Biochemical Engineering Journal 133 (2018) 59–65

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Performance, granule conductivity and microbial community analysis of upflow anaerobic sludge blanket (UASB) reactors from mesophilic to thermophilic operation

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ARTICLE INFO

Article history:
Received 16 November 2017
Received in revised form 5 January 2018
Accepted 26 January 2018
Available online 31 January 2018

Keywords:
Temperature variations
Conductivity
Microbial community
Methane
UASB

ABSTRACT

Effects of temperature variations from mesophilic to thermophilic on the performance of UASB reactors with glucose and ethanol as substrate were investigated. The reactor with ethanol as substrate had better resistance to temperature variations with over 93% COD degradation rate and 75% methane content. The conductivity of granular sludge that degraded ethanol was also considerably higher. High-throughput sequencing indicated that the abundance of *Methanobacterium* decreased, while *Methanosaeta* increased with increasing temperature. Moreover, the abundance of *Geobacter* and *Methanosaeta* were higher in the reactor with ethanol as substrate than that with glucose, whereas a higher abundance of *Klebsiella* was observed in the glucose reactor. More importantly, significant correlations were observed between granular sludge conductivity and COD removal rate ($R^2 = 0.73$), between the abundance of *Geobacter* and COD removal rate ($R^2 = 0.895$). These results indicate that the growth of *Geobacter* and *Methanosaeta* which promote transfer electrons in the manner of DIET in anaerobic methanogenic systems might alleviate the effect of temperature variation and facilitate reaction rate.

1. Introduction

Upflow Anaerobic Sludge Blanket (UASB) reactors have become more and more popular in wastewater treatment because of their many advantages including simplicity, low operational costs, low energy consumption, and low space requirements [1,2]. The natural turbulence caused by the rising gas bubbles which buoy the sludge, allows wastewater to reach a full contact with biomass [3,4]. Moreover, the formation of aggregated granular sludge maintains a relatively high biomass, which significantly improves the removal efficiency of chemical oxygen demand (COD).

The anaerobic digestion process in the UASB reactors is influenced by a large number of factors, such as temperature [5–7], pH [8,9], conductive material [10–12], organic loading rate (OLR) [13,14], and hydraulic retention time (HRT) [7]. Among these factors, the temperature is undoubtedly the most important because of the relatively large influence of temperature on the reaction rate and the activity of microorganisms [15,16]. There are three major operating temperature ranges, psychrophilic (<25 °C), mesophilic (25–40 °C), and thermophilic (>45 °C). Generally, mesophilic and thermophilic ranges are more efficient than psychrophilic in anaerobic digestion because low temperature is not conducive to the growth of methanogens [6,17,18]. Gao et al. discovered that temperature affected the richness and diversity of microbial populations [15]. Dessi et al. found that a UASB reactor at 55 °C achieved higher efficiency than at 30 °C [19]. However, other studies showed that both COD removal rate and methane production were dropped at thermophilic conditions [20,21]. A convincing explanation for the decrease of reaction rate under thermophilic condition is still unclear. Therefore, the effect of temperature change on the performance of UASB reactors needs to be confirmed.

More importantly, changes in temperature could affect the diversity of microbes and the conductivity of granule sludge, which

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https://doi.org/10.1016/j.bej.2018.01.029
1369-703X/

Table 1
The composition of artificial wastewater and trace elements.

	Composition	Unit	Concentration
Artificial wastewater	Ethanol/Glouse	g/L	2.875/5.623
	NH_4Cl	g/L	0.956
	$Na_2HPO_4{\cdot}12H_2O$	g/L	0.252
	KH_2PO_4	g/L	0.11
	$CaCl_2$	g/L	0.18
Trace elements	EDTA	mg/L	20
	$FeSO_4$	mg/L	5
	$NaMoO_4{\cdot}2H_2O$	μg/L	220
	$CoCl_2{\cdot}6H_2O$	μg/L	240
	$NiCl_2{\cdot}6H_2O$	μg/L	190
	$ZnSO_4{\cdot}7H_2O$	μg/L	430
	H_3BO_4	μg/L	14
	$MnCl_2{\cdot}4H_2O$	μg/L	990
	$CuSO_4{\cdot}5H_2O$	μg/L	250

affects the efficiency of the reactors directly. In general, the efficiency of methane production was significantly correlated with frequent electron transfer, which might lead to a higher conductivity of the granular sludge [11,22]. It has also been shown that the conductivity of the granular sludge and the electrical conductivity might be related to direct interspecies electron transfer (DIET), a rare way for electron transfer in methanogenic consortia [22,23]. Shen et al. reported DIET could be one pathway to generate methane in anaerobic granules because the distance between cells was reduced [24]. DIET has been demonstrated in defined methanogenic co-cultures of *Geobacter metallireducens* with *Methanosaeta* species [25] in a UASB reactor with an artificial brewery wastewater as carbon source. Therefore, analyzing the relationship of the performance, granule conductivity and microorganisms associated with DIET is crucial to understand the impacts of temperature on the performance of UASB reactors. Few studies have shown that there was a moderate correlation ($r = 0.67$) between the abundance of *Geobacter* species in the UASB granules and granule conductivity [23], however, the relationship of the performance, granule conductivity and microbial community must be further investigated to provide an explanation for the variation of reaction rate under mesophilic to thermophilic conditions. As thermophilic digesters are generally inoculated with mesophilic sludge, a good understanding of the variation of reaction rate under mesophilic to thermophilic conditions is necessary for full-scale process start-up, and to establish optimal operating conditions.

In this study, the variation of reaction rate under the temperature variations from a mesophilic (37 °C) condition to a thermophilic (45 °C, 50 °C) condition was investigated in UASB reactors with two different carbon sources. One carbon source was ethanol which previously found to be metabolized via DIET [22,26,27]. Another carbon source was a simple substrate such as glucose might be not metabolized via DIET. More importantly, with the increase of temperature, the relationship of the COD removal rate, the conductivity of the granular sludge and microbial community composition were examined, which could provide a better reference for practical applications.

2. Materials and methods

2.1. Experimental description and reactor operation

Two 2.3 L identical continuous-flow UASB reactors were used in this study. The influent was at the bottom through a peristaltic pump, the out fluent was at the top and there was a gas sampling bag of 2 L on the top of each reactor to collect the gas from the three-phase separator. The temperature of the two reactors was controlled by SPC biochemical incubator BSP-400. The sludge was inoculated from a sewage factory named Hengdianjingxing, in Jinhua city, Zhejiang province, China. The two reactors were fed with artificial wastewater and the carbon sources of each reactor were glucose and ethanol. The composition of artificial wastewater and trace elements was shown in Table 1. The pH was adjusted to approximately 7 by adding 0.5 g/L $NaHCO_3$.

The start-up time of the reactor was approximately one month with a low influent COD and the hydraulic retention time (HRT) was 12 h. Next, the influent COD was raised to approximate 6000 mg/L in approximately 55 d (This part of the data is not presented in this paper). All these were finished at 37 ± 0.5 °C. These conditions were maintained unchanged (COD, 6000 mg/L; 37 ± 0.5 °C) and after 10 days the temperature was raised to 45 ± 0.5 °C. In the same way, temperature was raised to 50 ± 0.5 °C after 15 days and held for 15 days. The hydraulic retention time (HRT) was 12 h and influent COD concentration was 6000 mg/L. Meanwhile, gas production, methane content, and the change of in and out of the water COD were measured. The conductivity of granular sludge, microbial community of each reactor was tested at the end of each temperature section.

2.2. Analytical methods

The suspended solid (SS), volatile suspended sludge (VSS), sludge volume index (SVI), settling velocity (SV), and chemical oxygen demand (COD) were all measured in accordance with the APHA Standard Methods [28]. The biogas volume was measured by its displacement of saturated sodium bicarbonate solution and the methane content in the produced biogas was determined with a gas chromatograph with a thermal conductivity detector (GC-TCD SP-2100A) [29].

The conductivity of granular sludge was detected as following steps: first, the granular sludge was cleaned three times with 0.1 M phosphate buffer (pH, 7.2) to remove impurities from the sludge surface; next, the sludge was dehydrated by 50%, 70%, 80%, 90%, 100% ethanol, respectively, and every concentration was dealt with for 15 min to remove the error of water conductivity. Lastly, impedance was measured by a device provided in the supplementary material. A solartron impedance analyzer (Solartron Mobrey, England) from Yang'Group-Materials for Electrochemical Energy, Department of Chemistry, Xiamen University was used. The parameter settings of the impedance measuring instrument was as follows: DC voltage, 0 V; frequency, 10^{-5}–10^{-1} Hz. The resistance value was calculated using the software of ZView2, the analog circuitry converted to electrical conductivity.

2.3. DNA extraction and MiSeq sequencing

DNA was extracted from approximately 1 g of granular sludge using the Fast DNA Spin Kit for soil (MP Biomedical) and DNA concentration and quality were checked using a NanoDrop Spectrophotometer. Extracted DNA was diluted to 10 ng/μL and stored at −40 °C for downstream use. Next, the V4–V5 hypervariable region of 16S rRNA genes was amplified with universal primers 515F (50-GTGYCAGCMGCCG CGGTA-3′) and 909R (50-CCCCGYCAA TTCMTTTRAGT-3′) with a 12 nt unique barcode at the 5′-end of 515F [30]. The details of PCR and related procedures were described elsewhere. All PCR products were quantified with Nanodrop and pooled together with an equal molar amount from each sample. The sequencing sample was prepared using TruSeq DNA kit per the manufacturer's instructions. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30% of final DNA amount) as described in the Illumina library preparation protocols, and then applied to an Illumina Miseq system for sequencing