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Study of microbial community and biodegradation efficiency for single- and two-phase anaerobic co-digestion of brown water and food waste

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HIGHLIGHTS

- First study on microorganisms involved in brown water and food waste degradation.
- Clear differences in bacterial communities between single- and two-phase CSTRs.
- *Methanosaeta* was the main contributor for methane production in both CSTRs.
- *Firmicutes* played an important role in solids reduction.

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ABSTRACT

The objective of this work was to study the microbial community and reactor performance for the anaerobic co-digestion of brown water and food waste in single- and two-phase continuously stirred tank reactors (CSTRs). Bacterial and archaeal communities were analyzed after 150 days of reactor operation. As compared to single-phase CSTR, methane production in two-phase CSTR was found to be 23% higher. This was likely due to greater extent of solubilization and acidification observed in the latter. These findings could be attributed to the predominance of *Firmicutes* and greater bacterial diversity in two-phase CSTR, and the lack of *Firmicutes* in single-phase CSTR. *Methanosaeta* was predominant in both CSTRs and this correlated to low levels of acetate in their effluent. Insights gained from this study would enhance the understanding of microorganisms involved in co-digestion of brown water and food waste as well as the complex biochemical interactions promoting digester stability and performance.

1. Introduction

Anaerobic digestion is a biochemical process that degrades biomass biologically and produces biogas consisting mainly of methane, which is a valuable source of renewable energy. Food waste is a suitable substrate for anaerobic digestion due to its high organic content. On the other hand, landfilling of food waste leads to uncontrolled emission of methane, and incineration could be inefficient due to the low calorific value of wet food waste (Bernstad and Jansen, 2012). In comparison with landfilling or incineration, the anaerobic digestion of food waste was found to be a more suitable and sustainable treatment method to address the growing concern over large amounts of food waste generated worldwide. Therefore, the treatment of food waste by anaerobic digestion process has attracted increasing attention in recent years (Wang et al., 2002; Ike et al., 2010).

The addition of co-substrate (e.g. brown water) to food waste could improve the anaerobic digestion process stability by providing additional nutrients and maintaining buffer capacity. The benefits of co-digesting brown water and food waste was described by Rajagopal et al. (2013). The authors observed higher biogas production and biodegradation efficiencies when brown water was added as a co-substrate to the anaerobic degradation of food waste. Production of methane via anaerobic digestion of organic pollutants not only provides a cheaper and greener alternative to food waste and brown water disposal, it also replaces fossil fuel-derived energy and reduces the impact of global warming (Abbasi et al., 2012).

Anaerobic digestion of organic matter is carried out syntrophically by microbial communities consisting of both bacterial and archaeal species. The degradation may be divided into three steps.

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During the first step, hydrolysis bacteria degrade polymeric organic matter into monomers, such as sugar and amino acid, which are further degraded in the second step by acetogenic bacteria into volatile fatty acids (VFAs), such as acetate. In the last step, methanogens produce biogas mainly from formate, hydrogen and acetate.

In conventional applications, anaerobic digestion processes usually occur in a single reactor system. However, acid- and methane-forming microorganisms have very different nutritional needs. When kept together in a single reactor system, some of such systems gradually gave rise to reactor instability problems (Demirel and Yenigun, 2002). The physical separation of acid- and methane-forming microorganisms in different reactors was first proposed by Poland and Ghosh (1971). Such systems provided optimum environmental conditions for each group of organisms and thus led to enhanced stability and control of the overall process.

Studies on bacterial and methanogenic archaeal community structures in anaerobic digesters treating food waste have been reported recently (Ike et al., 2010; Wang et al., 2010). However, the understanding of microbial aspects for co-digestion of brown water and food waste is still limited due to the lack of references on this topic. Comprehension of microbial community and its function is necessary to improve the efficiency and process stability of anaerobic digesters. 16S rRNA cloning and sequencing is the well known method used to characterize microbial community in an anaerobic reactor while fluorescent *in situ* hybridization (FISH) is a useful method to verify cloning findings and to visualize the different cells in anaerobic sludge. Therefore, these two methods were employed in the current study to determine the microbial populations. The objective of this work was to study the microbial community and reactor performance for the anaerobic co-digestion of brown water and food waste in single- and two-phase continuously stirred tank reactors (CSTRs). Insights gained from this study would enhance the understanding of microorganisms involved in the anaerobic co-digestion of brown water and food waste as well as the complex biochemical interactions that promote digester stability and performance. These could aid the selection of seeding sludge for rapid startup in future applications.

2. Methods

2.1. Feedstock and reactor operation

Food waste was collected from canteens on campus while brown water was collected from a specially designed source-separation toilet, where urine with 0.3 L flush water (as yellow water) and faeces with 2 L flush water (as brown water) were collected in separate tanks. The feed for this study consisted of a mixture of 300 g blended food waste and 2 L brown water, and had an average pH of 6.23 ± 0.07. The characteristics of the feed are as shown in Table 1. Anaerobic co-digestion of brown water and food waste was performed in laboratory scale (5 L) single- and two-phase CSTRs. The co-substrates were prepared daily and fed to the reactors, which included the acidogenic (R_A) and methanogenic (R_M) reactors of the two-phase CSTR system and the single-phase CSTR (R_S), in batch mode. The working volumes of R_A, R_M and R_S were 1.2 L, 4.1 L and 5.3 L, respectively, and the contents were mixed continuously (mixing time: 5 min ON followed by 5 min OFF) at 80 rpm by an overhead mechanical stirrer as reported previously by Rajagopal et al. (2013). R_A, R_M and R_S were initially inoculated with mesophilic anaerobic sludge collected from a local wastewater treatment plant (Ulu Pandan Water Reclamation Plant, Singapore). Reactor contents were gradually replaced by the brown water and food waste mixture. By the time this study started, anaerobic sludge was completely replaced by the brown water and food waste mixture. The single and two-phase CSTR systems were operated in parallel for 150 days at 35 °C with hydraulic retention time (HRT) as shown in Table 1. HRT was reduced by adding increased volumes of the brown water and food waste mixture into the reactors of fixed working volumes. The organic loading rate (OLR) was maintained at around 0.5–0.8 g-VS L^{-1} d^{-1} in this study. Both the single- and two-phase CSTRs were operated in the same way and had the same overall reactor working volume of 5.3 L. Both R_A and R_S were fed with brown water and food waste mixtures prepared daily while R_M was fed with the acidified effluent from R_A during the study. The reactor performances for R_A, R_M, and R_S were monitored weekly.

2.2. Chemical analysis

Biogas production was measured daily using a mass flow meter (McMillan Company, Model 50D-3E), while other parameters such as pH, total (TS) and volatile (VS) solids, total and soluble chemical oxygen demand (COD), VFAs and biogas composition were measured weekly. The biogas composition (i.e., methane, carbon dioxide, nitrogen and hydrogen contents) was analyzed by gas chromatograph (Agilent Technologies 7890A, USA) equipped with a thermal conductivity detector (TCD). pH value was measured using a compact titrator (Mettler Toledo) equipped with a pH probe (Mettler Toledo DGi 115-SC). TS and VS were analyzed according to the Standard Methods (APHA, 1998). Total and soluble COD were measured using COD digestion vials (Hach Chemical) and a spectrophotometer (DR/2800, Hach). Soluble COD was measured using the supernatant of samples after centrifugation (KUBOTA 3700, Japan) at 12,000 rpm for 10 min. The determination of VFAs was carried out using a gas chromatograph (Agilent Technologies 7890A, USA), equipped with a flame ionization detector (FID) and a DB-FFAP (Agilent Technologies, USA) column (30 m × 0.32 mm × 0.50 μm) and the samples were filtered through 0.45 μm cellulose acetate membrane filters (membrane solutions).

2.3. DNA extraction and construction of 16S rRNA gene clone libraries

Sludge samples were collected on day 150 and genomic DNA was extracted from sludge using chemical lysis and phenol–chloroform–isoamyl alcohol (25:24:1, v:v:v) purification protocol as described previously (Liu et al., 1997). Primer sets 530F (5′-GTGCCAGC(A/C)GCCGCGG-3′) and 1490R (5′-GGTTACCTTGTTACGACTT-3′) as well as Ar1F (5′-TCYGKTTGATCCYGSCRGAG-3′) and 1490R were used to amplify 16S rRNA gene from the total-community DNA, targeting total prokaryotes and Archaea, respectively. The thermal program used for amplification of 16S rRNA gene was as follows: hotstart 94 °C for 3 min, 30 cycles of denaturation (30 s at 94 °C), annealing (30 s at 54 °C) and extension (45 s at 72 °C) and a final extension at 72 °C for 5 min. TOPO TA cloning kit (Invitrogen, CA) was used for clone library construction according to the manufacturer's instructions. Approximately 100 and 50 clones were randomly selected from R_A, R_M and R_S for the members in the domain Bacteria (amplified by primer set 530F and 1490R), and Archaea (amplified by primer set Ar1F and 1490R), respectively. The amplified DNA insert was then PCR amplified with a vector-specific primer set (i.e., M13F and M13R). Restriction fragment length polymorphism (RFLP) was used to screen the 16S rRNA gene fragments to further remove the possible redundant clones. The M13-PCR products were separately digested to completion with tetramer restriction enzymes *Msp*I and *Rsa*I (New England BioLabs, UK), and separated by electrophoresis in a 3% agarose gel. Gels were visualized using the FireReader gel documentation (UVItec, Cambridge, UK) after staining with Gelred