BIO-FUEL FROM ANAEROBIC CO-DIGESTION OF THE MACRO-ALGAE *ULVA LACTUCA* AND *LAMINARIA DIGITATA*

Shiplu Sarker[1], Anette Bruhn[2], Alastair James Ward, Henrik Bjarne Møller
[1]Aarhus University, Department of Engineering, Blichers Allé 20, DK 8830, Tjele, Denmark
[2]Aarhus University, Department of Bioscience, Vejlsøvej 25, DK 8600, Silkeborg, Denmark

Abstract
World's energy crisis is becoming increasingly critical when emission from fossil fuel due to the rapid expansion of population and their growing energy consumption is considered. This leads us to reassess the potential of already available renewable energy sources and to step further in the exploration of marine biomass. So far, the progress in treating different groups of algae as a source of energy is promising although their utilization is still in early days. In the present study, green seaweed, *Ulva lactuca* and brown seaweed *Laminaria digitata* was co-digested with cattle manure at mesophilic (35±2^{o}C) and thermophilic (50±2^{o}C) condition for testing the biogas production at lab scale continuous reactors (3.2 liters working volume). Ulva was grown and harvested in Danish algae center and washed to reduce salt concentration and subsequently macerated to facilitate feeding in the digesters. *Laminaria*, however, was harvested from the sea (Fornæs, Denmark) although similar pretreatment method as *Ulva* was followed. The results show that the methane yield of *Laminaria* from mesophilic anaerobic digestion was fairly stable (average 138 lCH4/kgVSadded) over a different type of feeding. Methane generation from thermophilic reactors both for *Ulva* and *Laminaria*, on the other hand, were significantly varied, one opposite to the other, as the feeding rate varied. While the thermophilic treatment of *Laminaria* produced an average of 142 L/kgVS, Ulva yielded around 122 L/kgVS. Overall, it was found that algae are very interesting substrates for utilizing as gaseous bio-fuel through anaerobic co-digestion with cattle manure.
Key words: renewable energy, *Ulva lactuca, Laminaria digitata*, anaerobic digestion, cattle manure.

Introduction

The choice of algae as a bio-fuel is characterized by many fold advantages over conventional biomasses. Firstly, it has a huge potential as the source of energy crops due to its easily hydrolysable sugars and low lignin content (Nkemka and Murto, 2010). Secondly, it yields a higher amount of biomass per unit of light and area and grows in the seawater that saves a lot of agricultural lands which are continuously shrinking due to their use highly limited by the food production. In one study, it was estimated that the potential of algae for producing energy is 100 EJ (Exa-Joule) which corresponded to the land-based biomass was 22 EJ (Chynoweth et al. 2001). Much of these energy potential of seaweeds might be extracted through anaerobic digestion which has been a viable solution over the last few decades. Anaerobic digestion of seaweed, however, is challenged with the issue of inhibition due to the high concentration of sulfur, sodium chloride and heavy metal (Nkemka and Murto, 2010). But within the scenario where macroalgae is considered as a substrate together with the base feedstock such as cattle manure can overcome some of these problems. For instance, average species of macroalgae are rich in carbon content that can contribute to improve the overall C:N ratio while co-digested with basic feedstock (i.e. cattle manure). Additionally, the efficient operation of anaerobic digester can be enhanced by the higher C:N ratio in the range of 20-30 and the higher methane yield in the range of 20-30 $m^3 CH_4 m^{-3}$ as according to the literature study(H.B.Nielsen and S.Heiske, 2012). These values are unlikely to be achieved while the reactor is run by the digestion of cattle manure alone which has the C:N ratio equal to 8-12 and the yields approximately 13-14 $m^3CH_4 m^{-3}$ manure. Considering these, the purpose of this study, therefore, is to evaluate the feasibility of the anaerobic co-digestion of algae (*Ulva lactuca* and *Laminaria digitata*) with cattle manure by conducting experiment in the continuous reactors.

Materials and Methods

Inoculum

This study included both mesophilic and thermophilic inoculum. The thermophilic inoculum was collected from the biogas plant in the research center Foulum, Denmark, which operates with the thermophilic digester at 52±3^{o}C . The measured total solid (TS) and volatile solid (VS) of this inoculum were 2.83±0.5% and 1.43±0.5% respectively, whereas the approximate pH value and total ammonium nitrogen (TAN) were found as 8.12 and 1.82 g/L respectively. The mesophilic inoculum, on the other hand, was collected from the Bårnlev plant, Aarhus, Denmark, that works at 35±3^{o}C with an average TS, VS and ammonia content of 4.4±0.5%, 3.9±0.7%, and 3.8±0.3g/L respectively.

Substrates

Dairy cattle manure

Cattle manure was derived by providing the maize silage as the main feeding to the dairy cattle and was supplied to our facility in the Biogas plant, Foulum research center, Denmark. The 1m^3 container filled with cattle manure was placed inside the indoor storage in august 2011, in the vicinity of experimental premises, maintaining a constant temperature of 15±2 degree Celcius. Manure was supplied with the container as soon as it was generated and underwent no previous storage before delivering to us. The average TS, VS and pH of the cattle manure throughout the entire experimental period was 6.8±0.9%, 5.6±0.6% and 6.8±0.5 respectively.

Ulva lactuca and Laminaria digitata

The *Ulva lactuca* was grown and harvested at the Danish Algae center and *Laminaria digitata* was collected by hand from the sea (Fornæs, Denmark). After harvesting, algae were frozen in plastic bags at -18^{o}C until using for the biogas experiment. *Ulva* was washed by tap water and torn by hand before using for the experiment whereas *Laminaria* was washed by tap water and macerated by the kitchen scissors with an approximate size of 10x10 mm.

Analytical Methods:

Total solids (TS) were measured after heating the samples for 24 hours at 105^{o}C. TS was further heated and incinerated at 550^{o}C for 5 hours to turn into ashes. Volatile solid (VS) was calculated by subtracting the amount of ashes from the amount of TS (Møller et al. 2007). Volatile fatty acid (VFA) was determined by acidifying 1 ml of sample with 4 ml of pivalic acid and subsequently centrifuged for 20 minutes at 12,000 rpm. The centrifuged sample was then filtered to prepare for the Gas Chromatograph (Hewlett Packard 6850A) which analyzed the VFA by using its column, flame ionization detector and career gas. The dimension of the column was 30 m x 0.25 mm x 0.25 µm where Helium (He) was used as career gas. The temperature of the column was gradually increased from 110^{o}C to 220^{o}C with a rate of 10^{o}C/min. pH of the sample was determined by using a glass pH probe (Knick Portamess, 911 pH, Germany) whereas Total Ammonia Nitrogen was analyzed calorimetrically at 690®nm with Merck spectrophotometer (NOVA 60). The produced biogas from each reactor was measured by using the online gas measurement system that was composed with a water bath of 41x26x 25 cm (27L) dimension which further was equipped with gas measuring devices and the data acquisition system. The water bath system works according to the principle of water displacement by which the released biogas from the reactors passes through the tubes to its bottom where gas measuring devices or cells are located. The cells then generate the digital pulse of any defined biogas flow which is sent to the data acquisition system that calculates and stores the biogas production. Gas samples were analyzed for both CO_2 and CH_4 content using gas chromatograph Perkin Elmer Clarus 500 equipped with a Thermal Conductivity Detector and a Turbomatrix 16 Headspace auto sampler as described by Møller et al. (Møller et al. 2004). Methane and carbon dioxide was isolated by a 12' x 1/8" Haysep Q 80/100 Column. The temperatures of the injection port, oven and detector were 110, 40 and 150^{o}C respectively. Helium (He) was used as a carrier gas with a flow rate of 30 ml/min.

Co-digestion of *Ulva lactuca* and cattle manure in lab-scale reactors

Two reactors R_1 and R_2 with the capacity of 5 liters each and 3.2 liter working volume were simultaneously run at 22 days HRT. Both the reactors were placed in thermophilic incubator where the temperature was constantly maintained at 50±3 ^{o}C. The reactors were intermittently stirred for 5 minutes in every 45 minutes at 60 rpm by the steel rod fed with an electric motor (Zheng, China, 12 V) mounted on the top of the reactor lid. The experiment was initiated by feeding both the reactors with 3060 grams of inoculum in addition with 140 grams of cattle manure. R_1 was treated as reference reactor and fed only with cattle manure throughout the entire experimental period. Reactor R_2, on the other hand, was the experimental reactor that was also fed solely with cattle manure until the stable biogas production was achieved. The reactors had run and incubated for a very long time before the current experiment and therefore had reached the stabilization after a short period of 10 days incubation. This paper did not include the data before stabilization and reported the start of the experiment from the moment *Ulva lactuca* was added. Table 1 shows the variable parameters used in the co-digestion experiment of *Ulva* with cattle manure. After stabilization, R_2 was co-digested with cattle manure and algae *Ulva lactuca* with a variable OLR (Organic Loading Rate) of $0.31 gVSL^{-1}d^{-1}$ to $1.11 gVSL^{-1}d^{-1}$. The VS addition and hence OLR of *Ulva lactuca* in reactor R_2 was increased stepwise at three different concentrations. The OLR concentration of $0.31 gVSL^{-1}d^{-1}$ at 11% VS of *Ulva* was introduced at day 0 and was continued until day 31. The loading was further increased to $0.69\ gVSL^{-1}d^{-1}$ and continued between day 32 to day 58 at 24% VS of *Ulva*. In the final incubation period, the OLR was additionally increased to $1.11\ gVSL^{-1}d^{-1}$ corresponding to a 37% VS of *Ulva* that lasted from the day 59 to day 80.

Co-digestion of *Laminaria digitata* and cattle manure in lab-scale reactors

Unlike the experiment with *Ulva*, the continuous experiment for algae *Laminaria digitata* was conducted with four reactors (R_1, R_3, R_4, R_5) duplicated in two different treatments i.e. mesophilic (35^{o}C) and thermophilic (50^{o}C), each at HRT of 22 days. Reference reactor R_1 at thermophilic treatment was common for both the digesters running with *Laminaria* and *Ulva,* whereas reactor R_4 was used as reference for *Laminaria* at mesophilic condition. Both of these

Table 1

Experimental parameters (average) governing the anaerobic digestion of all the reactors

	R_1 (Control)			R_2 (Ulva)			R_3 (Laminaria)			R_4 (Control)			R_5 (Laminaria)		
Days	0-31	32-58	59-80	0-31	32-58	59-80	0-31	32-58	59-80	0-31	32-58	59-80	0-31	32-58	59-80
Temperature (^oC)		50			50			50			35			35	
TS/VS cattle manure (%)	6.01±1.5/5.4±0.6														
TS/VS *Ulva lactuca (%)*	20.4±7/15.0±0.4														
TS/VS *Laminaria digitata (%)*	15.5±1.5/10.5±1.05														
Feeding type (VS)	100% CM			11% Ulva	24% Ulva	37% Ulva	15% Lam	24% Lam	41% Lam	100% CM			15% Lam	24% Lam	41% Lam
pH	8.08	8.05	8.3	7.98	7.98	8.08	8.13	7.97	8.3	7.85	7.8	7.9	7.82	7.73	8.02
tVFA (mg/L)	306.6	64.4	88.4	73.4	118.6	1662	431	349.5	162.6	90.6	51.17	72.2	202	106.1	185.7
TAN (g/L)	2.08	2.0	1.85	2.14	2.11	1.15	2.03	1.48	1.69	2.13	2.28	1.78	2.3	2.1	1.6
CH_4 composition (%)	57.6	58.6	61.1	60	56.2	46.0	56.17	56.7	56.95	62.6	59.5	63.6	60.2	58	56.4
total CH_4 yield (L/kgVS)	154.1	142.2	126.2	157.6	133.5	70.8	119.6	165.6	185.7	143.6	130.6	124.3	139.2	136	139
CH_4 yield (L/L/d)	0.38	0.35	0.29	0.434	0.39	0.21	0.33	0.46	0.52	0.37	0.32	0.29	0.38	0.38	0.4
Algae CH_4 yield (L/kgVS)				164.9	129.6	72.1	35.6	192.3	197.7				128.5	142.2	142.0
Total OLR (gVS/L/d)	2.5	2.5	2.3	2.8	2.9	3.0	2.8	2.8	2.9	2.5	2.5	2.3	2.8	2.8	2.9
Laminaria OLR (gVS/L/d)							0.42	0.68	1.2				0.42	0.67	1.17
Ulva OLR (gVS/L/d)				0.31	0.69	1.11									
Laminaria feeding (g/d)							12	20	35				12	20	35
Ulva feeding (g/d)				5.8	13	25									
Cattle manure feeding (g/d)		140		134.2	127	115	128	120	105		140		128	120	105

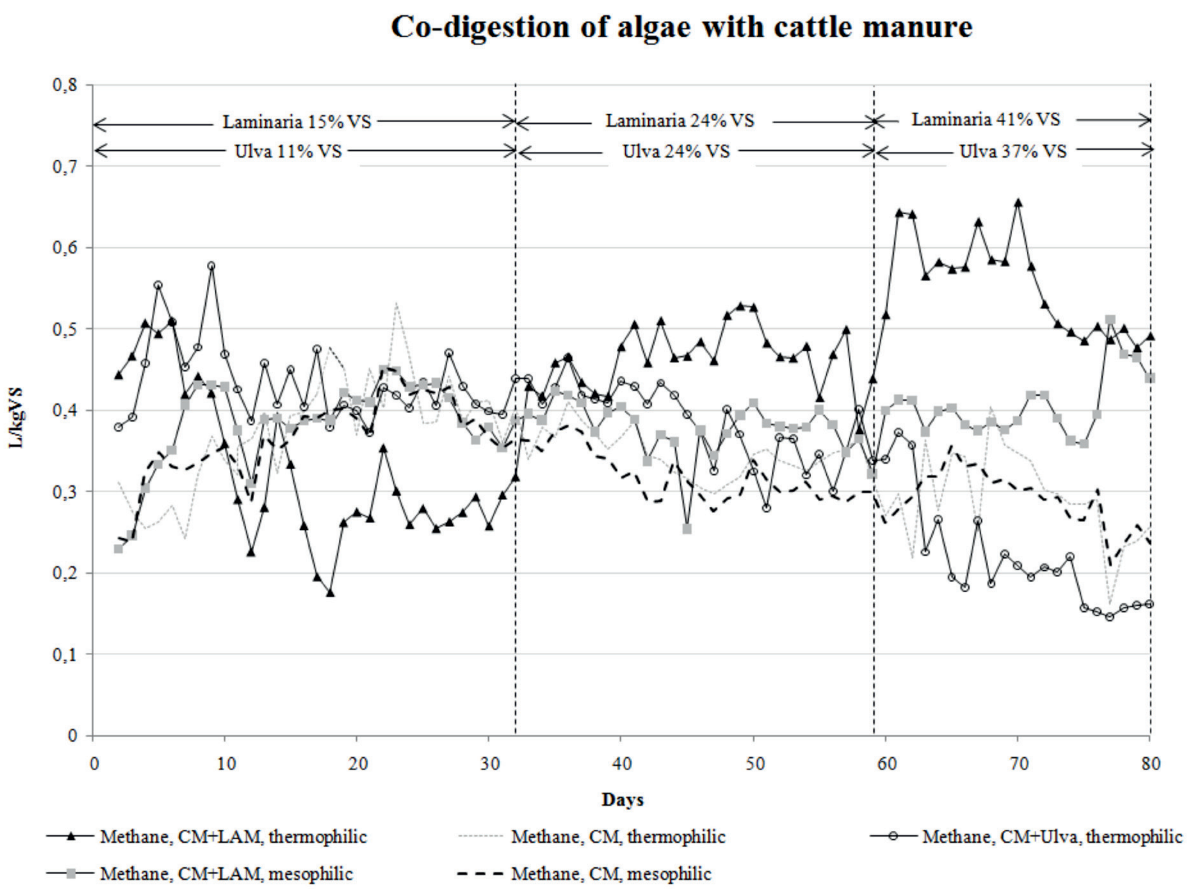
Source: made by the authors

reference reactors were fed exclusively with cattle manure throughout the entire experimental period, in contrast with the tested reactors which were fed only with cattle manure until the biogas production was stabilized. All the reactors (R_1 to R_5) were inoculated, stabilized and stirred in the same way, and reactors R_4 and R_5 were placed in the incubator that maintained temperature at 35±3 °C. R_1 and R_3, alternatively, were placed in the same thermophilic incubator (50±3°C) where R_2 (tested reactor for *Ulva lactuca*) was located. Co-digestion with *Laminaria digitata* and cattle manure for R_3 and R_5 were started after the stable biogas production was observed. Data during the stabilization period once again was not presented and the start of the experiment was determined by the start of the feeding of algae. Like the feeding of *Ulva*, the addition of *Laminaria* was also varied in three steps. In the first step (day 0-31), the R_3 and R_5 were supplemented with *Laminaria digitata* at 15% VS which corresponded to the OLR of 0.42 $gVSL^{-1}d^{-1}$ (table 1). The OLR was further augmented to 0.68 $gVSL^{-1}d^{-1}$ (table 1) with a corresponding 24%VS of *Laminaria digitata* from day 32 to day 58. The OLR was kept increasing and was fixed at 1.2 $gVSL^{-1}d^{-1}$ (table 1) between day 59 to day 80 at 41 % VS concentration of *Laminaria*.

Result and discussions

Co-digestion of *Ulva lactuca* and cattle manure in lab-scale reactors

The weight specific methane yield from the thermophilic co-digestion of *Ulva lactuca* with cattle manure for three different types of feeding is shown in figure 1. Generally, the methane yield from both R_1 and R_2 was gradually reduced throughout the experimental period. However, the difference of methane production between these two reactors was significantly expanded as the feeding concentration of *Ulva* in reactor R_2 was stepwise increased. For example, the control reactor (R_1) saw the three stage decrease in methane yield from 154.1 L/kgVS (between day 0-31) to 142.2 L/kgVS (day 32-58) and then to 126.2 L/kgVS (day 59-82) as the experiment proceeded. This perhaps can be attributed to the long storage of cattle manure contributing to the progressive decline in volatile solid content. Take R_2, on the other hand, at feeding volume of 11% *Ulva* (OLR of 0.31$gVSl^{-1}d^{-1}$), the average methane yield was 157.6 L/kgVS (0.43 L/L/day) between day 0 to day 31. Methane yield was first decreased to approximately 5% , from 157.6 L/kgVS to133.5 L/kgVS, when the feeding concentration of *Ulva* was raised to 24% (day32-58) and then to the highest 55%, from



Source: made by the authors

Figure 1. **Co-digestion of algae (*Laminaria digitata* and *Ulva lactuca*) with cattle manure**

133.5 L/kgVS to 70.8 L/kgVS, when the feeding was further augmented to 37% (day 59-80). The average methane yield and the value of other experimental parameters are tabulated in table 1. The high decrease in methane yield from R_2 was also reflected with a significant drop in methane composition (46%) and increase in total VFA (1662 mg/L) between day 59 to day 80. Clearly, the elevated feeding concentration of *Ulva* caused the instability in methanogenesis due to the overloading of VFA.

Co-digestion of *Laminaria digitata* and cattle manure in lab-scale reactors

Unlike R_2 (thermophilic reactor for *Ulva*), the methane yield from reactor R_3 (thermophilic reactor for *Laminaria*) was gradually increased as the feeding concentration of *Laminaria* was increased (figure 1). The methane yield from the reactor R_3 at feeding concentration of 15% *Laminaria* (0.42 $gVSl^{-1}d^{-1}$) was 119.6 L/kgVS which rose approximately 38% to 165.6 L/kgVS at *Laminaria* concentration of 24% (0.68 $gVSl^{-1}d^{-1}$) and approximately 55% to 185.7 L/kgVS (1.2 $gVSl^{-1}d^{-1}$) at the *Laminaria* concentration of 40% (of total feeding). This is in contrast with the reactor R_5 (mesophilic reactor for *Laminaria*) from which the methane yield was rather stable (average 138.06 L/kgVS) despite it was fed with similar variable *Laminaria* concentration for R_3. The mesophilic reference reactor R_4, on the other hand, followed the similar pattern (explained in previous section) of methane drop (from 143.6 to 130.6 and then to 124.3) like R_1 (thermophilic reference reactor) from the beginning till the end of the experiment. The higher average methane yield from the thermophilic *Laminaria* reactor (R_3) over the mesophilic *Laminaria* reactor (R_5) indicated the better methanogenesis and the adaptation of bacteria as an effect of higher temperature.

Conclusions

This study investigated the feasibility of methane production from the two algae species, *Ulva lactuca* and *Laminaria digitata.* The results from the co-digestion experiment of *Laminaria* with cattle manure at two different temperature conditions demonstrated that the gain in methane yield at thermophilic treatment is significantly higher than that from the mesophilic treatment as an effect of change in feeding. The deviation in methane yield from the sole temperature treatment of *Ulva* was also significantly large, but the trend is continuously downward as the feeding concentration was gradually increased. This work is yet to investigate the seasonal availability of algae based on which together with the findings so far, the choice of the type of algae for co-digestion with cattle manure can be determined.

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