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Continuous biohydrogen production from waste bread by anaerobic sludge

Wei Han [a], Jingang Huang [a], Hongting Zhao [a], Yongfeng 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

- Continuous biohydrogen production from waste bread by sludge was performed.
- The optimal hydrogen production rate of 7.4 L/(Ld) was obtained.
- Ethanol and carbon dioxide accounted for the largest parts of consumed carbon.
- It could be calculated that 1 g waste bread could produce 109.5 mL hydrogen.

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ABSTRACT

In this study, continuous biohydrogen production from waste bread by anaerobic sludge was performed. The waste bread was first hydrolyzed by the crude enzymes which were generated by *Aspergillus awamori* and *Aspergillus oryzae* via solid-state fermentation. It was observed that 49.78 g/L glucose and 284.12 mg/L free amino nitrogen could be produced with waste bread mass ratio of 15% (w/v). The waste bread hydrolysate was then used for biohydrogen production by anaerobic sludge in a continuous stirred tank reactor (CSTR). The optimal hydrogen production rate of 7.4 L/(Ld) was achieved at chemical oxygen demand (COD) of 6000 mg/L. According to the results obtained from this study, 1 g waste bread could generate 0.332 g glucose which could be further utilized to produce 109.5 mL hydrogen. This is the first study which reports continuous biohydrogen production from waste bread by anaerobic sludge.

1. Introduction

Hydrogen is regarded as an ideal energy source in the future since it is clean and renewable (Show et al., 2012). Furthermore, the energy density of hydrogen is 122 kJ/g which is 2.75 times higher than that of fossil fuel (Urbaniec and Grabarczyk, 2014). Currently, more than 70% of hydrogen is generated by steam reforming of natural gas which is not a clean and sustainable way because fossil fuels are used as feedstock (Acar and Dincer, 2015). Dark fermentation, which could produce hydrogen with mild operation, is considered to be an alternative way (Logan et al., 2002). At present, dark fermentative hydrogen production is still under investigation and could not apply into industrial application due to the low hydrogen yield and high cost (Guerrro-Lemus and Martinez-Duart, 2010). Utilization of raw organic waste (such as food waste) as feedstock for biohydrogen production seems to be a promising solution (Kim et al., 2011; Redondas et al., 2012).

Food waste, which is one of the largest parts of the municipal solid waste, is considered to be a serious global issue (De Gioannis et al., 2013; Van-Ginkel et al., 2005). It is reported that around 1.3 billion of food waste could be produced per year in the world (Alibardi and Cossu, 2015). So, it is important to take actions to alleviate the social, economic and environmental problems caused by the food waste (Elbeshbishy et al., 2011). Waste bread, which contains a great amount of nutrients for biofuels production, is one of the most common food wastes in Asian and Europe countries (Lee et al., 2010). So, utilization of no-value waste bread for valuable products, such as hydrogen, has attracted great attentions (Han et al., 2016a). However, compared to the soluble waste, it is difficult to directly use waste bread for hydrogen production since the solid waste bread should be solubilized to enhance the nutrient conversion efficiency (Alibardi and Cossu, 2016; Castillo-Hernandez et al., 2015). Furthermore, the nutrients stored in the waste bread are in the form of the

* Corresponding author.
E-mail address: dr_lyf@nefu.edu.cn (Y. Li).

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macromolecules (such as starch and protein) which have to be broken into monomers (glucose and free amino nitrogen) before being used by microorganisms for hydrogen production (Han et al., 2015b). Hydrolysis of macromolecules is considered to be the rate-limiting step in the overall biohydrogen production process (Kim et al., 2010). In light of the above technical analysis, there is little information about biohydrogen production from waste bread.

In this study, a novel bioprocess of hydrogen production from waste bread was developed. The waste bread was hydrolyzed by the crude enzymes which were generated by *Aspergillus awamori* and *Aspergillus oryzae* via solid state fermentation. The waste bread hydrolysate was then used as substrate for biohydrogen production by heat pretreated sludge in a continuous stirred tank reactor (CSTR). The effect of the chemical oxygen demand (COD) on the performance of the CSTR was also examined.

2. Materials and methods

2.1. Raw material and microorganism

The waste bread used in this study was provided by the local Wumei Supermarket and immediately brought to laboratory for processing. In order to get the smaller physical size of the substrate, the waste bread was grounded triplicate by the food blender. The pretreated waste bread was then stored at −4 °C. The characteristics of waste bread (Table 1) were measured according to Standard Method (APHA et al., 1998).

Microorganism strain of *A. awamori* and *A. oryzae* used in this study were purchased from Shanghai Beinuo Biotechnology Co., Ltd. and utilized in solid-state fermentation to produce crude enzymes (glucoamylase and protease). The hydrogen-producing sludge used in this study was collected from a local municipal wastewater treatment plant (Hangzhou, Zhi Jiang Wastewater Treatment Plant). The sludge was heat pretreated in a water bath at 100 °C for 6 h before inoculation to inhibit the activity of methane-producing bacteria. The volatile suspended solids (VSS) concentration of the seed inoculum was 3.6 g/L.

2.2. Solid-state fermentation and waste bread hydrolysate

Solid-state fermentation was carried out in two Petri dishes containing 5 g ground waste bread with 1 mL of cryopreserved spores of *A. awamori* (4×10^6 spores/mL) or *A. oryzae* (1×10^6 spores/mL) spreading evenly on waste bread. The mixtures were cultured in an incubator without stirring at 30 °C for 96 h to obtain the fermented solid mashes which were rich in glucoamylase and protease, respectively.

Fermented mashes obtained from solid-state fermentation 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. Waste bread (15%, w/v) was added into bioreactor when the temperature reached 55 °C. The conditions for solid-state fermentation and enzymatic hydrolysis have been approved to be optimal by our previous publications (Han et al., 2015a, 2016b). Samples were taken every hour to analyze the glucose and free amino nitrogen (FAN) production. The resultant broth was centrifuged at 10,000 rpm for 30 min and filtered by Whatman No. 1 filter paper to obtain the waste bread hydrolysate which was used as substrate for subsequent biohydrogen production. In order to investigate the effect of COD concentration on biohydrogen production, the waste bread hydrolysate was diluted by distilled water to certain COD concentration (2000–8000 mg/L).

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

Component	Value (g)	Component	Value (g)
Moisture	24.3 ± 0.8	Starch	48.6 ± 1.5
Protein ($N \times 5.7$)	9 ± 0.1	Phosphorous	0.08 ± 0.01
Total organic nitrogen	1.5 ± 0.1	Ash	2.3 ± 0.2

2.3. Continuous stirred tank reactor

Continuous biohydrogen production from waste bread hydrolysate was performed in a 3.5 L continuous stirred tank reactor (CSTR) with a working volume of 2.8 L. The CSTR was constructed by transparent plexiglas with a gas–liquid–solid separating device. The influent flow rate was controlled by a feed pump to regulate the HRT at 6 h in the CSTR. The generated gas was collected with a waterlock and measured by a wet gas meter. Fermentation pH was kept above 4 by using 5 M NaOH solution. The CSTR was operated in batch mode until gas was produced. Reactor was then switched to continuous mode (HRT = 6 h) with COD concentration of 2000 mg/L until steady state conditions were obtained. Steady state conditions were based on the constant products with a variation of less than 10%. The CSTR was sampled at a fixed COD over at least three days. The COD was then increased to the next level and the CSTR was operated until steady state conditions were achieved as noted above. All the samples obtained from this study were analyzed at least in triplicate.

2.4. Analytical methods

The glucose concentration produced in the waste bread hydrolysate was quantified using the high performance liquid chromatography (HPLC) system which was equipped with a BIO-RAD column (HPX-87H), a refractive index (RI) detector and a photodiode array (PDA) analyzer. The nynhidrin reaction method was used to analyze the free amino nitrogen (FAN) production in the waste bread hydrolysate. The detailed procedure of glucose and FAN measurements were described by our earlier publications (Han et al., 2015a, 2016b).

COD was monitored and measured daily according to Standard Methods (APHA et al., 1998). The produced gas was analyzed using a 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. Gas samples were compared to four standard levels of pure gas injections. Based on the percentage of hydrogen in the produced gas, the hydrogen yield could be calculated. 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 °C, 190 °C and 220 °C, respectively. The carrier gas was nitrogen at a flow rate of 30 mL/min. Liquid samples were refrigerated before analysis and compared to five standard levels of pure chemical injections.

3. Results and discussion

3.1. Enzymatic hydrolysis of waste bread

Fig. 1 showed the production of glucose and FAN in the enzymatic hydrolysis of waste bread. It was observed that both the glucose and FAN increased in the waste bread hydrolysate with time. The maximum glucose of 49.78 g/L and FAN of 284.12 mg/L production were obtained at 24 h, respectively. Linear regression