



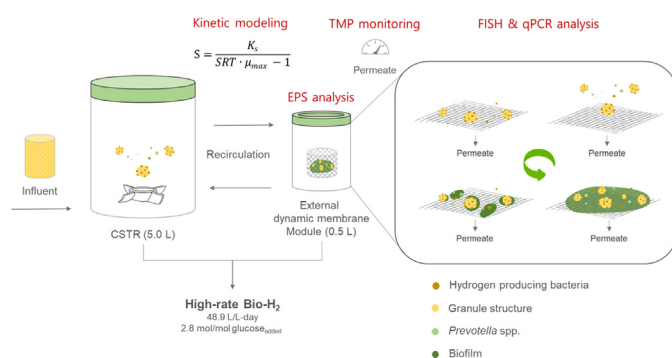
Kinetic modeling and microbial community analysis for high-rate biohydrogen production using a dynamic membrane

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GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the kinetic parameters of high-rate continuous performance and biofilm layer formation in a H₂-producing dynamic membrane bioreactor, composed of a continuously stirred tank reactor along with an external module containing polyester mesh with a pore size of 100 μm. A maximum H₂ production rate of 48.9 L/L-day and hydrogen yield of 2.8 mol/mol glucose_{added} were attained at a hydraulic retention time of 3 h. The maximum specific growth rate and Monod constant were estimated as 14.92 d⁻¹ and 1.02 g COD/L, respectively. During the entire operation without backwashing, the transmembrane pressure remained below 1.7 kPa, while tightly bound extracellular polymeric substances increased as the dynamic membrane was developed. Fluorescent *in situ* hybridization and quantitative polymerase chain reaction assays revealed that *Clostridium butyricum* was dominant in all samples; however, the biofilm had a higher proportion of *Prevotella* spp. than the fermentation liquor.

1. Introduction

Currently, climate change concerns and the energy crisis have prompted the development of renewable and eco-friendly fuels to meet global energy demands (Park et al., 2018). Hydrogen is an important

energy carrier with the potential to greatly reduce greenhouse gas emissions. Particularly, dark biohydrogen fermentation is a promising process that uses various substrates such as agricultural crops, residues, food and animal wastes, lignocellulose, wastewater with environmental friendly approaches (Kumar et al., 2018).

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However, the operating conditions for dark biohydrogen processes have not been completely established compared to conventional technologies such as anaerobic digestion (Kumar et al., 2017). For example, *Clostridium* spp., which is typically known as an anaerobic hydrogen producing bacterium, requires the maintenance of strict anaerobic conditions and is sensitive to changes in pH and temperature. Microbial cultures are used as a suspended biomass for reactor operations but exhibit washout problems and unstable production performances under a low hydraulic retention time (HRT), typically less than 6 h (Park et al., 2015).

To overcome these limitations, novel methods for hybrid immobilization were proposed for rapid granule formation and enrichment of microbial populations to achieve a high rate of hydrogen production (Sivagurunathan et al., 2014; Park et al., 2015). In contrast, immobilized cell inoculation can retain the active biomass in the system because of the formation of a protective layer of granules/biofilms, improving cell viability and enhancing production compared to the suspended biomass (Sivagurunathan et al., 2017). Continuous stirred tank reactors (CSTRs) are widely used for biohydrogen production using either suspended or immobilized cells. Mechanical mixing in CSTRs provides better mass transfer of the substrate into microorganisms and mitigates oversaturation of H_2 in the liquid phase. However, biomass washout can occur at a shorter HRT (less than 3 h) in a CSTR, even when immobilized cells are used (Kumar et al., 2016).

A dynamic membrane (DM), known as a secondary membrane, is a biofilm layer formed on an underlying support material, such as a membrane, mesh, or filter cloth, when the filtered solution contains suspended solid particles such as microbial cells and flocs (Erashin et al., 2012). The DM can replace costly membranes with low-cost materials to substantially lower investment and operational costs. A recent study reported the use of a dynamic membrane bioreactor (DMBR) for biohydrogen production (Park et al., 2017), showing that the effluent discharge through a mesh of 100 μm formed dynamic membrane on the support material and enhanced biohydrogen production performance. However, there is limited information available that can be used to develop processes for industrial applications.

Therefore, we investigated the kinetic parameters of high-rate continuous performance as well as the biofilm layer formation mechanism in a H_2 -producing DMBR. The DMBR system was modeled as a well-mixed continuous bioreactor equipped with a solid-liquid separating device, in this case the DM. To study biofilm formation, we investigated the evolution of trans-membrane pressure (TMP) and extracellular polymeric substances (EPS). The microbial community population was also monitored by quantitative polymerase chain reaction (qPCR) and fluorescent *in situ* hybridization (FISH) for both the DM and fermentation liquor to assess the population responsible for biofilm formation on the support material.

2. Materials and methods

2.1. Mesophilic hydrogen-producing microflora

Seed sludge was obtained from a mesophilic upflow anaerobic sludge blanket from a brewery industry wastewater treatment plant in Cheongju, South Korea. Characteristics of the inoculum were as follows: total suspended solids (TSS), volatile suspended solids (VSS), and pH were 9.7 and 5.9 g/L and 7.0, respectively. The sludge was heat-treated at 90 °C for 30 min to enrich hydrogen-producing bacteria and eradicate hydrogen-consuming or competitive microorganisms. The heat-pre-treated sludge was dried in a hot-air oven at 90 °C overnight, ground, and mixed with 2% Na-alginate, 2% activated carbon, 1% chitosan, and 1% SiO_2 . This mixture was then extruded with 2% CaCl_2 to form immobilized beads as previously described (Sivagurunathan et al., 2014).

2.2. Operation of dynamic membrane bioreactor

A lab-scale DMBR with a total working volume of 5.5 L (CSTR and an external DM module) was used in this study. The configuration is presented in the Supplementary information. The CSTR had a working volume of 5.0 L (19 cm in diameter and 27 cm in height). A mechanical stirrer was installed in the reactor and operated at 150 rpm. The effluent from the CSTR was flowed into the external DM module with a working volume of 0.5 L (8 cm in diameter and 15 cm in height) equipped with a cylinder type support material (4 cm in diameter and 10 cm in height) composed of polyester screen mesh (pore size 100 μm). The biomass was recirculated into the CSTR using a peristaltic pump at a flow rate of 10 times the feed flow rate. A pressure gauge ranging from 0 to 10 kPa was mounted on the front of the effluent peristaltic pump to monitor TMP.

The CSTR was inoculated with 0.5 L of seed sludge and the remaining working volume was filled with a carbon source of 15 g/L glucose with modified endo medium: 3 g/L NH_4HCO_3 , 0.125 g/L KH_2PO_4 , and 0.100 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.015 g/L $\text{MnSO}_4 \cdot 6\text{H}_2\text{O}$, trace elements 0.025 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.001 g/L $\text{CoCl}_2 \cdot 5\text{H}_2\text{O}$, and 6.72 g/L NaHCO_3 . Subsequently, the headspace of the reactor was flushed with nitrogen gas for 5 min. During operation, the pH was maintained in the range of 5.5 to 6.0 by adding 3 N NaOH to the CSTR to avoid acidification of the reactor at 35 ± 1 °C. HRT was varied from 12 to 2 h. Under each condition, the operation period was longer than 3 days and pseudo-steady-state performance with stable biohydrogen production ($\pm 10\%$) was maintained.

2.3. Analytical procedures

Biohydrogen generated from the CSTR and external DM module was connected to a wet-gas meter (JH-LMF-2-P, Jinghao Int., Shanghai, China). Hydrogen, carbon dioxide, and other components in the biohydrogen were analyzed by gas chromatography (GC, SRI 310, SRI Instruments, Torrance, CA, USA) as described previously (Park et al., 2017). Organic acid concentrations were determined by high-performance liquid chromatography (Waters 717, Waters Corp., Milford, MA, USA) equipped with an ultraviolet detector (Waters 2487) at 210 nm. Chemical oxygen demand (COD), TSS, and VSS were analyzed according to standard methods (APHA, 1998). EPS extractions and analysis were conducted as described previously (Zhen et al., 2018). The EPS samples (2 types, LB-loosely bound and TB-tightly bound) were filtered and analyzed for protein (PN) and carbohydrate (PS). Protein and carbohydrate analysis were explained in detail in a previous report (Anburajan et al., 2018).

2.4. Kinetic modeling

Kinetic models based on mass balance were constructed to describe the steady-state behavior of the DMBR system (Shemfe et al., 2018). As the system is composed of a CSTR and solid/liquid separating unit (DM), the mass balance for substrate utilization was constructed as follows (Chen et al., 2001):

$$S = \frac{K_s}{\text{SRT} \cdot \mu_{\max} - 1} \quad (1)$$

where μ_{\max} is the maximum specific growth rate (day^{-1}), K_s is the Monod constant (g COD/L), S is the glucose concentration in the reactor (g COD/L), and SRT is the solid retention time (day^{-1}). Biomass decay was not considered as high substrate concentration was applied (Chen et al., 2001). Unknown kinetic parameters, μ_{\max} and K_s , were estimated by numerical simulations with Sigmaplot 10 (Systat Software, San Jose, CA, USA).

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