Will different pretreatment methods influence the biogas production of seaweeds
Will different pretreatment methods influence the biogas production of seaweeds?

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Abstract
Global warming along with energy demand and rising prices of natural energy resources have motivated studies to find some renewable and clean energy. The use of algae as third generation biofuel can avoid the competition for farmland and algae can be considered as a potential future source of renewable energy. Algae can be used for biogas production through anaerobic digestion (AD). *Fucus vesiculosus* and *Fucus serratus* are the two dominating species of brown seaweed growing in the Baltic sea in the southwest of Sweden. Pretreatment can significantly affect the biogas production since hydrolysis of algae cell wall structure is a rate-limiting step in AD process. In this study, four different pretreatments: mechanical, microwave (600W, 2min), ultrasonic (110V, 15min), and microwave combined with ultrasonic (600W, 2min;110V, 15min) were applied to the seaweed and then co-digested with biogas plant leachate. The aim was to investigate the biogas production and methane yield from AD after these pretreatments. The results showed when comparing with mechanical pretreated only, that the ultrasonic, ultrasonic combined with microwave and microwave pretreatments could obtain increased cumulative methane yields with 167%, 185% and 156% , respectively. The maximum methane yield was 260 ml/g-VS with combined pretreatment after 20 days of digestion. The ultrasonic combined with microwave pretreatment showed a significant improvement of methane yield when comparing with mechanical pretreatment.

**Keywords:** methane; seaweed; pretreatments; anaerobic digestion
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1. Introduction
Nowadays, energy demand and global warming are major concerns in the society. One of the most important factors that contribute to the global warming is the increasing emission of greenhouse gases, such as carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O)(Abdeshabian et al., 2016).

Sweden government has declared high ambitions regarding reducing greenhouse gas emission. In 2017, the government proposed a new climate goals which contain:

- achieving no net emission of greenhouse gases into atmosphere by 2045 and thereafter achieve negative emission.
- Emissions in Sweden outside of the EU (European Union) ETS (emissions trading system) should, by 2030, be at least 63 percent lower than emissions in 1990, and by 2040 at least 75 percent lower.
- Emissions from domestic transport are to be reduced by at least 70 per cent by 2030 compared with 2010 (Proposal referred to the Council on Legislation on a climate policy framework for Sweden).

As a renewable resource, biogas has been getting a lot of attention due to its contribution to mitigation of global warming and solving the energy crisis. Biogas technology is widely used in many industrial sectors such as utilities, transportation, agriculture, and waste management (Karlsson et al., 2018). Raw biogas from biogas plant can be used for power generation and heating. Biomethane is the upgraded form of biogas which has properties equivalent to natural gas and can be used directly as vehicle fuel or injected into a natural gas grid (Federica Cucchiella et al., 2017; Lalander et al., 2018).

Biogas can contribute to a more renewable energy system and improved nutrient management (Ammenberg and Feiz, 2017). In 2014, the Sweden’s share of energy from renewable sources reached 52.6% in gross final use, while the transportation sector was only 19.2%. The Swedish government has declared high ambitions regarding transportation and has set a goal of a “fossil-independent” vehicle fleet by 2030. As a biofuel, biogas is a sustainable alternative of fossil fuel and natural gas (Ammenberg et al., 2018; Murray et al., 2017).

Up to 2013, the total number of biogas plants in Europe was 14 572 with a strong domination of Germany (9035 plants), followed by Italy (1 391 plants) and Switzerland (620 plants), Sweden was ranked 8th with 264 biogas plants (Torrijos, 2016). Biogas is generated through the anaerobic digestion of organic waste (Lalander et al., 2018). It
can be produced from different types of feedstock, including biomass containing various carbohydrates, lipids and proteins (Ammenberg and Feiz, 2017). According to different types of feedstock, biogas plant can be classified into (I) landfill; (II) plants treating sewage; (III) Agriculture based biogas plants that run on energy crops, manure and agricultural residues and; (IV) Other which refers to industrial food and beverage and biological waste (Torrijos, 2016).

1.1 Anaerobic digestion

Anaerobic digestion (AD) convert organic waste to renewable energy in the form of biogas and high-quality fertilizer (digested material). The cost of AD is relatively low compared to other techniques (Nilsson På ledal et al., 2018; Sárvári Horváth et al., 2016). Energy crops, agricultural waste, manure, municipal solid waste, waste oils, animal fat, food waste, sewage, wastewater, and various industrial effluents with a high organic load can also be used in anaerobic digestion (Appels et al., 2011; Lora Grando et al., 2017).

1.1.1 The process stage of AD

There are four key steps of the AD process involving hydrolysis, acidogenesis, acetogenesis and methanogenesis. The microorganisms dominating in the AD process can be classified as hydrolytic, fermentative, acetogenic, and methanogenic respectively (Li et al., 2011).

Hydrolysis occurs reducing high-molecular-weight polymeric components to simple soluble molecules such as simple sugars, fatty acids, and amino acids. The remaining components are further broken down by fermentative bacteria and generate short chain volatile fatty acids VFAs, along with ammonia, carbon dioxide, and other byproducts.

The break-down products created through the acidogenesis step are further digested by acetogens to produce acetate, carbon dioxide, and/or hydrogen which are the direct substrates for methane production. In the last step, methanogens use the intermediate products of the previous stages and convert them into methane, carbon dioxide, and water (Li et al., 2011; Yadvika et al., 2004).

1.1.2 Inhibitors and influence factors of AD process

There are still some negative factors influencing the AD process such as long retention
times, low removal efficiencies of organics, and the process may be unstable (Park et al., 2005a). Ammonia, sulfide, light metals ions (Na, K, Mg, Ca, and Al), heavy metals and organic compounds such as alkyl benzenes, halogenated, benzenes, phenol and alkyl phenols can cause the inhibition of the AD process (Chen et al., 2008).

The stability of the AD depends on biological activities of microorganisms and the biomass used as substrate (Mydin et al., 2014). Other factors affecting the AD process include temperature, pH and C/N ratio (Khalid et al., 2011).

There are two conventional temperatures of AD process, i) The optimum temperature for mesophilic digestion is approximately 30 to 38°C, ii) The optimum temperature for thermophilic digestion is around 49 to 57 °C. It is believed that the operation in mesophilic digestion is safer and more stable than the operation in thermophilic digestion (Mydin et al., 2014).

Various researchers have reported the optimal pH for methane production of the anaerobic digestion is round 7.0. The optimal pH for hydrolysis and acidogenesis is 5.5 and 6.5, respectively, while methanogenesis occurs efficiently at pH 6.5 - 8.2 (Khalid et al., 2011). Since this range of optimal pH is very wide, and the substrate and digestion technique will influence the optimal pH value. Liu et al. (2008) developed a mathematical model to obtain the optimal pH in the anaerobic digestion of the organic fraction of municipal solid waste. The result shows the optimal values of pH are 7.10, 7.21 under mesophilic and thermophilic temperature, respectively and differences in TS will cause little difference of optimal pH.

1.1.3 Enhancement of AD process

Biological hydrolysis was identified as the rate limiting step in anaerobic digestion. In order to reduce the impact of this rate-limiting step, different kinds of pretreatments are required (Park et al., 2005). The pretreatment not only remove the impurities such as metals, plastics or stones from the raw material but also decompose substrates with high lignocellulose content meanwhile increasing the bioavailability of microorganisms involved in the process (Lora Grando et al., 2017). Pretreatment systems includes ultrasonic, alkaline, thermal and mechanical disintegration systems (Park et al., 2005b).

If the C/N ratio of a mono-digestion system is in the low range from 10 to 25, the nutrient and trace elements will not be sufficient for the microorganisms, so co-
digestion is needed. Co-digestion of manure or food waste will improve the biogas production in AD process (Ammenberg and Feiz, 2017; Khalid et al., 2011). The use of a co-substrate system increases the biogas production in most cases due to the positive synergy established in the digestion system, and sometimes it can also help to establish the required moisture contents in AD process (Mata-Alvarez et al., 2000).

Guarino et al.(2016) found that the optimal range of C/N in order to maximize the biomethane yield was from 20 to 30. Optimal C/N ratio for anaerobic digestion of palm wastes and municipal solid wastes is 30 and 25, respectively.

1.2 Why use algae to generate biogas

The biofuel produced from energy crops such as soybean and palm oil called first generation biofuels. Second generation biofuel is produced from non-food biomass such as agricultural or forest residues (González-González et al., 2018). However, both of two generations of biofuel feedstock are not unsustainable mainly due to the consideration of food shortage and the scarcity of land resource (Zhu et al., 2018). The third generation biofuel can be produced from algae biomass, the use of algae can avoid the competition for farmland and can be considered as a potential future sources of renewable energy in the transport sector in Europe(Allen et al., 2015).

The use of algae to produce biofuel has a lot of advantages, for example to remove algae from the water body or the beach can diminish eutrophication of lakes, ponds or oceans and in the meantime mitigating CO₂ emission from degrading algae on the beach. Algae can also be grown in organic wastewater, so there is no competition for farmland; furthermore, efficient photosynthesis and high tolerance to wastewater also make it as a suitable feedstock for the biofuel production (Kothari et al., 2018; Zhu et al., 2018).

1.2.1 Microalgae and macroalgae

The algae can be divided by size into two groups: microalgae and macroalgae, commonly known as seaweed. Many microalgae exist as solitary cells, but colony formation by multiple cells is also common (Murphy et al., n.d.). In general, algal cell walls contain two main components: i) the cell wall skeleton which comprised by fibrillar component and ii) amorphous component such as polysaccharides, proteins and lipids (González-González et al., 2018).
Microalgae are rich in lipids which could lead to high methane yields (Allen et al., 2015). Both microalgae and macroalgae have no lignin in the cell. The cell wall of microalgae is mainly composed of carbohydrates (30-75%) and proteins (1-37%) (Kumar et al., 2016). The content of cell wall varies from species to species and depends on the availability of nutrients and the growing conditions (Murphy et al., n.d.). Species with protein-based cell wall or without cell walls are more easily digested than species with thick, hard cell walls (González-González et al., 2018).

Seaweed deposited on beaches always cause a significant waste disposal problem due to marine eutrophication (Nkemka and Murto, 2012) and tourists demand. With the characteristic of no lignin, high level of carbohydrates and low levels of cellulose and lipid content (Allen et al., 2015; Tedesco et al., 2013), seaweed is considered as one of the most promising renewable energy resources for biogas production (Romagnoli et al., 2017). Seaweeds can be divided into three broad types distinguished by color: brown, red and green seaweeds (Jard et al., 2013b). The composition of seaweed varies differently from species to species and will also be influenced by seasonal changes (Barbot et al., 2016). The biogas yield is related to the storage of sugars which vary with season, so it is necessary to find the best harvest time of seaweed when the sugar content is highest. Through evaluating the biogas production of brown seaweed Laminaria sp in Ireland, Montingelli et al. (2016a) found that autumn appeared the best harvesting period while spring represent the worst period for harvesting.

1.2.2 Brown seaweed

Brown seaweed contains various carbohydrates such as alginate, fucoidan, mannitol and laminarin, and the carbohydrates range from 30%~60% (Barbot et al., 2016; Lee and Lee, 2015). Alginate is a linear hetero polysaccharide that is composed of two unit monosaccharides, D-mannuronic acid (M) and L-guluronic acid(G). Alginate can provide both stability and flexibility for aquatic organisms exposed to flowing water (Barbot et al., 2016). Owning to its excellent rheological properties such as gelation, thickening and stabilization of dispersions, alginate can be used in various industries like paper, textile, biomedical, agri-food, cosmetic and pharmaceutical (Fertah et al., 2017).

Mannitol is the sugar alcohol corresponding to mannose, which can be oxidized to fructose Mannitol-2-dehydrogenase and producing NADH (Kawai and Murata, 2016). Mannitol can be used in food applications and produce rigid polyurethane foams, and
it can also be converted to isomannide (isomer of isosorbide) which have many applications, such as the synthesis of polymers and plasticizer components (van Hal et al., 2014). Laminarin is a low-molecular-weight polysaccharide and is composed of (1,3)-\(\beta\)-D-glucan and some \(\beta\)-(1,6)-intrachain links(Kadam et al., 2015). The result from Adams shows the laminarin and mannitol content of \textit{L. digitate} (a kind of brown algae) is highest in July and with the maximal methane and ethanol production (Adams et al., 2011).

Brown algal families includes Chordariaceae, Fucaceae, and Alariaceae. \textit{Fucus} belongs to the Fucaceae algae (Williams and Smith, 2007). \textit{Fucus vesiculosus} and \textit{Fucus serratus} are the two dominate species on rocky shores of southeast Sweden in the Baltic sea (Nilsson et al., n.d.). \textit{F. serratus} is a robust alga with olive-brown color, It does not have air bladders, which can be found at in \textit{Fucus vesiculosus}(Pycke et al., n.d.). Barbot et al. investigate the chemical element composition of \textit{Fucus vesiculosus}, and fond this kind of algae contained both high amounts of macro elements (P, Ca, K, et al in 450-21500ppm), and trace elements (Zn, Cu, Cr, Pb in 0.11-930ppm)(Balina et al., 2016). The table 1.2.2.1 shows the chemical composition of \textit{Fucus vesiculosus} and \textit{Fucus serratus}.

<table>
<thead>
<tr>
<th>Species</th>
<th>%MC(^{a})</th>
<th>%Nitrogen</th>
<th>%WSC(^{b})</th>
</tr>
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<tbody>
<tr>
<td>\textit{Fucus Vesiculosus}</td>
<td>78.2</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>\textit{Fucus Serratus}</td>
<td>80.0</td>
<td>2.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

\(^{a}\)MC =moisture content.  
\(^{b}\)WSC=Water-soluble carbohydrates

\section*{1.3 Pretreatment to enhance biogas production}

The decomposition of the complex algae cell wall structure is a rate-limiting step that significantly affects the bioconversion process in the AD process. Therefore, an effective and appropriate pretreatment is essential for the enhancement of biogas production (Kumar et al., 2017). The process of pretreatment should be simple and economic, the product should be highly fermented (Montingelli et al., 2016b). There are amounts of pretreatments such as sonication, thermal, alkaline(Ayala-Parra et al., 2018), acidic(Barbot et al., 2015), thermal(Tedesco et al., 2013), thermo-chemical(Jard et al., 2013a), beating(Montingelli et al., 2017), milling, grinding and extrusion(Tedesco et al., 2014), and biological pretreatment(Vidmar et al., 2017).
1.3.1 Sonication pretreatment

During sonication, microbubbles are formed due to the high pressure applied to the liquid (Rasapoor et al., 2016), the collapse of these microbubbles during the sonication process will lead to a change of the chemical structure by generating free radicals and a large amount of energy is released to a small area (Rasapoor et al., 2016; Zeynali et al., 2017). This physical disintegration will increase the microbial activity which in turn enhance the biogas production (Zeynali et al., 2017).

Ultrasound technology has been proved as one of the most effective methods compared with other pretreatments such as bacterial, thermal and chemical (Zeynali et al., 2017). It can promote cell disintegration which will lead to the release of intracellular soluble organic matter (OM) and enhance the efficiency of the AD process (Ayala-Parra et al., 2018). Kumar et al. (2017) found that higher energy input and longer duration will result in the increase of organic release, but better solubilization doesn’t directly contribute to the enhancement of biogas production, the formation of some by-product will have a toxic effect on the microbial activities and will affect the biogas production. Too long time of sonication will cause an increase of particle size due to the re-flocculation phenomena between the particles (Rasapoor et al., 2016).

1.3.2 Microwave pretreatment

Microwave pretreatment is one kind of thermal pretreatments, it can directly deliver heat into the material by using microwave energy (Sapci, 2013). The alternating electric field by microwave irradiation can rapidly change the dipole orientation of polar molecules and generate heat (Li et al., 2012; Sapci, 2013). Microwave pretreatment can add kinetic energy into water which is contained in the biomass and the heat and pressure generated by the process will benefit the hydrolysis of the cell wall and help release of components from the cell (Romagnoli et al., 2017).

The advantages of microwave include uniform heating of the material and increased energy efficiency, but can also reduce the processing time and have an exceptional control of heating process (Sapci, 2013). Microwave pretreatment shows better degradation performance when combined with other technologies (Yu et al., 2017). Yu et al. (2017) found that the combination of microwave and alkaline pretreatment could significantly increase the cumulative biogas production (Saifuddin et al., 2009).
Saifuddin et al. reported that the microwave in combination with ultrasonic pretreatment will increase the biodegradability of soluble organic material and can also improve the biogas production. But sometimes the microwave will have adverse effects on the AD process and lead to a decrease in biogas production. Sapci (2013) investigated the microwave pretreatment on biogas production from agricultural straws, and found that the increase of temperature led to lower biogas production, and the microwave pretreatment did not improve the AD process. Li et al. (2012) also found that the total methane produced after microwave pretreatment decreased by 12% when compared with conventional thermal pretreatment.

1.4 The aim of the study

The aim of the study was to investigate if different pretreatment methods could influence the biogas production of seaweeds. The study wanted to answer the following three questions:

- Do different pretreatments significantly influence biogas production of seaweed?
- What’s the value of maximum methane production?
- Which pretreatment will contribute most to the biogas production?
2. Methods

2.1 Substrate and inoculum

Fucus vesiculosus and Fucus serratus were collected on the shore outside Halmstad, Sweden. The leachate collected from a full-scale biogas plant in Laholm, Sweden, was used as a source of inoculum and the inoculum was incubated at 37°C before starting the experiment. The first experiment used the mixture of Fucus vesiculosus and Fucus serratus as substrate which was harvested at Rinqenäs on March 22th and the second experiment use Fucus serratus as substrate which was harvested at Tjuvahålan and Svärgarehålan on April 9th.

2.2 Different pretreatments

All the seaweeds were cut with a grinding mill after washing them.

The ultrasonic pretreatment was performed in an ultrasonicator (J.P.SELECTS s.a.), with 110V for 10, 15 or 20 min. The medium for ultrasonic pretreatment is the seawater collected from the sea in Halmstad.

The microwave pretreatment was performed in a glass plate and the samples were exposed to microwave at 400 W or 600W for 2 min. The weigh variation during the microwave pretreatment were measured by electronic scales (up to 0.01 gram).

The combined (ultrasonication + microwave) pretreatment was performed by ultrasonic pretreatment with 110V for 15 min, followed by microwave pretreatment at 600W for 2 min.

2.3 Batch biogas production

The biogas production tests were performed in a batch mode using 1000 ml bottles. Batch tests were prepared in triplicates. Each bottle was filled with 460 g of inoculum and 40 g of the pretreated seaweeds, the control bottles contained same amount of inoculum. After the bottles were filled with the substrate and inoculum they were kept in an incubator with a temperature of 37 ± 0.1 °C. Two experiments were performed. The pretreatments of each bottle are shown in the table 2.3.1 and table 2.3.2.
### Table 2.3.1 pretreatments for each bottle in first experiment

<table>
<thead>
<tr>
<th>Bottle number</th>
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<th>Ultrasonic 10 min</th>
<th>Ultrasonic 20 min</th>
<th>Microwave 400W, 2 min</th>
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### Table 2.3.2 pretreatments for each bottle in second experiment

<table>
<thead>
<tr>
<th>Bottle number</th>
<th>mechanical</th>
<th>Ultrasonic 20 min</th>
<th>Microwave 600W, 2 min</th>
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<td>13, 14</td>
<td>Control group</td>
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2.4 Analytical method

The seaweeds were stored in a refrigerator (±4°C) for future use. Measurement of total solids (TS) and volatile solids (VS), were performed according to the protocol described in the APHA standard method which means drying under 105°C for 24 h and further baking at 550 ± 25°C (American Public Health Association, 1998). Both TS and VS analyses were performed in triplicate. pH was measured by pH meter. The content of carbon and total N was determined by element analyzer (Mark FlashEA 1112 Series NC Soil Analyzer). The biogas composition was determined using a gas chromatograph (VARIAN CP-3800).

One-way ANOVA tests were performed with software SPSS 16.0 to check if there were statistical significant differences in biogas production between different pretreatment group. The statistical significance level was selected at p-value < 0.05.

Methane yield can be calculated as the equation 1

\[
\text{methane yield} \left( \frac{\text{ml}}{\text{g VS}} \right) = \sum_{n} \frac{A \times E}{B \times C \times D} \quad (\text{Equation 1})
\]

n: the time of biogas production, day
A = total biogas production
B = the amount of substrates
C = TS of substrate
D = VS of substrate
E = methane content in biogas, %

The net production of methane from algae equal to biogas yield of each bottle minus the methane yield from the control group.
3. Result
The experimental results are presented with the following figures and tables.

3.1 TS, VS, pH value, C/N ratio
The TS and VS of *Fucus vesiculosus* and *Fucus serratus* are presented in table 3.1.1.

<table>
<thead>
<tr>
<th></th>
<th><em>Fucus vesiculosus</em></th>
<th><em>Fucus serratus</em></th>
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<tbody>
<tr>
<td><strong>TS</strong></td>
<td>17.74%</td>
<td>19.98%</td>
</tr>
<tr>
<td><strong>VS</strong></td>
<td>85.24%</td>
<td>86.73%</td>
</tr>
</tbody>
</table>

The pH of inoculum in the first experiment was 7.8 and pH of the inoculum in the second experiment was also 7.8. The pH of each bottle after the experiment are presented in table 3.1.2 and table 3.1.3.

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<td><strong>pH</strong></td>
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<th>4</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<th>11</th>
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<th>14</th>
</tr>
</thead>
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<tr>
<td><strong>pH</strong></td>
<td>7.50</td>
<td>7.51</td>
<td>7.54</td>
<td>7.51</td>
<td>7.53</td>
<td>7.49</td>
<td>7.53</td>
<td>7.51</td>
<td>7.53</td>
<td>7.62</td>
<td>7.61</td>
<td>7.50</td>
<td>7.49</td>
<td>7.53</td>
</tr>
</tbody>
</table>

The C/N ratio was on average 9.3±1.6.

3.2 Biogas production in first experiment
Figure 3.2.1 shows the average biogas production from each pretreatment group. The groups under ultrasonic 10min, microwave 2min and mechanical pretreatment stopped producing biogas after five days of the experiment, only the group with the pretreatment of ultrasonic 20 min still produced biogas. This means the reaction of most bottles didn’t successfully start.
The average methane content and average cumulative methane yield of each group are presented in figure 3.2.2 and figure 3.2.3. After 6 days of digestion, the cumulative methane yield was very low, the maximum value was only 12.5 ml/g·VS from the group with the ultrasonic pretreatment for 20 min. The other three groups showed a very slow increase of methane production and the methane content of each group was only about 20%.
We found that the reason for the failure of this experiment was that we didn’t mix the leachate from the biogas plant very well, so the leachate was not homogeneous. This led to the microorganism were in the sediments of the leachate, and since we only used the top of the leachate as our inoculum the reaction didn’t start successfully.

3.3 Biogas production from the second experiment

Figure 3.3.1 shows the average biogas production from each pretreatment group in the second experiment. The biogas production was stable, continuous and lasted for 20 days. The maximum biogas production was 2920 ml with combined pretreatment, followed by 2644 ml with ultrasonic pretreatment, 2598 ml with microwave pretreatment, 1905 ml with mechanical pretreatment and 505 ml with control group.
The average methane content of each group is presented in figure 3.3.2. All the bottles showed the same trend of methane content, with a rapid increase during the first five days and a slight increase until the 13\textsuperscript{th} day of the digestion, and then the control group and the group with ultrasonic pretreatment for 15 min showed a little decrease of the methane content. The methane content of experimental groups finally stabilized at 75\%. The biogas content of control group was stabilized at 50\%.

![Graph showing average methane content of each group](image)

Figure 3.3.2 average methane content of each group in the second experiment

Figure 3.3.3 shows the cumulative methane yield of each group and we can see that the group 2 with pretreatment of 2 min of microwave plus 15 min of ultrasonic showed the best biogas production ability and obtained the highest cumulative biogas content of 260 ml/g\cdot VS during the 20 days in the second experiment.
Figure 3.3.3 Average cumulative methane yield of each group in the second experiment

The control group only contain 500 gram of inoculum in each bottle, so the methane yield was very low, which means the remaining biogas produced by the microorganisms. Except the control group, other groups show a large increase in methane yield during the first 5 days, but we can’t see obvious difference between the groups. The methane content of each group increased almost up to 60% during the first 5 days (Figure 3.3.2) meaning that the reaction was working.

At day 6-13, the methane yield of the groups with microwave and ultrasonic pretreatment increased rapidly while the group only with mechanical pretreatment showed a limited increase of biogas production. Compared with the mechanical pretreatment group, the groups with ultrasonic 15min, ultrasonic 15min +microwave 2min and microwave 2 min showed increasing methane yields of 167%, 185% and 156%, respectively.

At day 13-20, the biogas production rate of each group became more slow and the difference in methane yield of each group were obvious.

3.4 Statistical analysis

From one-way anova test, we conclude that the mean cumulative methane yield was significantly different for at least one of the different pretreatment groups \( F(3, 108) = \)
2.824, \( p = 0.042 \)). Table 3.4.1 shows the output from post Hoc test (equal variances assumed by Tukey). We can see the mean cumulative methane yield of group 2 was significantly different from group 4. Figure 3.4.1 shows the difference of mean methane yield between different pretreatments with standard deviations. Above all, we conclude that the pretreatment with ultrasonic for 15min+microwave 600W, 2min could significantly improve cumulative methane yield compare with the mechanical pretreated group.

Table 3.4.1 output from post Hoc test G1: group 1 with ultrasonic for 15min. G2: group 2 with ultrasonic for 15min+microwave 600W, 2min. G3: group3 with microwave 600W, 2min. G4: group 4 only with mechanical pretreatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 vs G2</td>
<td>0.883</td>
</tr>
<tr>
<td>G1 vs G3</td>
<td>0.990</td>
</tr>
<tr>
<td>G1 vs G4</td>
<td>0.141</td>
</tr>
<tr>
<td>G2 vs G3</td>
<td>0.728</td>
</tr>
<tr>
<td>G2 vs G4</td>
<td>0.027</td>
</tr>
<tr>
<td>G3 vs G4</td>
<td>0.241</td>
</tr>
</tbody>
</table>

Figure 3.4.1 difference of mean methane yield between different pretreatments with standard deviation. G1: group 1 with ultrasonic for 15min. G2: group 2 with ultrasonic for 15min+microwave 600W, 2min. G3: group3 with microwave 600W, 2min. G4: group 4 only with mechanical pretreatment.
4. Discussion

Figure 3.3.3 showed the influence of various pretreatments on cumulative methane yield. All the three pretreatments had positive effects on the methane yield. Methane yield was observed in the range of 122-260 ml/g-VS. The combined pretreatment showed the maximum biogas production of 260 ml/g-VS. This improvement was over 2-fold higher than the mechanical treated group of 122ml/g-VS.

Average cumulative methane production from mechanical pretreatment group showed the methane yield of 138 ml/g-VS, which was similar with the result from Allen et al.(2015), who found that the methane yield of *F. vesiculosus* and *F. serratus* harvested in August was 126.3 ± 11.4 ml/g-VS and 101.7 ± 9.4 ml/g-VS, respectively. This variability may be caused by different harvest location and time. Tedesco et al. (2017) reported the methane yields obtained from *Fucus spp*. Harvested in October and after extraction of high value compounds such as alginic acid and proteins was 100 ml/g-VS. The low methane yield could be explained by the fact that high value compounds had already been extracted from the biomass.

4.1 pH

The pH from the first experiment range from 7.14-7.24 which was low compared with the second experiment where pH ranged from 7.49-7.61. It was another indication that the first experiment was a failure.

During the first half of AD process, hydrolysis, acidogenesis produce a large amount of volatile fatty acids (VFA) which lead to a decrease of pH (Zeynali et al., 2017). Methanogens is very sensitive to pH, and the microbes can only survive in neutral or weak alkaline environment (Yu et al., 2017). If pH dropped within the acidic range, methanogenic activity would be hindered, which would result in the inhibition of biogas production. Montingelli et al.(2016) found the pH value between 7.4 and 7.6 after anaerobic digestion of *Laminaria spp.*, which wouldn’t cause the strong inhibition of methanogens. This result was consistent with our experimental result.

4.2 Ultrasonic pretreatment

Although the effect of ultrasonic pretreatment was not significantly different from the mechanical pretreated group, it had a positive effect on biogas yield (increased methane yield by 67%). Many studies showed that ultrasonic pretreatment of substrate could both reduce the processing time of AD process and enhance the biogas yield efficiency.
The effect of ultrasonic pretreatment depended on many factors such as: substrates type, the duration of ultrasonic power and frequency (Park et al., 2013; Zeynali et al., 2017). Karray et al. (2015) demonstrated that the 2.53g/L of reducing sugar in *Ulva rigida* was obtained through ultrasonic, compared with 0.6 g/L of reducing sugar in crude macroalgae. This means that ultrasonic pretreatment is important for hydrolysis of carbohydrate polymers to reducing sugar. Reducing sugar was a suitable substrate during digestion for biogas production. Park et al. (2013) also found that ultrasonic pretreatment could successfully improve the disintegration and anaerobic biodegradability of *Chlorella vulgaris*, but ultrasound at low doses (below 50 J ml⁻¹) couldn’t improve the biogas production. For specific energy higher than 50 J ml⁻¹, biogas production was proportional to specific energy.

When investigating biogas production from fruits and vegetable wholesale market waste, Zeynali et al. (2017) found that the highest methane production was obtained at 18 min sonication while longer time led to lower methane yield. A similar trend was reported by Hay et al. (2015). Longer ultrasonication time could increase the dissolved carbohydrate concentration, but no significant differences between ultrasonication times of 45 min or 60 min was shown. A decrease in the biohydrogen yield was observed at ultrasonication times longer than 30 min with amplitudes exceeding 90% (Hay et al. 2015). It’s important to find the optimum condition for ultrasonic pretreatment. Ayala-Parra et al. (2018) reported ultrasonication under optimized conditions (100 power level, 5 min) could obtain a marked increase in the biogas yield compared with the untreated algae (327 and 146 mLSTP CH₄/g VS, respectively).

### 4.3 Microwave pretreatment

Microwave pretreatment could improve methane yield by 56% in this study. The positive effect of microwave pretreatment on methane yield have also been shown by Romagnoli et al. (2017) Microwave pretreatment with power of 700w could improve biogas production in a range of 7.8%–43.7% when applied for 1.5 min, and in a range of 37.2%–45.2% when applied for 3min.

Several studies showed that microwave pretreatment had no or adverse impact on biogas production. Montingelli et al. (2016) showed the microwave pretreatment on the substrate of *Laminaria spp.* registered a reduction of 27% on biogas production when compared with untreated seaweeds, while the solubilization of the seaweed was improved by the pretreatment. Yoruklu et al. (2018) also found that the biogas
production of the seaweed decreased by 47.2% compared with untreated seaweeds.

This adverse effect was not only limited in seaweed, but also was found in the substrate of energy crops and organic waste. Li et al. (2012) made experimental analysis on the biogas production of energy grass and found the maximum production rate and total methane production of the grass decreased by 18% and 12%, respectively. Sapci (2013) also found the microwave pretreatment of agricultural residual straws couldn’t enhance their anaerobic digestion.

This reduction in biogas yield through microwave pretreatment may be explained by the change in osmotic pressure and the by-production from solubilization of lignin through microwave pretreatment which has detrimental effect on anaerobic bacteria (Civelek Yoruklu et al., 2018; Sapci, 2013). Since the substrate in this study didn’t have much lignin, the adverse effect is not found in this study. It needs to be emphasized that when using microwave as a pretreatment, it’s very important to pick the suitable substrate and optimum microwaving power and reaction time.

### 4.4 Combined pretreatment

Ultrasonic pretreatment in combination with microwave pretreatment obtained the maximum methane yield of 260 ml/g-VS. This was a significantly improve methane yield by 85% compare with mechanical pretreatment. Saifuddin et al. (2009) also found that the combination of ultrasonic and microwave pretreatment on sludge was a rapid and economic way for improving biogas production. In order to achieve similar improvement using only ultrasonic or microwave pretreatment, the energy required was more than doubled.

The ultrasonic and microwave pretreatment could be combined with other pretreatments to achieve the goal of improving biogas production. Kumar et al. (2017) investigated experimentally the effect of combining pretreatments of electrolysis and ultrasonication on biogas production from mixed microalgae biomass. He found the methane production from combined pretreatment was 257 ml/g-VS compare with untreated biomass which was 138 ml/g-VS, The value was a little bit lower than the individual ultrasonication pretreatment, but the energy input was relatively low. Yu et al. (2017) demonstrated microwave assisted alkaline pretreatment could not only significantly improve the biogas production of swine manure, but also shorten the time of achieving a stable biogas production rate.
Sometimes the combined pretreatment couldn’t improve or even have adverse effect on biogas production. Ayala et al. (2018) found the ultrasonic alone could lead to 2.2-fold increase of biogas production on *Chlorella protothecoides* biomass but the ultrasonic combined with alkaline pretreatment resulted in a moderate decrease on biogas production.

### 4.5 Environmental benefits and considerations

The seaweed is a valuable substrate for producing renewable energy, but also for dealing with the eutrophication problem which is the major environment problem in the Baltic Sea. To keep the beach clean, a large amount of seaweed could be removed, but the harvest and deposit of seaweed is a difficult problem. The use of seaweed as a substrate for anaerobic digestion could achieve the synergy of mitigating both climate change and coastal eutrophication. Furthermore, the digestate from AD process could also be a promising substitution for nitrogen from mineral fertilizers.

Czyrnek-Delêtre et al. (2017) showed a new environmentally friendly circular economy concept; farming of seaweed combined with salmon farming and bioenergy production. The nutrient waste discharged from fish farms could increase the growth of seaweed with over 27%. The system could also decrease CO₂ emission by 70% compared to gasoline. Asri et al. (2017) also proposed a new system applied in lagoon, which comprises anaerobic digestion of algae that could be used to produce green energy for a lagoon aerator to dissolve the atmospheric oxygen into the lagoon. This system could decrease the problem of eutrophication and minimize the cost for operation of the system.

However, there are still some negative considerations that should be noted. For example, our result showed that microwave pretreatment combined with ultrasonic pretreatment (600W, 2min; 110V, 15min) could significantly improve methane yield when compared with mechanical pretreated only. But the noise from ultrasound equipment may cause negative symptoms such as dizziness, tinnitus, excessive fatigue to exposed operators (Zeynali et al., 2017). Therefore, the control of ultrasonic pollution was essential to the full-scale implement of ultrasound pretreatment. The seaweed always contains heavy
metals which could limit the use of digestate as fertilizers. The pretreatments were very important, since it could enhance seaweed hydrolysis. The hydrolysis of seaweed could improve the mobilization of metal ion into a liquid phase and make the fertilizers quality better (Nkemka and Murto, 2012).

5. Conclusion
The study showed microwave pretreatment (600W,2min), ultrasonic pretreatment (110V,15min), and microwave combined with ultrasonic pretreatment (600W,2min;110V,15min) were effective at enhancing both biogas yield and methane content of anaerobic digestion of brown seaweed compared with only mechanical pretreatment of the biomass (milling and grinding). Combined pretreatment provided a significant increase in methane yield. Combined pretreatment, ultrasonic pretreatment, and microwave pretreatment can improve methane yield by 85%, 67%, 56%, respectively, when compared with mechanical pretreatment in AD process incubated for 20 days.

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Reference


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My name is Yining WU, I am from China, I study Halmstad university for master degree.