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Evaluation of *Laminaria digitata* and *Phragmites australis* for biogas production and nutrient recycling

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ABSTRACT

Eutrophication and climate change are major global problems. The sea weed *Laminaria digitata* and the reed *Phragmites australis* have the potential to absorb nutrients and CO₂ during growth, as well as being a source of renewable energy in the form of biogas. The aim of this study was to evaluate *Laminaria digitata* and *Phragmites australis* concerning biogas production and nutrient recycling using a two-stage pilot scale process. The plant has a total volume of 430 L and consists of a hydrolysis bed and an up-flow anaerobic sludge blanket reactor (UASB). Two experiments were performed; one with *Laminaria digitata* as the sole substrate and one with a mixture of *Laminaria digitata* and *Phragmites australis*. Frozen substrates were placed in the hydrolysis bed and digestion was performed at 305 K during 70 days for *Laminaria digitata* and 100 days for the mixture of *Laminaria digitata* and *Phragmites australis*. The methane yield achieved was approximately 170 L kg⁻¹ volatile substances (273.15 K, 101.3 kPa) in both experiments. These results suggest that *Laminaria digitata* can be efficiently digested in larger scale and has the potential to contribute to a future sustainable energy mix, considering its relatively high methane yield when anaerobically digested as the sole substrate. Digestion of *Phragmites australis* needs further development to make use of its full potential.

1. Introduction

Sweden has a goal to become a fossil fuel free country by 2045 [1]. To reach this goal all sectors need to move towards the use of more renewable energy. One way is to develop methods for the use of new biogas substrates. Biogas can be produced in smaller local or regional digesters and used to produce electricity to balance the electricity grids [2]. The ability to balance electricity grids is becoming more important as fluctuating and intermittent renewable solar and wind power are becoming more common. In Sweden, most biogas is upgraded to bio-methane and used as vehicle fuel; in 2016, the market share of biogas for bus gas vehicles in Sweden was 16% [3].

The use of sea-based substrates, such as the reed *Phragmites australis* and the sea weed *Laminaria digitata*, for biogas production is especially interesting because they absorb nutrients during their growth and thus contribute to decreasing overfertilization of the seas. Eutrophication of coastal waters is a global problem and is gaining much attention [4].

Nutrients are released from agricultural lands, industries, domestic effluents and through mariculture. Mariculture has expanded drastically during the last 40 years [5]. Between the 1980s and 90s, the annual growth rate was around 10%, which has since decreased to around 6% from 2001 to 2016 [5]. Due to this expansion, mariculture is one of the most important factors of increased pollution in the marine environment [6]. Water pollution is a serious threat to the environment and to the mariculture itself. In China, the largest mariculture producer in the world, 228,000 ha of coastal water had a decreased water quality and 40,000 ha had such a low quality that it could not be used for mariculture and recreation in 1998 [4]. The cultivation of a seaweed, *Laminaria japonica*, has successfully been used in China as a strategy to counteract pollution from scallop cultivations [4]. The growth of cultivated seaweeds is much better when nitrogen (N) and phosphorus (P) are abundant [7]. Furthermore, seaweed absorbs CO₂ during photosynthesis, which contributes to the reduction of greenhouse gases in the atmosphere, while supplying oxygen to the sea [8]. The total

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accumulated CO₂ in seaweed aquaculture beds was estimated to over 2.87×10^6 ton year⁻¹, for the year 2014 [9]. The primary productivity rate for macroalgae in terms of carbon capture has been estimated up to 1600 g C m⁻² year⁻¹, which can be compared to 470 g C m⁻² year⁻¹ for crop lands [10,11].

Seaweed biomass might be used in several applications: food, extraction of valuable chemicals and production of biofuels. Using seaweed biomass for the production of biofuels (third generation biofuels) has several advantages over terrestrial plants and agricultural residues. Seaweed biomass is not dependent on cultivable land, needs no addition of fertilizer or pesticides and does not compete for fresh water [12]. Further, the life cycles of seaweeds are shorter than for the terrestrial counterparts during cultivation [13]. The brown algae, to which *Laminaria digitata* belongs, contains very little lignin [14], which is an advantage for use in an anaerobic digestion process [15].

The co-digestion of two or more substrates is often advantageous, giving a higher methane production [16]. Vivekanand et al. [17] studied co-digestion of the brown seaweed *Saccharina latissima* and steam-exploded wheat straw, and concluded that co-digestion blends of seaweed and lignocellulosic materials has a promising potential for biogas production. Steam-explosion is, however, a pre-treatment that demands energy input. Therefore, it would be of interest to evaluate if a less energy demanding process might be used. A two-stage anaerobic digestion process is advantageous compared to a one-stage process for lignocellulosic materials, since the low pH in the first step (the acidogenic step) favors microbes that hydrolyze the material to H₂ and volatile fatty acids (VFA) [18].

A single stage co-digestion of *Laminaria digitata* and green peas showed that reactor stability was difficult to achieve [19]. A two-stage process might increase process stability and give a higher methane production than a one-stage process due to the increased hydrolysis of the lignocellulosic material in the acidogenic step [18].

Total solids (TS) and especially volatile solids (VS, a rough estimate of the organic content of the substrate) are critical to the amount of biogas that might be produced during the digestion process. The choice of technical process design is also vital. A two-stage process with a hydrolysis bed and a methane reactor, such as that used in the present study, is especially suitable for substrates with a TS content above 15%. Most processes in Sweden work at TS contents below 15% because they are based on the continuously stirred tank reactor (CSTR) concept, which demands substrates that are pumped into the reactor.

The amount of carbohydrates, fatty acids and proteins are also vital, since different amounts of biogas with different volume percentages of methane are produced depending on the chemical composition [20]. Fats and proteins give larger methane volumes than carbohydrates per kg of organic material, but high amounts of fats and proteins might inhibit the process due to the production of fatty acids and ammonia [21,22].

Phragmites australis (common reed) is an aquatic grass that grows under widely different conditions from the tropics to cold temperate regions [23]. The growth area in Sweden has been reported at approximately 100,000 ha [24]. Furthermore, its growth is correlated to high nitrogen loads that have led to an increased abundance in the Baltic Sea region in the past [25]. *Laminaria digitata* (sea weed) is a brown macroalgae belonging to a group, termed kelp, that grows in the Northern Atlantic ocean. It can grow up to 4 m in length and generate high amounts of dry biomass m⁻²year⁻¹ [26].

In areas where *Phragmites australis* is a non-native species, the reed growth can create problems in riparian environments [27]. The harvesting of *Phragmites australis* for the extraction of nutrients and energy might therefore contribute to decreased problems, such as eutrophication and increased availability of renewable energy. Risén et al. [28] studied biomethane production from reeds and got a considerable potential for greenhouse gas emissions savings in comparison to a fossil reference system. They also found a positive energy balance and a potential for nutrient removal, especially phosphorus. Hansson and

Fredriksson [29] have further evaluated the use of *Phragmites australis* as a nutrient source for organic crop production. They compared direct spreading of common reed on farmland, spreading after composting and spreading of digestate after biogas production. The biogas production scenario gave a very favorable energy balance (+4.6 MJ kg⁻¹ dm) due to the energy content of the gas. The largest amount of plant available nitrogen in the digestate was 56% of the harvested amount, compared to 30% for the compost scenario.

The present study focused on anaerobic digestion of *Laminaria digitata* and *Phragmites australis* in a pilot-scale (430 L), two-stage process consisting of hydrolysis bed and an up-flow anaerobic sludge blanket reactor (UASB) with granular sludge. The use of a reactor containing granular sludge often makes the plant smaller, needing less space compared to a conventional continuous stirred tank reactor process (CSTR) containing suspended sludge. The granular sludge is more efficiently retained in the reactor [30].

The aim of the present study was to evaluate the use of *Laminaria digitata* and *Phragmites australis* for biogas production and the creation of sustainable nutrient cycles using an adapted process in pilot-scale.

2. Material and methods

2.1. Inoculum

The inoculum used in the experiments was a sludge in the form of granules, collected from the wastewater treatment plant at Falkenberg Brewery (Falkenberg, Sweden). The granules consist of a mix of microorganisms naturally adapted and active in the digestion process in the specific brewery wastewater treatment plant. The granules had a size of 2–3 mm: The total solid (TS, weight % of wet sample) content was 8.5% and volatile solid (VS, weight % of TS) content was 83.2%. Soluble Chemical Oxygen Demand (SCOD) was 250 mg L⁻¹ and ammonium-N 5 mg L⁻¹. The pH was 7.0.

2.2. Substrate

The organic feedstock used for the anaerobic digestion was seaweed (*Laminaria digitata*) and reed (*Phragmites australis*). *Laminaria digitata* was collected in August 2015 on the west coast of Sweden (Lysekil in the Skagerrak strait), and the reed *Phragmites australis* was harvested in July 2015 in Kalmar on the east coast of Sweden (Baltic Sea). Both substrates were stored until use, in 253 K in the actual size that they were collected. The seaweed was frozen during transport. Both seaweed and reed were cut into approximately 100 mm pieces prior to use in the digestion experiments.

2.3. Analysis during the process

Liquid and gaseous samples were taken and analyzed from both the hydrolysis bed and the reactor during the process. Soluble chemical oxygen demand (SCOD), volatile fatty acids (VFA), ammonium-N, phosphate-P and alkalinity were analyzed in the liquid samples using Hach Lange test cuvettes LCK 114 or 314 (SCOD), LCK 365 (VFA), LCK 303 (ammonium-N), LCK 348 (phosphate-P) and LCK 362 (alkalinity). The analyses were performed using a Hach Lange spectrophotometer 2800 (Hach Lange AB, Sköndal, Sweden). The flow rate of produced biogas was measured by gas flow meters (Alicat scientific, Tuscon, AZ, USA) connected to a computer. Methane, carbon dioxide, hydrogen gas and hydrogen sulfide volume fractions were measured by a gas analyzer Biogas 5000 (Geotech, Coventry, United Kingdom). Temperatures were measured in the hydrolysis bed and reactor by PT 100 elements.

2.4. Analysis of seaweed

2.4.1. TS and VS

Total solids (TS) and volatile solids (VS) were analyzed according to

Ref. [31]. The samples were heated to 378 K and the weight loss was measured; the samples were then heated to 823 K and the weight loss was measured again. The measured weight losses were used to calculate the TS and VS for the samples. Multiple samples were analyzed.

2.4.2. Cadmium and nutrients

Cadmium (Cd), Phosphorous (P) and Sulphur (S) were analyzed according to NMKL no 161 [32], with Kjeldahl-N analyzed according to SS-EN13342. Ammonium-N was analyzed according to Eurofins standard method 1998 [33].

2.4.3. Total proteins

Total proteins were determined using the colorimetric Lowry based kit, DC™ protein (BioRad, Hercules, CA, USA), after hot TCA extraction followed by alkaline extraction, according to Slocombe et al. [34], with an additional bead beating step prior to extraction (2 min, 30 Hz, QIAGEN Tissuelyser II) to ensure proper disintegration of biomass.

2.4.4. Total fatty acids

The total fatty acid analysis was done according to Mayers et al. [35], based on transesterification and GC-MS detection.

2.4.5. Total carbohydrates

Total carbohydrates were determined using the colorimetric MBTH method after a two-step sulfuric acid hydrolysis, as described by Van Wychen et al. [36,37], and the National Renewable Energy Laboratory standard method (Technical Report Nr NREL/TP-5100-60957).

2.4.6. Laminarin and mannitol

Extraction was done according to Veide Vilg et al. [38], as described for laminarin with subsequent neutralization with CaCO_3 and analysis on HPLC for detection of mannitol using standard curve of $0.7\text{--}5\text{ g L}^{-1}$. The HPLC analysis was done using a Rezex ROA Organic acid H+ column ($300 \times 7.8\text{ mm}$) at $80\text{ }^\circ\text{C}$ with $5\text{ mM H}_2\text{SO}_4$ as eluent at a flow rate of 0.8 mL min^{-1} and refractive index detection.

2.5. The process set-up

The substrate was placed in the hydrolysis bed and the bed was filled with 350 L of tap water. The solution in the hydrolysis bed was continuously circulated by a submersible pump (75 L min^{-1}) (AL-KO drain 6001, Brønderslöv, Denmark). The temperature was maintained at 305 K with a 1 kW titan immersion heater (Weipro, Xiaolan town, China). The UASB reactor was filled with 10 L of inoculum granules and 30 L of tap water. The UASB reactor content was continuously recirculated by a Motive G71 BC pump (2 L min^{-1}) (Nova Rotors, Vicenza, Italy). Feeding from the hydrolysis bed to the UASB reactor was performed once a day with a pump (Watson Marlow 621 FX, Boston, USA) operated at 25 Hz (1.5 L min^{-1}). The pump was started manually and the amount of feeding was adjusted depending on the COD and VFA concentrations in the hydrolysis bed and UASB reactor content. The temperature was maintained at 305 K using a water heater and two heat exchangers placed in the recirculation of the reactor content; Fig. 1.

The processes were stopped after 70–100 days, when gas production was below 1 L day^{-1} during 4 subsequent days.

2.5.1. The experiment with *Laminaria digitata*

The first experiment was performed using *Laminaria digitata* as the sole substrate. Seaweed (25 kg) was placed into the hydrolysis bed and the bed was filled with 350 L of tap water. The process with *Laminaria digitata* was run for 70 days until the gas production had dropped to less than 1 L day^{-1} for 4 subsequent days. The organic loading was adjusted to not cause an overload of the process. The organic loading was kept between 1 and $2.5\text{ g COD L}^{-1}\text{ day}^{-1}$ during the experiment; Fig. 2.

2.5.2. The experiment with *Laminaria digitata* and *Phragmites australis*

The second experiment was performed according to the description above, but the substrate in this trial consisted of a mix of 7.2 kg of *Laminaria digitata* and 7.6 kg of *Phragmites australis*. The process was stopped after 100 days, when gas production was below 1 L day^{-1} during 4 subsequent days. The organic loading was lower in the

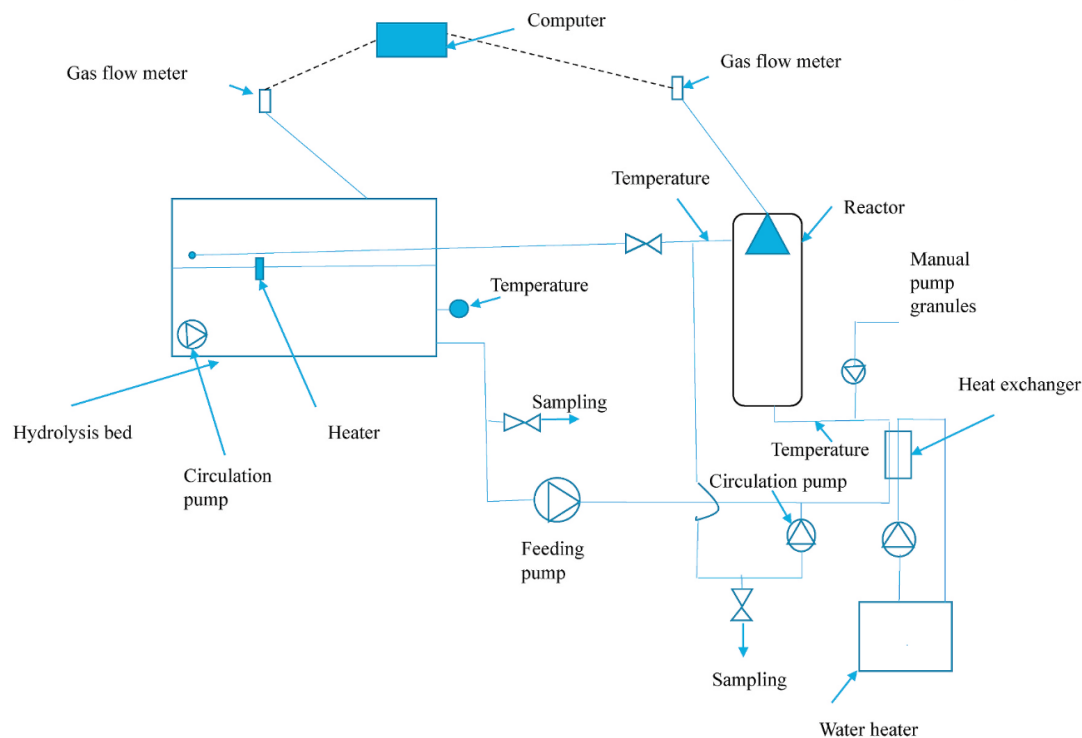


Fig. 1. The two-stage process.

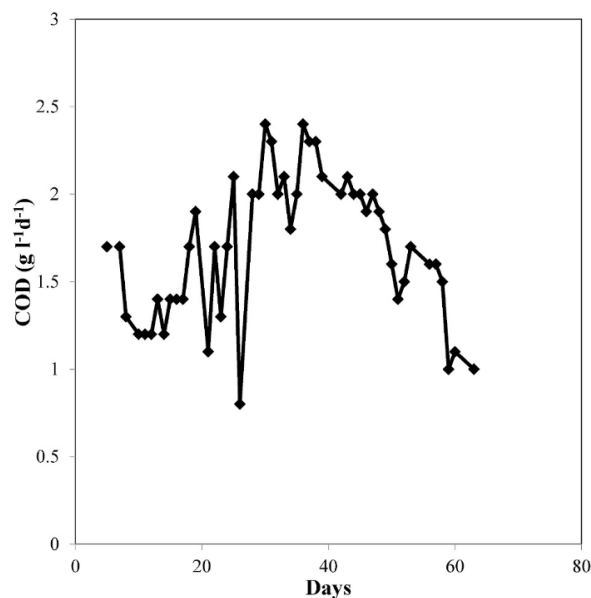


Fig. 2. Organic loading as a function of time during the seaweed experiment.

experiment with co-digestion of *Laminaria digitata* and *Phragmites australis*, Fig. 3, compared to the experiment with *Laminaria digitata* as the sole substrate; Fig. 2. The organic loading was purposely kept lower, since the COD and VFA concentrations in the UASB reactor varied drastically and did not stabilize during the first 50 days of the *Laminaria digitata* and *Phragmites australis* experiment; Fig. 8.

3. Results

3.1. The chemical composition of the substrates

The chemical composition of the substrates is shown in Table 1. Total solids (TS) was 21% for *Laminaria digitata* and 68% for *Phragmites australis*. *Phragmites australis* contained more nitrogen than *Laminaria digitata*.

Approximately 70% of the dry weight of the seaweed consisted of carbohydrates, proteins and fatty acids; Fig. 4. The carbohydrate fraction, which constitutes 60% of the dry weight material, was dominated by laminarin. The protein was about 8% of the dry weight and fatty acid content about 1.8% of the dry weight. Approximately 20% of the dry weight consisted of ash; Table 1. The mass balance shows that

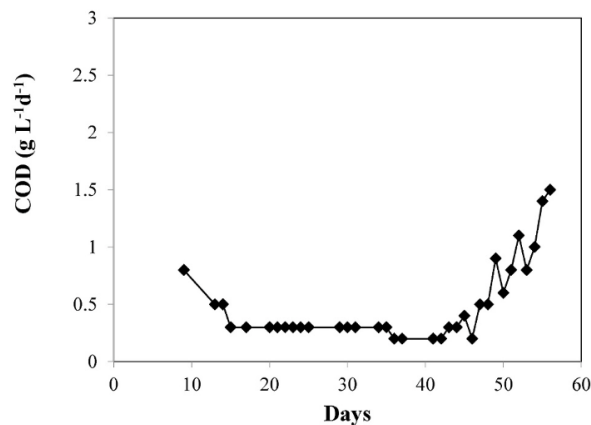


Fig. 3. Organic loading as a function of time during the common reed and seaweed experiment.

Table 1

The chemical composition of the substrates.

	<i>Laminaria digitata</i>	<i>Phragmites australis</i>
Total solids (TS% of wet weight)	21	68
Volatile solids (VS% of dry weight)	79	92
Kjeldahl-nitrogen (mg kg ⁻¹ wet weight)	2900	9300
Ammonium-nitrogen (mg kg ⁻¹ wet weight)	340	800
Phosphorous (mg kg ⁻¹ TS)	2800	2200
Sulphur (mg kg ⁻¹ wet weight)	2730	1930
Cd (mg kg ⁻¹ TS)	0.18	<0.095

approximately 90% of the dry weight consisted of carbohydrates, proteins, fatty acid and ash; Table 1.

3.2. The experiment with *Laminaria digitata*

COD, VFA and pH were measured in both the UASB reactor and the hydrolysis bed during the experiment; Figs. 5 and 6. The organic loading was between 1 and 2.5 g COD L⁻¹ day⁻¹ during the experiment, Fig. 2, to keep the COD and VFA concentrations in the UASB reactor low and stable at around 800 mg L⁻¹ (COD) and 200 mg L⁻¹ (VFA) during this experiment; Fig. 5.

The pH in the UASB reactor was relatively constant, between 6.8 and 7.2 (Fig. 6), and the ammonium-N concentration was 40–120 mg L⁻¹, values that will not contribute to inhibiting the process. The phosphate-P was 30 mg L⁻¹.

The COD concentration in the hydrolysis bed increased to approximately 9000 mg L⁻¹ on day 3, after which it decreased continuously and relatively rapidly during the experiment; Fig. 5. The VFA concentration in the hydrolysis bed increased during the first 10 days and reached approximately 2600 mg L⁻¹; Fig. 5. The ammonium-N concentration in the hydrolysis bed was 10–80 mg L⁻¹ and the phosphate-P concentration approximately 35 mg L⁻¹. The pH in the hydrolysis bed was between 4.2 and 5 during the first 40 days; Fig. 6.

The reactor started to produce biogas already on day 4 of the experiment; Fig. 7.

The methane content of the gas produced in the UASB reactor was around 55–65 vol % until day 43, after which it increased to 60–75 vol %. The carbon dioxide content was 30–35 vol % until day 43, and then decreased to 20–30 vol %. The hydrogen sulfide content varied between 20 and 470 ppm. The accumulated methane gas volume in the UASB reactor during the experiment reached 500 L; Fig. 7. The gas production in the UASB reactor dropped to below 1 L day⁻¹ for 4 subsequent days and after 70 days the process was stopped.

Gas containing high concentrations of hydrogen sulfide (>5000 ppm on one occasion), carbon dioxide 52–58 vol % and hydrogen gas (>5000 ppm) was produced in the hydrolysis bed during the first week, after which the volume of gas was too low to collect and measure until day 55, when biogas began to produce in the bed. The biogas production started in the hydrolysis bed when the pH reached 6.7 on day 55. The methane, carbon dioxide and hydrogen sulfide content of the gas produced in the hydrolysis bed during this experiment was approximately 55–58 vol % (methane), 39–40 vol % (carbon dioxide) and 370–400 ppm (hydrogen sulfide).

The largest amount of gas (methane), 79%, was produced in the UASB reactor as expected, not in the hydrolysis bed. The methane yield from the *Laminaria digitata* experiment was approximately 170 L kg⁻¹ VS.

3.3. The experiment with *Laminaria digitata* and *Phragmites australis*

The mixed seaweed/reed process was slow in the beginning compared to the seaweed process. The organic loading was kept lower compared to the experiment with seaweed as the sole substrate, since

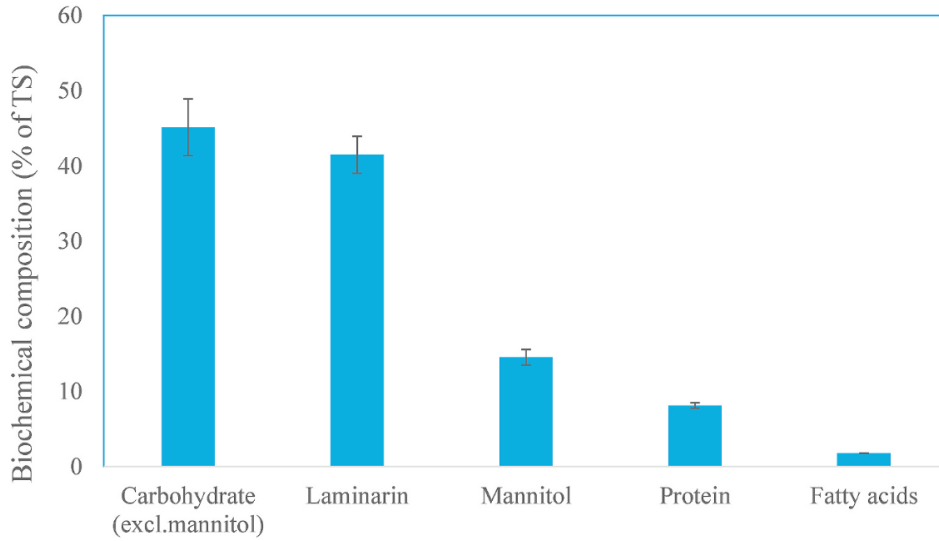


Fig. 4. The carbohydrate (excl. mannitol), laminarin, mannitol, protein and fatty acid content of *Laminaria digitata*.

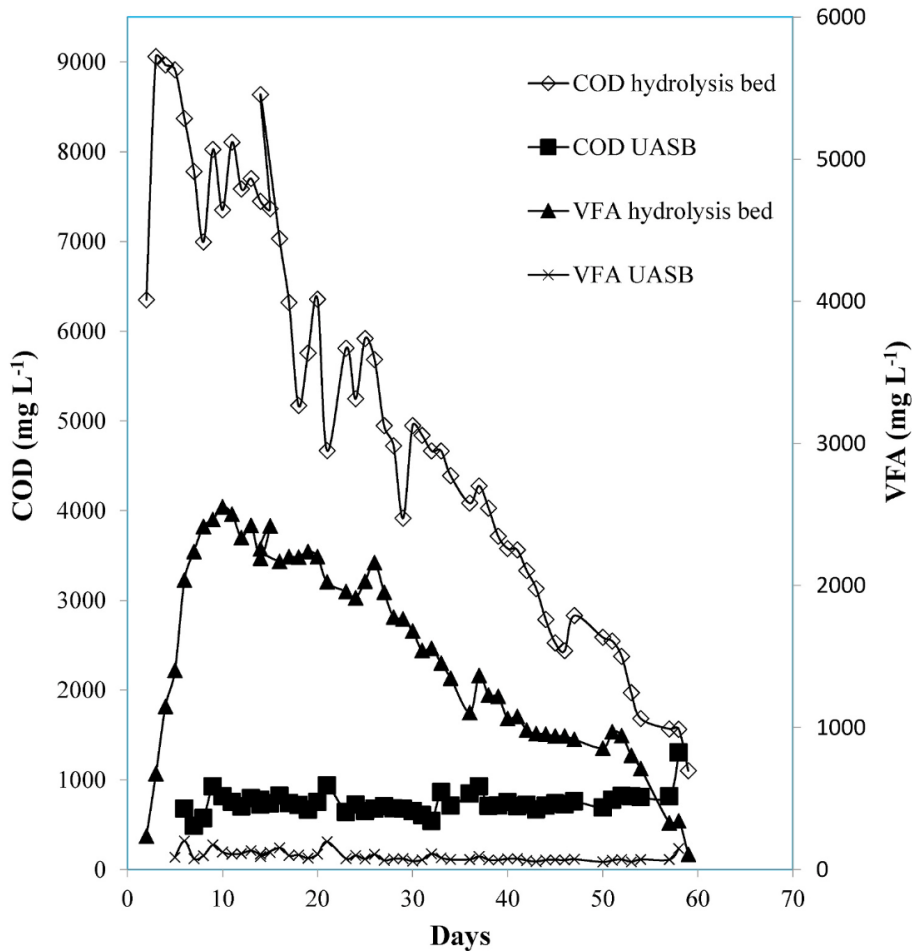


Fig. 5. COD and VFA concentrations in the hydrolysis bed and the UASB reactor during the experiment with *Laminaria digitata*.

the COD and VFA concentrations in the UASB reactor varied drastically during the first 50 days of the experiment; Fig. 8.

The ammonium-N concentration was 85–160 mg L⁻¹ in the UASB

reactor during the experiment. The pH in the UASB reactor varied between 6.2 and 7.2 during the experiment; Fig. 9.

The alkalinity was lower in the mixed substrate experiment

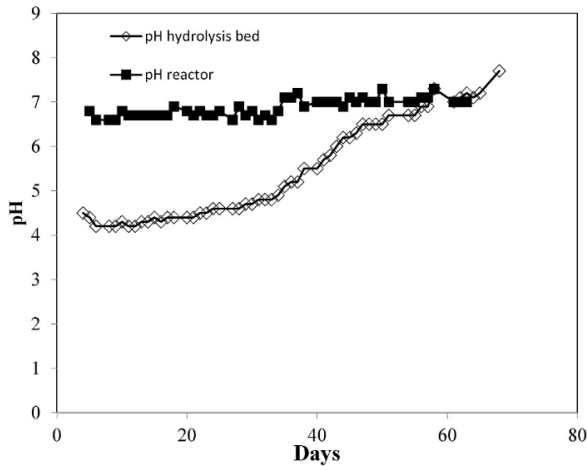


Fig. 6. pH in the UASB reactor and the hydrolysis bed as function of time during the seaweed experiment.

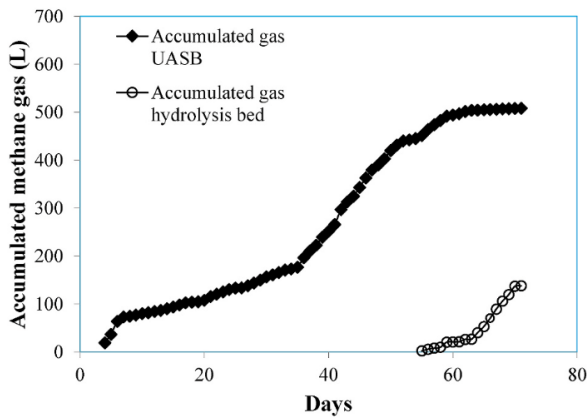


Fig. 7. Accumulated methane production in the UASB reactor and the hydrolysis bed as a function of time during the seaweed experiment.

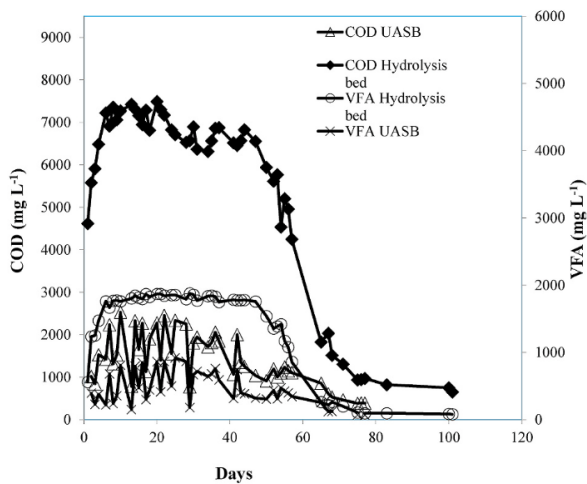


Fig. 8. COD and the VFA concentrations in the hydrolysis bed and the UASB reactor during the experiment with *Laminaria digitata* and *Phragmites australis*.

(490–1100 mg L⁻¹ HCO₃⁻) than in the seaweed experiment (1000–1500 mg L⁻¹).

The UASB reactor started to produce biogas already on day 6 of the experiment; Fig. 10. The gas produced in the UASB reactor contained 58–72 vol % methane, 20–35 vol % carbon dioxide, 30–150 ppm hydrogen sulfide, 15–50 ppm hydrogen gas.

The ammonium-N concentration in the hydrolysis bed was 30–170 mg L⁻¹. The pH in the hydrolysis bed was between 4.5 and 5 during the first 50 days of the seaweed reed experiment, a 10-day delay in pH increase compared to the seaweed experiment; Figs. 6 and 9.

Gas containing high concentrations of CO₂ (approximately 50 vol %), H₂S (2200–>5000 ppm) and H₂ (>5000 ppm) was produced in the hydrolysis bed during the first week, after which the volume was too low to measure until day 52 when biogas began to be produced; Fig. 10. The hydrolysis bed started to produce biogas when the pH was approximately 6.5; Figs. 9 and 10.

The largest amount of gas (methane) 66%, was produced in the hydrolysis bed, not in the UASB reactor as desired. The methane content of the gas produced in the UASB reactor was between 58 and 72%, while it was 51–63% for the gas produced in the hydrolysis bed.

The methane yield was approximately 170 L kg⁻¹ VS in both experiments. However, there was a difference in the location of the biogas production in the two experiments. Approximately 34% of the methane gas was produced in the UASB reactor during the co-digestion of *Phragmites australis* and *Laminaria digitata*, whereas the corresponding number was 79% for the experiment in which only *Laminaria digitata* was digested; Figs. 7 and 10.

4. Discussion

The biomethane yield of the seaweed in the experiment with *Laminaria digitata* as the sole substrate was relatively high: approximately 170 L kg⁻¹ VS. This result can be compared to the biomethane yield achieved by Vanegas and Bartlett [39] in a semi continuous co-digestion of *Laminaria digitata* and manure, where the highest yield was 184 L kg⁻¹ VS, obtained during mesophilic digestion. In another study performed by Vanegas and Bartlett [40], the highest methane potential obtained during laboratory scale batch experiments was 246 mL g⁻¹ VS after 109 days. The degradation process was slow and 152 mL g⁻¹ VS was produced during the first 39 days, compared to the present study in which the process ran for 70 days. Adams et al. [26] investigated anaerobic digestion of *Laminaria digitata* in biomethane potential (BMP) experiments using wild biomass that was harvested during different months. The highest potential 219 L kg⁻¹ VS was achieved for biomass harvested in July. Late summer and early autumn have shown to be the

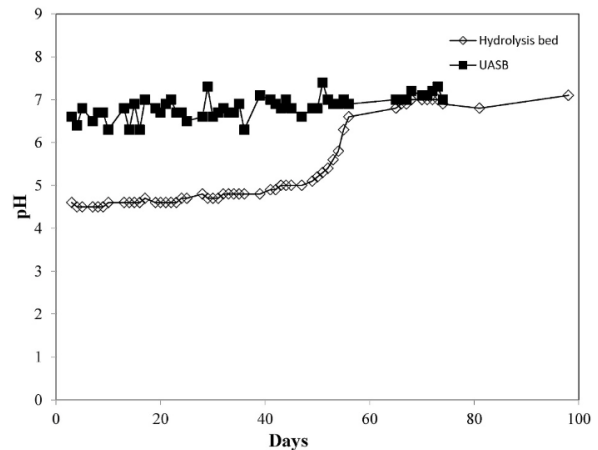


Fig. 9. pH in the UASB reactor and the hydrolysis bed as a function of time during the *Laminaria digitata* and *Phragmites australis* experiment.

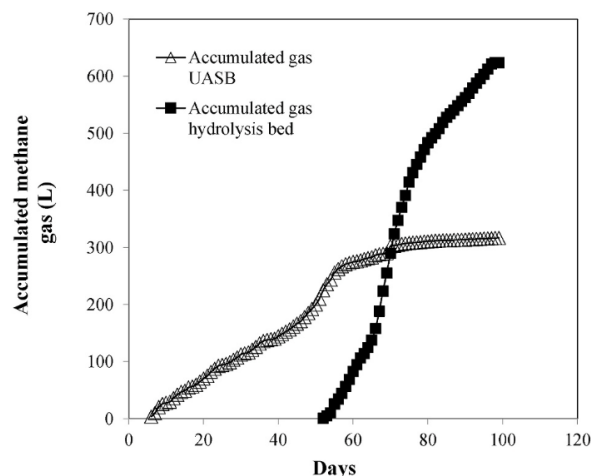


Fig. 10. Accumulated methane production in the UASB reactor and the hydrolysis bed as a function of time during the seaweed common reed experiment.

best periods for harvesting concerning biomethane potential [26], with the biomass used in the present study collected in late summer. For example, the potential is dependant on the C/N ratio, whose range 20–30:1 is suitable during the summer months. The macroalgae accumulates carbohydrates during the summer, whereas the protein content increases during the winter [26]. The relatively high methane content of the gas 55–75 vol % for the experiment with *Laminaria digitata* and 51–72 vol % for the experiment with mixed substrate is of a special interest because the dry weight of the seaweed is highly dominated by carbohydrates; Fig. 4. Carbohydrates have previously been reported to give a gas with a relatively low methane content, around 50 vol % [20]. A high methane content is vital to lower the cost when the gas should be upgraded to transport fuel, especially important when much of the produced biogas is upgraded to transport fuel, as in Sweden [41]. The use of biomethane as transport fuel is still low in the EU and concentrated to a few countries, though it has a high rate of development [41].

The two-stage process was most suitable for the digestion of *Laminaria digitata*, without the addition of *Phragmites australis*, since 80% of the methane in this experiment was produced in the UASB reactor. *Laminaria digitata* was efficiently hydrolyzed in the hydrolysis bed, as seen by the high COD values in the first part of the experiment.

The process was more stable during this experiment than the second part of the study with mixed substrate, which are shown by the COD, VFA and alkalinity results; Figs. 5 and 8. The alkalinity was low in both experiments, indicating that co-digestion with some substrates that increase alkalinity might stabilize the process so that the organic loading might be increased. Blue mussels (*Mytilus edulis*) might be an interesting alternative. Wollak et al. [42] digested *Mytilus edulis* from the Baltic Sea using the same process as in the present study. The digestion of the mussels was considerably more stable than the digestion with *Laminaria digitata* and *Phragmites australis*. The alkalinity was around 3000 mg L⁻¹, in the optimal range [20]. The shells might have contributed to the alkalinity in this experiment. Another interesting alternative might be solid cow manure. Nkemka et al. [43] studied the anaerobic digestion of beach cast seaweed and solid manure in a laboratory scale two-stage process and found that co-digestion of the substrates to stabilize the process. The methane yields obtained in their study correspond with the yields obtained during the present study, suggesting that anaerobic digestion of seaweed can be performed in a larger scale without loss of efficiency.

An earlier study using single- and two-stage fermentation of *Laminaria digitata* were compared, and showed the two-stage process to reduce the hydraulic retention time (HRT) by 33%, while improving the energy conversion by 9.8% [44]. Further, the methane content of the gas

produced in the two-stage process was significantly higher [44]. However, the use of seaweed as biogas substrate needs further process development because of the risk for disruption to methane production due to inhibitory compounds. Akunna and Hierholtzer [19] got process instability even when only 2% of the feed-stock (green peas) was exchanged for seaweed. The inhibition was found to depend on different process parameters, such as the organic loading rate prior to the addition of seaweed [19]. The methanogens were more sensitive to the inhibition than the acidogens/acetogens. In the present study, an up-flow anaerobic sludge blanket reactor (UASB) was used for methane production, with both Gunterman et al. [44] and Akunna and Hierholtzer [19] using continuously stirred tank reactors (CSTR). Because the CSTR depends on suspended biomass that is not retained in the reactor, but has to grow fast enough to not be washed out, the UASB is based on biomass that grows in the form of granules and are effectively retained in the reactor so that a high biomass concentration is maintained. Consequently, the UASB can handle higher organic loading rates, typically from 2 to 20 kg COD m⁻³ day⁻¹ depending on the substrate, while the corresponding figures for the CSTR are normally from 0.5 to 2.5 kg m⁻³ day⁻¹ [45,46]. Higher loading rates make it possible to use smaller reactors, potentially leading to less space and lower investment costs. Furthermore, the granules are more efficiently protected against toxic compounds [47], resulting in a more insensitive and stable process. Therefore, an UASB process is suitable for the digestion of substrates containing, for instance, high amounts of sulphur such as seaweeds [48]. The relatively low concentrations of ammonium-N and VFA in the UASB reactor during the seaweed experiment indicate that the organic loading might be increased.

The instability of the process during the second experiment of the present study led to a very low organic loading rate of the UASB reactor, and a prolonged time for methanogens to establish in the hydrolysis bed. High methane production in the hydrolysis bed during the seaweed common reed experiment might partly be due to refractory polymers in the reed. The result agrees with the study performed by Nkemka and Murto [49]. Nkemka and Murto [49] studied the digestion of reed in a laboratory scale two-stage leachbed-UASB process and got a methane yield of approximately 220 L CH₄ kg⁻¹ VS. In their study, approximately 80% of the methane was produced in the leach bed [49]. In Risén et al. [28], the digestion of *Phragmites australis* with a mixture of agricultural substrates in continuously stirred tank reactors gave the same result (220 L CH₄ kg⁻¹ VS). The use of *Phragmites australis* as biogas substrate was found to contribute to considerable savings in greenhouse gases compared to a fossil reference system in their study [28]. Furthermore, the harvesting of reed might supply agriculture with substantial amounts of phosphorus and nitrogen. Risén et al. [28] harvested 74 tons of *Phragmites australis* in a pilot area of 5 ha (Municipality of Kalmar), where the reed used in the present study came from. Based on this result and the results of the phosphorous and nitrogen analyses performed during the present study, the biomass of 1 ha of *Phragmites australis* will contain enough nitrogen to supply 0.5 ha of farmland and phosphorous to supply 1.0 ha of farmland; Appendix A. Nutrient losses were considered in the calculations. Approximately 99% of the phosphorous and 60% of the nitrogen might be recovered in plant available form in the digestate [28].

The absorption of phosphorous during the cultivation of seaweed is especially interesting, since phosphorous is a limited resource and when released into the seas is difficult to recycle due to dilution [50]. Pedersen et al. [51] found a productivity of *Laminaria hyperborea* (a species related to *Laminaria digitata*) to be 12.5 kg wet weight m⁻² year⁻¹. A calculation based on this result and the analyses results of Kjeldahl-N and phosphorous gained in the present study shows that the biomass from 1 ha of seaweed biomass would be enough to fertilize 3.3 ha of agricultural land with phosphorous and 1.3 ha with nitrogen; appendix A. The calculations are based on the maximum allowed amounts of phosphorous and nitrogen added per ha year [52]. The nitrogen amount is based on the regulation for sensitive areas. The calculations are based on the amount

of phosphorous and nitrogen in the seaweed; appendix A. Losses of nutrients during handling of the digestate are included according to Ref. [28].

Further, seaweeds contain a mixture of carbohydrates, such as arabinose, galactose and sugar acids, that suit the production of biogas, which is a process with mixed cultures, compared to the fermentation of, e.g. glucose to ethanol with conventional microorganisms [53]. Macroalgae might further enhance the digestion in a biogas process due to their content of trace metals. Cogan and Antizar-Ladislao [54] studied the anaerobic digestion of food waste, and the addition of even a relatively low amount of macroalgae enhanced the efficiency of the digestion. Alvarado-Morales et al. [55] compared biogas production and biogas plus ethanol production from *Laminaria digitata* under Nordic conditions, and found the scenario with only biogas production to be most favorable from an environmental point of view. Energy consumption during downstream and purification processes for the ethanol production seem to be the major difference between the scenarios. The methane yield during the scenario with only biogas production was 200 L CH₄ kg⁻¹ VS [55]. Seghetta et al. [56] studied biogas and protein production from the off-shore cultivation of *Laminaria digitata* and *Saccharina latissima* and found biogas production from dried *Laminaria digitata* to be the most favorable scenario, saving 18.7 × 10² kg CO₂ eq. ha⁻¹.

The methane yield obtained during the second experiment with *Laminaria digitata* and *Phragmites australis* was 170 L kg⁻¹ VS, which is close to the 180 L kg⁻¹ VS assumed by Hansson and Fredriksson [29] in their evaluation of common reed. Their evaluation shows the anaerobic digestion of common reed to give a favorable energy balance, as reported in the introduction. *Phragmites australis* from the Baltic Sea has previously been shown in a BMP experiment to have a methane yield of 400 L CH₄ g⁻¹ VS (unpublished results). The reed was cut into smaller pieces in that experiment, which was performed with an inoculum from a biogas process receiving a complex mixture of substrates from agriculture and food industry.

5. Conclusions

Laminaria digitata has a substantial potential to contribute to the biological cycle through absorption of nutrients and CO₂ during growth and as biomass for bioenergy production. The present study shows that it can be digested in a larger scale with a methane yield of 170 L kg⁻¹, stability in the process, and a potential to further increase the organic loading.

The co-digestion of *Phragmites australis* and *Laminaria digitata* did not increase the methane yield and showed process instability. *Phragmites australis* is relatively refractory substrate, which might be suitable for a two-stage process with a more optimized separate hydrolysis step. There is, however, a need for further process development to decrease the establishment of methanogens in the hydrolysis bed and to optimize a pre-treatment step.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2020.105670>.

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