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Role of sludge volume index in anaerobic sludge granulation in a hybrid anaerobic reactor

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HIGHLIGHTS

- Relating granulating ability with start-up SVIs.
- Self-immobilized fluidized granules developed between start-up SVI index of 150–210.
- SVI based DGGE fingerprints and Q-PCR analysis.
- Archaeal diversity was related to better granulation.
- Granulating reactors were rich in aceticlastic methanogens.

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ABSTRACT

This work focuses on microbial granulation in hybrid anaerobic reactors (HAR). Five HARs were used in two sets of experiments and all were seeded with the same inoculum. The volume of inoculum added was 10%, 20%, 30%, 40% and 50% in the first and 13%, 15%, 18%, 23%, and 27% in the second set of experiment. The volume of inocula added affects the sludge volume index, which in the present study proves to be important parameter for granulation. The results suggest that if SVI during the reactor start-up is kept between 150 and 210, reactors were able to form granules. Outside this range in the present set-up, no granulation was observed due to increased biomass wash-out, causing decreased SRT. Higher methane production was achieved in reactors with good granulation. The DGGE profiles show that the non-granulating systems had lesser diversity, due to increased wash out. Archaeal profile gave better correlation for granulation. Granulating reactors were rich in aceticlastic methanogens over hydrogen utilizing groups, especially *Methanosaetaceae*. Both *Methanosarcina* and *Methanosaeta* are required for better granulation as confirmed quantitatively. Bacterial profile of granulating reactors were rich in acetogens. SEM (Scanning electron microscopy) pictures show that granules are dominated by *Methanosaeta*-like microbes. Thus, the sludge characteristics at the start-up are of vital importance as they influence the sludge quality developed during the reactor operation and also the microbial communities retained.

1. Introduction

Anaerobic digestion is widely used to treat wastewaters from various sources with simultaneous production of biogas and reduction in amount of waste. The reduction in amount of sludge generation is of major importance as half of the total operating cost of a wastewater treatment plant is due to sludge treatment [1]. It is also promising in terms of energy production in the form of biogas. The amount of biogas and the quality of sludge obtained will vary according to the wastewater treated. More gas will be produced if the waste is more liable to decompose. Anaerobic treatment process has some problems associated with it like longer start-up time, instability, requirement of large volume digester with retention of sensitive and slow growing microbes. To overcome these problems high cell density reactors are used like upflow anaerobic sludge blanket reactor (UASB), expanded granular sludge blanket reactor (ESBR), fluidized bed reactor (FBR), and hybrid anaerobic reactor [2]. They have uncoupled hydraulic retention time (HRT) and solid retention time (SRT). These high cell density reactors develop granules, which helps in increasing biomass retention times and decreasing sludge washout. Granulation is based on the natural capability of anaerobic microorganisms to form aggregates. Thus, granulation is very important for the successful start-up of these anaerobic reactors. The granules possess higher

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settling velocities and retention time with better ability to withstand high gas pressure and upward liquid velocities. They provide maximum microorganisms to space ratio along with higher specific methanogenic activity i.e. in a given volume of digester maximum microbes can be retained in granular form. There were various problems faced during the granulation using the digested sludge as the seeding material like very long start up time of the reactor (which may be due to very high susceptibility of methanogens and acetogens), poor settling of biomass lead to frequent wash out of the sludge which required re-start of the reactor.

The diversity and abundance of different microorganisms present in the anaerobic system are not considered as typical design parameters [3,4]. Generally, methane production or activity of methanogens is considered as the key parameter for monitoring the performance of anaerobic digester [5–8]. A few studies have been done to explore the effect of different microbial population present in a digester on the granulation process. Very less is known about bacterial communities affecting granulation and making anaerobic treatment stable [9]. Aceticlastic *Methanosaeta* species are important for stable anaerobic digester function and anaerobic granulation [10–12]. While developing granules, focus has mostly been given on chemical parameters of the sludge and reactor system, neglecting the microbial community diversity retained inside during reactor operation. Knowing the community would help in a better understanding of the potential, as well as limitations, for granules development in a system. Moreover, the study would help in developing strategy for increasing the rate of granulation of the sludge. The present study aims at understanding granulation process in high-rate anaerobic reactor with the help of both the physico-chemical parameters and microbial characteristics of the granules using DGGE (denaturing gel gradient electrophoresis) profiling and qPCR (quantitative polymerase chain reaction). This will also help to better understand the importance of SVI (Sludge Volume Index) as a selective pressure and relate this with other parameters. DGGE profiles were generated by resolving 16S rRNA gene amplicons of archaea as well as bacteria to understand the important communities required for granulation to occur. The quantitative real time PCR was performed for universal bacteria and five methanogenic groups to estimate the abundance and the diversity of major microbial guilds.

Granulation of digested sludge can take anything from three to eight months [13–15]. So, while studying granulation, the focus was also on increasing the rate of granule formation and thus reducing the start up time. Even with the same wastewater and inoculum being used for all the HARs, sludge characteristics differed with variation in the volume of inoculum added to the reactors. It also depends on various sludge properties like SVI of the seed sludge, VSS and SS content, SLR and SRT maintained in the system.

However, very little is reported on granule development during reactor start-up under different SVIs but at the same OLR and using the same wastewater in the high rate anaerobic reactor. In the present work, an attempt is made to evaluate the effects of different SVIs maintained in the reactor by varying the concentration of the seed sludge added to the reactor, during primary start-up for enabling granulation in the high rate anaerobic reactors. SVI tells about the sludge settling ability. Based on this study the favorable range of SVI or inocula (% v/v) to be added, can be predicted to develop a sludge with good granule forming characteristics.

Molecular analysis also concluded the requirement for some important microbes for granulation to occur and this study was able to relate favorable SVI range with the presence of these microbes, which were important for granulation. Outside the favorable SVI range, important microbial communities were washed out of the reactors. Thus, it can be said that the sludge characteristics at the start-up are of vital importance as they influence the sludge quality developed during the reactor operation and also the microbial communities retained, influencing granulation ability.

2. Materials and methods

2.1. Experimental set-up

Five laboratory scale HAR [2] reactors (R1, R2, R3, R4 and R5) were used in this study with upflow velocities maintained between 4 and 5 m/h. Two sets of experiments were carried out. Synthetic wastewater which was used had the following composition: glucose (1% w/v), yeast extract (0.34 g/l), ammonium chloride (0.84 g/l), potassium di-hydrogen phosphate (0.136 g/l), di-potassium hydrogen phosphate (0.23 g/l), magnesium chloride hexa hydrate (0.084 g/l), ferric chloride (0.05 g/l) and calcium chloride (100 mg/l). The feed was prepared daily and the reactors were maintained at optimum conditions of temperature (37 ± 2 °C) by housing in a walk-in constant temperature incubator and pH in all the reactors was maintained at 6.8–8.0. A mixture of fresh cow dung and digested sludge (1:3) from the anaerobic digester of the sewage treatment plant at Okhla, New Delhi, was used as inoculum in all experiments. The above inoculum was sieved using a mesh of 1.0 mm to remove large debris and other suspended impurities prior to inoculation. This was then enriched and mother inoculum was prepared, with which all the HARs were seeded.

The inoculum quantity (v/v) added was 10%, 20%, 30%, 40% and 50% of the working volume (1.2 L) of the reactors R1, R2, R3, R4 and R5, respectively for the first set of experiment. Based on the results obtained from the first experiment, it was found that reactor with 20% seeding inoculum showed good granule formation. Therefore, the second set of experiments were conducted by considering inoculum quantity around 20% ± 7% (i.e. 13%, 15%, 18%, 23% and 27% of the inoculum quantity) (Table 1). VSS/SS ratio for the inoculum was 0.447. In each experiment, the reactors were operated for about 90 days (run time depends upon the stability and performance of the reactor or observations about the probability of forming granules) with constant OLR throughout. Operating conditions were kept the same for each reactor throughout the experiment.

2.2. Analytical methods

For estimating the methane content in the biogas produced, a gas chromatograph (AIMIL-NUCON, India, Series 5700) equipped with a 6-ft. Porapack-Q column and thermal conductivity detector (TCD). A calibration mixture of methane and carbon dioxide (EDT Research, England) was used for standardization. H_2 gas was used as carrier. The temperatures of injector, oven and detector were maintained at 80 °C, 60 °C and 80 °C, respectively. The current was maintained at 80 mA. To analyze VFAs, a gas chromatograph (AIMIL–NUCON, India, Series 5765) equipped with flame ionization detector (FID) and Chromosorb 101 column was used. N_2 was used as carrier gas at a flow rate of 30 mL/min and a mixture of hydrogen and air was used as fuel to maintain the flame in the detector. Calibration was done with standard solutions of volatile fatty acids in distilled water. Sludge samples (2 mL) were withdrawn from the sludge bed of the reactors for analyzing different sludge characteristics. Suspended solids (SS), volatile suspended solids (VSS), mixed liquor suspended solids (MLSS), sludge volume index (SVI), sludge wash out, sludge retention time (SRT) and pH were analyzed as per standard methods [16]. The sludge characteristics were determined at the beginning and after 30 days of operation, in duplicate. Intermediate sludge analysis was carried out for only once per month.