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Characterization of the planktonic microbiome in upflow anaerobic sludge blanket reactors during adaptation of mesophilic methanogenic granules to thermophilic operational conditions

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A B S T R A C T

Upflow anaerobic sludge blanket (UASB) technology refers to reactor technology where granules, i.e. self-immobilised microbial associations, are the biological catalysts involved in the anaerobic digestion process. During the start-up period, UASB reactors operate at relatively long HRT and therefore the liquid phase of the reactor becomes a favourable environment for microbial growth. The current study aimed to elucidate the dynamicity of the suspended microbial community in UASB reactors, during the transition from mesophilic to thermophilic conditions. High throughput 16S rRNA amplicon sequencing was used to characterize the taxonomic composition of the microbiome. The results showed that the microbial community was mainly composed by hydrolytic and fermentative bacteria. Results revealed relevant shifts in the microbial community composition, which is mainly determined by the operational conditions and the reactor performance. Finally, shared OTUs between the microbial consortia of the suspended and the granular sludge showed that planktonic microbiota is significantly influencing the granule microbial community composition.

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1. Introduction

The global dependence of fossil fuels is considered to be unsustainable due to the limited resource availability and the negative environmental impact. To mitigate this problem, various alternative renewable energy technologies have been proposed including those exploiting biomass degradation. Bioenergy (i.e. energy from biomass) is gaining increased attention and is estimated to be the fourth largest energy resource in the world [1]. A number of different technologies were developed for various forms of bioenergy extraction and utilization. Anaerobic digestion (AD) is one of the processes utilizing various kinds of biomass producing biogas as energy and digestate as fertilizer. Biogas covers an important fraction means for bioenergy production especially for slurries and wastewaters [2].

In the context of AD, up-flow anaerobic sludge blanket (UASB) reactors are extensively used for treatment of industrial effluents and conventionally operated at mesophilic conditions [3]; however thermophilic operation was proved to be feasible at certain conditions exhibiting a number of advantages such as better sanitation capacity and higher rates [4,5]. For example, Kaparaju et al. [6] reported a successful thermophilic UASB reactor operation with wheat straw stillage as substrate. Thermophilic UASB reactors can achieve a higher CH_4 production rate [7] and an analogous COD removal [8] compared to the mesophilic ones. Nevertheless, the main obstacle for the large-scale implementation of thermophilic UASB application is the availability of thermophilic methanogenic granule seeds for the start-up process. Without active thermophilic granule seeds, granulation from suspended sludge is extremely time consuming. Previous studies reported that it typically takes several months and the process requires intensive control of many parameters [9]. Therefore, attempts to initiate a thermophilic UASB

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reactor with mesophilic methanogenic granules were investigated [10,11]. It was proven that a shift of the operational temperature from mesophilic to thermophilic range led to an extreme modification of the microbial consortium residing inside the granules [13]. This alteration of the microbiota was determined by many operational parameters, such as pH, temperature and hydraulic retention time (HRT).

Up to now, all the microbial analyses performed on the UASB reactors focused on the granule microbiota because it was assumed that the vast majority of the active cells resided in the granules. This assumption would be valid in case that the reactor operates at very short HRT and with low-strength wastewater, which promotes wash out of the suspended non-immobilised microbes. However, the UASB reactors can also operate at a relatively long HRT. A feedstock for UASB reactors, which has high methane potential, is a by-product of potato-starch industry, namely potato juice. A previous study demonstrated that the AD process of potato juice in UASB reactors collapsed after applying HRT shorter than 5 days [14]. Furthermore, during the start-up period, it is a general strategy to maintain long HRT in order to avoid organic overloading of the reactor. Therefore, under such operational conditions, the microbial community evolving in the liquid phase of the UASB reactor would also contribute to the substrate degradation and might influence the granule microbial consortium.

To characterize the planktonic microbial community in UASB reactors, next generation sequencing technology targeting the 16S rRNA gene was employed in the current study, which is a continuation of our previous work [13]. Correlations between the suspended (in the liquid phase) microbial profiles and the reactor performance were investigated, as well as the interaction between the planktonic and the granule microbial consortium. To the best of our knowledge, this is the first study aiming to elucidate the microbial diversity and dynamicity in the liquid phase of a UASB reactor during the adaptation of mesophilic methanogenic granules to thermophilic operational conditions.

2. Materials and methods

2.1. Reactor operation description

A lab scale UASB reactor fed with potato juice was started with mesophilic methanogenic granules (G1) and operated at thermophilic conditions. The granules used in the present study were obtained from a wastewater treatment plant in Netherlands (Colsen) and raw potato juice used as substrate was obtained from potato-starch processing factory (Karup Kartoffelmelfabrik, Denmark). After arrival to the laboratory, the raw potato juice was frozen at −20 °C. Before usage, the juice was thawed at 4 °C and diluted 7 times with distilled water. The experiment consisted of 3 periods namely start-up, operation 1 and operation 2. During start-up period, only 40 mL potato juice was fed to the reactor to provide basic nutrition and carbon source for development of new thermophilic microbial community. During both periods, the reactor operated at organic loading rate of 3.97 gVS/(L-reactor.d) and 7 days HRT. The operation conditions were chosen based on previous research analysing the UASB reactor operation with potato juice as substrate [14]. During the 2nd operation period, sodium bicarbonate ($NaHCO_3$) was added in the reactor to maintain the pH higher than 6.5. The daily biogas production was recorded using an automated displacement gas metering system with 100 ml cycle. The detailed reactor operation was previously described [13] and essential reactor and substrate information are listed in Table 1.

2.2. Theoretical biomethane potential

In order to calculate theoretical biomethane potential of potato juice, the concentration of main degradable digestible organic matter (i.e. total sugar protein and lactate) was measured and the results were listed in Table 2.

The theoretical biomethane potential of the substrate and methane equivalent of digestion products (i.e. volatile fatty acids and alcohols) was calculated according to stoichiometric degradation to biogas. Under strictly anaerobic conditions, organic material will convert to CH_4 and CO_2 with H_2O as the only external source and the methane equivalent can be calculated from the following equation [15].

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2$$

According to the equation, the calculated yields for digested products are listed in Table 3.

2.3. Analytical methods

The concentration of total solids (TS), volatile solids (VS), total

Table 2
Organic compound concentration in potato juice.

Compound	Concentration (g/L)
Protein	6.75 ± 0.08
Sugar	9.65 ± 0.55
Lactate	2.27 ± 0.01

Table 1
Reactor configuration and operational parameters.

	Start-up	Period 1	Period 2
Working Volume (L)	1.4		
Upflow velocity (m/h)	2.2		
Temperature (°C)	55		
Volatile solids for potato juice (g/L)	163.98 ± 2.78		
pH for potato juice	5.23		
Duration (days)	6	21	14
Sodium bicarbonate (g/L-reactor)	n.a.	n.a.	4
Organic loading rate (gVS/L-reactor.d)	0.79	3.97	3.97
HRT (day)	35	7	7
pH	7–7.5	5.4–5.7	6.5–6.8
Granule sample	G2	G3	G4
Liquid sample	L2	L3	L4
Sample ID	SUB2036508	SUB2036505	SUB2036499

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