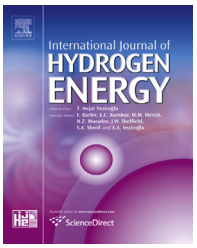
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2015) 1–11

Available online at www.sciencedirect.com

ScienceDirect

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



Feasibility of enriched mixed cultures obtained by repeated batch transfer in continuous hydrogen fermentation

Periyasamy Sivagurunathan [a,e], *Gopalakrishnan Kumar* [b], *Jeong-Hoon Park* [c,d], *Jong-Hun Park* [c,e], *Hee-Deung Park* [c], *Jeong-Jun Yoon* [d], *Sang-Hyoun Kim* [a,e,*]

[a] *Department of Environmental Engineering, Daegu University, Gyeongsan, Gyeongbuk 712-714, South Korea*
[b] *Center for Materials Cycles and Waste Management Research, National Institute for Environmental Studies, Tsukuba, Japan*
[c] *Civil, Environmental and Architectural Engineering, Korea University, Anam-Dong, Seongbuk-gu, Seoul 136-714, South Korea*
[d] *IT Convergence Materials R&D Group, Korea Institute of Industrial Technology, Chungnam 330-825, South Korea*
[e] *Sustainable Environmental Process Research Institute, Daegu University, Gyeongsan, Gyeongbuk 712-714, South Korea*

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form
23 June 2015
Accepted 24 June 2015
Available online xxx

Keywords:
Hydrogen
Enriched mixed cultures (EMC)
Galactose
Soluble metabolic products
Next generation sequencing

ABSTRACT

This research investigated the suitability of enriched mixed cultures (EMC) for anaerobic hydrogen fermentation in continuous operation. EMC was prepared after four successive transfers in PYG (peptone, yeast extract and galactose) medium in batch cultivation. The peak hydrogen production rate (HPR) and hydrogen yield (HY) of 770 ± 10 mL H_2/L-d and 1.05 ± 0.06 mol H_2/mol $galactose_{added}$, were attained respectively. There forward a continuously stirred tank reactor (CSTR) has been operated with the substrate concentration of 15 g/L at a hydraulic retention time (HRT) of 12 h for more than 15 days by using EMC. The performance showed that HPR and HY were fluctuated significantly during the operation and the average values were 1710 ± 250 mL H_2/L-d and 0.82 ± 0.12 mol H_2/mol $galactose_{added}$, respectively. The soluble metabolic products analysis revealed that butyrate, lactate and acetate were the dominant metabolic products with less quantity of propionic and formic acids. The microbial community structure has been determined by next generation DNA sequencing technique and revealed *Clostridium* sp. was the dominant microbial consortium during repeated batch transfer, whereas *Sporolactobacillus* sp. was the major population in continuous operation. This study demonstrates that operational mode (batch and continuous) significantly influence the microbial diversity and hydrogen production, and EMC obtained by repeated process may not be suitable for continuous hydrogen fermentation.

* Corresponding author. Jillyang, Gyeongsan, Gyeongbuk 712-714, South Korea. Tel.: +82 53 850 6691; fax: +82 53 850 6699.
E-mail address: sanghkim1@daegu.ac.kr (S.-H. Kim).

http://dx.doi.org/10.1016/j.ijhydene.2015.06.133

Introduction

Although fossil fuels remain as dominant primary energy source, its increasing demand is unsustainable due to the depletion of available natural resources and the environmental impacts associated with combustion. Hydrogen is considered as a potential alternative fuel due to its clean nature during combustion process (no CO_2 emission) and higher energy content with a value of 122 kJ/g. It can be produced by biological (dark fermentation, photofermenation, and biophotolysis) and non-biological (steam reforming) methods. Currently, steam reforming of fossil fuels is the prevailing hydrogen production process; however, this process is considered as neither sustainable nor clean [1]. In recent years, dark fermentative hydrogen production method has been proposed as a sustainable and attractive process mainly because of its mild operational conditions and high productivity without light requirement. Additionally, hydrogen can be produced from wide varieties of renewable organic wastes, which can reduce the operational cost and provide sustainable waste management [2].

Dark fermentative hydrogen production can be conducted by using either pure cultures or mixed microflora. Mixed cultures operation is beneficial to the industrial and economical process due to the robust performances and efficient conversion of wide range of non-sterile organic waste materials with the aid of diverse group of bacteria [3,4]. These diverse group of mixed microflora can be obtained from various natural sources such as sludge, soil, lake sediment, hot spring, and treatment plants [5]. However, along with the hydrogen producing microflora, hydrogen consuming microflora also exists in the natural eco-system; this can be suppressed by using appropriate pretreatment methods such as heat, acid, alkali, chemical agents to enrich the hydrogen producing microflora [5,6]. Heat treatment is widely used technique to enrich the spore forming hydrogen producers [7,8]. Apart from the pretreatment methods culture enrichment methods also applied to obtain the stable hydrogen producing microbial consortia [9].

Galactose is one of the most abundant carbohydrates, and the major component of the marine red algae and dairy industry wastes [10,11]. Galactose have the same molecular formula with glucose, however the direct conversion of galactose to hydrogen production is slow when compared to the glucose, due to the additional energy input for the conversion of galactose to glucose-1-phosphate via Leloir pathway [12] and subsequent metabolism to produce hydrogen and soluble metabolic products [12,13]. Biohydrogen production from glucose has been widely studied; however the studies on galactose are quite limited [13–15]. The key species responsible for the hydrogen production from galactose has to be identified in order to obtain the efficient hydrogen production from the galactose based monomers.

In our recent study it is indicated that cluster I *Clostridium* sp. was the major hydrogen producing bacteria from galactose in a batch mode operation [13] and the relationship between the microbial species and physiological dynamics changes during the hydrogen fermentation of galactose is still unknown/partially known. Therefore, this study used a synthetic medium containing galactose as a sole sugar. Additionally, previous investigations reported that enriched mixed cultures showed significant improvement in hydrogen production due to the selective population of the hydrogen producers [16,17]. However, most reported investigations were conducted in batch mode operation only [18,19]. Lab-scale continuous operation is a prerequisite step to design a full-scale hydrogen production facility using an enriched mixed culture. In addition checking the stability of the EMC in both batch and continuous operation could provide the new insights to the field and also aid in the development of efficient H_2 fermentation process using galactose. Up to author's knowledge, this is the first report using the enriched mixed cultures in the continuous galctose-H_2 fermentation process. Hence, this study was aimed to monitor the microbial species composition of an enriched mixed culture as well as hydrogen and metabolic products formation from galactose in a repeated batch and continuous fermentation process.

Materials and methods

Feedstock and seed inoculum

The monosaccharide sugar, galactose (Daejung, Korea) was used as the sole carbon source for the hydrogen production. The seed sludge (anaerobic mixed cultures) was collected from an anaerobic digester in a local wastewater treatment plant. The characteristics of the sludge were TCOD: 22.6 g COD/L, VSS: 12.6 g/L and pH: 6.8. Heat treatment (90 °C for 30 min in a water bath) was applied as pretreatment method to cultivate/enrich spore forming hydrogen producers and also to prohibit the activity of hydrogen consumers (methanogens).

Enrichment of hydrogen-producing bacteria and batch hydrogen fermentation

Enrichment of H_2 producing mixed cultures was carried out in 125 mL serum vials with a 50 mL working volume following a method described elsewhere [19]. The sterile pre-reduced peptone-yeast-galactose (PYG) medium contained the following nutrients (in g/L): 10, peptone; 10, yeast extract; 0.001, resazurin; 0.5, L-cysteine-HCl; 10, galactose. The pH was adjusted to 7.0 using either 1 N HCl or NaOH prior to autoclave. 10 mL of (12.6 g VS/L) Seed sludge was directly used in the first transfer, further enrichment was done by adding 10% (v/v) of 24 h grown mixed culture in to the pre-reduced batch vials. The vials were purged with nitrogen gas to make the headspace empty and also to remove the oxygen. Then, the vials were placed in an incubator maintained at 35 ± 1 °C and a rotating speed of 150 rpm. All the data were obtained from triplicate experiments and the mean values are reported. The enriched cultures were stored at 4 °C. Freshly grown (24 h) enriched mixed cultures after the fourth transfer were used as the inoculum for the continuous fermentation experiments.

Please cite this article in press as: Sivagurunathan P, et al., Feasibility of enriched mixed cultures obtained by repeated batch transfer in continuous hydrogen fermentation, International Journal of Hydrogen Energy (2015), http://dx.doi.org/10.1016/j.ijhydene.2015.06.133