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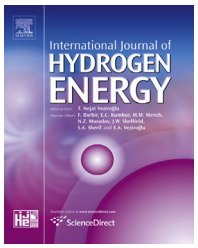

Effect of acid-pretreatment on hydrogen fermentation of food waste: Microbial community analysis by next generation sequencing

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ABSTRACT

This work presents the effect of acid-pretreatment on H_2 fermentation of food waste with detailed microbial information by next generation sequencing. The pretreated food waste at pH 1.0–4.0 was cultivated under mesophilic conditions without external inoculum addition. From the food waste acid-pretreated at pH 1–3, H_2 yields in the range of 1.37–1.74 mol H_2/mol $hexose_{added}$ were achieved, attaining the highest value at pH 2. *Clostridium* sp. such as *Clostridium acetobutylicum* ATCC 824 and *Clostridium perfringens* occupied more than 70% of total number of sequences at pH 1–3. On the other hand, in the control (no pretreatment) and at pH 4, lactic acid bacteria such as *Lactobacillus* and *Streptococcus* were found to be the dominant genus (>90% of total number of sequences), resulting in a low H_2 yield. In addition, the effect of substrate concentration on H_2 fermentation was investigated, and the maximum H_2 productivity was estimated to be 27.2 L H_2/L/d by Andrew's model.

Introduction

Hydrogen (H_2) is widely regarded as one of the most promising energy carriers since it produces only water when combusted, has a high energy capacity of 33 kWh/kg H_2, and can be easily converted to electricity by fuel cells [1]. Currently, most H_2 is made by splitting natural gas, heavy oil, naphtha, and coal, which are all fossil fuels, under high temperature and pressure conditions [2]. However, it is necessary to use renewable

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and sustainable resources as a feedstock, and to employ an environmentally friendly method of H_2 production.

Biological H_2 production processes are more environmentally friendly and less energy consumptive than physicochemical ones. They provide a wide range of approaches to generate H_2, including direct biophotolysis, indirect biophotolysis, photo-fermentation, and dark fermentation, which uses water and organic substances as feedstock [3]. In particular, dark fermentation is considered the most practically applicable method since its H_2 production rate is much faster than that of other processes, and problematic organic wastes such as food waste can be treated along with H_2 production [4,5]. Food waste is a topic of concern worldwide, with its problems of decay, odor, and leachate during collection and transportation [6]. However, as food waste has organic content, in particular, carbohydrate substances (30–60% of total organics), the choice of dark fermentation seems appropriate [7].

In dark fermentation using mixed cultures, the microbial community has often been analyzed to support the experimental results of H_2 fermentation performance. As tools for analysis, fluorescence *in situ* hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), quantitative Real-Time PCR (qPCR), and 16S rRNA gene clone library analysis have been employed [8]. These methods could provide a better understanding on microorganisms involved in dark fermentation by identifying predominant microorganisms, exploring the diversity of community compositions, and the functions of certain groups of microorganisms. However, they are extremely laborious, time consuming, and expensive when using a large number of samples, have low sensitivity, and often lead to misidentification [9].

Recent advances in sequencing technology, called next generation sequencing (NGS) have revolutionized the field of microbial ecology and genomics. NGS could determine microbial communities of environmental samples with high sequencing depth at a much faster speed with low cost. NGS has been widely used in large, complex, and dynamic microbial communities such as marine water, soil, human distal intestines, and wastewater treatment plants [10,11], but have never been applied in dark fermentation.

To increase the H_2 yield from solid-type feedstock, various pretreatments have been applied for two purposes: (1) increase hydrolysis, and (2) suppress indigenous non-H_2-producers. Steam explosion and acid/alkali shock have been applied to lignocellulosic biomass to remove lignin, and hydrolyze hemicellulose and cellulose to fermentable reducing sugars [12]. Waste activated sludge, which is mainly composed of cell walls, has been pretreated by mechanical, chemical, thermal, and biological attack for better contact of intracellular organics [13]. On the other hand, food processing waste is easily biodegradable, but contains non-H_2-producers, which impede stable H_2 fermentation performance. Noike et al. [14] reported that continuous H_2 production from bean curd manufacturing waste was not sustained due to the existence of lactic acid bacteria (LAB) in the feedstock, and they could overcome it by heat treatment (50–90 °C for 30 min). In the continuous treatment of food waste, a gradual drop of H_2 yield was observed in spite of high substrate removal, which was solved by alkaline pretreatment of the food waste [15,16].

In this study, food waste was pretreated by acid-shock to enhance H_2 fermentation performance. The pretreated food waste at pH 1.0–4.0 (intervals of 1.0) was cultivated under mesophilic conditions without external inoculum addition. We investigated microbial communities at the end of fermentation using an NGS tool, and correlated the results with the fermentation performance. In addition, the effect of substrate concentration on H_2 production from food waste was investigated using Andrew's model.

Materials and methods

Feedstock

Food waste collected from a cafeteria at the Korea Institute of Energy Research was shredded to a size smaller than 5 mm in diameter. As the food waste was not collected at the same time, the characteristics of the food waste were different at each batch test, as shown in Table 1. The main components of food waste were mainly rice (>50%), vegetables, fruits, etc. The acidic condition of the food waste indicates the presence of indigenous microorganisms that generate acids.

Fermentation

The first batch test investigated the effect of pretreatment pH on the H_2 fermentation performance. After diluting the ground food waste by 1.3 times using tap water, it was pretreated at pH 1–4 (at intervals of 1.0) using a 6 N HCl solution for 12 h. During the pretreatment process, the food waste was agitated at 100 rpm using a mechanical stirrer with four impellers, and the pH was maintained by a pH sensor and 3 N HCl solution addition under room temperature condition (20 °C). The acid-pretreated food waste corresponding to 30 g CCOD (Carbohydrate COD)/L was then added to the batch fermenter with an effective volume of 300 mL, while the remainder of the effective volume was filled with tap water. Neither external inoculum nor basal medium was added. In one batch fermenter, the food waste without pretreatment was added as a control. Before fermentation, the pH was readjusted to 8.0 ± 0.1 by 6 N KOH addition, which was found to be the optimal initial pH value in our previous work [17]. After

Table 1 – Characteristics of food waste used (Batch test I and II were to investigate the effect of pretreatment pH and substrate concentration on H_2 fermentation of food waste, respectively.).

Item	Unit	Food waste	
		Batch I	Batch II
Total COD	g COD/L	195 ± 11	194 ± 7
Total solids (TS)	g/L	166 ± 13	170 ± 5
Volatile solids (VS)	g/L	160 ± 13	158 ± 2
Carbohydrate	g COD/L	125 ± 12	117 ± 14
Total nitrogen (TN)	mg N/L	4330 ± 60	3180 ± 70
Ammonia	mg NH_4–N/L	120 ± 9	133 ± 15
pH	–	4.8 ± 0.2	4.5 ± 0.1