 mL of isopropanol for the second experiment. The extraction was allowed to proceed for 2 h at room temperature in a 13-L steel reactor with stirring (300 rpm). After extraction, the microalgae residues were removed by filtration through a Büchner funnel with a cellulose filter (Whatman filter papers no 541, Cytiva, Massachusetts, USA). The microalgae residues were washed in the funnel with the addition of 500 mL hexane, and the extracted microalgae biomass was, thereafter, kept in the freezer until further experiments.

The separation of the two liquid phases (hexane and isopropanol) was performed using 500 mL of liquids in 1 L separating funnels until all material was separated in hexane and isopropanol phases. The hexane phases containing the lipids were collected, and the isopropanol phases were washed with the addition of hexane. All hexane phases containing the lipids were pooled, and the hexane was evaporated using a 10-L evaporator (Rotavapor R-220 SE, Büchi, Flawil, Switzerland). The evaporator was run at 40 °C, 70 rpm, and 200 mbar, and the vacuum was decreased to 80 mbar towards the end of the evaporation to remove the last portion of hexane. A small volume of hexane was added to the final material to be able to transfer the lipids from the evaporator round bottom flask to a pre-weighed 250 mL bottle. The bottle containing the lipids was left to evaporate at room temperature in a fume hood.

2.2.2. FAMEs quantification and characterization

Crude lipids obtained in the previous step were purified and separated into neutral and polar fatty acids with solid-phase extraction (SPE). Hypersep SI SPE columns with a capacity of 3 mL (Thermo Scientific, Waltham, Massachusetts, USA) were used. Subsequently, the fatty acids were transmethylated into FAMEs according to Lage and Gentili (2018), based on Christie and Han (2010). FAMEs extracts were re-suspended with heptane and injected into a TRACE™ 1310 gas chromatography system (Thermo Fisher Scientific, Hägersten, Sweden) equipped with a flame ionization detector and a 30 m FAMEWAX column (Restek Corporation, Bellefonte, Pennsylvania, USA) (Lage and Gentili, 2018). The crude lipids purification was performed in triplicate. FAMEs were identified by comparison of retention times with authentic standards. Real response factors were used to determine FAMEs concentrations. Data were normalized against the internal standard methylated heptadecanoic acid (C17:0-Me).

2.3. Biochemical methane potential (BMP)

The BMP assays were conducted using two units of Automatic Methane Potential Test System (AMPTS) II (Bioprocess Control, Lund, Sweden). The two units were run simultaneously; thus, the same sample of inoculum was used in all samples. The tests were done following VDI4630 (2006) and previous studies (Angelidaki et al., 2009; Holliger et al., 2016; Raposo et al., 2012). The total solids (TS) and volatile solids (VS) were determined according to standard methods (APHA-AWWA-WEF, 1998). The water bath was set to 55 °C during the test. The sample amount in each bottle was 400 g in 500 mL bottles, resulting in a headspace of approx. 200 mL, with an inoculum to substrate ratio (I/S-ratio) (based on VS) of 2. According to the literature, this is the optimal I/S-ratio of microalgae BMP (Raposo et al., 2012; VDI4630, 2006). AMPTS II is equipped with a CO_2-adsorption unit, with a bottle containing 3 M NaOH with pH indicator (0.4% thymolphthalein) for every sample and flow cell. Only CH_4 is assumed to pass through the CO_2-adsorption unit to the measuring unit, which works by the principle of water displacement (Fig. S2). The measurements are automatically converted to standard conditions (0 °C and 1 bar), as the device monitors both temperature and pressure continuously in the room. The agitation

Table 1
The mean VS in the sample bottles from substrate and inoculum as well as the I/S-ratio and VS concentration at the end of the BMP assays; ($n = 3$).

Substrate	VS in bottle from substrate [g]	VS in bottle from inoculum [g]	IS-ratio	VS start substrate [%]	VS start inoculum [%]	VS end [%]
E Ext algae	2.35	4.70	2	45.70	2.41	2.80
25% E Ext algae and 75% sludge	3.52	7.04	2	3.26	2.41	2.17
50% E Ext algae and 50% sludge	3.84	7.68	2	4.72	2.41	2.21
75% E Ext algae and 25% sludge	4.23	8.45	2	8.56	2.41	3.13
NE Ext algae	4.52	9.04	2	18.31	2.41	3.13
25% NE Ext algae and 75% sludge	3.50	6.99	2	3.18	2.41	2.18
50% NE Ext algae and 50% sludge	3.78	7.56	2	4.38	2.41	2.29
75% NE Ext algae and 25% sludge	4.12	8.24	2	7.07	2.41	2.79
Microalgae biomass	2.67	5.34	2	2.35	1.54	1.53
Sludge	3.25	6.50	2	2.49	2.41	2.03

was constant at 80% of maximum speed (160 rpm) and set to switch direction at intervals of 5 s. At the beginning of the experiment, each reactor was flushed with nitrogen gas for 30 s to achieve anaerobic conditions, following manufacturer instructions. The software of the device has a function for eliminating overestimation of gas production from the gas in the headspace at the start, which was used. The assays were stopped after 27 days when the daily methane flow dropped below 1% of the accumulated volume for 3 consecutive days for that particular sample (Fig. 2), as previously recommended (Angelidaki et al., 2009; Holliger et al., 2016; VDI4630, 2006). All samples were done in triplicate, as well as blank samples for subtracting the methane production of the inoculum in each test (Fig. 2). The BMP is reported as the average of the triplicates and their standard deviation, also taking into consideration the standard deviation of the blank samples since they are used to remove the indigenous gas production. The BMP is expressed as Normalized mL of methane produced per gram of VS of substrate added (NmL CH_4/g VS).

The extracted microalgae biomass (i.e., after lipids extraction) still contained some of the solvents. The sample was divided into two portions. One portion of approx. 200 g (wet weight) was evaporated in a heating oven (Thermo Scientific Heraeus UT 20 P, Thermo Electron LED GmbH, Germany). The oven was set at 20 °C and 40% of maximum ventilation. After 6.5 h, the weight and solids content had changed and stabilized. The non-evaporated extracted microalgae biomass portion was stored and handled to minimize evaporation.

Mixing of different substrates can cause a synergistic or antagonistic effect through, for example, dilution of inhibiting substances or better balance of nutrients (Sialve et al., 2009). In the present study, the synergistic effect of co-digestion was determined by Eq. (1):

$$\propto = \frac{Experimental\ production}{Theoretical\ production} \quad (1)$$

The "experimental production" refers to the measured result from the performed experiment, and "theoretical production" is the calculated BMP from mono-digestion of the separate substrates related to the VS from the substrates in the co-digestion mixture. If the experimental BMP is higher than the calculated BMP (i.e., $\alpha > 1$), the effect of mixing substrates is synergistic (Nielfa et al., 2015).

The BMP co-digestion assays were carried out with six different mixtures of sludge and microalgae residues; three with evaporated

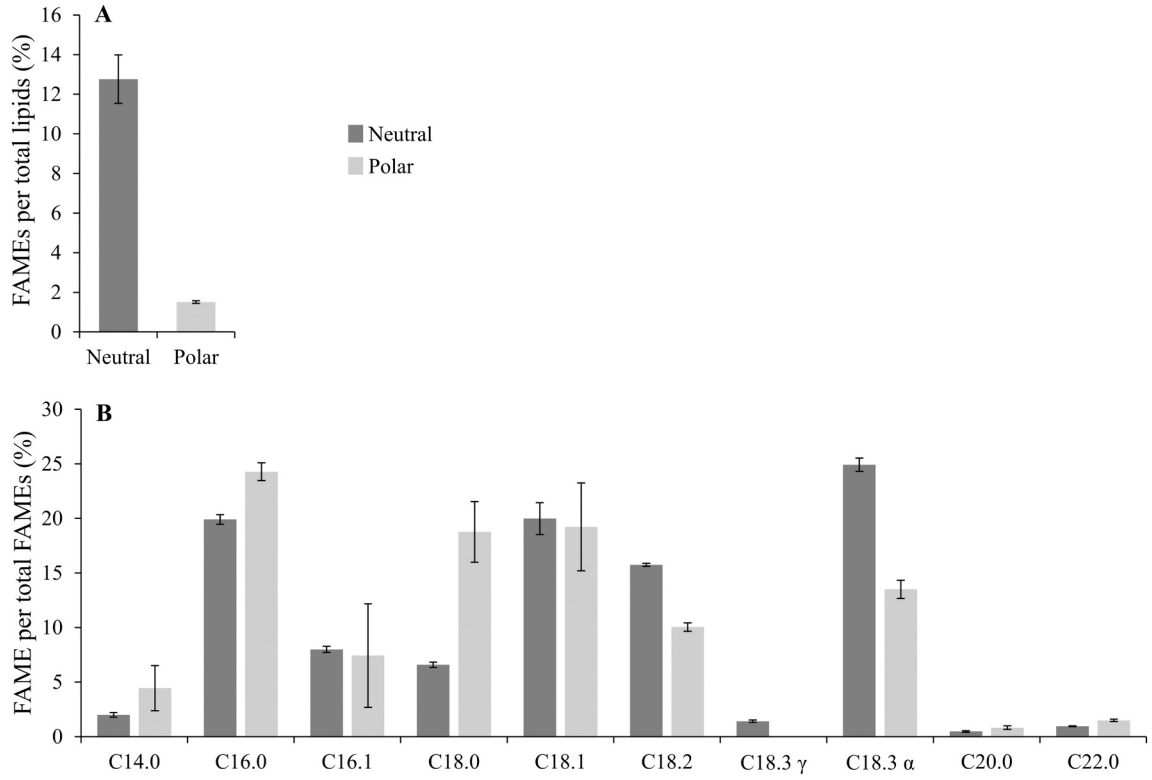

Fig. 1. A, Total neutral and polar FAMEs (% total lipids) and B, neutral and polar FAMEs profiles of microalgae biomass. Error bars express the standard deviation of the mean ($n = 3$).

Table 2
BMP is expressed as Normalized mL of methane produced per gram of VS of substrate added (NmL CH_4/g VS). Effect of co-digestion and solvent on the anaerobic digestion of sludge, microalgae biomass, evaporated extracted microalgae biomass (E Ext algae), non-evaporated extracted microalgae biomass (NE Ext algae), and their mixtures. The BMP values represent the mean and the standard deviation of the mean ($n = 3$).

Substrate	BMP (NmL CH_4/g VS)	Effect of co-digestion	Effect of solvent
Sludge	251.0 ± 10.2		
E Ext algae	91.4 ± 32.7		
25% E Ext algae and 75% sludge	228.6 ± 3.8	1.08	
50% E Ext algae and 50% sludge	198.5 ± 8.3	1.16	
75% E Ext algae and 25% sludge	188.7 ± 4.3	1.44	
NE Ext algae	−64.6 ± 3.8 (0)		−170%
25% NE Ext algae and 75% sludge	194.0 ± 16.8	1.13	−15%
50% NE Ext algae and 50% sludge	114.3 ± 6.3	1.22	−42%
75% NE Ext algae and 25% sludge	−45.0 ± 4.7 (0)	2.99	−124%
Microalgae biomass	122.0 ± 15.0		

extracted microalgae biomass (E Ext algae) and three with non-evaporated extracted microalgae biomass (NE Ext algae). The co-digestion mixing ratios were 75–25, 50–50, and 25–75%VS (% total subtract VS fed to the reactor) of sludge and microalgae residues, respectively. The VS in the sample bottles, the I/S-ratio, as well as the total VS (inoculum + substrate) at the end of the BMP assays are shown in Table 1. The amounts are calculated based on the total sample amount of 400 g (wet weight) and I/S-ratio of 2.

Additionally, for comparison with the co-digestion mixtures, all substrates (i.e., sludge, E Ext algae, NE Ext algae, and microalgae biomass) were digested separately. Thereafter, the effect of the solvent on the mono- and co-digestion of the extracted microalgae biomass was determined with Eq. (2):

$$Effect\ of\ solvent = \frac{NE\ Ext\ algae - E\ Ext\ algae}{E\ Ext\ algae} \quad (2)$$

3. Results and discussion

3.1. FAMEs quantification and characterization

The neutral and polar FAMEs extraction yield (% total lipids) of the microalgae biomass is shown in Fig. 1A. The neutral FAMEs were the largest part of the total FAMEs with approximately 13% of the total lipids, while polar FAMEs were about 1.5% of the total lipids (Fig. 1A). Under optimal conditions of growth, microalgae synthesize only about 5–20% fatty acids per dry cell weight (Hu et al., 2008; Orr et al., 2016); however, under unfavourable environmental or stress conditions for growth, microalgae can alter their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids to 20–50% per dry cell weight, reaching up to 80% of the total lipids (Hu et al., 2008; Yao et al., 2015). The accumulation of neutral lipids begins at the end of the log phase or in the stationary phase when the nutrient content of the growth medium is limited (Ferro et al., 2018; Kudahettige et al., 2018). In this study cultivation system, the total volume of the pond was removed after 6 days; i.e. at each biomass harvest day 1/3 of the volume of the microalgae culture was removed and the biomass was harvested every 2 days. Therefore, the biomass was harvested, when the microalgae were still under the exponential phase with less accumulation of lipids if compared to microalgae kept for a longer time to reach the stationary phase. This explains the reduced neutral lipids concentrations. Biomass with low-lipid productivities from microalgae cultivated in open ponds has been previously reported (Lundquist et al., 2010; Sialve et al., 2009). The main fatty acids belong to the 16 and 18 carbons fatty acids (Fig. 1B). Accordingly, these fatty acids are the most commonly synthesized fatty acids by microalgae. Specifically, C16:0 and C18:1 are the major fatty acids produced by green algae (Cobelas, 1989; Hu et al., 2008; Lage and Gentili, 2018; Niemi et al., 2019). In neutral FAMEs, the most represented fatty acids were 18:3, 18:1, and 16:0, while in polar FAMEs, the most represented fatty acids were 16:0, 18:1, and 18:0 (Fig. 1B).

3.2. Biochemical methane potential

3.2.1. Microalgae biomass and residues after lipid extraction

In the present study, the BMP of microalgae biomass mono-digestion was 122 NmL CH_4/g VS (Table 2). This BMP is comparable to a previous study on microalgae biomass cultivated at the same facilities but under a different period and growth conditions, in which the BMP of the mono-digestion of the microalgae under mesophilic anaerobic conditions was 118.2 NmL CH_4/g VS (Olsson et al., 2018). Olsson et al. (2018) also measured the theoretical methane potential of the microalgae biomass based on the organic fraction composition, showing a 27% degree of degradation. Thus, theoretically, most of the methane output remained in the microalgae biomass after digestion. Indeed, the BMP of the microalgae biomass in the current study and Olsson et al. (2018) is low compared to the literature (Neves et al., 2016). This might be attributed to the reduced biodegradability of the predominant microalgae genus, considering that microalgae biodegradability is highly species-dependent (Mussgnug et al., 2010; Passos et al., 2013) and it has been shown that the cell wall of *Scenedesmus* sp. can stay intact during anaerobic digestion (Mussgnug et al., 2010).

The microalgae lipid extraction ruptures the cell wall and makes the biomass more available for degradation, with most studies measuring higher methane yields on microalgae biomass after lipid extraction compared to biomass without extraction (Neves et al., 2016). Accordingly, it was observed that the lipid extraction process increased the methane yield of the microalgae biomass of a culture of *Scenedesmus* sp. and of a mixed culture enriched with *Scenedesmus* sp. (Keymer et al., 2013; Passos et al., 2013). Also, in *Nannochloropsis gaditana*, an increase in the BMP was observed after drying and lipids extraction. This effect was compared to pre-treatment methods, and based on the results, the extraction of lipids could be considered a pre-treatment step (Alzate et al., 2014). However, this positive effect of lipid extraction was not observed in the present study; the BMP of the biomass residues after lipid extraction (E Ext algae and NE Ext algae) mono-digestion was lower than the BMP of the non-extracted microalgae biomass (Table 2). This suggests that either the cell wall was not the cause of the low BMP or that the gain was outweighed by the loss in lipids. The organic fraction resulting in the highest methane concentration is theoretically the lipids fraction, but simultaneously, the kinetics of the digestion of lipids is slower than of the other organic fractions (Alzate et al., 2014; Nielfa et al., 2015). Moreover, Alzate et al. (2014) used a different microalgal species than the present study, which can play a pivotal role in the anaerobic digestion process, as previously discussed (Mussgnug et al., 2010; Passos et al., 2013). Additionally, contrary to the present study, in the two previous studies with *Scenedesmus* sp. as the predominant microalgae, a pre-treatment to break microalgae cell walls by mechanical means was applied (Keymer et al., 2013), which might have increased the biodegradability of the microalgae biomass and, subsequently, increased both the lipids and methane yields (Lee et al., 2013). Nevertheless, as in this study, a higher methane productivity for untreated microalgae biomass than lipid-extracted microalgae residues has been previously reported. Out of five microalgae species investigated (*Chlorella vulgaris*, *Phaeodactylum tricornutum Nannochloropsis* sp., *Nannochloropsis salina*, and *Nanofrustulum* sp.), four of them had higher methane production in biomass samples without lipids extraction (Zhao et al., 2014). Ehimen et al. (2009) also observed higher methane

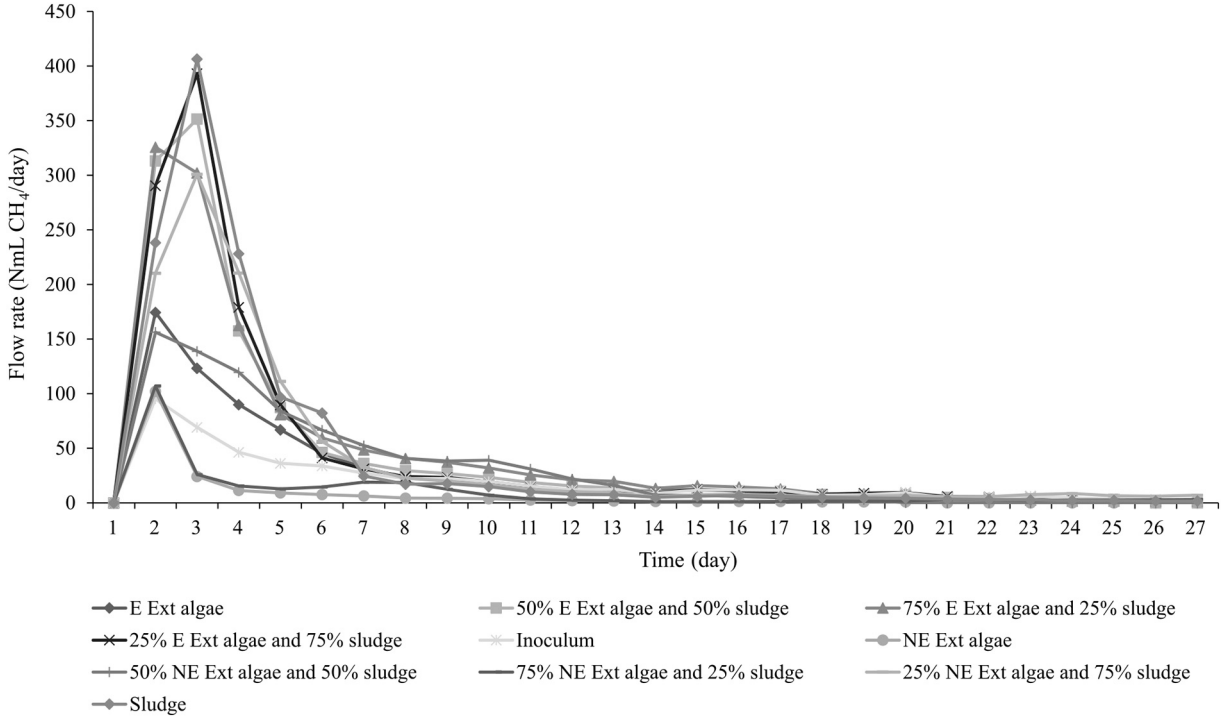

Fig. 2. Daily methane production of the samples with inoculum. The gas flow (NmL CH_4/day) data points express the mean values ($n = 3$).

productivity for *Chlorella* sp. biomass than lipid extracted microalgae residues. Thus, although lipid extraction methods can increase the biodegradability of microalgae biomass, depending on the solvent used in the conventional transesterification process, methane production can be inhibited (Ehimen et al., 2009; Zhao et al., 2014).

3.2.2. Inhibitory effect of solvents

Overall, the evaporated extracted microalgae residues, E Ext algae, had higher BMPs than the non-evaporated, NE Ext algae (Table 2), thus suggesting that organic solvents retained in the residual biomass after lipid extraction, i.e., hexane and isopropanol, had a strong inhibitory effect on the microbial activity and, consequently, the methane yield. In a previous study, it was shown that n-hexane inhibited methanogenesis (Yun et al., 2016a); however, the combination of n-hexane and isopropanol was not investigated. A particular, severe inhibition of the microbial activity was observed in two of the NE Ext algae samples, where a negative BMP was measured (Table 2). The negative values of net biogas production are an indication that no biogas was produced from those samples and that inhibition occurred during the anaerobic digestion (Suhartini et al., 2019). Hence, the BMP for both samples is given as 0 NmL CH_4/g VS in parentheses, and the negative values should be indicative and used mainly to evaluate the effect of the inhibition (Table 2).

In the literature, there are examples of negative methane production where the only biogas production is attributed to the inoculum. In those cases, the substrate can be a high-strength waste, such as textile wastewater (Suhartini et al., 2019). Although, to the authors' knowledge, negative BMP values for anaerobic digestion of microalgae have not been previously reported, there are reports of low biogas yields (Ehimen et al., 2009; Neves et al., 2016; Yun et al., 2014).

In comparison with the corresponding evaporated samples, i.e., E Ext algae, 75% E Ext algae and 25% sludge, the non-evaporated samples with the negative BMP had 170 and 124% lower BMP, respectively (Table 2). The general lower BMPs in the digestions of non-evaporated samples suggest that the evaporation of the organic solvents by the heating oven removed the residual solvents and their inhibitory effect. Our results underline the importance of organic solvents evaporation from the microalgal residues after lipid extraction. Of course, the evaporation should be performed in a safe and close space to avoid environmental pollution.

3.2.3. Synergistic effect of co-digestion

Overall, the co-digestion of the microalgae biomass residues with sludge leads to higher BMPs than the mono-digestion of each microalgae substrate (Table 2). In the co-digestion of 75% of the evaporated biomass residue with 25% sludge, the synergistic effect of co-digestion on the BMP was 1.44, while in the co-digestion with 25% of the evaporated biomass residue and 75% of sludge, it was 1.08 (Table 2). In the non-evaporated residue, the co-digestion also promoted higher BMPs than the mono-digestion; however, the negative BMP of NE Ext algae mono-digestion makes it difficult to interpret the results. The co-digestion with sludge enhanced the anaerobic digestion of microalgae biomass residues, leading to higher substrate biodegradability and biogas production. In addition, the co-digestion partly reduced the organic solvent inhibition. Accordingly, an increase in methane yield was shown in the co-digestion of sewage sludge with microalgae slurry compared to microalgae mono-digestion at thermophilic conditions (Olsson et al., 2014). However, the highest biogas production was obtained under a mesophilic condition than a thermophilic condition (Olsson et al., 2014). Also, the co-digestion of microalgae and primary sludge (25/75%) led to a 65% increase in the methane production compared to the microalgae mono-digestion under mesophilic conditions (Solé-Bundó et al., 2019). A possible explanation of the positive effect of the co-digestion is due to the balancing of the C/N ratio (Lage et al., 2018). Considering that whole microalgae biomass in the exponential growth phase, as in this study, has a high N content, the same biomass after lipid removal will have an even higher N content. Hence, the mixture with a high carbon source such as municipal sludge can balance the C/N ratio, increasing biogas production.

The average daily production of methane calculated from triplicate samples is shown in Fig. 2. The sample with only sludge shows the largest production at start. The lowest gas production is from the sample containing only mono-digested, lipid-extracted microalgae biomass with non-evaporated extraction solvent (NE Ext algae) and the following

sample with the highest proportion of non-evaporated biomass (75% NE Ext algae +25% sludge), which is in line with the results reported in the previous section. Since the NE Ext algae and 75% NE Ext algae +25% sludge samples have a lower gas production than the inoculum (blank sample), the gas production would be negative if the gas production of the inoculum was removed. In addition, the sludge sample contains the lowest concentration of VS (Table 1), which means that if the VS amount in the samples is normalized, the difference between the sludge and the samples containing mostly or only NE Ext algae would be more pronounced.

The present study used a thermophilic condition, while most of the studies in the literature use a mesophilic condition. Thermophilic bacteria have been found to be more sensitive than mesophilic bacteria (Appels et al., 2008). For digesting substrates that can cause inhibition, such as lipid extracted microalgae, mesophilic digestion can be a more feasible option than thermophilic digestion.

4. Conclusion

The extraction of lipids for biofuel production or high-value-added products will result in residual algal biomass. This residual biomass can be digested to produce biogas. This study highlights the importance of removing residual organic solvents after lipid extraction of algal biomass followed by anaerobic digestion, which can improve biogas production. Moreover, the co-digestion of algal biomass after lipids extraction with wastewater sludge could, to some extent, reduce the organic solvent inhibition. Future studies should determine the concentration at which the solvents become toxic to the anaerobes and the possibility of selecting tolerant anaerobes through continuous digestion.

CRediT authorship contribution statement

S. Lage: Investigation, Visualization, Writing – original draft, Writing – review & editing. **A. Willfors:** Funding acquisition, Investigation, Formal analysis, Writing – review & editing. **A. Hörnberg:** Funding acquisition, Investigation, Writing – review & editing. **F.G. Gentili:** Funding acquisition, Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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