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Effects of low-strength ultrasonication on dark fermentative hydrogen production: Start-up performance and microbial community analysis

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HIGHLIGHTS

- Low-strength ultrasonication enhanced bio-hydrogen production by dark fermentation.
- Effluent recirculation also affected hydrogen production performance.
- *Ethanoligenes*, *Aeromonas* and *Clostridium* were assumed as key H_2 producers.
- Some *Clostridium* spp. responded differently upon ultrasonication treatment.

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ABSTRACT

Our previous studies have demonstrated the positive impacts of low-strength (LS)-ultrasonication on biological methane production via anaerobic digestion. In this study, the effects of LS-ultrasonication and effluent recirculation on dark fermentative hydrogen production using upflow anaerobic sludge blanket reactor were investigated. Significant increase in hydrogen (H_2) yield (up to 0.89 mol H_2/mol hexose) was observed under LS-ultrasonication conditions. This enhanced H_2 yield seems to be associated with the decrease in dissolved H_2 concentration via a degassing effect, as evident from increased H_2 content (up to 20%). Effluent recirculation alone led to positive effects. However, applying both LS-ultrasonication and effluent recirculation decreased H_2 yield compared to the trial with only LS-ultrasonication (0.89–0.72 mol H_2/mol hexose), possibly due to the cumulative shear stress and sludge bed expansion above the ultrasonication zone. Microbial community analysis revealed that the presence/absence of LS-ultrasonication and/or effluent recirculation significantly affected the microbial communities. *Ethanoligenes cloacae*, *Aeromonas hydrophila* and *Clostridium pasteurianum* were identified as principal H_2 producers under the LS-ultrasonic conditions, while *C. butyricum*, the most commonly reported H_2 producer, was more abundant in the controls. The energy balance of the LS-ultrasonication system was evaluated and future research directions were suggested.

1. Introduction

Ultrasound is defined as a sound wave at a frequency above human hearing and has been widely applied to various research fields, such as welding, degassing of solutions, detection of defects, medical diagnosis and treatments [1]. During ultrasonication, an acoustic wave propagates in aqueous media, and then, microbubbles (cavitation bubbles) are generated in the rarefaction region. These cavitation bubbles get bigger in successive cycles, reach an unstable diameter, and then finally collapse [2]. As a result of cavitation ruptures, the hydro-mechanical shear stress localizes, the temperature increases up to 5000 K and highly reactive OH^- radicals are generated [3]. Additionally, an increase in mixing and mass transfer, namely acoustic streaming, is also, reportedly, created in an aqueous solution or suspension [1]. To exploit these useful effects, ultrasound has also been applied to various biologically driven environmental fields, but its application is completely different regarding its intended purpose and main mechanism.

The application of ultrasonication on biological processes can be classified as high-strength (HS) and low-strength (LS), based on where ultrasonication is irradiated [4]. HS-ultrasonication is referred to when ultrasonication is irradiated to a feedstock as a pretreatment, whereas LS-ultrasonication is irradiated to the reactor itself to target microorganisms involved in the biological process. Various mechanisms are suggested to explain the performance enhancement between LS- and

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HS-ultrasonication. While the former is attributed to an increase of substrate availability by solubilizing inert and slowly biodegradable sections, the enhanced performance by LS-ultrasonication is attributed to increased microbial activity through the stimulation of biological enzymes [5]. Given that LS-ultrasonication has economic advantages over HS-ultrasonication, LS-ultrasonication has been receiving increasing attention over the last decade [6].

Many research results have demonstrated the positive effects of LS-ultrasonication on aerobic, anaerobic and nutrient removal processes. Aerobic processes with LS-ultrasonication showed increased chemical oxygen demand (COD) removal rate (up to 6% with 10 min ultrasonication every 8 h at 0.3 W/cm^2 of ultrasonic density), reduced sludge production (by 91.1% with 15 min ultrasonication at 120 kW/kg dried solids of ultrasonic intensity) and/or accelerated aerobic digestion (shortened from 17 days to 3–7 days at 0.53 W/cm^2 of ultrasonic density) compared to the control [7,8]. Furthermore, enhanced nitrogen (up to 16% with 10 min ultrasonication every 5 h at 0.2 W/cm^2 of ultrasonic density) and phosphorus (up to 50% with 10 min ultrasonication at 0.2 W/cm^2 of ultrasonic density) removal rates were achieved from a biological nutrient removal process [9,10]. LS-ultrasonication has also been successfully applied to anaerobic procedures, such as the anaerobic digestion system [4] and the anaerobic hydrogen (H_2) fermentation process [11].

To the best of our knowledge, only two research groups have reported the effects of LS-ultrasonication on anaerobic methane (CH_4) [4,12] and/or H_2 production [11]. In the former studies, which were the first reports of the application of LS-ultrasonication on CH_4 production, 40% higher (2 s ultrasonication every 30 s at 0.0025 W/mL of ultrasonic intensity) and 43% higher (1 s ultrasonication every 1 min at 0.05 W/mL of ultrasonic intensity) CH_4 yields were achieved from dry anaerobic and upflow anaerobic sludge blanket reactors (UASBR), respectively, than the control reactors [4,12]. The latter group [11] applied 1 s ultrasonication every 1 min at 0.25 W/mL of ultrasonic intensity to a continuous stirred tank reactor; H_2 production rate was enhanced from 2.8 to 5.6 L/L/d while H_2 yield increased from 1.0 to 1.9 mol H_2/mol glucose. H_2 is considered as one of the most promising alternative renewable energies due to many advantages, such as its clean nature (no CO_2 emission during the combustion process) and high energy content of 122 kJ/g [13]. Biohydrogen-producing granules have been known to be advantageous, based on their ability to treat a high organic loading rate at high productivity [14]. However, no further study has been attempted on the biohydrogen process using a granular process, such as an upflow anaerobic stage reactor.

Therefore, the present work investigated the effects of LS-ultrasonication on H_2 production using UASBr, by dark fermentative process, for the first time. Furthermore, the influences of effluent recirculation with/without ultrasonication on H_2 yield were also analyzed. The start-up performance was monitored, and the microbial community structure was thoroughly characterized in the presence/absence of ultrasonication, using next-generation sequencing (NGS). The energy balance of the LS-ultrasonication system was evaluated and future research directions were suggested.

2. Material and methods

2.1. System setup and operation conditions

Seed sludge was taken from a pilot scale anaerobic digester treating foodwaste leachate located in D city, Korea. The pH, alkalinity, total solids (TS), volatile solids (VS), and volatile suspended solids (VSS) concentration of sludge were 7.8, 3.1 g $CaCO_3$/L, 33.9 g/L, 18.3 g/L and 12.4 g/L, respectively. As shown in Fig. 1, UASBr had the lower part of 810 mm height × 76 mm inside diameter (ID) and the upper part of 165 mm height × 110 mm ID with 3.8 L of effective volume. To investigate the both effects of ultrasonication and recirculation on dark fermentative H_2 production (DFHP), two UASBr were installed with

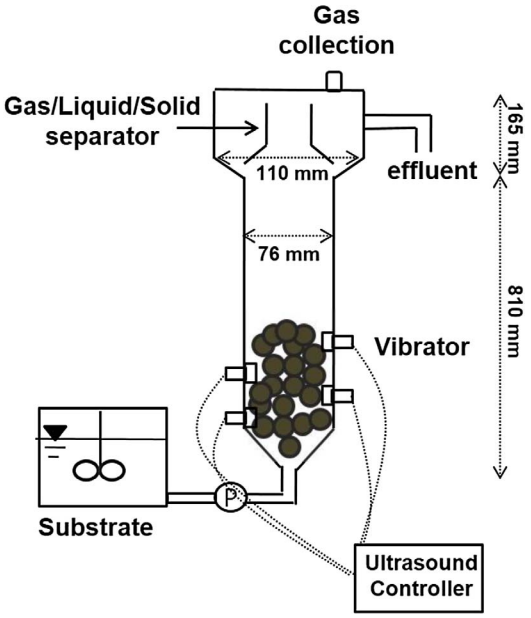

Fig. 1. Schematic diagram of the UASBr with low strength ultrasonication.

four vibrators (50 W, 20 kHz, customized device) while the effluent of two UASBr was recirculated with 2.5 m/h of upflow velocity from bottom to top. Thus, four reactors in total were used for this study: C (Control, without ultrasonication and recirculation), CR (Control with Recirculation, without ultrasonication but with recirculation), U (Ultrasonication, with ultrasonication but without recirculation), UR (Ultrasonication with Recirculation, with both ultrasonication and recirculation).

After inoculation without any pretreatment, each reactor was purged with N_2 gas for 10 min to provide anaerobic conditions. During continuous mesophilic (38 °C) operation, LS-ultrasonication was applied with following conditions: 200 W of ultrasonication strength, 0.1 W/L of ultrasonication strength (i.e., 2 L effective volume for ultrasonication), 1 s of ultrasonication time per every 1 min, and hydraulic retention time (HRT) was kept at 8 h corresponding of 45 g COD/L/day of organic loading rate (OLR). Glucose was used as a substrate at 15 g chemical oxygen demand (COD)/L or 14.06 g glucose/L concentration. For the 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 substrate solution also contained trace nutrients and 5 g of $NaHCO_3$ to provide the buffer capacity as described previously [15]. The DFHP performance showed a steady state after day 5, and the reactors were operated continuously until day 25.

2.2. Analytical methods

The concentrations of TS, VS, VSS and alkalinity were measured according to standard methods [16]. Glucose was measured by colorimetric method [17] and COD was measured using a commercial kit (Cat. #01013-01, Humas, Korea). The biogas was collected using gas-tight bags and sampled with a 1-mL gas-tight syringe to analyze H_2 content. Measured biogas production was adjusted to standard temperature (0 °C) and pressure (760 mm Hg) (STP). The liquid samples were pretreated with a 0.20 μm membrane filter before injection to HPLC. Organic acids were analyzed by a high-performance liquid chromatograph (HPLC) (LC-10A model, SHIMADZU Co.) with an ultraviolet (215 nm) detector (UV1000, SHIMADZU) and a 100 mm × 7.8 mm Fast Acid Analysis column (HPX-87H, Bio-Rad Lab.) using 0.005 M H_2SO_4 as a mobile phase. Flow rate was 0.6 ml/min, column was maintained at 35 °C. H_2 gas content was analyzed using a gas chromatograph (Gow Mac Series 580) equipped with a thermal