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Performance and microbial community analysis in alkaline two-stage enhanced anaerobic sludge digestion system

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ABSTRACT

This study investigated an alkaline two-stage enhanced anaerobic sludge digestion system, which simultaneously combined biological and chemical mode of degradation. The alkaline enhanced mesophilic stage-1 was operated at pH 8 with 3 days hydraulic retention time (HRT), while the mesophilic stage-2 was without pH adjustment at 17 days HRT. The system achieved higher chemical oxygen demand (COD) removal, volatile solids (VS) reduction, and methane yield than the conventional 20 days HRT single-stage system. Further enhancement was obtained by moving the stage-1 from 35 to 55 °C, but it did not yield better energy balance with the 3 + 17 days HRT configuration implemented in this study. 454 pyrosequencing revealed the acclimation of specialized communities in the alkaline two-stage system. *Methanosarcina*, *Methanobrevibacter* and *Methanothermobacter* could survive at pH 8 in the alkaline enhanced stage-1 and contributed to regulating the potentially inhibitory volatile fatty acids (VFA) or hydrogen levels under the enhanced sludge solubilization and acidogenesis condition. Various fermentative populations, distinct to those in the single-stage system, were also enriched in the stage-1s. These populations could grow at pH 8, were transferred into the stage-2, and ensured continuity of the biochemical reactions under mild alkaline condition, leading to the enhanced sludge digestion process.

1. Introduction

Anaerobic digestion (AD) is a widely used method for the treatment of sewage sludge, being able to reduce the volatile solids (VS) content and generate methane, a source of renewable energy. The process involves solubilization or hydrolysis of the particulate organic substrate, acidogenesis, acetogenesis and methanogenesis. These are performed by a wide range of microorganisms [1,2]. Hydrolysis has been known to be the rate-limiting step in anaerobic sludge digestion process and various strategies had been introduced to improve the hydrolytic rate. However, cost associated with energy needs have led to the reconsideration of pretreatment methods with such needs (e.g., thermal and ultrasound pretreatment), and to consider less costly microbial manipulation options (e.g., two-stage AD configuration) [3]. A recent review had found the two-stage anaerobic system to have good degradation efficiency compared to other methods [1].

The first stage of a two-stage anaerobic system is typically operated in acidic or near neutral condition [4,5], but process enhancement by operating the stage-1 in mild alkaline condition can be applied to enhance the hydrolysis step. Higher pH had been demonstrated to give better sludge hydrolysis compared to neutral or acidic conditions [6]. Its mechanism is one of protein denaturation, disrupting the extracellular polymeric substances (EPS) matrix and transferring the organic components (protein and carbohydrate) from the pellet and tightly-bound EPS fractions into the loosely-bound EPS and soluble fractions, thus making these organics more accessible to microorganisms [7]. Microbial activity is, however, reduced when sludge hydrolysis is conducted at pH

Abbreviation: AD, anaerobic digestion; COD, chemical oxygen demand; CSTR, continuous stirred tank reactor; DNA, deoxyribonucleic acid; EPS, extracellular polymeric substances; HRT, hydraulic retention time; OLR, organic loading rate; OTU, operational taxonomic unit; PCR, polymerase chain reaction; rRNA, ribosomal ribonucleic acid; SRT, solid retention time; TPAD, temperature phased anaerobic digestion; TSS, total suspended solids; TVFA, total volatile fatty acids; VFA, volatile fatty acids; VSS, volatile suspended solids.

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9 and above, indicating the mechanism is indeed more abiotic than biotic [7–9]. Because of this impact on bioprocesses, pH correction after alkaline treatment at pH 9 and above is necessary prior to AD and this would result in additional chemical cost [1,10]. High molecular weight organics were also released into the soluble fractions after treatment at pH 9 and above, which were associated with poor biodegradability [9]. Therefore, this study built on previous research [9], which found the optimal stage-1 operation to be pH 8 with 35 °C and 3 days HRT to enhance sludge hydrolysis and acidogenesis. This method leveraged chemically mediated hydrolysis by alkaline dosage with the cultivation of biologically active microbial communities tolerant to mild alkaline condition. A novel alkaline two-stage enhanced anaerobic sludge digestion system was then conceptualized and tested in this study. The stage-1 would be operated at 3 days hydraulic retention time (HRT) [11], followed by the stage-2 operated with a longer HRT. The extended HRT in the stage-2 was necessary to cultivate the slower-growing methanogens. Additionally, temperature of 55 °C has been found to be optimum in the first reactor of a two-stage system when treating a mixture of primary and secondary sewage sludges [12]. Both mesophilic and thermophilic conditions would be tested on the alkaline enhanced stage-1 to compare performance under these two conditions.

Microbial communities underpin the success of AD process and these communities involve many microorganisms conducting the various biochemical functions, i.e., hydrolysis, acidogenesis, acetogenesis and methanogenesis [13]. The microbial ecology in AD processes has still not been adequately elucidated due to the complexity of these biochemical interactions by various different species, in which, many still remained unclassified and their functions unknown. Investigation in the AD's microbial ecology is required to better understand the functions of the various species, the interactions between species, and how these are influenced by the operating or environmental conditions of the process. The alkaline enhanced two-stage anaerobic system as described in this study would obviously exert its set of operating conditions, which had not been evaluated previously. Utilization of molecular techniques have helped to advance the microbiological understanding of AD process and revealed that reactor performance is linked to the microbial communities established [3,14–16]. A better understanding on the dynamics of microbial communities in AD process could be applied to improve the system performance or even to design predictive tools for process monitoring. Recently, next generation sequencing (NGS) method such as 454 pyrosequencing has been used to sequence higher numbers of reads than other cloning-related technique. As a result, the analytical confidence level becomes higher and a more detailed representation of the microbial community structure can be obtained [3,17].

The objectives of this study were to: (1) evaluate the extent of sludge solubilization and acidification achieved in the alkaline enhanced stage-1 under mesophilic and thermophilic conditions, (2) determine if the overall sludge digestion efficiency could be enhanced in the alkaline two-stage system with the stage-1 under mesophilic (35 °C) and thermophilic (55 °C) conditions compared to the conventional single-stage AD system, and (3) characterize the microbial communities in the alkaline two-stage systems by 454 pyrosequencing and correlate microbial community shifts in relation to reactor performance.

2. Materials and methods

2.1. Bioreactor operation

Two continuous stirred tank reactor (CSTR) systems were operated in parallel. The single-stage AD system had a working volume of 50 L, while the alkaline two-stage AD system comprised a 7.5 L stage-1 and 42.5 L stage-2 reactor. Both systems were operated at 20 days overall HRT, with the stage-1 at 3 days HRT and the stage-2 at 17 days HRT for the alkaline two-stage system. Since the system was operated in complete-mix mode, HRT was equal to solids retention time (SRT). pH was controlled automatically with 5 M sodium hydroxide and hydrochloric acid as required. The single-stage system was maintained at 35 °C, without pH control and remained between pH 6.9 and 7.1 throughout the study. The operational pH and temperature of the alkaline two-stage system were altered according to the operational periods described in Table 1.

Table 1
The operational parameters of the alkaline two-stage system.

Reactor operational period	Stage-1 reactor		Stage-2 reactor	
	pH	Temperature	pH	Temperature
Period 1 (0–89 days)	8	35 °C	6.8–7.6 (without control)	35 °C
Period 2 (90–165 days)	8	55 °C	7.6–7.8 (without control)	35 °C
Period 3 (166–252 days)	8	55 °C	6.9–7.1	35 °C

All reactors were seeded with anaerobic sludge collected from an anaerobic digester at a municipal water reclamation plant in Singapore. The feed sludge was a mixture of primary and secondary sludges from the same plant, collected weekly, and stored at 4 °C before use. The characteristics of the feed sludge were 32,300 ± 3400 mg total chemical oxygen demand (COD) L^{-1}; 1800 ± 600 mg soluble COD L^{-1}; 25.3 ± 1.8 g total solids (TS) L^{-1}; 20.2 ± 1.3 g volatile solids (VS) L^{-1}; and pH 5.7 to 6.1.

2.2. Chemical analysis

Fifty millilitre samples were collected twice weekly from each reactor's sampling valve. COD, TS and VS were measured in accordance with standard methods [18]. Soluble fraction of a sludge sample was prepared by centrifugation at 12,000 × *g* for 5 min and filtering the supernatant through a 0.45 μm nylon filter. Dissolved total ammonia nitrogen from the soluble fraction was measured with the Nessler method kit (Hach, USA). Concentrations of various volatile fatty acids (VFA) and biogas compositions were measured using gas chromatography (GC) (7890A, Agilent, USA) as described by Maspolim et al. [19]. Daily biogas volume produced was measured with a thermal-based gas flowmeter (McMillan, USA) fitted on the bioreactor in period 1 and with a wet gas meter (Ritter, Germany) in period 2 and 3. Single-factor analysis of variance (ANOVA) and Tukey's test were calculated in statistical analysis of the COD removal, VS reduction and methane yield for the two reactor configurations and operating conditions.

The extent of solubilization was calculated following Ge et al. [12], and is expressed as:

$$\text{Extent of solubilization}\% = \frac{\text{COD}_{CH_4} + \text{COD}_{So} - \text{COD}_{Si}}{\text{COD}_{Ti} - \text{COD}_{Si}} \times 100\% \quad (1)$$

where COD_{CH_4} = methane produced in a day, as mg COD based on 0.38225 L CH_4 g COD^{-1} at 25 °C (temperature during biogas volume measurement); COD_{Si} = soluble COD concentration at inlet; COD_{So} = soluble COD concentration at outlet; COD_{Ti} = total COD concentration at inlet.The total COD and VS reductions were calculated based on mass balance, which is expressed as

$$\text{Reduction}\% = \frac{C_{in} - C_{out}}{C_{in}} \times 100\% \quad (2)$$