Anaerobic Co-digestion of Cast Seaweed and Organic Residues

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1. Final report

1.1 Project details

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1.2 Executive summary

Abstract

This reports summarizes the major findings and results on the utilization of cast seaweed as substrate for energy recovery through anaerobic digestion in Denmark. Overall, results from this project shown that due to its low biodegradability (143 ± 27 NmLCH_4 gVS^{-1}) co-digestion strategies with dissimilar substrates are necessary. In addition to that, sand content is of major importance if cast seaweed is going to be dedicated as feedstock for anaerobic digestion. Depending on the collection site and season, cast seaweed samples exhibited different extent of biodegradability, and this eventually has an impact on the pretreatment to be applied. However, for the particularly cast seaweed samples used in this work, suitable pretreatments were identified. Co-digestion ratio of 80 % manure and 20% cast seaweed showed the best performance in terms of net methane yield (232 ± 39 NmLCH_4 gVS^{-1}), compared to mono-digestion of cast seaweed. Co-digestion ratio of 80% manure, 10% cast seaweed and 10% sugar beet pulp was also identified to boost the methane yield (281.0 ± 92 NmLCH_4 gVS^{-1}). Co-digestion

ration of 80 % manure and 20% cast seaweed was successfully tested at continues experiments at lab scale and pilot scale. A proof of concept of a bio-sand separation technology was successfully demonstrated at lab scale. A complete Life cycle assessment revealed that the co-digestion scenario with the best performance in the majority of the impact categories considered followed corresponded to co-digestion of cast seaweed with cattle manure at VS ratio 20:80, thereby validating the experimental results. While the highest negative environmental impact was found for co-digestion of cast seaweed, manure and sugar beet pulp at VS ratio 10:10:80. Finally, economic evaluation showed that cost of transportation and labor in collecting and conditioning the cast seaweed are of major importance and have to be reduced so that the NPV can be positive.

1.2.1 Introduction

The negative environmental impact, associated with the huge algae masses that are washed up smothering the coastline and forming rotting piles on the shores, is well documented[1]. AD process has been proposed as potential solution for the final disposal problem of the beach seaweed so that two issues are tackled, the environmental benefits such as nutrient removal from coastal areas and the recovery of energy. It is the aim of the Danish energy policy to reduce fossil fuels, and biomass is an important source of renewable energy. Aquatic biomass, in particularly seaweed, is an emerging option for meeting this target in the long-term. Due to high carbohydrate content (60% dry weight), seaweed can be dedicated for biofuels production, such as biogas (methane). The production of methane from cast seaweed has recently drawn attention due to interest in new biomass resources for increasing biogas production. Several studies have been concerned either cast seaweed which washes up at beaches, or have focused on growing macroalgae as energy crop. Methane yield from cast seaweed is influenced by environmental conditions, i.e. inert content (mainly, sand), period of collection and level of degradation. Methane yield from batch test and continuous process has been reported to be 0.17 and 0.16 L/gVS, respectively[2], which is in the lower range for manure (0.15-0.35 NL/gVS).

However, the idea of using cast seaweed as substrate for anaerobic digestion (energy recovery) possess two important technical challenges that must be considered:

- High content of sand and other debris in the cast seaweed. This problem needs to be solved before beach seaweeds can be used as feedstock into a biogas plant. Anaerobic digestion (AD) systems are particularly susceptible to sand accumulation and equipment wear. Sand accumulation results in a reduction of the working volume and consequently leads to work-

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[1] Smetacek V, Zingone A, Green and golden seaweed tides on the rise. Nature 2013; 504: 85-88

[2] Nkemka, V.N., Arenales-Rivera, J., Murto, M., 2014

ing with retention times lower than expected. Additionally, high risk of clogging and abrasion problems can cause the accelerated wear of process equipment, thus leading to unexpected shutdowns of the plant.

- Heavy metal content of cast seaweed (particularly Cadmium-Cd). It is possible that not all collected seaweed can be used at the biogas plant when the maximum concentration level of heavy metals is exceeded[3] due to their inhibitory effect on the AD process.

On the other hand, cast seaweed has high content of nutrients (N, P, K) and thus removing beach seaweed will improve the local environmental indicators (marine and terrestrial eutrophication) and potentially provide an alternative biofertilizer (AD process digestate).

The cast seaweed could be used in anaerobic co-digestion process to significantly increase the biogas productivity and yield of manure in full scale biogas reactors, especially in Denmark, where the amounts of manures are abundant. In a recent study the methane yield of the anaerobic co-digestion of cast seaweed with manure and waste from pectin and carrageenan production were estimated to be 227.2 mL/gTS. However, the optimal co-digestion conditions were not determined as well as the possibility to co-digest cast seaweed with other types of organic waste. Therefore, it seems that co-digesting cast seaweed and organic waste in the existing manure based biogas reactors could be an attractive alternative to the conventional co-digestion processes. Furthermore, this new co-digestion scenario could be implemented in the short term using the existing infrastructure of biogas plants minimizing investment and operational costs.

1.2.2 Objectives

The goal of this project was to propose a bioenergy concept in which cast seaweed collected from beaches will be used as substrate in co-digestion with manure and other organic wastes in biogas plants for energy production purposes. The specific objectives of this project were:

1) To evaluate the collection technologies for cast seaweed.
2) To quantify and chemically characterize the cast seaweed and the organic wastes.
3) To evaluate the different pretreatment methods.
4) To evaluate different options to utilize digestate as fertilizer.
5) To perform environmental and economical assessments of the developed concept.

1.2.3 Project activities

To match the objectives, the following methodology was carried out in a systematic way:

1) Collection and quantification of cast seaweed samples. Collection of cast seaweed was performed at Solrød beach and Skodsborg. Also, beach seaweed collected at Hanstholm and Skive was used during the time frame of the project.

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[3] Fredenslund AM (2010). Udnyttelse af tang og restprodukter til production af biogas. Solrød Kommune. Tekniske Administration.

2) **Evaluation of collection technologies for cast seaweed.** Information were retrieved from different sources, including published literature, databases, and personal communication with representatives of the Solrød municipality.

3) **Collection of organic wastes.** Cattle manure was collected at Snertinge centralized biogas plant (Denmark) whilst other organic residues such as sugar beet pulp, grønt, and vinasses were provided by Nordic Sugar. This was necessary to comply with the Task 3.2 as was not possible to obtained the pectin and carrageenan residues from CP-Kelco factory.

4) **Chemical characterization of cast seaweed samples and organic wastes.** Complete physicochemical characterization of the different cast seaweed samples and organic wastes was performed. Physicochemical characterization was performed in terms of total solids (TS), volatile solids (VS), ash, and volatile fatty acids for all the organic wastes. In addition to that, N, P, S and metal content determinations were performed only for cast seaweed samples.

5) **Evaluation of different pretreatment methods and co-digestion strategies.** The effect of different pretreatments -such as mechanical, thermal, acid, alkaline, enzymatic hydrolysis -on the biodegradability and ultimate biomethane potential of cast seaweed samples were evaluated. Depending on the type of sample (location site and physicochemical characterization), the suitable pretreatments and/or combination of them were identified at lab scale. As main of the drawbacks in using cast seaweed in anaerobic digestion process is the high content of sand and some other debris, a novel concept at which sand separation and pretreatment take place simultaneously was developed and a lab scale prototype was constructed for validation of the concept. The pretreated cast seaweed samples were used in co-digestion with organic substrates in batch reactors at lab scale to identify the best co-digestion ratios. The best co-digestion ratios from batch experiments were tested in continuous reactors at lab scale. The best co-digestion ratios from continuous experiments in terms of process stability was tested in continuous reactors at pilot scale.

6) **Evaluation of possibilities for utilization of the digestate as fertilizer.** The fertilizer potential of the anaerobic digestate was directly assessed from the amount of mineralized nutrients, which play a vital role in determining the suitability of a digestate as fertilizer, through performance comparisons with commercial organic and inorganic fertilizers.

7) **Environmental and economical assessments.** Five scenarios were evaluated through a LCA to quantify environmental impacts; where four scenarios refer to AD of the cast seaweed and one to composting:
   i. Anaerobic digestion of the cast seaweed (AD-SW).
   ii. Anaerobic co-digestion of cast seaweed with cattle manure (COAD-M).

iii. Anaerobic co-digestion of cast seaweed with manure and sugar beet pulp (COAD-MSBP).
iv. Anaerobic co-digestion of cast seaweed sugar beet pulp (COAD-SBP).
v. Composting, using only the cast seaweed as substrate (COMP).

The LCA modelling was facilitated in EASETECH software. Inventory data were collected from the experimental work performed during the time frame of this project, different sources, including published literature, databases (i.e. ECOINVENT 2.0), unpublished experimental data and personal contacts with professionals with expertise on the topic. When process data were not available, assumptions were made and/or process engineering calculations were performed and data obtained. An economic evaluation of the sand separator prototype together with the most suitable co-digestion scenario was performed.

8) **Evaluation and exploitation of the results.** The main results obtained during the time frame of this project are summarised in the current report. More details can be found in the attached scientific manuscripts:

- Anaerobic co-digestion of orange peels and seaweed with cow manure
  Viviana Negro, Merlin Alvarado-Morales, Debora Fino, Bernardo Ruggeri and Irini Angelidaki (Under revision in Biomass and Bioenergy)-see Appendix.
- Biogas production from macroalgal species native to Nordic conditions and effect of pretreatment on their biodegradability Merlin Alvarado-Morales, Irini Angelidaki (Being resubmitted to Energy and Fuels) – see Appendix.
- Life Cycle Assessment of Anaerobic Co-digestion of cast Seaweed with organic residues
  Merlin Alvarado-Morales, Avraam Symeonidis, Irini Angelidaki (In preparation)-see Appendix.

1.3 Project results

1.3.1 Collection and quantification of cast seaweed

In framework of this project historical data related to annual collection rate of cast seaweed was performed at Solrød municipality. The cleaning of the beach is performed three times a year during the period from 1st of May to 1st September. The first cleaning begins about 1st of June and ends just after Sankt Hans (23th June). The second one is performed during a week in early August, and final cleaning at the end of August.

Two collections were performed at Solrød beach and Skodsborg beach during the time frame of this project. Both took place during the months of June in 2014 and 2016. During 2015 was not possible to perform a collection at Solrød beach as we were informed by the representative of the Solrød beach homeowners that a shortage of seaweed took place. To overcome this problem, cast seaweed from other locations in Denmark (already in stock at our lab facilities), namely, Hanstholm and Skive were used. Unlike the samples collected at Solrød beach, these samples were collected manually a mainly consisting of *Laminaria digitata* and *Ulva lactuca*, respectively.

Based on cast seaweed volumes treated in Solrød Municipality in 2009, where the amounts collected on a 3.7 km stretch corresponded to approximately 4,000 tonnes, an available quantity of 1081 tonnes yr^{-1} km^{-1} beach has been estimated[4]. However, more precise data on annual collection of cast seaweed were obtained directly from Solrød Municipality. This annual average collection of cast seaweed corresponds to 1,500 tonnes yr^{-1}[5]. However, considering the entire Køge Bay (38.6 km of coast line) an annual average collection of cast seaweed has been estimated to be 42,000 tonnes yr^{-1} which corresponds to 1 141 tonnes yr^{-1} km^{-1} of coast line.

1.3.2 Technologies for cast seaweed collection

Currently, cast seaweed at the Solrød beach is collected and cleaned by means of a back-hoe with big shovel in front, with a loader tractor, and with a special beach cleaning machine. Algae waste is removed from the beach with a dump truck and put in a temporary place where they stay for a few days and get drained there. Afterwards, the algae waste is taken away from the beach by trucks. Data on this technology respect to fuel consumption, working hours, type of fuel were retrieved so that can be included in the LCA.

1.3.3 Physicochemical characterization of cast seaweed

Samples collected at Solrød and Skodsborg beaches mainly consisted of debris, a considerable amount of sand, *Zostera marina* (eelgrass), *Pilayella littoralis* and *Ectocarpus* (filamentous brown algae). After sorting and cleaning the cast seaweed, a complete characterization was performed including the samples previously collected at Hanstholm and Skive.

Results from the physicochemical characterization showed that C:N ratio of cast seaweed were out of the range (C:N 20:1-30:1) for optimal anaerobic digestion process; particularly the cast seaweed collected at Solrød beach which exhibited the lowest C:N ratio (17.8:1). This potentially can lead to problematic digestion due to excess levels of ammonia nitrogen which may inhibit the methanogenic community. Therefore, co-digestion with dissimilar substrates is highly recommendable to shift the C:N ratio.

Another important ratio that needs to be taken into account, particularly when seaweed is considered as potential substrate for biogas production, is the C:S ratio. A substrate with a C:S below 40 will tend to have larger accumulations of H_2S gas as experienced by seaweed digestion trails in other studies. Results from characterization showed a C:S ratio of cast seaweed collected at Solrød below 40; therefore, problems due to inhibition caused by H2S could be expected.

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[4] Fredenslund AM (2010). Udnyttelse af tang og restprodukter til production af biogas. Solrød Kommune. Tekniske Administration.

[5] Personal communication with Solrød municipality

Another important result is the quantification of metals and nutrients (K, N, P) in the cast seaweed samples. Cadmium content has been relatively constant in previous years (in the range 0.50-0.80 mg kgTS^{-1})[6], however, it has been shown that the content in recent years were somewhat higher than 1 mg kgTS^{-1}. This fact coincides with the cadmium content report in this study (1.47 mg/kgTS). As also observed, the sample taken in January 2009 also exceeded the maximum level for cadmium. On the other hand, the cadmium content for samples collected at Hanstholm was lower of the limit of detection

Table 1 Characterization of cast seaweed from different locations

	06/2015[§]	2014/2015[§,1]	01/2010[§,2]	01/2009[§,2]	05/2009[§,2]	08/2012[*]
Al ± SD (mg/kgTS)	2240.54 ± 138.12					37.55 ± 2.94
As ± SD (mg/kgTS)	3.86 ± 0.53					32.82 ± 1.79
Ba ± SD (mg/kgTS)	34.07 ± 1.42					12.76 ± 0.86
Ca ± SD (g/kgTS)	30.24 ± 2.61		5.92 ±	n.m.	n.m.	9.37 ± 0.007
Cd ± SD (mg/kgTS)	1.47 ± 0.05	> 1	0.46 ±	2 ±	0.25 ±	<LOD
Co ± SD (mg/kgTS)	1.81 ± 0.43					0.43 ± 0.03[†]
Cr ± SD (mg/kgTS)	7.20 ± 0.84					11.64 ± 0.46
Cu ± SD (mg/kgTS)	13.69 ± 0.65					4.44 ± 0.58
Fe ± SD (g/kgTS)	2.17 ± 0.05					0.19 ± 0.00
K ± SD (g/kgTS)	1.59 ± 0.11		1.26 ±	n.m.	n.m.	9.42 ± 0.00
Li ± SD (mg/kgTS)	1.26 ± 0.08					<LOD
Mg ± SD (g/kgTS)	4.23 ± 0.15					5.07 ± 0.00

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[6] Personal communication with Solrød municipality

Mn ± SD (mg/kgTS)	75.67 ± 1.45					6.89 ± 0.47
Mo ± SD (mg/kgTS)	2.48 ± 0.13					0.46 ± 0.25[†]
Na ± SD (g/kgTS)	1.84 ± 0.05					14.26 ± 0.28
Ni ± SD (mg/kgTS)	6.65 ± 0.40		1.4 ±	0.9 ±	8.9 ±	6.15 ± 0.05
P ± SD (g/kgTS)	0.903 ± 0.017		0.34 ±	1.2 ±	0.53 ±	0.58 ± 0.02
N ± SD (g/kgTS)	5.2 ± 0.017		3.10 ±	7.1 ±	4.1 ±	
Pb ± SD (mg/kgTS)	5.98 ± 0.33		2.5	6	< 3	13.73 ± 0.63
S ± SD (g/kgTS)	18.07 ± 1.73		0.99 ±	n.m.	n.m.	6.47 ± 0.27
Sb ± SD (mg/kgTS)	7.19 ± 2.58					2.03 ± 0.06[†]
Se ± SD (mg/kgTS)	3.20 ± 1.49					2.75 ± 2.05[†]
Si ± SD (mg/kgTS)	1186.29 ± 652.85					
Sr ± SD (mg/kgTS)	422.60 ± 7.09					640.38 ± 11.03
V ± SD (mg/kgTS)	5.66 ± 0.26					0.99 ± 0.24
Zn ± SD (mg/kgTS)	120.55 ± 3.94					117.74 ± 4.35

[†] Concentration is higher than limit of detection but lower than quantification limit. Value has therefore high uncertainty

[‡] LOD, limit of detection

[§] Solrød

*Hanstholm

[1] Personal communication with Preben Larsen

[2] Fredenslund AM (2010). Udnyttelse af tang og restprodukter til production af biogas. Solrød Kommune, Tekniske Administration.

1.3.4 *Pretreatment assessment and biodegradability test*

The aim of these experiments was to increase the biodegradability of cast seaweed samples by means of the application of different pretreatment methods and/or combination of them. Then biogas potential tests were conducted to evaluate the effect of the applied pretreatments. Pretreatments such as alkaline, acid, mechanical, enzymatic hydrolysis and combination of them were applied.

Results obtained (Figure 1) clearly showed that the alkali (pH 12) and enzymatic treatment resulted in 12-28 % increase of biomethane potential compared to the unwashed and to the washed seaweed biomass. Methane potential of the liquid fraction (after enzyme addition) was highest, which can be explained with presence of easy degradable oligosaccharides as a result from enzymes action on the solid fraction of seaweed. Also, a significant effect was mainly observed in the biodegradability rate when enzymatic hydrolysis was applied. There was no significant difference between effect of the enzymes and alkaline pretreatments respect to the ultimate methane potential. Hereby, with respect to process economy of seaweed bioconversion, alkaline hydrolysis would be the preferable option over enzymes which are very costly substances.

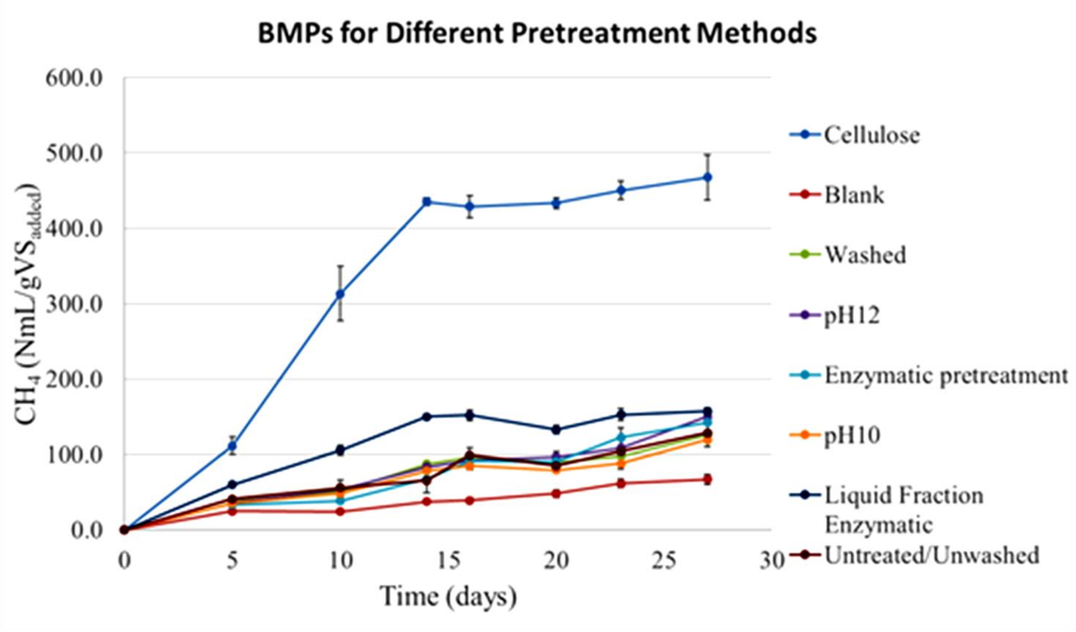

Figure 1 Effect of pretreatment on the ultimate methane potential and biodegradability of cast seaweed collected at Solrød beach. (values are mean ± SE).

In the case of the cast seaweed samples collected at other locations, results showed that, most of the pretreatments investigated showed to be suitable for samples collected at Skive, except thermal pretreatment (UL4) which resulted in detriment in the ultimate methane potential.

However, dried/grounded (UL2) and enzymatic (UL7) pretreatments have limitations for further implementation due to high cost associated to energy provision and enzymes, respectively.

Ultimate methane yield reached by *Laminaria digitata* (samples collected at Hanstholm) pretreated fractions was lower compared to untreated fraction indicating that no pretreatment is required. The exception was the screw pressed liquid fraction (LD5). This increase in the ultimate methane potential is explained by the fact that in the screw pressed liquid fraction high concentrations levels of glucose (main product from the hydrolysis of the linear polysaccharide laminarin) and mannitol were present. A soluble sugar determination revealed a glucose concentration of 59.7 g L^{-1} and a mannitol concentration of 9.85 g L^{-1} in the liquid fraction.

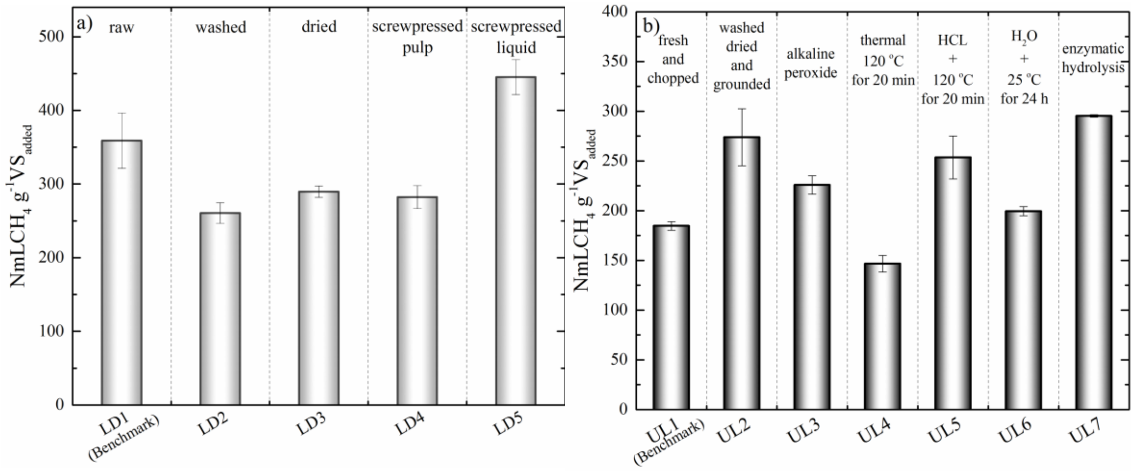

Figure 2 Effect of the pretreatment on ultimate methane potential for samples collected at Hanstholm (LD1-5) and Skive (UL1-7). (values are mean ± SE).

In order to investigate the effect of the enzymatic hydrolysis on the biodegradability on cast seaweed, different enzymes blends were tested so that the best combination was determined. We chose samples collected from Solrød to test the different enzymes blends as they presented the lowest biodegradability compared to samples collected at Skive and Hanstholm. In spite of the best enzyme combination (0.1 mL Celluclast + 0.01 g β-glucosidase) was determine, overall results clearly demonstrated that enzymes were not efficient enough for saccharification of the cast seaweed (Figure 3).

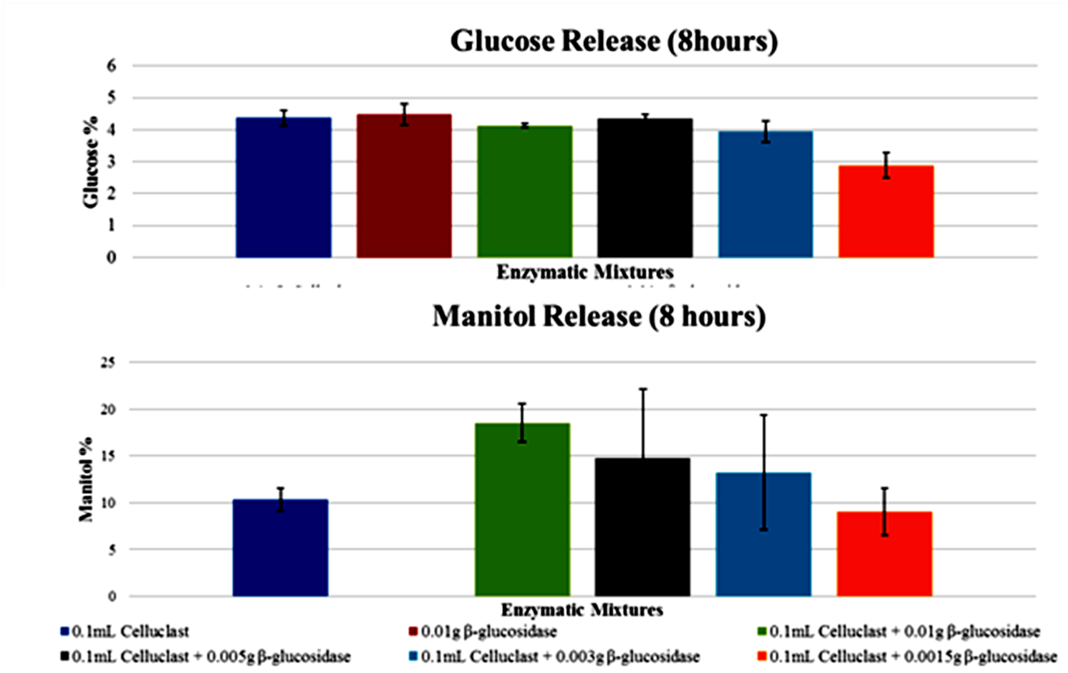

Figure 3 Effect of different enzymes blends of the saccharification. (values are mean ± SE).

No significant differences were detected on basis of glucose release. Highest glucose release (up to 4.2% of the theoretical maximum) was registered when β-glucosidase was used alone. No need for Celluclast can be possibly explained with the fact that cellulose from seaweed was already degraded to cellobiose which was easily available for conversion to glucose by β-glucosidase. An interesting finding was the mannitol release (up to 18.2% of the maximum) which can be due to hydrolysis of the algal cellulose fibers resulting in release of mannitol.

1.3.5 Development of a process for simultaneous pretreatment and sand separation

As the main drawback in using beach seaweed as feedstock for biogas plants is the high sand-debris content when it is collected. Therefore, the aim of this task was to develop a unique process in which both sand-debris removal and the pretreatment steps take place simultaneously. As proof of concept a lab scale prototype was design and constructed. Then, trials were performed to investigate:

- The optimal operational conditions of the prototype aiming at maximizing seaweed recovery (and removal of sand).
- The combined effect of pretreatments and sand-debris removal on the biodegradability of cast seaweed through BMP assays.
- The evaluation of the fertilizer potential of the digested effluents from the BMP assays.

1.3.5.1 Determination of the optimal operation conditions of the lab-scale prototype

The so-called *Bio-Sand-Separator* prototype is shown in Figure 4 and was designed to sufficiently remove sand, small stones and debris from CSW that is collected from shores; without requiring huge amounts of energy and by utilizing water that can be recycled in the same process. During the experiment five operation variables were considered to be varied, namely, a) water load, b) seaweed load, c) rotational velocity (rpm), d) water temperature and e) time of rotation. Several tests were performed in order to identify the operational variables that provided the highest sand separation whilst maximizing the cast seaweed recovery.

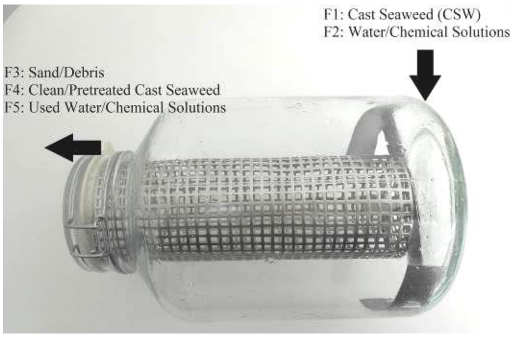

Figure 4 Bio-Sand-Separator *prototype*

The optimum operating conditions in terms of seaweed recovery and sand removal were determined. Thus, the optimum loading ranged from 60 to 75 % of the total volume of the device. The maximum recovery (84.3 ± 12.5%) of clean (sand free) seaweed was obtained under following operational conditions: 40 ± 20 g CSW/L device volume, 0.4 L water/L device volume, temperature 20 °C, and rotation parameters: 17 rpm for 10-20 min. Results obtained showed that sand was sufficiently removed (95-100%) and the amount of seaweed that was lost was equal to 15.7 ± 12.5 % of the initial load.

Figure 5 Left) water, seaweed debris, and sand after cleaning process, right) clean seaweed

1.3.5.2 Investigation of the combined effect of pretreatment and sand-debris removal on the biodegradability

Optimal operation conditions for cast seaweed recovery and sand removal were further tested in combination with a set of pretreatments:

i. Only washing (with 2 L of water).
ii. Washing combined with HCl treatment at pH 1.5 (0.6 mL 1 M hydrochloric acid per g of CSW).
iii. Washing combined with acetic acid treatment at pH = 4.0 (0.425 mL 99 % acetic acid per g of CSW).
iv. Washing combined with NaOH alkaline treatment at pH = 12 (10 mL 0.01M NaOH per g of CSW).

After this, a set of BMP´s tests were conducted to evaluate the combined effect of the sand removal and pretreatments. Results are shown in Figure 6. Overall, the combined effect of sand removal and pretreatment had a positive effect on the biodegradability, except when acetic acid was used which clearly shows a negative effect. Acid (hydrochloric acid) and alkaline pretreatments (sodium hydroxide) improved methane production by 109% and 38% respectively, compared to untreated seaweed assays. However, caution should be taken respect to the acid pretreatment as shows a high standard deviation indicating uncertainty in the ultimate methane yield.

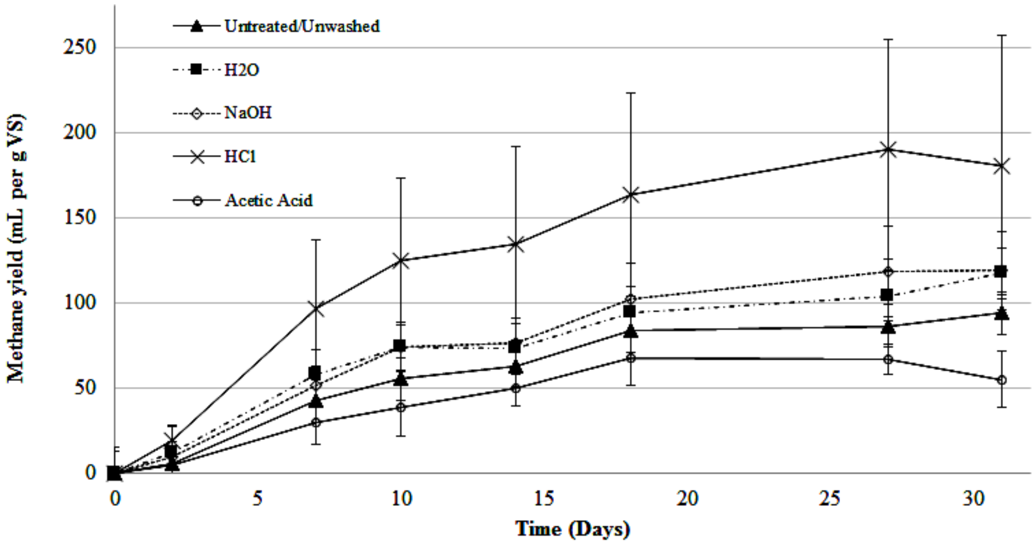

Figure 6 Simultaneous effect of the pretreatment and sand removal on the ultimate methane potential and biodegradability of cast seaweed. (values are mean ± SE).

1.3.5.3 Biofertilizer properties of the digested effluents

The fertilizing value of the anaerobic digestate was directly assessed from the amount of mineralized nutrients, which play a vital role in determining the suitability of a digestate as a fertilizer, through performance comparisons with recognized organic or inorganic fertilizers. Prior to anaerobic digestion nitrogen existed mainly in organic form, while after digestion nitrogen was mostly present (80-94%) in the form of NH_4^+ due to the anaerobic conditions in the assays, and the mineralization that took place.

Effect of different pretreatments (washing with H_2O, and HCL addition) on the nitrogen and phosphate content also was investigated. It was observed that compared to the blanks (no biomass, only inoculum), the seaweed digestate had a slightly decreased NH_4^+ concentration (Table 1). That can be attributed to the higher pH of these samples leading to higher part of NH_3 compared to NH_4^+, compared to the blank assays. High pH values in the digestate can promote losses of N as ammonia (NH_3, gaseous phase) during the sample preparation. The losses were insignificant as there was not observed a significant change in the TKN content of the digestate.

Concerning the phosphate concentration, an increase of 17% and 21% was observed, for the washed with water and HCl pretreated seaweed assays, respectively. Thus, the phosphate content of the digestate, compared to the inoculum, was improved. Moreover, CSW contained 0.5 to 0.8 mg kg TS^{-1} of cadmium, which was slightly higher the limits of the Danish regulation for fertilizers (0.5 mg kg^{-1}), rendering it unusable as a source of nutrients for soils.

Compared to typical commercial fertilizers, the nitrogen and phosphorus content of digested seaweed was significantly lower (50 and approximately 3600 times for nitrogen and phosphorus respectively) which hinders the practical applications on agricultural land. The complete data sets are presented in Table 2. It can be assumed that 50 kg of bio-fertilizer can replace 1 kg of commercial fertilizer in terms of nitrogen content, or 3633 kg can replace 1 kg of commercial fertilizer in terms of phosphorus. The Danish legislation allows 126-147 kg of N, and 20kg of P per hectare for the cultivation of spring wheat. This means that 44.75 tons of bio-fertilizer should be used in a hectare in order to cover the N needs or 1111.11 tons to cover the P limits set by Danish laws. That amount of effluent is sizable and the costs of transportation would be substantial. Therefore, we evaluate that the bio-fertilizer capacity of cast seaweed digestate is very low. Cast seaweed digestate cannot be used as sole source of nutrients for agricultural crops and that it can only be used in mixtures with commercial products.

Table 2 TKN, NH^+_4 and phosphate amounts and pH of digestate samples

	TKN (g/kg)±SD	**NH_4^+-N (g/kg)±SD**	**PO_4^{3-} (mg/kg)±SD**	**pH**
Blank (inoculum only)	3.01±0.04	2.81±0.15	45.1±1.6	7.98
Washed with H_2O	3.08±0.05	2.55±0.02	52.9±0.6	8.10
Pretreated with HCl	3.05±0.18	2.53±0.03	54.4±0.5	8.08

Table 3 Comparison between the commercial and biofertilizers

	Commercial fertilizer	**Digested seaweed as bio-fertilizer**
Nitrogen (g/kg)	150.0[7]	3.1
Phosporus (mg/kg)	65,400[8]	18

1.3.6 Co-digestion of cast seaweed with organic residues

1.3.6.1 Batch assays

A set of co-digestion experiments were performed at which the aim was to investigate the best co-digestion ratio with organic residues to increase biogas productivity and yield. As starting point, co-digestion of cast seaweed with cattle manure was performed in batch experiments in order to determine the best co-digestion ratio. Among the co-digestion ratios tested, 80% cattle manure/20% cast seaweed was identified as the best co-digestion ratio (232 ± 39 NmLCH_4 gVS^{-1}) compared to mono-digestion of cast seaweed (Figure 7).

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[7] Solrodbiogas, 2016. Solrodbiogas. [Online] Available at: http://www.solrodbiogas.dk/en/solroed-biogas/facts.aspx

[8] Solrodbiogas, 2016. Solrodbiogas. [Online] Available at: http://www.solrodbiogas.dk/en/solroed-biogas/facts.aspx

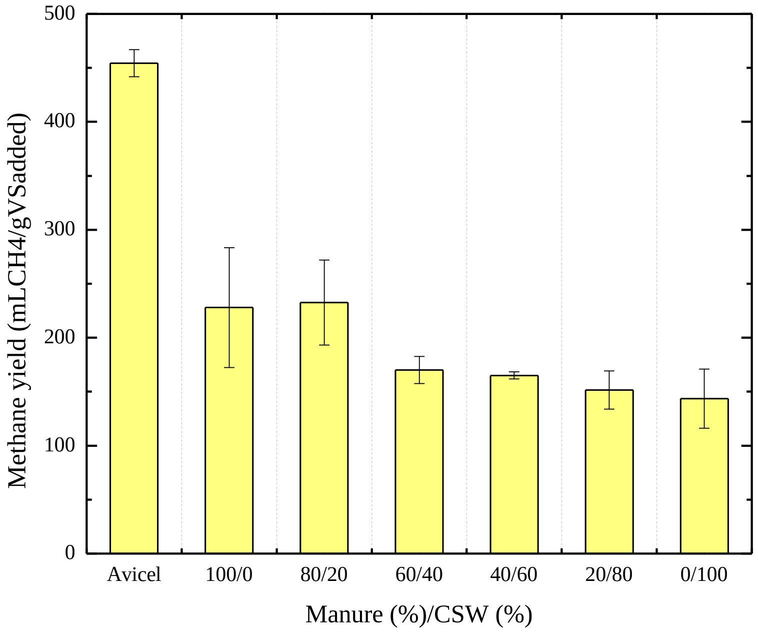

Figure 7 *Methane yields of different manure-cast seaweed (CSW) co-digestion ratios.* (values are mean ± SE).

A second set of experiments was performed to determine the best co-digestion ratio with sugar beet pulp Figure 8. Co-digestion of certain ratios of CM, CSW and SBP seems to have a synergistic effect. This is present both when comparing the achieved results to the yields obtained from mono-digestion of each substrate, and with respect to co-digestion of only CM and CSW. Best co-digestion ratios identified were Man (80%)/SBP (10%)/CSW (10%), Man (60%)/SBP (20%)/CSW (20%), Man (50%)/SBP (30%)/CSW (20%) and Man (25%)/SBP (60%)/CSW (15%).

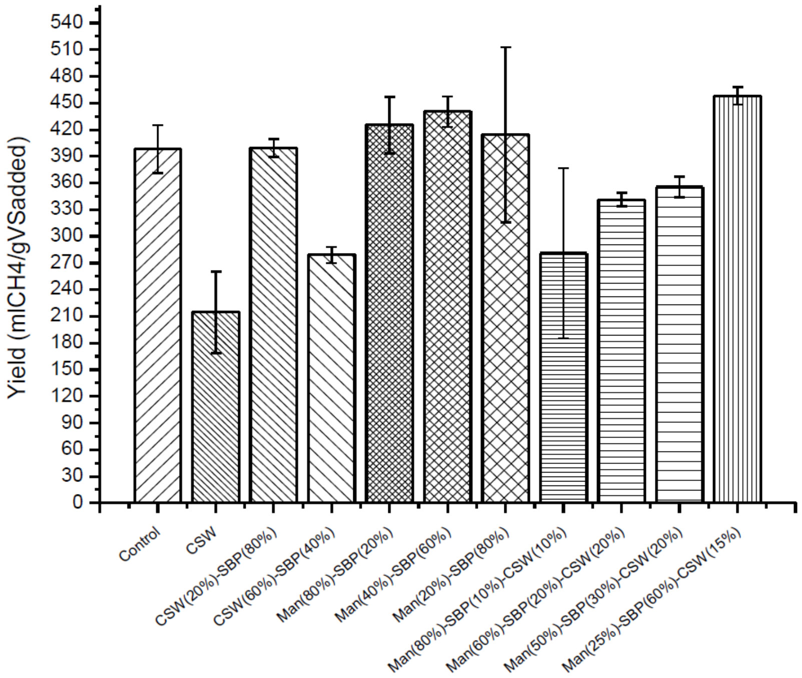

Figure 8 BMP of SBP, manure and CSW co-digestion at different volatile solids ratios. (values are mean ± SE).

1.3.6.2 Continuous experiments

The aim of these experiments was to investigate the stability and process performance at two different organic loading rates (OLR) and one hydraulic retention time (HRT) at 55 °C. The reactor had a working volume of 3.5 L and a feed system calibrated for delivery of two daily pulses at a set point of 175 mL/day, giving a HRT of 20 days. The HRT of 20 days and OLR of 2.5 gVS L^{-1} day^{-1} were set in Phase I (P-I). It is of mention that during P-I, cast seaweed was blended with water in ordinary kitchen blender to make slurry suitable to be pumped. During P-II the cast seaweed was directly blended with manure. Additional water was not needed indeed as in P-I, thereby increasing slightly the VS content in the final blend. This caused a slightly increment in the OLR (2.58 ± 0.01 gVS L^{-1} day^{-1}) as observed in Figure 9.

Methane productivity during the first HRT was recorded at 277.0± 22.6 mL CH_4 $L^{-1}d^{-1}$ with a yield of 123.85± 10.1 $mLCH_4$ gVS^{-1}. The increase in OLR in the second HRT corresponds to higher methane output, consisting in an average productivity of 448 ± 47 mL CH_4 $L^{-1}d^{-1}$ and a methane yield of 178.7± 18.7 $mLCH_4$ gVS^{-1}. Similar results were obtained during the third HRT, when no changes were caused. Average productivity and yield were found at 463.2 ± 42.8 mL CH_4 $L^{-1}d^{-1}$ and 184.8± 17.1 $mLCH_4$ gVS^{-1}, respectively. Addition of sugar beet pulp took place on day 89 so that the co-digestion was to CM (80%)-SBP (10%)-CSW (10%). SBP supplement had a very quick effect on the digestion performance. A major increase in methane productivity and yield was observed, along with decrease in total VFA. Acclimation period, following SBP addition lasted around 10 days. Steady conditions of biogas production were achieved on day 109. Methane productivity and yield throughout the first HRT were recorded at 389.5 ± 42.67 mLCH$_4$ L^{-1} d^{-1} and 210.8 ± 16.4 mLCH$_4$ gVS^{-1}, respectively. Methane content in the biogas increases up to 70%.

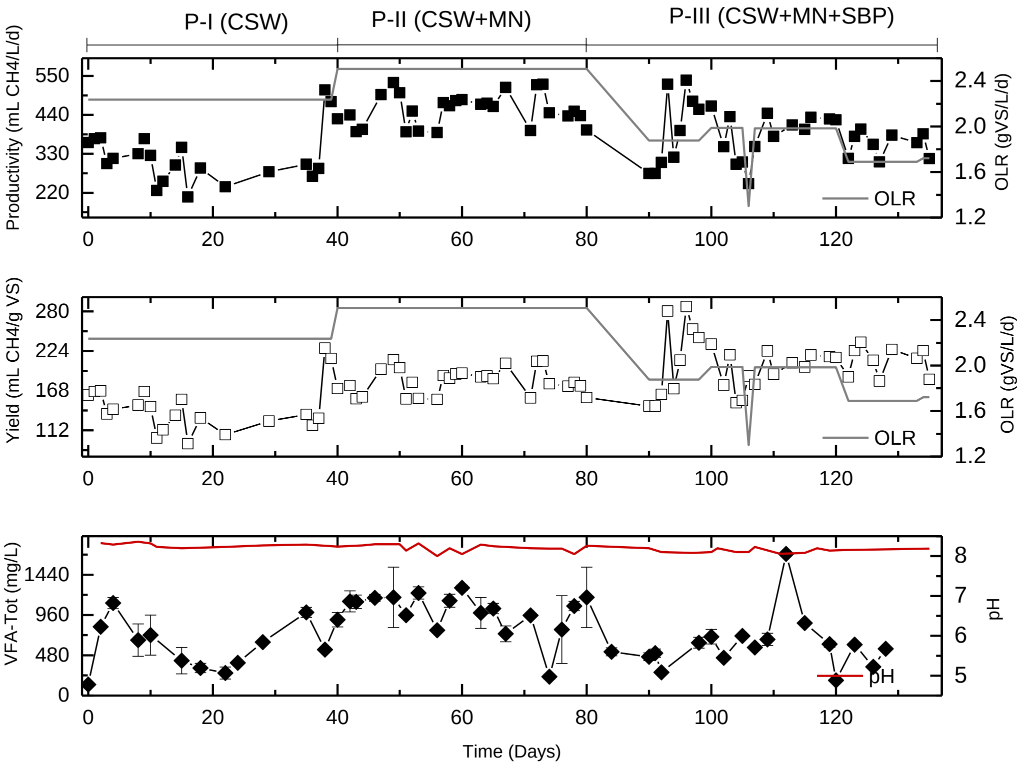

Figure 9 a) Gas production, b) methane yield and c) VFA-tot and pH.

1.3.6.3 Pilot scale experiments

The aim of these experiments was to investigate the stability and process performance of co-digestion of cast seaweed and manure at larger scale –pilot scale (150 L, operating temperature 53°C, OLR 1.4 gVS L^{-1} d^{-1} and HRT 30 days). Production of biogas from anaerobic co-digestion of cast seaweed with manure was demonstrated at pilot scale facilities (DTU). As starting phase, the reactor was fed only with cattle manure until steady state conditions were reached (day 41-42) and subsequently the best co-digestion ratio found at lab scale experiments was tested. Feedstock with a VS ratio of 80%(Manure)/20% (cast seaweed) was fed at approximately day 42. As was expected an increase in biogas productivity was observed thereby validating our findings at lab scale trials. Afterwards, feedstock with high ratio of pre-treated cast seaweed (30 % from volatile solids in the feedstock) in the mixture was fed at day 73. Because of this, a significant increase in biogas productivity was observed. Overall, the co-digestion of cast seaweed and cattle manure was demonstrated to be feasible at pilot scale (Figure 10).

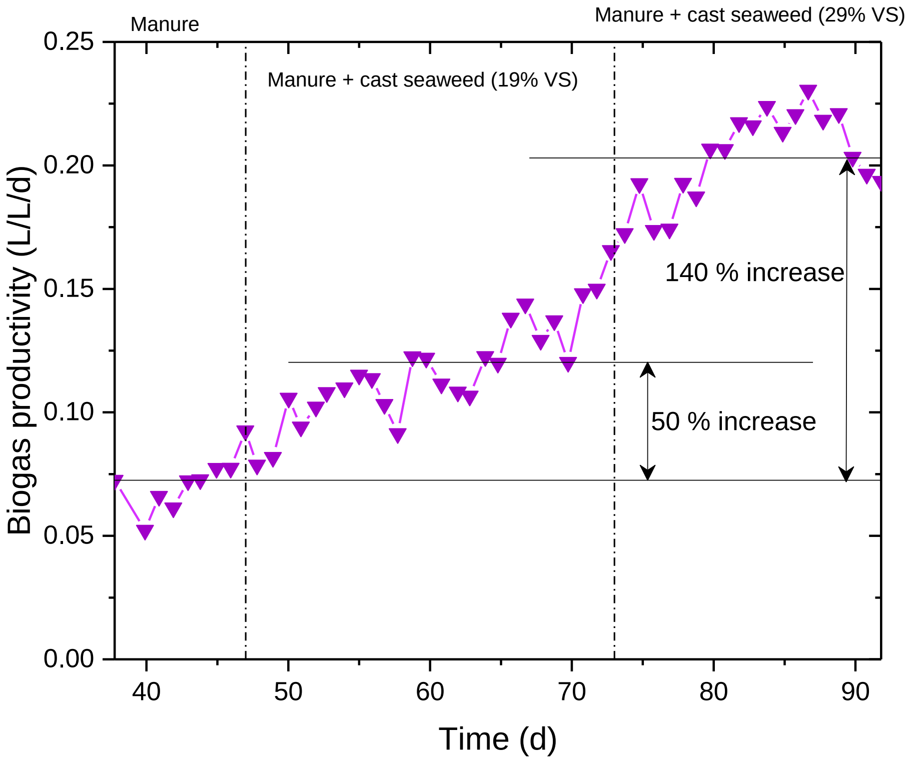

Figure 10 Results from pilot - scale AD experiments

1.3.7 Life Cycle Assessment and Economic Evaluation

The aim of this task was to perform a complete life cycle assessment to quantify the potential environmental impacts arising from handling one Mg of cast seaweed. As mentioned before, five different waste management scenarios were modelled, where there are 4 co-digestion scenarios and one composting scenario. The scenarios were: anaerobic digestion of cast seaweed (AD-CSW); co-digestion of cast seaweed with cattle manure at VS ratio 20:80 (COAD-M); co-digestion of cast seaweed, manure and sugar beet pulp (residual stream from sugar beet processing industry) at VS ratio 20: 50:30 (COAD-MSBP); co-digestion of cast seaweed and sugar beet pulp at VS ratio 20:80 (COAD-SBP) and composting (COMP) of the cast seaweed.

Overall, COAD-M (co-digestion of cast seaweed with cattle manure at VS ratio 20:80) exhibited the best performance in most the impact categories considered, followed by AD-SW (anaerobic digestion of cast seaweed), while the highest negative environmental impact was found for COAD-SBP (codigestion of cast seaweed, manure and sugar beet pulp). In fact, the use of manure in anaerobic digestion, and hence avoiding its storage, results in large environmental savings mostly related to Global Warming Potential (GWP), Terrestrial Acidification (TA) and

Terrestrial Eutrophication (TE). Substrate consisting of seaweed with SBP seems to be a promising substrate for biogas production as it is highly biodegradable with a biological methane potential (BMP) value approx. 99% of the respective theoretical one. However, removing SBP from the animal feed market induces activities (i.e. production of maize and soy) that proved not to be environmentally friendly. The results obtained are of high importance for Denmark where large quantities of manure are produced annually and they can be utilized for co-digestion with casted seaweed for sustainable bioenergy production.

For economical evaluation of the casted seaweed anaerobic digestion process, variable costs such as biomass transportation & collection, and expenses associated with anaerobic digestion and capital costs were calculated. Lab data generated previously (reported in previous interim reports) were used for estimation of chemical pretreatments, sand separation and bioconversion costs. The main finding was that economic feasibility cannot be achieved, in any seaweed bioconversion scenario, if the biomass transportation costs are considered. This occurs due to high amounts of cast seaweed on the beach, and the extensive manual labour (truck-drivers) that is required in Danish conditions.

1.4 Utilization of project results

The results obtained could be further utilized by up-scaling and development of a full-scale anaerobic co-digestion system placed near the biomass collection point-coastal environment, e.g. to reduce as much as possible the cast seaweed transportation costs. Results obtained were used for dissemination-preparation of scientific publications, and as part of master thesis. In addition, strong networking was developed with biogas plant managers in Denmark upon communication of the promising results for seaweed based biogas production.

1.5 Project conclusions and perspectives

The project demonstrated technical and environmental feasibility of co-digestion of cast seaweed with organic residues, particularly with cattle manure. Pilot scale co-digestion of cast seaweed with manure was successfully demonstrated at DTU. The best process performance from environmental point of view was obtained with mixture cast seaweed and manure in ratio 20:80 matching the experimental findings. The economic feasibility analysis clearly showed that a positive Net Present Value (NPV) cannot be achieved if seaweed biomass transportation costs are taken into consideration. The process technology derived in this project can be part of the integrated energy system package for high efficiency energy production and resources optimisation.

1.6 Annual export of electricity (only ForskVE)

1.7 Updating Financial Appendix and submitting the final report

APPENDIX

Anaerobic co-digestion of orange peels and seaweed with cow manure

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Abstract

Anaerobic digestion (AD) is a promising technology for production of energy and treatment of waste streams. Orange peels (OP) and seaweed (SW) are found in large amounts and could with advantage be used for biogas production. However, their content of toxic for the AD process compounds presents a challenge. In this study we examined possibility of using co-digestion of OP, SW with manure (MN) as a feedstock for successful anaerobic co-digestion. Batch toxicity tests showed that limonene and gallic acid at 6 g L^{-1} were entirely hindering biogas production, while concentrations at 3 g L^{-1} of both compounds significantly increased the lag phase before methane was produced. In bio methane potential (BMP) assays we found that the maximum values for OP and SW were found to be 398.4 ± 9.36 and 348.6 ± 10.15 $NmLCH_4$ g^{-1} VS_{added}, respectively, at thermophilic conditions. In addition, four continuous experiments with Stirred Tank Reactor (CSTR) were carried out in order to investigate the performance and process dynamics when OP, SW and MN were co-digested at different organic loading rates (OLR) and different substrate ratios. The results showed that OP or SW can be successfully co-digested with manure until an organic loading rate of 4 $gVSL^{-1}d^{-1}$ and hydraulic retention time (HRT) of 15 days reaching a methane yield of 266.65 ± 15.69 and 218.47 ± 13.17 $NmLCH_4$ $g^{-1}VS_{added}$, respectively. Continuous co-digestion tests (OP + SW + MN) confirmed the contribution of OP to boost the methane yield.

Keywords: anaerobic co-digestion, organic waste, orange peels, seaweed, manure

1. Introduction

Landfilling organic waste, such as citrus waste and seaweed, is not an option in European countries, due to regulations. Moreover, these organic wastes have to be collected, transported and disposed treated for avoiding local nuances offering a great cost and load to the local municipalities. In the perspective of a proper waste management, organic waste can be considered as a renewable resource for energy recovery, complying with the Waste-to-Energy concept [1]. Depletion of fossil fuels and the negative environmental impacts related to their use are paving the road towards alternative energetic sources, able to answer the increasing need of a growing world-wide population in a sustainable way.

Co-digestion of agro-industrial waste with MN is a widely spread technology in Denmark, where 40 million tons of manure are produced every year [2]. Even though AD of MN is a well-established technology for energy recovery, co-digestion strategy is a solution for some substrates which are difficult to digest, as OP and SW, due the presence of inhibiting chemicals. Moreover, co-digestion gives the possibility to face the disposal issue of voluminous industrial waste and reduce toxic compounds.

Every year, the disposal of citrus waste leads to economic and environmental problems. In Europe, more than 10 Mton of citrus waste are yearly generated [3] and AD is an attractive candidate technology to treat this waste stream. Citrus waste is characterized by a high organic content with a ratio of volatile solid (VS) to total solid (TS) of more than 95% (VS/TS ≥0.95) [4]. The main issue for using this waste stream in fermentative processes is its content of compounds such as D-limonene, a terpenic compound found in citrus peels and characterized by antimicrobial activity [5] and [6]. Mesophilic AD is inhibited at concentrations of 400 µL/L, while the thermophilic operation is inhibited in the range between 450-900 µL/L [7]. D-limonene needs to be removed/recovered or, a co-digestion strategy as suggested by [8] and [5] could be evaluated in order to treat by AD the OP.

On the other hand, every year approximately 42 000 tons of cast SW are washed out on Danish beaches. Intense academic and industrial research efforts are devoted to exploit this abundant biomass for biofuels production. AD of SW does not compete with food chain and the low lignin content in SW avoids energy-intensive pre-treatments [9]. However, the presence of high concentration of sulfur, sodium chloride and heavy metal present in SW can hamper the biological process [10] and, in addition, polyphenolic compounds are widely accepted to be toxic for fermentative bacteria because of their anti-oxidant properties [11] and [12]. In order to focus attention of the inhibition of polyphenolic compounds, Disley et al. [13] reported the threshold concentrations (expressed in g L^{-1}) for the single components in case of thermophilic anaerobic digestion: 2.28 for gallic acid, 1.45 for pyrogallol, 0.05 for tannic acid, 2.1 for mimosa and 4.1 for quebracho. Furthermore, MN is a suitable substrate to dilute the toxic compounds, found both in citrus waste and seaweed; in addition it provides nutrients for the microbial growth and buffering capacity [14].

Anaerobic co-digestion of seaweed and food waste has been successfully performed in a previously work [15], which highlighted feedstock C/N ratio as a key parameter to ensure a stable process. Furthermore, C/N ratio for AD lies usually in the range of 20-30, with the optimum at 25 [16].

ThBMP indicates the maximum methane production achievable of such specific waste [17]. It could be estimated using different approaches: based on chemical oxygen demand (COD) [18], elemental composition [19] and by organic fraction characterization [20]. Nevertheless, ThBMP overestimates the experimentally obtainable BMP due several reasons such as not considering consumption of carbon for built of microbial biomass, or due to the low biodegradability of lignocellulosic materials present in the refuses. Besides, BMP tests are a powerful tool not only to estimate the maximum achievable biogas production but also to discern the optimum ratio among the different substrates when anaerobic co-digestion is under evaluation [21].

In the present study, co-digestion strategies of OP, SW and MN have been investigated for biogas production following different approaches. Firstly, ThBMP and experimental BMP values were determined for OP and SW as single substrates. The preliminary results were used to set-up the parameters for the continuous anaerobic co-digestion tests, which were performed at different organic loading rates (OLR) and different OP, SW and MN ratio.

2. Materials and method

2.1. Analytical methods

Total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen (TKN) and ammonium nitrogen NH^{+4} (NH_4-N) were evaluated according to Standard Methods [22] for OP, SW, diluted MN and inoculum. The total VFA (volatile fatty acids) content was analyzed using a gas chromatograph (Smimadzu GC-2010AF, Kyoto, Japan) equipped with a flame ionization detector (FID). For total sugars analysis, samples were subjected to a hydrolysis process (4 % H_2SO_4 for 60 min at 30 ºC) and autoclaved (72 % H_2SO_4 for 40 min at 121 ºC). Total sugar analysis was performed by HPLC (Hewlett Packard, CA, USA), according to Kongjan and Angelidaki (2010). The elemental analysis of OP and MN were evaluated by means of CHNOS elemental analyzer (Macro cube, Hanau, Germany). Total phenolic compounds were determined according to Wang et al. [24] and measured through a spectrophotometer (Thermo Spectronic, Helios Epsilon, USA). Amino acid determination was performed by the following procedure: 50 mg of lyophilized material was hydrolyzed with 6 mL of 6 M hydrochloric acid in a microwave (Microwave 3000 SOLV, Anton Paar, Austria) at 150 °C and 500W for 30 minutes. Quantification of amino acids was done by in-needle derivatization HPLC-FLD (Dionex UltiMate 3000, Thermo Scientific). Amino- acids were separated in a c18 reversed phase column (Eclipse Plus C18, Agilent Technologies, USA) with an in-line guard column (EC 4/2 Universal RP, Macherey-Nagel, Germany) and mobile phases A (10mM Na2HPO4, 10 mM Na2B4O7) and B (methanol:acetonitrile:water, 45:45:10). The flow rate was 0.420 mL min^{-1}. Quantitative analyses were performed by means of calibration curves using a commercial amino-acid mix standard (AAS18 Fluka). The pH was measured by a PHM 92 Lab pH-meter. All these measurements were performed in triplicates.

Analytical measures were performed in duplicates on CSTR samples, including pH, VFA, methane content, TKN, (NH_4 N) analysis. Biogas composition was measured through a gas chromatograph (Mikrolab, Aarus A/S, Denmark) with a thermal conductivity detector (TCD). Methane content in BMP tests were analyzed by a gas chromatograph (Thermo scientific, Trace 1310, Denmark), equipped with a FID. TKN, NH_4 -N and pH were determined as above mentioned.

Biogas production in the CSTR experiments was monitored by gas meters measuring gas by the volumetric water displacement method [25]. Operation pressure was around 1 atm. The biogas production was recorded daily, while liquid samples for analytical measurements of pH, VFA, TKN and ammonia nitrogen were taken 2-3 times per week.

2.2. Substrate characteristics

Three types of substrates were used as a feedstock for AD: OP, SW and MN. OP were separately gathered at kitchen food waste, while SW sample consisted of *Laminaria digitata* collected during summer period at Hamborg Strand, north of Hanstholm at the Danish North Sea coast in 2012. Both OP and SW were dried at 70 °C for 24 hours and then milled with Cutter Mill (Retsch 8M 2000), in order to obtain a homogenous sample for analytical analysis and anaerobic co-digestion trials. Cow manure was obtained from the feedstock stream for Snertinge biogas plant in Denmark. All the substrates were stored at -18 °C and kept refrigerated at + 4 °C prior to use. Thermophilic inoculum (55 °C ± 1) was used for the batch assays and was obtained from the effluent of CSTR reactors fed with only diluted manure (3:2 manure to water *w/w*). Effluent from the CSTR reactors, flushed with nitrogen gas for ten minutes and then incubated for degrading remaining organic matter in the inoculum, for 10 days at 55 °C, was used as inoculum for BMP reactors. Table 1 reports the characteristics of OP and SW used, while Table 2 shows those of manure and inoculum used.

Table 1. Orange Peels (OP) and seaweed (SW) characteristics.

Parameters	Units	OP	SW
TS	gTS/g_{sample}	0.93 ± 0.07	0.92 ± 0.001
VS	gVS/g_{sample}	0.90 ± 0.07	0.82 ± 0.001
pH	-	4.04 ± 0.08	5.33 ± 0.17
Total Kjeldahl nitrogen (TKN)	g/kgTS	9.97 ± 0.2	6.78 ± 0.1
Ammonium nitrogen (NH_4 N)	g/kgTS	1.17 ± 0.1	0.87 ± 0.1
Total Aminoacids (AA)	% TS	3.50 ± 0.15	3.14 ± 0.01
of which essential	% AA	32.09 ± 0,07	37.98 ± 0.09
Total sugars	% TS	56.86 ± 1.01	52.45 ± 3.30
Total phenolics	mg gallic acid equivalents/100gTS	-	27.20 ± 3.34
C	% on TS	43.22 ± 0.16	37.01 ± 0.63
H	% on TS	5.31 ± 0.26	5.25 ± 0.10
N	% on TS	1.18 ± 0.01	0.87 ± 0.10

S	% on TS	0.21 ± 0.09	0.43 ± 0.06
O	% on TS	41.04 ±0.02	39.82 ±0.02
C/N	-	36.62	42.54

Table 2. Manure and inoculum characteristics.

Analysis	Units	Inoculum for BMP	Diluted Manure
TS	gTS/g_{sample}	0.14 ± 0.00	0.029 ± 0.01
VS	gVS/g_{sample}	0.10 ± 0.00	0.027 ± 0.00
pH	-	8.54 ± 0.10	7.58 ± 0.10
TKN	g/L	1.31 ± 0.01	1.28 ± 0.01
Ammonium nitrogen	g/L	1.15 ± 0.01	0.90 ± 0.01
Proteins*	g/L	0.9 ± 0.10	2.38 ±0.08
VFA	mg/L	65.7 ± 11.70	4,122.6 ± 86.60
Acetate	mg/L	60.7 ± 10.60	2,806.2 ± 63.44
Propionate	mg/L	3.3 ± 0.50	768.9 ± 18.41
Isobutyrate	mg/L	0.2 ± 0.00	94.8 ± 1.08
Butyrate	mg/L	0.3 ± 0.10	254.3 ± 5.08
Isovalerate	mg/L	0.4 ± 0.10	113.9 ± 1.97
Valerate	mg/L	0.1 ± 0.20	80.6 ± 1.09
n-hexanoate	mg/L	0.7 ± 0.20	4.1 ± 0.01

2.3. Batch reactors

Batch assays, conducted to evaluate the ultimate methane potential, were designed according to suggested protocol at thermophilic conditions (55 °C ± 1) [26] for 30 days. The assays were carried out at different initial organic loads 2.5, 3 and 5 gVS L^{-1}, for OP and 3 and 4 gVS $L^{-}1$ for SW, respectively as reported in Table 3. Flasks of 547 mL total volume and 150 mL were used for the assays. The flasks were initially flashed with nitrogen, inoculated and the samples were added, before inserted to thermostated incubators. The flasks were shacked once a day manually in order to allow good contact of substrate to microorganisms and homogeneous conditions; each assay of Table 3, was performed in triplicate. BMP values for single substrate were compared to the ThBMP ones, according to Sheng's (1) and Buswell's equation (2) [19] and [18]:

$$HHV_{biomass} *= -1.3675 + 0{,}3137\ C + 0.7009\ H + 0{,}0318\ O \qquad (1)$$

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{a} - \frac{c}{2} + \frac{3d}{b} + \frac{e}{4}\right) * H_2O \rightarrow \qquad (2)$$

$$\left(\frac{a}{2} - \frac{b}{8} - \frac{c}{4} + \frac{3d}{b} + \frac{e}{4}\right) * CO_2 + \left(\frac{a}{2} - \frac{b}{8} - \frac{c}{4} + \frac{3d}{b} + \frac{e}{4}\right) * CH_4 + c * NH_3 + d * H_2S.$$

($HHV_{biomass}{}^*$ is the High Heating Value of the considered biomass, expressed in MJ $kg_{TS}{}^{-1}$.)

Table 3. Experimental conditions used on the BMP tests.

Substrate	Organic load (gVS L^{-1})	VS contribution tested	C/N ratio
OP	2.5		36.73
	3		36.73
	5		36.73
SW	3		43.11
	4		43.11
OP + SW	3	70 % SW, 30 % OP	41.20
		50 % SW, 50 % OP	39.92
		30 % SW, 70 % OP	38.64

A second set of batch assays was conducted for the anaerobic co-digestion of OP and SW at an initial organic load of 3 gVS L^{-1} with different SW and OP ratios as reported in Table 3.

Toxicity assays were performed in order to verify the inhibitor effect of limonene and acid gallic on thermophilic anaerobic digestion, designed according to Fang et al. [26]. For this scope, 3 g/L and 6 g/L of limonene or acid gallic was added to the batch reactors fed with cellulose (initial organic load of 3 gVS L^{-1}). The presented concentrations are higher than the threshold concentrations reported in Forgács et al. [7] and Disley at al. [13], respectively for limonene and gallic acid 1 and 2.28 g L^{-1}. This test was performed in triplicate.

2.4. CSTR experiments

Four CSTR experiments were performed on co-digestion of MN, OP and SW at different ratios and OLR´s. For the experiments 2 L-CSTR reactors, with a working volume of 1.8 L, were used and were operated at hydraulic retention time (HRT) of 15 days and at thermophilic conditions (55 °C ± 1). The volumetric flow rate of 120 mL d^{-1} was reached in two times per day by means of a peristaltic pump. CSTR reactors as well as the feed bottles were equipped with magnetic stirrers in order to maintain mixing homogenous conditions inside the reactors.

3. Results and discussion

3.1. Characterization of substrates

Table 1 and Table 2 show the experimental characterization for OP and SW as well as for the MN and the inoculum used. Considering that the composition of dry matter of OP is: glucose (14.6 ± 0.4), fructose (15.5 ± 0.5), sucrose (10.9 ± 0.3), pectin (7.9 ± 0.1), cellulose (8.1 ± 0.46), hemicellulose (13.8 ± 0.3), lignin (1 ± 0.01) and ash (1.7 ± 0.1) [27], we can observe that compared to other agricultural wastes, the content of lignin is low, [28]. Moreover, the content of fermentable sugars is high, which makes OP as an attractive feedstock for AD. However, its content of limonene causing potential inhibition to the AD process [29], a challenge which has to be addressed in order to use AD as means of treatment of this waste stream with simultaneous production of biogas. As far as SW is concerned, the composition of *L. digitata,* with 31.6 ± 7.1 % of ash, 70.7 ± 11.6 % of carbohydrates, 6.9 ±1.1 % of proteins and a low content of lipid (% TS) [30], and with absence of lignin content, makes this biomass attractive for biogas production. This without considering the presence of phenolic compound which are highly inhibiting the AD process. In fact, taking into account the experimentally determined quantity of phenolic compound of 27.20 ± 3.34 mg gallic acid equivalents/100 gTS (Table 1) and the antimicrobial activity of them [31] the AD of SW could strongly be inhibited. In addition, the pH value for the examined OP is low (around 4) and when OP is used as feedstock this parameter may be crucial for the overall process [32]; moreover, C/N ratio for both substrates is largely above the AD value recommended of 37 and 45 for OP and SW, respectively (Table1). Put into the foreground all the above considerations in order to reduce the concentration of antimicrobial chemicals such as limonene and phenols while keeping the optimal C/N ratio, co-digestion with cow manure seems to be a feasible alternative.

3.2. Batch reactors

Table 4 compares BMP values with the ThBMP ones evaluated according the equations (1) and (2) in the Section 2.3. For the BMP assays a large amount of inoculum was used, in order to give optimal conditions for the AD. In this way possible toxicants contained in the tested biomasses were brought below the inhibition level. Therefore, inhibition by limonene or phenolics contained in OP and SW respectively would not appear at the tested conditions. In merit to the BMP values found for OP either by equations or by experimental tests, it is important to remark that they are in the same range reported in literature: 450 $mLCH_4$ $g^{-1}VS$ [33]. As far as SW characterization is strongly influenced by seasonal variation, the experimental results of the examined SW- collected during the summer time period- are trustful when compared with samples obtained during the same season. In fact, Tabassum et al. [34] found the highest BMP using samples recovered in August (327 ± 26 $NmLCH_4$ $g^{-1}{}_{VS}$).

Table 4. BMP and ThBMP values for OP and SW.

($NmLCH_4$ g^{-1} VS)	OP	SW	70 % SW 30 % OP	50 % SW 50 % OP	30 % SW 70 % OP
BMP	398.4 ± 9.36	348.6 ± 10.15	354.46 ± 5.26	322.95 ± 7.49	375.23 ± 14.84
ThBMP [19]	424.74 ± 5.13	407.92 ± 6.92	412.7 ± 4.74	415.83 ± 3.82	418.97 ± 3.72
ThBMP [18]	454.58 ± 7.21	428.65 ± 6.90	437.7 ± 4.81	443.5 ± 4.36	449.3 ± 4.98

From Table 4 appears that BMP of OP is higher than that of SW, while in both cases they are lower that the estimated values of the ThBMP ones using equation (1) and (2). Calculation of the theoretical methane potential is based on stoichiometric conversation of organic matter (balanced with water) to CH_4 and CO_2 and is not taking into account that a part of carbon varying between 5-10% is used for microbial biomass formation. Therefore, the theoretical value is always overestimated compared to the practical achievable by BMP tests. Another major reason for discrepancy between theoretical and practical methane potential is of course low biodegradability of the organic matter, or presence of inorganic electron acceptors than organic compounds, taking up the electrons resulting in lower methane production. According to Labatut et al. [35], about 12 % of the total carbon is consumed for cell formation and in case of OP the difference between T_hBMP and BMP is about 12 %, while in case of SW is 18 %. In addition, Table 4 shows the methane yield for OP-SW anaerobic co-digestion at different VS ratio. In this case, a higher concentration of OP in the fermentative broth at the expense of SW is translated into to an increase in the BMP. This goes in the same direction of the results of Cogan and Antizar-Ladislao [15], which performed co-digestion tests of SW and food waste and observed the lowest BMP value when only SW was used as feedstock.

Batch tests focused on the determination of the inhibitory effect of limonene and gallic acid showed lag phase of more of 20 days before any methane production was recorded, while controls without addition of the toxicants did not show any lag phase. After the initial lag phase methane was produced at low rate which reached the same level as in the controls after 70 days of incubation with 3 g L^{-1} gallic acid added, while for the batch reactors with 3 g L^{-1} limonene, only 60 % of the expected methane was produced. Higher concentrations of the two toxicants entirely inhabited the AD process, and no methane was detected after 70 days of incubation.

3.3. CSTR experiments

Codigestion experiements with different mixtures of MN, OP and SW were performed in four CSTRs (R1, R2, R3 and R4) (Table 5). All reactors were operated at HRT of 15 days and each test lasted around 10 times the HRT (the first 3 HRTs are not shown).

Table 5. Experimental conditions used in the CSTR tests and experimental results.

Reactor	Operation	Days	OLR	OP	SW	Diluted	Average methane	Yield increase
			$gVSL^{-1}d^{-1}$	**% VS**	**% VS**	**% VS**	**$NmLCH_4\ g^{-1}VS$**	**%**
1	I	1-15	1.88			100	112 ± 9.68	-
	II	16-45	1.95	3.5		96.5	109.68 ± 1.21	-2
	III	46-74	3.0	37.3		62.7	130.42 ± 2.53	+16.44
	IV	74-100	4.12	37.3	37.3	25.4	207.82 ± 6.78	+85.55
2	I	1-15	1.88			100	112 ± 9.68	-
	II	15-45	2.08	9.6		90.4	148.53 ± 5.05	+32.64
	III	45-100	4.0	53.5		46.5	264.52 ± 5.17	+118.32
3	I	1-15	1.88			100	112 ± 9.68	-
	II	16-45	1.95		3.5	95.6	125.65 ± 0.99	+12.10
	III	46-74	3.0		37.3	62.7	137.20 ± 7.24	+22.5
	IV	74-100	3.56	15.73	31.56	53.6	212.34 ± 9.38	+89.59
4	I	1-15	1.88			100	112 ± 9.68	-
	II	15-45	2.08		9.6	90.4	142.14 ± 1.5	+27.26
	III	45-100	4.0		53.5	46.5	220.53 ± 5.19	+96.9

Figure 1 presents the performance of the four reactors operated in parallel. The reactors performance at different operating conditions was compared with the reactor operated with MN alone at OLR = 1.88 gVS L^{-1} d^{-1} (Table 5).

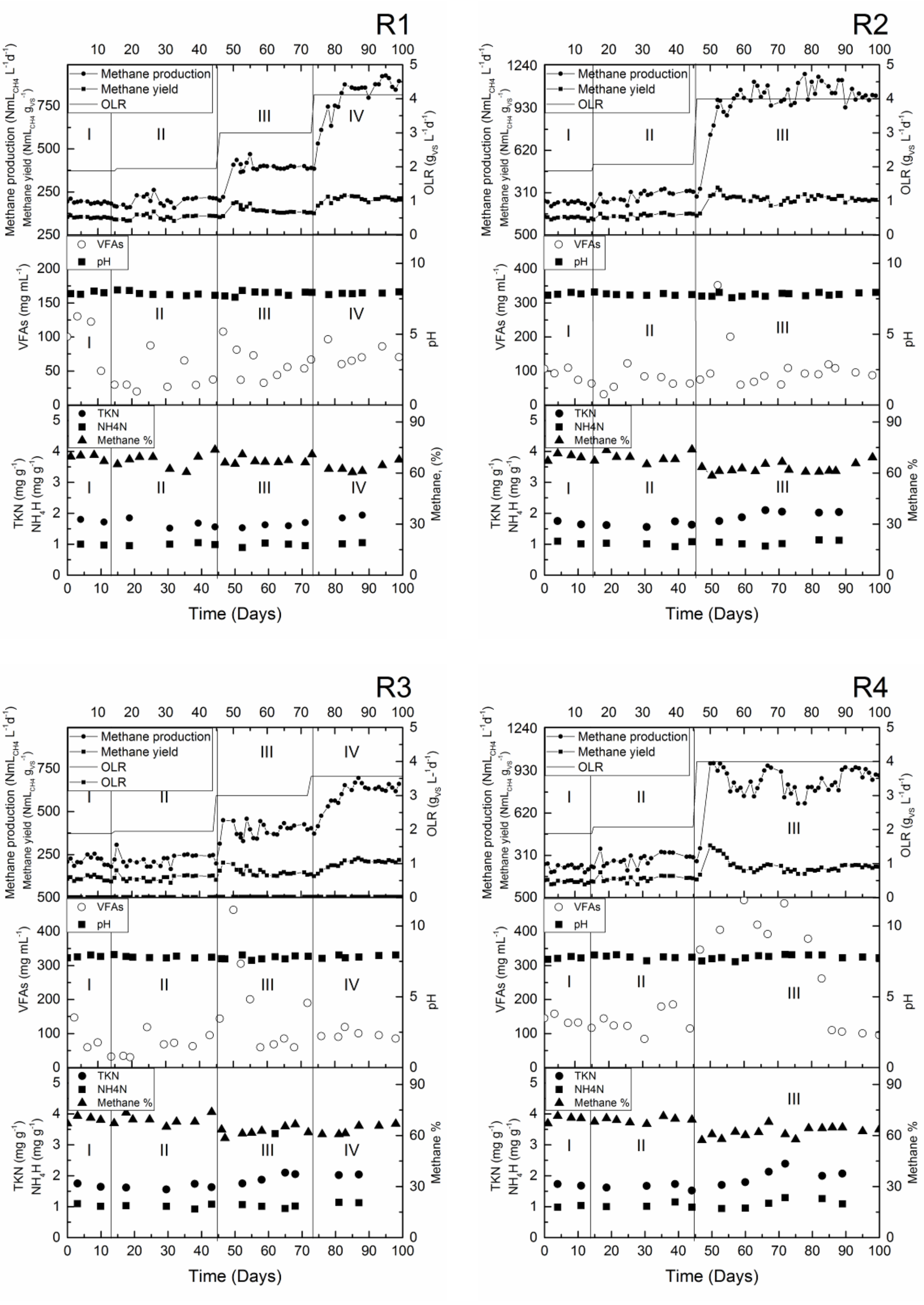

Figure 1. Performance for R1 R2 R3 and R4; a) methane production, methane yield and OLR; b) total VFA and pH; c) TKN, HN_4H and methane percentage.

In phase I all reactors were operated identically OLR = 1.88 gVS L^{-1} d^{-1}, and showed an average methane yield of 112 ± 10 $NmLCH_4$ $g^{-1}VS$. Furthemore, the comparisons reported in Table 5 for the operational phases II, III and IV refer to this above mentioned value. The CSTR experiments highlighted that methane yield of the co-digestion mixtures was significantly higher when compared with the yield achieved by manure alone: hence, the co-digestion ehnaced notably the energetic recovery (Table 5).

For determining the optimal operation conditions in respect to mehtane yield and process stability, different OLRs and mixture ratios were tested. In the first reactor (R1), the OLR was firstly increased from 1.88 to 1.95 and then to 3 gVS L^{-1} d^{-1} by the addition of only OP and consequently to 4.12 gVS L^{-1} d^{-1}, adding the equal quantity of OP and SW (% VS). When the increase of OLR from 1.88 to 1.95 gVS L^{-1} d^{-1} was operated in R1, no yield increase was observed, considering the values of standard deviations on the yields reported in Table 5.

Hence, it is possible to argue that there was no effect on methane yield when OP are added to the manure at low percentage. Instead, in case of operation phases III for R1 and II-III for R2, an addition of OP higher than about 4% in the MN, provoked an increase of the methane yield, confirming the beneficial effect of the co-digestion compared to digestion only with MN. As shown in **Table 6** , when co-digestion of OP with MN is performed, the methane yield obtained in this study is lower when compared to other studies [36], [5], [7] and [37] (AD of OP, AD of treated OP and co-digestion with other substrate, for instance OP with glycerol (1:1 on COD basis) and OP with municipal solid waste (30: 70 on VS basis). However, the less intensive operative conditions (lower HRT and higher OLR) and the avoided use of oil extractive techniques, which require an energetic expenditure, are definitely the advantages of the investigated process. The same behavior was observed in the case of SW addition and the increases in the % of SW in manure were translated into higher methane yields (II and III for R3, II and III for R4 of Table 5).

Table 6. Anaerobic digestion of OP in different conditions.

Organic loading rate (gVS L^{-1} d^{-1})	HRT (d)	Methane yield m^3CH_4 kg^{-1} VS	Pre-treatment	Temp. (°C)	Reactor type	Reference
2.85	32	0.290	Peel oil extraction	37	CSTR	[36]
2.57	25	0.290	Size-reduction & steam distillation	55	CSTR	[5]

3.00	21	0.555	Steam explosion +Co-digestion with municipal solid waste	55	CSTR	[7]
1.91	30	0.330	Co-digestion with glycerol	55	CSTR	[37]
4.00	15	0.266	Co-digestion with manure	55	CSTR	This work

Concerning mixtures of the three substrates MN, OP and SW, only two situations were tested: same quantity of OP and SW (37.3 % VS), and OP as half of SW (15.73 % VS and 31.56 % VS, respectively) in the operational phases IV for R1 and R3. In this case, modest differences were recorded in the methane yield (86 % and 90%, respectively for OP and SW). Figure 2 shows the yield of the OP and SW addition in co-digestion with MN. The yield increased as a result of addition of OP or SW. Clearly (Figure 2) when addition of OP and SW was low, only a modest increase of yield was observed (about 40%), while at higher concentrations the yield increased over 100% compared to yield with only manure.

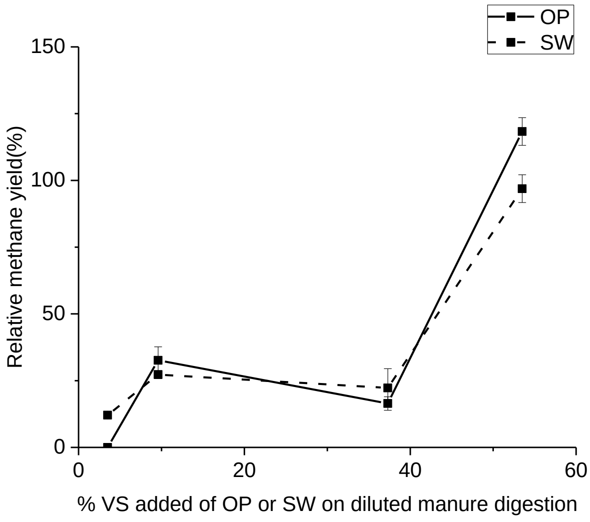

Figure 2. Co-digestion tests under CSTR conditions: enhanced methane yield of MN plus either OP or SW at different concentrations.

The high increase could be explained by the higher individual yield of OP and SW, but also, probably due to beneficial effect linked to the buffering capacity and content of several important nutrients and minerals in manure [38]. In fact, as reported in Figure 1 for all the tested situations, the VFA concentrations remained constant, probably for the presence of an adequate microbial population able to remove hydrogen and other intermediates [39]. For this reason, the co-digestion of OP, SW and MN can favor the microbial dynamics, and, consequently, the activity of methanogens for the last step of methanogenesis [40]. This hypothesis needs to be verified experimentally by counting the different bacteria species by advanced molecular techniques not covered by this article.

4. Conclusion

OP and SW have high content of organic matter which can be used for biogas production. As the difference of the ThBMP values for both OP and SW were only slightly higher than experimentally determined BMP, we could conclude that the organic content of these biomasses is easily biodegradable. Co-digestion of OP and SW with manure resulted in high methane yields and stable AD process probably due to the buffering capacity and dilution effects offered by the manure. The results showed that OP or SW can be successfully co-digested with manure until an organic loading rate of 4 $gVSL^{-1}d^{-1}$ at HRT of 15 days, obtaining the maximum methane yield of 266.7 and 218.5 $mLCH_4\,g^{-1}VS$, respectively. Content of toxic compounds in OP and SW could inhibit the AD process as shown in batch assays. However, no inhibition was noticed for OP and SW, at the applied mixtures with cattle manure in continuous experiments. Furthermore, continuous anaerobic co-digestion of MN, OP and SW follows the same trend noticed in batch assays, where the highest percentage of OP (in terms of VS) at the expenses of SW at equal organic load had a positive effect on the methane yield.

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Pretreatment of macroalgal species native to Northern Europe conditions for enhanced biogas production

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Abstract

Three macroalgae species harvested in Denmark, Ulva lactuca (UL), Laminaria digitata (LD) and Saccharina latissima (SL) were anaerobically digested for biogas production and the effect of various pretreatments on biodegradability was investigated. Biological methane assays showed that pretreatments applied to UL increased the final methane yield as follows: washed/dried/grounded-UL2 by 48 %, alkaline-UL3 by 22 %, thermochemical acid-UL5 by 37 %, soaked in water-UL6 by 8% and enzymatic-UL7 by 59 %. Pretreatments applied to LD had a negative effect on the biodegradability and lower ultimate methane potentials were achieved. The only exception was the liquid fraction (LD5) whose ultimate methane yield was increased by 24 %. SL pretreated fractions presented inhibition mainly caused by the presence of soluble polyphenols compounds which caused a delay of 20 days at least in the methane production. Silage pretreatment did not cause inhibition and improved greatly the biodegradability of SL suppressing the lag phase.

Keywords: Anaerobic digestion, Methane, Macroalgae, Bioenergy, Polyphenol

1 Introduction

Gaseous biofuels, particularly biogas - which is generally composed of 50-75% CH_4 and 25-50% CO_2 - are important renewable energy source for combined heat and power (CHP) generation. Moreover, their role as transport fuel is also expected to increase in the coming years (Holm-Nielsen et al., 2009). Biogas can be produced through anaerobic digestion of organic materials such as wood and their wood wastes, energy crops, aquatic plants (macro, microalgae), agricultural crops and their waste by-products, animal wastes and certain fractions of municipal wastes. Among these, macroalgae biomass offers the advantage that during their production the use of arable land and fertilizers is avoided compared for instance to energy crops, thereby minimizing competition over land, food and feed production. Moreover, macroalgae are often washed out in coasts; this decreases the appeal of the coasts as recreation areas and constitutes a burden to the environment besides. Therefore, many municipalities have implemented programs for collecting and disposing this cast seaweed biomass. Consequently, use of this biomass for biogas production is relevant as a sustainable gaseous biofuel.

Several studies have been performed in different species of marine macroalgae assessing their methane potential (**Table 1**). The research focused on the production and conversion efficiencies to investigate, whether this biomass can be competitive to the conventional non-renewable energy sources. Early studies concluded that macroalgae are a suitable substrate for biogas production (Srivastava et al., 1988). Nevertheless, C:N and C:S ratios must be considered as preliminary criteria to assess a specific substrate for biogas production. For an optimal performance of anaerobic digestion process, C:N ratio should be in the range 20:1–30:1; whilst a substrate with a C:S ratio below 40:1 will tend to accumulate H_2S gas as experienced by macroalgae digestion trials (Allen et al., 2015, 2013).

Pretreatments in most of the cases can improve the methane yields either they are physical or physicochemical or combination of both. When *Saccharina latissima* underwent steam explosion pretreatment (130 °C/10 min) higher methane yields (285 mL g VS^{-1}) were achieved (Vivekanand et al., 2012). Mechanical and thermal pretreatments were also performed in *Ulva lactuca*. Mechanical pretreatment had a positive effect in the biodegradability of *U. lactuca*, thereby increasing the methane yield, while thermal pretreatment (110 °C/20 min) resulted in a decrease (Bruhn et al., 2011). Although several reports on effects of pretreatment have been published the recent years, it is still unclear how pretreatment affects macroalgal biodegradability, as the experimental conditions were unclear and some results have been so far contradictory. Even the same pretreatment had different effect in the biodegradability of same macroalgae species.

Hence, considering the benefits gained in methane production from marine macroalgae (Chynoweth, 2005), it is worthwhile to investigate the effect of different types of pretreatments or combination of them in the biodegradability of this particular biomass. Therefore, the aim in the actual study was to assess the effect of different types of pretreatments (such as, size reduction, alkali-peroxide, thermo-chemical, ensiling and enzymatic) in the ultimate methane potential of three genera of macroalgae – *Laminaria digitata* (brown algae), *Saccharina latissima* (brown algae) and *Ulva lactuca* (green algae) – which are growing in North Sea European conditions.

Table 1. Overviwe of pretreatment studies on the selected macroalgae species

	Pretreatment	Conditions	mLCH$_4$ g^{-1}VS	Source
Laminaria digitata	Raw and chopped	53 ± 1 °C, min 30 days	359 ± 37[a]	This study
	Washed		261 ± 14	
	Dried and milled		289 ± 8	
	Screwpressed pulp		282 ± 15	
	Screwpressed liquid		445 ± 24	
	Macerated	37 °C, 30 days	218	Allen et al. (2015)
	Dried and milled	35 °C, 36 days	196-254	Adams et al. (2011)
	-	-	260-280	Chynoweth (2005)
Sac-cha-	Raw and chopped	53 ± 1 °C, min 30 days	220 ± 16	This study
	Screwpressed pulp		227 ± 13	

	Chopped silage		323 ±13	
	Chopped silage		258 ± 5	
	Dried		168 ± 3	
	Untreated	37 °C, 119 days	223	Vivekanand et al. (2012)
	Ground, SE[b], 130°C/10 min		268	
	Ground, SE, 160°C/10 min		260	
	Co-digestion (wheat straw)		270	
	Washed chopped	55 °C, 34 days	340 ± 48	Nielsen and Heiske (2011)
	Washed, macerated		333 ± 64	
	Milled	35 °C, 32-35 days	25-200	Østgaard et al. (1993)
		35 °C, 34-40 days	220-270[c]	
	Chopped	36.5 °C, 30 days	230	Hanssen et al. (1987)
		35 °C, 24 days	230[c]	
Ulva lactuca	Fresh and chopped	53 ± 1 °C, min 30 days	185 ± 4	This study
	Washed, dried and grounded		274 ± 28	
	Alkaline		226 ± 9	
	121 °C/20 min		147 ± 8	
	HCL + 121 °C/20 min		254 ± 22	
	Water + 25 °C/24 h		199 ± 9	
	Enzymatic hydrolysis		295 ± 1	
	Macerated	37 °C, 30 days	190.1	Allen et al. (2015)
	Fresh		183.2	
	Wilted and unwashed		165	
	Washed, macerated and dried/80 °C	37 °C, 30 days	250.2	Allen et al. (2013)
	Washed and wilted		221.1	
	Fresh		205	
	Dried		226	
	Unwashed, roughly chopped	55 °C, 42 days	174	Bruhn et al. (2011)
	Unwashed, macerated		271	
	Washed, roughly chopped		171	
	Washed, macerated		200	
	Washed, 110 °C/20 min		157	
	Washed, 130 °C/20 min		187	
	Dried, ground		176	
	Unwashed, roughly chopped	37 °C, 58 days	162	

	Washed chopped	55 °C, 34 days	152 ± 19	Nielsen and Heiske (2011)
	Washed, macerated		255 ± 48	
Ulva sp.	Screw pressed (hydrolysate juice)	35 °C, 10 days	340^d	Morand et al. (2006)
	Non-washed	35 °C, 23 days	110	Briand and Morand (1997)
	Washed	35 °C, 44 days	94	
	Non ground	35 °C, 42 days	145	
	Ground	35 °C, 64 days	177	
	Ground	35 °C, 15 days	203^e	

2 Materials and Methods

2.1 Chemicals and gases

All chemicals and enzymes used in this study were of analytical grade and were purchased from Sigma Aldrich ApS (Brøndby, Denmark) and gases were supplied by AGA A/S (Copenhagen, Denmark).

2.2 Preparation of macroalgae substrates

Ulva lactuca samples were collected in mid-August 2011 at Skive, Denmark and exposed to different types of pretreatments. Basically mild pretreatments were chosen as the algal biomass is not containing lignin which is often the reason of the high recalcitrance of land based biofibers. Moreover, mild pretreatment conditions would have the advantage of lower costs, and lower potential of formation toxicants such as furfurals and hydroxymethylfurfural (HMF).

In pretreatment 1 (UL1), –size reduction –the samples of unwashed fresh *U. lactuca* (directly sampled from the shoreline) were chopped in small pieces (< 2 mm) with scissors to enlarge the functional surface. Pretreatment 2 (UL2) –washed, dried and grounded –consisted of washing and later drying the fresh *U. lactuca* macroalgae at 80 °C until moisture content was < 10 %. Afterwards the dried macroalgae was ground into powder using a Siebtechnik Screening disc mill TS 250. In pretreatment 3 (UL3) –alkaline peroxide pretreatment –the fresh biomass was mixed with 0.5 g of Mg_2SO_4 (1 g L^{-1}) and 2.5 mL H_2O_2 (1% v v^{-1}) and incubated in shaker at 50 °C and 120 rpm for 24 hours. Before incubation, the pH was adjusted to 11.5 using 0.5 M NaOH solution. Pretreatment 4 (UL4) –thermal pretreatment –consisted of exposing the fresh chopped macroalgae to thermal treatment at 120 °C for 20 minutes. In pretreatment 5 (UL5) –acidic thermal pretreatment –the fresh macroalgae was treated as in pretreatment 4. Before the fresh macroalgae was exposed to thermal treatment (120 °C for 20 min) the pH was adjusted to 1.0 using 0.2 M HCL solution. In pretreatment 6 (UL6), –pre-soaked thermal pretreatment –the chopped fresh macroalgae was soaked in water and incubated in shaker at 25 °C and 120 rpm for 24 hours. In pretreatment 7 (UL7) –enzymatic pretreatment –the fresh chopped *U. lactuca* was treated with an enzyme blend (3 mL of Celluclast 1.5 L (cellulase) and 1.5 mL of cellobiase) and incubated in shaker at 50 ºC and 120 rpm for 24 hours.

Macroalgae *L. digitata* was harvested during early-August 2012 at Hamborg Strand, north of Hanstholm at the Danish North Sea coast. The different pretreated fractions of *Laminaria digitata* were stored at -20 °C until analysis and experiments were performed. *L. digitata* pretreated fractions consisted of the following qualities: untreated raw (LD1), washed (LD2), dried (LD3), screwpressed pulp (LD4) and screwpressed liquid (LD5).

A second batch of macroalgae material consisted of different pretreated fractions of *Saccharina latissima*. Samples of *S. latissima* were harvested in May 2013 and received the 11th June, 2013. The delivered material fractions were stored at -20 °C prior to further use. *S. latissima* material was pretreated to the following fractions: raw chopped (SL1), screwpressed pulp (SL2), chopped silage added wheat bran (SL3), chopped silage (SL4), dried (SL5) and screwpressed liquid (SL6).

2.3 Set up of biomethane potential (BMP) assay

Biomethane potential (BMP) assay was conducted according to .Angelidaki et al. (2009) in 320 mL serum glass vessels (batch reactors). Thermophilic (53 ± 1 ºC) methanogenic inoculum which was digested manure, was collected from Hashøj centralized biogas plant in Denmark, and was used (80 mL) in the batch reactors. The inoculum was allowed to degas for seven days in an incubator prior to use. Batch reactors were filled with the degassed inoculum and the samples to be tested were added. Two different concentrations of the pretreated biomass fractions (2 and 4 g VS L^{-1}, respectively) were tested and water was added up to final working volume of 100 mL. Batch reactor containing avicel® PH-101 cellulose (Sigma Aldrich) as substrate, was used (2 g VS L^{-1}) as positive control to validate the accuracy of the BMP assay process. Batch reactors only with inoculum and water (blanks) were included to determine the residual methane production from the inoculum. Finally, the batch reactors were flushed with a N_2/CO_2 (80/20 % v v^{-1}) gas mixture, closed with rubber stoppers and aluminium caps, and incubated for a minimum of 30 days. All BMP assays were performed at least in triplicates.

2.4 Preparation macroalgal biomass for analysis of phenols

Preparation of macroalgal extracts was performed according to Wang et al. (2012) with some modifications. Freeze-dried and grounded macroalgae aliquots (400 mg dry weight) were extracted with 10 mL of 70 % aqueous acetone solution. Samples were incubated in a platform shaker for 24 h at 200 rpm and at room temperature in the darkness. Afterwards, samples were centrifuged for 10 min at 3500 g at 4 °C. The extract phase was collected in a separate tube and solvent was removed in the fume hood. After solvent removal, both macroalgal extract and solid residue were freeze-dried and weighted, respectively. Solid residue was stored at -80 C and the freeze-dried macroalgal extract was redissolved in distilled water to yield a concentration of 5 mg mL^{-1}. This stock solution was used for determination of total phenolic content (TPC).

2.5 Analytical methods

Total solids (TS) or dry matter (DM), volatile solids (VS), ash contents and total Kjeldahl nitrogen were determined as described in Standard Methods (APHA, 2005). Elemental analysis (C, H, N, and S) was performed in a vario MACRO cube (Elementar Analysensysteme GmbH, Germany). Total sugar determination was performed according to the NREL protocol with slight modifications for determination of structural carbohydrates in biomass (Sluiter et al., 2008). Soluble sugars were all detected and quantified using high performance liquid chromatography, HPLC. The HPLC (Agilent) had a refractive index detector (detection of sugars, VFAs and ethanol) and a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm) with 0.04 M H_2SO_4 as eluent and flow rate was set to 0.6 mL min^{-1} with column oven temperature set to 63.5 ºC. Methane production in the headspace of batch reactors was determined using a gas-chromatograph (GC Shimadzu 14A, Shimadzu, Kyoto, Japan) (Fotidis et al., 2013). Determination of Total phenolic content (TPC) of the macroalgal extracts was performed following the procedure described by Wang et al. (2012) with slight modifications. An aliquot of macroalgal extract (0.5 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10 diluted) in a test tube. After 5 min, 2 mL of sodium carbonate (7.5% w v^{-1}) were added to each tube, the test tubes were cap-screwed and vortexed for 1 min. Following this, test tubes were incubated for 2 h at room temperature in the darkness and centrifuged for 10 min at 1600 g. Then, absorbance was measured at 725 nm in a Jenway 6405 UV/VIS spectrophotometer. A standard calibration curve with gallic acid solutions (ranging from 20-100 μg mL^{-1}) was plotted and used for quantification of total phenolics and the results were expressed as gallic acid equivalents.

2.6 Statistical analysis

An ANOVA analysis followed by Fisher's Least Significant Difference test (LSD, $p < 0.05$) was used to evaluate if any significant differences were observed in experimental measurements. All statistical analyses were carried out using OriginPro 9.0.0 SR2 software (OriginLab Corporation, USA).

3 Results and Discussion

3.1 Macroalgae characterization

TS/VS content, C/N and C/S ratios

Differences in dry weight or total solids (TS) content determined in raw macroalgae fractions of *Laminaria digitata* (LD1), *Saccharina latissima* (SL1) and *Ulva lactuca* (UL1) were found to be significant ($p < 0.05$) from each other. The highest TS content was found in *L. digitata* (LD1) followed by *U. lactuca* (UL1), and finally *S. latissima* (SL1). Differences in volatile solids (VS) and ash content were observed only to be significant ($p < 0.05$) between *S. latissima* (SL1) and *L. digitata* (LD1) and between *U. lactuca* (UL1) and *L. digitata* (LD1); whilst no significant differences ($p > 0.05$) were observed between *U. lactuca* (UL1) and *S. latissima* (SL1) fractions as reported in **Table 2**.

As was expected for macroalgae species of the same type (brown), *L. digitata* fraction (LD1) harvested in August exhibited lower ash content (higher VS content) compared to *S. latissima* (SL1) harvested in May. This is in agreement to what has been documented in literature with respect to seasonal variations in VS and ash content for these species. The highest ash (lowest VS content) content for *L. digitata* and *S. latissima* occurs during winter and spring (February to June) followed by a decline into the autumn months (Schiener et al., 2014). Nevertheless *U. lactuca* (UL1) fraction had a lower VS content compared to *L. digitata* (LD1), thereby resulting in high ash content as reported in Table 2.

Taking as benchmark the LD1 –fraction sampled directly from field – the effect of the different pretreatments in TS and VS content was compared. As result of the washed pretreatment a decrease in TS content of 19 % was observed in LD2 (washed fraction) whilst no differences were observed in the VS content, as apparently salt and other inorganics were washed away. Screwpressed pretreatment resulted in a decrease in TS content of 19 and 78 % for LD4 (screwpressed pulp) and LD5 (screwpressed liquid) fractions, respectively. A decrease in VS of 9 % was observed in LD5 fraction; whilst for LD4 fraction no significant difference was observed. This decrease observed in TS and VS content was mainly attributed to the removal of sand and debris. Conversely as expected, increase in TS and VS content in LD3 fraction (dried macroalgae) as result of moisture removal, was found to be significant in comparison to TS and VS content in LD1 fraction (raw macroalgae). Overall, one-way ANOVA and pairwise comparison of TS and VS content highlighted the significant differences in TS and VS content among all pretreated fractions with the exception of LD2 (washed) and LD4 (screwpressed liquid) fractions where differences in TS and VS content were found not to be significant ($p > 0.05$) from each other.

Screwpressed pretreatment in *Saccharina latissima* resulted in a 46 % increase in TS content for SL2 (screwpressed pulp) fraction compared to the TS content in SL1 fraction; at the same time, the TS content in SL6 fraction (screwpressed liquid) was found not to be significantly different to TS content in SL1 fraction. As result of the different silage pretreatments a decrease in TS content of 41 % was observed in SL3 (chopped silage wheat bran added fraction) whilst on the contrary, TS content in SL4 (chopped silage fraction) was increased by 45 % when compared to SL1 (raw chopped) fraction. Increase in TS content in SL5 fraction (dried macroalgae) as result of moisture removal, was found to be significant in comparison to TS content in SL1 fraction (raw chopped macroalgae). As regards ash content only significant differences were observed for fractions SL3, SL4, SL5 and SL6 where compared to SL1. Only exception was SL2 for which ash content was found not to be significantly different compared to SL1. Effect on VS content followed the same statistically trend as ash content.

C:N ratio values for all fractions of *S. latissima* were far below 20:1 which reflects an imbalance between carbon and nitrogen requirements for the anaerobic micro-flora. This can result in increased levels of ammonia in the reactor which can eventually lead to process failure (Allen et al., 2013; Nielsen and Angelidaki, 2008). All *L. digitata* fractions exhibited a C:S ratio superior than the minimum recommended (40:1); contrary to this, C:S ratio for all *S. latissima* fractions were below than the minimum ratio, thereby expecting to have large accumulation of H_2S in the biogas.

Table 2. TS,VS, ash and elemental characterization of the different selected macroalgae species

Macroalgae fractions	% TS ± SD[a]	% VS ± SD	% Ash ± SD	% C ± SD	% N ± SD	% S ± SD
UL1	22.9 ± 0.5	66.9 ± 0.8	33.1 ± 0.8	n.d.[b]	2.0 ± 0.0	n.d.
LD1	34.0 ± 2.7	90.2 ± 1.7	9.8 ± 1.7	36.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.0
LD2	28.3 ± 0.1	91.3 ± 0.8	8.7 ± 0.8	36.9 ± 0.1	0.6 ± 0.0	0.5 ± 0.0
LD3	91.1 ± 0.3	90.7 ± 0.6	9.3 ± 0.6	38.3 ± 0.2	0.7 ± 0.0	0.5 ± 0.1
LD4	27.7 ± 2.2	92.2 ± 0.7	7.8 ± 0.7	36.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.2
LD5	7.5 ± 0.8	81.7 ± 1.9	18.3 ±1.9	n.d.	n.d.	n.d.
SL1	10.1 ± 1.0	65.3 ± 0.5	34.7 ± 0.5	29.2 ± 0.5	4.4 ± 0.1	0.8 ± 0.1
SL2	14.7 ± 0.6	66.5 ± 0.6	33.5 ± 0.6	28.3 ± 0.1	5.0 ± 0.0	1.1 ± 0.4
SL3	6.0 ± 0.2	59.4 ± 1.2	40.6 ± 1.2	32.6 ± 0.1	3.4 ± 0.0	0.9 ± 0.2
SL4	14.7 ± 0.3	70.2 ± 0.3	29.8 ± 1.1	31.0 ± 0.1	3.6 ± 0.0	0.8 ± 0.0
SL5	98.0 ± 0.1	62.4 ± 1.3	37.6 ± 1.3	n.d.	n.d.	n.d.
SL6	10.3 ± 0.5	46.8 ± 1.5	53.2 ± 1.5	n.d.	n.d.	n.d.

[a]Standard deviation; [b]No determined

3.2 Biomethane potential of pretreated macroalgae fractions and effect of pretreatment

Ulva lactuca

As shown in Fig. 1, a positive effect in the ultimate methane potential was observed when fresh biomass was exposed to dried and ground pretreatments (UL2). This resulted in an increase of the methane yield of 48 % (from 185 ± 4 to 274 ± 28 $NmLCH_4$ $g^{-1}VS_{added}$) in the ultimate methane potential. Similarly, Allen et al. (2013) reported a boost of 36 % (from 183.2 to 250.2 $NmLCH_4$ g^{-1} VS_{added}) in the final methane yield when untreated fresh *U. lactuca* was washed, macerated and further dried at 80 °C. However, it has been reported in some other studies a minor and/or opposite effect of this pretreatment in the biodegradability of *U. lactuca*. Bruhn et al. (2011) reported a negligible increase (from 174 to 176 $NmLCH_4$ g^{-1} VS_{added}) in the ultimate methane yield when unwashed and roughly chopped macroalgae *U. lactuca* was dried at 45 °C and subsequently grounded (< 1 mm).

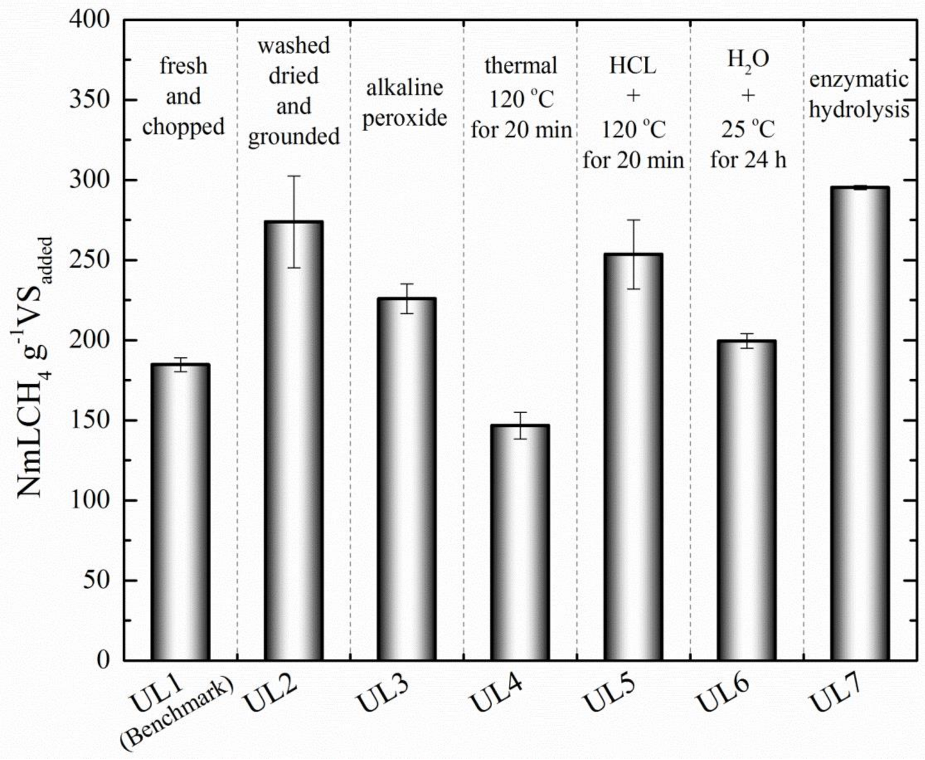

Fig. 1. Effect of pretreatment in the ultimate methane potential of macroalgae *Ulva lactuca*

A more modest increase of 22 % (from 185 ± 4 to 226 ± 9 NmLCH$_4$ g^{-1}VS$_{added}$) was observed in the ultimate methane yield when alkaline peroxide pretreatment (UL3) was applied.

As observed in Fig. 1, thermal pretreatment at 121 °C for 20 min (UL4) had a negative effect in the biodegradability of *U. lactuca*, resulting in a significant decrease of 20 % (from 185 ± 4 to 147 ± 8 NmLCH$_4$ g^{-1} VS$_{added}$) in the ultimate methane potential. Bruhn et al. (2011) also reported a decrease of 9.8 % in the methane yield when *U. lactuca* was exposed to 110 °C for 20 min.

However it is interesting to observe that addition of an acid catalyst (HCL) –pretreatment UL5 –did shift the effect of the thermal pretreatment in the final methane potential. This effect resulted in a significant increase of 37 % (from 185 ± 4 to 254 ± 22 NmLCH$_4$ g^{-1} VS$_{added}$) in the ultimate methane yield. Pretreatment UL6 (soaked in water at 25 °C and 120 rpm for 24 h) had slightly positive effect on the ultimate methane yield resulting in an increase of 8 % (from 185 ± 4 to 199 ± 9 NmLCH$_4$ g^{-1} VS$_{added}$). Finally, enzymatic pretreatment UL7 had a positive effect on the methane potential which increased by 59 % (from 185 ± 4 to 295 ± 1 NmLCH$_4$ g^{-1} VS$_{added}$).

Laminaria digitata

All pretreated *Laminaria digitata* fractions from different pretreatments exhibited a decrease in the ultimate methane potential when compared to the one achieved by LD1 (raw fraction), with the exception of LD5 fraction (screwpressed liquid). For instance, by washing *L. digitata* (LD2 pretreatment) a decrease of 27 % (from 359 ± 37 to 261 ± 14 NmLCH_4 $g^{-1}VS_{added}$) was observed in the final methane yield. It is evident that the washed pretreatment not only removes sand and other debris, but it also washes out some carbohydrates such as laminarin and mannitol.

Drying and grounding of the *L. digitata* (LD3 fraction) had a negative effect, thereby resulting in 19 % decreased (from 359 ± 37 to 289 ± 8 NmLCH_4 $g^{-1}VS_{added}$) in the final methane yield as depicted Fig. 2a. On the other hand, ultimate methane potential of LD4 fraction (pulp fraction from screwpressed pretreatment) was found to be 21 % (from 359 ± 37 to 282 ± 15 NmLCH_4 $g^{-1}VS_{added}$) lower than the raw macrolagae (LD1 fraction). This decreased is explained by the loss of some soluble carbohydrates into the liquid fraction (LD5) due to the action of the screwpressed pretreatment. As mentioned. the only exception was the LD5 fraction (screwpressed liquid) whose ultimate methane potential was higher than LD1 fraction and higher than the maximum methane yield achieved by the control (345 ± 13 NmLCH_4 g^{-1} VS_{added}). This increase in the ultimate methane potential is explained by the fact that in the screwpressed liquid fraction high concentrations levels of glucose (main product from the hydrolysis of the linear polysaccharide laminarin) and mannitol were present. A soluble sugar determination revealed a glucose concentration of 59.7 g L^{-1} and a mannitol concentration of 9.85 g L^{-1} in the liquid fraction.

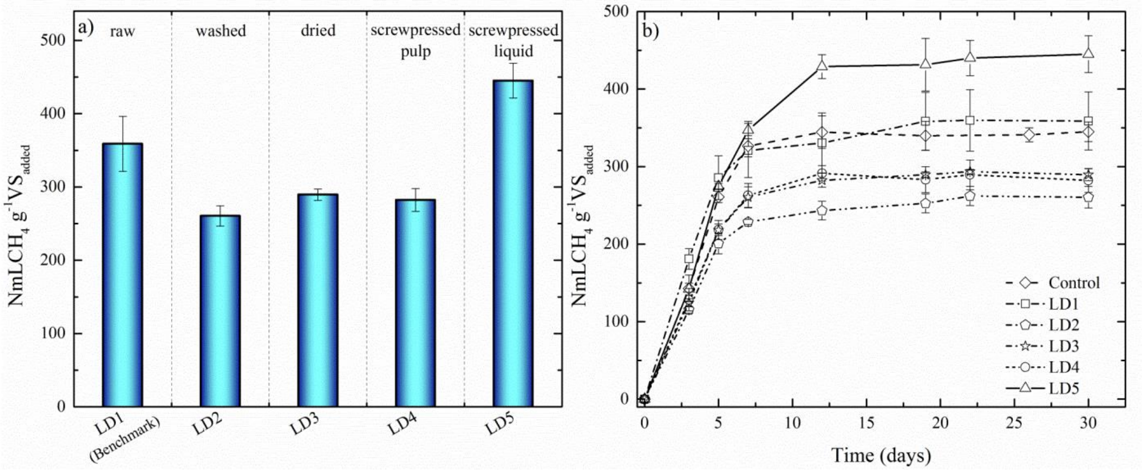

Fig. 2. a) Effect of pretreatment in the ultimate methane potential, b) Methane profiles for different fraction of macroalgae *Laminaria digitata*

Saccharina latissima

Saccharina latissima fractions presented inhibition during anaerobic digestion process as depicted in Fig. 3. Fig. 3c shows that methane production was initiated after a long lag-phase period ranging from 20-35 days.

A possible explanation of this lag phase observed for *S. latissima* fractions, probably might be due to polyphenolic compounds inhibited the methanogenesis. Experimental evidence of this lag phase has been reported before for *Laminaria hyperborea* and *Ascophyllum nodosum* where the dominant factor for the conversion of these macroalgae species during anaerobic digestion was the inhibitory effect of the polyphenols on alginate lyases activity and methanogens. Alginate lyases are required to perform depolymerization of the alginate molecule into its monomers. On the other hand, polyphenols are known to be potent inhibitors of methanogenesis (Moen et al., 1997a, 1997b).

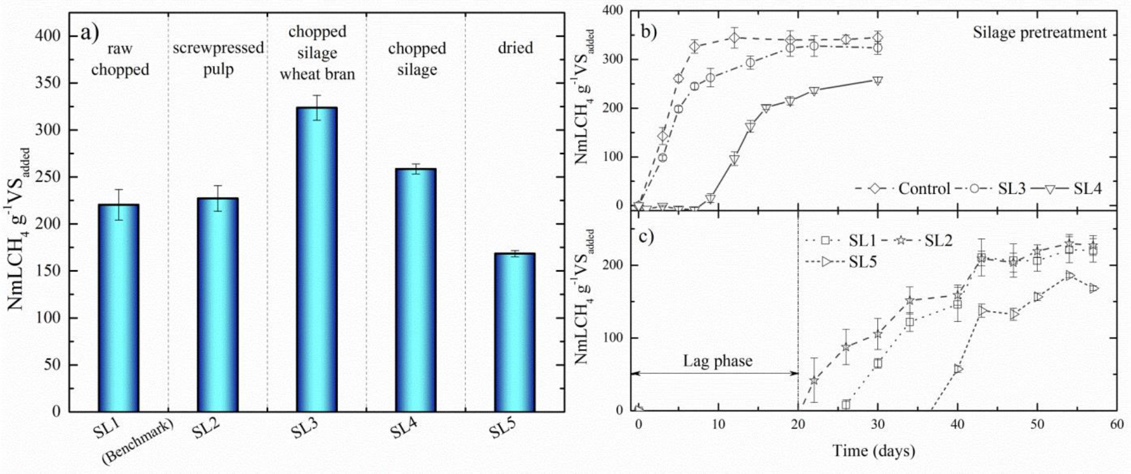

Fig. 3. a) Effect of pretreatment in the ultimate potential, b) methane profiles of silage pretreatments, c) lag phase exhibited by SL1, SL2 and SL5 *Saccharina latissima* macroalgae fractions

Determination of total phenolic content (TPC) of LD1 (*L. digitata* raw) and SL1 (*S. latissima* raw) fractions revealed a much higher content of polyphenols in *S. latissima* (17.7 ± 0.33 mgGA-eq 100 g^{-1}DM) than in *L. digitata* (2.6 ± 0.09 mgGA-eq 100 g^{-1}DM), thereby reducing the biodegradability of *S. latissima*. For an organic loading of 4 g VS L^{-1}, this correspond to have a soluble polyphenol concentration ranging from 0.03 ± 0.02 g L^{-1} for *L. digitata* to 1.07 ± 0.00 g L^{-1} for *S, latissima*. These values are comparable to what has been reported in literature for inhibitory levels of soluble polyphenols (0.14-1.2 g L^{-1}) in anaerobic digestion of *A. nodosum* (Moen et al., 1997b). Besides polyphenolic compounds affecting the biodegradability of the macroalgae, inhibition may occur due to high concentrations of substances such as heavy metals, sulphides, salts and volatile acids. This can be explained by looking at seasonal variation in the chemical composition of *S. latissima*. The highest content of polyphenols in *S.*

latissima is observed to occur during May-July (Schiener et al., 2014). Variation in ash content exhibits an opposite trend to VS content, being highest in early spring and winter (low carbohydrate content) and lowest in the summer (where the carbohydrate content peaks). Furthermore, the assimilations products, mannitol and laminaran show a variation opposite to that of ash and alginate content. Contents of ash and alginate are higher in the first (January-June) than in the second half of the year. Therefore, is most likely expected to have a low biodegradability due to high polyphenols content, high recalcitrant fraction (alginate), high ash and low carbohydrate contents (mannitol and laminarin).

Also as result of the low C:S ratio exhibited by *S. latissima* fractions high concentrations of hydrogen sulphide are expected to be present in the biogas thereby inhibiting the anaerobic digestion process.

Fig. 3b shows the effect of the silage pretreatment in the methane production. SL3 pretreatment (chopped silage added wheat bran) had a positive effect in the biodegradability of *S. latissima* reaching a methane yield as high as the control (323 ± 13 $NmLCH_4\ g^{-1}\ VS_{added}$).

In addition, this pretreatment reduces the lag phase exhibited in the other *S. latissima* fractions as shown in Fig. 3c. A lag phase of 7 days was observed for the chopped silage fraction (SL4), which may be explained as the time needed for adaptation of the inoculum to the substrate. After this lag phase, methane production started taking place and after 30 days the SL4 fraction reached a methane yield of 258 ± 5 $NmLCH_4\ g^{-1}VS_{added}$. For SL6 fraction, (screw-pressed liquid) methane production did not even take place after 60 days (data not shown), concluding that methanogens community was completely inhibited. Finally, compared to other studies, methane yields for *S. latissima* obtained in this work are in the range to what has been reported before (230–340 $NmLCH_4\ g^{-1}\ VS_{added}$ (Nielsen and Heiske, 2011; Vivekanand et al., 2012)).

4 Conclusions

In the actual study, three genera of macroalgae suitable to grow in Northern Europe climate conditions were anaerobically digested for methane production and the effect of various pretreatments on biodegradability was investigated. Based on the results obtained in this study, the following conclusions can be drawn:

- Dried and grounded, thermal with acid catalyst and enzymatic pretreatments were suitable for macroalgae *Ulva lactuca*. However, from an economical point of view dried and enzymatic pretreatment have a limitation for further implementation due to the high cost associated to energy provision and enzymes, respectively. Therefore a promising pretreatment option could be alkaline pretreatment.

- Ultimate methane yield reached by the *Laminaria digitata* pretreated fractions was lower compared to untreated fraction, thereby suggesting that no pretreatment is required for further methane production. No inhibition effects were observed during anaerobic digestion batch experiments.
- *Saccharina latissima* fractions underwent inhibition during anaerobic digestion batch experiments. This was due to the high concentration of polyphenols present in the macroalgae and the poor biodegradability related to high concentrations of recalcitrant compounds (alginate) as well as ash content.
- Silage pretreatment overcome inhibition and enhances greatly the biodegradability of *Saccharina latissima*.

Acknowledgements

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Technical University of Denmark

Avraam Symeonidis
S141619

LCI formulation of a biogas plant.

Special Project, December 2016

DTU Environment
Department of Environmental Engineering

A depth of flavor is what I covet.

T.K.

A true master is an eternal student.

M.Y.

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Introduction

Life Cycle Assessment (LCA) is a structured, comprehensive and internationally standardised method. It quantifies all relevant emissions and resources consumed and the related environmental and health impacts and resource depletion issues that are associated with any goods or services ("products") (EC, 2010a).

LCA is greatly used in waste management systems in order to identify the environmental impacts of the different management techniques. (Laurent et al., 2014a). In addition the application of it can provide a robust solution at assessing and quantifying the environmental impacts of different waste management techniques (Laurent et al., 2014b).

Aim of this study is to use the LCA methodology in order to evaluate the full life cycle of the energy produced in an anaerobic digestion plant dedicated for co-digestion of casted seaweed (CS) and manure.

In particular, this report is focused on the collection of the data related to the processes performed in the aforementioned anaerobic digestion plant. This process is the second phase (ISO, 2006, EC, 2010b) of a complete life cycle assessment in accordance with the more recent framework (EC, 2010a). The data (Inventory Analysis) will be later used to model the operation of the biogas plant and consequently estimate the environmental burden or/and possible benefits.

The initial idea was born when the problem of CS begun to be disturbing for the local communities of the southern Copenhagen area. Solrød municipality eventually proceed at collecting and treating the CS waste in an anaerobic treatment plant. This decision is in accordance with the sustainability that is promoted by the public agenda (EC, 2011). Co-digestion with manure was chosen to be the waste management technique in order to mitigate the problems associated with the CS accumulation. This report is forming the LCI of the processes currently in operation as well as exploring the alternative waste management techniques in order to create a prismatic view of all the options and possibly compare the environmental burden or benefits.

Since CS is considered as waste, the functional unit could be 1 tone of wet macro algae. This should be the reference among all the scenarios tested.

Ultimately the assessment of the overall environmental impacts of different management techniques (scenarios) for CS will be performed in the LCA. Thus the results can be used to support a decision making mechanism. (Fredenslund et al., 2010)

Objectives of this report is to highlight and present the different processes that take place. From the very initial waste that are used as raw materials till the final product of energy services. Analysis of the different steps of the processes take place in order to include every single emission or resource consumed until the final product is delivered.

The main steps that were identified are the collection of the raw materials, transportation, pretreatment of the CS and biogas production.

Collection

Collection of the seaweed is performed three times per year from May the 1st (1.05) till September the 1st (1.09). According to information provided by officials working in the municipality of Solrød the collections approximately last for one week each and take place around the dates that are presented in table 1.

Table 1: Typical Collection dates for Cast seaweed

Collection	Dates
1st	1.06-23.06
2nd	1.08-7.08
3rd	24.08-31.08

The collection is a very costly process and is performed with machinery that uses fossil fuels. Since there is not solid experience in project like this, it is expected that a continuous adjustment of collection methods and strategy is necessary in order to achieve an efficient collection while minimizing environmental and financial burdens.

Although change on the collection practices is expected, the LCI is formulated taking into account the current collection techniques that were used this year (2015) (Fredenslund et al., 2010). On an average basis the trucks travel a distance of 8km till they reach the biogas plant and unload the respective biomass. Approximately 22200 tons of CS are collected on an annual basis from the shore. After the collection the CS biomass is transported to the biogas plant where treatment takes place prior to use in the digesters.

Figure 1: Seaweed collection at Solrød shore.

Transportation

Transportation is inevitably linked with almost all industries and is mainly accounted for supply of raw materials as well as delivery of the final products. In this case transportation of materials and products takes place in the following cases (Fredenslund et al., 2010).

a) From the seaweed collection point (shore) to the plant
b) From CPKelco facilities to the plant
c) Fertilizer from the plant to the farmers and manure from farmers to the plant
d) Manure from farmers to the plant

In all cases transportation is performed with trucks powered by fossil fuels. In cases "a","c" and "d" (Table 2) the trucks have a 30 and 28 tons (Fredenslund et al., 2010) of nominal and working load capacity, whilst it is assumed that trucks with similar capacity are used to transport the Pectin waste from CPKelco.

Table 2: Transportation cases with respective distances

Case of Transportation	Starting Point	Destination	Distance (km)
a	Collection	Biogas Plant	8*
b	CPKelco	Biogas Plant	2.7
c	Biogas Plant	Farmers	8
d	Farmers	Biogas plant	8

**Average*

The collection of manure takes place in an area with a radius of 15km from the biogas plant (Fredenslund et al., 2010). In the same area the digestate is advertised. For simplicity reasons it is assumed that the average distance covered for transportation of manure and digestate is 8 km on average basis.

In an effort to minimize the environmental burden of the process, for transportation cases "c" and "d" trucks carry the digestate from the plant to the farmers and after unloading the product they are again loaded with manure. Having that in mind it is concluded that when the trucks carry the manure to the plant are not filled till their working capacity is reached. Hence, the planning is made using the total quantity of the digestate that is transported.

Finally, it worth mentioning that the distances presented in Table 3, are representative for one way trips.

Table 3: Total distances of the transportation processes

Case of Transportation	Quantity Transported (tons)	Distance (km)	Trucks (28 tons each)	Total Distances (km)
a	22200	8*	793	6344
b	77000	2.7	2750*	7425
c	49700	8	1775	14200
d	99346	8	3548	28384

**assumed nominal capacity of the truck to be 28 tons.*

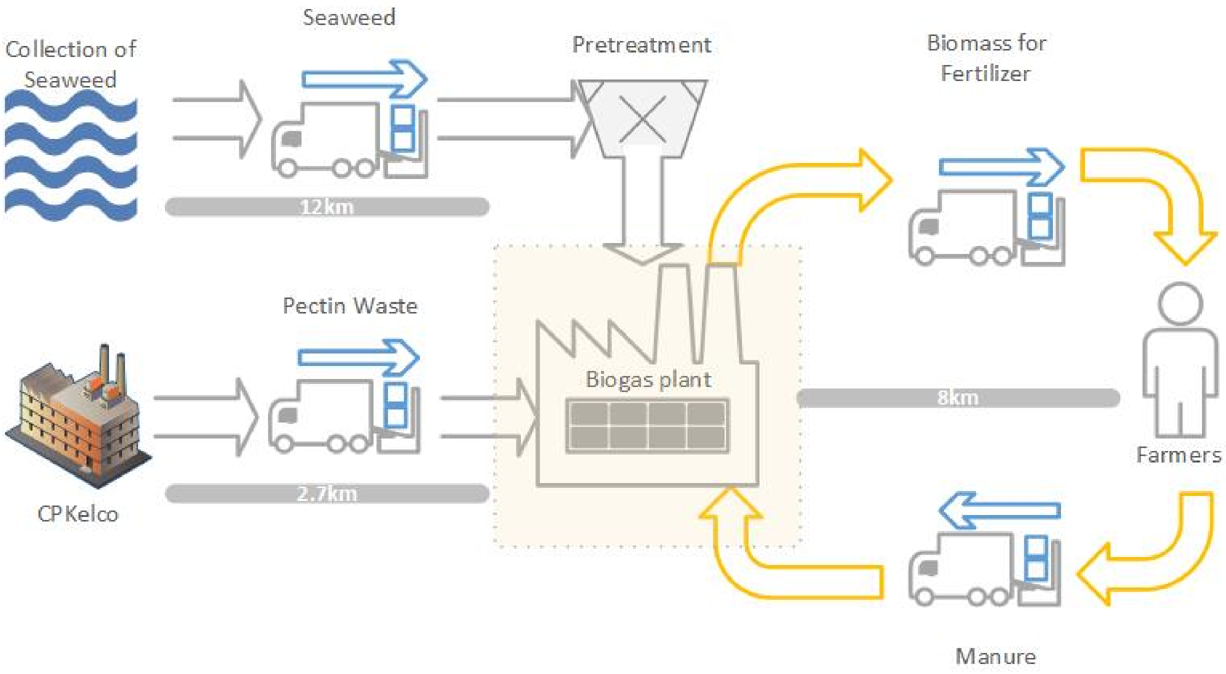

Figure 2: Schematic Representation of transportation flows to and from the biogas plant.

The above figure (Figure 1) summarizes all the transportation processes that are related to the biogas plant and that were explained earlier on. The respective quantities transported are shown in Table 2 and in Table 3.

Pretreatment

Pretreatment of the collected seaweed is considered essential for the safe and efficient operation of the biogas plant. Furthermore, different stages of pretreatment as well as combination of pretreatment methods can greatly increase the quality of the methane yield. Many, methods have been reported in the existing literature whilst this study focus on washing, mechanical pretreatment and enzymatic pretreatment.

Washing

Seaweed is sometimes filled with sand which can negatively affect the reactors. Washing of the seaweed initially takes place in order to clean the seaweed biomass from sand.

According to the stages of the pretreatment a float/sink separator as seen in *Picture 2,* is used for the initial stage of washing. The main byproducts of the washing pretreatment is sand and water. The sand is returned and placed back to the shore where initially the seaweed was collected (Fredenslund et al., 2010).

Figure 3: Float/Sink separator from ReTec Miljø ApS.

The aforementioned separator is powered by an electric motor. Electricity consumed should be calculated in regard to the functional unit. Furthermore, if the water used for that process needs special treatment, the resources consumed for that treatment should also be calculated.

Mechanical Pretreatment

Thereafter the washing, mechanical treatment takes place in an effort to reduce and homogenize the size of the biomass in order to ensure a smooth feed to the reactor. As Li et al. documented, mechanical pretreatment can increase the biogas potential of seaweed. According to that study biomethane potential was measured with BMP tests for 2 different algae species, *Fucus vesiculosus* and *Filamentous red algae.* It was found out that it was increased from 67 NL CH4/kg VS to 92 NL CH4/kg VS for the first species and from 212 to 223 NL CH4/kg VS to the second species (Li et al., 2013). Practically resulting to a 27% and 5% increase for *Fucus vesiculosus* and *Filamentous red algae* respectively. It worth mentioning though, that mechanical pretreatment was coupled with thermal pretreatment at 50oC. Finally the authors observed a tendency of the biomass to degrade faster when it was pretreated mechanically (Li et al., 2013).

In the aforementioned study though, mechanical pretreatment took place with a prototype machine. Since this study is meant to evaluate a whole plant, different machinery should be chosen and the resources consumed should be documented precisely in reference with the functional unit.

Thermal Pretreatment

This kind of pretreatment is meant to increase the soluble COD of the substrate and thus facilitate the anaerobic digestion process. In an experiment conducted by Vivekanand et al., steam explosion was used for 10 minutes in two different temperatures and the pretreated material was later used to assess the biomethane potential. It was documented that methane yiled rose from 223 223 Nm3 CH4/tn VS for the unpretreated SW to 268 and 260 223 Nm3 CH4/tn VS for 130 and 160 oC respectively (Vivekanand et al, 2012).

Enzymatic Pretreatment

Pretreatment with enzymes can be performed with various types of enzymes in different doses. In addition it can also be coupled with other types of pretreatment such as mechanical. Li et al., examined the the effect of enzymatic pretreatment in *Fucus vesiculosus* and *Filamentous red algae.* Initially the enzymatic pretreatment did not exhibit great results but was observed that had a positive effect on the methane yield when it was combined with mechanical pretreatment. In particular an increase in the methane yield was observed from 67 Nm3 CH4/tn VS (raw algae) to 131 Nm3 CH4/tn VS after mechanical and enzymatic pretreatment (results for *Fucus vesiculosus*) (Li et al., 2013).

Though enzymatic pretreatment appears to have a positive impact on the methane yield, a financial assessment of the method is considered crucial in order to evaluate the viability of a possible industrial application (Merola, 2014).

Biogas Production

As widely known, the most desired product of anaerobic digestion is biogas that contains the rich energy carrier of methane (Christensen, 2011). Methane yield of the plant is really important since it can almost directly translated into energy or financial terms.

As was investigated experimentally the methane yield of CS is generally low in comparison with regular substrates currently being used commercially. Alvarado-Morales et al., report a value of of approximately 200 Nm^3 CH_4 $tnVS^{-1}$ of the species *Laminaria digitate* (Alvarado-Morales, 2013) in thermophilic conditions. Similarly to the same range Vivekanand et al, report a value of approximately 223 Nm^3 CH_4 $tnVS^{-1}$ (Vivekanand et al, 2012) dry for *Saccharina latissimi.*

In align to the aforementioned findings, Nkemka and Murto report a value of Nm^3 CH_4 $tnVS^{-1}$ added (Nkemka and Murto, 2010). This study though was contacted with seaweed collected at the southern shores of Sweden. Therefore, the results are considered very similar to the seaweed found to the Danish shores. Additionally, in this study experiments were performed in order to evaluate the biomethane potential of seaweed collected in Koge bay. The findings report a value of 118 Nm^3 CH_4 $tnVS^{-1}$ added (Fredenslund et al., 2011).

On the other hand a master thesis performed at Denmark's Technical University (DTU) assessed experimentally the methane yield of CS consisted of a mixture of *Zostera marina*, *Pilayella littoralis* and *Ectocarpus*. The results indicate an even lower values than the aforementioned literature with a methane yield of approximately 143 Nm^3 CH_4 $tnVS^{-1}$ (Merola, 2014).

The latter thesis examined also co-digestion options with various cattle manure to CS dilution rates where no apparent difference was observed when comparing digestion of only manure as a substrate and co-digestion with manure and CS.

On the other hand, Troelstrup reports that a mixture of 20% CS and 80% cattle manure can actually can actually achieve higher methane production than a case where only manure is used as a substrate (Troelstrup, 2014).

However a value of 21,5* m^3 CH_4 $tnTS^{-1}$ of CS whilst the respective value of the pectin residues is approximately 410* m^3 CH_4 $tnTS^{-1}$ (Fredenslund et al., 2010). Similarly the respective value for the manure derived by the literature but also reported at Fredenslund et al., is 370 m3 CH4/ton TS.

In addition to the seaweed substrates, anaerobic digestion can performed in forms of co-digestion substrates. Many authors found that co-digestion of CS is a better option and yields higher amounts of biogas in comparison to one only substrate.

Merola investigated co-digestion options at different ratios. It is reported that a mixture of sugar beet pulp (SBP) and CS with proportions of 60 and 40% respectively yielded a value of 271 Nm^3 CH_4 $tnVS^{-1}$ while a mixture with CS and cattle manure (80 and 20% respectively) yielded 232 Nm^3 CH_4 $tnVS^{-1}$ (Merola, 2014)

**Note the different units!*

Substitution of fertilizers

One of the by-products of the anaerobic digestion plant are *digestion residues* (digestate) as defined in the respective legislation (EC, 2002).

Proper treatment of the digestate is performed in ordered to ensure that diseases are not transmitted during its application to the land. This step is required by the respective legislation in regard to animal by-products (EC, 2009). Hence the digestate is sanitized for 1 hour at 70oC (Fredenslund et al., 2010).

Sanitation, practically allows the safe use of it in land. Hence, the digestate is transported to the local farmers that support and provide manure to the biogas plant where is used as fertilizer. This step is quite important since the farmers use this digestate instead of industrial made fertilizers.

Subsequently, an avoided production and use of artificial fertilizers can be attributed to the digestate.

This avoided production can be estimated according to the quality of the digestate produced along with its ability to substitute the artificial fertilizers. (Kramer et al., 1999)

According to (Alvarado-Morales et al., 2013) The nutrients recovered in the digestate were estimated to be 7.98, 8.75 and 36.6 kg of N, P, and K, respectively per one tonne of dry seaweed biomass.

In addition, the quality of the digestae as by product, can also be estimated according to the initial elemental composition of the substrates used in the anaerobic digestion process. *Merola* has documented the content of N, P and K that is presented bellow.

Table 4: Nutrient characterization in terms of N,P and K (Merola, 2014).

Nutrient	g kgTS^{-1} ± SD
N	5.2 ± 0.017
P	0.903 ± 0.017
K	1.59 ± 0.11

Conclusions

In this study the LCI of an anaerobic digestion plant was formulated. The vital processes that are part of the plant were reported and analyzed. Hence, collection of raw materials, transportation of them as well as pretreatment of the respective biomass substrates. In addition to the above, the distribution of products and by-products was also analyzed. This study does not take into account the environmental burden that is generated by the production of the machinery used in the plant, as well as the environmental impact of building facilities. In addition, energy consumption of the plant is not analyzed in this report.

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