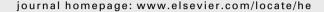
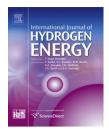


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Direct fermentation of Laminaria japonica for biohydrogen production by anaerobic mixed cultures

Xueqing Shi a , Kyung-Won Jung a , Dong-Hoon Kim b , Yong-Tae Ahn c , Hang-Sik Shin a,*

- ^a Department of Civil and Environmental Engineering, KAIST, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
- ^b Wastes Energy Research Center, Korea Institute of Energy Research, 102 Gajeong-ro, Yuseong-gu, Daejeon 305-343, Republic of Korea
- ^cDepartment of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA 16802, USA

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ABSTRACT

A few studies have been made on fermentative hydrogen production from marine algae, despite of their advantages compared with other biomass substrates. In this study, fermentative hydrogen production from Laminaria japonica (one brown algae species) was investigated under mesophilic condition (35 \pm 1 $^{\circ}$ C) without any pretreatment method. A feasibility test was first conducted through a series of batch cultivations, and 0.92 mol H_2/mol hexoseadded, or 71.4 ml H_2/g TS of hydrogen yield was achieved at a substrate concentration of 20 g COD/L (based on carbohydrate), initial pH of 7.5, and cultivation pH of 5.5. Continuous operation for a period of 80 days was then carried out using anaerobic sequencing batch reactor (ASBR) with a hydraulic retention time (HRT) of 6 days. After operation for approximately 30 days, a stable hydrogen yield of 0.79 \pm 0.03 mol H_2/mol hexoseadded was obtained. To optimize bioenergy recovery from L. japonica, an up-flow anaerobic sludge blanket reactor (UASBr) was applied to treat hydrogen fermentation effluent (HFE) for methane production. A maximum methane yield of 309 \pm 12 ml CH4/g COD was achieved during the 90 days operation period, where the organic loading rate (OLR) was 3.5 g COD/L/d.

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1. Introduction

Energy supply and environmental protection are two crucial issues for the sustainable development of global prosperity. Over 80% of the energy consumed today in the world is derived from fossil fuels [1]. However, this current energy system is now facing two fundamental problems: gradual depletion and environmental pollution. This lack of sustainability has led researchers to search for new alternative energy sources [2]. Among various kinds of energy sources, H_2 is regarded as the most promising future energy carrier, because it produces only water upon combustion, generating a higher energy yield (122 kJ/g), which is 2.75 times greater than that of hydrocarbon fuels. In addition, hydrogen can be

easily used as an automotive fuel in conventional internal combustion engines, and also can be applied in proton exchange membrane fuel cell vehicles [3].

 $\rm H_2$ is commercially produced by either electrolytic or thermo-chemical processes, both of which are energy intensive [4]. From an environmental engineering point of view, $\rm H_2$ made from renewable resources seems to be more promising, since it meets the goal of sustainable development. In this regard, fermentative hydrogen production, where carbohydrates are directly fermented into $\rm H_2$, $\rm CO_2$, and organic acids/alcohols without any external energy or electron acceptors, is considered a feasible biological process to produce $\rm H_2$ [5].

One of the main concerns in fermentative hydrogen production (FHP) is the high cost of the feedstock. In efforts to

^{*} Corresponding author. Tel.: +82 42 350 3613; fax: +82 42 350 8640. E-mail address: hs48@kaist.ac.kr (H.-S. Shin).

resolve this problem, many researchers have recently focused on lignocellulosic materials, which are composed of cellulose, hemicelluloses, and lignin, as new fermentative H_2 production substrates [5–9].

Lignocellulosic biomass in nature is by far the most abundant raw material, originating from hardwood, softwood, grasses, and agricultural residues. The annual yields of lignocellulosic biomass residues worldwide are estimated to exceed 220 billion tons, equivalent to 60-80 billion tons of crude oil [10]. However, yields of H_2 produced by direct fermentation of lignocellulosic biomass are very low, mainly due to the complex structure of these substrates [11]. In order to enhance the digestibility of lignocellulosic material, different pretreatment methods have been applied, such as thermal, mechanical, acid, alkaline pretreatment, etc. However, various kinds of inhibitors are generated during these processes [6].

Marine algae is an aquatic group of cellulosic biomass. Although it has not been actively explored as an energy crop, it has many advantages for FHP, including the followings: 1) The main components of marine algae are cellulose and hemicellulose, not lignin, and thus fewer inhibitors will be generated during the pretreatment or fermentation process; 2) It has higher carbohydrate content compared with lignocellulosic biomass; and 3) It is massively abundant and easy to obtain or harvest [12,13]. Among this group, Laminaria japonica, a brown algae species, is a potential candidate for H_2 fermentation [14]. In 2006, the production of L. japonica in South Korea was estimated at around 0.8 million tons [14]. Like other brown algae, the main carbohydrate constituents of L. japonica are mannitol, laminaran, cellulose, and fucoidan and alginic acid, some of which are already known as good substrates for FHP [15,16]. Moreover, Jung et al. [16] reported that among various marine algae candidates, L. japonica showed the highest potential for FHP.

The aim of this study was to establish a stable FHP system using *L. japonica* as a feedstock. After a feasibility test under a series of batch cultivations, continuous operation was conducted using an anaerobic sequencing batch reactor (ASBR). To optimize bioenergy recovery, an up-flow anaerobic sludge blanket reactor (UASBr) was applied to treat the hydrogen fermentation effluent (HFE) for methane production.

2. Materials and methods

2.1. Seed sludge and substrate

The seed sludge was taken from an anaerobic digester in a local wastewater treatment plant in South Korea. The pH, alkalinity, and volatile suspended solid (VSS) concentration of the sludge were 7.6, 2.83 g $CaCO_3/L$, and 5.5 g/L, respectively.

For screening hydrogen producing bacteria (spore-forming anaerobic bacteria such as *Clostridium sp.*) and inactivating hydrogen consumers, 20 min heating at 90 °C was applied as a pretreatment step.

The feedstock was first dried at room temperature and then ground into 0.5 mm (diameter) particles by a normal blender. There was no external nutrient addition. The composition of L. japonica is shown in Table 1.

2.2. Batch test

To investigate the feasibility of utilizing L. japonica for biohydrogen production and to determine optimal operation parameters, three batch tests were conducted under mesophilic condition (35 \pm 1 $^{\circ}$ C). Batch reactors with a working volume of 3 L were seeded with heat-pretreated sludge, equivalent to 30% of the working volume, and filled with a specific amount of L. japonica particles and tap water. The reactor was purged with N₂ for 5 min to provide an anaerobic condition and agitated at 150 rpm pH was monitored by pH sensors and controlled by the addition of 3N KOH solution. The produced gas was collected by gas collectors and sampled using a 1 ml syringe to analyze H2 content. Three operation parameters, substrate concentration, initial pH, and cultivation pH (initial pH was controlled at the beginning of experiment, to provide suitable growth environment for H2 producing microbes, and cultivation pH was controlled as constant during the fermentation process to ensure the microbial metabolic pathway was suitable for H₂ production and also inhibit H₂consuming methanogenic activity), were evaluated in terms of their effect on hydrogen production. In the first batch test, the substrate concentrations were 5, 10, 20, 30, and 40 g Carbo. COD/L (calculation based on carbohydrate content and TCOD/TS ratio of the substrate); in the second batch test, the initial pH values were 7.0, 7.5, 8.0, 8.5, and 9.0; and in the third batch test, the cultivation pH values were 5.0, 5.5, 6.0, and 6.5, respectively. In the first batch test, the initial pH and cultivation pH values were kept at 8.0 and 5.5, while for the second and third batch tests, the operation parameters were selected as the optimal values based on the previous batch results (as shown in the result part, for the second batch test, the substrate concentration was 20 g Carbo. COD/L, and cultivation pH was 5.5; for the third batch test, the substrate concentration was 20 g Carbo. COD/L, and initial pH was 7.5).

To describe the hydrogen production, cumulative H_2 production curves were obtained using the modified Gompertz Eq. (1) [17].

$$H(t) = P \times exp \left\{ -exp \left[\frac{R \times e}{P} (\lambda - t) + 1 \right] \right\}$$
 (1)

Table 1 — Composition of Laminaria japonica.								
Name Composition (%)								
Laminaria japonica	Carbohydrate					Protein	Lipid	Etc.
	Total	Cellulose	Hemi- cellulose	Lignin	Etc.			
	56.4	16.9	31.0	0	8.5	8.4	1.6	33.6

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