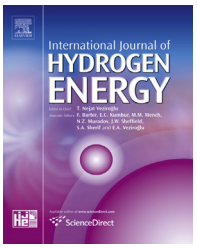
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2014) 1–6

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he



Inhibitory effect of chloroform on fermentative hydrogen and methane production from lipid-extracted microalgae

Yeo-Myeong Yun [a], *Si-Kyung Cho* [a], *Kyung-Won Jung* [b], *Mi-Sun Kim* [c], *Hang-Sik Shin* [a], *Dong-Hoon Kim* [c,*]

[a] *Department of Civil and Environmental Engineering, KAIST, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea*

[b] *Center for Water Resources Cycle Research, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seonbuk-gu, Seoul 136-791, Republic of Korea*

[c] *Biomass and Waste Recycling Laboratory, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea*

Keywords:
Lipid-extracted microalgae
H_2 production
CH_4 production
Chloroform
Inhibitor concentration

ABSTRACT

To improve the sustainability of microalgae as a bioenergy feedstock, lipid-extracted microalgae (LEM) are often further treated by anaerobic digestion (AD). However, the residual chloroform used for extracting lipids as a solvent could inhibit this process, an aspect that has not been studied to date. In this study, the inhibitory effect of chloroform on H_2 and CH_4 production was investigated by performing batch tests. To prepare the feedstock, *Chlorella vulgaris* was ultrasonicated and the supernatant was discarded after centrifugation. In case of H_2 production, it was found that the H_2 yield fell to almost half that of the control (15.6 mL H_2/g COD_{added}) at 100 mg $CHCl_3$/L. The reason for the decrease of the H_2 yield with the increase of chloroform level was due to the change of metabolites from acetate and butyrate to lactate via a non-hydrogenic reaction. In comparison with H_2 production, a much more severe inhibitory effect of chloroform on CH_4 production was observed. The inhibitor concentration ($IC_{30,\ 60,\ and\ 90}$) on H_2 production was 138, 319, and 622 mg $CHCl_3$/L, respectively, while concentrations of 15, 37, and 86 mg $CHCl_3$/L were obtained on CH_4 production. When the chloroform concentration was $\geq$25 mg/L on CH_4 production, more than 2 g COD/L of organic acids remained, resulting in a decrease of CH_4 yield. These findings indicate that the residual chloroform in LEM should be seriously considered to prevent possible microbial inhibition when designing a process for additional energy recovery from microalgae via AD.

* *Corresponding author*. Tel.: +82 42 860 3707; fax: +82 42 860 3739.
E-mail address: dhkim77@kier.re.kr (D.-H. Kim).

http://dx.doi.org/10.1016/j.ijhydene.2014.04.167

Introduction

With increasing interest in sustainable development, huge efforts are now being made to exploit renewable energy sources including biomass. Biomass is a carbon neutral resource during its life cycle and is found throughout the world, which is not subject to global price fluctuations or supply uncertainties [1]. Biomass can be divided into terrestrial and aquatic sources, and the latter has advantages of fast growth rates with high biodegradability [2]. In addition, as a non-edible feedstock it is free from ethical issues, and requires little land and irrigation water. In this regard, microalgae are considered one of the most promising future bioenergy sources [3].

Microalgae are more than two times efficient than terrestrial plants in converting sunlight to biochemical energy and fixing CO_2 [3]. Under certain conditions, they can store neutral lipids, 20–70% of their body, which can be used as an industrial chemical and bioenergy feedstock [4]. In general, to obtain lipids, a microalgae broth is dewatered, cells are ruptured, and finally organic solvents such as methanol and chloroform are added to extract the lipids. During this process, approximately 45–85% of raw microalgae remains as residues, and recent reports have pointed out that attaining more energy from the residues is essential for the feasibility and sustainability of microalgae-based bioenergy systems [5].

One of the viable technologies to convert lipid-extracted microalgae (LEM) to biofuel is anaerobic digestion (AD). AD is a multistage biological conversion route, consisting of hydrolysis, acidogenesis, and methanogenesis, which finally converts organic polymers to methane (CH_4). Raw and pretreated LEM are often treated by AD, showing a CH_4 yield of 20–70% of their maximum biological potential [6]. In addition, during the acidogenesis step, hydrogen (H_2) can be obtained, unless the methanogenesis step is active. This process, often called dark fermentation, is attractive in terms of producing carbon-free energy and reacting in a fast manner [7].

Chloroform is used as an organic solvent to extract lipids from microalgae, but it is known to be toxic to microbial cells. It disrupts the microbial cell membrane and compromises cell viability, resulting in inactivation of essential membrane function and denaturation of essential enzymes [8,9]. The inhibitory effects of chloroform have been studied in the field of anaerobic microbial processes. According to Narayanan et al. (1993), chloroform inhibits the activity of methanogenic microorganisms even at a very low concentration of 1.67 μM [10]. More recent study has shown that chloroform can inhibit not only the activity of methanogenic archaea but also the activity of acidogenic bacteria and sulfate-reducing bacteria [11]. These findings indicate that special care is required in utilizing LEM as a feedstock for AD.

This study sought to evaluate the inhibitory effect of chloroform on the fermentative H_2 and CH_4 production from LEM. The batch tests were performed using ultrasonicated *Chlorella vulgaris* as a model feedstock with varying concentration of chloroform in ranges of 0–800 mg $CHCl_3$/L and 0–200 mg $CHCL_3$/L for H_2 and CH_4 production, respectively. The inhibitor concentrations affecting the microbial activity by "x" percent (IC_x) were estimated using a non-competitive inhibition model.

Materials and methods

Feedstock and inoculum preparation

As microalgae, *Chlorella vulgaris* with carbohydrate content of 12.5%, protein 66.9%, lipid 13%, ash 6%, and others 1.6% was used. The chemical oxygen demand (COD) concentration of *C. vulgaris* was 1.3 g COD/g dry cell weight (dcw). To prepare LEM, it was diluted with distilled water to 50 g COD/L, and ultrasonicated to disrupt the cell walls at the specific energy input of 100,000 kJ/kg TS using an ultrasonicator with a frequency of 20 kHz and a power of 150 W (VCX-750, Sonics and Materials, USA). The broth was then centrifuged at 7000 rpm for 10 min, and the supernatant was discarded. Finally, the sediment (195 g TS/L) used for the experiment was diluted with distilled water to 108 g TS/L, 91 g VS/L, 115 g COD/L, 22 g carbo./L, and pH 7.1.

The inoculum used in this study was obtained from an anaerobic digester at a local wastewater treatment plant. The pH, alkalinity, and volatile suspended solid (VSS) concentration of the sludge were 7.2, 2.8 g $CaCO_3$/L, and 40 g VSS/L, respectively. While raw sludge was used for CH_4 production, heat-treated sludge (90 °C for 20 min) was used for H_2 production to inactivate H_2-consuming methanogenesis [12].

Experiments

Batch tests were carried out using 250 mL serum bottles with a working volume of 150 mL. In the H_2 production experiment, 35 mL of heat-treated sludge and 100 mL of LEM were added to the bottles, corresponding to a substrate concentration of 76 g COD/L and an inoculum-substrate (I/S) ratio of 0.15 [12]. The rest of the working volume was filled with distilled water, and no external nutrients were added. After adding all substances, initial pH was adjusted at 7.5 ± 0.1 by adding 5 N HCl solution and the bottles were purged with N_2 gas for 5 min to provide an anaerobic condition. Finally, specified amounts of 10 N chloroform (CAS no. 67-66-3, $CHCl_3$, MW: 119.39) were added to make the desired chloroform concentrations at 0, 10, 25, 50, 200, 400, and 800 mg $CHCl_3$/L. All bottles were placed in a shaking incubator controlled at 35 °C and agitated at 100 rpm.

In case of CH_4 production, considering that CH_4-producers prefer a lower substrate concentration and a higher I/S ratio compared to H_2-producers [13], the experiment was conducted at a substrate concentration of 10 g COD/L and an I/S ratio of 2 [13]. Chloroform concentration was adjusted at 0, 2.5, 5, 10, 25, 50, 100, and 200 mg/L, and the rest of the experimental procedure was the same as that employed in the H_2 production test. All experiments including a blank (adding only inoculum) were carried out in triplicate, and the results were averaged.

Analytical methods

The amounts of CH_4 and carbon dioxide in the biogas were analyzed by a GC (Gow Mac series 580) equipped with a thermal conductivity detector (TCD) and a 2-m × 2-mm stainless steel column packed with Porapak Q (80/100 mesh). To determine the H_2 content in the biogas, a gas chromatograph

Please cite this article in press as: Yun Y-M, et al., Inhibitory effect of chloroform on fermentative hydrogen and methane production from lipid-extracted microalgae, International Journal of Hydrogen Energy (2014), http://dx.doi.org/10.1016/j.ijhydene.2014.04.167