Journal of Cleaner Production xxx (2015) 1–6

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

A combined bioprocess based on solid-state fermentation for dark fermentative hydrogen production from food waste

Wei Han [a], Min Ye [a], Ai Jun Zhu [a], Jin Gang Huang [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

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
15 August 2015
Accepted 16 August 2015
Available online xxx

Keywords:
Solid-state fermentation
Food waste hydrolysate
Dark fermentation
Carbon recovery

ABSTRACT

In this study, the feasibility of hydrogen production from food waste using a combined bioprocess of solid-state fermentation (SSF) and dark fermentation was investigated. Food waste was first used to produce glucoamylase and protease enzymes via SSF using *Aspergillus awamori* and *Aspergillus oryzae*. The produced enzymes were then used to release glucose and free amino nitrogen (FAN) from the food waste. Both glucose and FAN increased with increasing of food waste mass ratio from 5% to 15% (w/v). However, the glucose yield and starch conversion decreased from 0.434 g glucose/g food waste and 96.2% to 0.307 g glucose/g food waste and 68.1%, respectively, when the food waste mass ratio increased from 5% to 15% (w/v) probably because of the deactivation caused by high temperature or protease. The food waste hydrolysate was then used as the nutrient source for dark fermentative hydrogen production by *Biohydrogenbacterium* R3. The best hydrogen yield of 52.4 mL H_2/g food waste was achieved at food waste mass ratio of 5% (w/v). The modified Gompertz model could be used to describe the cumulative hydrogen production for the food waste hydrolysate. The results demonstrated a promising combined bioprocess for hydrogen production from food waste.

1. Introduction

Due to the emission of greenhouse gas and depletion of fossil fuel, interest in the development of sustainable and non-pollution energy is increasing (Urbaniec et al., 2010). Hydrogen is considered to be one of the most promising alternative energy carriers of the future since it is clean and renewable. Furthermore, the energy yield of hydrogen is 122 kJ/g which is 2.75 times higher than that of fossil fuel (Sharma et al., 2011). Biological hydrogen production which could deal with the conversion of low cost residues or organic waste/wastewater to hydrogen has attracted considerable attention by the researchers (Miltner et al., 2010). Dark fermentation and photosynthesis are the two main biological methods for hydrogen production (Lay et al., 2012). Dark fermentation could produce hydrogen without light limitation which is considered to be a promising way (Sagnak et al., 2011). However, it is difficult to realize dark fermentative hydrogen production for industrial scale since the problem of low hydrogen yield and high cost should be overcome (Han et al., 2012). Waste and wastewater show great potential for economical production of hydrogen because producing a valuable product from waste could reduce disposal cost and enhance the economic benefit (Kim et al., 2009; Urbaniec and Grabarczyk, 2014).

Every year, nearly 1.3 billion ton of food waste is produced which accounts for 40% of the total municipal solid waste. The food waste consists of around 60% carbohydrates and 20% proteins which make it a promising source of nutrient for the production of high value products, such as hydrogen and methane (Han et al., 2015a). However, the nutrients need to be broken into fermentable forms before utilized for hydrogen production. Therefore, the hydrolysis of macromolecules (starch and protein) to micromolecules (glucose and free amino nitrogen) is considered to be the limiting step for dark fermentative hydrogen production (Zhang et al., 2012). Physical and chemical pre-treatments could convert the macromolecules into utilizable forms, but various inhibitory products (such as furfural) could also be produced (Gustavo et al., 2009). Enzymatic hydrolysis would be a promising way because it could release the nutrients (glucose and free amino nitrogen) from food waste with advantage of high hydrolysis speed (Leung et al., 2012). However, studies on dark fermentative hydrogen production from enzymatic hydrolysis of food waste are limited.

* Corresponding author. Tel.: +86 13903614476; fax: +86 451 82192120.
** Corresponding author. Tel.: +86 13738020870; fax: +86 571 86919158.
E-mail addresses: hzhao@hdu.edu.cn (H.T. Zhao), dr_lyf@nefu.edu.cn (Y.F. Li).

http://dx.doi.org/10.1016/j.jclepro.2015.08.072
0959-6526/

Therefore, the purpose of this study is to investigate the potential of a combined bioprocess of solid-state fermentation (SSF) and dark fermentation for hydrogen production from food waste. *Aspergillus awamori* and *Aspergillus oryzae* were utilized to produce glucoamylase and protease via SSF, respectively. Food waste at three different values of the mass ratio was hydrolyzed by the obtained enzymes to produce the food waste hydrolysate rich in glucose and free amino nitrogen (FAN). The food waste hydrolysate was then used as substrate for dark fermentative hydrogen production by *Biohydrogenbacterium* R3. The effect of the glucose concentration in the food waste hydrolysate on the performance of hydrogen production was also examined.

2. Materials and methods

2.1. Microorganisms

The microorganisms of *A. awamori* and *A. oryzae* used in this study were purchased from Shanghai Beinuo Biotechnology Co., Ltd. Prior to the experimental work, they were prepared according to the previous publication (Pleissner et al., 2014) and stored at −80 °C until used for SSF. The hydrogen-producing bacteria of *Biohydrogenbacterium* R3 were isolated from anaerobic sludge of a fermentative hydrogen production fermenter and stored in cryopreservation vials at −80 °C. The 16S rRNA gene sequence of *Biohydrogenbacterium* R3 has been deposited in the NCBI nucleotide sequence database with the accession number of AY363375 (Ren et al., 2012). The defined medium used for *Biohydrogenbacterium* R3 culture and fermentative hydrogen production was prepared according to Ren et al. (2012) except the carbon and nitrogen sources were replaced with food waste hydrolysate. The microorganism was cultivated in a 250 mL shake flask containing 150 mL defined medium at 37 °C. In order to ensure anaerobic condition, the culture flask was purged with N_2 for 10 min prior to inoculation. The rotation speed of incubator for seed culture was 200 rpm. The active culture in the mid-log phase was stored at −80 °C and used as inoculum for fermentative hydrogen production.

2.2. Solid-state fermentation and food waste hydrolysate

Food waste was collected from the canteen of Hangzhou Dianzi University and immediately brought to laboratory for processing. It was mixed thoroughly in a blender after removing bones and shells by hand. Table 1 showed the composition of food waste which was measured according to Standard Method (APHA et al., 1998). Solid-state fermentation was performed in two Petri dishes containing 15 g blended food waste with 1 mL of spore solution of *A. awamori* (4×10^6 spores per mL) and *A. oryzae* (1×10^6 spores per mL), respectively. The plates were then incubated under static condition at 30 °C for 4 days to obtain the fermented solid mashes of glucoamylase and protease.

Enzymatic hydrolysis of food waste was carried out in a 3 L bioreactor with the fermented solid mashes at agitation speed of 500 rpm and temperature of 55 °C. The total volume of the blend was adjusted to 1 L by addition of demineralized water. After the hydrolysis process, solid content of the paste was centrifuged at 10,000 rpm for 30 min and filtered by Whatman No. 1 filter paper to produce the food waste hydrolysate. Small amount of oil was also removed. The aqueous food waste hydrolysate was kept frozen at −20 °C until used as the substrate for dark fermentative hydrogen production.

The solid-state fermentation for enzymes production and subsequent enzymatic hydrolysis of food waste were performed in triplicate.

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

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

2.3. Dark fermentative hydrogen production

Dark fermentative hydrogen production was performed at 37 °C in a 3 L fermentor with working volume of 500 mL using food waste hydrolysate. The inoculum size of bacteria (*Biohydrogenbacterium* R3) was 2% (v/v). Dark fermentation was conducted with external N_2 sparging into the broth at a rate of 0.5 vvm and agitated at 300 rpm by magnetic stirrer. Fermentation pH was automatically controlled between 4.0 and 4.6 using 5 M $NaHCO_3$ and 0.005 M H_2SO_4. The fermentative hydrogen production from food waste hydrolysate was carried out in duplicate.

2.4. Analytical methods

The cell dry mass (CDM) was determined from the steady state of the optical densities at each initial glucose concentration using Eq. (1) for *Biohydrogenbacterium* R3. The procedure of glucose and FAN measurements were described by our earlier publication (Pleissner et al., 2013; Han et al., 2015b).

$$\mathrm{CDM(g/L)} = (0.258 \times \mathrm{Optical\ Density_{600}}) + 0.001 \quad (1)$$

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) (including ethanol, acetate, butyrate and propionate) 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 °C, 190 °C and 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min.

2.5. Kinetic analysis

The starch and protein hydrolysis by glucoamylase and protease with different initial food waste mass ratios (5%, 10% and 15%) (w/v) followed the first-order (Eq. (2)) reaction rate equation.

$$C(t) = C_\infty\left(1 - \mathrm{e}^{-kt}\right) \quad (2)$$

where $C(t)$ is the glucose/FAN concentration at time t, C_∞ is the saturation concentration of glucose/FAN and k is the kinetic constant (1/s).

A modified Gompertz equation (Eq. (3)) was used to describe the cumulative hydrogen production curve.

$$\mathrm{CHP} = \mathrm{Pexp}\{-\exp[(R_m e/\mathrm{P})(\lambda - t) + 1]\} \quad (3)$$

where CHP is the cumulative hydrogen production (L), P is the maximum potential hydrogen production (L), R_m is the maximum hydrogen production rate (L/h), λ is the lag-phase time (h), e is

Please cite this article in press as: Han, W., et al., A combined bioprocess based on solid-state fermentation for dark fermentative hydrogen production from food waste, Journal of Cleaner Production (2015), http://dx.doi.org/10.1016/j.jclepro.2015.08.072