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Impact of abrupt temperature increase on the performance of an anaerobic hybrid bioreactor and its intrinsic microbial community

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HIGHLIGHTS

- Microbial community structure and reactor performance under temperature shocks (45–65 °C).
- Both diversity and relative abundance of methanogenic groups were significantly affected.
- Emergence of thermophilic strains prevented system deterioration upto 55 °C.
- Absence of any thermophilic acetoclastic methanogens caused system failure at 65 °C.

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ABSTRACT

This study aimed to analyse the effect of sudden temperature increases (in the range of 45–65 °C) on the performance and the microbial community structure of a hybrid anaerobic reactor. The reactor recovered with time after every temperature shock up to the operating temperature of 55 °C. At 55 °C, a 10 °C shock resulting in an operating temperature of 65 °C, deteriorated the reactor's performance. At this condition, both, the diversity and the relative abundance of methanogenic groups, especially of *Methanosaetaceae*, were significantly affected as observed by DGGE fingerprinting and quantitative PCR. In contrast, at lower temperatures (i.e., 45 and 55 °C), thermal shocks seemed to have less effect due to the presence and maintenance of thermophilic strains, which prevented system deterioration. At 65 °C, the absence of any acetoclastic methanogen is assumed to be the cause of system failure.

1. Introduction

Anaerobic digestion (AD) is an attractive option for sustainable organic waste management because of production of methane as a by-product, which can be used as an alternative fuel. AD of organic matter is dependent on the concerted action of at least four metabolic groups of microorganisms, namely hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria, and methanogenic archaea. The maintenance of the complex metabolic interactions between different trophic groups of anaerobic microbial consortium in the system is crucial for sustained anaerobic digestion. As the growth of microorganisms is determined by several operational parameters such as temperature, pH and carbon sources, the overall performance of anaerobic reactors is also dependent on the same factors. Especially the operating temperature is known to have a fundamental effect on the inherent microbial communities (e.g., Rademacher et al., 2012). Generally, there are three optimal temperature ranges for the operation of AD reactors – psychrophilic (<25 °C), mesophilic (25–40 °C) and thermophilic (>45 °C) (El-Mashad et al., 2004). Operating anaerobic reactors at mesophilic temperature is commonly applied and seems to be highly effective due to the fact that it allows optimal growth of a broad range of microorganisms essential for AD.

Generally, AD processes are considered highly sensitive to environmental perturbation or changes in operating conditions (Leitão et al., 2006). When a system is operating at steady state condition, all trophic microbial groups are well balanced, and there is no accumulation of intermediates in the reactor. On the other hand, when the process is subjected to a sudden change, e.g., in temperature, the different level of response of particular trophic groups of microorganisms may lead to an imbalance in the entire microbial consortium and its trophic network; subsequently resulting in reduced performance of the system (Cha and Noike, 1997; Leitão et al., 2006).

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Fluctuations in temperature occur commonly in field application of anaerobic treatment plants. The discharge of effluents from different units of a given industrial facility, with wide ranges of temperature and temporal variations, can affect the performance of AD; therefore, demands robustness of the process. For example, in the pulp and paper industry, wastewater is discharged at high strengths in terms of the chemical oxygen demand (COD, 10,000–50,000 mg L^{-1}) and wide temperature range (20–70 °C) (Jahren and Rintala, 1997; Morgan-Sagastume and Allen, 2003). In such cases, temperature variations may arise by frequent temperature transits of wastewater streams or their accordant junction (Gao et al., 2011). Furthermore, the seasonal variation in temperature of a certain geographical area can affect the operating temperature. If the temperature is maintained in a treatment plant by automated control, failure in control (unexpected cooling and heating problems) may also cause temperature fluctuation. Therefore, the impact of temperature fluctuation on AD is regarded as an important topic, especially in industrial and field applications, where a stable AD is crucial despite varying temperature conditions.

The effect of temperature shock depends on the degree of shock, exposure time, and microbial composition of the anaerobic sludge (El-Mashad et al., 2004). In mesophilic anaerobic reactors, temperature shock is reported to result in unstable performance to the extent of complete failure (Lau and Fang, 1997; Ahn and Forster, 2002). Most of the previously published studies have been focused on temperature fluctuation in thermophilic reactors as such processes are believed to be more sensitive to operational fluctuation than the mesophilic ones (Ahring et al., 2001; Iranpour et al., 2002). Unfortunately, there is little information on the transition of underlying methanogenic communities with respect to temperature shock in case of a change from mesophilic to thermophilic range (Gao et al., 2011). However, if in AD a wide range of operation temperatures is demanded for biomethanation due to the particular industrial application, the effect of temperature shocks on both, the reactor performance and the methanogenic community structure, needs to be addressed more in detail, especially with respect to the apparent operating temperature.

In a previous study of the group, high strength simulated wastewater was successfully degraded in three hybrid anaerobic reactors operating at mesophilic (37 °C), moderate thermophilic (45 °C) and thermophilic (55 °C) temperatures (Kundu et al., 2012). Advanced molecular microbiology tools, viz. denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993) and quantitative PCR (Yu et al., 2005), were applied to characterize the microbial community structure with respect to the different process conditions. The microbial community structure was found to be significantly different in the three reactors leading to differences in systems' efficiency.

To extend the knowledge on the impact of sudden thermal fluctuation, in this study, now the tolerance of hybrid anaerobic reactors to temperature shocks was assessed by monitoring the performance of the reactor with respect to the process stability and recovery, and the microbial community structure.

2. Methods

2.1. Reactor design and experimental setup

A 1.5 L laboratory scale hybrid anaerobic reactor (Saravanan and Sreekrishnan, 2008; Kundu et al., 2012), developed at the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, was used in this study. In this reactor, the granular sludge bed is maintained to completely fluidized condition like fluidized bed reactor (FBR), eliminating dead zones and resulting in better sludge-wastewater contact. However, the liquid superficial velocity used for fluidization in this reactor is in the range of 4–8 m h^{-1}, which is lower than that in FBR (>10 m h^{-1}). Also, this reactor involves enrichment of the microflora by formation of self-immobilized granules (without carrier particle unlike FBR) as upflow anaerobic sludge blanket (UASB) reactor. In this manner, the reactor has the features of both UASB and FBR, thus termed as hybrid anaerobic reactor. An effluent recirculation facility was used to maintain a liquid upflow velocity of 5 m h^{-1} to fluidize the bed in this study. All experiments were carried out in a temperature-controlled room, maintained at 37 °C. Temperature shock was applied by increasing the temperature with a heating tape equipped with a thermostat. The reactor was fed with synthetic wastewater consisting of glucose (10 g L^{-1}), yeast extract (0.34 g L^{-1}), ammonium chloride (0.84 g L^{-1}), potassium di-hydrogen phosphate (0.136 g L^{-1}), di-potassium hydrogen phosphate (0.23 g L^{-1}), magnesium chloride hexa hydrate (0.084 g L^{-1}) and ferric chloride (0.05 g L^{-1}). The reactor was seeded with active anaerobic culture from a laboratory scale batch reactor (50% v/v) using simulated wastewater, as described above. The batch reactor was in turn seeded with anaerobic culture from fresh cow dung as well as anaerobically digested sludge from a sewage treatment plant at Okhla, New Delhi, India. The volatile suspended solids (VSS) and suspended solids (SS) concentration in the inoculum were 18.3 and 53.8 g L^{-1}, respectively. The reactor was operated at an organic loading rate (OLR) of 2.22 kg COD m^{-3} reactor d^{-1} with a hydraulic retention time (HRT) of 5 days. Three consecutive temperature shocks increasing the operating temperature to 45, 55 and 65 °C were given to the reactor. Each shock was induced after the system reached steady state under the pre-shock condition. Samples of the reactor effluent and biogas were routinely taken on a daily basis for determination of volatile fatty acids (VFA), COD and CH_4 content.

2.2. Chemical analysis

Gas composition was analyzed using a gas chromatograph (AIMIL-NUCON, India, Series 5700). A 6 ft. Porapack-Q column and a thermal conductivity detector were used. The liquid samples were centrifuged at 1400×*g* for 20 min and were analyzed for VFA using gas–liquid chromatography (AIMIL–NUCON, India, Series 5765) fitted with a Flame Ionization Detector (FID). A 6 ft. Chromosorb 101 column was used. COD (using dichromate closed reflux method), total suspended solids (TSS) and volatile suspended solids (VSS) were estimated as suggested by standard methods (APHA, 1995).

All statistical analyses were done using SPSS software version 20.0 by one-way analysis of variance (ANOVA) to determine the significance of temperature shocks on the measured parameters at the 5% level.

2.3. DNA extraction

Samples were collected under each temperature shock at transient phase and/or steady state. DNA was extracted in triplicate, using FastDNA SPIN kit for soil (MP Biomedicals, Cambridge, UK) according to the manufacturer's instructions and quantified with a Nanovue™ spectrophotometer (GE Healthcare, USA).

2.4. Polymerase chain reaction (PCR)

For the generation of DGGE profiles, archaeal partial 16S rRNA genes (*rrs*) were amplified from the extracted genomic DNA using mastermix (Bioline, USA). For amplifying methanogens the primers 109F and 515r (Sigma, USA) were used as described by de Bok et al. (2006). The amplicon size was checked by electrophoresis using 1%