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Batch dark fermentation from enzymatic hydrolyzed food waste for hydrogen production

Wei Han [a], Min Ye [a], Ai Jun Zhu [a], Hong Ting Zhao [a], Yong Feng Li [b,*]

[a] College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou 310018, China
[b] School of Forestry, Northeast Forestry University, Harbin 150060, China

HIGHLIGHTS

- A combination bioprocess for hydrogen production was developed.
- Food waste was used in solid-state fermentation to produce enzyme solids.
- The resulting solids were utilized to hydrolyze food waste.
- The food waste hydrolysate was used as feedstock for biohydrogen production.
- The best hydrogen yield of 39.14 ml H_2/g food waste was achieved.

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ABSTRACT

A combination bioprocess of solid-state fermentation (SSF) and dark fermentative hydrogen production from food waste was developed. *Aspergillus awamori* and *Aspergillus oryzae* were utilized in SSF from food waste to generate glucoamylase and protease which were used to hydrolyze the food waste suspension to get the nutrients-rich (glucose and free amino nitrogen (FAN)) hydrolysate. Both glucose and FAN increased with increasing of food waste mass ratio from 4% to 10% (w/v) and the highest glucose (36.9 g/L) and FAN (361.3 mg/L) were observed at food waste mass ratio of 10%. The food waste hydrolysates were then used as the feedstock for dark fermentative hydrogen production by heat pretreated sludge. The best hydrogen yield of 39.14 ml H_2/g food waste (219.91 ml H_2/VS_{added}) was achieved at food waste mass ratio of 4%. The proposed combination bioprocess could effectively accelerate the hydrolysis rate, improve raw material utilization and enhance hydrogen yield.

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1. Introduction

Hydrogen is considered to be a promising fuel because it is clean and renewable. Moreover, the energy yield of hydrogen is 122 kJ/g which is 2.75 times higher than the fossil fuel (Abbasi and Abbasi, 2011). Biological hydrogen production has attracted considerable attention since it could deal with the conversion of low cost residues or organic waste/wastewater to hydrogen (Show et al., 2012; Van-Ginkel et al., 2005). Generally, biological hydrogen production can be divided into two categories: photosynthesis and dark fermentation (Han et al., 2015b). Dark fermentation is seemed to be a more feasible biotechnology for hydrogen production than the photosynthesis due to less energy consumption and no light limitation (Tawfik et al., 2011). However, low hydrogen production rate and high cost are the dominant obstacles for large scale of dark fermentative hydrogen production (Han et al., 2012). Utilization of raw waste/wastewater as substrate for fermentative hydrogen production (such as food waste) could effectively enhance the economic benefit which is regarded as a promising solution (Karagiannidis and Perkoulidis, 2009).

Food waste is one of the most severe environmental problems all over the world (Zhang et al., 2012). Over a billion tones of food waste is generated per year which accounts for 33% of annual global food production (Lee and Chiu, 2012). Therefore, disposal and utilization of food waste is becoming to be one of the major global challenges. Food waste consists mainly of starch and protein which make food waste to be an economical source for biofuels production (Wang et al., 2011). Utilization of food waste for hydrogen production could not only solve the food waste problem, but also produce the alternative energy source simultaneously (Han and Shin, 2004; Lee et al., 2008). However, nutrients stored in food waste are in the form of macromolecules (such as starch and

* Corresponding author. Tel.: +86 13738020870; fax: +86 571 86919158.
E-mail address: dr_lyf@nefu.edu.cn (Y.F. Li).

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protein) which have to be broken into utilizable forms (glucose and free amino nitrogen) before utilized by microorganisms for fermentative hydrogen production (Han et al., 2015a; Lay et al., 2005). Although some reported pretreatments were able to convert the macromolecules into utilizable forms, various inhibitory products (such as furfural) for fermentative hydrogen production could also be produced (Gioannis et al., 2013; Sagnak et al., 2011). Enzymatic hydrolysis could release the nutrients (glucose and free amino nitrogen) from food waste with advantage of high hydrolysis speed which would be a promising way. However, there is little information about fermentative hydrogen production from enzymatic hydrolysis of food waste.

Therefore, this study investigated the feasibility of a combination bioprocess of solid-state fermentation (SSF) and fermentative hydrogen production from food waste. Food waste was first utilized in solid-state fermentation by *Aspergillus awamori* and *Aspergillus oryzae* to produce glucoamylase and protease, respectively, which were used to hydrolyze food waste to obtain the food waste hydrolysate rich in glucose and free amino nitrogen (FAN). Then, the food waste hydrolysate was used as substrate for fermentative hydrogen production by heat pretreated sludge. The effect of the glucose concentration in the food waste hydrolysate on the performance of hydrogen production was also investigated. The data obtained from this study could provide basic information for the large scale of hydrogen production from food waste.

2. Methods

2.1. Microorganisms

A. awamori and *A. oryzae* were purchased from Shanghai Beinuo Biotechnology Co., Ltd. and used for production of glucoamylase and protease, respectively. They were prepared according to the previous publication (Pleissner et al., 2014) and stored at −80 °C until used for solid-state fermentation.

The anaerobic sludge was collected from a local municipal wastewater treatment plant and screened by a sieve (diameter: 2 mm) to eliminate large particulate materials. It was heat pretreated in a water bath at temperature of 100 °C for 6 h to inhibit the methane-producing bacteria activity (Ren et al., 2012). The heat pretreated sludge was then used as inoculum for fermentative hydrogen production.

2.2. Raw material

The food waste used in this study was collected from the canteen of Hangzhou Dianzi University. After removing the undesirable materials (such as bones and shells) by hand, food waste was mixed thoroughly in a blender. The characteristics of food waste were analyzed according to Standard Method (APHA et al., 1998) and shown in Table 1.

2.3. Solid-state fermentation by A. awamori and A. oryzae and food waste hydrolysate

The blended food waste (15 g) was spread in a Petri dish with 1 ml of spore solution of *A. awamori* (4×10^6 spores/ml) or *A. oryzae* (1×10^6 spores/ml) for solid-state fermentations, respectively. The mixtures were cultured in an incubator at 30 °C for 4 days to achieve the fermented solid mashes which are abundant in glucoamylase and protease, respectively.

Table 1
Characteristics of food waste used in this study (per 100 g food waste).

Component	Value (g)	Component	Value (g)
Moisture	78.3 ± 1.5	Starch	40.6 ± 0.6
Total solid (TS)	19.6 ± 1.2	Protein	10.5 ± 0.5
Volatile solid (VS)	17.8 ± 0.9	Total phosphorus	1.6 ± 0.06
Carbohydrate	42.7 ± 0.8	Lipid	6.2 ± 0.7

The fermented solid mashes were transferred into a 3 L bioreactor which was equipped with automatic temperature controller and stirrer for enzymatic hydrolysis. The agitation speed was 500 rpm. Various food waste mass ratios (4%, 6%, 8% and 10%) were added into bioreactor when the temperature reached 55 °C. Samples were taken every hour for the glucose and FAN measurements. The resultant broth was centrifuged at 10,000 rpm for 30 min and filtered by Whatman No. 1 filter paper to obtain the food waste hydrolysate.

2.4. Fermentative hydrogen production

Fermentative hydrogen production was carried out at 37 °C in a 3 L fermentor with working volume of 500 ml using food waste hydrolysate. The initial volatile suspended solid (heat pretreated sludge) concentration of the seed inoculum was 4.3 g/L. The food waste hydrolysate was filtered by 0.2 μm PTFE membrane filter before adding to the fermentor. The broth was sparged with 0.5 vvm N_2 to achieve the anaerobic condition for fermentative hydrogen production and agitated at 300 rpm by magnetic stirrer. The pH of the fermentation was automatically maintained at 4.0–4.6 by adding 5 M $NaHCO_3$ and 0.005 M H_2SO_4. Samples from the culture medium and headspace were regularly taken for the glucose and hydrogen analysis.

2.5. Analytical methods

The activities of glucoamylase and protease were determined as described by Du et al. (2008). One unit (U) of glucoamylase or protease was the amount of enzyme required to produce 1 mg glucose per minute. The procedure of glucose and free amino nitrogen (FAN) measurements were described by our earlier publication (Pleissner et al., 2013).

The gas products (mainly H_2 and CO_2) were analyzed by gas chromatography (GC) equipped with a thermal conductivity detector (TCD) and a stainless steel column (2 m × 5 mm) filled with Porapak Q (50–80 meshes). Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. A dose of injected sample was 0.5 ml each time.

The soluble microbial products (SMPs) in the fermentation solution were analyzed by another GC using flame ionization detector (FID). A 2-m stainless steel column was packed with the supporter GDX-103 (60–80 meshes). The temperatures of the injection port, oven and detector were 220, 190 and 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 30 ml/min.

3. Results and discussion

3.1. Fermentative feedstock production from enzymatic hydrolysis of food waste

Fig. 1 shows the profiles of glucose and free amino nitrogen (FAN) concentrations in the hydrolysate with initial food waste mass ratio of 4–10% (w/v). It was observed that the glucose and FAN concentrations in the hydrolysate increased with food waste mass ratio increased from 4% to 10%. The maximum glucose concentration of 36.9 g/L and FAN concentration of 361.3 mg/L were obtained at the food waste mass ratio of 10%. It was found that the duration of the hydrolysis reaction was similar to the hydrolysis of bakery waste as reported in our previous study (Zhang et al., 2012). But, the maximum glucose yield obtained from this study is 0.389 g glucose/g substrate (food waste mass ratio of 4%) which is higher than that

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