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## Three-stage anaerobic digester for food waste

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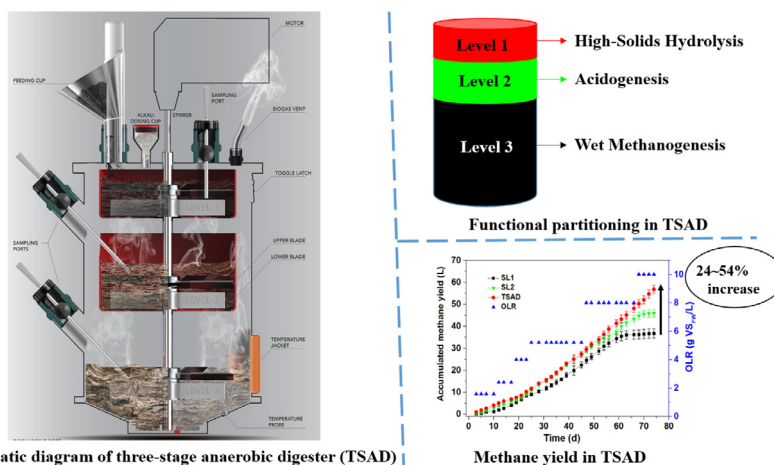
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### HIGHLIGHTS

- A compact three-stage anaerobic digester (TSAD) was developed for food waste treatment.
- TSAD combined the advantages of high solids AD and Wet AD.
- Methane yields in TSAD was increased by 24–54% compared to traditional anaerobic digesters.
- Functionalized partitioning in TSAD significantly improved hydrolysis/acidogenesis efficiency.
- TSAD has higher treatment capacity and solid reduction rate with less reactor volume requirement.

### GRAPHICAL ABSTRACT



Schematic diagram of three-stage anaerobic digester (TSAD)

Methane yield in TSAD

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### ABSTRACT

A novel compact three-stage anaerobic digester (TSAD) was developed for high-efficiency anaerobic digestion of food waste and methane production. Through structure optimization by having three separate chambers in a single-stage anaerobic digester, hydrolysis, acidogenesis and methanogenesis were independently optimized with concomitant improvement in anaerobic digestion performance. Compared to traditional one-stage and two-stage anaerobic digesters, TSAD had a 24–54% higher methane yield at a high organic loading rate of 10 g VS<sub>FW</sub>/L. A higher volatile solid reduction rate of 83.5 ± 1.3% was also achieved in TSAD. Even at high organic loading, TSAD still presented a high buffering ability when the one-stage and two-stage digesters had already soured and failed. Pyrosequencing analysis indicated that the bacterial community in TSAD is more diverse than the control digesters. Multi-function methanogens *Methanosarcina* and some dominant populations with the function of acetogenesis, amino-acid-utilization and symbiosis were found to selectively enrich in TSAD.

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## 1. Introduction

Research on sustainable and renewable bioenergy derived from organic solid wastes is increasingly important and popular because of increasing global population, depletion of natural energy resources, and the environmental burden brought on by the

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generation of organic solid wastes [1,2]. Among the many waste-to-bioenergy technologies being explored, anaerobic digestion (AD) is commonly used for organic waste treatment, renewable energy production and the recycling of nutrients [3,4]. Through AD, biodegradation of organic wastes generates biogas (55–65% CH<sub>4</sub>; 35–45% CO<sub>2</sub>) that can be converted to electricity and/or heat [5–7]. To a limited extent, AD bioenergy can be a good supplement to conventional fossil energy, as well as mitigate greenhouse gas emissions.

On the basis of total solids (TS) content, AD can be categorized into wet AD (TS < 15%) or high-solids AD (15% < TS < 40%) [8–10]. Wet AD is more widely applied in practice as the high moisture content promotes the growth of the microorganisms through enhancement of mass transfer between substrates and microorganisms [11,12]. However, wet AD suffers from the need for larger reactor volume, high consumption of water, as well as expensive post-treatment [9]. High-solids AD, on the other hand, is attracting more attention because of its reduced capital and operating costs, as well as its amenability to tackle a wide range of organic wastes [10,13]. Nevertheless, high-solids AD also suffers from some technical bottlenecks such as high inoculum demand, long retention time and accumulation of volatile fatty acids (VFAs) [14–17]. The specific objective of this research was to design a novel anaerobic digester combining the benefits of wet AD and high-solids AD to enhance organic waste processing and methane production.

Given that the optimum pH for hydrolysis and acidogenesis were 5.5 and 6.5 [9,18], respectively, and the suitable range of pH for methanogenesis was 6.5–8.2 [19], a novel three-stage anaerobic digester (TSAD) was developed and tested using food waste (FW) as feedstock. Structurally, the TSAD is separated into three vertical components i.e. high-solids hydrolysis, acidification and wet methanogenesis, respectively. Based on this, it was anticipated that the optimal condition of pH can be applied in each stage, thereby improving overall efficiency. In addition, vertical integration in the TSAD resulted in a small footprint, saving space and created favorable environments for the growth of the different functional microorganisms of hydrolyzing bacteria, fermenting bacteria and methanogenic archaea.

Hitherto, most studies focus specifically on either wet AD or high-solids AD [20–23], and little research has been conducted to design and operate a compact AD reactor that combines both wet AD and high-solids AD in one digester. In this study, the treatment performance of TSAD and the synergistic effect of microorganisms in the TSAD were investigated. Further to a proof-of-concept for the TSAD operation on food waste, an in-depth study of the microbial community structure using high-throughput 16S rDNA pyrosequencing was also conducted.

## 2. Materials and methods

### 2.1. Inocula and substrates

Seed sludge was collected from a large-scale anaerobic digester from the Ulu Pandan Water Reclamation Plant (UPWRP) in Singapore. The anaerobic digester at UPWRP currently treats waste activated sludge from the secondary wastewater treatment plant for domestic sewage wastes. In this study, each reactor was inoculated with this seed sludge at approximately 80% (v/v). The ratio of volatile suspended sludge (VS) to total suspended sludge was 0.65 with initial TS of 11.8 g/L.

FW was obtained from a canteen at the National University of Singapore. This comprised mainly rice, noodles, meat, vegetables, and condiments. After removing any bones and non-biodegradable waste like plastic bags and utensils, the FW was homogenized by a blender and then stored at –20 °C freezer to

prevent biological decomposition. Detailed characteristics of FW are listed in Table S1 (see Supplementary Material).

### 2.2. Reactor specifications and operation

Fig. 1 shows the schematic diagram of this TSAD system fabricated for this research. Chambers level 1, level 2 and level 3 correspond to high-solids hydrolysis stage, acidification stage and wet methane-production stage, respectively. Food waste was first fed into chamber level 1 for hydrolysis. After that, the hydrolyzed FW was transferred to chamber level 2 for acidogenesis. Finally, the hydrolyzed and acidified FW was dropped to chamber level 3 for methanogenesis. The baffle at the bottom of each chamber can be opened by a connecting rod from the outside of the digester. In this way, food waste was gravity transferred from one chamber to another. The optimized pH in each chamber was adjusted and controlled individually. To demonstrate the performance of the TSAD, baseline studies involving 1 L AD reactors were carried out to examine the individual stages of hydrolysis, acidogenesis and methanogenesis, before fabricating a 20 L steel TSAD for proof-of-concept. Essentially, three 1 L glass reactors (effective working volume of 0.8 L) was set up in parallel, to simulate a one-stage AD (SL1), a two-stage AD (SL2) and the TSAD. FW was adjusted to 20% TS through the addition of seeding sludge and then stored in the –20 °C freezer as feedstock for SL1. During the hydrolysis stage, SL1 was operated at 35 °C with a stirrer speed of 150 rpm. After 2 days of operation, the hydrolyzed FW was stored in the freezer at –20 °C to be used as feedstock for SL2. For SL2 operation, this hydrolyzed FW was mixed with seeding sludge to a TS of 10%. pH was controlled at 6.5 ± 0.2, to provide the optimal conditions for acidogenesis. After 2 days of operation, the acidified FW was stored in the freezer at –20 °C to be used as feedstock for TSAD. After adding the seed sludge, the three reactors (SL1, SL2 and TSAD) were operated in a semi-continuous mode (feeding every two or three days with the respective feedstocks) with gradual increase in organic loading rates (OLR) of 1.6, 2.4, 4.0, 5.2, 8.0 and 10.0 g VS<sub>FW</sub>/L. All the experiments were conducted in triplicates at the same experimental conditions.

After the baseline studies, a steel TSAD (diameter 250 mm \* height 400 mm) was fabricated and operated. The internal structure of this TSAD system is shown in Fig. 1. Fresh FW was added into the top chamber every day. The digestate was used as inocula for hydrolysis and acidogenesis and recirculated from the bottom of the TSAD to the top chamber; details of each step as described above. The operating steps and conditions were the same as that of the 1 L lab-scale ADs. The OLR was maintained at 2 g VS<sub>FW</sub>/L.

### 2.3. Analytical methods

COD and ammonia were determined using HACH colorimeter (HACH DR900, USA) according to the manufacturer's instructions. The pH was recorded using a pH analyzer (Agilent 3200 M, USA). TS and VS were determined based on the weighing method after being dried at 103–105 °C and burnt to ash at 550 °C. Methane (CH<sub>4</sub>) production was determined using a gas chromatograph (Clarus 580 Arnel, PerkinElmer, USA) equipped with a thermal conductivity detector. Volatile fatty acids (VFA) such as acetic acid, propionic acid and butyric acid were determined by a gas chromatograph (Clarus 580GC, PerkinElmer, USA) equipped with a flame ionization detector. C and N elemental analyses in FW were determined using the vario MICRO cube (Elementar, Germany). The abundance of bacteria and archaea were determined by real-time PCR according to the method described by Zhang et al. [24].

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