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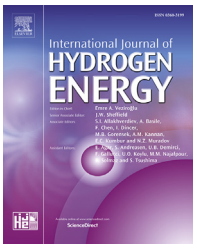

Co-digestion of untreated macro and microalgal biomass for biohydrogen production: Impact of inoculum augmentation and microbial insights

Periyasamy Sivagurunathan [a,b,*], *Gopalakrishnan Kumar* [c], *Takuro Kobayashi* [d], *Kaiqin Xu* [d,e,**], *Sang-Hyoun Kim* [c], *Dinh Duc Nguyen* [f], *Soon Woong Chang* [f]

[a] *Green Energy Technology Research Group, Ton Duc Thang University, Ho Chi Minh City, Viet Nam*
[b] *Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam*
[c] *School of Civil and Environmental Engineering, Yonsei University, Seoul, 03722, Republic of Korea*
[d] *Center for Materials Cycles and Waste Management Research, National Institute for Environmental Studies, Tsukuba, Ibaraki, 305-8506, Japan*
[e] *School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai, China*
[f] *Department of Environmental Energy Engineering, Kyonggi University, 94 San, Iui-dong, Youngtong-gu, Suwon-si, Gyeonggi-do, 16227, Republic of Korea*

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ABSTRACT

This study assessed the co-digestion of macro and microalgal biomass towards the improvement of hydrogen production. The red macroalgal biomass (*Gelidium amansii*) and green mixed microalgal biomass was mixed in a ratio of 8:2, with an initial substrate concentration of 10 g/L, and various amount of inoculum addition range from 3 to 15% (v/v) was evaluated to assess the feasible substrate to inoculum ratio for the effective co-digestion of the algal biomass. The results showed that the co-digestion with 6% inoculum addition provided the peak hydrogen yield of 45 mL/g dry biomass added with a high hydrogen content of 24% in the gas phase. The other tested conditions showed moderate hydrogen content in the range of 17–22%, respectively. These results suggest that anaerobic co-digestion of macro and microalgal biomass, with appropriate initial biomass loading (6%) is essential for enhanced hydrogen production.

Introduction

Presently, the global hydrogen market of USD 117.94 Billion is expected to grow up to USD 152.09 Billion by 2021, with its main use in fuel cells and hydrogen powered vehicles [1,2] According to International Energy Agency (EIA, 2011), 53% rise in energy consumption is expected between 2008 and 2035, with a moderate annual growth of 1.6%. Recent projections (EIA, 2011) indicates that renewable energy is the

* *Corresponding author*. Green Energy Technology Research Group, Ton Duc Thang University, Ho Chi Minh City, Viet Nam.
** *Corresponding author*. National Institute for Environmental Studies, 16-2, Tsukuba, Ibaraki, 305-8506, Japan.
E-mail addresses: periyasamy.sivagurunathan@tdt.edu.vn (P. Sivagurunathan), joexu@nies.go.jp (K. Xu), sanghkim@yonsei.ac.kr (S.-H. Kim).

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fastest-growing energy and its contribution can escalate up to 14% in 2035 against the 3% in 2010, with an estimated average rate of 3% per annum [3,4].

Renewable hydrogen production via dark fermentation by assessing various organic waste streams such as wastewater, lignocellulose, agricultural biomass and recently algal biomass has been widely focused to meet the energy demands of the future due to the cleaner nature and easier production methods with higher production rates of the process and well established commercial avenues [5–8]. Therefore, in the present proliferation of global economy, biohydrogen fuel production has emerged as a promising approach to fulfil the energy needs by utilizing the massive biological wastes generated in the fast-growing industrial society [9]. Co-digestion of various substrates towards boosting hydrogen production performances has been practised recently as an alternative option of the mono-fermentation of the organic biomass that shows quite lower production performances. A well improved synergistic effect has been shown with the fermentation of micro and macroalgae biomass along with various carbohydrate sources and also avoiding the over accumulation of C/N ratio which is considered as an important factor for the higher H_2 production turnouts [10–13].

Biohydrogen production from galactose (a major fraction of macroalgae biomass) has been well explored as a feasible model substrate for the biohydrogen production [14–16]. However, the major issue with the red algae biomass is their digestion efficiency and by co-fermentation with microalgae biomass this might be improved. Up to author's knowledge, Co-digestion of untreated micro and macro algae has not been well explored [10,13,17–20]; especially in the aspect of studying the impact of inoculum augmentation ratio of these untreated biomasses. Therefore, in this study, inoculum augmentation of macro and microalgae biomass has been documented with the framework of microbial community information for the strong support towards the utilization of algal biomass concerning enhanced energy production performances. In addition, the organic matter removal and the relative energy yield by means of hydrogen production have also been well archived.

Materials and methods

Inoculum source and algal biomass

The inoculum used for the experiment was procured from the granular digester sludge obtained from our laboratory. Table 1 shows the initial characteristics of the inoculum and algal biomass used in the study. The anaerobic seed sludge was used as an inoculum for hydrogen producing experiments. Thermal conditioning of the inoculum at 90 °C for 30 min was applied to promote the spore-forming hydrogen producing bacteria, meanwhile reducing the methanogenic populations [21]. The microalgal biomass used in the experiment was grown in a bolds basal medium and harvested using sedimentation for 24 h, air dried, and further used for the hydrogen producing experiments [22]. The dried biomass

Table 1 – Characteristics of inoculum and algal biomass used in this study.

Parameters	Inoculum	Macroalgae [23]	Microalgae [34]
pH	7.4	6.5	7.9
TS (g/L)	42.9	0.96	5.6
VS (g/L)	32.5	0.89	5.05
TS/VS ratio (%)	75.8	92.8	90.8
Total Protein (%-VS)	N.A	15.6	69.32
Total Carbohydrates (%-VS)	N.A	67.3	6.49

N.A- Not available/detected, data for algal biomass obtained from Refs. [23,34].

powder of *Gelidium amansii* with a particle size of 300 μm was used as a macroalgal biomass source for this study. The detailed composition of the *Gelidium amansii* was mentioned elsewhere [23] with a total carbohydrate content of 67.3% and protein of 15.6%.

Biohydrogen production

Hydrogen fermentation was performed in a batch vial with a holding capacity of 150 mL and 65 mL was used as a working volume for the experiment. Each vial was added to 50 mL of the slurry contains the macroalgal and microalgal biomass mixed in a ratio of 8:2, with an initial substrate concentration of 10 g/L, followed by addition of 5 mL of the modified Endo nutrient medium, and 10 mL of inoculum with varied volume of 3–15% (v/v) of the heat treated digester sludge. The headspace oxygen in the vial was removed by sparging the inert pure N_2 (99.9%) gas for 3 min to maintain the strict anaerobic environment during the initial startup of the batch vials. The initial pH of the media was adjusted to 7.0 by adding 6 N NaOH or HCl solution. At last step, the batch reactors were kept in an incubator under a mesophilic temperature regime 35 ± 0.1 °C with a stable rpm of 150. The experimental sets were run as duplicates and the average mean and median values were reported.

Analytical methods

Biogas volume was analyzed using an air-tight glass syringe with an adjustable volume. The biogas content (H_2 and CO_2) was checked with a gas chromatograph (GC-8A, Shimadzu) equipped with a thermal conductivity detector and a stainless steel column packed with Shin carbon ST (Shimadzu GLC) [24]. The final pH after the end of the fermentation was analyzed by using a pH meter (IM-55G, DKK-TOA). The TS (total solids) and VS (volatile solids) analysis were followed as per the APHA standard method [25]. Chemical Oxygen Demand (COD) was analyzed by HACH Digest Vials. Volatile fatty acids (VFA) concentration was measured with a gas chromatograph (GC14B, Shimadzu) equipped with a flame ionization detector and a StabiliwaxR-DA capillary column (Resteck) and the operational conditions were followed as reported elsewhere [26]. Protein and carbohydrate analysis was followed by Lowry and phenol-sulfuric standard methods [27,28].

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