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Converting mesophilic upflow sludge blanket (UASB) reactors to thermophilic by applying axenic methanogenic culture bioaugmentation

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ABSTRACT

The application of thermophilic conditions in anaerobic digesters leads to higher methane production rates and better sanitation of the effluents compared to mesophilic operation. However, an increase in operational temperature is challenging due to the tremendous selective pressure imposed on the microbial consortium. The adaptation of microbial community to a new environment or condition can be accelerated by a process known as "bioaugmentation" or "microbial community manipulation", during which exogenous microorganisms harbouring specific metabolic activities are introduced to the reactor. The aim of the current study was to rapidly convert the operational temperature of up-flow anaerobic sludge blanket (UASB) reactors from mesophilic to thermophilic conditions by applying microbial community manipulation techniques. Three different bioaugmentation strategies were compared and it was proven that the injection of axenic methanogenic culture was the most efficient approach leading to improved biomethanation process with 40% higher methane production rate compared to the control reactor. Microbial community analyses revealed that during bioaugmentation, the exogenous hydrogenotrophic methanogen could be encapsulated in granular structures and concomitantly promote the growth of syntrophic fatty acid oxidizing bacteria. The results derived from the current study indicated that microbial community manipulation is an efficient alternative method to speed up transition of UASB reactors from mesophilic to thermophilic conditions.

1. Introduction

Anaerobic digestion (AD) is a popular technology for simultaneous production of energy and treatment of wastewaters. Various reactor types and configurations have been developed in order to increase the efficiency of the process and to be able to utilize a wide variety of substrates. Up-flow anaerobic sludge blanket (UASB) reactors are extensively used to treat high strength liquid wastes such as leachate from food waste, cheese whey and textile dyeing wastewater [1–3]. In a UASB reactor, the liquid waste flows through the sludge bed at a significantly shorter hydraulic retention times (HRT) compared to the growth rates of key microorganisms for the biomethanation process. The granular sludge with excellent settling properties confines the active microbes and prevents them from washout [4]. UASB reactors are widely used in tropic countries where the reactors operate at ambient temperature (21–24 °C) or at mesophilic conditions [5,6].

It is postulated that the conversion rate increases with temperature in AD process [7]. Nevertheless, especially in applications where UASB reactors are used, it is extremely difficult to find thermophilic granular sludge. This is mainly because most of the UASB reactors are operated at mesophilic conditions to avoid heating costs and to increase the tolerance of the process towards specific inhibitors such oleate [8]. Previous researchers analysed the process stability during temperature transition from mesophilic to thermophilic operation; however, the outcomes were inconclusive [9,10]. Results showed that in specific cases the methane production was inhibited without providing an obvious explanation regarding this failure. Denaturing gradient gel electrophoresis (DGGE) of 16s rRNA gene was applied to analyse the granular microbial community changes from mesophilic to thermophilic treatment of palm oil mill effluent, and the results suggested significant differences in microbiota compositions [11]. However, the microbial community was not explored quantitatively and comprehensively due to the technical limitations of DGGE technique. Recent studies based on high throughput amplicon sequencing remarked that during temperature transition, the thermophilic bacterial community evolved spontaneously in liquid phase of UASB reactors and were found inhabiting in the granular structures [12]. However, it was demonstrated that the thermophilic archaeal group grew slowly and

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concomitantly led to process imbalance [13].

The key postulate of successful temperature conversion is the ubiquity of thermophiles. Therefore, the most commonly used practice in biogas reactors involves a stepwise increment of the operating temperature, which allows a spontaneous but slow microbial community transition. A method to accelerate the microbial adaptation into new or extreme conditions is known as "bioaugmentation" or "microbial community manipulation" and relies on the provision of exogenous microbial consortia into certain microbial ecosystems to manipulate the existing metabolic activities towards a desired effect or process. Recently, the bioaugmentation strategies are gaining increased attention in different AD applications. For example, bioaugmentation was used to increase hydrolysis of cellulose [14], overcome ammonia inhibition [15] and restore methane production from overloaded reactor [16]. Microbes encoding proteins involved in crucial steps of biomethanation were injected into biogas reactors leading to significant enhancement of methane production. One important consideration about bioaugmentation in continuous reactor operations is the duration of the bioaugmentation effect, which is related to the maintenance and proliferation of the exogenous microbes inside the reactor. It is often reported that the positive bioaugmentation effect occurs for a limited time period after the inoculation due to the wash out of the exogenous strains [14,17]. This obstacle could potentially be overcome in sludge retaining reactors due to the fact that the microbes can be immobilised into the granules, and thus, stay in the reactor for longer periods.

In the current study, three bioaugmentation strategies were examined by comparing both reactor performance and microbial community dynamics. Two laboratory-scale mesophilic UASB reactors were successfully changed to thermophilic operation with and without applying selected bioaugmentation. Methane production rate and yield were monitored in order to evaluate the process enhancement, and high throughput amplicons sequencing was employed to reveal the microbial dynamics in the granules. The outcomes of the current work allowed the proposal of an alternative method to enhance biomethanation in UASB reactors and also provided insights regarding the shifts of microbial composition during process optimization.

2. Materials and methods

2.1. Characteristics of substrate and granules

Mesophilic granules used in this study were obtained from a full scale UASB reactor of a wastewater treatment plant (Colsen) processing potato juice in Netherlands. The granules were stored at 4 °C and moved to room temperature for 24 h before use. Potato juice used as substrate was obtained from potato-starch processing factory (Karup Kartoffelmelfabrik, Denmark) in two batches. Batch 1 was used as substrate in the experiment related to the selection of bioaugmentation strategy and Batch 2 was used during biomethanation enhancement by thermophilic operation and bioaugmentation. The raw potato juice was diluted with distilled water before usage in order to achieve the desired the organic loading rate (OLR) and HRT. The characteristics of raw and diluted potato juice are presented in Table 1.

2.2. Experiment 1-testing various bioaugmentation strategies

Three different bioaugmentation strategies were investigated using lab scale UASB reactors with working volume of 1.4 L. The reactor configuration was previously described by Zhu et al. [13]. Briefly, the liquid feedstock was supplied from the bottom of the reactor every h according to the designed HRTs and OLRs. In addition to the feedstock influent, internal liquid recirculation was applied to create an up flow velocity of 2.2 m/h, providing efficient mixing of the feedstock and hydraulic condition for granular structure maintenance. Initially, four UASB reactors were inoculated with 600 mL mesophilic granular sludge and 800 mL basal anaerobic (BA) medium [18]. The reactors operated at 55 °C and the bioaugmentation was performed at the first day of operation. One reactor operated without any bioaugmentation practice serving as control (Control 1).

In Strategy 1, the practice previously proposed to develop thermophilic adapted granules by replacing the 800 mL BA medium with AD digestate was followed [19]. The digestate used for bioaugmentation was obtained from a conventional lab scale continuous stirring tank biogas reactor (CSTR) treating cattle manure at 55 °C. The rationale beyond this strategy was that the digestate from CSTR would provide a "complete" thermophilic microbial consortium mediating the entire AD process from hydrolysis to methanogenesis. Besides the biotic parameters i.e. the active methanogenic microbial community, digestate from cattle manure degradation provides abiotic enhancement, such as the necessary nutrients for optimal microbial growth, the high pH buffer capacity and the absence of substances that could inhibit the biomethanation process (e.g. high ammonia or fatty acid concentration). It is expected that the abiotic benefits will be gradually weakened with long operation due to the wash out of digestate; however, it was hypothesized that till that time an adapted microbial consortium would be fully shaped. Strategy 2 relied on the injection of 40 mL of AD digestate (same origin as described previously) into the UASB reactor for 6 consecutive days. During the bioaugmentation period the reactor was daily fed with 40 mL of potato juice to proliferate specialised microbes for substrate degradation. Comparing with previous strategy, the low proportion of AD digestate in the liquid phase limited the abiotic enhancement and therefore we were able to focus our evaluation on the manipulation of microbial community composition. Strategy 3 relied on the injection of a specific axenic thermophilic methanogenic culture in the liquid phase of the UASB reactor. The daily injected amount of axenic methanogens (40 mL) consisted of isovolume archaeal culture containing *Methanothermobacter thermautotrophicus* (DSM No. 3720) and *Methanosarcina thermophila* (DSM No. 1825). The two strains were chosen based on their ubiquity in AD system and their metabolic capacities to cover the most known methanogenic pathways, namely acetoclastic and hydrogenotrophic. The supplied archaeal strains were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures and were always injected into the reactor under exponential growth phase. The cultivation procedure was previously described by Tsapekos et al. [14]. The composition of the cultivation media and growth curve for both strains are presented in Supplementary Information (Tables S1, S2 and Figs. S1, S2). The cell yields in both cultures were assumed proportional to methane production and the biomass concentrations were calculated from substrate-specific coefficient: Y_{H2+CO2} = 8.7 mg dry cell mass/mmol CH_4 and $Y_{acetate}$ = 2.1 mg dry cell mass/mmol CH_4 [20]. As a result, each bioaugmentation portion (40 mL) contained 3.8 mg *M. thermautotrophicus* and 5.0 mg *M. thermophila* as dry cell mass. The bioaugmentation period lasted for 6 days and the operation details for each strategy are listed in Table 2. In order to evaluate effect of bioaugmentation, we operated the reactor using potato juice as substrate for 21 days i.e. 3 HRTs at the OLR of 4 gVS/L-reactor.day after the bioaugmentation.

2.3. Experiment 2-elucidation of the microbial composition alterations as a result of biomethanation with axenic cultures

The enhancement and persistence of bioaugmentation was tested in 2 laboratory scale UASB reactors, each one having a working volume of 1.2 L. The reactor configuration was previously described by Fang et al. [6]. Initially, the reactors operated at mesophilic conditions (37 °C) for 27 days until the achievement of stable (less than 10% variance for 1 HRT) methane production. The OLR and HRT were 3 gVS/L-reactor.day and 8 days respectively. Subsequently, the operational temperature was changed to 55 °C and one reactor was bioaugmented according to the Strategy 3, while the same amount of BA medium was added to the other reactor serving as control. Only bioaugmentation by