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Acidogenic fermentation characteristics of different types of protein-rich substrates in food waste to produce volatile fatty acids

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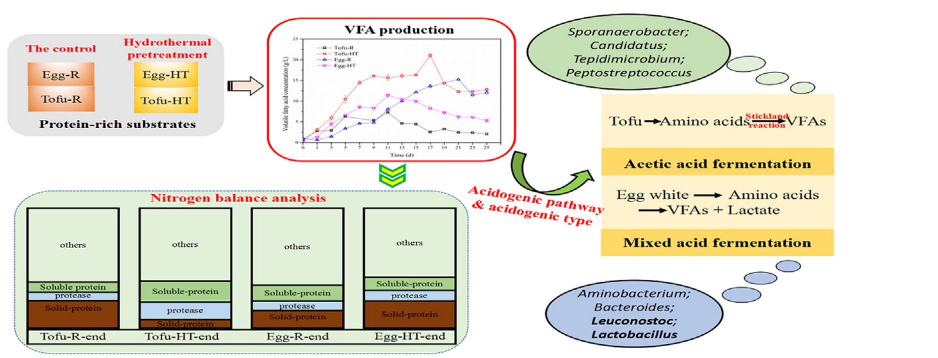
HIGHLIGHTS

- Two kinds of protein-rich substrates (tofu and egg white) were first investigated for producing VFA.
- Hydrothermal pretreatment had a positive impact on the VFA production from tofu but not for egg white.
- Tofu and egg white had different metabolic pathway for VFA production.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, tofu and egg white, representing typical protein-rich substrates in food waste based on vegetable and animal protein, respectively, were investigated for producing volatile fatty acids (VFAs) by acidogenic fermentation. VFA production, composition, conversion pathways and microbial communities in acidogenesis from tofu and egg white with and without hydrothermal (HT) pretreatment were compared. The results showed HT pretreatment could improve the VFA production of tofu but not for egg white. The optimum VFA yields were 0.46 g/g VS (tofu with HT) and 0.26 g/g VS (egg white without HT), respectively. Tofu could directly produce VFAs through the Stickland reaction, while egg white was converted to lactate and VFAs simultaneously. About 30–40% of total protein remained in all groups after fermentation. Up to 50% of the unconverted soluble protein in the HT groups was protease. More lactate-producing bacteria, mainly *Leuconostoc* and *Lactobacillus*, were present during egg white fermentation.

1. Introduction

Renewable processes based on biomass are being developed to generate more kinds of high-value products. One of these processes involves the acidogenic fermentation, in which acidogenic bacteria convert organic substrates to volatile fatty acids (VFAs). VFAs are valuable substrates for several applications: the production of biodegradable plastics (Cai et al., 2009), electricity, biofuels (Uyar et al., 2009; Choi et al., 2011), and the biological removal of phosphorus and nitrogen from wastewater (Zheng et al., 2010). Generally, VFAs are produced commercially by chemical routes, which need a large amount of non-renewable petrochemicals as the raw material. Comparatively, acidogenic fermentation could provide an opportunity to recycle organics while producing the value-added chemicals VFAs, thus attracting wide interest. The potential of many solid and liquid organic wastes has been studied

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in VFA production by anaerobic fermentation. Due to the fact that food waste (FW) accounts for the major proportion of municipal solid waste and has a high total chemical oxygen demand (TCOD) content, it has been the desirable solid waste for VFA production.

Three main components of FW are carbohydrates (simple sugars and polysaccharides), proteins, and lipids. Lipids found in FW are highly resistant to biodegradation and contribute to high COD levels (Nakhla et al., 2003). Consequently, lipids are usually separated to produce biodiesel, and not used by anaerobic fermentation. The total sugar and protein contents in FW are normally in the range of 35.5%–69.0% and 3.90%–21.9%, respectively (Kiran et al., 2014). Carbohydrates (except for lignin, cellulose, and hemicellulose) are easily converted to glucose by enzymes, and then subsequently fermented to high-value organic acids (De Gioannis et al., 2013; Wang and Wan, 2009; Ni et al., 2006). In the Chinese diet, the essential proteins come from foods, such as pork, eggs, fish, beef, legumes, nuts, and dairy products. The proteins obtained from egg whites are termed as egg protein and from dairy products as whey protein. However, in Chinese food waste, there are less dairy products, and mainly animal protein (such as egg white) and vegetable protein (such as tofu or legumes). According to Jiang et al. (2014) survey results, tofu was the main protein-rich substrate in FW. Lay et al. (1999) also used egg white and lean (two protein-rich substances) to produce hydrogen by anaerobic fermentation. Because of its unique three-dimensional structure, protein is not susceptible to protease cleavage in its native folded conformation (Herman et al., 2006; Carbonaro et al., 2012; Yin et al., 2016). A previous study has found that the conversion efficiency of total sugar in FW was up to 88%, but of protein was 40–70% (Yin et al., 2016). Therefore, the conversion of proteins in FW is usually the rate-limiting step during acidogenic fermentation.

Hydrothermal (HT) pretreatment with no added chemicals has been widely used to dispose of protein-rich wastes, such as FW and waste activated sludge (Takata et al., 2013; Wang et al., 2016). The HT process increases the quantity of ionized products in water under elevated temperatures and high pressure. Macromolecules can be hydrolyzed by these ionized products along with organic dissolution (Yin et al., 2014). Nevertheless, Liu et al. (2012) found that only 40–50% of protein in the sludge was converted into VFAs even after thermal-alkaline pretreatment. Yuan et al. (2006) used bovine serum albumin (BSA) as the substrate for alkaline fermentation, but the residual BSA was as high as about 54%. Interestingly, some soluble proteins still accumulated in the fermentation broth after acidogenic fermentation with HT pretreatment (Yin et al., 2014; Wang et al., 2014). Enhancement of the conversion efficiency of proteins would contribute to achieving a higher VFA production from FW.

Nowadays, little attention has been devoted to studying the effect of HT pretreatment on acidogenesis from protein-rich substrates in FW, with studies about the hydrolysis and acidogenesis of single proteins also being limited. Therefore, the present study aims to evaluate VFA production from different types of protein-rich substrates (tofu and egg white) in food waste. Then, the effect of HT pretreatment on hydrolysis and acidogenesis from proteins will be investigated. In addition, the compositions of the residual proteins in FW will be determined. The type of fermentation, the acidogenic pathway, the critical enzyme activity and key microbes during fermentation will be discussed.

2. Materials and methods

2.1. Substrate and inoculum

In the present study, two kinds of protein-rich substrates, tofu and egg white, were used to simulate vegetable protein and animal protein in food waste, respectively. They mainly consist of 50–60% protein and 5–6% carbohydrate on a dry weight basis. The tofu and egg white were purchased from the same vendor at Cui Yuan farmers' market (Hangzhou, China), and then immediately crushed using a mangle for the subsequent experiment. The inoculum (anaerobic granular sludge) was withdrawn from an up-flow anaerobic sludge blanket (UASB) reactor of the Xihu Brewery (Hangzhou, China). The main characteristics of the inoculum and protein-rich substrates are listed in Table 1. Before being added into the fermentation system, the anaerobic sludge was reactivated in a culture medium.

2.2. Hydrothermal pretreatment

The HT pretreatment of tofu and egg white is described in a previous study (Yin et al., 2014). Briefly, air-tight pressure digestion vessels each with a volume of 80 mL were used for the HT pretreatment of crushed FW (85% moisture content). The temperature and duration of the HT pretreatment were set at 160 °C and 30 min, respectively. Substrates without HT pretreatment were used as the controls.

2.3. Fermentation experiments for VFA production

The hydrothermally pretreated tofu and egg white (Egg-HT and Tofu-HT) were fermented in amber wide-mouth bottles with a working volume of 500 mL. Substrates with no pretreatment (Egg-R and Tofu-R) for acidogenic fermentation were used as the controls. The total chemical oxygen demand (TCOD) of the protein-rich substrate for fermentation in each reactor was controlled at 50 g/L, keeping food waste-to-microorganism ratio (F/M) at 5.0 g volatile solids (VS)/g VS. All reactors were stirred mechanically at 120 rpm using a magnetic stirrer and maintained at 30 ± 2 °C and at a pH of 6.0 by adding 4.5 M HCl or NaOH. All the fermentation tests were conducted in duplicate and lasted for 25 days.

Samples were taken from the reactors every 2 days. The fermented broth was separated from the residue by centrifuging at 11,000g for 5 min then filtered using a microfiltration membrane (0.45 μm). The supernatant was used to determine the soluble chemical oxygen demand (SCOD), soluble protein, ammonia nitrogen (NH_4^+-N), amino acid, lactate, and VFAs contents. The total solids (TS), VS, volatile suspended solids (VSS), total nitrogen (TN), total protein (T-protein), total sugar (T-sugar), and TCOD were measured before and after fermentation. Microbial samples were taken for bacterial community analysis from all reactors at the beginning and end of fermentation, as well as at the time of maximum VFA production. There were three replicas of the DNA samples taken from each reactor. After the extraction of DNA, three replicas were mixed into one DNA sample to determine the microbial community for each reactor.

Table 1
The characteristics of two protein-rich substrates and inoculum.

Characteristic	Protein wastes		Anaerobic sludge
	Tofu	Egg White	
pH	6.12	9.44	6.80
TS (%)	12.6	13.5	5.7
VS (%)	95.4	95.0	94.3
TCOD (g/g dry sample)	1.11	0.88	–[a]
SCOD (g/L)	14.8	31.7	15.0
soluble protein (g/L)	2.8	9.9	0.2
NH_4^+-N (mg/L)	43.7	79.2	24.8
T-protein (g/g dry sample)	0.46	0.58	–[a]
T-sugar (g/g dry sample)	0.06	0.05	–[a]

[a] Undetected.