



# Enhancement of volatile fatty acid production by co-fermentation of food waste and excess sludge without pH control: The mechanism and microbial community analyses

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## HIGHLIGHTS

- The high VFA production was obtained the first time without pH control.
- Variations of DOM were quantitatively assessed by EEM and FRI technique.
- Co-fermentation effectively enhanced hydrolysis yield and acidogenesis yield.
- pH was self-maintained at beneficial range of 5.2–6.4 for VFA accumulation.
- Co-fermentation raised hydrolytic/acidogenic bacteria while inhibited methanogens.

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## ABSTRACT

The study provided a cost-effective and high-efficiency volatile fatty acid (VFA) production strategy by co-fermentation of food waste (FW) and excess sludge (ES) without artificial pH control. VFA production of 867.42 mg COD/g-VS was obtained under the optimized condition: FW/ES 5, solid retention time 7 d, organic loading rate 9 g VS/L-d and temperature 40 °C. Mechanism exploration revealed that the holistic biodegradability of substrate was greatly enhanced, and proper pH range (5.2–6.4) was formed by the high buffering capacity of the co-fermentation system itself, which effectively enhanced hydrolysis yield (63.04%) and acidification yield (83.46%) and inhibited methanogenesis. Moreover, microbial community analysis manifested that co-fermentation raised the relative abundances of hydrolytic and acidogenic bacteria including *Clostridium*, *Sporanaerobacter*, *Tissierella* and *Bacillus*, but suppressed the methanogen *Anaerolineae*, which also facilitated high VFA production. These results were of great guiding significance aiming for VFA recovery from FW and ES in large-scale.

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## 1. Introduction

Efficient treatment and disposal of solid organic wastes has become an urgent task due to strict legal regulations, insufficient lands, rising costs, and public concerns along with fast urbanization and economic development. Food waste (FW) as the largest fraction of municipal solid waste (37–55%) is growing rapidly in China (Xu et al., 2014) and its annual output has exceeded 30 million (Chen et al., 2012). Meanwhile, there are about 1.3 million tons of excess sludge (ES) (dry weight) produced every year from wastewater treatment plants (WWTPs) in China, and the cost of treating and disposing ES takes 40–60% of the total operation costs in WWTPs (Yang et al., 2012). The improper dispose of FW and ES

could cause many serious environmental problems such as the pollution of water, soil, air, and thus threatening to food safety and human health (Chen et al., 2015). Therefore, finding an eco-friendly and economical treatment method of FW and ES is now a critical issue worldwide.

Both FW and ES are the ideal substrates for anaerobic fermentation for their natures of high moisture, easily biodegradation and abundant organic compositions (Jiang et al., 2013; Chen and Wu, 2010). The generated volatile fatty acid (VFA) from anaerobic fermentation of ES and FW have been widely considered as a resource utilization of transforming “waste” into “treasure” (Zhang et al., 2016), and have been an excellent carbon source for wastewater biological nutrient removal (Feng et al., 2009). Recently, co-fermentation has been an more attractive method compared with sole FW and sole ES fermentation as it can simultaneously deal with two wastes in one facility, which can not only reduce facility

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construction investment, dilute potential hazardous compounds, guarantee balanced nutrients, but also promote synergistic effects of microorganisms (Chen and Wu, 2010; Chen et al., 2013a). So, co-fermentation can significantly enhance product yields and economic efficiencies. Moreover, co-fermentation of FW and ES could be a strategic solution to realize beneficial synergies for the wastewater treatment industry and FW management authorities.

To achieve practical large-scale application of VFA as external carbon sources for biological nitrogen and phosphorus removal, it is necessary to maximize VFA productivity meanwhile avoiding the high cost of VFA production. Anaerobic fermentation is a complex organic matter degradation and energy generation pathway that mainly involves in three successive stages, names hydrolysis, acidogenesis and methanogenesis. To enhance VFA production, the first two stages should be accelerated to produce more soluble organics and promote the transformation of hydrolyzate into VFA. At the same time, the methanogenesis must be prevented to reduce the VFA consumption from methanogens (Wang et al., 2014). To date, various methods based on the aforementioned principles have been investigated, which included plentiful pre-treatment methods (Zhang et al., 2016; Fu et al., 2015), optimization of key operation parameters (Jiang et al., 2013; Chen and Wu, 2010; Chen et al., 2013a; Luo et al., 2014), and addition of methanogen inhibitor (Huang et al., 2015; Chen et al., 2013b). However, all of the above-mentioned researches were based on adjusting pH to alkalinity or weak acidity by adding a large amount of NaOH or HCl. In this way, as the external carbon source, the obtained VFA have failed to gain practical application in WWTPs due to the specific drawback of large consumption of chemicals (for pH adjustment). In fact, free pH adjustment could lower the production cost, reduce operation complexity, and avoid the potential negative impacts of chemicals on microorganisms. Therefore, a free pH control anaerobic fermentation may be more economically feasible for preparing VFA to enhance nitrogen and phosphorus removal in WWTPs.

The complex anaerobic fermentation process was involved in large amounts of interacting microorganisms. To enhance the VFA production, it is instructive to characterize the microbial community structure since hydrolysis and acidogenesis are carried out by enormous bacteria groups (Jie et al., 2014). To date, the microbial studies for producing VFA were mainly based on a certain pH (Jie et al., 2014; Yang et al., 2014). But aiming at the practical application of VFA production without artificial pH adjustment, it is more significant to explore the effect of un-controlled pH on bacterial community structures and dominant bacterial species. Besides, the microbial community information concerning the sole- and co-fermentation of FW and ES for VFA production without pH control have been rarely investigated and compared.

Consequently, without pH artificial control, the objectives of this study are (1) to investigate the optimum conditions for VFA production from co-fermentation of FW and ES; (2) to reveal the mechanism of efficient VFA production without pH control; (3) to quantitatively characterize the specific microbial community structure with the optimal hydrolysis/acidification performance. It is expected that the results obtained in this study can provide some useful information for future investigation of VFA production without pH control.

## 2. Materials and methods

### 2.1. FW and ES

FW was obtained from a cafeteria in Harbin Institute of Technology (Harbin, China), which mainly contains rice, noodles, vegetables and meat. The FW after removing the superficial oil was

crushed by an electrical blender and stored at 4 °C for use. The ES was collected from the secondary sedimentation tank of Wenchang wastewater treatment plant (Harbin, China). The sludge was washed three times with tap water to remove impurities and then concentrated by settling at 4 °C for 24 h. The main characteristics of FW and ES are shown in Table 1.

### 2.2. Operation conditions optimization and mechanisms investigation

Response surface methodology (RSM) based on a five-level-four-variable central composite design (CCD) was applied to obtain the optimal conditions. The variations of FW/ES (based on the volatile solid content), temperature (T), solid retention time (SRT) and organic loading rate (OLR), and their levels are shown in Table S1 (Supporting Information, SI). VFA production was regarded as a dependent output variable. Thirty identical anaerobic fermentation reactors, with working volume of 500 mL, were operated in a semi-continuous mode (once-a-day draw-off and feeding) for VFA production. Each reactor was inoculated with designed amount of FW and ES, and the final volatile solid (VS) concentrations were  $17,820 \pm 635$  mg/L. During anaerobic fermentation process, according to the designed SRT and OLR, each reactor was withdrawn a certain amount of fermentation substrate everyday and then added the same volume fresh mixture accordingly. The pH values in all reactors were not controlled due to most anaerobic microorganisms could not tolerate the hostile environment such as alkaline or acidic. All the reactors were flushed with nitrogen gas (99.9%) for 5 min to remove oxygen after daily feeding, and then stirred in a water-bath shaker (180 rpm).

To investigate the mechanisms of higher VFA production from co-fermentation of FW and ES without pH control, three identical semi-continuous reactors inoculated with sole FW, sole ES, and the mixture of FW and ES with the optimum FW/ES, respectively. The T, SRT and OLR in the three reactors were controlled with the optimum conditions obtained by RSM.

During fermentation experiments, the liquid samples were taken from reactors daily for the analysis of soluble chemical oxygen demand (SCOD) and VFA concentrations. When the SCOD and VFA concentrations remained relatively stable, reactors were considered to be in a steady state and then the related investigations were carried out.

### 2.3. Analytical methods

The analyses of total solid (TS), VS, chemical oxygen demand (COD) and ammonia were conducted according to Standard Methods (APHA, 2005). The pH was measured by a pH probe (Germany WTW Company pH meter). VFA was analyzed by a gas chromatograph (GC) (HP 7890, Agilent Technologies, USA) with a flame ionization detector (FID). The sample injection volume was 1.0  $\mu$ L, and the carrier gas was nitrogen at the flux of 25 mL/min. The COD conversion factors of acetic, propionic, butyric and valeric acids were 1.066, 1.512, 1.816 and 2.036, respectively. For the analysis

**Table 1**  
Characteristics of FW and ES.

Parameters	FW	ES
Total solid (TS) (g/L)	66.17 $\pm$ 0.81	27.63 $\pm$ 0.53
Volatile solid (VS) (g/L)	65.15 $\pm$ 1.32	15.96 $\pm$ 0.74
Total chemical oxygen demand (TCOD) (g/L)	104.37 $\pm$ 10.40	25.10 $\pm$ 3.41
Soluble chemical oxygen demand (SCOD) (g/L)	50.64 $\pm$ 6.63	1.19 $\pm$ 0.37
Total biological oxygen demand (TBOD) (g/L)	72.46 $\pm$ 5.34	9.67 $\pm$ 0.55
Soluble NH <sub>4</sub> <sup>+</sup> -N (mg/L)	340.38 $\pm$ 9.45	160.76 $\pm$ 1.07
pH	5.62 $\pm$ 0.10	7.03 $\pm$ 0.13

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