Thermal hydrolysis and steam explosion pretreatment of different lignocellulosic aquatic weeds by anaerobic digestion

熱加水分解と水蒸気爆砕による異なる水草についてのメタン発酵処理能向上に向けた基質可溶化

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SYNOPSIS

Overgrowth of aquatic weeds has been occurring worldwide, leading to various social and environmental problems. Therefore, it is necessary to harvest and utilise these aquatic weeds. Anaerobic digestion (AD) is considered suitable for treating aquatic plant weeds as it has a lower impact on the environment and can recover energy in the form of biogas; further, the digestate can be used for various purposes. Previous studies pointed out that lignin in the lignocellulose structure of aquatic weeds limits the biodegradability and methane potential of the substrate. Therefore, before AD, a suitable pretreatment is required to make the lignocellulose structure more accessible to produce higher methane. Therefore, in this study, we conducted thermal hydrolysis (TH) and steam explosion (SE) pretreatment on different aquatic weeds having different chemical compositions. Chapter 2 (Study 1) clarifies the physical and chemical differences between the lignocellulosic aquatic weed pretreated by TH and SE. Chapter 3 (Study 2) evaluated the effect of TH and SE on the anaerobic digestion of lignocellulosic aquatic weeds. In Study 1, when results were interpreted, it was seen that substrate degradation leads to higher TOC readily available, which increases the amount of inhibitory compounds in the pretreated liquid substrate. For TH, this had an increasing linear trend regardless of the substrate's chemical composition. For SE, as the lignin concentration of the substrate increased, the TOC kept increasing, whereas the phenolic compounds reached an optimum point, leading to a different trend than TH. Study 2 revealed that all the three substrates' methane yield was enhanced after the TH and SE pretreatment. *In Eichhornia crassipes* and *Ludwigia grandiflora* the methane yield enhancement from the untreated condition was much higher for both TH and SE pretreatment than *Hydrilla verticillata*.

Keywords: Aquatic weed, anaerobic digestion, lignocellulose, steam explosion, thermal hydrolysis.

Introduction

Generally, aquatic weeds play a vital role in freshwater ecosystems by maintaining water quality and providing habitat for marine life[1]. However, within a few decades, due to poor agricultural and waste management practices, the overgrowth of these aquatic weeds has been taking place worldwide[2]. Overgrowth of aquatic weeds is causing environmental issues and economic losses such as ecosystem change and sailing disturbance in lakes, dams and reservoirs[3,4]. Therefore, there is an urgent need to harvest and utilise these weeds by cost-effective and sustainable methods.

Anaerobic digestion (AD) is a method that can be used for harvesting aquatic weeds due to its relatively simple process and lower processing cost. AD allows bioenergy recovery in the form of methane (CH_4) from plant biomass with high moisture content. Also, the nutrient-rich digestate can be used as a liquid fertilizer[5]. Previous studies have investigated the AD of various plant biomass and reported that the lignin content of the biomass mainly limits CH4 recovery[5].

Plant biomass has a lignocellulose matrix which consists of cellulose, hemicellulose and lignin. Due to their high biodegradability, cellulose and hemicellulose are the primary carbon sources for CH_4 recovery. In contrast, lignin is the most resistant polymer having a complex three-dimensional structure[6,]7. Koyama et al., 2014[5] revealed that the CH_4 yield of aquatic weeds varies due to their lignin composition and content. Therefore, before performing AD, a suitable pretreatment is required to disrupt the lignocellulose structure and make it more accessible, enhancing CH_4 production.

Pretreatments on various plant biomass have been widely attempted to improve biomass degradability[8]. Generally, pretreatments work differently to break the lignocellulosic material's complex structure, leading to different products and yields. While some pretreatments have successfully transitioned from a research platform to the industrial stage, significant challenges remain, including the generation of environmentally hazardous wastes and/or high energy inputs; there is a pressing need for green technology solutions to this challenge[9,10].

Among the various pretreatments, thermal pretreatments, i.e., thermal hydrolysis (TH) and steam explosion (SE), can be considered the best choice as green and easily scalable pretreatment methods for breaking the lignocellulosic structure of aquatic weeds. TH and SE use chemical and physical reactions to degrade lignocellulosic biomass8. Due to the high temperature and pressure imposed, cellulose and hemicellulose degradation occurs, and lignin transformation occurs, enhancing the overall hydrolysis and making the surface more accessible. Both pretreatments lead to similar autohydrolysis, the sole difference between the two being the rapid decompression (explosion) that takes place only at the end of the SE pretreatment (Figure 1). This decompression forces the fibrous material to expand rapidly. The fibres and bundles separate, creating a solid fraction with a more open structure[11] and transferring sugars and inhibitors to the liquid fraction may improve the effectiveness of subsequent treatments.

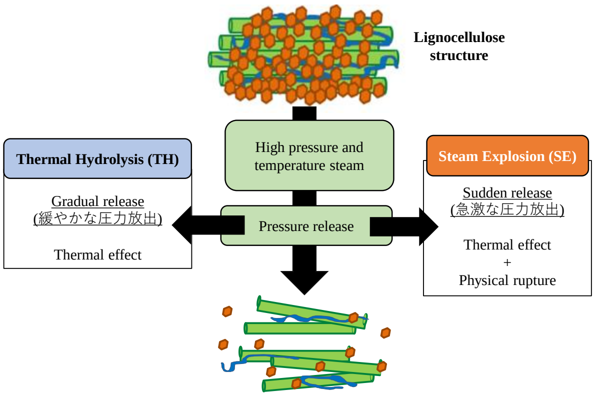

Figure 1. Schematic diagram of Thermal Hydrolysis and Steam explosion pretreatment

When TH and SE pretreatment was compared in previous studies, the trend observed when SE was used led to higher substrate degradation. Various studies mention that the concentration of inhibitors can increase with the increase in substrate degradation, negatively affecting the AD process[5,12,13]. The effect of explosion contributes to the efficiency of the process, but different researchers claim different views[14,15]. Brownell et al., 1986[16] pointed out that the importance of explosion effects in SE pretreatment was minimal when using high temperatures. On the other hand, another study by Wang[17] comparing TH and SE showed a significant difference in digestibility of pretreated substrate, and explosion played a vital role in pretreatment. Therefore, this study will de-couple TH from SE pretreatment. Both the pretreatments can be used on the same machine under the same conditions. This is the first study to clarify the effects of SE and TH on the AD of different lignocellulosic aquatic weeds. Therefore, the gap in the mechanism of these pretreatments and the parameters affecting each pretreatment will be studied further to clarify the effect of inhibitors released during the process.

This PhD thesis aims to reveal the appropriate pretreatment between TH and SE upon the AD of different lignocellulosic aquatic weeds. The specific objectives are Chapter 2) To clarify the physical and chemical differences of lignocellulosic aquatic weeds pretreated by SE and TH. Chapter 3) To examine the effect of TH and SE on the anaerobic digestion of lignocellulosic aquatic weeds. Therefore, Chapters 2 and 3 will compare the SE and TH mechanism, which has not been studied before. Therefore, Study 1 and Study 2 compare the TH and SE mechanisms, which have not been studied before. After that, a proposal of an appropriate pretreatment based on biomass type is discussed.

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Materials and Methods

Study 1. Comparison of physical and chemical differences of substrates pretreated by SE and TH pretreatment

Substrate: Three different aquatic weeds were used as substrates in this study; *Eichhornia crassipes* (floating type), *Hydrilla verticillata* (submerged type) and *Ludwigia grandiflora* (emergent type). *E. crassipes* was harvested from a pond in Saitama in December 2019. *H. verticillata* was harvested from the southern basin of Lake Biwa in September 2020. *L. grandiflora* was harvested from the southern basin of Lake Biwa in July 2018 (for SE pretreatment) and September 2020 (for TH pretreatment). The substrates were milled into 3–5 cm using a milling machine and stored at −20 °C to be used in the experiment.

Pretreatment: TH and SE pretreatment were conducted in a 3-L reactor equipped with an electric heater and a flash tank (Nitou Kouatsu Co. Ltd, Japan). Pretreatments were performed under eight different conditions for each substrate. The pretreatments were conducted at two retention times of 10 and 30 min with temperatures of 150, 165, 180 and 210 °C. The severity/intensity of SE and TH is defined through the severity factor (SF) used to characterise the combined effects of temperature and time[18]. This factor can be determined from Eq. as follow:

$$SF = \log\left[t \cdot \exp\left(\frac{T\text{-}100}{14.75}\right)\right]$$

where t is the retention time in minutes, T is the temperature in degrees centigrade, and the value 14.75 is the activation energy under first-order process kinetics, following the Arrhenius law.

Each substrate (500 g-wet weight (g-wwt)) and Milli-Q water (750 mL) were added to the reactor boiler to obtain a solid to liquid ratio of 1:1.5 (w/v). Steam was supplied from the electric steam boiler at the top of the reactor and heated until the desired temperature for pretreatment was reached. At the end of the retention time, *for SE pretreatment,* the pressure was rapidly reduced to atmospheric pressure for disrupting the substrate structure, whereas, for *TH pretreatment,* the pressure was gradually reduced, avoiding the explosion effect. The pretreated slurry was instantly transferred into a flash tank. The slurry was mixed, weighed, and separated into solid and liquid fractions using a sieve with a mesh opening of 500 μm and stored at −20 °C for future use.

Total solids (TS) and volatile solids (VS) for solid and liquid fractions were measured by following standard methods from APHA (2005).

For Solid fraction: For Solid fraction: Scanning electronic microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were measured to see the morphological difference. Carbon, Nitrogen was measured by a CHN analyser (2400 CHNS/O Series II System, Perkin Elmer). Lignocellulose (cellulose, hemicellulose and lignin) contents were measured by a detergent method using a fibre analyser (Model: A-200, Ankom Co. Ltd, USA).

For liquid fraction: Total organic carbon (TOC) and (DOC) were measured by a total organic carbon analyser. Phenolic compounds were measured according to the Folin-Ciocalteau method. Dissolved lignin concentration in the liquid fraction was measured following the same method described in Koyama et al. 2017[19].

Study 2. Effect of pretreatments on the anaerobic digestion of pretreated lignocellulosic aquatic weeds

Substrate: All the 3 untreated and pretreated aquatic weeds used in study 1 will be used as substrates.

Inoculum: Mesophilic anaerobic sludge was collected from the Hokubu Sludge Treatment Center, Kanagawa Prefecture, Japan. The collected sludge was stored for 2 days at 37 ± 1 °C.

Bio-methane potential tests (BMP) were carried out at a mesophilic temperature of 37 ± 1 ◦C for 15-20 days. The substrate (1.7 g-VS) of combined solid and liquid fractions was added to a 500-mL medium bottle. The substrate to inoculum ratio in the reactor was 1:2 based on the VS content. The VS content of 1.7 g was adjusted with the help of a mixed VS and L/S ratio for every condition of the BMP reactor. All tests and blanks were conducted in triplicate, and the net methane production values were calculated. BMP tests were conducted by an automatic AD system (AMPTS II, Bioprocess Control AB, Sweden) with agitation/no agitation cycles of 10 s.

The following parameters were measured to monitor the experiment: Methane yield, methane production rate, and modelling were done by fitting the methane production to the modified Gompertz model[20].

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Results and Discussion

Study 1. Clarification of physical and chemical difference of substrates pretreated by SE and TH pretreatment

Solid fraction: The lignocellulose values in the solid fraction of aquatic weeds were measured (Figure 2). The values of *E. crassipes* and *H. verticillata* differed from previous studies. For example, a study by Castro et al., 2021[22] shows that the hemicellulose content in *H. verticillata* was 50.3 %, whereas, in this study, the hemicellulose was not detected whereas the values of cellulose and lignin were quite similar. Also, for *E. crassipes*, the hemicellulose value was similar to Kaur et al., 2019[23]. However, the values of cellulose and lignin varied a lot, with lignin being 7.72 % in the study by Kaur et al., 2019[23]. Fujiwara et al., 2022[24] revealed that the harvesting time could significantly change the values of these components, which can further affect the anaerobic digestion of these substrates.

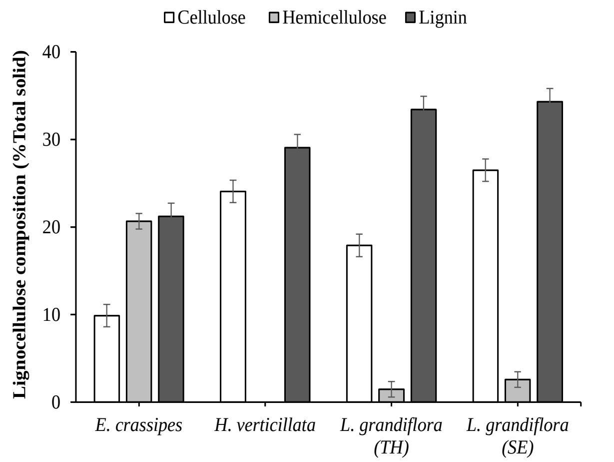

Figure 2. Lignocellulose components of the untreated substrates used in this study

Liquid fraction: To check the solubilisation of the liquid fraction by TH and SE pretreatment, the total organic carbon (TOC) relationship with the phenolic compounds was plotted (Figure 3). Interestingly, with the increase in the TOC value, the phenolic compounds were increasing. The Figure explains that for *E. crassipes* and *H. verticillata*, the relationship of phenolic compounds increased with the increase in the total organic carbon

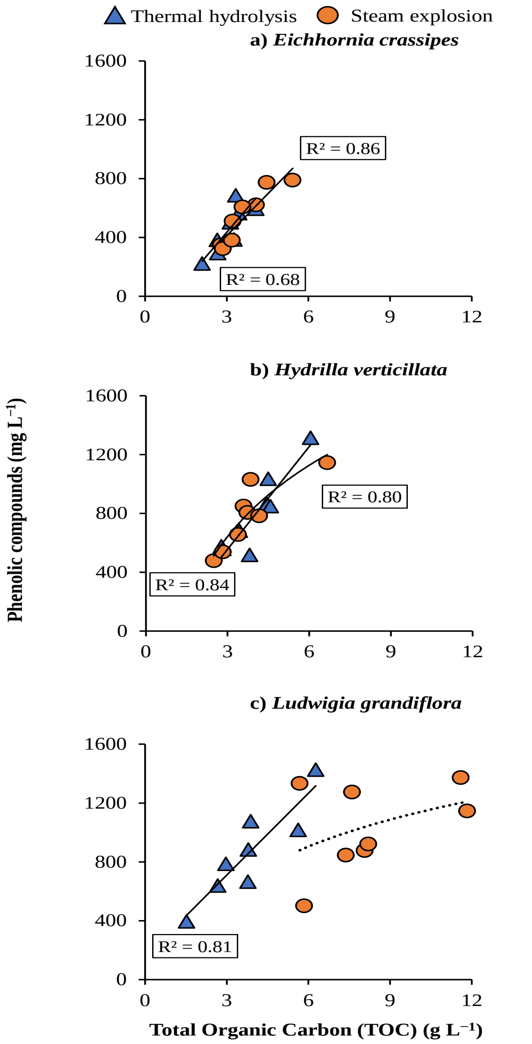

Figure 3. Phenolic compounds concentration relationship with total organic carbon in liquid fraction during thermal hydrolysis and steam explosion pretreatment

concentration for both TH and SE. The trend was a little different for *L. grandiflora*; for TH pretreated biomass, it had a positive linear relationship with the increase in the total organic carbon, whereas, for SE, there was no linear relationship with the total organic carbon, but the values of phenolic compounds were even higher at the low temperatures which can also be seen from Figure 3. It can be seen that TH for all the substrates had an increasing trend despite the lignin in the untreated substrate kept on increasing. The substrate degradation led to higher TOC readily available, increasing the amount of phenolic compounds in the pretreated liquid substrate. For TH, this had an increasing linear trend regardless of the substrate's chemical composition. For SE, as the lignin concentration of the substrate increased, the phenolic compounds reached an optimum point, whereas the TOC kept increasing, leading to a different trend than TH.

Study 2. Effect of pretreatments on the anaerobic digestion of pretreated lignocellulosic aquatic weeds

The methane yield for the untreated *E. crassipes* is 166.57 mL g-VS-1, compared with a study done by Kist et al. (2018)[25], which shows that the methane yield (140-193 mL g-VS-1) is similar to this study. The value for the untreated *H. verticillata* in this study was 231.81 mL g-VS-1as shown in Table 3-2, whereas in a previous study, the value was 81 mL g-VS-1 (Abbasi et al., 1990). This difference can be attributed to factors such as harvesting period, growth origin, and degradability (Li et al., 2014; Fujiwara et al., 2022) [24,26]. The untreated methane yield for *L. grandiflora* was 85.84 mL g-VS-1. *L. grandiflora* is considered an emergent aquatic plant with a rigid body because it emerges from the water[27]. *L. grandiflora* has a lignin amount of 34.3%TS. Lignin strengthens a plant's cell walls; thus, rigidity can be shown by lignin content[28] which may have affected the methane yield.

The methane yield improvement was evaluated after TH and SE pretreatment for all the substrates used. The methane yield for *E. crassipes* was improved by 89.91% (TH) and 56.83% by SE pretreatment. Compared with Enrique 2019 (master study)[29], the methane yield was improved by 16.3% (TH) and 37.9% by SE pretreatment. For *H. verticillata*, the improvement was 24.44% for TH and 11.33 for SE pretreatment. For both *E. crassipes* and *H. verticillata*, the improvement was more significant for thermal hydrolysis pretreatment, but as the lignin concentration increased to 34.2% for *L. grandiflora*, the methane yield improvement was higher for SE (216.13%) than TH (140.72%) pretreatment. This is further discussed in the general discussion.

Conclusion and Summary

The present study compared SE and TH pretreatment to the physical and chemical differences of different aquatic weeds (Study 1) and the effect of these pretreatments on anaerobic digestion (Study 2). The key findings are listed below: Methane yield improvement. Figure 4 compares the methane yield improvement with the lignin concentration of different types of lignocellulosic biomass from previous studies. The methane yield improvement was more significant for TH pretreatment when lignin concentration was lower at 21.2% for *E. crassipes* and 29.1% for *H. verticillata*. In contrast, when the lignin concentration was a maximum of 34.3% for *L. grandiflora*, SE pretreatment significantly enhanced the methane yield improvement (216.13 %). Both TH and SE pretreatment enhanced the methane yield of aquatic weeds significantly. TH pretreatment was sufficient to improve the methane yield for a wide range of lignocellulosic biomasses, whereas SE pretreatment helped disrupt the substrate structure with maximum lignin content (*L. grandiflora*= 34.2%TS). The value of the lignin polymer in the untreated substrate can help to evaluate the methane yield improvement yield. This evaluation of methane yield improvement can be helpful for new studies/research as only the lignin concentration is used for the evaluation. This is the first study which proposes a methane yield improvement estimation model for both TH and SE pretreatment. Also, as no equipment is needed for TH pretreatment, its application/feasibility needs to be studied further.

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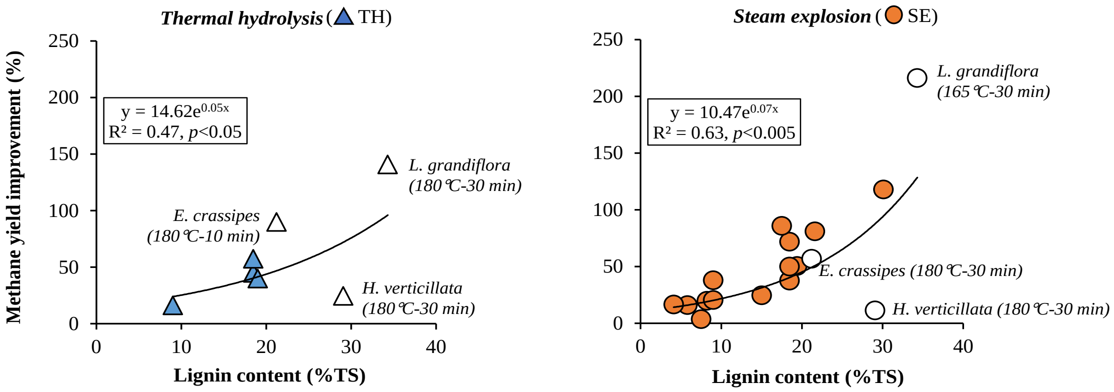

Figure 4. The methane yield improvement relationship with the lignin concentration of different lignocellulosic biomass from previous studies