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Two-stage co-fermentation of lipid-extracted microalgae waste with food waste leachate: A viable way to reduce the inhibitory effect of leftover organic solvent and recover additional energy

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ABSTRACT

This study aimed to mitigate the inhibitory effect of leftover organic solvent (CH_3Cl, chloroform) on anaerobic digestion of lipid-extracted microalgae waste (LEMW). Food waste leachate (FWL) was added as a co-substrate and a two-stage fermentation process (H_2 production with acidogenesis + methanogenesis) was adopted. The result of the first batch experiment, conducted in the absence of chloroform, showed that as the FWL addition ratio increased up to 60% on chemical oxygen demand (COD) basis, there was a gradual increase in the amount of H_2 produced. At 80% addition, however, there was a huge drop in H_2 yield, accompanied by a drop in pH. In the presence of chloroform (100–900 mg CH_3Cl/L), the mixture (LEMW:FWL = 40:60) exhibited a much higher tolerance than that of LEMW alone, which could be ascribed to the co-metabolic degradation of chloroform by FWL addition. At 600 and 900 mg $CHCl_3$/L, the degradation efficiency dropped below 40% for LEMW alone, while it was maintained above 90% in the mixture. A H_2 yield of 36 mL H_2/g COD, equivalent to 2.6% of the energy content in the feedstock, was attained from the mixture at 600 mg $CHCl_3$/L. The H_2 fermented effluent was then fed to a continuous methanogenic reactor at HRT 40 d, and 82% of energy content in the feedstock was further gained in the form of CH_4. Although the energy gained from H_2 production was negligible, most of the chloroform was degraded during acidogenesis, which resulted in a successful CH_4 conversion.

Introduction

Given dwindling fossil fuel reserves in the face of rapidly increasing energy consumption, coupled with concerns on global warming, there is a pressing need for the development of sustainable and clean energy sources [1]. Biomass – the plant matter formed by photosynthetic capture of solar energy and stored as chemical energy – is a carbon-neutral resource in its

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life cycle, and thus, its conversion to energy is gaining significant political and scientific momentum [2]. The traditional biomass for energy generation was terrestrial one including sugar-rich energy crops and municipal solid wastes. However, aquatic biomass, such as microalgae, has newly emerged as an outstanding biomass, since it grows with light and carbon dioxide, and stores lipid with high content, in general 30–40% of their body [3]. After the collection and extraction of microalgae, the lipid can be transesterified and fed to vehicles.

Meanwhile, it is also important to generate more energy from the lipid-extracted microalgae waste (LEMW) (>50% of the microalgae dry weight), to make microalgae-based bioenergy systems fully sustainable [4,5]. One of the viable options is anaerobic digestion (AD), which could provide an economic benefit of $114–129 per ton of dry LEMW [6,7]. AD is a biological process in which organic matter is degraded and converted to clean biogas, methane (CH_4), under anaerobic conditions. The produced CH_4 can be utilized for heat or electricity generation, replacing fossil fuels and thereby reducing carbon dioxide emissions [8].

During the extraction step of lipids, an organic solvent such as chloroform ($CHCl_3$) is used, which might cause an inhibition of microbial activity [9]. Previous research has shown that the activity of both acidogens and methanogens was significantly inhibited when chloroform dose increased. In particular, methanogens were much more vulnerable to chloroform: a complete inhibition was observed at 100 mg $CHCl_3$/L while only 30% inhibition (based on specific hydrogenic/methanogenic activity) was observed for acidogens at the same concentration [6]. The application of evaporating and washing steps prior to AD could be the options to reduce chloroform toxicity. However, those methods have the limitation that they require a large amount of energy and are difficult to scale-up [6,10].

Anaerobic co-digestion is a traditional method that improves digestibility and biogas production by synergistic and complementary effects, which offset the lack of nutrients and dilute harmful substances [11]. In addition, the degradation of harmful substances, such as chloroform, can occur via co-metabolism. The addition of easily biodegradable organics can provide sufficient energy to break solid structure of chloroform in a short time [12]. Furthermore, the adoption of a two-stage digestion process is advantageous in treating LEMW, owing to the fact that acidogens have a higher tolerance to chloroform than methanogens. The degradation of chloroform in the first-stage can reduce the chloroform loading in the second-stage of CH_4 production. In addition, hydrogen (H_2) can be obtained in the first-stage, which is a clean energy carrier, producing only water when combusted [13]. There have been a lot of researches on H_2 production during acidogenesis, and various feedstocks including microalgae have been tested [6,13].

This is the first study investigating two-stage anaerobic co-digestion of LEMW with food waste leachate (FWL) to mitigate the inhibitory effect of leftover organic solvent and recover additional energy. FWL is a residue generated from food waste recycling facilities in Korea, which has a high organic carbon content and good biodegradability [14]. A series of batch co-fermentation experiments for H_2 production were performed at various mixing ratios of LEMW to FWL and chloroform concentrations (0–900 mg $CHCl_3$/L). The H_2 fermented effluent (HFE) was then fed to an intermittent continuously-stirred tank reactor (iCSTR) for CH_4 production.

Materials and methods

Feedstock and inoculum preparation

To prepare LEMW, *Chlorella vulgaris* consisting of lipid (13%), protein (66.9%), carbohydrate (12.5%), ash (6%), and unknown (1.6%) was diluted with tap water to 50 g chemical oxygen demand (COD)/L. It was ultrasonicated at the specific energy input of 100,000 kJ/kg TS using an ultrasonicator with 20 kHz frequency and 150 W (VCX-750, Sonics and Materials, USA) to disrupt the cell walls [6]. After centrifugation at 7000 rpm for 10 min, the supernatant was discarded while the residual sediment (210 g COD/L) was used for experiment after dilution to 150 g COD/L. The lipid content in LEMW was 1%. FWL was collected from a food-waste resource facility (Daejeon, Republic of Korea) and strained through a sieve (1 × 1 mm). It was then diluted to 150 g COD/L by adding tap water. The carbohydrate content of LEMW and FWL were 19% and 35%, respectively, on COD basis.

The seed sludge was taken from an anaerobic digester at a local wastewater treatment plant (Daejeon, Republic of Korea). Heat treatment (90 °C for 20 min) was conducted to inactivate H_2-consuming methanogens while raw sludge was utilized for CH_4 production [6]. The characteristics of the feedstock and inoculum are summarized in Table 1.

Experiments

Two sets of batch tests were performed for H_2 production using a 250 mL serum bottle with an effective volume of 100 mL. The feedstock concentration and an inoculum/substrate ratio (I/S) in all tests were controlled to be 75 g COD/L and 0.2, respectively. In Batch Test I, the mixing ratio of LEMW to FWL was set at 100:0, 80:20, 60:40, 40:60, and 20:80 on a COD basis in the absence of chloroform (Table 2). Meanwhile, Batch Test II was performed at two mixture ratios of LEMW to FWL (100:0 and 40:60). Seed sludge (40 mL) and feedstock (50 mL) were added to each bottle, and the rest of the working volume was filled with distilled water. No external nutrients were added. After adding all substances, initial pH was adjusted to be 7.5 ± 0.1 by adding 4 N KOH solution. The bottles were

Table 1 – Characteristics of seed sludge and feedstock.

Item	Units	Seed sludge	LEMW[a]	FWL[b]
TS	g/L	36 ± 2.3	114 ± 0.9	122 ± 3.9
VS	g/L	–	104 ± 2.0	106 ± 4.7
TCOD	g COD/L	38 ± 0.5	150 ± 3.1	150 ± 1.7
SCOD	g COD/L	–	21 ± 3.0	96 ± 8.1
Carbohydrate	g COD/L	–	19 ± 2.0	35 ± 2.1
TN-N	mg/L	1360 ± 40	3680 ± 120	6850 ± 50
pH	–	7.2 ± 0.1	6.8 ± 0.0	4.6 ± 0.1

[a] LEMW: Lipid-extracted microalgae waste.
[b] FWL: Food waste leachate.