



## Effect of lipase addition on hydrolysis and biomethane production of Chinese food waste



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### HIGHLIGHTS

- Two different lipase addition groups had an improvement for the hydrolyses of FW.
- The VFA increased and the LCFA decreased with the lipase addition.
- The pre-treated digester with 0.5% lipase addition obtained the best performance.
- The max methane yield was 4.97–26.5% higher than the lipase addition directly group.

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### ABSTRACT

The lipase obtained from *Aspergillus niger* was applied to promote the hydrolysis of food waste for achieving high biomethane production. Two strategies of lipase additions were investigated. One (Group A) was to pre-treat food waste to pre-decompose lipid to fatty acids before anaerobic digestion, and another one (Group B) was to add lipase to anaerobic digester directly to degrade lipid inside digester. The lipase was used at the concentrations of 0.1%, 0.5%, and 1.0% (w/v). The results showed that Group A achieved higher biomethane production, TS and VS reductions than those of Group B. At 0.5% lipase concentration, Group A obtained experimental biomethane yield of 500.1 mL/g VS<sub>added</sub>, 4.97–26.50% higher than that of Group B. The maximum  $B_d$  of 73.8% was also achieved in Group A. Therefore, lipase pre-treatment strategy is recommended. This might provide one of alternatives for efficient biomethane production from food waste and mitigating environmental impact associated.

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### 1. Introduction

Approximately  $6.0 \times 10^7$  tons of food wastes were generated in China annually. For example, in Beijing city, approximately 1600 tons of food wastes are currently collected daily (Meng et al., 2014). Traditional incineration, composting, and landfill were not suitable for food waste disposal due to its specific characteristics of high moisture, organic content, and high salinity. If the food waste is not treated properly, serious environmental pollution and health threat would be caused (Chen et al., 2012; Meng et al., 2014; Shen et al., 2013).

Anaerobic digestion is widely accepted as suitable technology to treat food waste to produce biogas (Li et al., 2013a,b; Shen et al., 2013; Zhou et al., 2014). Food waste is characterized by high moisture and organic matter content. On the dry base, the crude

lipid and crude protein contents of food waste are in the ranges of 22.8–31.45% (Chen et al., 2012; Li et al., 2013a,b; Shen et al., 2013; Zhang et al., 2013; Zhou et al., 2014) and 14.71–28.64% (Chen et al., 2012; Li et al., 2013a,b; Shen et al., 2013; Zhou et al., 2014), respectively. The other compositions were mainly carbohydrates (Li et al., 2013a,b; Shen et al., 2013; Zhou et al., 2014). The order of the hydrolysis rate was given as lipids < proteins < carbohydrates (Christ et al., 2000). This implies that lipids hydrolysis is limiting-step for the whole anaerobic process for food waste, and crucial for achieving high production of biomethane. In addition, the high content of lipids in food waste could possibly result in lipid residue accumulation in one digester, and pose adverse effect on digestion process (Sun et al., 2014; Zhang et al., 2013).

The lipids are first hydrolyzed to glycerol and free long-chain fatty acids (LCFAs) in an anaerobic environment. LCFAs are main intermediates from lipid degradation, which are further converted by acetogenic bacteria ( $\beta$ -oxidation process) to hydrogen and acetate, and finally to methane by methanogenic archaea (Palatsi

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et al., 2012). As lipids hydrolysis is limiting-step for anaerobic digestion of food waste, it would be critical to improve hydrolysis rate, meanwhile, to minimize adverse effect of lipid accumulation. A number of studies on various lipase hydrolyses have been conducted for achieving the goal (Cavaleiro et al., 2013; Cirne et al., 2007; Gomes et al., 2011; Rosa et al., 2009; Silva et al., 2012). However, the previous studies were mainly focused on other lipid-rich wastes such as meat-processing by-products (Luste et al., 2009; Valladão et al., 2007, 2011), fish-processing plant wastewater (Alexandre et al., 2011), and dairy wastewater (Adulkar and Rathod, 2014; Gomes et al., 2011). Very limited references are available for food waste, especially, Chinese food waste. Chinese food waste has specific characteristics. It includes various kinds of animal lipids and vegetable oils. These diversities are mainly determined by cooking ways and food materials traditionally used (Sun et al., 2014). Therefore, it would be more difficulty to achieve high hydrolysis rate for Chinese food waste. Lipase could be used to promote hydrolysis of lipid. This has been proven to be one of effective methods. Kameswari et al. reported that the biogas production was increased by 15% when the enzymatic pretreatment was applied on the co-digestion of fleshings and sludge (Sri Bala Kameswari et al., 2011). Prabhudessai et al. found that the enzymatic pretreatment on the cottage cheese could lower the hydraulic retention time and increase the methane production (Prabhudessai et al., 2014).

In this study, two different strategies of lipase hydrolysis were advanced. One was to pretreat food waste by lipase to predecompose lipid to LCFAs (and VFAs) before anaerobic digestion. Another one was to add lipase to anaerobic digester directly to degrade lipid inside digester. Two strategies were then evaluated and compared in terms of LCFAs (and VFAs) and biomethane production. The aim was to determine suitable method for efficient biomethane production from Chinese food waste, and provide one of alternatives for treating and reutilizing food waste.

## 2. Methods

### 2.1. Substrate and inoculum

The employed food waste was collected from a cafeteria at Beijing University of Chemical Technology. The indigestible materials, such as chopsticks, metals, glasses and plastics were initially separated from food waste by hands. Then the food waste was crushed by using a mechanical mixer (SS2600, WasteKing, Anaheim America) and kept in  $-20\text{ }^{\circ}\text{C}$  to prevent biological decomposition. Inoculum was obtained from the effluent of an anaerobic digester treating pig manure (Nanwu biogas plant at Shunyi District, Beijing, China). The characteristics of food waste and inoculum are presented in Table 1.

**Table 1**  
Characteristics of food waste and inoculum.

Characteristics	Food waste	Inoculum
TS (%)	20.26 ± 0.04	8.69 ± 0.01
VS (%)	17.98 ± 0.40	4.83 ± 0.01
VS/TS (%)	88.77 ± 2.17	55.58 ± 3.57
Ash (%)	2.28 ± 0.44	3.86 ± 0.50
C (%) <sup>a</sup>	52.66	29.41
N (%) <sup>a</sup>	1.66	2.87
H (%) <sup>a</sup>	7.60	4.05
O (%) <sup>a</sup>	26.74	11.56
S (%) <sup>a</sup>	0.09	0.52
C/N	20.61	9.80
Crude fat (%) <sup>a</sup>	27.3 ± 0.8	ND
Crude protein (%) <sup>a</sup>	13.5 ± 0.6	ND

ND: not determined.

<sup>a</sup> As TS of sample.

The employed lipase powder with high lipase activity of 50 U/mg was purchased from Jen's enzyme co., LTD (Shandong province, China). The lipase was obtained from *Aspergillus niger*. The highest activity of the lipase powder was shown at 20–45 °C and pH 6–13. The lipase powder was stored at ambient temperature (lower than 20 °C) in a dry place.

### 2.2. Enzymatic hydrolysis of the food waste

Two different strategies of enzymatic hydrolysis were applied in this work. One was to pretreat food waste before anaerobic digestion by the lipase (Valladão et al., 2007) with addition of 0.1%, 0.5%, and 1.0% (w/v). This group was called as Group A. Lipase used was in power state. Lipase concentration was defined as the weight (kg) of lipase powder added per liter (L) of food waste (w/v). The pH of the food waste was adjusted to 7.4–7.6, and then the food waste was kept in an incubator at 40 °C for 24 h. Another one was to add lipase to anaerobic digester directly. This group was called Group B. The same amount of lipase was added as Group A. Control experiments for both groups were conducted without lipase addition. All experiments were conducted in triplicate.

### 2.3. Anaerobic biodegradability tests

The food waste prepared in Group A, and B was anaerobically digested to test their biodegradability. Serum bottles with 150 mL working volume were used as batch digesters. The organic load of 20 g VS/L and food-to-microorganism ratio (F/M) of 1.0 were employed for all digesters. After loading the required amounts of food waste, lipase and seed sludge, each digester was filled up to 150 mL with tap water. Then the bottles were incubated in a shaker with temperature of  $35 \pm 1\text{ }^{\circ}\text{C}$ , and shaken 24 times a day and lasted 5 min for each shaking.

In order to evaluate the effects of the hydrolysate, the different fatty acids were measured on the fourth and eighth day after the digestion process started up. The biogas production and biomethane content were measured daily during the digestion time. All experiments were conducted in triplicate.

### 2.4. Analytical methods

The H<sub>2</sub>, N<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub> content in the biogas were analyzed by a gas chromatograph (SP2100A, Beifen-Ruili) equipped with a thermal conductivity detector (TCD) and a stainless steel column (2 m × 3 mm). The final results from gas chromatography were the average of three reads. The biomethane production can be calculated according to the biogas production and biomethane content. The concentration of volatile fatty acids (VFAs), such as acetic acid, propionic acid, butyric acid, valeric acid, and ethanol, was determined by a gas chromatograph (SHIMADZU, GC2014) equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm × 0.25 μm column (Agilent, DB-WAX). The detailed protocols for the determination of biogas content and VFAs were followed with the reference (De La Rubia et al., 2009).

The long chain fatty acids (LCFAs) in effluent were determined after transesterification by a gas chromatography (SHIMADZU, GC2014) equipped with a FID and a 30 m × 0.25 mm × 0.25 μm column (Agilent, DB-WAX). The carrier gas was N<sub>2</sub> at 1.0 mL/min. Temperature of the injection port and detector were 250 °C and 300 °C, respectively. Initial oven temperature was 120 °C for 3 min, with a 20 °C min<sup>-1</sup> ramp to 220 °C, and finally isothermal for 12 min (Meng et al., 2014; Neves et al., 2009).

Total solids (TS), volatile solid (VS) and pH were determined according to the standard methods (APHA, 1998). The crude lipids content was measured by weight difference after extraction with diethyl ether in a soxhlet system. Crude protein content was

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