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This is an electronic version of an article published in:

Ometto, F., Berg, A., Björn, A., Safaric, L., Svensson, Bo H., Karlsson, A., Ejlerstsson, J., (2018), Inclusion of *Saccharina latissima* in conventional anaerobic digestion systems, *Environmental technology*, 39(5), 628-639. <https://doi.org/10.1080/09593330.2017.1309075>

Original publication available at:

<https://doi.org/10.1080/09593330.2017.1309075>

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Inclusion of *Saccharina latissima* in conventional anaerobic digestion systems

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ABSTRACT

Loading macroalgae into existing anaerobic digestion (AD) plants allows us to overcome challenges such as low digestion efficiencies, trace elements limitation, excessive salinity levels and accumulation of volatile fatty acids (VFAs), observed while digesting algae as a single substrate. In this work, the co-digestion of the brown macroalgae *Saccharina latissima* with mixed municipal wastewater sludge (WWS) was investigated in mesophilic and thermophilic conditions. The hydraulic retention time (HRT) and the organic loading rate (OLR) were fixed at 19 days and 2.1 g l⁻¹ d⁻¹ of volatile solids (VS), respectively. Initially, WWS was digested alone. Subsequently, a percentage of the total OLR (20%, 50% and finally 80%) was replaced by *S. latissima* biomass. Optimal digestion conditions were observed at medium-low algae loading (≤50% of total OLR) with an average methane yield close to 220 Nml g VS_{algae}⁻¹ and 250 Nml g VS_{algae}⁻¹ in mesophilic and thermophilic conditions respectively. The conductivity values increased with the algae loading without inhibiting the digestion process. The viscosities of the reactor sludges revealed decreasing values with reduced WWS loading at both temperatures, enhancing mixing properties.

Highlights

- Efficient algae digestion was observed at organic loading rates $< 1 \text{ gVS l}^{-1} \cdot \text{d}^{-1}$.
- Thermophilic digestion allowed higher algae loading.
- Conductivity values increased over time (+300%) without affecting AD efficiency.
- Algae addition reduced digester material viscosity enhancing its mixing properties.

Keywords

Seaweed, co-digestion, salinity, viscosity, biomethane.

1. Introduction

Macroalgae are a valuable biomass for the production of a number of profitable products including biofuels. Having a potential energy content between 10 and 15 MJ kg⁻¹ of dry matter (DM), a value similar to that of energy crops such as sugarcane (11 MJ kg DM⁻¹), sunflower and rapeseed (19 MJ kg DM⁻¹), together with the absence of lignin (non-fermentable component), deems macroalgae a promising substrate for anaerobic digestion (AD) [1,2]. The Environment Committee of the European Parliament foresees the possibility of macroalgae-derived biofuels covering at least 1.25% of the total European energy consumption in the transport sector by 2020 [3]. However, there are still a number of obstacles limiting the exploitation of algae for biofuel production at a commercial scale [4,5]. Focusing on biomethane production via AD, these are related to biomass availability, AD process efficiency and profitability.

While high-value algae derived products fulfil the demand of a small market with a relatively low amount of algae biomass (0.2 million tonnes of macroalgae harvested in the EU in 2015 [6]), biofuel production requires significantly higher biomass availability. For macroalgae to satisfy the 1.25% target of the total European energy consumption for transport, more than 150 million tonnes per year would be required [7]. Therefore, despite the large amount of natural algae forests available, commercial utilisation of this biomass requires sustainable production facilities (e.g. algae farms). Algae farms have huge potential, but have to date suffered severe technical and biological difficulties such as determining optimal cultivation and harvesting techniques, experiencing adverse offshore weather conditions and a growth cycle mostly allowing for one harvest per year [7,8]. Once harvested, the algae biomass decays quickly [9]. Therefore, the biomass has to be stored and preserved until it is required, or it has to be processed in a short time [7].

Storage of algae biomass is costly and still not applicable to intensive production systems, while processing of extensive biomass quantity in a short time implies high organic loading rates (OLRs) in the AD unit often resulting in low digestion performances. For example, *Laminaria digitata*, a brown macroalgae available in large quantities in the natural environment and easy to mass cultivate [10], has a theoretical methane potential close to 500 ml g⁻¹ of volatile solids (VS), but experimental data on semi-continuous digestion give in average half of this amount [11,12]. This low yield would, assuming an annual biomass availability of 10,000 ton and a total solids (TS) content of 12% (75% VS of TS), give a yearly production of 225 000 Nm³ CH₄ from *L. digitata*. Applying a selling price at the production facilities equal to 0.75 € Nm⁻³ CH₄ [13], this gives a potential revenue of 140 € ton DM⁻¹, a value 1.5 times lower than the sole estimated harvesting cost for this algae (equal to 250 € ton DM⁻¹) [14]. Therefore, a better profitability for production of biogas from macroalgae is needed. This can be achieved by higher biomass yields from algae cultivation and use of optimised AD conditions to obtain efficient degradation rates and high methane yields. In the example with *L. digitata*, a 90% anaerobic degradation efficiency (instead of 50%) would increase the biomethane production by 80% giving a potential income close to 250 € ton DM⁻¹ offsetting the harvesting costs. This higher degradation grade, combined with low cost harvesting and cultivation techniques, will be required for macroalgae to be considered as an economically competitive biomass for AD [7,8].

Co-digestion of algae with complementary substrates has shown promising results by enhancing the efficiency of the algal digestion. For instance, co-digestion of *Saccharina latissima* with wheat straw (75:25 algae:straw ratio) at a total OLR of 1.5 g VS l⁻¹ d⁻¹ in batch mode, increased the methane yields by 40% from 200 ml g VS⁻¹ to 275 ml g VS⁻¹

[15]. More recently, digestion of a mixture of fresh *Ulva lactuca* with dairy slurry in a semi-continuous system (25:75 algae:slurry ratio) with a total OLR at 2 g VS l⁻¹ d⁻¹ resulted in an algal methane yield at 95% of its batch potential value [16]. This suggests that it is beneficial to co-digest algal biomass in existing AD plants to increase digestability and methane yields. The co-digestion might however influence the fluid properties of the AD sludge which might lead to changes in rheological properties (e.g. apparent viscosity, limit viscosity and yield stress) possibly leading to inadequate mixing and heat transfer, breakdown of stirrers, and/or foaming. These kind of problems, which can differ in mesophilic and thermophilic conditions, may lead to deteriorations in the overall process performance and increase in energy consumption [17,18,19].

The aim of the study is to investigate the performance of *S. latissima* in co-digestion with municipal WWS under different conditions including different algae:sludge VS-ratios (0:100, 20:80, 50:50 and 80:20), at both mesophilic (37°C) and thermophilic (52°C) conditions. Tests will be performed in lab-scale semi-continuously stirred tank reactors (CSTRs). This will assess the impact of algal biomass on the reactor sludge viscosity at low (<1 gVS l⁻¹ d⁻¹) and high (1-1.7 gVS l⁻¹ d⁻¹) algae loading for the first time.

2. Material and methods

2.1 Substrates

S. latissima was cultivated by SINTEF in Kristiansund, Norway, and harvested at the end of its growth cycle in June 2014. The algae biomass (ca. 200 kg wet weight) was homogenised in a blender to a particle size $\leq 1 \text{ cm}^2$ and stored at -20°C until utilisation. The TS was 10% and VS 50% of TS. WWS (primary sludge mixed with waste activated sludge from a recirculation tank) was collected every two weeks from a local municipal

wastewater treatment plant (Linköping, Sweden) and stored at 4°C until utilisation. The TS concentration varied between 4% and 6%, while the VS concentration was 78% of TS.

2.2 Semi-continuous AD reactors

Four 5 l semi-CSTRs, with a working volume of 4 l, were operated in duplicate for 295 days; two in mesophilic (37°C) and two in thermophilic (52°C) conditions. The reactors were inoculated with active anaerobic biomass (4 l to each reactor) from a full scale mesophilic AD plant treating municipal WWS in Linköping (Sweden), and a thermophilic plant treating municipal food waste in Borås (Sweden), respectively. The OLR and HRT were 2.1 gVS l⁻¹ d⁻¹ and 19 days, respectively. For the first three HRTs, the reactors were fed with WWS. Subsequently, 20% (Phase 1 – 20:80 algae:sludge VS-ratio), 50% (Phase 2 – 50:50 algae:sludge VS-ratio) and 80% (Phase 3 – 50:50 algae:sludge VS-ratio) of the WWS was replaced (on VS-bases) by *S. latissima* biomass, with a minimum interval of three HRTs between the shifts. Mixing was provided at 350-400 rpm for 15 min every 3 h to reproduce mixing condition for commercial scale AD plants operated by Scandinavian Biogas Fuels AB (Stockholm, Sweden). Biogas production was measured continuously by Ritter MCG meters (Bochum, Germany). Gas composition measurements (CH₄, CO₂, O₂ and H₂S) were undertaken using a Geotech Biogas analyser (Royal Leamington Spa, United Kingdom) once a week on a 24h collected sample. Measurements were verified using a standard gas mixture (CH₄ and N₂). All gas yields are given at standard pressure and temperature (0°C; 100 kPa) and as a mean average value of the duplicate. Statistical analysis (ANOVA) of biogas production was carried out as per <http://www.r-project.org/>, with significance accepted at $p \leq 0.05$.

2.3 Batch digestion

The biomethane potential (BMP) of the substrates was determined after 60 days of mesophilic batch digestion and performed in triplicate, following the procedure reported by Ekstrand et al. [20]. Briefly, a 320 ml glass bottle was filled with 20 ml of fresh inoculum, obtained from the local mesophilic AD plant (Linköping, Sweden), substrate (8 g for *S. latissima* and 15 g for the WWS, giving an substrate:inoculum ratio close to 2:1 of VS), 2 ml of saline solution (450 mM NH_4Cl , 410 mM NaCl , 60 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 29 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), 0.3 ml Na_2S (100 mM solution) and water to reach 100 ml final volume. Biogas production was measured at regular intervals (day 1, 2, 5, 10, 20, 30, 45 and 60) using a Testo 312-3 pressure gauge (Testo AG, Germany). Methane content was determined by GC-FID following the procedure reported by Karlsson et al. [21]. All gas yields are given as average value of a triplicate $\pm$ SD at standard pressure and temperature (0°C; 100 kPa).

2.4 Analytical procedures

The VFAs content was determined from 1 ml of liquid samples centrifuged at 12,000 rpm for 10 min. The supernatant was then injected on a GC-FID, following the method reported in Jonsson and Borén [22]. The detection limit was 0.2 mM and the quantification limit was 0.6 mM. An EcoSense® EC300 (Yellow Springs, USA) was used for conductivity measurements. TS, VS, ammonium concentration and pH were measured in duplicate according to the APHA [23] standard methods. Substrate elemental composition analysis was completed in accordance with the SS 028150-2/ICP-AES method and performed by Eurofins Environment Sweden AB (Lidköping, Sweden). Sugar content and composition analysis were performed by SINTEF (Kristiansund, Norway) according to the APHA [23] standard methods.

Samples for viscosity characterisation were withdrawn at the end of each experimental

phase (1-3) and analysed immediately or stored at 4°C prior to analyses for a maximum of 24 h. Limit and apparent viscosity were measured at shear rates of 800 and 300 s⁻¹, respectively, in triplicate following the three-step protocol described by Björn et al. [18]. A rotational rheometer (RheolabQC SN80609650) equipped with a CC27-SN19237 measuring system and a C-LTD 80/QC cell, coupled with Rheoplus software was used for the analyses. The temperature was fixed at 37°C and 52°C for the meso- and thermophilic samples respectively. The certified viscosity reference standard Cannon®RT1000 was used for quality control.

3. Results and discussion

3.1 Substrate characterisation

Cultivated *S. latissima* had an average TS content of 10%. Visual observation of the algae identified the presence of fouling biomass (small shellfish and marine microorganisms) as a result of the late harvesting. No fouling was observed on the non-cultivated *S. latissima*. The fouling resulted in an increased ash content (50% of TS), related to higher contents of elements including iron, copper, magnesium, manganese and titanium in comparison with the same algae specie harvested from the non-cultivated wild biomass (Table 1).

The main inorganic components of the algae biomass was sodium (75 g kg TS^{-1}) and potassium (55 g kg TS^{-1}) followed by sulphur, magnesium, calcium, iron and phosphorus (ranging 3.3 to 19 g kg TS^{-1} ; Table 1). The sum of arsenic, cadmium, chromium, lead and mercury constituted $0.03 \text{ g kg TS}^{-1}$. The concentrations of trace elements essential for AD, such as cobalt, molybdenum, nickel, selenium, were low in relation to the requirements presented by Montingelli et al. [24]. For the WWS, iron was the main inorganic compound (33 g kg TS^{-1}) followed by phosphorus (19 g kg TS^{-1}), calcium (15 g kg TS^{-1}) and aluminium (12 g kg TS^{-1}). The concentration of cobalt was similar to that of the algae while molybdenum and nickel levels were two times higher, and selenium four times lower, while the other heavy metals contents were below $0.02 \text{ g kg TS}^{-1}$ (Table 1).

The BMP of the cultivated algae biomass was $240 \pm 5 \text{ ml g VS}^{-1}$, which is similar to the value of $220 \pm 61 \text{ Nml g VS}^{-1}$ reported by Vivekanand et al. [15] for *S. latissima* biomass cultured in the same area and environment. However, higher values, $340 \pm 36 \text{ Nml g VS}^{-1}$, were reported for processing the same algae, non-cultivated and harvested from a

different location [12]. In addition, the processed algae biomass was characterised by a low sugar content. Compared with expected values exceeding 10% of the dry weight, the measured laminarin and mannitol content was equal to 0.2% and 3.8%, respectively [cf. 9,25]. The low sugar content partly explains the low BMP value obtained in the study, confirming the impact of cultivation location and conditions, and harvesting time, on the overall biomass composition [26].

Compared with the algae biomass, the WWS showed a significantly higher (+48%) methane potential equal to $360 \pm 9 \text{ Nml g VS}^{-1}$. Therefore, while replacing an increasing amount of the OLR with algae biomass, a decrease in the potential methane production occurred. An increasing proportion of VS with a high methane yield ($360 \text{ Nml g VS}_{\text{sludge}}^{-1}$) was replaced with VS with a low methane yield ($240 \text{ Nml g VS}_{\text{algae}}^{-1}$). When changing the ratio from 100% WWS (which yielded $360 \text{ Nml g VS}^{-1}$) to 20:80 algae:sludge, 50:50 and 80:20, the estimated BMP value decreased to 350, 300 and 270 Nml g VS^{-1} , respectively.

3.2 Digestion performance

During the first three HRTs municipal WWS was the sole substrate and a biogas production of 470 and 510 $\text{Nml g VS}^{-1} \text{ d}^{-1}$ was reached at mesophilic and thermophilic conditions, respectively (Figure 1 – Control). With a methane content of 61% (Table 2) this gave a methane production close to 300 and 350 $\text{Nml g VS}^{-1} \text{ d}^{-1}$ at mesophilic and thermophilic conditions, respectively. The total solids content in the reactor was 2.7% (65% VS of TS) resulting in a VS reduction close to 60% in both systems (Table 2).

On day 65, the substitution of 20% of the OLR with algae biomass caused a drop in the biogas production of about 15% in accordance with the lower BMP value of the new feeding (Figure 1 – Phase 1, 20% algae). After 3 HRTs, the total biogas production at

37°C had stabilised at 450 Nml g VS⁻¹ d⁻¹. This value is equal to 280 Nml CH₄ g VS⁻¹ d⁻¹ and it covers 80% of the estimated BMP value for this level of co-digestion resulting in a calculated total VS reduction of 50%. Assuming that the VS-reduction of the WWS is still 60%, this means that the algal biomass was subjected to 25% VS reduction. Higher yields and VS reduction were observed at 52°C, where biogas and methane production were close to 470 and 300 (+7%) Nml g VS⁻¹ d⁻¹, respectively. This corresponds to 52% total VS reduction and 30% VS reduction of the algae biomass (60% VS reduction applied to WWS).

The additional algae biomass loading introduced into the system from day 145 (Figure 1 – Phase 2, 50% algae) did not impact the average biogas/methane production for the first HRT. However, from day 170 a decrease in production was observed for all reactors followed by an increase during the third HRT (days 198-215). All reactors showed similar methane production; 260 Nml g VS⁻¹ d⁻¹, which corresponds to 86% of the estimated BMP value for this phase. The total VS reduction was stable at 50% and 52% for the mesophilic and thermophilic processes, respectively, but a greater percentage of the algal biomass was now digested (48-50% algal VS-reduction).

During the final phase of the experiment (Figure 1 – Phase 3, 80% algae) the behaviour of the duplicate reactors at both mesophilic and thermophilic conditions differed significantly ($p < .05$ between all 4 reactors) indicating inefficient AD process. This was confirmed by the shift in the total VFA and pH values between days 215 and 295 (Figure 2). During the instable period the methane yield of the most affected mesophilic reactor dropped to 60-100 Nml g VS⁻¹ d⁻¹, after 2 HRTs. However, from day 265 and onwards (3rd HRT), the reactor increased the production to values between 230 and 250 Nml CH₄ g VS⁻¹ d⁻¹, equal to 85-95% of the BMP value. A similar behaviour occurred at

thermophilic conditions, however, higher methane yields (+30% compared to the mesophilic reactors) were maintained (Figure 1 - Phase 3, 80% algae). Between days 216 and 265 the methane production gradually decreased to 200 Nml CH₄ g VS⁻¹ d⁻¹. After day 265, the methane yield increased to over 250 Nml CH₄ g VS⁻¹ d⁻¹ as a result of higher digestion rates with total and algae VS reduction between 50% and 55%.

Overall, these results confirm that anaerobic co-digestion of *S. latissima* and WWS is a feasible way forward for the use of algal biomass as a substrate in AD. The estimated methane production from the solely algae biomass at 37°C during the first phase (20% algae) was equal to 200 Nml g VS⁻¹ representing 83% of the BMP value of *S. latissima*. When using a 50:50 algae:sludge ratio condition, the estimated production increased to 220 Nml g VS⁻¹ d⁻¹ (92% of the BMP value) suggesting that this combination is close to optimal conditions for co-digestion of these two substrates. The instability observed at levels above 50% VS algae (50% of OLR from algae), is in agreement with previous studies. For instance, Jard et al. [27] reported strong inhibition when processing *S. latissima* and *Palmaria palmata* in mesophilic conditions with an OLR equal or higher than 2.1 g VS l⁻¹ d⁻¹, with more than 50% methane yield reduction. Similarly, co-digesting efficiency of *Ulva lactuca* and dairy slurry (OLR of 2 g VS l⁻¹ d⁻¹) decreased from 95% to 76% and 18% when increasing the algae:slurry ratio from 25:75 to 50:50 and 75:25, respectively [16]. This was also confirmed in thermophilic conditions, where moving from an algae loading rate of 3.3 g VS l⁻¹ d⁻¹ to 2.5 g VS l⁻¹ d⁻¹ in co-digestion with manure, the average methane yield of *Ulva* sp. decreased from 250 to 150 ml g VS⁻¹algae [28]. Process instability caused highly fluctuating biogas yields (between 50 and 200 ml g VS_{algae}⁻¹) over the 7 HRTs of observation (115 days), which is similar to the reactor behaviours during phase 3 of the current work. Here, the estimated production after 3

HRTs ranged 180-250 Nml g VS⁻¹ d⁻¹ at both mesophilic and thermophilic conditions with a biodegradability efficiency varying between 80% and 95% of the BMP value.

3.3 pH and VFA accumulation

The mesophilic reactors operated at a pH of around 7.5 until day 215 (Figure 1). Subsequently, during the highest algae loading phase (80% algae), in correspondence to the low methane yields observed previously, VFA accumulation up to 8 g l⁻¹ reduced the pH value below 7.0. The observed reduction of acetic acid after day 265 and the consequent pH increase confirmed microbial adaptation to the new feedstock composition enhancing digestion performances (Figure 2).

The effect of the 80:20 algae:sludge load was less pronounced in the thermophilic reactors; the pH value remained at 8.0 and total VFA did not exceed 3 g l⁻¹. Temperature is known to impact the microbial community and therefore higher diversity occurs at mesophilic versus thermophilic conditions [29], and higher activity take place at thermophilic conditions [30,31]. This partially justifies the lower VFA accumulation, together with the higher methane yields observed at 52°C compared to 37°C. However, the thermophilic digester behaviour suggests a process capable of digesting higher algae loading despite observed decreasing concentrations of trace elements and high S with increased algae load (additional information in Table A1). To illustrate, the replacement of WWS with algae over time reduced the concentration of Co, Ni and Mo measured in the digested sludge with over 50%, while the loading of S into the system increased from 8 g kg TS⁻¹ (sludge only) to 17 g kg TS⁻¹ (80:20 algae:sludge).

3.4 Hydrogen sulfide

The initial H₂S concentration was below 5 ppm and increased gradually eventually exceeding 1000 ppm at the end of Phase 2 (50:50 algae:sludge) and 10 000 ppm during

Phase 3 (80:20 algae:sludge) (Table 2). This confirms the findings of Venages and Bartlett [32] on H₂S formation when digesting seaweed. However, during the first phase of the experiment, the presence of iron in the WWS gave a Fe:S ratio in the mixed substrate close to 3:1, enabling precipitation of the formed sulfide to FeS [33]. When the iron loading was reduced (lower ww sludge load), the Fe:S ratio decreased to 2:1 and 1:1, after Phase 2 and 3, respectively, and H₂S formation occurred.

Although similar H₂S concentrations were shown not to give process disturbance during AD of macroalgae [32], the presence of H₂S has a major impact on the economy of the biogas upgrading process and downstream utilisation [14]. This is in contrast to previous work [34]. Despite high H₂S concentrations, the higher methane yields observed at thermophilic conditions during the first HRT in Phase 3 (Figure 1 – 80% algae), show that this process is more suitable for degradation of high loadings of algae biomass than for mesophilic digestion.

3.5 Ammonium

The ammonium concentration increased over time in both systems without exceeding 1.7 g NH₄⁺-N l⁻¹ (Table 2). This is the result of the additional proteins introduced with the algae (between 3% and 21% of the dry matter [2]). Higher values were recorded at thermophilic conditions which is likely to be due to the higher degradation of the nitrogenous organic compounds at 52°C than at 37°C [cf. 35]. Similar behaviour was observed during co-digesting *Ulva* sp. (26% proteins) with mixed WWS in batch mode (15:75 algae:sludge VS-ratio) [16]. Ammonium concentrations of up to 1400 mg NH₄⁺-N l⁻¹ did not affect the AD process [36]. However, when processing protein-enriched algae (50-60% of dry matter), higher levels of ammonium will occur which may result in the inhibition of the methanogenic population. Nevertheless, the loading of Na⁺, K⁺, Ca²⁺

and Mg^{2+} , introduced into the system with the algae biomass, will hamper the ammonium inhibition by supporting methanogenic activity [cf. 37,38].

3.5 Conductivity

The conductivity value was highly effected by the algae biomass addition (Figure 3). Within the reactor, the initial conductivities of the mesophilic and thermophilic conditions were $7.5 \pm 0.1 \text{ ms cm}^{-1}$ and $8.7 \pm 0.2 \text{ ms cm}^{-1}$, respectively. The conductivity increased linearly with the TS content of algae added by 2, 3 and 5 times for the 20:80, 50:50 and 80:20 algae:sludge VS-ratio, respectively. This reached 37 ms cm^{-1} in both systems after 295 days. In both reactors, stable conductivity values were observed after 2 HRTs after the new feeding composition was started. Constant conditions are a characteristic of efficient and stable AD processes [39]. Therefore, the observed conductivity increment will mainly be due to salt loading (Na^+ and K^+), suggesting a stress of the system as a result of the algae addition. In all reactors the concentration of Na^+ and K^+ increased over time to reach 0.5 g l^{-1} and 5 g l^{-1} respectively at the end of phase 3 (80:20 algae:sludge). However, these values are below the 6 g Na l^{-1} and 17 g K l^{-1} identified by Jard et al. [27] to inhibit the methanogenic activity when processing the same alga species *S. latissima* (50% inhibition).

Although salinity has been proved to inhibit the AD by impacting the methanogens [40], the levels achieved in this work did not seem to affect the methanogenic activity as the methane level in the produced gas remained above $\text{CH}_4 \geq 60\%$. (Table 2).

3.5 Viscosity

The viscosity values (apparent and limit viscosity) differed between the two digestion systems (Table 2). Higher limit viscosities, between 6.1 and 7.4 mPa s, were measured in mesophilic conditions (thermophilic 4.6 - 5.6 mPa s). In both systems, the increase in TS

from 3.5% to 4.5% between phase 1 (20:80 algae:sludge) and phase 2 (50:50 algae:sludge) did not impact on the limit viscosity values. However, the additional TS increment (>5%) in Phase 3, due to increased algae loading (80:20 algae:sludge), reduced limit viscosities by 20%. These behaviours confirm that the TS content alone is not a reliable parameter to predict and evaluate changes in viscosity in AD processes. This is as previously reported by the authors in earlier viscosity studies of full-scale biogas reactors with different substrate profiles [18,41]. As the structure of digested WWS is governed by steric interactions [42], a high impact on viscosity can be influenced by the presence of extracellular polymeric substances (EPS) in the WWS including filamentous cells, lipids, proteins and biosurfactants [43]. Therefore, together with TS content, the monitoring of these parameters could be an optimal strategy to predict changes in viscosity of the digester fluid and the extent of the mixing demand and associated required energy supply .

4 Economic feasibility

Current cultivation practise of *S. latissima* in Norway would supports an algal biomass production of 100-150 ton (wet weight) ha⁻¹ y⁻¹ [9,44,45]. For the purpose of this analysis, a 80 ha algae farm has been considered, thus resulting in a production of 10,000 ton y⁻¹. This corresponds to 700-980 ton VS y⁻¹ depending on the biomass' VS content (Table 3). In co-digestion with mixed municipal WWS, this amount of algae could provide between 154 000 and 245 000 Nm³ CH₄ depending on the digestion condition applied (Table 3). Hence, with a methane market value of 0.75 € Nm⁻³ [13], 10,000 ton of algae could provide a revenue between 0.12 and 0.19 million Euro when used for biomethane production (Table 3). This value covers only 30-50% of the estimated price for macroalgae biomass delivered at the AD site exceeding 0.35 million Euro (250 € ton DM⁻¹

¹ as harvesting costs only [14]), confirming the processing of macroalgae for biomethane production non-yet profitable [14].

To turn macroalgae biomass as a profitable biomass for biomethane production, we see three feasible alternatives: (1) algae biomass production costs (cultivation and harvesting) are significantly reduced, or (2) biomethane production from algae is integrated in a biorefinery approach, or (3) innovative strategies to compensate the biomass costs are considered. For (1), based on current costs for the operation of a conventional AD unit treating multiple substrates in Sweden [13], the maximum acceptable selling price for macroalgae delivered at the AD plant premises is estimated to range between 4 and 12 € ton DM⁻¹ (Table 3). This value is similar to the one reported by Murthy Konda et al. [8] when considering *S. latissima* for ethanol production (23 € ton DM⁻¹). Such low biomass costs can only be achieved with the implementation of low costs harvesting techniques that require new technologies not yet available on the market [7,24].

For (2), the biomass should first be processed for high valuable products (e.g. extraction of proteins and pigments) and the residues then used for biomethane production [7,8]. The profit related to the commercialisation of the high-value products will contribute to offset cultivation/harvesting costs supporting commercial competitiveness of algae based biofuels. However, this will impact expected methane yields and the related revenue value as part of the methane potential will be lost in the extracted material (e.g. proteins). For (3), policy makers should consider economically compensated algae farms for the environmental benefits related to macroalgae phytodepuration characteristics [45,46]. A macroalgae farm producing 10,000 ton wet biomass has a nutrient uptake equal to 35 ton of nitrogen [9,47]. Cultivated in the premises of fish farms, algae have been demonstrated to mitigate nitrogen emissions otherwise untreated [9]. The fixed nitrogen can after

anaerobic digestion be concentrated using evaporation technic and sold as a liquid fertiliser. To compare, such nitrogen mitigation via conventional European wastewater treatments (waste activated sludge) would cost between 0.4 and 2.7 million Euro [49], enough to offset algae biomass production costs.

5. Conclusions

Wastewater sludge is a suitable co-substrate to optimise AD of *S. latissima*. An algae biomass loading between 0.4 and 1 g VS l⁻¹ d⁻¹ (OLR_{algae} below 50% of total OLR) showed a digestion efficiency up to 92%, with average methane yields close to 220 Nml g VS⁻¹, equal to 15 Nml g⁻¹ of wet biomass. This potentially allows a revenue generation of between 10 and 18 € ton⁻¹ wet treated biomass, depending on the VS content. The highest algae loading tested (1.7 g VS l⁻¹ d⁻¹) initially gave an instable digestion process that suffered from VFA accumulation as well as low trace elements concentration. However, both the mesophilic and the thermophilic processes adapted to the high algae load leading to a recovery of VFA concentrations below 2 g l⁻¹ and re-established methane yields, with the thermophilic process, allowing a faster recovery. The salt loading introduced into the AD systems with the algae, did not reduce the process efficiency. Furthermore, the highest inclusion of macroalgae reduced the viscosity of the digester material, likely enhancing its mixing properties both at mesophilic and thermophilic conditions.

Acknowledgments

The authors would like to thank the European project ATBEST (grant agreement n. 316838), the Swedish Biogas Research Centre (BRC) hosted by Linköping University (Sweden), Biokraft AS (Norway) and SINTEF (Norway) for their financial and intellectual support.

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Figure 1 – Reactors' performance under different experimental phases. The algae:sludge VS-ratios changed from 0:100 (Control) to 20:80 (Phase 1), 50:50 (Phase 2) and 80:20 (Phase 3). The OLR was constant at 2.1 gVS d^{-1} . Biogas yields (A), total VFAs (B) and pH (B) values are reported at mesophilic (grey marks) and thermophilic (black marks) conditions, as an average of duplicate ($p < .05$ between duplicates). Vertical lines at day 67, 145 and 215 mark the different experimental phases.

Figure 2 - Specific VFAs composition between days 217 and 295 (80:20 algae:sludge VS-ratio) at mesophilic (A) and thermophilic (B) conditions. Values are reported as average of duplicate samples.

Figure 3 - Conductivity and TS correlation over time at mesophilic (empty markers) and thermophilic (filled markers) conditions. Black arrows mark the end of each experimental phase corresponding to different algae:sludge VS-ratios: circle (0:100), triangle (20:80), square (50:50) and diamond (80:20). Data are presented as an average of duplicate values.

Table 1 - Elemental composition analysis of processed biomass: comparison between *S. latissima* and wastewater sludge (SS 028150-2/ICP-AES method applied giving 95% accuracy).

Element ^a	<i>S. latissima</i> cultivated and harvested in Kristiansund (NO) ^b	<i>S. latissima</i> harvested in Kristiansund (NO) ^c	<i>S. latissima</i> harvested in Clachan Sound (UK) ^d	Municipal ww sludge Linköping (SE)
Al	130	63	1877	12000
Sb	< 1	< 1	-	1
As	24	68	73	2
Ba	67	14	22	120
Pb	< 1	< 1	2	9
B	140	120	-	25
P	3300	2400	-	19000
Fe	3400	230	1159	33000
Cd	2	1	-	< 1
Ca	11000	16000	18000	15000
K	55000	64000	42000	2100
Co	< 3	< 3	-	3
Cu	43	3	4	170
Cr	5	< 3	5	8
Hg	-	-	-	-
I	-	-	3193	-
Mg	12000	8200	8000	1500
Mn	23	4	35	130
Mo	1	< 1	1	4
Na	75000	48000	38000	980
Ni	6	< 3	2	10
Se	4	< 1	-	1
Ag	< 1	< 1	-	1
Rb	-	-	33	-
S	19000	11000	-	7900
Sr	-	-	645	-
Ti	12	< 5	135	130
V	< 5	< 5	-	7
W	< 1	< 1	-	1
Zn	85	38	29	360
Dry weight	14%	18%	15%	6%
Ash content	50%	29%	35%	22%

^adata reported in mg kgDM⁻¹; ^bbiomass cultivated by SINTEF (Norway), harvested in June 2014 and utilised in the present study; ^cbiomass harvested by SINTEF from the wild environment in July 2014 (Norway); ^dbiomass harvested from the wild environment in late May 2011 in the UK (Schiener et al., 2015); "-" means below detectable limit.

1 Table 2 - Digestate characterisation and biogas composition (mean±SD) observed at the end of each experimental phase under different
2 algae:sludge VS-ratios. Limit and apparent viscosity are reported at shear rate of 800 and 300 s⁻¹, respectively.
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Parameter	Unit	37 °C				52 °C			
		Control	20:80 algae:sludge	50:50 algae:sludge	80:20 algae:sludge	Control	20:80 algae:sludge	50:50 algae:sludge	80:20 algae:sludge
Digestate									
pH		7.4 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.4 ± 0.1	7.9 ± 0.1	7.9 ± 0.1	8.0 ± 0.1	7.8 ± 0.1
TS	%	2.7 ± 0.1	3.5 ± 0.1	4.5 ± 0.1	5.5 ± 0.2	2.6 ± 0.1	3.4 ± 0.1	4.4 ± 0.1	5.3 ± 0.3
VS	% of TS	65 ± 1	58 ± 1	44 ± 1	34 ± 1	64 ± 1	57 ± 1	44 ± 1	34 ± 1
Ammonia	mgNH4+-N/l	1100 ± 51	1200 ± 20	1400 ± 46	1600 ± 20	1500 ± 52	1500 ± 25	1700 ± 44	1690 ± 127
Conductivity	ms/mc	7.6 ± 0.1	13.7 ± 0.5	24.2 ± 0.7	35 ± 0.5	8.7 ± 0.2	15.1 ± 0.4	24.8 ± 1	37 ± 0.5
Limit viscosity		-	7.2 ± 0.6	7.4 ± 0.3	6.1 ± 0.5	-	5.3 ± 0.4	5.6 ± 0.3	4.6 ± 0.2
Apparent viscoisty	mPa·s	-	10 ± 2	10 ± 2	7.2 ± 1.4	-	6.6 ± 0.3	6.0 ± 0.9	3.0 ± 0.3
Shear stress	mPa·s	-	2.2 ± 1.8	1.8 ± 1.2	1.1 ± 0.5	-	0.8 ± 0.3	0.7 ± 0.2	0.3 ± 0.1
Biogas									
Total yiled	ml/gVS·d	470 ± 23	440 ± 5	410 ± 12	230 ± 24	520 ± 15	460 ± 16	430± 11	310 ± 21
CH ₄	%	65 ± 1	64 ± 1	61 ± 2	59 ± 3	65 ± 1	64 ± 1	62 ± 1	61 ± 4
CO ₂	%	35 ± 1	36 ± 1	40 ± 2	41 ± 1	35 ± 1	35 ± 1	39 ± 2	39 ± 2
H ₂ S	ppm	-	-	1100 ± 296	> 10000	< 5	< 2	1100 ± 278	9350 ± 100

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Table 3 – Economic assessment for the AD of 10,000 ton of *S. latissima* in co-digestion with mixed municipal wastewater sludge at mesophilic and thermophilic condition.

Parameters	Unit	Degestion condition					
		Mesophilic (37°C)			Thermophilic (52°C)		
<i>S. latissima</i> VS content ^a	% of TS	50	60	70	50	60	70
Biomass ^b	tonVS/y	700	840	980	700	840	980
Processing time ^c	days	43	53	61	43	53	61
Methane ^d	m ³ CH ₄ /y	154 000	185 000	216 000	175 000	210 000	245 000
Revenue ^e	million€/y	0.12	0.15	0.16	0.14	0.16	0.19
	€/ton wet biomass	12	15	16	14	16	19
Maximum biomass costs ^f	€/ton DM	4-7	5-9	6-10	4-8	6-10	6-12

^adata based on observed VS variation for *S. latissima* as reported in Table 1 (current work).

^bconsidering an annual biomass availability of 10,000 ton wet biomass (14%TS) [45].

^cconsidering an existing AD plant (two reactors of 8000 m³) with enough spare capacity to accept an algae biomass loading equals to 1 g VS l⁻¹ d⁻¹.

^dbased on a methane yield equal to 220Nml gVS⁻¹ and 250 Nml gVS⁻¹ at mesophilic and thermophilic condition, respectively (current work).

^econsidering a selling price for methane equals to 0.75€ Nm⁻³ (mean value based on the Swedish market 2016 where the price for methane purchased at the AD site varies between 0.65 and 0.85 € Nm⁻³ [13]).

^fconsidering maximum biomass costs to secure a margin of profitability equal to 40-55% of the revenue from sold methane at the AD site [13].

Table A1 – Digestate characterisation observed at the end of each experimental phase under different algae:sludge VS-ratios, under mesophilic and thermophilic conditions. Data are presented as an average of duplicate reactors (SS 028150-2/ICP-AES method applied giving 95% accuracy).

Element ^a	37°C				52°C			
	Control	20:80 algae:sludge	50:50 algae:sludge	80:20 algae:sludge	Control	20:80 algae:sludge	50:50 algae:sludge	80:20 algae:sludge
Al	9600	9575	5650	3300	9050	9525	5700	3500
Sb	1	1	1	1	1	1	1	1
As	4	11	20	25	4.58	12.5	20	25
Ba	247	135	125	54	208	133	104	57
Pb	17	18	8	4	17	18	9	4
B	25	52	105	115	25	40	100	115
P	31250	23500	16000	9900	32500	23750	16000	10000
Fe	65750	38500	26250	11000	56250	37000	25750	11500
Cd	1	1	1	2	1	1	1	2
Ca	30500	47500	79000	90000	44500	48750	75250	86500
K	5575	21500	46250	58000	12800	23000	46750	63000
Co	4	3	2	2	3	3	2	2
Cu	320	203	130	60.5	270	202.5	150	65
Cr	40	25	14	9	17	13	20	16
Mg	3125	6000	11000	12000	3425	6100	10750	12000
Mn	238	150	98	51	225	148	97	53
Mo	10	7	4	3	8	6	6	4
Na	3375	27500	61250	75000	9975	29750	61250	80500
Ni	34	20	12	7	18	14	16	11
Se	2	2	4	5	2	3	4	4
Ag	2	2	1	1	1	2	1	1
S	11975	15250	19500	15500	11250	15750	18500	14000
Ti	308	110	73	38	248	105	69	38
V	12	8	6	5	10	7	6	5
W	2	1	1	1	2	1	1	1
Zn	583	430	298	170	530	428	303	175
TS ^b	3	4	5	5	2	3	4	5
VS of TS ^b	66	58	45	38	64	57	44	37
Chloride ^b	2	6	12	16	2	6	12	17
Total Kjeldahl nitrogen ^b	8	7	6	5	9	7	6	6
Ammonium nitrogen ^b	4	3	3	2	5	4	4	3

^adata reported in mg kgDM⁻¹; ^bdata reported as a percentage of dry mass.