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Carbon source — A strong determinant of microbial community structure and performance of an anaerobic reactor

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ABSTRACT

Industrial effluents differ in their organic composition thereby providing different carbon sources to the microbial communities involved in its treatment. This study aimed to investigate the correlation of microbial community structure with wastewater composition and reactor's performance. Self-immobilized granules were developed in simulated wastewater based on different carbon sources (glucose, sugarcane molasses, and milk) in three hybrid anaerobic reactors operated at 37 °C. To study archaeal community structure, a polyphasic approach was used with both qualitative and quantitative analysis. While PCR-denaturing gradient gel electrophoresis of 16S rRNA gene did not reveal major shifts in diversity of archaea with change in substrate, quantification of different groups of methanogens and total bacteria by real-time PCR showed variations in relative abundances with the dominance of *Methanosaetaceae* and *Methanobacteriales*. These data were supported by differences in the ratio of total counts of archaea and bacteria analyzed by catalyzed reporter deposition – fluorescence in situ hybridization. During hydraulic and organic shocks, the molasses-based reactor showed the best performance followed by the milk- and the glucose-based reactor. The study indicates that carbon source shapes the microbial community structure more in terms of relative abundance with distinct metabolic capacities rather than its diversity itself.

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1. Introduction

Anaerobic digestion is an attractive option for biological treatment of wastewater due to low investment together with the production of a valuable end product – methane, which can be used as an alternative fuel. Both high and medium strength wastewaters can be very efficiently treated by anaerobic digestion. This process is driven not by a single species, but rather by four functionally different trophic groups of microorganisms – hydrolytic, acidogenic, acetogenic bacteria, and methanogenic archaea. The existence of strong syntrophic interaction between different microbial consortia is favored by the formation of microbial aggregates such as granules or flocs in which participating organisms share a common spatial microniche (Thiele et al., 1988). However, due to different kinds of substrate degradation kinetics, the types of microorganisms present and their relative abundance in the biomass varies with variation in wastewater composition and other operational conditions (McHugh et al., 2006). Therefore, effluents from different industries, which also differ in their organic composition, grossly affect the performance of anaerobic reactors by influencing the composition and activity of the microbial communities. Though the major difference in the composition of wastewater lies in the source of carbon, the presence of fat and protein, as in the case of dairy or food processing industries, can cause an imbalance between different microorganisms due to inhibitory intermediates (Vidal et al., 2000; Cirne et al., 2007). Moreover, temporal changes in manufacturing processes that result in heterogeneous wastewater composition have been another concern. Such sudden changes and any imposed stress may alter the diversity, relative abundances of species and microbial activities, which subsequently affect the reactor's performance (McHugh et al., 2006). Therefore, the structure, dynamics and distribution of the microbial community with differences in wastewater composition and temporal variations are important to achieve stable performance of anaerobic reactors.

Developments in molecular microbiology, both in qualitative and quantitative methods such as denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993), fluorescence in situ hybridization (FISH) (Amann et al., 1995), and quantitative PCR (q-PCR) (Yu et al., 2005) have led to deeper insights into the microbial community structure involved in wastewater treatment. Using DGGE, dominant phylotypes in microbial communities can be determined whereas q-PCR provides valuable knowledge about relative abundances of different microbial phylotypes. To

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quantify total cell numbers of different phylogenetic groups the FISH approach can be used. Applying qualitative and quantitative analysis, microbial community structures can be coupled with functional stability of reactors treating different wastewater compositions. In several studies significant differences in the performance of reactors were observed due to different carbon sources (Yang and Anderson, 1993; Fukuzaki et al., 1995). The spatial organization of microbial phylotypes inside aggregates is also influenced by the wastewater composition (Batstone et al., 2004). Though, some studies reported an influence of different wastewater compositions on the microbial community structure (Akarsubasi et al., 2005; McHugh et al., 2006), it remains to be investigated how carbon sources can shape the microbial community structure in terms of diversity, abundance and functional characteristics.

In this light, the aim of this study was to characterize the microbial community structure, function, and relative abundances of different methanogenic groups in a novel hybrid anaerobic reactor treating wastewaters with different organic content using a polyphasic approach with three different culture-independent techniques – DGGE, catalyzed reporter deposition (CARD)-FISH and q-PCR to overcome limitations of each method. Moreover, the effects of shock loads were also studied to establish a link between the community structure and the system's performance under stressed conditions.

2. Materials and methods

2.1. Reactor design and experimental setup

Three laboratory scale hybrid reactors (Saravanan and Sreekrishnan, 2008) developed at the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, were used with simulated wastewater based on different carbon sources (glucose, milk and sugarcane molasses). The reactors had the features of both, a fluidized bed reactor and an upflow anaerobic sludge blanket reactor, which involved an enrichment of the microflora by the formation of self-immobilized (without carrier particle) granules. An effluent recirculation facility was used to maintain the liquid upflow velocity of 4–5 m h^{-1} to fluidize the bed. All the experiments were carried out in a temperature-controlled room maintained at 37 °C. The reactors were fed with synthetic wastewater consisting of glucose or molasses (1% w/v), 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 hexahydrate (0.084 g l^{-1}) and ferric chloride (0.05 g l^{-1}). The milk-based wastewater was prepared by diluting milk containing 1.5% total fat, ten times using tap water. All reactors were seeded with an active anaerobic culture from a laboratory-scale batch reactor using the same synthetic wastewater. The batch reactor itself was seeded with an anaerobic culture from fresh cow dung, ruminal fluid as well as anaerobically digested sludge from the sewage treatment plant at Okhla, New Delhi, India. The volume of the reactors was maintained at 1500 ml during the operation. The initial hydraulic retention time (HRT) was set at 5 d with an organic loading rate (OLR) of 2.22 kg COD m^{-3} d^{-1} for each reactor during start-up. Effects of HRT were studied by decreasing it gradually from 5 d till acidification occurred. An individual effect of OLR on the performance of the reactors was observed by varying the influent COD concentration along with an increase in carbon source concentration and other compositions as well at a fixed HRT of 2.5 d. Steady state conditions were achieved in each new OLR before any further increase in OLR. Steady state condition was defined as condition prevailing in the reactor at which constant COD reduction of the outlet liquid and biogas production occurred at a fixed OLR and HRT. Samples of the reactor effluent and biogas were routinely taken to determine the concentrations of glucose, volatile fatty acids (VFA), COD and methane.

2.2. Analytical methods

Gas composition was analyzed using a gas chromatograph (AIMIL-NUCON, India, Series 5700). A Porapack-Q column (6 ft) and a thermal conductivity detector (TCD) 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 (dichromate closed reflux method) and sludge volume index (SVI) were estimated as suggested by Standard Methods (APHA, 1995).

All statistical analyses were done using SPSS software by one way analysis of variance (ANOVA) to determine the significance of carbon source effect, HRT and OLR on measured parameters at 1% level.

2.3. Sampling and DNA extraction

Samples were collected at steady state at each HRT and OLR level or during transient phase while system deteriorated. DNA was extracted in triplicates, using the FastDNA SPIN kit (for soil) (MP Biomedicals, Cambridge, United Kingdom) according to the manufacturer's instructions and quantified with a NanoVue™ spectrophotometer (GE Healthcare, USA).

2.4. Polymerase chain reaction

For generation of DGGE profiles, archaeal partial 16S rRNA genes were amplified from extracted genomic DNA using the 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% (wt/v) agarose gels stained with ethidium bromide using a 100-bp DNA ladder (MBI Fermentas, Vilnius, Lithuania).

2.5. Denaturing gradient gel electrophoresis (DGGE)

DGGE was performed with the Bio-Rad DCode™ system (Bio-Rad, Hercules, CA, USA). Archaeal PCR amplicons were loaded on 0.75 mm thick 8% (w/v) polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels with a 30–60% linear gradient (100% denaturant is 7 M urea and 40% (v/v) deionized formamide). Archaeal gels were run at 60 °C and 60 V for 12 h in @@1 × TAE buffer (40 mM tris-acetate, 1 mM Na-EDTA, pH 8.0). Gels were stained in 1× TAE buffer containing SYBR Gold (Sigma-Aldrich, Inc., USA; 1:10000 diluted) and images were taken using a Gel Doc imaging system (Bio-Rad, Hercules, CA, USA).

2.6. Analysis of DGGE pattern

Gel images were analyzed using the GelCompar II software version 6.5 (Applied Biomath, Sint-Martens-Latem, Belgium). Similarities in banding patterns were calculated as Dice coefficient (unweighted data based on presence or absence of bands). Community relatedness dendrogram of hierarchical clustering with the observed similarity values between profiles at different conditions were performed. Linkage type applied was the unweighted pair group method with arithmetic mean (UPGMA).

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