Effect of lignocellulosic components on the anaerobic digestibility of aquatic weeds and development of a predictive model of methane production

水草のメタン発酵特性に与える細胞壁成分の影響とメタン生成予測モデルの構築

18D5702　藤原 正明　　指導教員　戸田 龍樹

SYNOPSIS

世界各地で過剰繁茂し、深刻な生態系の撹乱ならびに経済的損失を与える水草バイオマスの処理方法としてメタン発酵が試みられている。湖沼から刈り取られた水草には複数種が混在するため、メタン発酵特性は、季節的に変化する種組成ならびに各種水草の化学組成に影響を受けると考えられる。本博士論文では、化学組成からメタン発酵特性が推定可能なモデルの構築を目的とし、水草の化学組成ならびにメタン生成ポテンシャルの季節変化の解析 (Study 1)と、水草の化学組成と嫌気分解特性の関係性の解析 (Study 2)、処理性能の推定が可能な嫌気性消化モデル (ADM1)の確立 (Study 3)を実施した。Study 1 では、6 月から 10 月の各月に採取した優占種 3 種のうち、2 種で季節的に化学組成が変化し 1 種は変化せず、化学組成の季節変化の有無は、水草各種の季節に伴う生活史の違いに起因することが示唆された。一方でメタン生成量は、各種ごとに季節変化は見られたものの、種による差が大きいことから、刈り取られた水草のメタン生成ポテンシャルは主に種組成によって予測できることが明らかとなった。Study 2 では、メタン生成量とセルロース/リグニン比との間に、最大メタン生成速度とセルロース/ヘミセルロース比との間に、そして T80 (Technical digestion time)とヘミセルロース量の間にそれぞれ有意な相関が見られ、水草のリグノセルロース組成およびその比率からメタン発酵特性を推定できることを明らかにした。Study 3 では、これらの関係性を組み込んだ、水草バイオマスを対象とした ADM1 を作成しシミュレーションを行った。その結果、本 ADM1 によって高精度で水草バイオマスのメタン生成が推定できることを示した。総合考察では、本処理の社会実装化に向けて、連続処理時の運転安定性をモデルによって推定し、議論した。

Keywords: Aquatic weed, anaerobic digestion, lignocellulose, modified Gompertz model, ADM1.

Introduction

Recently, overgrowth of aquatic weeds is causing severe environmental issues and economic losses such as ecosystem change, interruption of hydraulic power generation, sailing disturbance and fishery catches decreasing in lakes, dams and reservoirs worldwide (Mailu, 2001; Jain and Kalamdhad, 2018). Therefore, cost-effective and sustainable valorization of aquatic weeds removed from water bodies is urgently needed. Anaerobic digestion is considered as an appropriate technology to treat aquatic weed biomass because of recoveries of the bioenergy from substrate with the high moisture content and the nutrient-rich digestate usable for liquid fertilizer with low energy input and cost.

For the plant biomass, seasons are main factors to affect growth speed, biomass yield, and maturity (Dragoni et al. 2015). Methane yields could be affected by seasonal changes in the chemical composition, especially the amount of lignocellulosic components. Kandel et al. (2013) reported the relationships between the seasonal changes in the chemical composition and methane yield of reed canary grass and found that the seasonal changes in the chemical components, such as lignin and cellulose, significantly affected the methane yield. In addition, temperate lakes experience four distinct seasons, and the optimal temperature and light intensity allowing the highest aquatic weed growth rates differ between species (Imamoto et al. 2008), leading that the species composition can vary seasonally. Therefore, the seasonal variation of both of chemical composition and species composition might affect to the methane potential of harvested aquatic weeds. To assess the possibility for implementation of anaerobic digestion, approximate methane potential and its seasonality should be evaluated.

Plant biomass including aquatic weeds contains cellulose, hemicellulose and lignin which are the main component of cell walls. Cellulose and hemicellulose are macromolecules composed of different sugars, whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors. Especially, lignin is a key component that affects digestibility as it has a low degradability and coats cell walls, thereby limiting the attachment of enzymes and microbes to cell wall components, such as cellulose and hemicellulose (Mussatto et al. 2008; Sawatdeenarunat et al. 2014). Koyama et al. (2014) revealed that the methane yield of aquatic weeds differs between species due to differences in lignin contents. In addition to the different degradability of each lignocellulosic component, lignocellulose has structural features such as coverage and complicated linkage among cellulose, hemicellulose and lignin. Therefore, the ratio of each lignocellulosic components might regulate not only the methane yield but also anaerobic digestibility such as production rate and required period to finish methane production.

To evaluate the anerobic digestibility for various substrate, kinetic model such as modified Gompertz model and Anaerobic Digestion Model No.1 (ADM1) was applied in numerous studies (Xie et al. 2016). Clarifying the relationships between lignocellulosic components and not only methane yield but also these kinetic parameters make it possible to understand the characteristics of anaerobic digestibility by only analyzing its lignocellulosic composition of aquatic weeds. Furthermore, in previous ADM1, cellulose, hemicellulose and starch, which show different degradability, were treated as a single component of carbohydrates. Because the content of cellulose, hemicellulose and starch varies with species and season, it is necessary to develop ADM1 for aquatic weed biomass.

Therefore, this thesis investigated seasonality of the lignocellulosic components and methane yield of harvested aquatic weeds (Study 1) and the quantitative relationships between lignocellulosic components and their anaerobic digestibility (Study 2). For Study 3, a mathematical model for estimating annual methane recovery from the lignocellulosic components was established and the reliability of this model was validated in General Discussion part.

Materials and Methods

Study 1. Seasonal variation in the chemical composition and methane potential of harvested aquatic weeds

The 19 samples of aquatic weed including six macrophyte species (four submerges macrophytes and two floating macrophytes) used for the substrates were harvested from Lake Biwa, Shiga Prefecture, Japan (35° 20′ N, 136° 10′ E). *Egeria densa*, *Elodea nuttallii*, and *Potamogeton maackianus*, and *Hydrilla verticillate* were harvested monthly from June to October 2015, and *Spirodela polyrhiza* and *Trapa japonica* were harvested in August 2018. The harvested macrophytes were dried at 80 °C for 12 h and milled into particles smaller than 3 mm and used for the biological methane potential (BMP) test and chemical composition analysis. 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.

The BMP tests were conducted to evaluate the anaerobic digestibility of aquatic weeds. An automatic methane potential test system (AMPTS II) supplied by Bioprocess Control Co. Ltd, Sweden was used for the BMP tests. Identical medium bottles with an effective volume of 300 mL were used as reactors. The substrate and seed sludge were added to the medium bottle, leading to a ratio of 1 to 2 on VS basis. The BMP tests were performed at 37±1 °C for 14 days. All tests and blanks were conducted in triplicate, and the net methane production values were calculated.

Total solids (TS), volatile solids (VS) and methane yield were measured. Standard methods from APHA (2005) were applied to the analysis of TS and VS. Lignocellulose (cellulose, hemicellulose and lignin) contents were measured by a detergent method using fiber analyzer (Model: A-200, Ankom Co. Ltd, USA). Methane yield was measured by using AMPTS II. For the estimation of monthly methane potential, seasonal species compositions of aquatic weeds were cited from Ohmi Environment Conservation Foundation (2015).

Study 2. Relationships between the lignocellulosic components and anaerobic digestibility

The cumulative methane yield of each test was fitted to a modified Gompertz equation to investigate the anaerobic digestibility of aquatic weeds,

$$\mathrm{B} = \mathrm{B}_0 \exp\{-\exp[\mu_\mathrm{m}\, e \,/\, \mathrm{B}_0(\lambda - t) + 1]\}$$

where B_0 is the cumulative methane production (mL g-VS^{-1}), μ_m is the maximum methane production rate (mL g-VS^{-1} day^{-1}), λ represents the lag phase (days), and t stands for the digestion time (days). T80 defined as the required period to finish 80% of the ultimate methane yield was calculated from the obtained parameters.

Study 3. Development of ADM1 for aquatic weed biomass

ADM1 was developed to take account into the effect of lignocellulosic components for the simulation of anaerobic digestion of aquatic weeds as follows.

$$f_{cell,xc} = m_{cell,xc} \times [m_{cell,xc} /(m_{cell,xc} + f_{lig,xc} \times 3.383)]$$

$$f_{hemi,xc} = f_{hem,xc} \times [m_{hemi,xc} /(m_{hemi,xc} + f_{lig\_xc} \times 1.123)]$$

$$f_{xI,xc} = f_{xIm,xc} + (f_{cell,xc} - m_{cell,xc}) + (f_{hemi,xc} - m_{hemi,xc})$$

where, $f_{cell,xc,}$ $f_{hemi,xc}$, $f_{lig,xc}$, and $f_{xI,xc}$ indicate the distribution ratio from fed substrate to each component of cellulose, hemicellulose, lignin, and solid inert without lignin, respectively. $m_{cell,xc,}$ $m_{hemi,xc}$, $m_{lig,xc}$, and $m_{xI,xc}$ indicate the percentage of each component in the chemical composition of aquatic weeds. The parentheses [ ] on the right side indicate the magnitude of coverage by lignin to cellulose and hemicellulose, and the higher coverage by lignin led cellulose and hemicellulose to undegraded components ($f_{xI,xc}$). 3.383 and 1.123 are the correction factors calculated by the least-squares method. As a comparison with the ADM1 in this study, original ADM1 designed for wastewater (Batstone et al. 2002) and ADM1 for *H. verticillate* (Chen et al. 2016), a species of aquatic weeds, were also simulated.

Results and Discussion

Study 1. Seasonal variation in the chemical composition and methane potential of harvested aquatic weeds

For *E. densa*, the lignin concentration in October demonstrated a significantly higher value, of 101 mg g-VS^{-1}, compared with those of other months, which ranged from 32 to 46 mg g-VS^{-1} ($p < 0.01$). The hemicellulose content in August exhibited a significantly lower value compared with those in September and October ($p < 0.01$), though the others did not display the variation. For *E. nuttallii*, the lignin concentration in October showed the statistically significant highest value (153 mg g-VS^{-1}) compared with the values of all other months (59–114 mg g-VS^{-1}, $p < 0.01$). The hemicellulose content of this species also tends to drop in August and increase in October. These variations in the lignocellulosic components of *E. densa* and *E. nuttallii* might be related to annual life events such as get rid of their own leaves, roots, and apical buds in fall (cite). However, *P. maackianus* is a perennial plant and does not defoliate during fall and winter (Imamoto et al. 2008). Therefore, the chemical composition of *P. maackianus* varied very little in comparison with the other two species. The chemical composition of aquatic weed species with an annual lifecycle might change seasonally.

The seasonal variations in methane yields differed in each macrophyte species. The methane yield of *E. nuttallii* varied significantly, within the range of 189.2–284.1 mL g-VS^{-1} ($p < 0.01$). This fluctuation is related to the seasonal changes in the chemical composition. The methane yield of *E. densa* also varied, exhibiting a significantly lower value in August ($p < 0.05$) although its range was smaller than *E. nuttallii*, between 211.6–251.5 mL g-VS^{-1}. The methane yield of *P. maackianus* varied only slightly from 139.8 to 164.7 mL g-VS^{-1}, while the chemical composition of *P. maackianus* did not change seasonally. The methane yield of *P. maackianus* was remarkably lower than that of the other two species, indicating that the differences in the methane yields between different species were larger than those between different months for the same species. According to these results, the methane yields of the seasonally harvested aquatic weeds mainly varied depending on the species composition.

Estimation of monthly methane potential from BMP results and seasonal aquatic weeds species composition were performed (Figure 1). The monthly methane potential varied from 171 to 231 mL g-VS^{-1}. *P. maackianus* is predominant from fall to spring, while most of the other species are predominant during summer. Notably, with an increase in the abundance of *P. maackianus*, which had a low BMP, the monthly methane potential decreased to 75% in October.

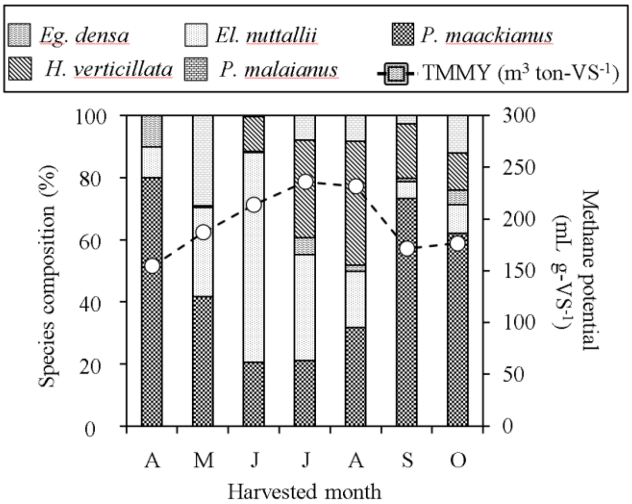

Figure 1. Seasonal changes of species composition and monthly methane potential of harvested aquatic weeds from Lake Biwa.

Study 2. Relationships between the lignocellulosic components and anaerobic digestibility

Kinetic parameters (ultimate methane yield, maximum methane production rate, lag phase and T80) were obtained by fitting to the modified Gompertz model. Ultimate methane yields in this study showed 139 – 281 mL g-VS^{-1}. Maximum methane production rate showed 30 – 119 mL g-VS^{-1} day^{-1}. Relatively short lag phase of 0.00 – 0.66 days also suggests all aquatic weed species were easily degradable. All the sample showed short T80 of 1.9 – 4.2 days than other lignocellulosic biomass (Cite).

The relationships among lignocellulosic components (cellulose, hemicellulose and lignin) and anaerobic digestibility parameters obtained by fitting the timeseries of methane yield to the modified Gompertz model were investigated. Lignin content showed a significant negative correlation with the methane yield ($p < 0.05$) as same as Koyama et al. (2014). Cellulose showed significant positive relationship with ultimate methane yield ($p < 0.05$) and maximum methane production rate ($p < 0.05$). Since cellulose is composed of only glucose which is degradable substance for anaerobic digestion (Mussatto et al. 2008), the amount of cellulose in the biomass would have strongly contributed the methane production. Hemicellulose was not correlated with ultimate methane yield. Hemicellulose is short-branched chain hetero-polysaccharides consisting of

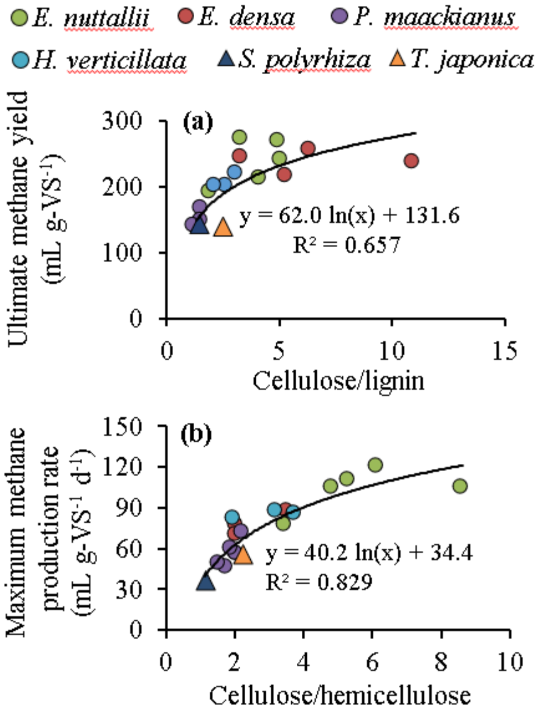

Figure 2. Relationship between the ratio of lignocellulosic components and kinetic parameters. Solid lines represent $p < 0.05$.

various sugar units such as arabinose, xylose, mannose, glucose and galactose. Unexpectedly, hemicellulose had a significant negative correlation with maximum methane production rate ($p < 0.01$) and positive correlation with T80 ($p < 0.05$). These results indicate that hemicellulose is not easily degradable so much. Because cellulose, hemicellulose and lignin were complicatedly linked, the degradability might be affected each other. To clarify these synergetic effects, relationship between the ratio of lignocellulosic components (cellulose/lignin, hemicellulose/lignin and cellulose/hemicellulose) and kinetic parameters (ultimate methane yield and maximum methane production rate) were analyzed (Figure 2). The ultimate methane yield of aquatic weeds was well expressed by cellulose/lignin ($y = 62.0 \ln(x) + 131.6$, $R^2 = 0.657$) and the maximum methane production rate by cellulose/hemicellulose ($y = 40.2 \ln(x) + 34.4$, $R^2 = 0.829$), respectively. This apparent relationship between cellulose/lignin and ultimate methane yield may have resulted from the resistance of lignin to degradation covering cellulose, and adsorption of cellulase (an enzyme that decomposes cellulose) by lignin (Koyama et al. 2016). The correlation of cellulose/hemicellulose and maximum methane production rate might be related to the coverage of cellulose by hemicellulose, taking important part in recalcitrance of cell walls.

Study 3. Development of ADM1 for aquatic weed biomass

ADM1 treating cellulose, hemicellulose and starch as a different component was developed in Study 3. The estimation results of ADM1 developed in this study were compared with experimental value and ADM1 developed in the previous study (Figure 3).

The original ADM1 (Backstone et al. 2002), which treats cellulose and hemicellulose as carbohydrates, showed higher methane productions of 500-700 mL g-VS^{-1} than the experimental value of 150- 250 mL g-VS^{-1}. The ADM1 developed in the previous study (Chen et al., 2016), which treat cellulose and lignin as undegraded components, showed closer values than the original model, but the final production tended to be lower than the experimental values. The simulation result from ADM1 developed in this study showed almost the same to the measured value. This result indicated that the lignocellulose coating structure of aquatic weeds determines the anaerobic digestibility. Furthermore, this model may be applicable to estimate the performance in semi-continuous anaerobic digestion of harvested aquatic weeds. This thesis is the first to simulate the effect of lignocellulosic components structure to reaction rate in ADM1.

Conclusion

The chemical compositions of the aquatic weeds varied depending on their lifecycles. Although chemical composition and methane yield changed seasonally, the monthly methane potential of the harvested aquatic weeds varied mainly affected by the species composition. The monthly methane potential varied from 171 to 231 mL g-VS^{-1}, and the necessity of pretreatment was when the less-biodegradable aquatic weeds became the dominant species (Study 1). It is revealed that cellulose/lignin and cellulose/hemicellulose were regulating factors to determine ultimate methane yield and maximum methane production rate, respectively (Study 2). Furthermore, this study successed to modifying ADM1 considering the effect of lignocellulosic coating structure with high accuracy. By applying this model, anaerobic digestibility of aquatic weeds can be estimate from chemical compositions (Study 3).

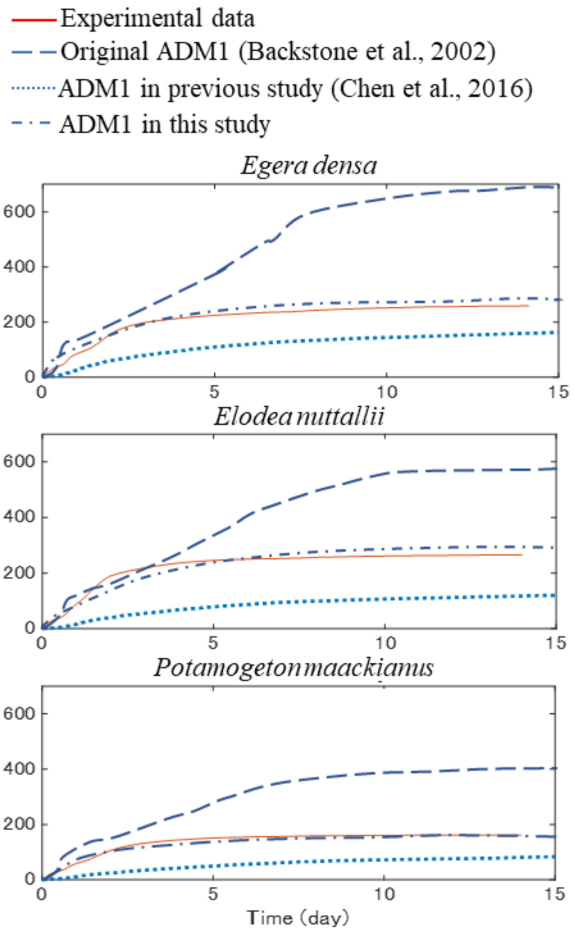

Figure 3. Comparison of batch and simulation results by ADM1 treating aquatic weeds. Aquatic weeds were harvested from Lake Biwa in July 2015.

References

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