Journal of Environmental Management 144 (2014) 143–151

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Analysis of microbial community adaptation in mesophilic hydrogen fermentation from food waste by tagged 16S rRNA gene pyrosequencing

Thanaporn Laothanachareon [a], Suwimon Kanchanasuta [b], Wuttichai Mhuanthong [a], Chantaraporn Phalakornkule [b, c, d], Nipon Pisutpaisal [b, d, e, f, **], Verawat Champreda [a, *]

[a] Enzyme Technology Laboratory, Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, Khlong Luang, Pathum Thani 12120, Thailand
[b] The Joint Graduate School for Energy and Environment (JGSEE), King Mongkut's University of Technology Thonburi, Thungkru, Bangkok 10140, Thailand
[c] Department of Chemical Engineering, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[d] The Research and Technology Center for Renewable Products and Energy, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[e] Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand
[f] The Biosensor and Bioelectronics Technology Centre, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand

A R T I C L E I N F O

Article history:
Received 27 January 2014
Received in revised form
12 May 2014
Accepted 20 May 2014
Available online

Keywords:
Bacterial community
Dark fermentation
Food waste
Hydrogen
Pyrosequencing
16S rRNA gene

A B S T R A C T

Dark fermentation is an attractive process for generation of biohydrogen, which involves complex microbial processes on decomposition of organic wastes and subsequent conversion of metabolic intermediates to hydrogen. The microbes present in an upflow anaerobic sludge blanket (UASB) reactor for waste water treatment were tested for application in batch dark fermentation of food waste at varying ratios of feedstock to heat-treated microbial inoculum (F/M) of 1–8 (g TVS/g TVS). Biohydrogen yields between 0.39 and 2.68 mol H_2/mol hexose were obtained, indicating that the yields were highly dependent on the starting F/M ratio. The highest H_2 purity of 66% was obtained from the first 8 h of fermentation at the F/M ratio of 2, whereas the highest H_2 production was obtained after 35 h of fermentation at the F/M ratio of 5. Tagged 16S rRNA gene pyrosequencing showed that the seed culture comprised largely of uncultured bacteria with various *Proteobacteria*, *Bacteroidetes*, and *Firmicutes*, while the starting food waste contained mainly lactic acid bacteria. Enrichment of *Firmicutes*, particularly Clostridia and lactic acid bacteria occurred within 8 h of the dark fermentation and the H_2 producing microcosm at 35 h was dominated >80% by *Clostridium* spp. The major H_2 producer was identified as a Clostridial strain related to *Clostridium frigidicarnis*. This work demonstrated the adaption of the microbial community during the dark fermentation of complex food waste and revealed the major roles of Clostridia in both substrate degradation and biohydrogen production.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Hydrogen (H_2) is widely known as an ideal clean energy source. It has the highest specific energy content and its combustion releases water as the only by-product (Edwards et al., 2008). There are many strategies to generate H_2 from various inexpensive renewable feedstocks by thermochemical, electrochemical, and biological processes (Chen et al., 2006). Biological H_2 (biohydrogen) production is less energy intensive than non-biological processes, and so is considered more environmentally friendly. Hydrogen is generated as a product of several different metabolic pathways including direct water bio-photolysis by green algae, indirect water bio-photolysis by cyanobacteria, photo-fermentation by photosynthetic purple non-sulfur bacteria, and dark fermentation by heterotrophic anaerobic bacteria. The light-dependent processes generate H_2 at lower rates and the spectrum of the substrates is narrower than that of the dark fermentation (Hallenbeck and

* Corresponding author. Tel.: +66 2564 6700x3473; fax: +66 2564 6707.
** Corresponding author. The Joint Graduate School for Energy and Environment (JGSEE), King Mongkut's University of Technology Thonburi, Thungkru, Bangkok 10140, Thailand. Tel.: +66 2 943 5549; fax: +66 2 587 8257.
E-mail addresses: npp@kmutnb.ac.th (N. Pisutpaisal), verawat@biotec.or.th (V. Champreda).

http://dx.doi.org/10.1016/j.jenvman.2014.05.019
0301-4797/© 2014 Elsevier Ltd. All rights reserved.

Benemann, 2002; Levin et al., 2004; Wakayama and Miyake, 2001). Feedstocks such as municipal waste, livestock manure, crop residues, and industrial wastes have been demonstrated to support H_2 production by dark fermentation (Cakır et al., 2010; Datar et al., 2007; Noike and Mizuno, 2000; Xing et al., 2010). In addition, dark fermentation can occur under mesophilic, thermophilic, or extreme thermophilic conditions. With its process flexibility, simple reactor design, and capability on degrading wastes that are difficult to break down by other means, H_2 production by dark fermentation has increasingly attracted attention for industrial-scale implementation (Ren et al., 2011).

Efficient production of biohydrogen depends on many factors including operating conditions, substrate compositions, and microbial community. The key biohydrogen-producing microbes may originate from substrates, microbial seed or both. The use of complex microbial seed cultures as starting inocula is advantageous for biohydrogen production from complex organic substrates. These advantages include higher operating stability and tolerance to indigenous microbes present in the feedstock, as well as capability for producing a wide range of hydrolytic enzymes (Argun and Kargi, 2009). Food waste is a common substrate for biogas production. Typically, it comprised of starch and fiber polysaccharides i.e. cellulose and hemicellulose, proteins and lipids as major constituents and vitamins and ash as minor constituents (Kapdan and Kargi, 2006). Over the last decade, H_2 production from dark fermentation of food waste has been reported using lab-scale bioreactors under mesophilic and thermophilic conditions (Ismail et al., 2009; Li et al., 2008).

The community structures of microbes and their metabolic capability play important roles in biohydrogen fermentation processes. Molecular diversity analysis of 16S rRNA as a general phylogenetic markers and more specifically, the [Fe–Fe] hydrogenase gene (*hyd*A) has been used to explore organismic diversity in H_2 fermentation. Various culture-independent molecular methods have been used to explore microcosm structures and dynamics in dark fermentation niches based on diversity of these phylogenetic markers e.g. denaturing gradient gel electrophoresis (Jo et al., 2007; Mariakakis et al., 2011; Ueno et al., 2001), capillary gel electrophoresis-single strand conformation polymorphism (Quéméneur et al., 2010, 2011), clone library (Chaganti et al., 2012; Tomazetto and Oliviera, 2013), and pyrosequencing of biodiversity marker genes. (Im et al., 2012; Lu et al., 2012). The tagged amplicon pyrosequencing approach is the current method of choice as it allows for high-throughput quantitative parallel analysis of microbial community structures and functions from different environmental and engineered systems (Dowd et al., 2008). In this study, the microbes present in an UASB reactor were used as an inoculum for mesophilic fermentation of food waste. The production of biohydrogen was monitored during fermentation under different conditions and the microbial diversity investigated using tagged 16S rRNA gene pyrosequencing. The data reveal an abundance of biohydrogen producing microcosms among the microbial communities of the complex inoculum and food waste suggestive of adaptation. This study provides an important basis for process monitoring and further improvement of hydrogen production efficiency from complex organic wastes.

2. Materials and methods

2.1. Feedstock and microbial seed inoculum

Synthetic food waste (F) was prepared from typical locally-produced food waste with the composition of 65% carbohydrate (rice), 17% vegetable and 18% meat (w/w). The feedstock was ground in a blender to particles of diameter approx. 0.5 mm and used as the feedstock with no prior sterilization. Hexose content in the food waste was analyzed by the phenol-sulfuric acid method. Anaerobic granules were obtained from a full scale UASB reactor for treating fruit juice processing wastewater (Malee Industry Co. Ltd., Nakornpathom, Thailand). Coarse matter >0.5 mm diameter was removed by sieving and the granules were washed twice with tap water. The fine granules were boiled at 100 °C for 1 h to deactivate methanogens. The heat-treated granules were re-cultivated in 0.5% (w/v) glucose solution and used as a seed microbial inoculum (S) for dark fermentation. The characteristics of the feedstock and seed culture are shown in Table 1.

2.2. Batch fermentation

The batch fermentation system was set up in 500 mL screw-cap bottles with a working volume of 500 mL (375 mL of food waste and 125 mL of inoculum) and the head space of 100 mL. The microbial seed inoculum in all conditions was fixed at 4.75 g total volatile solids (TVS) per batch. The food waste was added at varying concentrations between 3.74 and 29.94 g COD/L, corresponding to F/M ratios between 1 and 8. The initial pH was adjusted to 6.0 with 6 N NaOH or concentrated H_3PO_4. The system was flushed with nitrogen gas to generate anaerobic conditions. H_2 fermentation was conducted at 37 °C with rotary shaking at 150 rpm. During the fermentation experiment, total gas volume and composition were periodically monitored by gas counters and gas chromatography, respectively. The liquid samples were analyzed for pH and metabolic products, i.e., volatile fatty acids (VFA) and ethanol every 4–6 h according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). In this study, each experiment was carried out in triplicate and the data shown for each condition are representative results of independent tests that were done in triplicate.

2.3. Gas analysis

Biogas content (H_2, CH_4, and CO_2) was measured periodically every 5 h using a gas chromatograph (Shimadzu GC-8A, Kyoto, Japan) equipped with a thermal conductivity detector (TCD) with a Unibeads C 60/80 column (GL Sciences, Inc., Tokyo, Japan). Helium was used as a carrier gas. The temperatures of the injection port and the detector were 150 °C and 80 °C, respectively. VFAs and ethanol were analyzed by gas chromatography (Shimadzu GC-7A system equipped with a flame ionization detector and a Stabilwax DA capillary column (Restek Corporation, PA, USA). The temperatures of the injection port and detector were maintained at 240 °C (Kanchanasuta and Pisutpaisal, 2012). The modified Gompertz equation (eq. (1)) was used to fit cumulative hydrogen production data obtained from each batch experiment (Lay et al., 1999).

Table 1
Characteristics of the seed culture and feedstock.

Parameter	Heat-treated anaerobic granule (M)	Food waste (F)
Total solids (TS, mg/L)	7333.3	7000.0
Total volatiles solids (TVS, mg/L)	6333.3	6666.7
Total COD (mg/L)	5030.7	3742.3
Soluble COD (mg/L)	2061.4	785.3
Acetic acid (mmol/L)	0.54	1.70
Butyric acid (mmol/L)	0.11	0.12
Propionic acid (mmolL)	0.06	0.05
Ethanol (mmol/L)	0.60	0.85

Download English Version:

https://daneshyari.com/en/article/7483656

Download Persian Version:

https://daneshyari.com/article/7483656

Daneshyari.com