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Enhancing anaerobic digestion performance of crude lipid in food waste by enzymatic pretreatment

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HIGHLIGHTS

- Three lipases were applied to hydrolyze the floatable grease in the food waste.
- Animal fat and vegetable oil are applied as substrates for anaerobic digestion.
- Three lipids are hydrolyzed in the conditions of 24 h, 1000–1500 μL and 40–50 °C.
- The digestion time was shortened by 10–40 d for three lipids treated by lipases.
- The biomethane production rate of the hydrolysis products was enhanced 26.9–157.7%.

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ABSTRACT

Three lipases were applied to hydrolyze the floatable grease (FG) in the food waste for eliminating FG inhibition and enhancing digestion performance in anaerobic process. Lipase-I, Lipase-II, and Lipase-III obtained from different sources were used. Animal fat (AF) and vegetable oil (VO) are major crude lipids in Chinese food waste, therefore, applied as substrates for anaerobic digestion tests. The results showed that Lipase-I and Lipase-II were capable of obviously releasing long chain fatty acid in AF, VO, and FG when hydrolyzed in the conditions of 24 h, 1000–1500 μL and 40–50 °C. Compared to the untreated controls, the biomethane production rate were increased by 80.8–157.7%, 26.9–53.8%, and 37.0–40.7% for AF, VO, and FG, respectively, and the digestion time was shortened by 10–40 d. The finding suggests that pretreating lipids with appropriate lipase could be one of effective methods for enhancing anaerobic digestion of food waste rich in crude lipid.

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1. Introduction

Chinese food waste is characterized by high amount of organic matters, including crude lipid (22.8–31.45%), crude protein (14.71–28.64%), and carbohydrates on dry matter bases (Meng et al., 2015a). Food waste is therefore suitable to be used to produce biogas through anaerobic digestion. Anaerobic digestion has received increasing attentions in recent years for its advantages of reducing waste pollution and produce clean energy as well (Cho et al., 2016; Dhamodharan et al., 2015; Naroznova et al., 2015).

The lipid is one of major contents in food waste. It is a mixture of vegetable oils and animal fats (Meng et al., 2015a). The production of vegetable oils and animal fats were about 160 million tons per year in global level (Lin et al., 2013). Approximately 80% of total oils and fats were used for human food consumption (Rosillo-Calle et al., 2009). Compared to the food waste in other countries, Chinese food waste contains higher crude lipids due to specific dietary habits in China. The crude lipids in Chinese food waste were found to be mainly comprised of animal fat and vegetable oil. Although food waste contains high contents of readily biodegradable compositions such as protein, carbonhydrates, the direct digestion without any pretreatment of crude lipids could lead to low digestion efficiency and severe inhibition to anaerobic process due to the slow degradation rate and the accumulation of crude lipid in digester (Chen et al., 2008; Meng et al., 2015b). It was found that the digestion of lipid is the rate-limiting step in the digestion process of food waste (Sun et al., 2014). Therefore, it would be critical to find effective methods to eliminate the limitation for achieving high performance of food waste anaerobic

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digestion. The lipase pre-hydrolysis of crude lipids could be one of options.

Lipase has been applied in the treatment of lipid-rich wastewater by anaerobic process. The pre-hydrolysis stage could enhance the anaerobic digestion efficiency of wastewater from meat-processing plant (Cavaleiro et al., 2013; Luste et al., 2009; Masse et al., 2003) and babassu oil processing industry (Valladão et al., 2007). High chemical oxygen demand removal efficiency was obtained in the lipase-treated dairy wastewater (Mendes et al., 2006; Rosa et al., 2009) and fish-processing plant wastewater (Alexandre et al., 2011). Additionally, lipase pretreatment could increase the organic load of lipid-rich waste water in a hybrid UASB reactor (Gomes et al., 2011).

It was also found that treatment with pancreatic lipase significantly reduced the size of pork fat particles, enhanced the concentration of long chain fatty acid (LCFA) and decreased the digestion time in slaughterhouse wastewater (Masse et al., 2003; Mendes et al., 2006). In a recent study, we found that lipase addition could enhance the biomethane production of food waste (Meng et al., 2015a). These studies have provided important insight that lipase pretreatment is a possible strategy to minimize the inhibition of lipid accumulation in food waste digester. What remained unclear is if and how the lipases from different sources influence the digestion performance of crude lipid in Chinese food waste.

In this study, three lipases were applied to hydrolyze the floatable grease (FG) in Chinese food waste for eliminating FG inhibition and enhancing digestion performance in anaerobic process. The purpose of the study is to investigate the feasibility of lipase pretreatment and also determine lipase pretreatment parameters.

2. Materials and methods

2.1. Substrate and inoculum

Animal fat (AF), vegetable oil (VO), and floatable grease (FG) skimmed from food waste are collectively called FOG in this study. FOG was employed to investigate the effect of lipase pretreatment on biomethane production in anaerobic digestion process. AF was lard that rendered down dryly by hands. VO was arachis oil purchased from the supermarket. FG was skimmed from food waste after centrifuging (Meng et al., 2015b). The food waste was collected from cafeteria at Beijing University of Chemical Technology (BUCT). The saponification values of AF, VO and FG were 184.9 ± 0.3 mg KOH/g, 185.3 ± 0.5 mg KOH/g and 188.6 ± 0.2 mg KOH/g, respectively. The AF, VO and FG were sealed up and stored at 4 °C to prevent biological decomposition. The inoculum was obtained from the effluent of an anaerobic digester treating pig manure (Nanwu biogas plant at Shunyi District, Beijing, China). The contents of total solid (TS) and volatile solid (VS) were 3.53 ± 0.06% and 1.95 ± 0.03%, respectively.

The properties of lipases used are presented in Table 1. Lipase-I, Lipase-II and Lipase-III are purchased from different companies listed in Table 1. The sources of three lipases are *Aspergillus*, *Candida* and Porcine pancreatic, respectively. Both Lipase-I and Lipase-II are produced by submerged fermentation. But the carbon sources of Lipase-II during the fermentation are refined vegetable oil and vegetable protein. The enzyme activity values of Lipase-I and Lipase-II are same (100 μ/mg) which could be applied in wide range of pH and temperature. Lipase-III is natural lipase that extracted from porcine pancreatic, which is purchased from Sigma-Aldrich are effective at pH 7.4–7.7 at 37 °C.

2.2. Anaerobic digestion tests of hydrolyzed FOG

2.2.1. Enzymatic hydrolysis of the FOG

To obtain appropriate application conditions for Lipase I–III, the hydrolysis time, volume used and temperature are investigated when FOG are hydrolyzed. In consideration of subsequent work about anaerobic digestion of FOG, pH was not adjusted during the hydrolysis process. Because initial pH of the mixture of FOG and lipase was nearly 7.0 which is in the suitable range (Table 1). Lipase-I, Lipase-II and Lipase-III are dissolved in deionized water (5.0 g lipase powder in 100 mL deionized water). Firstly, the hydrolysis time (0 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and 36 h) was analyzed by adding 0.5 g FOG and 500 μL enzymatic solution in incubator shaker (40 °C, 200 rpm). Then the volume of enzymatic solution (50 μL, 100 μL, 200 μL, 500 μL, 1000 μL, 1500 μL and 2000 μL) was investigated at 40 °C. Lastly, hydrolysis reactions were taken place at four temperatures (30 °C, 40 °C, 50 °C and 60 °C) to obtain the maximum hydrolysis rate. The FOG would be hydrolyzed by the lipases at appropriate conditions (hydrolysis time, volume of addition and temperature). The hydrolysis products were analyzed by GC. All experiments were conducted in triplicate.

2.2.2. Anaerobic biodegradability tests

The anaerobic digestion of lipase treated FOG was conducted. When the FOG was hydrolyzed by lipase, the hydrolysis products were anaerobically digested in scrum bottles. The organic loads of 10 gVS/L and food-to-microorganism ratio (VS/VS) of 1.0 were employed for all digesters. After loading the required amounts of hydrolytic FOG and seeding sludge, each digester was filled up to 150 mL with tap water. Then the bottles were incubated in a shaker with temperature of 35 ± 1 °C (5 min/h).

In order to evaluate the effects of the lipase addition on the hydrolysis of FOG, same amount of control FOG was digested in the same conditions without adding lipase. The daily biogas production and biomethane content were measured during entire digestion time. All experiments were conducted in triplicate.

2.3. Analytical method

TS and VS were determined according to Standard Methods for the Examination of Water and Wastewater (Wef, 1998). The saponification and acid value of FOG were determined according to Chinese National Standards (Ma et al., 2015).

The hydrolysis yields with LCFA were obtained after transesterification and extraction using n-Hexane. The amount of extracts were measured by a gas chromatography (SHIMADZU, GC2014) equipped with a DB-WAX column and a FID detector.

Table 1
The sources of three lipases purchased from different companies and their properties.

Lipase	Sources	Companies	Application conditions	Enzyme activity
Lipase I	*Aspergillus*	JieNuo Enzyme Co., Ltd (Shandong province, China)	pH: 6.0–13.0 Temperature: 20–45 °C	100 μ/mg (50 °C, pH 6.0)
Lipase II	*Candida*	Beijing CAT New Century Biotechnology Co., Ltd (China)	pH: 6.5–9.5 Temperature: 25–55 °C	100 μ/mg
Lipase III	Porcine pancreatic	SIGMA-ALDRICH (China)	pH: 7.4–7.7 Temperature: 37 °C	100–400 μ/mg protein (using olive oil (30 min incubation))

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