Waste Management xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Improvement of acidogenic fermentation for volatile fatty acid production from protein-rich substrate in food waste

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ARTICLE INFO

Article history:
Received 23 June 2017
Revised 7 November 2017
Accepted 27 November 2017
Available online xxxx

Keywords:
Hydrothermal pretreatment
Maillard reaction
Sodium oxamate
Lactate dehydrogenase
Volatile fatty acids

ABSTRACT

Based on our previous study, the low volatile fatty acid (VFA) production from egg white in food waste was mainly attributed to more acidogenic substrates (proteins and amino acids) consumed in the Maillard reaction and more organics converted into lactic acid. In this study, two methods were employed to improve VFA production: (1) reducing Maillard reaction with a drop in pH during hydrothermal (HT) pretreatment, and (2) inhibiting the conversion from protein to lactic acid. HT pretreatment under weakly acidic condition significantly promoted the hydrolysis and degradation of protein and the hydrolytic enzyme (protease) activity, thus increasing VFA yield by 45.8% from 0.24 to 0.35 g/g protein for HT pretreated egg white. Addition of sodium oxamate increased the maximal VFA yield from 0.24 to 0.29 g/g protein for HT pretreated egg white and from 0.32 to 0.67 g/g protein for egg white with no pretreatment in which there was more protein converted through the lactic acid metabolism pathway. Sodium oxamate improved the acidification step by inhibiting the reaction from pyruvates to lactic acid, and thereby accelerating the process of conversion from pyruvates to VFA.

1. Introduction

Food waste (FW) consists of carbohydrates, proteins and lipids, and is a potentially suitable substrate for anaerobic fermentation. Some metabolic products (e.g. organic acids, and enzymes) produced during FW fermentation can create greater value, \$1000/ton biomass, than generating electricity (\$60–150/ton biomass), producing fuel (\$200–400/ton biomass), and providing animal feed (\$70–200/ton biomass) (Sanders et al., 2007). Volatile fatty acids (VFA) are short-chain organic acids, which can be used for biological removal of nitrogen and phosphorus (Zheng et al., 2010) and for the production of bioenergy and bioplastics (Uyar et al., 2009; Choi et al., 2011; Cai et al., 2009). Of all substrates in FW, proteins are relatively non-susceptible to cleavage by proteases due to their native folded conformations (Herman et al., 2006; Carbonaro et al., 2012). Therefore, enhancing the hydrolysis rate of protein has a great impact on VFA production during FW fermentation.

Several studies have shown that pretreatments, like crushing, heat, Fenton oxidation, ozone, acid, alkali, and ultrasonic method, can improve the biodegradability of organic materials (Carrere et al., 2010; Lee et al., 2014; Liu et al., 2012). In the current engineering practice, hydrothermal (HT) pretreatment with no added external chemicals, has been widely used to dispose of FW (Takata et al., 2013). HT conditions are typically provided by pressurized liquid-phase water at a temperature above its normal boiling point. With hot water as the reaction solvent, higher solubility of organic compounds in water are possible than at ambient conditions, which can increase rates of hydrolysis. Some reports have showed that hemicellulose can be hydrolyzed to sugar with HT pretreatment (Sasaki et al., 2003; Matsunaga et al., 2008). Yin et al. also demonstrated the soluble substance in FW increased and VFA accumulation significantly enhanced after HT pretreatment (Yin et al., 2014).

Our previous study used egg white and tofu (two types of model proteins in Chinese FW) to investigate hydrolytic and acidogenic characteristics of the protein-rich during anaerobic fermentation (Shen et al., 2017). The study showed that fermentation of egg white without HT pretreatment resulted in higher yield of both VFA and lactic acid. And HT pretreatment improved VFA production greatly from tofu, but not from egg white. Compared to tofu, the lower VFA production from HT pretreated egg white, was mainly attributed to differences in initial pH and the type of amino acids, which would have a great effect on the level of Maillard reaction.

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https://doi.org/10.1016/j.wasman.2017.11.047
0956-053X/

Please cite this article in press as: Yu, X., et al. Improvement of acidogenic fermentation for volatile fatty acid production from protein-rich substrate in food waste. Waste Management (2017), https://doi.org/10.1016/j.wasman.2017.11.047

During heat processing, the Maillard reaction occurs which involves a series of very complex reactions between reducing sugars and the free amino groups of proteins or amino acids (especially arginine and glycine) (Einarsson et al., 1983). During the process, the so-called Maillard reaction products (MRPs) will be produced, which are brown and non-biodegradable. The number of different MRPs depends on the pH value during the Maillard reaction, the reaction time, as well as the carbohydrate and amino acid components used to generate the MRPs (Taure et al., 2004). In our previous study, the initial pH of the egg white and tofu were 9.44 and 6.12, respectively (Shen et al., 2017). It has been reported that a higher pH value contributes to a more active Maillard reaction (Dong et al., 2011). Besides, arginine, a main reactant in the Maillard reaction, was the predominant amino acid in egg white (Shen et al., 2017), which could contribute to a more efficient Maillard reaction during HT pretreatment (Dong et al., 2011; Einarsson et al., 1983). These reactions are accompanied by a reduction in nutritive value (protein or sugar loss) and the formation of toxic compounds (Ledl and Schleicher, 1990). From heat-processed fruits and vegetables, Wilson and Brown isolated substances that could inhibit various types of bacteria (Wilson and Brown, 1953). Ingram et al. reported that orange juice that had turned brown was not readily fermented by *Saccharomyces ellipsoideus* (Ingram et al., 1955). Reducing Maillard reactions would decrease MRPs production and protein loss. However, whether controlling initial pH at about 6.5 (similar to that of tofu) before HT pretreatment could alleviate Maillard reaction and improve VFA production from egg white needs to be studied.

The other byproduct of egg white degradation is lactic acid. Various approaches have been taken to decrease lactic acid production. Arioli et al. found that adding 20 mM sodium oxamate decreased the production of lactic acid by 31% (Arioli et al., 2013). Huang et al. used sodium oxamate to restrict the metabolism of lactic acid, thus enhancing the production of hydrogen by anaerobic digestion (Huang et al., 2013). However, it is unknown whether the decline in lactic acid content would increase VFA production after oxamate addition during egg white protein fermentation.

The present study aims to explore two different ways to convert egg white into VFA more optimally. First, the differences in color and chemical structure of egg white under various treatments (the original, HT pretreatment, and HT pretreatment under weakly acidic conditions) were analyzed to find if weakly acidic condition could reduce Maillard reaction. Next, the influence of HT pretreatment at about pH 6.5 (similar to that of tofu) on egg white acidogenic fermentation was studied. Finally, the effect of sodium oxamate addition on reducing lactic acid accumulation and improving VFA production was investigated.

2. Materials and methods

2.1. Substrate and inoculum

Egg white from chickens was used as a model protein-rich substrate in Chinese FW. It consisted of 50–60% protein and 5–6% carbohydrate on a dry weight basis. The egg white was purchased from the Cuiyuan farmers' market (Hangzhou, China), and then was immediately crushed using a mangle for subsequent experiments. The inoculum (anaerobic granular sludge) was withdrawn from an up-flow anaerobic sludge blanket (UASB) reactor at the Xihu Brewery (Hangzhou, China). The main characteristics of the inoculum and egg white protein are listed in Table 1. Before being added to the fermentation system, the anaerobic sludge was reactivated in a culture medium (Supplementary Information).

Table 1
The characteristics of protein-rich substrates and anaerobic sludge.

Characteristic	Protein-rich substrates			Anaerobic sludge
	Egg-R[b]	Egg-HT[c]	Egg-pH-HT[d]	
TS (%)	13.5	13.7	14.3	5.7
VS (g/g dry sample)	0.95	0.95	0.93	0.78
TCOD (g/g dry sample)	0.9	–[a]	–[a]	1.3
SCOD (g/L)	31.7	51.0	41.5	15.0
S-protein (g/L)	9.9	36.2	31.6	0.2
NH_4^+-N (mg/L)	79.2	174.0	166.5	24.8
S-sugar (g/L)	–[a]	2.1	1.3	0.1
T-protein (g/g dry sample)	0.6	–[a]	–[a]	0.3
T-sugar (g/g dry sample)	–[a]	–[a]	–[a]	0.1
C/N ratio	5.3	–[a]	–[a]	8.6

[a] –: Undetected.
[b] Egg-R: The sample that was without HT pretreatment.
[c] Egg-HT: The sample that was without pH adjustment before HT pretreatment.
[d] Egg-pH-HT: The sample that was with pH adjustment before HT pretreatment.

2.2. Hydrothermal pretreatment

The HT pretreatment of egg white has been 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 HT pretreatment of the crushed egg white (85% moisture content). The temperature and duration of the HT pretreatment were set at 160 °C and 30 min, respectively.

2.3. Experimental design

The experiments were carried out in five pairs of identical amber wide-mouth bottles (each with a working volume of 500 mL). The total chemical organic demand (TCOD) of the egg white for fermentation in each reactor was controlled at 50 g/L, and the substrate to inoculum ratio (S/I) was 5.0 g volatile solids (VS)/g VS (Wang et al., 2014). The experimental conditions are shown in Table 2. In one group, before HT, pH of egg white was adjusted to about 6.5 without sodium oxamate (Egg-pH-HT). Egg white without sodium oxamate or pH adjustment served as the control (Egg-R, Egg-HT) in two pairs of reactors. For the other two pairs of reactors, 15 mM sodium oxamate (≥98%, Sigma-Aldrich, China) was added after day 7 of fermentation when lactic acid production had begun in reactors (Egg-R(+) and Egg-HT(+)). All reactors were stirred mechanically at 120 rpm using a magnetic stirrer and maintained at 30 ± 2 °C and at pH 6.0 by adding 4.5 M HCl or NaOH (Wang et al., 2014). All the fermentation tests were conducted in duplicate for 25 days.

2.4. Analytical methods

Samples were taken from the reactors every 2 days. The fermented broth was separated from the residue by centrifuging at 11,000*g* for 5 min then filtered using a 0.45 μm microfiltration membrane. The supernatant was used to determine the soluble chemical oxygen demand (SCOD), soluble protein, ammonia nitro-

Table 2
Experimental design for acidogenic fermentation from egg white.

Reactor	HT pretreatment	Initial pH	Sodium oxamate (mM)
Egg-pH-HT	+	6.5	0
Egg-R(control)	/	9.4	0
Egg-R(+)	/	9.4	15[a]
Egg-HT(control)	+	9.4	0
Egg-HT(+)	+	9.4	15[a]

[a] Sodium oxamate was added after day 7 of fermentation when lactate began to produce in the reactors.