Ultrasonics Sonochemistry 32 (2016) 198–203

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Short communication

Low-strength ultrasonication positively affects methanogenic granules toward higher AD performance: Implications from microbial community shift

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 8 March 2016
Accepted 8 March 2016
Available online 9 March 2016

Keywords:
Low-strength ultrasonication
Upflow anaerobic sludge blanket
Pyrosequencing
Evenness
Syntrophism
δ-*Proteobacteria*

ABSTRACT

To elucidate the enhanced methane yield from organic wastes, the effects of low-strength ultrasonication on the microbial community structures in upflow anaerobic sludge blanket reactors were for the first time analyzed using pyrosequencing. Interestingly, a more even microbial community was observed in the ultrasonicated granules than in the control, which could compensate for the decreased richness and resulted in comparable (archaea) or even higher (bacteria) diversity. The ultrasonicated granules contained higher levels of δ-*Proteobacteria*, of which many are reportedly potential syntrophs, as well as methanogenic genera *Methanosaeta*, *Methanotorris*, and *Methanococcus*. The increased presence of syntrophic bacteria with their methanogenic partners was discussed with respect to hydrogen flux; their selective proliferation seems to be responsible for the enhanced anaerobic performance. This study is the first research shedding light on the novel function of low-strength ultrasound shifting the microbial structure towards better biogas production performance, and will facilitate application of low-strength ultrasound to other bioprocesses.

1. Introduction

Ultrasound, a sound wave at a frequency above human hearing, has been widely applied to welding, degassing of solutions, detection of defects, medical diagnosis and treatment etc. [1]. When ultrasound is applied to an aqueous phase, microbubbles (cavitation bubbles) are generated in the rarefaction region. These cavitation bubbles grow in successive cycles, reach an unstable diameter, and then finally collapse [2]. The hydro-mechanical shear stress localizes, temperature increases to 5000 K, highly reactive OH^- radicals are generated, and mixing and mass transfer in an aqueous solution or suspension are increased, namely acoustic streaming effects [1]. To exploit these beneficial effects, ultrasound has been applied to various environmental processes, such as intracellular material recovery, enzyme extraction, pollutant removal and anaerobic digestion etc. [3].

Anaerobic digestion (AD) is considered as an attractive technology for the treatment and recycling of organic waste on the basis of the following advantages: (1) reduction of waste volume, (2) generation of energy-rich gas in the form of methane (CH_4) and (3) generation of nutrient-containing final products. AD is a biotransformation process consisting of hydrolysis, acidogenesis, acetogenesis and methanogenesis [4]. Among these, hydrolysis and is the rate-limiting step of the entire process, particularly when solid organic waste is being treated. To accelerate the hydrolysis step, various pretreatments (i.e., thermal, ultrasound, microwave, and chemical pretreatments, and combinations thereof) have been introduced, and improved solubilization with subsequent enhancements of the AD performance has been reported under case-specific conditions [5]. Among them, ultrasound is one of the most widely used pretreatment method for laboratory-, pilot- and full-scale AD processes due to its aforementioned beneficial effects [6].

Ultrasound is generally divided into three regions based on its frequency; i.e., power ultrasound (20–100 kHz), high

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http://dx.doi.org/10.1016/j.ultsonch.2016.03.010
1350-4177/

frequency ultrasound (100 kHz–1 MHz) and diagnostic ultrasound (1–500 MHz) [2], the application of ultrasound to AD can be split into high- and low-strength ultrasound based on the target of the ultrasonication [7]. High-strength ultrasonication is applied to only feedstock to accelerate the rate-limiting step of AD by increasing the solubility of substrates, while low-strength ultrasonication is targeted at the bioreactor itself to stimulate the microorganisms involved in AD [7].

The majority of ultrasound-mediated AD research has employed high-strength ultrasonication to produce energy-rich biogas such as CH_4 and hydrogen (H_2); in these studies, only the physical and chemical properties (particle size, turbidity, dewaterability, soluble chemical oxygen demand (SCOD), volatile solid (VS) concentration etc.) of the feedstock were evaluated to determine the effect on AD [3,5]. In contrast, few studies have focused on low-strength ultrasonication, for example, the biosynthesis of shikonin [8], nitrogen removal [9] etc. Regarding AD, to our knowledge, only two research groups have reported the effects of low-strength ultrasonication on H_2 production [10] and CH_4 production by our group. The former group [10] applied intermittent ultrasonication (0.25 W/mL, 1 s per 1 min) to H_2 production in a continuous stirred tank reactor. An enhanced H_2 production rate (2.8 L/L/d → 5.6 L/L/d), H_2 yield (1.0 mol H_2/mol glucose → 1.9 mol H_2/mol glucose) and glucose conversion efficiency (76% → 84%) were reported with an organic loading rate of 32.1 g COD/L/d.

In our previous research, the first report of application of low-strength ultrasonication to an upflow anaerobic sludge blanket reactor (UASBr) for CH_4 production, 43% higher CH_4 production was achieved under continuously intermittent ultrasonication (0.05 W/mL, 1 s per 1 min) [11]. To elucidate the positive effects of low-strength ultrasonication on methanogenic granules, the physicochemical characteristics of the methanogenic granules after low strength ultrasonication were investigated through settling experiments, scanning electron microscopy (SEM) analysis, and Brunauer–Emmett–Teller (BET) analysis [12]. By SEM, low-strength ultrasonication resulted in numerous craters and cracks on the granular surface. As a consequence, ultrasonicated granules had a 2.3-fold higher specific surface area and 37% higher permeability than the control granules. Therefore, enhanced penetration of nutrients and substrates into the granules could be expected, which would enhance AD performance.

In this study, the direct effects of low-strength ultrasonication and/or the sequential effects of the alterations in physicochemical properties due to ultrasonication on the microbial community were investigated using the 454-pyrosequencing technique for the first time. In addition, to elucidate the correlation between enhanced CH_4 production yield and alterations in the microbial community, ultrasonicated bacterial and archaeal communities were compared to the control sample with a focus on the enrichment of syntrophic bacteria and the shift in methanogens, because the associations of syntrophic bacteria and methanogenic archaea are indispensable for complete and high-rate methanogenesis.

2. Material and methods

2.1. System setup and operation of UASBr and analytical methods

Information on reactor configurations, operating conditions, and analytical methods is described elsewhere [12]. Briefly, two identical UASBrs, fed with an acidified mixture (food waste and livestock waste; $v{:}v = 6{:}4$) at a 2.5 g chemical oxygen demand/L/day, were operated with or without low-strength ultrasonication.

2.2. Sampling and pyrosequencing analysis

Sludge samples were taken at steady state from both UASBrs and DNA was extracted using the Ultraclean Soil DNA Kit (Mo Bio Laboratory). The extracted DNA was purified using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories). A 20 ng aliquot of each sample DNA was used for a 50 μl PCR reaction. The V1–V3 regions of 16 s rRNA genes for bacteria and archaea were amplified using universal primers 27F (5′ GAGTTTGATCMTGGCTCAG 3′) and 518R (5′ WTTACCGCGGCTGCTGG 3′), and Arc8F (5′ TCCGGTTGATCCTGCC 3′) and Arc519R (5′ TTACCGCGGCKGCTG 3′), respectively. A Fast Start High Fidelity PCR System (Roche) was used for PCR under the following conditions: 94 °C for 3 min, followed by 35 cycles of 94 °C for 15 s, 55 °C for 45 s, and 72 °C for 1 min, with a final elongation step at 72 °C for 8 min. The PCR products were purified using AMPure beads (Beckman Coulter).

A library was prepared using the products according to the GS FLX Titanium library guide and quantified using the Picogreen assay (Life Technologies). Pyrosequencing was conducted using a GS FLX Titanium (454 Life Sciences) according to the manufacturer's instructions, with a commercial sequencing facility (Macrogen). The emPCR was carried out using a GS-FLX titanium emPCR Kit (454 Life Sciences). The sequences generated from pyrosequencing were analyzed mainly with the MOTHUR software for pre-processing (quality-adjustment, barcode split), identification of operational taxonomic units (OTUs), taxonomic assignment, community comparison, and statistical analysis [13]. To minimize errors, sequences were filtered against those with more than one ambiguous base-call and those with shorter than 300 nt. Sample-specific sequences were collected according to the barcode sequences tagged to each sample. The barcode and the primer sequences were trimmed from the result. The trimmed sequences were aligned using Infernal, and associated covariance models were obtained from the Ribosomal Database Project Group [14]. The sequences spanning the same region were then realigned with the Silva database. The OTUs defined by a 3% distance level were phylogenetically classified with a modified bacterial RDP II database containing 164,517 almost full-length 16S rRNA gene sequences prepared using TaxCollector (http://www.microgator.org). The Shannon and Simpson diversity indices and the Pielou's evenness indices were calculated using the OTU tables with the R software package, employing the vegan library. The sequences reported in this study were deposited in the National Center for Biotechnology Information GenBank database (accession number: KT807958–KT808246).

3. Results and discussion

3.1. Summary of microbial community analyses

Overall, 3884 high-quality reads (2226 bacterial and 1658 archaeal) were obtained after screening (Table 1). The Bray–Curtis dissimilarity measures for bacteria and archaea were 0.462 and 0.444, respectively, between the control and the ultrasonicated samples, implying that the two samples share considerable number of OTUs. The apparent richness was higher for the control granules (248 for bacteria, 60 for archaea, 308 overall) than for the ultrasonicated granules (92 for bacteria, 28 for archaea, 120 overall) (Table 1). The rarefied richness of the control, estimated after rarefying to the minimum number of reads (i.e., the ultrasonicated), was also higher than that of the ultrasonicated, but the difference was not distinct for archaea. Likewise, the rarefied bacterial Shannon and Simpson diversity indices for the control were significantly lower than those for the ultrasonicated after rarefaction,