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Enhancing anaerobic digestibility of lignin-rich submerged macrophyte using thermochemical pre-treatment

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ABSTRACT

The present study investigated the effect of alkaline thermochemical pre-treatment on anaerobic digestibility of two submerged macrophyte species which have significantly different lignin content. The highest hydrolysis efficiency was achieved at NaOH loading rate of 0.20 g g-$TS_{substrate}^{-1}$, 80 °C, 3.0 h for both species. Alkaline delignification was much conspicuous in lignin-rich macrophyte (*Potamogeton maackianus*) as compared with lignin-poor macrophyte (*Egeria densa*). Ferulic acid, which is cross-linked with lignin polymer and polysaccharides, remarkably declined with increase of NaOH loading rate. It suggests that alkali removed lignin–ferulate complex from the surface of polysaccharides. The CH_4 yield of pre-treated *P. maackianus* was 243 mL g-VS^{-1}, which is 51% higher than the un-treated (161 mL g-VS^{-1}). In contrast, the CH_4 yield of pre-treated *E. densa* was 24% higher than the un-treated. These results indicated that alkaline thermochemical pre-treatment could be an effective method for anaerobic digestion of lignin-rich macrophytes. However, it was also suggested that the high NaOH addition to lignin-rich macrophyte possibly inhibit the methane recovery, due to the increase of solubilized lignin in the digestate.

1. Introduction

The excessive propagation of vascular aquatic weeds including submerged macrophytes has lately been posing threats in lakes, dams and reservoirs throughout the world due to the eutrophication, and the treatment of the harvested phytomass has been explored [1–4]. In Japan, the infestation of submerged macrophytes is problematic in Lake Biwa, which is the largest lake in Japan [5]. In Lake Biwa, submerged macrophytes are responsible for a number of impacts including water stagnation, foul odor, fishing interference, ecosystem change and landscape fouling [6,7]. Anaerobic digestion (AD) is expected for the effective alternative treatment of harvested macrophyte wastes, since it recovers bioenergy from the substrate with high moisture content. The reported CH_4 yields of aquatic weeds greatly fluctuate from 38 to 361 mL g-volatile solids $(VS)^{-1}$ with species [1,3,4,8,9]. Plant biomass is considerably recalcitrant for anaerobic biological degradation mainly due to the lignin barrier on cellulose and hemicellulose [10]. Previous studies have revealed the negative relationship between CH_4 yield and the lignin content of various plant biomass including submerged macrophytes [9]. Thus delignification pre-treatment should be applied to obtain the sufficient CH_4 recovery from lignocellulosics.

Amongst various pre-treatments, thermochemical pre-treatment using alkali is noted as one of the most promising methods for hydrolysis of lignocellulose, mainly owing to the strong and rapid delignification ability [11,12]. It is known that the degradability of lignin by chemicals depends on the composition of lignin [13]. Lignin polymer consists of three different phenylpropane unit; guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) lignin. Angiosperm lignin is generally dominated by S and G lignin, while gymnosperms contain fewer S lignin. Within angiosperm, non-woody tissues (e.g., leaf and pollen) and herbaceous plants have all G, S and H lignin. In addition, a number of studies have stated that lignin of herbaceous plants and non-woody angiosperm tissues contain hydroxycinnamic acids (*p*-coumaric and ferulic acids). G, S and H lignin are combined with each other by alkali-stable ether bonds; whereas, hydroxycinnamic acids has alkali-labile ester bonds, forming lignin/phenolics-carbohydrate complexes (LCCs) by combining with lignin polymer and/or hemicellulose [13]. Koyama et al. [9] revealed that submerged

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macrophytes contain substantial amount of hydroxycinnamic acids, which accounts for 27.2–59.4% of total lignin phenols. Accordingly, submerged macrophytes are expected to be easily delignified by using alkali under relatively mild pre-treatment conditions (e.g., low temperature, low alkali dose and short treatment time), as compared with woods.

Previous studies about alkaline thermochemical pre-treatment coupled with AD has investigated terrestrial herbaceous plants such as grass silage [12], sunflower stalk [14], smooth cord-grass [15] and asparagus stem [16]. Xie et al. [12] reported that alkaline thermochemical pre-treatment enhanced the CH_4 yield of grass silage from 325.8 to 452.5 mL g-VS^{-1} at 100 °C, NaOH loading rate of 7.5% (w/w) against the VS of the substrate. For aquatic weeds, alkaline thermochemical pre-treatment was applied to co-digestion of water hyacinth (floating macrophyte) and cow manure [8], and two-stage (H_2–CH_4 fermentation) AD process of water hyacinth [17]. However, no studies have investigated the effect of alkaline thermochemical pre-treatment on lignocellulose degradation and CH_4 recovery of aquatic weeds. Submerged macrophytes tend to have more flexible body structure as compared with terrestrial herbaceous plants and floating/emergent macrophytes, because they are mostly submerged under water [18]. Thus, the effect of alkali on lignocellulose degradability and CH_4 recovery of submerged macrophytes may be significantly different from other plants.

The objective of the present study was to investigate the effect of alkaline thermochemical pre-treatment on mesophilic AD of submerged macrophytes. The present study used two dominant submerged macrophyte species in Lake Biwa, which have remarkably different lignin content. *Potamogeton maackianus* is a lignin-rich indigenous macrophyte species which is dominated approximately 56% in Lake Biwa [5]. An invasive species *Egeria densa* (dominance 11%) was also used to compare/contrast the results with *P. maackianus*, since the lignin content of *E. densa* is much lower than *P. maackianus* [9]. In the present study, hydrolysis test and batch AD test of submerged macrophytes after thermochemical pre-treatment were conducted.

2. Materials and methods

2.1. Substrates and inoculum

P. maackianus and *E. densa* were harvested from the Southern Basin of Lake Biwa, Shiga prefecture, Japan. Fresh macrophytes were shredded to the particle size of 0.5–1.5 cm and preserved at −20 °C for the experiment. The chemical components were shown in Table 1. The lignin content of *P. maackianus* was 20.7%-TS, which was more than fourfold as compared with *E. densa*. For the inoculum of batch AD test, the mesophilic anaerobic sludge was obtained from full-scale biogas plant (6800 m^3) treating domestic sewage in Hokubu Sludge Treatment Center, Yokohama, Japan. The total solids (TS) and volatile solids (VS) contents of the sludge were 3.0%-wwt and 2.1%-wwt, respectively. The anaerobic sludge was degassed at 37 °C for 2 days before batch AD test, to digest the residual organics left in the inoculum.

Table 1
Chemical composition of submerged macrophytes used in the experiments of thermochemical pre-treatment.

Parameter	Unit	*Potamogeton maackianus*	*Egeria densa*
Total solids (TS)	%-wwt	9.7	4.9
Volatile solids (VS)	%-wwt	8.2	4.0
VS/TS	%	84.5	81.6
Total COD	g kg-wwt^{-1}	110.3	50.4
Cellulose	%-dwt	36.2	36.2
Hemicellulose	%-dwt	11.4	1.9
Lignin	%-dwt	20.7	4.4
Ash	%-dwt	15.5	18.4

2.2. Hydrolysis test of submerged macrophytes using thermochemical pre-treatment

Thermochemical pre-treatment was conducted on *P. maackianus* and *E. densa* in order to investigate the optimum pre-treatment conditions. The NaOH loading rate over total solids (TS) of the substrate was 0, 0.02, 0.05, 0.10, 0.20 g g-TS$_{substrate}^{-1}$, respectively. 10.0 g-wwt of substrate was added with 10.0 mL of NaOH solution to 50 mL centrifuging tubes. Previous studies have investigated a wide range of pre-treatment temperature of 50–150 °C [12,14]. However, low temperature below 100 °C should be more preferable since production of inhibitory substances may occur at high temperature [19]. In the present study, the substrates with the NaOH solution were heated in a convection oven (EYELA, WFO-700, Japan) at two different temperature (60 and 80 °C), for four different treatment time (0.5, 1.0, 2.0 and 3.0 h), respectively. For *P. maackianus* at NaOH 0.20 g g-TS$_{substrate}^{-1}$ and 80 °C, 4.0 and 5.0 h was also conducted. After heating, substrates were immediately centrifuged at 25 °C, 5000 rpm for 10 min using by high-speed centrifugation (KUBOTA, Compact high speed refrigerated centrifuge 6500, Japan). The supernatant was filtered through 0.45 μm glass-fiber filter paper (ADVANTEC, GC-50, 47 mm, Japan) and preserved at −20 °C in the freezer until the chemical analysis. The solid residues were rinsed with 1 M H_2SO_4 for neutralization and dried in the convection oven at 60 °C for more than 24 h. The dried residue was finely milled to pass the sieve pore of 1.0 mm and used for lignocellulose analysis. All experiments were conducted in triplicate.

2.3. Batch AD test of pre-treated macrophytes

Pre-treated and un-treated submerged macrophytes were anaerobically digested in a batch mode, by using the method of Koyama et al. [9]. *P. maackianus* and *E. densa* were pre-treated at 80 °C, NaOH 0.10 and 0.20 g g-TS$_{substrate}^{-1}$ for 3.0 h, followed by neutralization to around pH 7 using HCl. The CH_4 yield of inoculum was also measured and subtracted from the results. Substrate: inoculum ratio was adjusted to 1:2 based on volatile solids (VS). 500 mL flask was used for the batch reactors, and the reactors were purged with N_2 to make anaerobic environment and sealed by silicon stopper. Produced biogas was collected using 1-L aluminum gas bag (GL Sciences, AAK-2, Japan). A shaker (TAITEC, NR-150, Japan) was used to agitate the reactors at 100 rpm. Batch AD test was performed at 37 ± 1 °C in triplicate for 14 days.

2.4. Analytical methods

pH, TS, VS, chemical oxygen demand (COD), lignocellulose and biogas were measured. The pH of the samples was measured using a pH meter (HORIBA, B-212, Japan). Standard methods from APHA [20] were applied to the analysis of TS, VS and COD. Lignocellulose (cellulose, hemicellulose and lignin) content was measured by detergent system [21] by using fiber analyzer (ANKOM Technology, A-200, USA). Lignin composition was analyzed by using the tetramethylammonium hydroxide (TMAH) derivatization method proposed by Clifford et al. [22]. Detailed lignin phenol contents were identified by GC/MS (Agilent Technologies, 6890 N GC/5973MS, USA). DB-5MS capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness) was used for the GC column. The temperature of injector and ion source were 310 °C and 230 °C, respectively. The oven temperature was gradually increased from 60 °C to 310 °C. In the mass spectrometry, the temperature of ion source and quadrupole were 230 °C and 150 °C, respectively. Helium was used as carrier gas with the flow rate of 1.0 mL min^{-1}.