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Research paper

Characterization of microalgae cultivated in continuous operation combined with anaerobic co-digestion of sewage sludge and microalgae

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The present study reveals characteristics of microalgae cultivated in continuous laboratory-scale experiments combined with anaerobic co-digestion of sewage sludge and microalgae for as long as 10 months. Filtrate from dewatering of anaerobically digested sludge was used for continuous cultivation of microalgae. The effect of hydraulic retention time (HRT) on continuous microalgal cultivation was investigated, and HRT 7.8 d was selected for the continuous experiment. 16S/18S rRNA gene analysis confirmed that indigenous wild type *Chlorella* was stably cultivated without microalgal species control, and no cyanobacteria were cultivated. Average volatile solids (VS) per total solids and chemical oxygen demand per VS mass ratios of the cultivated microalgal mixture were 0.918 g g^{-1} and 1.39 g g^{-1}, respectively. A measured higher heating value of the cultivated *Chlorella* mixture was around 22 MJ kg^{-1}. Fatty acid methyl ester analysis showed that the obtained microalgal mixture cultivated without controlling species is useful for anaerobic digestion rather than biodiesel production. A biochemical methane potential assay for 28 d showed that methane conversion from the cultivated *Chlorella* mixture was approximately 0.26 ± 0.02 m^3 kg^{-1} of VS-fed at standard conditions of 101.3 kPa and 273.15 K. This indicates that approximately 46% of the energy was recovered by anaerobic digestion as methane. No specific elemental accumulation was observed during the continuous operation. Combined anaerobic co-digestion of sewage sludge and microalgae, and microalgal cultivation is a promising energy recovery approach.

1. Introduction

Anaerobic digestion of sewage sludge at wastewater treatment plants (WWTPs) is widely used for energy recovery, but nitrogen and phosphorus loadings into recycle flow from anaerobic digestion are of great concern in terms of nutrient control. If such plentiful nutrients included in sewage are used to cultivate microalgae, WWTPs produce more energy. The temperature of sewage is usually higher than the external ambient, and waste heat can be recovered from anaerobic digesters, generators and incinerators at WWTPs. Anaerobic digestion and incineration produces carbon dioxide, which can be utilized for microalgal cultivation [1]. The availability of heat and carbon dioxide can be another advantage for microalgal cultivation at WWTPs.

Cultivation of hydrocarbon-rich microalgae has been intensively investigated as an efficient new source of oil [2]. However, the cultivation of specific microalgae using nutrients in sewage without pre-treatment is difficult because sewage contains other types of microalgal seeds, and the growth of unintended microalgal species results in a failure of efficient lipid production. Contrary to lipid production, various types of microalgae can be potential substrates for anaerobic digestion, without requiring biomass drying [3]. Sialve et al. [4] suggested that direct anaerobic digestion of microalgae is energetically more favorable than removal of lipids from microalgae when the total lipid content is less than 40%. Alcántara et al. [5] reported that the integration of microalgal growth with anaerobic digestion can significantly improve the economics and energy balance at WWTPs.

Ficara et al. [6] reported that *Botryococcus* cells were

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outcompeted by *Chlorella* and *Scenedesmus* when cultivated under continuous operation using centrate from dewatering. Khanh et al. [7] compared the dilution ratio of 1–20 in cultivation of *C. vulgaris* using the filtered (0.2-μm pore size membrane filter) digestate containing NH_4^+-N and PO_4^{3-}-P of 866 and 613 g m^{-3}, respectively, and concluded that the dilution ratio of 5 resulted in the highest growth rate. Hidaka et al. [8] proved the applicability of recycle flow from dewatering processes to indigenous wild type *Chlorella* sp. cultivation without filtration. Cultivation of indigenous wild type *Chlorella* sp. is a possible candidate for a new energy source at WWTPs. Hirata et al. [9] reported the optimum conditions for *Chlorella* sp. UK001 were 30 °C and pH 5.7. Mayo [10] reported that *C. vulgaris* can be cultivated at pH 4–6, although the optimum pH was 6.3–6.8. Biller and Ross [11] reported a higher heating value (HHV) of freeze dried *Chlorella* obtained from a commercial source was 23.2 MJ kg^{-1}. Total fatty acid methyl ester (FAME) contents are reported to be 100 mg g^{-1} dry in *C. vulgaris* [12], and 180 mg g^{-1} dry in *C. sorokiniana* [13]. He et al. [14] reported an increase of FAME content in *C. vulgaris* with a decrease of NH_4^+-N concentrations in the culture. In anaerobic digestion, the methane yields of 0.26 m^3 kg^{-1} of VS-fed from pure culture *Chlorella* spp. cultivated in synthetic media [15], and 0.23 m^3 kg^{-1} of VS-fed from wild type *Chlorella* sp. cultivated in real wastewater [16] are reported. Wang et al. [17] reported that anaerobic co-digestion of waste activated sludge (WAS) and *Chlorella* cultivated using synthetic medium promoted methane production and dewaterability.

There are several research papers on the anaerobic digestion of microalgae cultivated using various types of sewage, but most researches on anaerobic co-digestion are based on microalgae cultivated using synthetic media or filtered sewage, which is not realistic on site. Real sewage contains various kinds of microorganisms and suspended solids (SS), which may affect the growth of microalgae [18]. Thermophilic anaerobic digestion can reduce potential microbial contaminations in microalgal cultivation more than mesophilic operation [19]. Wang et al. [20] reported that thermophilic operation was more stable than mesophilic operation for co-digestion of grass with sewage sludge. Thermophilic anaerobic digestion is suitable for combining with microalgal cultivation at WWTPs. However, there have been few reports on continuously operated combined thermophilic anaerobic digestion and microalgal cultivation. Optimum operational conditions for microalgal cultivation and characteristics of continuously cultivated microalgae are uncertain.

The objective of this study is to investigate the feasibility of the proposed system using unfiltered sewage samples. Most experiments were conducted in continuous mode rather than in batch mode, except for a biochemical methane potential (BMP) assay. In this study, continuous indigenous wild type microalgal cultivation using recycle flow from thermophilic anaerobic digestion of sewage sludge was investigated. Firstly, the effect of hydraulic retention time (HRT) on continuous microalgal cultivation was investigated. Then continuous operation of combined anaerobic digestion and microalgal cultivation was performed for as long as 10 months. The characteristics of the cultivated microalgae were investigated by various methods, including energy evaluation and 16S/18S rRNA gene analysis, and its anaerobic digestion performance was evaluated.

2. Materials and methods

2.1. Sewage sample

Samples were obtained from a municipal WWTP in Japan. Its conventional activated sludge process was operated treating approximately 10 dam^3 d^{-1} domestic sewage under the conditions of HRT of 10 h, solids retention time of 11 d and a mixed liquor suspended solids concentration of 1.3 kg m^{-3}. Its thermophilic anaerobic digester was operated under the conditions of temperature of 55 °C, HRT of 11 d and volatile solids (VS) loading of 2.0 kg m^{-3} d^{-1}. A recycle flow from dewatering anaerobically digested sludge, containing secondary effluent that was used for washing the dewatering machine, was used for the work described in section 2.2, and mixed sludge (concentrated mixture of primary sludge and WAS) whose total solids (TS) concentration was 20–30 g dm^{-3} was used for the work described in section 2.3.

2.2. Microalgal cultivation under different HRT

The effect of HRT on continuous microalgal cultivation was investigated under three HRT conditions. Three bottle reactors with an effective volume of 4 dm^3 were operated in semi-batch mode with aeration and fluorescent lighting under similar conditions to those reported by Inoue and Uchida [21]. The reactors were irradiated by a fluorescent lamp with a photo flux density of approximately 120 μmol m^{-2} s^{-1} from the side for 12 h every day. The culture process was carried out at approximately 25 °C. Air flowed into the reactor at a flow rate of approximately 1 dm^3 min^{-1} during the cultivation. The collected recycle flow sample was diluted by four times. At the beginning, indigenous microalgae were cultivated using the diluted recycle flow sample without seeding, and the liquor became green in one week. Then, semi-continuous cultivation under three different HRT conditions was started. In each reactor, 90% of the volume of the cultivated liquor was replaced with the raw diluted recycle flow sample once, twice and three times a week, which corresponds with HRT of 7.8, 3.9 and 2.6 d, respectively. This semi-continuous cultivation was performed for 42, 37 and 30 d, respectively.

2.3. Continuous operation of combined anaerobic digestion and microalgal cultivation

Two continuous anaerobic digestion reactors fed with sewage sludge and cultivated algae, and sewage sludge only (AD_AL and AD_SS, respectively) were operated for as long as 10 months. The anaerobic digestion experiments were conducted according to almost the same method as Hidaka et al. [22]. The working volumes of AD_AL and AD_SS were 10 and 2 dm^3, respectively. The temperature was controlled at 55 °C using a water bath.

AD_SS was a control reactor, fed with only the mixed sludge every weekday (five times a week). The HRT based on the amount of the mixed sludge was 28 d. AD_AL was fed with the mixed sludge every weekday at the same loading rate as AD_SS, and the cultivated microalgae using a recycle flow sample from AD_AL once a week. The withdrawn digested sludge from AD_AL was stored at 4 °C. A recycle flow sample from AD_AL was obtained by adding a polymer coagulant at a concentration of 1–2% (TS) to the stored digested sludge and dewatering using a dewaterability test kit (Tokyo Metropolitan Sewerage Service Corporation, Japan) once a week. Cultivation reactors were operated by replacing 90% of the volume of the cultivated liquor with the diluted recycle flow sample once a week using the same reactors as the work described in section 2.2. This corresponds with HRT of 7.8 d. At the first cultivation, seed microalgae were taken from the previously cultivated reactor in the work described in section 2.2. The withdrawn microalgae from each reactor were 36 times concentrated by centrifuge at 3000 g, and then the concentrated microalgae was fed into AD_AL once a week. The volume ratio of added cultivated microalgae to the mixed sludge was 4–15%.

One cultivation reactor was operated before Day 176, two reactors were operated between Day 176 and 189, and four reactors