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Effect of a high strength chemical industry wastewater on microbial community dynamics and mesophilic methane generation

Harish Venkatakrishnan[1,2], Youming Tan[2,3,*], Maszenan bin Abdul Majid[4], Santosh Pathak[2], Antonius Yudi Sendjaja[2], Dongzhe Li[1], Jerry Jian Lin Liu[5], Yan Zhou[2], Wun Jern Ng[1,2,4,*]

1. School of Civil and Environmental Engineering, Nanyang Technological University, 639798, Singapore. E-mail: harish2@e.ntu.edu.sg

2. Advanced Environmental Biotechnology Center, Nanyang Environment and Water Research Institute, Nanyang Technological University, 637141, Singapore

3. School of Public Health, Shanghai Jiaotong University, Shanghai 200025, China

4. Nanyang Environment and Water Research Institute, Nanyang Technological University, 637141, Singapore

5. Sembcorp Industries Ltd., 627876, Singapore

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ABSTRACT

A high strength chemical industry wastewater was assessed for its impact on anaerobic microbial community dynamics and consequently mesophilic methane generation. Cumulative methane production was 251 mL/g total chemical oxygen demand removed at standard temperature and pressure at the end of 30 days experimental period with a highest recorded methane percentage of 80.6% of total biogas volume. Volatile fatty acids (VFAs) analysis revealed that acetic acid was the major intermediate VFAs produced with propionic acid accumulating over the experimental period. Quantitative analysis of microbial communities in the test and control groups with quantitative real time polymerase chain reaction highlighted that in the test group, Eubacteria (96.3%) was dominant in comparison with methanogens (3.7%). The latter were dominated by Methanomicrobiales and Methanobacteriales while Methanosarcinaceae in test groups increased over the experimental period, reaching a maximum on day 30. Denaturing gradient gel electrophoresis profile was performed, targeting the 16S rRNA gene of Eubacteria and Archaea, with the DNA samples extracted at 3 different time points from the test groups. A phylogenetic tree was constructed for the sequences using the neighborhood joining method. The analysis revealed that the presence of organisms resembling Syntrophomonadaceae could have contributed to increased production of acetic and propionic acid intermediates while decrease of organisms resembling *Pelotomaculum* sp. could have most likely contributed to accumulation of propionic acid. This study suggested that the degradation of organic components within the high strength industrial wastewater is closely linked with the activity of certain niche microbial communities within eubacteria and methanogens.

Introduction

Anaerobic degradation has been used as a high strength organic waste and wastewater treatment process for several decades (Ahring, 2003). The degradation process is complex and includes sub-processes like hydrolysis, acidogenesis and methanogenesis, each of which is delicately balanced with each other for optimum substrate degradation. A consortium of Eubacteria converts organic carbon into acetic acid and/or carbon dioxide, which is then further converted to methane by a group of specialized microbes, the methanogens (Ueno et al., 2001). The con-

* Corresponding authors. E-mail: youmingtan@ntu.edu.sg (Youming Tan); wjng@ntu.edu.sg (Wun Jern Ng)

version of organics to methane under anaerobic conditions is reliant on the actions of microbial communities on the substrate. Therefore, it is useful to study the microbial flora and its members which are involved not only in order to improve the overall anaerobic process but also to track process changes which may lead to failure of system (Fernández et al., 1999).

Anaerobic degradation has widespread applications in today's pursuit of renewable energy sources. But before determining its effective potential, it would be better served if a key element of the process, i.e., the ultimate biogas generation potential of a given substrate can be better understood. In the past and up until a few years ago, research was focused more on solving the issue of determining the biogas generation potential. The biochemical methane potential (BMP) test does indeed provide a sufficient base for determining the potential of a particular substrate to generate methane. Past research on the BMP test had often focused on either optimizing the substrate to inoculums ratio (Fernandez et al., 2001; Neves et al., 2004; Raposo et al., 2006) or had used specific substrates (Raposo et al., 2006). Some research has also been dedicated to understanding the various factors which may affect the degradation process like temperature, pH and particle size of substrate (Pabon-Pereira et al., 2012). However, substantial research has not been reported on understanding the microbial community dynamics during the process of a BMP test.

Microbial community shifts occur over a period of time during the anaerobic degradation process with niche members possibly growing to dominate over the other members. However, tracking these changes may be difficult with the standard practices of culturing organisms. This may arise from the niche organism's inability to propagate ex-situ and hence leading to false results. Recent developments in molecular techniques targeting the 16S rRNA gene can possibly provide greater insight into such anaerobic community shifts in response to different process settings (Lee et al., 2009). Monitoring microbial communities in an anaerobic degradation process can possibly provide information for process optimization and system configuration. The quantitative real time polymerase chain reaction or q-PCR test allows for targeting microorganisms in an anaerobic degradation process and so can facilitate tracking of community dynamics and shifts as the process undergoes change (Yu et al., 2005). The changes in microbial community structure can be monitored using denaturing gradient gel electrophoresis (DGGE) in combination with investigation of formation and degradation of certain reaction products. The DGGE technique has proven effective in detecting microbial community shifts and also identifying the phylogenetic affiliates of microbial populations in mixed culture systems (Ueno et al., 2001; Calli et al., 2005). Lee et al. (2008) also elucidated that DGGE and qPCR are ideal techniques to study microbial transitions in batch systems operating with mixed microbial cultures. The phylogenetic analysis would help in the in-depth better understanding of how the communities at the species or strain level react to the presence of a particular substrate. This would prove especially helpful in the study of acidogens, whose community dynamics are relatively unknown.

This article describes a study which used the BMP test coupled with microbial community analysis, to investigate anaerobic degradation process challenged with a high strength chemical industry wastewater at mesophilic (35°C) temperature. Process performance was investigated in terms of chemical oxygen demand (COD) reduction, biogas production, and microbial community responses. Duration of the BMP test in this study was 30 days. Community shifts of individual methanogen families were monitored with the q-PCR technique and DGGE combined with phylogenetic analysis provided the community structure within the systems. The research also provided an insight on impact of wastewater on methane yields with changes in community structure.

1 Materials and methods

1.1 Wastewater characteristics

The chemical industry wastewater was initially profiled in terms of parameters shown in **Table 1**. The wastewater was defined as high strength in terms of its high total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) value.

1.2 Seed biomass

Anaerobic sludge was collected from a mesophilic anaerobic digester at a local municipal sewage treatment plant treating primary and secondary sludges. This seed sludge, identified as AnSL, was profiled as shown in **Table 2**.

Table 1 Chemical industry wastewater properties

Parameter	Value
Total chemical oxygen demand (TCOD)	(343.12 ± 3.56) g/L
Soluble chemical oxygen demand (SCOD)	(294.35 ± 2.78) g/L
Volatile fatty acids (VFA's)	(3.6 ± 0.02) g/L
Acetic acid	0.83 g/L
Valeric acid	1.23 g/L
Other organic components present	Glutarate, adipate, and succinate
pH	9.23
Total dissolved solids (TDS)	(9914 ± 46) mg/L
Sodium	44.1 g/L
Colour (visual)	Deep red