



Anaerobic digestion of submerged macrophytes: Chemical composition and anaerobic digestibility



Mitsuhiro Koyama^{a,*}, Shuichi Yamamoto^a, Kanako Ishikawa^b,
Syuhei Ban^c, Tatsuki Toda^a

^a Graduate School of Engineering, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan

^b Lake Biwa Environmental Research Institute, 5-34 Yanagasaki, Otsu, Shiga 520-0022, Japan

^c School of Environmental Science, University of Shiga Prefecture, 2500 Hassaka-cho, Hikone, Shiga 522-8533, Japan

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ABSTRACT

Aquatic weeds including submerged macrophyte have been excessively propagated and causing environmental issues in freshwater environment of many countries, and the sustainable treatments have been investigated. In the present study, five submerged macrophyte species dominant in Lake Biwa, Japan, *Ceratophyllum demersum*, *Egeria densa*, *Elodea nuttallii*, *Potamogeton maackianus* and *Potamogeton malaianus* were used as a substrate for anaerobic digestion to investigate the chemical composition and the anaerobic digestibility. The lignin content of the submerged macrophyte widely ranged from 3.2 to 20.7%–TS depending on species. The lignin of all macrophytes contained 27.2–59.4% of hydroxycinnamic acids, suggesting they are relatively alkali-labile as compared with woody plants. The total CH₄ yield of submerged macrophytes greatly varied from 161.2 to 360.8 mL g-VS⁻¹ depending on species. The CH₄ conversion efficiency of *C. demersum*, *El. nuttallii*, *Eg. densa*, *P. maackianus* and *P. malaianus* was 57.1, 61.4, 60.6, 33.9 and 72.2%, respectively. The results showed that *C. demersum*, *El. nuttallii*, *Eg. densa* and *P. malaianus* are feasible for anaerobic digestion due to the high methane recovery, whereas *P. maackianus* was not preferable for anaerobic digestion. The present study revealed that the methane recovery of submerged macrophytes is regulated by the lignin content, as well as other lignocellulosic biomass.

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1. Introduction

The vascular aquatic weeds including floating, emergent and submerged macrophytes have been excessively grown and causing various environmental problems in lakes, dams and reservoirs worldwide (Abbasi et al., 1990; Moorhead and Nordstedt, 1993; O'Sullivan et al., 2010; Muñoz Escobar et al., 2011; Haga and Ishikawa, 2011). For instance, the invasion of submerged macrophyte *Elodea nuttallii*, originated in North America, is serious in many countries of Europe (Muñoz Escobar et al., 2011). In the case of Japan, Lake Biwa, which is the largest lake in the country (674 km²), submerged macrophytes have been excessively propagated, covering approximately 90% of the Southern Basin since 1994 (Haga and Ishikawa, 2011). The large quantity of phytomass has been causing water stagnation, foul odor, fishing interference,

ecosystem change and landscape fouling (Haga et al., 2006a,b). Every year, more than 2600 tons (wet weight: ww) of the submerged macrophytes are removed from the lake but the harvesting cost reaches more than USD 2.0 million per annum (Kawanabe et al., 2012).

Effective and low-cost treatments are needed for treating the excessively propagating macrophytes. Anaerobic digestion is one of the effective and low-cost bioenergy recovery technology from the harvested aquatic weeds (Abbasi et al., 1990; O'Sullivan et al., 2010). Anaerobic digestion generates methane-rich biogas from organic wastes with high moisture content like submerged macrophytes (80–95%-wwt), and the nutrient-rich digested fluid can be used for liquid fertilizer. Furthermore, the operation of anaerobic digester is very simple, and it requires low energy input and cost.

A number of previous studies reported that the methane yields of floating aquatic weeds, emergent plants and submerged macrophytes greatly varied depending on the species from 38 to 333 mL g-VS⁻¹ (Table 1), but lower than labile substrate such as food waste (364–489 mL g-VS⁻¹) (Verrier et al., 1987; Heo et al.,

* Corresponding author. Tel.: +81 426919455; fax: +81 426914086.
E-mail address: mkoyama1113@soka.gr.jp (M. Koyama).

Table 1CH₄ yields from floating, emergent and submerged macrophytes. All data was obtained by batch anaerobic digestion.

Aquatic weeds	Type	CH ₄ yield (mL.g-VS ⁻¹)	CH ₄ yield (mL.g-TS ⁻¹)	CH ₄ yield (mL.g-wwt ⁻¹)	CH ₄ conversion efficiency (%-COD)	Country	Literature
<i>Azolla pinnata</i>	Floating	132	107	6.3	–	India	Abbasi et al. (1990)
<i>Cabomba caroliniana</i>	Floating	173	–	–	–	Australia	O'Sullivan et al. (2010)
<i>Ceratopteris</i> sp.	Floating	204	164	6.7	–	India	Abbasi et al. (1990)
<i>Cyperus</i> sp.	Emergent	38	30	4.8	–	India	Abbasi et al. (1990)
<i>Eichhornia crassipes</i>	Floating	209	–	–	–	India	Chanakya et al. (1993)
		140–180	120–154	8.4–10.8	–	USA	Moorhead and Nordstedt, 1993
		190	–	–	–	Australia	O'Sullivan et al. (2010)
		182–193	–	–	–	India	Patel et al. (1993)
<i>Elodea nuttallii</i>	Submerged	333	–	–	–	Germany	Muñoz Escobar et al. (2011)
<i>Hydrilla verticillata</i>	Submerged	81	66	5.3	–	India	Abbasi et al. (1990)
<i>Salvinia molesta</i>	Floating	242	204	11.9	–	India	Abbasi et al. (1990)
<i>Scirpus</i> sp.	Emergent	66	53	7.4	–	India	Abbasi et al. (1990)
<i>Utricularia reticulata</i>	Emergent	132	108	4.2	–	India	Abbasi et al. (1990)
<i>Ceratophyllum demersum</i>	Submerged	249	191	12.2	57.1	Japan	The present study
<i>Elodea nuttallii</i>	Submerged	361	299	20.9	61.4	Japan	The present study
<i>Egeria densa</i>	Submerged	287	234	11.5	60.6	Japan	The present study
<i>Potamogeton maackianus</i>	Submerged	161	136	13.2	33.9	Japan	The present study
<i>Potamogeton malaianus</i>	Submerged	278	156	14.2	72.2	Japan	The present study

Some literature values were missing due to the lack of data (TS, VS, and/or COD values) from the literature.

2004). In general, hydrolysis of lignocellulose is a limiting step during anaerobic digestion of plant materials, since recalcitrant lignin protects cellulose and hemicellulose against microbial/enzymatic attack by coating them (Taherzadeh and Karimi, 2008). Lignin is scarcely degraded under anaerobic digestion, demonstrating only 2–17% of methane conversion efficiency even after 300 days of digestion (Benner et al., 1984; Tuomela et al., 2000; Barakat et al., 2012). Previous studies have already reported that the methane recovery of terrestrial woody and herbaceous plants is regulated by the lignin content (Gunaseelan, 2007; Triolo et al., 2011; Frigon et al., 2012). However, the chemical composition in relation to the methane recovery of aquatic weeds, especially submerged macrophytes, has not been investigated yet. The body rigidity and structure of submerged macrophytes is different from other plants. Total phenolic content of submerged macrophytes is lower than that of other aquatic weeds (Smolders et al., 2000). Thus submerged macrophytes have more flexible and softer body structure in order to adapt to the water flow, while terrestrial herbaceous plants and floating and emergent macrophytes normally have more rigid body, since they are predominantly emerged from water (Asaeda et al., 2005).

Therefore, the objective of the present study was to investigate the relationship between chemical composition and anaerobic digestibility of five dominant submerged macrophyte species *Ceratophyllum demersum*, *Egeria densa*, *Elodea nuttallii*, *Potamogeton maackianus* and *Potamogeton malaianus* collected from Lake Biwa. *Eg. densa* and *El. nuttallii* are widely known as invasive species in many countries (Yarrow et al., 2009; Muñoz Escobar et al., 2011), and accounting for 13 and 6% of the total phytomass in Lake Biwa, respectively (Haga and Ishikawa, 2011). *C. demersum*, *P. maackianus* and *P. malaianus* are native species in Japan and accounts for 6, 56 and 4% of the total phytomass, respectively. In the present study, batch anaerobic digestion was conducted using these five macrophyte species.

2. Materials and methods

2.1. Substrates and inoculum

For the substrate, five dominant submerged macrophyte species *C. demersum*, *Eg. densa*, *El. nuttallii*, *P. maackianus* and *P. malaianus* were harvested from the Southern Basin of Lake Biwa,

Shiga Prefecture, in Japan. Fresh samples were roughly shredded to the particle size of approximately 0.5–1.5 cm and preserved at –20 °C for the experiment. Before the batch anaerobic digestion and chemical component analysis, substrates were defrosted at room temperature. For the inoculum of batch anaerobic digestion, mesophilic anaerobic sludge treating domestic sewage was used. After we obtained the anaerobic sludge, we preserved the sludge at 37 °C for 2 days before batch anaerobic digestion, in order to digest the sewage sludge left in the anaerobic sludge. The anaerobic sludge was obtained from Hokubu Sludge Treatment Center, Yokohama, Japan.

2.2. Batch anaerobic digestion of submerged macrophytes

Batch anaerobic digestion was performed at mesophilic temperature of 37 ± 1 °C in a temperature controlled laboratory for 14 days. The mix ratio of the substrate to the inoculum was adjusted to 1:2 based on volatile solids (VS) contents, and the mixture was loaded to 500 mL Erlenmeyer flask. The batch reactors were sealed by silicon stopper with two sampling ports to allow gas and slurry samples to be collected. 1-L aluminum gas bag (GL Sciences, AAK-2, Japan) was attached for the biogas collection. The batch reactors were purged with N₂ to make anaerobic environment in the reactor. pH was not controlled throughout the operational period. The reactors were constantly agitated at 100 rpm using a shaker (Taitec, NR-150, Japan). All experiments were conducted in triplicate for *El. nuttallii*, *Eg. densa*, *P. maackianus*, *P. malaianus* and controls, and duplicate for *C. demersum*. The methane yield of inoculum was also measured as control, and subtracted from that of each reactor to determine the methane yield from submerged macrophytes.

2.3. Analysis parameters

pH, total solids (TS), VS, chemical oxygen demand (COD), lignocellulose and biogas (CH₄, CO₂) were measured. The pH of the samples was measured using a pH meter (Horiba, B-212, Japan). Standard methods from APHA (1998) were applied to the analysis of TS, VS and COD. Lignocellulose (cellulose, hemicellulose and lignin) content was measured by detergent system (Van Soest et al., 1991) using fiber analyzer (Ankom, A-200, USA).

Lignin composition was analyzed using GC/MS by following the method of Clifford et al. (1995). Lignin phenols were derivatized

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