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# Comparison of hyper-thermophilic–mesophilic two-stage with single-stage mesophilic anaerobic digestion of waste activated sludge: Process performance and microbial community analysis

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## HIGHLIGHTS

- Hyperthermia at 70 °C and recycling were employed to upgrade mesophilic digestion.
- The hyper-thermophilic–mesophilic process improved the solid reduction by over 10%.
- Hyperthermia solubilized 34.4% of the COD in waste activated sludge.
- *Firmicutes* was the most dominant in the mesophilic stages of two-stage systems.
- Recirculation improved the diversity of species in the hyper-thermophilic stage.

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## ABSTRACT

The conventional mesophilic digestion of waste activated sludge (WAS) is challenged by the updated wastewater treatment process. A hyper-thermophilic (70 °C)–mesophilic (35 °C) temperature-phased two-stage systems without and with recirculation were employed to upgrade the single-stage mesophilic digestion of waste activated sludge in this study. The solid reduction increased by more than 10% in the two-stage systems. Similar methane production was observed in the single-stage system and two-stage systems, with a methane yield of 0.24 L/g VS added. In the case of two-stage system without recirculation, hyper-thermophilic stage played an important role in COD solubilization, with 34.4% of the WAS solubilized in that stage, while mesophilic stage contributed more in methane production, with 36.2% of COD in the WAS produced as methane in the mesophilic stage. *Firmicutes* became the most dominant bacteria phylum in the mesophilic stages of two-stage systems as compared to the dominant phylum *Proteobacteria* in single-stage system, with the genus *Methanothermobacter* cardinal in the hyper-thermophilic stage. The recirculation in the two-stage system increased the microbial community diversity in the hyper-thermophilic stage, which was one factor to result in improved methane production in the hyper-thermophilic stage.



## 1. Introduction

Sewage sludge is a byproduct, generated in the sewage treatment in wastewater treatment plants (WWTPs). The disposal of sludge has represented up to half the operating costs in a WWTP [1]. The necessary treatment and disposal is needed to reduce its volumes, to improve its character, and decrease its potential health hazards. Some technologies, such as landfilling, incineration and composting have been worldwide employed [2]. Among them, anaerobic digestion is promising, having been extensively applied to sludge stabilization in WWTPs for decades. It is characterized by (a) the capability to stabilize large volumes of dilute organic slurries at low cost, (b) low biomass production, (c) a high kill rate of pathogenic organisms, and (d) the capability to produce solids residues suitable for use as soil conditioners [3,4].

The ultimate biodegradability of waste activated sludge (WAS), generated in the biological treatment unit of WWTPs, is reasonably high [5], but only 30–45% of organic fractions are digestible in conventional anaerobic digestion [5–7]. In addition, new wastewater treatment processes confront WWTPs with new challenges for

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anaerobic digestion, for poor degradability of activated sludge requires long digester retention times, higher mixing costs, and also results in poor gas production [3]. In the upgrading and revamping of WWTPs to adapt to the reduced nitrogen discharge limits, primary settling tank is often removed, and retention times for biological nutrient removal (BNR) processes are extended. As a consequence, WAS is generated more, and digestibility of the WAS becomes lower in the updated WWTPs, than when the activated sludge treatment for carbon removal only [8,9]. Therefore, it is urgent to develop novel anaerobic technologies to enhance the degradation of WAS.

Anaerobic digestion of WAS is limited by the poor hydrolysis of particulates to soluble substrate [10]. Therefore, the enhancement of hydrolysis is the critical issue to upgrade the conventional digestion of WAS [11,12]. Pretreatment is the widely used technique to enhance hydrolysis, and facilitate the application of anaerobic digestion [13]. These pretreatment techniques include mechanical, thermal, chemical and biological methods [14,15]. Among those pretreatment methods, thermal pretreatment is suitable in improving the stabilization, enhancing the dewaterability, and reducing the number of pathogens at relatively low costs [16]. However, thermal pretreatment at higher temperature (>100 °C) causes higher energy requirements and operating difficulties. Thus, pretreatment below 100 °C is appropriate [17], especially when treating wastewater or waste containing high concentration of protein [18]. It is reported that pretreatment at 50–70 °C with the hydraulic retention time (HRT) 1–3 days promoted the solubilization of WAS with 15–27% [3]. In particular, the studies of pretreatment at 70 °C suggested that the solubilization of the particulate chemical oxygen demand (PCOD) could attain approximately 40% [19–21]. Moreover, in addition to solubilization, methanogenesis, especially hydrogenotrophic methanogenesis, can also make a contribution at 70 °C [22,23]. When mesophilic digestion of WAS followed the pre-fermentation at 70 °C, both the methane potential and production rate could be improved [17]. Unfortunately, little attention is paid to the investigation on the continuous operation of a hyper-thermophilic (70 °C)–mesophilic two-stage anaerobic digestion to treat WAS. Also, effects of hyper-thermophilic stage as pre-fermentation on microbial community of mesophilic digestion of WAS are hardly found.

In recent years, the introduction of recirculation from the end stage to the front stage in a two-stage system has attracted considerable attention [24–26]. Particularly, Recirculation was introduced to a hyper-thermophilic–thermophilic continuous process to study the co-digestion of polylactide and kitchen waste [27]. It is found that recirculation exerts considerable influence on the overall process performance, and it is feasible in degrading high-solid waste. The advantages of recirculation in two-stage digestion can be concluded as the supplement of alkalinity, ammonia and microorganism to the front stage and the dilution for the influent [28,29]. Nevertheless, few investigations have been carried out on the upgrading of anaerobic digestion of WAS by a temperature-phased two-stage process with recirculation, especially for a hyper-thermophilic–mesophilic two-stage process.

Continuous hyper-thermophilic (70 °C)–mesophilic (35 °C) temperature-phased two-stage processes without and with recirculation were synchronously operated in this study, with a single-stage mesophilic digestion as the control, aiming at investigating the operation performance of two-stage processes in treating WAS and at evaluating the feasibility in upgrading single-stage digestion. The comparative results between the two-stage process without and with recirculation also allow the effects of recirculation to be investigated. In addition, the microbial community analysis in each reactor was conducted to evaluate and validate the process effects.

## 2. Materials and methods

### 2.1. Substrate and inoculum

The concentrated WAS after centrifugation was used to the substrate in this study. It was fetched from a WWTP in Miyagi-ken, Japan. Immediately the WAS was transferred to the laboratory, it was stored in a refrigerator at 4 °C to maintain the stability of the characteristics of substrate. The characteristics of the WAS are shown in Table 1. The mesophilic inoculum was from the mesophilic digesters in a WWTP for sewage sludge treatment. In the case of microbial community of the mesophilic inoculum, the phylum *Proteobacteria* was the most bacteria, at 43.9%, and the genera *Methanosaeta* and *Methanolinea* had nearly equal weighting in the archaea community, at about 50%. The hyper-thermophilic reactor was inoculated with thermophilic (55 °C) inoculum, which was originally used for WAS treatment. *Firmicutes* and *Proteobacteria* was the most primary bacteria in the thermophilic inoculum, accounting for about 45% and 30%, respectively, while *Methanosarcina* and *Methanoculleus* constituted the most significant compositions of the archaea, with a similar percentage.

### 2.2. Experimental setup and operation

A single-stage system, a two-stage system without recirculation and a two-stage system with recirculation were constructed at a laboratory. They shared the same substrate tank by different outlets of the tank. The schematic diagram of the whole system is shown in Fig. 1. The working volume of the single-stage system was 5 L. The working volumes of hyper-thermophilic phase (HTP, the front stage) and mesophilic phase (MP, the end stage) of the two-stage systems were set as 3 L and 12 L, respectively. Thus, the total volume of the two-stage systems was 15 L. Each tank or reactor consisted of the reactor itself, a stirrer, a gas measuring unit (wet gas meters, WNK-0.5, Shinagawa Corporation, Japan), a temperature control device and inlets & outlets. The temperature of the substrate tank was controlled by a water jacket and a cooler (4 °C). The temperatures of reactors were assured by water jacket and heaters. Each reactor was equipped with a submerged thermometer to indicate the temperature in the reactors. The feeding and withdrawal was carried out using peristaltic pumps. The HTP of the two-stage systems and single-stage system was fed with WAS from the substrate tank. The MP was fed with the effluent from the front stage. In the case of two-stage system with recirculation, the recirculation was introduced from the MP to the HTP. The experimental conditions set for each system are shown in Table 2. Since the inocula were originally used for sewage sludge treatment, immediately the systems were operated with the HRT 30 days synchronously until the steady state reached.

**Table 1**
Characteristics of the WAS used in the experiment.

| Items | Unit | |
|---|---|---|
| TS | % | 4.66 ± 0.13 |
| VS | % | 3.46 ± 0.20 |
| T[a]-COD | g/L | 53.3 ± 0.9 |
| T-carbohydrate | g/L | 5.5 ± 0.7 |
| T-protein | g/L | 16.8 ± 1.3 |
| T-lipid | g/L | 4.0 ± 0.2 |
| $NH_4^+$-N | mg/L | 430 ± 40 |
| VFA | mg HAc[b]/L | 1420 ± 280 |

*Note:*
[a] 'T' is designated as 'Total'.
[b] All individual VFAs were calculated as acetic acid.