Bioresource Technology 171 (2014) 132–138

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste

Jun Wei Lim [a,b,1], Jun An Chiam [b,2], Jing-Yuan Wang [a,b,*]

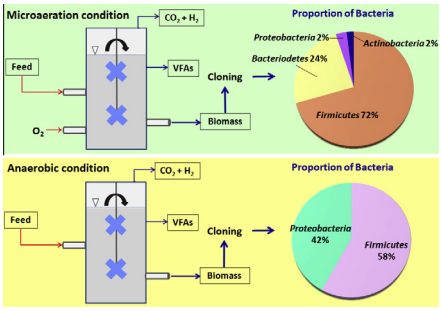
[a] Residues and Resource Reclamation Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, #06-08, Singapore 637141, Singapore

[b] Division of Environmental and Water Resources, School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

## HIGHLIGHTS

- Microaeration gave rise to a significantly more diverse bacterial population.
- Higher proportion of clones affiliated to *Firmicutes* in microaeration reactor.
- Microaeration led to a shift in fermentation production pattern.
- Microaeration enhanced fermentation during co-digestion of BW and FW.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 17 June 2014
Received in revised form 6 August 2014
Accepted 9 August 2014
Available online 19 August 2014

*Keywords:*
Microaeration
Bacterial community structure
Brown water
Food waste

## ABSTRACT

The purpose of this study was to investigate the impact of microaeration on the fermentation process during anaerobic co-digestion of brown water (BW) and food waste (FW). This was achieved by daily monitoring of reactor performance and the determination of its bacterial consortium towards the end of the study. Molecular cloning and sequencing results revealed that bacteria within phyla *Firmicutes* and *Bacteriodetes* represented the dominant phylogenetic group. As compared to anaerobic conditions, the fermentation of BW and FW under microaeration conditions gave rise to a significantly more diverse bacterial population and higher proportion of bacterial clones affiliated to the phylum *Firmicutes*. The acidogenic reactor was therefore able to metabolize a greater variety of substrates leading to higher hydrolysis rates as compared to the anaerobic reactor. Other than enhanced fermentation, microaeration also led to a shift in fermentation production pattern where acetic acid was metabolized for the synthesis of butyric acid.



## 1. Introduction

Anaerobic digestion (AD) refers to the fermentation process that produces biogas (composed of mainly methane and carbon dioxide) from the degradation of organic material. Due to the production of useful energy in the form of biogas, AD has been widely applied for the treatment of organic waste such as brown water

* Corresponding author at: Residues and Resource Reclamation Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, #06-08, Singapore 637141, Singapore. Tel.: +65 67904102/67927319; fax: +65 67927319.

*E-mail addresses:* jwlim3@e.ntu.edu.sg (J.W. Lim), jachiam1@e.ntu.edu.sg (J.A. Chiam), jywang@ntu.edu.sg (J.-Y. Wang).

[1] Tel.: +65 67904102/67927319.

[2] Tel.: +65-67927319; fax: +65-67927319.

http://dx.doi.org/10.1016/j.biortech.2014.08.050
0960-8524/

(BW) and food waste (FW) (Rajagopal et al., 2013; Curry and Pillay, 2012; Zeeman et al., 2008). AD is a complex degradation pathway that proceeds in four successive stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis. Acid-forming bacteria carry out hydrolysis, acidogenesis and acetogenesis, while methane-forming archaea produce biogas during methanogenesis. Due to the different nutritional needs of the microorganisms involved in each stage, the physical separation of acid- and methane-forming microorganisms in two separate reactors was first proposed by Pohland and Ghosh (1971) to provide optimum environmental conditions for each group of microorganisms. Recent studies have indeed shown that two-phase systems could lead to enhanced stability and control of the overall AD process (Lim et al., 2013; Demirel and Yenigun, 2002).

Though the AD technology existed for more than 100 years, there are still unresolved challenges faced by AD operators, thus limiting the implementation of more AD plants. In view of current global concerns over environmental sustainability, AD is regarded as a promising process due to its potential in renewable energy generation and waste stabilization. However, one of the main problems faced by AD operators is its inherent instability since AD is alarmingly sensitive to changes in operation and feed conditions. Accidental or unavoidable oxygen loading is one aspect of this problem. Theoretically, the AD process will be inhibited when exposed to oxygen. However, several studies have shown that partial aeration did not cause any inhibition, but enhanced AD performance instead (Botheju and Bakke, 2011). According to Botheju and Bakke (2011), the presence of oxygen led to a higher yield and population of facultative acidogens and therefore higher amount of enzymes excreted. It was hypothesized that more acidogenic biomass leads to more hydrolysis, since hydrolysis is carried out by the extracellular enzymes excreted by acidogens.

The term microaeration refers to the controlled introduction of small amounts of oxygen into an anaerobic biochemical process to enable both anaerobic and aerobic biological activities to occur within a single bioreactor. The term microaeration will be used in this study to describe the conditions of adding oxygen to the acidogenic reactor. The study by Rolfe et al. (1978) was among the first few that investigated the oxygen tolerance level of anaerobic bacteria. Zitomer and Shrout (1998) subsequently reported that oxygen addition did not inhibit the growth of methanogens, but increased their initial activity. More recently, several studies reported advantages of microaeration in terms of higher degree of solubilization and acidification of organic matter (Xu et al., 2014a,b; Lim and Wang, 2013; Díaz et al., 2011a; Jagadabhi et al., 2010). Therefore, microaeration has been regarded as a potential pre-treatment method for improving the hydrolysis stage during the AD process. Another reported benefit of microaeration was the cleaning of biogas by removing more than 99% hydrogen sulfide ($H_2S$) (Díaz et al., 2011b; Tang et al., 2004). As compared to the other chemical and physical pre-treatment methods or biological processes for the desulphurization of biogas, microaeration of AD system has a relatively smaller footprint and require lower investment costs as well as small modification to the existing process (Ramos et al., 2014).

An earlier study on the anaerobic co-digestion of BW and FW (Lim et al., 2013) reported the unexpected predominance of aerobic bacteria species – *Acetobacter peroxydans* in the acidogenic reactor of a two-phase continuously stirred tank reactor (CSTR). As the acidogenic reactor was operated under anaerobic conditions, the predominance of an aerobic bacteria species suggested the reactor might be unknowingly exposed to partial aeration. Despite the predominance of *A. peroxydans*, the AD system achieved high degrees of COD solubilization and VFA production. This study illustrated that in case of accidental or unavoidable oxygen loading, the fermentation process of AD was not compromised.

In view of the tendency for accidental or unavoidable oxygen loading, as well as the benefit of microaeration in terms of enhanced fermentation and desulphurization of biogas, it is important to understand how AD systems respond to small amounts of oxygen stress. Determining the microbial diversity of reactors will provide more insights on the changes in the biochemical processes due to microaeration conditions. However, information on the microbiology of anaerobic digesters operated under microaeration conditions is limited (Ramos et al., 2014; Zhou et al., 2007; Tang et al., 2004).

Tang et al. (2004) reported that microaeration led to a decrease in *Methanosarcina* and increase in *Methanoculleus* populations while Zhou et al. (2007) found that limited aeration caused the predominant microorganisms to change from rod-shape to cocci-shaped methanogens in the UASB reactor. The DGGE analysis carried out by Ramos et al. (2014) showed that oxygen affected the richness, evenness and structure of the bacterial and the archaeal communities in the long term. These studies mainly discussed the effect of microaeration on the archaeal populations and very little is known about the bacterial community shifts due to oxygen. Since one of the main benefits of microaeration was reported to be enhanced hydrolysis, it is essential to have a more detailed understanding of how microaeration affects the bacterial population. Therefore, the objective of this study was to investigate how microaeration affected the fermentation process in the anaerobic co-digestion of BW and FW. This aim was achieved by determining the bacterial community structure of the acidogenic reactor of a two-phase CSTR and correlating the microbial structure to the reactor's performance.

## 2. Methods

### 2.1. Experimental set-up

The feed for this study consisted of a mixture of 56.25 g blended food waste (FW) and 0.75 L brown water (BW) with an average pH of 5.96 ± 0.22. The FW/BW mixture was prepared and fed daily to the acidogenic reactor of a two-phase CSTR system, in batch mode. The working volume of the reactor was 3 L and the contents were mixed continuously at 80 rpm by an overhead mechanical stirrer. The reactor was initially inoculated with sludge collected from another acidogenic reactor treating BW and FW at 35 °C for more than one year. With hydraulic retention time (HRT) of 4 days, the organic loading rate (OLR) for the acidogenic reactor was maintained at 5.15 ± 0.44 g-VS/L/d in this study. As shown in Table 1, the study consisted of three operating conditions. The reactor was operated under anaerobic conditions (AN) from week 1 to 6, low microaeration conditions (MA1) from week 7 to 13, and higher microaeration conditions (MA2) from week 14 to 20. Oxygen was added to the liquid part of the reactor at a rate of 3 mL/min daily one to two hours after feeding.

### 2.2. Chemical analysis

The reactor performance was monitored daily for pH, oxidation reduction potential (ORP) and biogas while total solids (TS), volatile solids (VS), chemical oxygen demand (COD), volatile fatty acid (VFA) and ammonia ($NH_3$-N) were monitored weekly. All analysis were carried out in duplicates.

pH value was measured using a compact titrator (Mettler Toledo) equipped with a pH probe (Mettler Toledo DGi 115-SC). ORP was determined using a platinum ORP combination electrode (Fisher Scientific Accumet® $Ca^+$ Model 13-620-81). Biogas was collected and stored in a Tedlar gas sampling bag (Sigma–Aldrich, USA) and its volume was monitored daily using a rotary displacement