Stable Fermentative Hydrogen Production by Polyvinyl Alcohol (Pva) Gel Beads Fluidized Bed Reactor

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Abstract : A novel hydrogen fermentation technique by using polyvinyl alcohol (PVA) gel beads as a biomass carrier was investigated. The hydrogen gas was stably produced throughout the experimental period in a continuous reactor. Even though the hydrogen productivity was suddenly decrease by experimental troubles, the bacteria attached to the PVA gel beads played as an inoculum, it was promptly recovered. The hydrogen yield per glucose was not very high (1.0 - 1.2mol-H_2/mol-glucose), thus the optimization of the experimental conditions such as ORP and HRT should be considered to improve the hydrogen productivity. Bacterial community was stable during experimental period after the PVA gel beads applying, which indicated that applying of biomass carrier was specific to keep not only the biomass but also the bacteria commonly. Clostridium species were phylogenetically detected, which suggested that these bacteria contributed to the hydrogen production in the biofilm attached to the PVA gel beads..

Keyword : Hydrogen fermentation, PVA gel beads, Fluidized bed, Biofilm, Bacterial community

Introduction

Recently, problems of waste disposal, e.g., shortage of waste disposal site and hazardous chemicals generated in incineration process, have become more serious. It is desired to develop new technologies for waste reduction and recycling of waste materials in order to establish a recycling society. Since effective utilizations of organic waste have been desired, the biological fermentation technologies to recover energy like a ethanol, methane and hydrogen from the waste have received considerable attention. Among them, hydrogen is expected as one of the promising future energy replaced with the fossil fuel because that it is a material for fuel cell and the clean energy which produce no carbon dioxide in combustion. As for the technologies to recover hydrogen from organic waste and wastewater, anaerobic hydrogen fermentation and photo-synthetic hydrogen production have been known (Kawagoshi, Y.). Anaerobic hydrogen fermentation is especially studied as a pretreatment process in a methane fermentation. In the methane fermentation of the organic waste containing low water-soluble materialslike a cellulose, solubilization step is commonly needed (Ohba, M. *et al.*, 2005; Goto, M. *et al.*, 2005). If hydrogen fermentation can be used as an alternative of this step, hydrogen recovery is expected as well as the solubilization reaction. There are many reports about anaerobic hydrogen fermentation from a pure substrates like a glucose (Mizuno, O. *et al.*, 1996) and real wastes like a kitchen garbage (Mizuno, O. *et al.*, 1997).

So far, we have studied on the hydrogen fermentation condition (pH, temperature, Nitrogen concentration) to obtain the high hydrogen productivity by using a suspended biomass (Kawagoshi, Y. *et al.*, 2005). Commonly, genus Clostridium and genus *Enterobacter* have been known as anaerobic hydrogen fermentating

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bacteria (Levin, B.D. *et al.*, 2004). The growth rate of these bacteria was usually higher than that of methane bacteria which consume the hydrogen produced during fermentation. Therefore, a continuous stirred tank reactor (CSTR) was commonly applied for hydrogen fermentation in order to wash out the methane bacteria from the reactor by shortening hydraulic retention time (HRT). However, it is difficult to hold the suspended biomass at high density in the reactor, and hydrogen fermentation reaction likely to be unstable (Noike, T. *et al.*, 2004). In this study, we aim to establish a stable hydrogen fermentation technique using a hydrogen fermentating biofilm formed in PVA gel beads as biomass carrier, and discussed interaction between hydrogen fermentation properties and the bacterial community.

Materials and methods

Continuous hydrogen fermentation

Schematic diagram of continuous hydrogen fermentation reactor is shown in Figure 1. A 1.6l of the medium (Glucose 40g/l, NH_4HCO_3 2.5g/l, K_2HPO_4 125mg/l, Na_2CO_3 2g/l, $CuSO_4 \cdot 5H_2O$ 5mg/l, $MgCl \cdot 6H_2O$ 100mg/l, $MnSO_4 \cdot 4H_2O$ 15mg/l, $CoCl_2 \cdot 6H_2O$ 0.125mg/l, $FeSO_4 \cdot 7H_2O$ 25mg/l) containing the anaerobic digested sludge of 3.0 g/l was put in a 2l of grass-made reactor. The medium was purged with argon gas for 10 min to remove oxygen and maintained anaerobic condition by connecting a gas-bag containing argon gas as shown in Figure 1. Continuous culture was carried out at pH of 6.0 (controlled), HRT 24hours, agitation rate 200rpm and temperature 35℃. At the beginning, the culture was performed with no PVA gel beads. After that, PVA gel beads were put into the reactor at the filling rate of 32 % (v/v).

Analysis

The gas composition (H_2, CO_2, CH_4, N_2, and O_2) was analyzed using a gas chromatograph equipped with thermal conductivity detector. Argon gas was used as a carrier and other conditions were set according to the manufacturer' sprotocol. The volatile fatty acids(VFAs; acetic

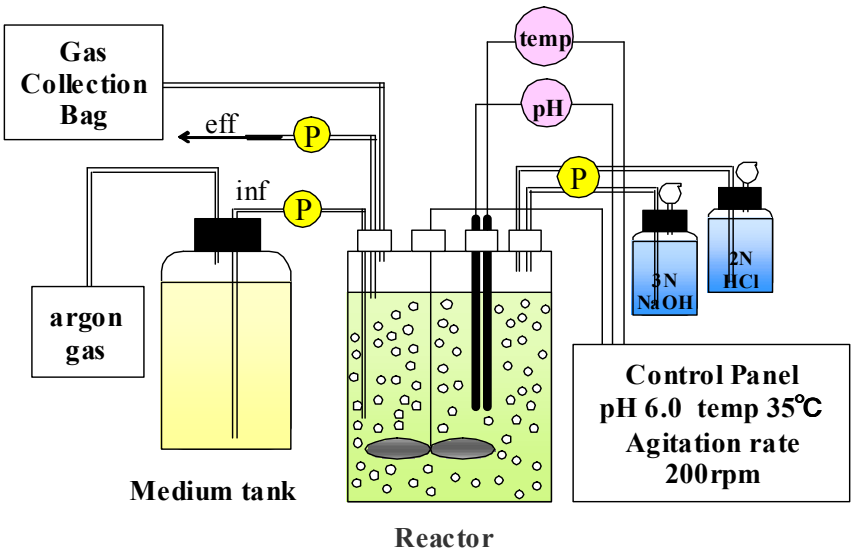

Fig. 1 Schematic diagram of the continuous hydrogen fermentation

acid, lactic acid, butyric acid, propionic acid, isobutyric acid, valeric acid and isovaleric acid) were analyzed using a high-performance liquid chromatography (HPLC) system equipped with UV-detector (210nm). The glucose concentration was measured by phenol- sulfuric acid method.

Bacterial community analysis by denaturing gradient gel electrophoresis (DGGE)

A 1ml of culture was centrifuged at 12,000 x g for 5 min and the harvested cells were used for DNA extraction.. DNA extraction and purification were performed using UltraClean Soil DNA Isolation Kit (Mo Bio, West Carlsbad, CA, USA). The bacterial universal primer combination was used (forward primer was attached with the GC-clamp for DGGE), i.e., forward primer: GM5F-GC-clump 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3'), reverse primer: DS907R (5'-CCCCGTCAATTC CTTTGAGTTT-3'). PCR reaction mixture was prepared with 2 μl of the extracted DNA, 10 μl of Taq-& Go ready mix (Qbiogene, Irvine, CA, USA), 1 μl of each 25 μM forward and reverse primer solution. PCR was performed according to hot-start (Choou, Q. *et al.*, 1992) and touch-down (Don, R. *et al.*, 1991) method. The PCR tubes were set in PC708 thermal cycler (ASTEC,Fukuoka) and kept at 94°C for 2 min, then the temperature was held at 80°C and 2 μl of the above extracted DNA was added as a template. The reaction was continued as follows: annealing (65°C for 30 sec), elongation (72°C for 1.5 min) and denaturation (94°C for 1 min); the PCR cycle was repeated with annealing temperature being lowered to 55°C in 1.0 °C increments (total of 10 cycles) and 10 cycles were continued at 55°C for annealing.

The amplified DNA was verified by an agarose gel electrophoresis by using Mupid S electrophoresis system (Advance, Tokyo). DGGE gel was made by SJ-1060GF gradient gel maker (ATTO, Tokyo) with 20–60% formamide concentration gradient. Electrophoresis was performed using AE-6290E system (ATTO) at 200 V for 6 h according to the manufacturer's protocol. After electrophoresis, the gel was stained with SYBR GREEN I for 30 min and DNA bands were verified using EM-20E UV-transilluminator (UVP, Upland, CA, USA). Distinct DNA bands were taken from the gel. Each gel section was used for DNA templates to reamplify the DNA fragments. DNA sequencing was performed using DTCS Quick Start Kit and CEQ8000 (Beckman Coulter, Fullerton, CA, USA). The sequences of DNA bands were compared with FASTA and BRAST DNA database (Person, W.R. *et al.*, 1988).

Results and Discussion

Hydrogen productivity in PVA gel beads fluidized bed reactor

Figure 2 shows hydrogen yield per glucose before and after applying PVA gel beads. hydrogen yield was unstable and varied in the range of 0.2 to 0.8 mol-H_2/mol-glucose before applying PVA gel beads. However, after PVA gel beads were applied, hydrogen yield gradually increased and became stable in the range of 1.0 to 1.2. Hydrogen yield was temporarily decreasedon 2400 h cultivation due to the trouble of medium-making, but it was promptly recovered within one day. On our previous study, the recovery of hydrogen productivity needed a few days at least if biomass in the reactor decreased due to the some kind troubles. Therefore, this result indicated that the biofilm attached to PVA gel beads played as the inoculums, and contributed the prompt hydrogen recovery.

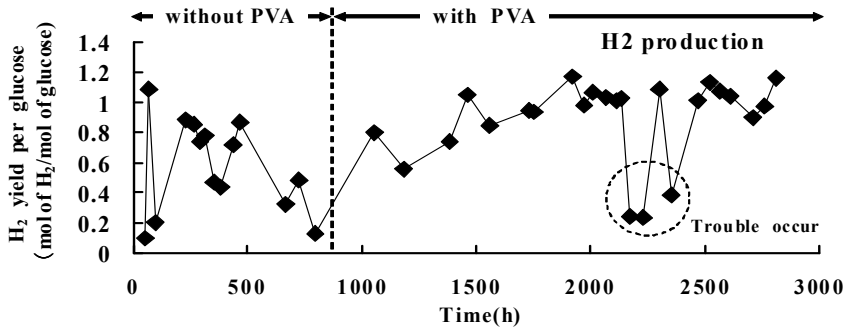

Fig. 2. Effect of PVA gel beads applying on H_2 production

In this experiment, increase of methane bacteria due to a long sludge retention time (SRT) by using the biomass carrier was apprehended,but methane was not detected throughout the experimental period. We have already demonstrated that the growth of methane bacteria was inhibited at pH 6.0. From this result, we concluded that the pH control was quite effective to inhibit methane bacteria in the biofilm..Noike *et al.* investigated the hydrogen fermentation by using a membrane bioreactor (MBR), which accumulate the biomass in the reactor and obtained the stable hydrogen production for more than 35 days (Noike, T. *et al.*, 2004). Hariklia et al reported that high and stable hydrogen production was obtained in an upflow anaerobic sludge blanket (UASB) compared to CSTR (Harikli N.G. *et al.*, 2005). We also obtained good result of stable hydrogen production by using PVA gel beads as a biomass carrier to hold the biomass in the reactor, however, the hydrogen yield was not very high (1.0 - 1.2 mol-H_2/mol-glucose) in this experiment. As the reason for this result, we consider the effect of inoculums and fermentation condition such as HRT and ORP as follows: 1) The digestion sludge used for inoculums contained no excellent hydrogen producing bacteria, 2) HRT of 24 hours was not appropriate for high hydrogen yield (Ohba, M. *et al.*, 2005; Harikli N.G. *et al.*, 2005), 3) the other operation conditions such as ORP and pH were not optimized.

The main gas products were hydrogen and carbon dioxide. Hydrogen content was ranged from 40 % to 57 % through the experimental period. Before applying the PVA gel beads, glucose consumption rate fluctuated between 50% and 80%,and after applying the PVA gel beads, it was increased to 80%, which suggested that the PVA gel beads held the enough biomass to consume the supplied glucose.

Effect of PVA gel beads applying on hydrogen production and metabolites

Relationships between hydrogen production and acetic or butyric acid before and after the applying PVA gel beads

VFAs like acetic acid were as major metabolites in anaerobic hydrogen fermentation. Among VFAs, acetic acid and butyric acid were preferable, while propionic acid and lactic acid were comonly undesirable for hydrogen production. Figure 3 shows the relationships between hydrogen production and the concentrations of acetic or butyric acid. These VFAs concentrations were fluctuatedbefore apply-

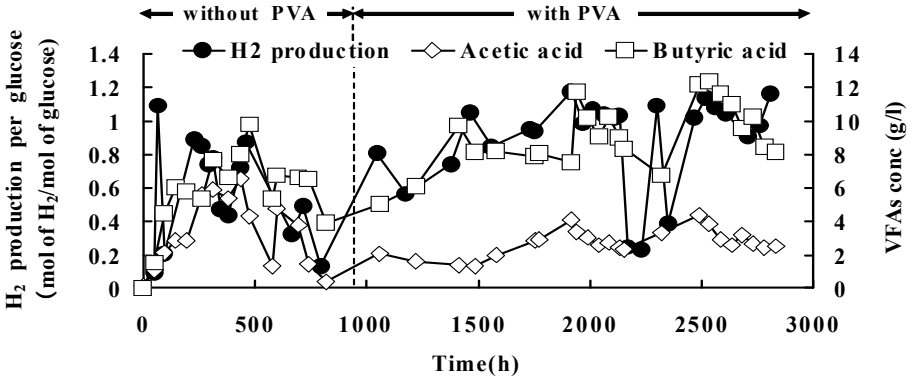

Fig. 3 Relationships between H_2 yield and acetic or butyric acid before and after applying PVA gel beads

ing PVA gel beads. But they were stable in the range of 2 − 4 g/l and 8 − 12 g/l, respectively after applying PVA gel beads. As shown in Figure 3, the butyric acid concentration changed according with the hydrogen production, which indicated that the butyric acid fermentation was main metabolic pathway in our reactor.

Relationships between hydrogen production and lactic acid before and after applying PVA gel beads

Figure 4 shows the relationship between hydrogen production and lactic acid concentration. The lactic acid concentration varied more drastically in the range of 2~14g/l compared to other VFAs. The high hydrogen production was obtained when the lactic acid concentration was low, and while it became low when the lactic acid concentration was high.

Result of bacterial community analysis by PCR-DGGE is shown in Figure 5. Although ten or more DNA bands have been observed at the beginning, about only 8 DNA bands were observed throughout the experimentalperiod. The

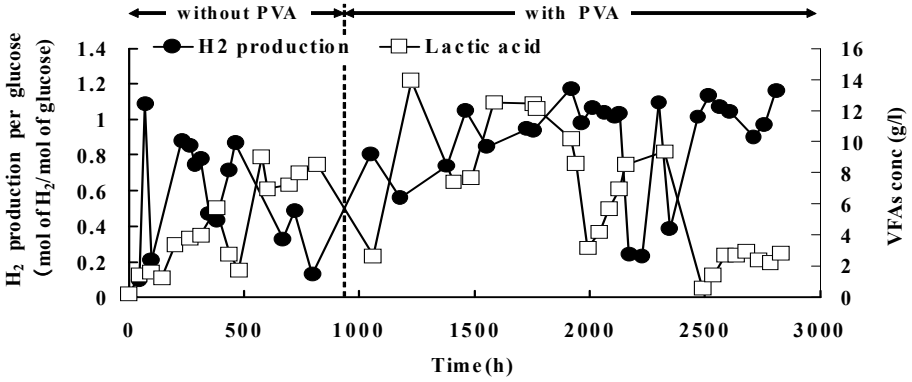

Fig. 4. Relationship between H_2 production and lactic acid before and after applying PVA gel beads

Results of bacterial community analysis by PCR-DGGE

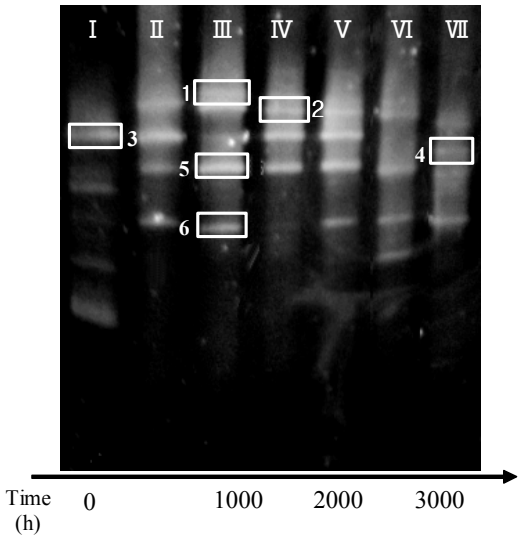

Fig. 5. Result of bacterial community analysis by PCR-DGGE

list of the DNA bands identified phylogenetically by the sequence similarity to the data in FASTA DNA database was shown in Table 1. The DNA bands of no.1 - 4 were phylogeneticaly identified to *Clostridium* species. Astable transition of DNA bands pattern was observed compared to that in our previous study in hydrogen fermentation with suspended biomass. On the other hand, the DNA bands of no.5 and no.6 showed high sequence similarities to *Sporolactobacillus* and *Lactobacillus*, respectively.

Commonly it was known that the *Clostridium* and *Enterobacter* species were main hydrogen producing bacteria in a hydrogen fermentation. In this study, the main DNA bands showed high sequence similarly to *Clostridium* species, which indicated that *Clostridium* species mainly contributed to the hydrogen production in our reactor. On the other hand, theDNA bands of no.5 and 6 were presumed to be lactic acid bacteria, and they were clearly observed throughout the experimental period. Therefore, it was considered that the continuous lactic acid production was by these bacteria. The DNA band of no.6 decreased rapidly on 1560 hours, but appears clearly again on 2180 hours. These result indicated that the biomass carrier held the bacteria nonspecifically, thus the elimination of unfavorable able bacteria was the key to improve the hydrogen productivity in the biofilm-reactor.

Table 1. The list of DNA bands on DGGE showing high sequence similarity to those registered on FASTA database

Band no.	Closest relative (accession number)	Similarity(%)
1	*Clostridium tyrobutyricum* (L08062)	98.7
2	*Clostridium tyrobutyricum* (L08062)	99.3
3	*Clostridium* FRB1 (AY925092)	97.7
4	*Clostridium tyrobutyricum* (L08062)	94.2
5	*Sporolactobacillus terrae* (AJ634662)	97.7
6	*Lactobacillus paracasei* (AY369076)	100.0

Conclusions

1. Stable hydrogen production was achieved for a long time in PVA gel beads fluidized bed reactor.

2. When hydrogen production suddenly decreased by experiment troubles, the bacteria quickly attached to PVA-gel beads played a role of inoculums and hydrogen production was recovered.

3. On DGGE analysis, the DNA bead pattern was stable by the applying PVA-gel beads, and the main DNA bands were phylogenically identified to *Clostridium* and *Sporolactobacills* species.

4. *Clostridium* species were the main hydrogen fermentating bacteria and these bacteria were stably maintained by PVA gel beads. However, the lactic acid bacteria were also maintained, therefore, it was suggested that the control of lactic acid bacteria was quite important to improve the hydrogen productivity in the biofilm-reactor.

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