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Bacterial community structure in maximum volatile fatty acids production from alginate in acidogenesis

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HIGHLIGHTS

- The bacterial community structure was monitored in acidogenesis from alginate.
- *Bacteroides*-related organism might be contributed to hydrolysis of alginate.
- *Clostridium* spp. corresponded to alginate-fermenting were mainly detected.
- Bacterial community shifts corresponded to VFA profiles was statistically verified.

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ABSTRACT

Alginate as biomass feedstock for bioconversion into volatile fatty acids (VFAs) is limited primarily by the low solubility in water or little utilization as microbial substrate and yet unknown about the microbial community structure for acidogenesis. The bacterial community structure was demonstrated the reflected changes in VFAs profiles in the maximized acidogenic process from alginate. *Bacteroides*- and *Clostridium*-related microorganisms were suggested to be mainly responsible for the hydrolysis of alginate and VFAs production, respectively. And the bacterial community shifted corresponded to VFAs producing was statistically demonstrated. A number of features discussed in this research can stimulate further interests on bioconversion of alginate into anaerobic biofuels production.

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1. Introduction

Marine algae (including macroalgae and microalgae) are receiving increasing attention as attractive renewable resources for biofuels production with many advantages over biomass from the crop-based or cellulosic matters (Wei et al., 2013). In particular, macroalgae (i.e., seaweed) has the growth rate with high productivity, greater potential for carbon dioxide fixation, and abundant content of carbohydrates which are easily converted to biofuels (Pham et al., 2013). Among the macroalgae, massive brown algae are primarily composed of polysaccharides such as alginate, laminaran, fucoidan, mannitol, and trace cellulose (Chang et al., 2010). The main polysaccharide, alginate, accounts for up to 40% dry wt. in brown algae as a principal material of the cell wall (Draget et al., 2005; Jung et al., 2013) and has a chemical structure that consists of two uronic acids (i.e., manuronic acid and gulonic acid) each containing a carboxyl group (Yang et al., 2011). A high yield of organic acids can be theoretically available by simple decomposition of alginate and maintaining its carboxyl group structures (Aida et al., 2012). However fermentative production of organic acids from alginate is still challenge due to its low solubility in water or little utilization as microbial substrate (Pham et al., 2013).

Volatile fatty acids (VFAs) including short-chain fatty acids from C2 to C6 (i.e., acetate, propionate, and butyrate, etc.) are valuable chemical compounds which have diverse industrial applications such as feedstock resources for biofuels (i.e., biohydrogen, biomethane, and mixed alcohols, etc.) (Singhania et al., 2013). Recently, a study of VFAs production from alginate has been firstly reported (Pham et al., 2013). Although the study has been showed a potential of VFAs production in anaerobic alginate fermentation, information is still lacking on the behavior of microbial communities for better understanding of the process.

In this research, we performed to operate acidogenic process using alginate and especially focused on the changes in bacterial

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communities as functions of performance and operating conditions for insight understanding of the process. Therefore, the aim of this research was to investigate bacterial community structure, in relation to changes in VFAs profiles, throughout an acidogenic process from alginate.

2. Methods

2.1. Microorganism

Anaerobically digested sludge was obtained from a municipal wastewater treatment plant in Busan, Korea. Acid pre-treatment (2 N HCl) was applied at 35 °C for 24 h in waterbath in order to enhance the activity of acidogenic bacteria (Lee et al., 2009). Continuous acidogenic reactor as an inoculum system was operated at 35 °C and 150 rpm in a 3 L LiFlus GX bioreactor (BioTron Inc., Puchon, Korea) with a working volume of 2 L and equipped with temperature and pH controller. Acidogenic microorganism was cultivated in feed medium with 20.0 g/L of glucose as a sole carbon source and nutrient solution containing: 5.2 g/L NH_4HCO_3; 0.12 g/L K_2HPO_4; 0.1 g/L $MgCl_2 \cdot 6H_2O$; 0.015 g/L $MnSO_4 \cdot 6H_2O$; 0.025 g/L $FeSO_4 \cdot 7H_2O$; 0.005 g/L $CuSO_4 \cdot 5H_2O$; 0.0005 g/L $CoCl_2 \cdot 5H_2O$; and 6.7 g/L $NaHCO_3$. The pH value was maintained at 5.5 with buffer solution of 5 N NaOH or 5 N HCl to enhance acidogenic bacteria activity and suppress methanogenic bacteria activity. The operation of acidogenic reactor was continued in a fill and draw once a day and hydraulic retention time (HRT) was kept at 1 day. The acidogenic biomass was taken before feeding and the concentrations of TVFAs and alcohols in the system were remained at 12–14 g/L.

2.2. Experimental set-up

Sodium alginate (80–120 mPa s, Wako Pure Chemical Industries Ltd., Osaka, Japan) was dissolved in distilled water and autoclaved (121 °C for 15 min) then used as a microbial growth substrate. Alginate was the sole carbon source in the medium, which also contained NH_4HCO_3, 2.0 g/L; KH_2PO_4, 1.0 g/L; $MgSO_4 \cdot 7H_2O$, 0.01 g/L; NaCl, 0.001 g/L; $Na_2MoO_4 \cdot 2H_2O$, 0.001 g/L; $CaCl_2 \cdot 2H_2O$, 0.001 g/L; $MnSO_4 \cdot 7H_2O$, 0.0015 g/L; and $FeCl_2 \cdot 4H_2O$, 0.00388 g/L as nutrient additives. The initial pH was adjusted as required using 5 N NaOH or 5 N HCl. The effluent including acidogenic biomass was added to distilled water with volume ratio of 1:1 and centrifuged at 1000 rpm for 3 min. This procedure was repeated three times for removal of residue in the effluent and then was used as seed culture (equivalent to 10% of working volume), resulting in a volatile suspended solids (VSS) concentration of approximately 7500 mg/L as cell concentration. The acidogenic process using the alginate was operated at 35 °C and 120 rpm. Chloroform ($CHCl_3$; 100 μM) was used as a methanogenic inhibitor from both H_2/CO_2 and acetate. It also inhibited acetate consumption by sulfate reducers (Hu and Chen, 2007; Pham et al., 2013).

The effects of changing alginate concentration (4–9 g/L) and pH (6.0–10.0) on the production of VFAs in acidogenic batch process were evaluated used a 2 × 2 (Conc. × pH; two levels each) orthogonal central composite cube (CCC) design. The optimum condition was estimated by response surface methodology (RSM) using Minitab software (version 15.1.1.0, Minitab Inc., State College, Pennsylvania, USA). Changes in VFA profiles and in acidogenic bacterial communities were investigated under the estimated optimum condition as validated points.

2.3. DNA extraction

Raw samples were collected from the reactor for optimum condition. Samples were centrifuged at 10,000×g for 1 min. The supernatant was removed and resuspended in 1 mL of distilled water. Total genomic DNA in the suspension was immediately extracted using PowerSoil™ DNA kit (Mo Bio Labs, Carlsbad, USA). Purified DNA was eluted with 100 μL of Tri-HCl buffer (pH 8.0) and stored at −80 °C for further analyses. DNA extraction of was performed in duplicate.

2.4. PCR–DGGE analysis

The extracted DNA was amplified using a Mastercycler gradient automated thermal cycler (Bio-Rad Laboratories Inc., Hercules, USA) with primer set consisting of a GC-clamp eub 341F (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and eub 518R (5′-ATT ACC GCG GCT GCT GG-3′) for V3–V5 region of bacterial 16S rRNA gene at the following program: an initial denaturation at 95 °C for 2 min; 30 cycles of denaturation (30 s at 95 °C), annealing (40 s at 55 °C), extension (30 s at 72 °C); and a final extension at 72 °C for 10 min. The reactions were carried out in a 25 μL volume containing 1 μL of template DNA, 0.25 μL of the forward and reverse primers (10 pmol), 2.5 μL of 10× Taq buffer, 10 μL of 10 mM dNTP, and 0.125 μL of DNA polymerase (Solgent Co., Seoul, Korea). Polymerase chain reaction (PCR) products were checked electrophoretically and were purified using a PCR purification kit (Bioneer, Alameda, USA).

The denaturing gel gradient electrophoresis (DGGE) was then performed with a DCode™ Universal Mutation Detection System (Bio-Rad Laboratories Inc., Hercules, CA) using a 6% polyacrylamide gel with denaturing gradient ranging from 20% to 55% denaturant in 0.5× TAE buffer. The electrophoresis was run at a constant voltage of 150 V at 60 °C for 7 h. After the electrophoresis, the gel was stained in ethidium bromide solution for 30 min, rinsed for 30 min in water and visualized by UV transillumination. For identification of DGGE bands, each band was eluted into 40 μL of deionized and distilled water and then mixture was incubated overnight at 4 °C to extract the DNA from DGGE bands. And then solution was used as the template in the reamplification reaction using same primer without GC-lamp, the specific primers, eub 341F (5′-TAC GGG AGG CAG CAG-3′) and eub 518R (5′-ATT ACC GCG GCT GCT GG-3′).

PCR fragments were purified using a purification kit (Solgent Co., Seoul, Korea). Sequencing was performed using an ABI 3730XL capillary DNA sequencer (Applied Biosystems, Foster City, USA) and an ABI Prism Bigdye terminator cycle sequencing ready reaction kit (version 3.1, Applied Biosystems, Foster City, USA). The obtained sequences were finally compared with the related sequences in the Genbank DNA database using BLAST program in the National Center for Biotechnology Information (NCBI). Sequences were aligned with the closest 16S rRNA gene found in Genbank Database with Clustal X2 software (version 2.1, www.clustal.org) and the phylogenetic tree was constructed using the neighbor-joining method with MEGA 5.2 software package (version 5.2, www.megasoftware.net). Bootstrap analysis with 500 replicates was performed to estimate the confidence of the tree topologies. Statistical analyses were performed using the Statistical Package for Social Sciences, fingerprinting II informatix software (version 3.0 for windows, Bio-Rad Laboratories Inc., Hercules, USA) to analyze Pearson correlation coefficient. Also, principle component analysis (PCA) was performed, to analyze the relationships among the bacterial sequence structures derived from DGGE gels, with SPSS Statistics software (version 14.0 for windows, SPSS Inc., Chicago, USA).

2.5. Analytical methods

The liquid sample was measured for pH values, and supernatant extracted from centrifugation at 2500 rpm for 10 min was filtered

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