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Effect of hydraulic retention time on lactic acid production and granulation in an up-flow anaerobic sludge blanket reactor

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HIGHLIGHTS

- Lactic acid (LA) production performance with granulation in an UASBr.
- As HRT decreased, the diameter and hydrophobicity of the granules increased.
- LA productivity increased up to 16.7 g LA/L-fermenter/h at HRT 0.5 h.
- Rod-shaped organisms with pores and internal channels at granule surface.
- Next generation sequencing revealed that *Lactobacillus* was dominant.

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ABSTRACT

In the present work, lactic acid (LA) production performance with granulation was investigated at various hydraulic retention times (HRTs), 8–0.5 h. Glucose was used as a feedstock, and anaerobic mixed cultures were inoculated in an up-flow anaerobic sludge blanket reactor. As HRT decreased, the average diameter and hydrophobicity of the granules increased from 0.31 to 3.4 mm and from 17.5% to 38.3%, respectively, suggesting the successful formation of granules. With decreasing HRT, LA productivity increased up to 16.7 g LA/L-fermenter/h at HRT 0.5 h. The existence of rod-shaped organisms with pores and internal channels at granule surface was observed by scanning electron microscope. Next generation sequencing revealed that *Lactobacillus* was the dominant microorganism, accounting for 96.7% of total sequences, comprising LA-producing granules.

1. Introduction

Lactic acid (LA) is the simplest type of fermentation products and an industrially important product due to its attractive properties and versatile applications. About 70% of the demand for LA is in the food related applications but recently there has been increasing demand related to non-food industrial applications such as bioplastics, pharmaceuticals, cosmetics, leather, textiles, and other chemical industries (Dusselier et al., 2013).

LA can be produced by either biotechnological fermentation or chemical synthesis, but the latter always leads to a racemic mixture, which is a major disadvantage. Presently, over 90% of LA is commercially produced by a fermentation route, specifically using pure cultures in a batch manner. However, this type of fermentation has weaknesses including the high cost of sterilization and low volumetric productivity due to long fermentation time required (Abdel-Rahman et al., 2013). Meanwhile, a continuous process using mixed cultures has advantages of high productivity, ease of operation, and the decomposition of complex materials (Kleerebezem and van Loosdrecht, 2007). In a continuous LA production process, huge effort has been made to maximize the productivity by immobilizing and recycling cells using a matrix, centrifugal system, and membrane. In particular, by using a

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membrane, high volumetric productivity ranging from 5 to 10 g LA/L/h has been reported (Lu et al., 2012; Ramchandran et al., 2012). However, technical issues such as fouling and the economic burden imposed by membrane installation and cleaning are obstacles impeding practical application.

The up-flow anaerobic sludge blanket reactor (UASBr) has been widely used in wastewater treatment owing to its fast reaction rate with specific bio-granules having high settling velocity. Microbial granules form through self-immobilization of microorganisms in the UASBr and reactors with similar regimes (Schmidt and Ahring, 1996). The formation of granules and the UASBr performance have been extensively investigated in the fields of methane fermentation, aerobic nitrogen removal, sulfate reduction, and ammonium oxidation, but little is known about LA production (Liu and Tay, 2004). The success of UASBr is highly dependent on the granulation process, which is affected by various operating parameters such as pH, temperature, hydraulic retention time (HRT), and metal ions. Among them, HRT is particularly important because it directly relates to the efficiency of reactor operation.

In the present work, anaerobic mixed cultures in a LA-producing continuous stirred tank reactor (CSTR) were inoculated to UASBr and HRT was shortened to maximize productivity. The effects of HRT on LA production performance with granulation were investigated in detail. In addition, a microbial community analysis was conducted by next generation sequencing (NGS).

2. Methods

2.1. Experimental procedure

In this study, an UASBr with a height of 900 mm at the main body, a height of 100 mm at the upper part, and a diameter of 60 mm was used. The effective volume of UASBr was 2.9 L. As there is no mechanical mixing in the UASBr, pH is not uniform. Therefore, to assess the change of pH, five pH probes were prepared and positioned at different locations: four at the main body of the reactor at every 200 mm, and one at the upper part of the reactor. The pH probes were connected to a pH monitoring device and data were collected every 10 min.

The inoculum used in the UASBr was taken from the CSTR showing a high LA conversion yield of 1.84 mol LA/mol $glucose_{added}$ (Kim et al., 2012). After seeding 1 L of mixed liquor in the CSTR to UASBr, the reactor was purged with N_2 gas for 10 min to provide anaerobic conditions. Glucose of 10 g chemical oxygen demand (COD)/L (=9.375 g glucose/L) was used as a substrate and the temperature was controlled at 50 °C using a water bath circulator and a built-in water jacket. In order to supplement macro nutrients, NH_4Cl, KH_2PO_4, and $FeCl_2{\cdot}4H_2O$ were added to yield a COD:N:P:Fe ratio of 100:5:1:0.33. The feed also contained the trace nutrients as described in our previous work (Kim et al., 2012). The feeding rate was gradually increased, 8.7, 17.4, 34.8, 69.6, and 139.2 L/d, corresponding to an HRT of 8, 4, 2, 1, and 0.5 h, respectively. At each HRT, the reactor was operated for more than five times of HRT after reaching a steady state.

2.2. Microbial community analysis by next generation sequencing

To analyze the microbial diversity, DNA in the mixed samples from the reactor was extracted using an Ultraclean Soil DNA Kit (Cat #12800-50; Mo Bio Laboratory Inc., USA). The extracted DNA was subsequently purified with an UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, CA, USA). A library was then prepared using PCR products according to the GS FLX titanium library prep guide. Libraries were quantified using Picogreen assay (Victor 3). The emPCR, corresponding to clonal amplification of the purified library, was carried out using a GS-FLX titanium emPCR Kit (454 Life Sciences). A 20 ng aliquot of each sample DNS was used for a 50 ul PCR reaction. The 16S universal primers 27F (5′ GAGTTTGATCMTGGCTCAG 3′) and 800R (5′ TACCAGGGTATCTAATCC 3′) were used for amplifying 16s rRNA genes. A Fast Start High Fidelity PCR System (Roche) was used for PCR as previous described (Nam et al., 2012). After the PCR reaction, products were purified using AMPure beads (Beckman coulter). Then sequencing was performed using a 454 pyrosequencing Genome Sequencer FLX Titanium (Life Sciences, CT, USA), according to the manufacturer's instructions, by a commercial sequencing facility (Macrogen, Seoul, Korea). The sequences generated from pyrosequencing were mainly analyzed with the software MOTHUR for pre-processing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs), taxonomic assignment, community comparison, and statistical analysis. The method of sequences filtration and trimming were performed as previous described (Nam et al., 2012).

2.3. Analysis

Organic acids including volatile fatty acids (VFAs, C2–C6) and lactate were analyzed by a high performance liquid chromatography (HPLC) (Finnigan Spectra SYSTEM LC, Thermo Electron Co.) with an ultraviolet (210 nm) detector (UV1000, Thermo Electron) and an 100 × 7.8 mm Fast Acid Analysis column (Bio-rad Lab.) using 0.005 M H_2SO_4 as mobile phase. The liquid samples were pretreated with a 0.45 μm membrane filter before injection to HPLC. COD and glucose concentration was measured according to the method described in Kim et al. (2012).

The granule size was analyzed with the free UTHSCSA Image Tool program, a program developed at the University of Texas Health Science Center at San Antonio, Texas. The sludge sample (0.2 mL) was spread over a petri dish and fixed within a transparent 25 g-gelatin/L gelatin solution (5 mL). After the gelatin solidified, the sample dishes were placed over the scanner surface. Eight-bit grayscale images were obtained, and then analyzed. The software provided the information of area, particle number, diameter, and other characteristics of the particles in the digital image. The microstructures of granules were investigated by scanning electron microscope (SEM, LEO 1455VP) equipped with a secondary electron and quadrant back-scattering detector (QBSD). Cell hydrophobicity was measured by conventional microbial attached to hydrocarbons (MATH) methods using n-hexadecane as the hydrocarbon and PUM buffer as the water phase. To disperse the cells in granular sludge, grinding and low sonication (50 W for 2 min with 5 s pulse and 5 s interval) were applied.

3. Results and discussion

3.1. UASBr performance

The LA fermentation performance of the UASBr at various HRTs is shown in Fig. 1. Over 90% substrate degradation was observed immediately after seeding, and over 95% LA conversion yield was achieved within 5 days, suggesting a successful start-up. The accumulation of microorganisms was observed from the bottom, and it gradually increased as operation continued. At the time when HRT was shortened, there was a sudden decrease of substrate removal with a drop of LA conversion yield. However, it recovered within two days and showed a stable performance during the entire experiment.

As HRT decreased, it appears that the LA concentration slightly decreased with low substrate degradation, from 9.7 g COD/L at HRT 8 h to 8.9 g COD/L at HRT 0.5 h. However, considering a huge

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