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Thermophilic Hydrogen Production from Microbial Consortia Using PVDF Membrane Bioreactor

YOU-KWAN OH*, DONG-YEOL LEE, MI-SUN KIM*†**

**Bioenergy Research Center, Korea Institute of Energy Research, 71-2 Jang-dong Yuseong-gu Daejeon 305-343, Korea*
***Woorhitech, 49-6 Munpyeong-dong Daedeok-gu Daejeon 306-220, Korea*

PVDF 여과막 생물막 반응기를 이용한 혐기 세균 복합체의 고온 수소생산

오유관*, 이동렬, 김미선*†**

*한국에너지기술연구원 바이오에너지연구센터, **(주)우리텍

ABSTRACT

여과막 생물반응기를 이용하여 60℃에서 혐기 세균 복합체가 포도당으로부터 수소를 생산할 수 있는 최적조건을 연구하였다. 여과막 생물반응기는 연속교반 탱크반응기와 외부에 장착된 PVDF (polyvinylidene fluoride) 중공사막 여과장치로 구성되었다. 접종슬러지는 하수처리장 소화 슬러지조에서 얻었고, 포자형성 수소생산 미생물을 얻기 위해 90℃에서 20분 간 열처리하였다. 16S rRNA PCR-DGGE(polymer chain reaction-denaturing gradient gel electrophoresis) 분석을 통해 열처리 전후의 미생물상 변화를 조사하였다. 열처리 후 DGGE 밴드의 수는 감소하였고, 주요 밴드는 *Clostridium perfringens*와 유사한 염기서열을 나타내었다. 운전 기간 동안 바이오가스 내 수소함량은 60%(v/v)를 유지하였고, 메탄은 검출되지 않았다. 연속교반 탱크반응기를 여과막 없이 수력학적 체류 4시간에서 운전하였을 때 공급된 포도당의 95.0%가 제거되었고, 이때 균체농도 및 수소생산속도는 각각 1.35 g cell/L 및 7.4 L H_2/L/day이었다. 동일한 체류시간에서 PVDF 중공사막 여과장치를 장착하여 연속교반 탱크반응기를 운전하였을 때, 균체농도는 1.62 g cell/L로 증가하였고 높은 포도당 제거율(99.5%) 및 수소생산속도(8.8 L H_2/L/day)가 관찰되었다. 40 nm 및 100 nm의 공극크기를 가진 여과막은 균체농도 및 수소생산 측면에서 유사한 성능을 나타내었다. 여과막 생물반응기는 여과막의 반복적인 세척을 통해 30일 이상 안정적으로 운전될 수 있었다.

KEY WORDS : hydrogen production(수소생산), thermophilic fermentation(고온발효), PVDF hollow-fiber membrane(PVDF 중공사막), membrane bioreactor(여과막 생물반응기)

1. Introduction

Hydrogen is an efficient energy carrier with a high energy content per unit mass. It is considered to be the cleanest energy carrier because the combustion by-product is only water. It does not produce any green-house gases and neither does it contribute to

†Corresponding author : bmmskim@kier.re.kr

global warming[1]. Hydrogen is also an important raw material in various chemical industries, and is gaining increasing attention[2].

Microbial H_2 production can be either photosynthetic or non-photosynthetic. Photosynthetic H_2 production is carried out by either algae[3] or photosynthetic bacteria[4]. Non-photosynthetic or fermentative H_2 production is performed by either facultative[5,6] or obligate anaerobes[7,8]. The fermentative H_2 production is generally faster than photosynthetic H_2 production and does not rely on the availability of light. However, the H_2 conversion yield(mol H_2/mol substrate) is lower than that observed in photosynthetic processes[9].

The economic feasibility of fermentative H_2 production mainly depends on the rate and yield of H_2 production with various substrates. Most researchers have focused on developing proper biocatalysts and/or efficient production processes. Both pure[5,9] and mixed cultures[10,11] have been investigated as biocatalysts. When microbial consortia are used, mixed substrates can be utilized. However, the H_2 production yield is generally low since H_2-consuming bacteria and many non-H_2 producing bacteria, such as lactate- and propionate-producing bacteria, coexist in the reactor.

Continuous processes for fermentative H_2 production can be divided into suspended and immobilized systems[12]. Suspended systems, represented by a continuous stirred tank reactor (CSTR), are simple and easy to operate. However, when operated at a high dilute rate, the system may be unstable occasionally and result in washout of the cells. The immobilized system uses a support matrix onto which biofilms are established. The immobilized system maintains a higher cell concentration and H_2 production rate than a suspended one. However, it has been reported that excessive gas hold-up and severe fluid-channeling are most challenging problems in the immobilized systems[13].

Membrane bioreactors(MBR) have been used for treating wastewater under aerobic and anaerobic conditions for many years. The use of the membrane in a reactor allows fluid, but not bacteria, to leave the reactor. Although not extensively studied thus far, MBR is expected to offer many advantages to fermentative H_2 production, such as high cell density, high organics removal rates, high-quality effluent by the membrane, and easy control of pH and temperature[14,15].

This study focuses on continuous thermophilic H_2 production in a PVDF(polyvinylidene fluoride)-based MBR system with mixed cells as inoculum.

2. Materials and Methods

2.1 Seed Culture

Inoculum for MBR was obtained from an anaerobic digester in a local municipal wastewater treatment facility(Daejeon, Korea). In order to inactive non-spore-forming, H_2-consuming bacteria and harvest spore-forming, H_2-producing bacteria, the sludge was heat-treated at 90℃ for 20 min. The cells were acclimated to synthetic wastewater containing glucose(1% w/v)[15] in serum-bottle cultures at 60℃ under anaerobic conditions and then used as inoculum.

2.2 Hollow-Fiber Membrane Preparation

PVDF(Kynar grade 740) was used for the hollow-fiber membrane material and solubilized in DMAc(N, N-dimethylacetamide, Grade>99%). Inorganic materials were added as additives for forming the pores of membrane. Hollow-fiber

Table 1 Characteristics of the hollow-fiber membrane

Characteristics	Value/comment
Membrane surface(m^2)	0.121
Membrane material	polyvinylidene fluoride
Normal pore size(nm)	40 or 100
Membrane dimension(mm)	Inner diameter, 1.1 - 1.2 Outer diameter, 2.0 - 2.1
Flow direction	Out to inside

membranes were spun using spinning dope (completely mixed PVDF/DMAc/additives) by phase inversion method. The hollow-fibers were washed by distilled water at 50℃ in order to remove residual solvent. The characteristics of the hollow-fiber membrane used in this study were summarized in Table 1.

2.3 MBR System and Operation

Fig. 1 shows a schematic diagram of the MBR system used in this study. The reactor was a 5 L cylindrical Pyrex glass jar(working volume, 2 L),

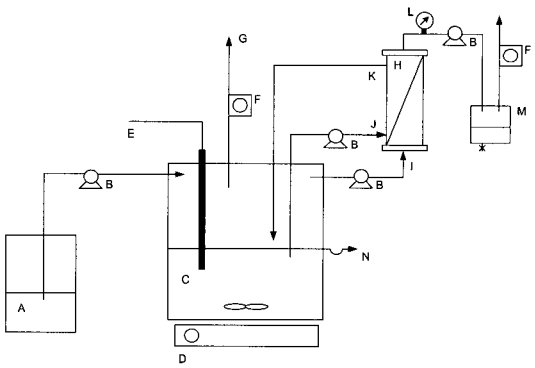

Fig. 1 Schematic diagram of the MBR system (A) feed tank, (B) peristaltic pumps, (C) reactor, (D) magnetic stirrer, (E) pH controller, (F) gas meters, (G) biogas out, (H) membrane filtration unit, (I) biogas recirculation, (J) liquid recirculation, (K) recirculation circuit of liquid and gas, (L) pressure gauge, (M) permeate storage tank, and (N) liquid waste stream

which was equipped with headtop, outlet appendage, and water jacket, and constantly agitated at 100 rpm. There were 7 ports in the headtop, 1 for medium supply, 2 for pH control, 1 for biogas removal, and 3 for membrane filtration. The bundle of hollow-fiber membranes was used to filter culture broth and packed in a 95 mL flexi-glass column.

Culture broth in the reactor was recirculated at 540 mL/min into the lower part of membrane filtration unit to generate cross-membrane flow, while the headspace gas was introduced to the bottom part of the unit to reduce the fouling of membrane at 770 mL/min. The liquid broth and biogas collected from the upper part of the filtration unit were returned to the reactor. A permeate flow was controlled by adjusting the peristaltic pump on the permeate side of the unit. Pressure across membrane was measured at the permeate side of the filtration unit. Fresh medium was stored at 4℃ and continuously bubbled with an argon gas(99.999%) to maintain the anaerobic condition. The temperature was maintained at 60℃ and pH at 5.5. Membrane was cleaned by sequentially rinsing it in 1 wt%(weight percent) HNO_3 for 2 h, 2 wt% NaOCl for 2 h and tap water. Synthetic wastewater containing 1%(w/v)[15] glucose was fed into MBR.

2.4 Analytical Methods

The volume of biogas produced was measured by a wet gas meter. The contents of H_2, CO_2 and CH_4 in the biogas were analyzed by a gas chromatograph(14-B, Shimadzu, Japan), which was equipped with a thermal conductivity detector. A stainless steel column packed with a Molecular Sieve 5A(80/100 mesh; Alltech, Deerfield, USA) was used for H_2 and one packed with a Hayesep Q(80/100 mesh; Alltech) was used for CO_2 and

CH_4, respectively. Glucose in the culture broth was measured by dinitrosalicylic acid(DNS) method[8]. Biomass(dry cell weight, dcw) was measured according to Standard Methods. Detailed procedure is available elsewhere[12]. Permeate flowrate was measured gravimetrically.

Microbial communities in the sewage sludge before and after heat-treatment were determined by PCR-DGGE(polymer chain reaction-denaturing gradient gel electrophoresis) analysis. DNAs from the sludge were extracted by using an Ultraclean DNA kit(Mo Bio Labs. Inc., Solana Beach, USA). The 16S rDNA gene fragments were amplified with PCR primers 968f-GC and 1492r. PCR protocol was: initial denaturation for 5 min at 95℃ and 30 cycles of denaturation for 30 s at 95℃, annealing for 30 s at 72℃, extension for 90 s at 72℃, followed by a final extension for 10 min at 72℃. DGGE was carried out using a Dcode Universal Mutation Detection System (Bio-Rad, Hercules, USA) in accordance with the manufacturer's instructions. PCR products were separated at 100 V, 60℃ for 6 h. The denaturing gradient in the gel was generated by mixing two stock solutions of 6% polyacrylamide containing 40% and 60% denaturants, respectively. After electrophoresis, the gel was stained with EtBr for 30 min, and DNA was visualized on a UV transilluminator. Major DNA bands were excised from DGGE gels and re-amplified by PCR. Nucleotide sequences were analyzed and screened against GenBank database using BLASTN to identify the most similar sequences in the database.

3. Results and Discussion

3.1 Inoculum Preparation

In order to use sewage sludge as inoculum for MBR, the sludge from municipal wastewater treatment facility was heat-treated at 90℃ for 20 min. The number of DGGE bands detected from heat-treated sludge decreased compared to that of non-heat-treated sludge(data not shown). Most DGGE bands observed in the fresh sludge were affiliated with the *Lactobacillus* sp. and *Bifidobacterium* sp. In contrast, nucleotide sequences of the strongly stained bands in the heat-treated sludge were most similar to the 16S rRNA gene of *Clostridium perfringens*, which is known as a spore-forming, H_2-producing bacterium from carbohydrates[16].

3.2 Effect of Hydraulic Retention Time(HRT) on CSTR Performance and Operation with PVDF Membrane Filtration Unit

A CSTR was inoculated with the heat-treated sludge, fed with synthetic wastewater containing 1%(w/v) glucose, and initially operated in a batch mode at 60℃ under anaerobic condition. When cell growth reached the late exponential phase, the culture was switched to the chemostat mode. Fig. 2 shows the effect of HRT on CSTR performance in the range of 4.0 - 10.3 h. As HRT decreased, volumetric H_2 production rate increased from 4.5 L H_2/L/day to 7.4 L H_2/L/day, while the H_2 production yield and glucose removal efficiency decreased from 1.6 mol H_2/mol glucose and 99.4% to 1.0 mol H_2/mol glucose and 95.0%, respectively. Biomass concentration increased from 1.11 g/L to 1.31 g/L by decreasing the HRT from 10.3 h to 6.7 h, but did not increase further at 4 h.

Fig. 2 shows also H_2 production performance at a HRT of 4.0 h with PVDF hollow-fiber membrane filtration unit. The effective surface area of the membrane was 0.121 m^2 and culture

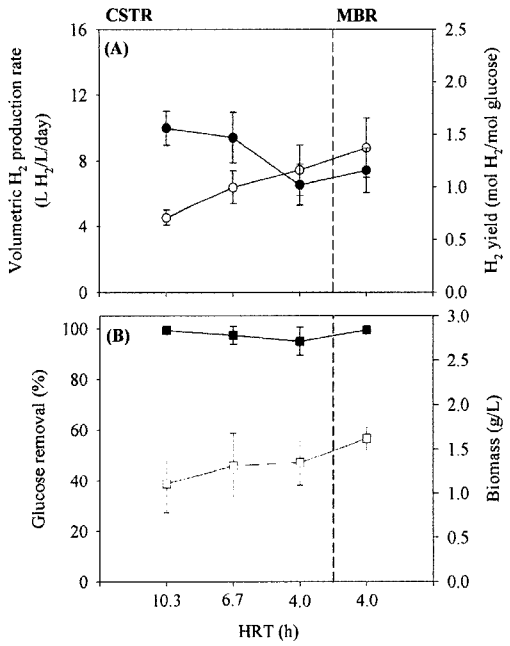

Fig. 2 Hydrogen production performance using CSTR and MBR: (A) volumetric H_2 production rate (○) and H_2 yield (●); (B) glucose removal (■) and biomass concentration (□). For MBR, the effective surface of membrane, gas recirculation flowrate, and permeate flowrate were 0.121 m^2, 770 mL/min, and 1.5 mL/min, respectively

broth was filtered at 1.5 mL/min. The headspace gas in the reactor was introduced into the bottom of the membrane unit to reduce the fouling of the membrane at 770 mL/min. Filtering the culture broth across the membrane could increase biomass concentration by 20% to 1.62 g/L compared to that without filtration. This also increased the glucose utilization efficiency(from 95.0% to 99.5%) and the rate and yield of H_2 production(from 7.4 L H_2/L/day and 1.02 mol H_2/mol glucose to 8.8 L H_2/L/day and 1.16 mol H_2/mol glucose, respectively). Hydrogen content (60%) in the biogas was not affected by the change in HRT or filtration of culture broth, and no appreciable CH_4 was detected under all conditions.

3.3 Effect of Membrane Pore-Sizes on MBR Performance

Two different membrane pore-sizes(40 and 100 nm) were studied for MBR(Fig. 3). Effective surface of membrane, gas recirculation flowrate, and permeate flowrate were kept at 0.121 m^2, 770 mL/min and 1.5 mL/min, respectively. Biomass concentration was maintained at 1.6 g cell/L regardless of the pore-sizes of membranes. When 100 nm pore-size membrane was used for MBR, the sharp build-up of pressure(0.1 kg_f/cm^2) across the membrane was observed initially, while maintained below 0.2 kg_f/cm^2 for further 13-days operation. In the case of 40 nm pore-size membrane, the pressure gradually increased and reached in 0.1 kg_f/cm^2 for 14-days operation. Other performances of MBR were not affected by the pore-sizes of membranes used(volumetric H_2 production rate, 8.8±1.8 L H_2/L/day; H_2 yield, 1.16±0.21 mol H_2/mol glucose; and glucose removal, 99.5±0.3%). These results indicate that the hollow-fiber membranes in the range of 40 - 100 nm pore-sizes could be used for MBR without affecting significantly overall H_2 fermentation performance.

Membrane fouling would ultimately result in a need for frequent membrane cleaning and/or replacement[15]. In this study, the long-term operation of MBR using 40 nm pore-size membrane was challenged by cleaning the membrane sequentially in 1 wt% HNO_3 for 2 h, 2%(wt/wt) NaOCl for 2 h and tap water(see arrow in Fig. 3). Although the faster build-ups of pressure across the membrane were observed compared to that of initial operation(0 - 14 days), the cleaned membrane could be used to filtrate the culture broth without significant fouling (below 0.15 kg_f/cm^2) for further 17 days.

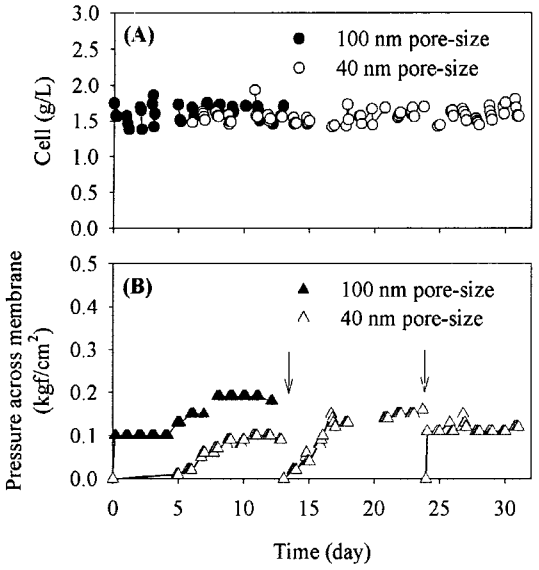

Fig. 3 Effect of membrane pore-sizes(40 nm, ○ and ▲; 100 nm, ● and ▲) on biomass concentration (A) and pressure across membrane (B). Effective surface of membrane, gas recirculation flowrate, and permeate flowrate were kept at 0.121 m^2, 770 mL/min, and 1.5 mL/min, respectively. Arrows indicate cleaning of membrane by sequentially rinsing it in 1%(wt/wt) HNO_3, 2%(wt/wt) NaOCl, and tap water

Based on the concept of reaction kinetics, increasing organic loading rate(or decreasing HRT) in a continuous culture leads to a higher volumetric H_2 production rate. However, if the organic loading rate gets too high, the elevated hydraulic pressure may affect the stability of the system and result in washout of the cells[14]. In this study, the PVDF-based MBR system was able to be stably operated at a short HRT of 4.0 h without suffering significant membrane fouling problem. Therefore, the MBR could be considered as one of the most promising systems for continuous H_2 production.

4. Conclusion

MBRs using PVDF hollow-fiber membranes with 40 and 100 nm pore-sizes were studied at 60℃ for thermophilic H_2 production from glucose with mixed culture. The reactors could increase biomass concentration by 20% compared to that without membrane filtration, resulting in higher H_2 production rate and yield(8.8 L H_2/L/day and 1.16 mol H_2/mol glucose). The MBR could be also operated for an extended period of 31 days with the repeated cleanings of membrane.

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