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Biohydrogen production from food waste hydrolysate using continuous mixed immobilized sludge reactors

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HIGHLIGHTS

- The effect of packing ratio on biohydrogen production was investigated.
- The substrate loading rate on biohydrogen production was investigated.
- The food waste hydrolysate was used as feedstock for biohydrogen production.
- The best hydrogen production rate of 353.9 ml/h/L was obtained.

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ABSTRACT

A continuous mixed immobilized sludge reactor (CMISR) using activated carbon as support carrier for dark fermentative hydrogen production from enzymatic hydrolyzed food waste was developed. The effects of immobilized sludge packing ratio (10–20%, v/v) and substrate loading rate (OLR) (8–40 kg/m^3/d) on biohydrogen production were examined, respectively. The hydrogen production rates (HPRs) with packing ratio of 15% were significantly higher than the results obtained from packing ratio of 10% and 20%. The best HPR of 353.9 ml/h/L was obtained at the condition of packing ratio = 15% and OLR = 40 kg/m^3/d. The Minitab was used to elicit the effects of OLR and packing ratio on HPR (Y) which could be expressed as $Y = 5.31$ OLR + 296 packing ratio + 40.3 ($p = 0.003$). However, the highest hydrogen yield (85.6 ml/g food waste) was happened at OLR of 16 kg/m^3/d because of H_2 partial pressure and oxidization/reduction of NADH.

1. Introduction

Environmental pollution caused by the use of fossil fuels makes it necessary to find alternative energy sources that are clean and efficient (Sharma et al., 2011). Hydrogen satisfies the above requirements when it is combusted as a fuel (Park et al., 2010; Zhang et al., 2003) since only water is produced with energy yield of 122 kJ/g (Wang and Wan, 2009). However, conventional hydrogen production processes are neither sustainable nor environmentally friendly because fossil fuels are used as the raw materials (Chu et al., 2012; Van Ginkel and Logan, 2005). Dark fermentation is reported to be an economical and sustainable way which could produce hydrogen and treat waste/wastewater simultaneously (Kim and Kim, 2013).

Food waste is a promising raw material for biofuels production because of its high organic content and availability. It mainly consists of starch, protein and fat which are good carbon sources for biohydrogen production. Fermentative bacteria hydrolyze and ferment carbohydrates, protein and lipids to volatile fatty acids which are then further converted into acetate, carbon dioxide and hydrogen by acetogenic bacteria. Hydrogen and ATP are produced by fermentative bacteria such as *Clostridium sp*. during the degradation process. The limiting factor for biohydrogen production from food waste is the hydrolysis rate (Yasin et al., 2013). Kim et al. (2009) found that heat-pretreated food waste could celebrate the hydrolysis rate of food waste and produce high biohydrogen yield when compared to untreated food waste. Similarly, sonication of food waste with heat and without inoculum was applied by Elbeshbishy et al. (2011) for biohydrogen production. These researches showed that pretreatment of food waste could enhance biohydrogen production efficiency and therefore can be regarded

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as an important parameter influencing biohydrogen production. Enzymatic hydrolysis could release nutrients (such as glucose and free amino nitrogen) from food waste with advantage of high hydrolysis rate and mild reaction conditions (Lau et al., 2014). At present, using food and bakery waste hydrolysates produced by enzymatic hydrolysis for biodiesel and succinic acid production have been reported (Pleissner et al., 2013; Leung et al., 2012). However, studies on dark fermentative hydrogen production from enzymatic hydrolyzed food waste are limited.

On the other hand, it is of great importance to maintain a sufficient amount of hydrogen-producer in the biohydrogen production system to ensure a high hydrogen production rate (HPR) while operating at high organic loading rate (OLR) or low hydraulic retention time (HRT) (Barros et al., 2010). However, it is difficult to achieve that in the suspended sludge system (such as continuous stirred tank reactor, CSTR) since the washout of biomass or products inhibition usually occurs at high OLR (Jung et al., 2011; Gustavo et al., 2009). Attempts to enhance biomass retention time by physical immobilization of sludge are applied and some outstanding H_2 productivities reported to date have been achieved using immobilized reactors (Sharma and Li, 2009). Lin et al. (2011) operated a draft tube anaerobic fluidized-bed reactor (AFBR) containing silicone gel as support carrier to immobilize hydrogen-producing bacteria with the highest HPR of 94.5 ml/h/L. Recently, we have successfully developed a novel continuous mixed immobilized sludge reactor (CMISR) using activated carbon as support carrier for continuous hydrogen production (Han et al., 2012). Nevertheless, the packing ratio of the activated carbon used for sludge immobilization inevitably occupies significant space in the CMISR which could limit sludge density and possibly generate mass transfer barriers. Therefore, detailed information regarding design of CMISR (such as packing ratio) for effective hydrogen production needs to be further investigated.

This study demonstrates stable and efficient hydrogen production in the CMISRs with packing ratios of 10%, 15% and 20% (v/v), respectively. Solid-state fermentation was carried out by *Aspergillus awamori* and *Aspergillus oryzae* from food waste to generate glucoamylase and protease which were then used to hydrolyze food waste. Food waste hydrolysate was used as substrate and diluted by distilled water to certain organic loading rate (OLR) (8–40 kg/m^3/d) for continuous hydrogen production. The CMISRs with different packing ratios were operated at various OLRs to investigate the performance of hydrogen production. The data obtained from this study was expected to provide basic information for the bioreactor design of practical application.

2. Methods

2.1. Microorganisms and feeding

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 (SSF) to produce glucoamylase and protease, respectively. Prior to experimental work, *A. awamori* and *A. oryzae* spores were prepared according to Du et al. (2008) and Wang et al. (2005) and stored at −20 °C until used for SSF. The H_2-producing sludge used in this study was collected from a local municipal wastewater treatment plant (Hangzhou, Zhi Jiang Wastewater Treatment Plant). The sludge was aerated for 30 days before inoculation to inhibit the methane-producing bacteria activity. The volatile suspended solids (VSS) concentration of the seed inoculum was 8.6 g/L.

Food waste used in this study was collected from the canteen of Hangzhou Dianzi University campus and immediately brought to laboratory for processing. It was mainly consisted of rice, noodles, vegetables and meat after removing bones and shells by hand. The food blender was used to grind the food waste which was then stored at −4 °C. The characteristics of food waste (Table 1) were measured according to Standard Method (APHA, 1998).

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

Component	Value (g)	Component	Value (g)
Moisture	80.4 ± 1.2	Starch	30.6 ± 0.6
Total solid (TS)	19.6 ± 1.2	Protein	11.2 ± 0.4
Volatile solid (VS)	17.8 ± 0.9	Total phosphorus	1.6 ± 0.04
Carbohydrate	42.7 ± 0.8	Lipid	6.7 ± 0.8

2.2. Solid-state fermentation and food waste hydrolysate

Solid-state fermentation was carried out in two Petri dishes containing 15 g food waste 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 food waste. The mixtures were cultured in an incubator without stirring at 30 °C for 96 h to obtain the fermented solid mashes which are rich in glucoamylase and protease, respectively.

Fermented mashes obtained from solid-state fermentation were transferred into a 3 L bioreactor which equipped with automatic temperature controller and stirrer for enzymatic hydrolysis. The agitation speed was 500 rpm. Food waste (20%, w/v) was added into bioreactor when the temperature reached 55 °C. 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 food waste hydrolysate which was used as substrate for subsequent biohydrogen production. In order to investigate the effect of substrate concentration on biohydrogen production, the food waste hydrolysate was diluted by distilled water to certain organic loading rate (8–40 kg/m^3/d).

2.3. Continuous mixed immobilized sludge reactor (CMISR)

Three identical continuous mixed immobilized sludge reactors (CMISRs) with effective volume of 3.2 L were used in this study for biohydrogen production from food waste hydrolysate. The CMISRs were initially packed with activated carbon as support carrier for sludge immobilization with packing ratio of 10%, 15% and 20%, respectively. The particles of activated carbon were sieved for uniformity of approximately 1.5–2 mm in diameter. The main physical characteristics of granular activated carbon are as follows: media real density = 1420 g/L; surface area = 1200–1350 m^2/g; bulk density = 450–500 g/L (Hainan Wen Chang Qiu Chi Activated Carbon Co., Ltd.). The CMISR was constructed with 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 CMISR. The generated gas was collected with a waterlock and measured by a wet gas meter. Fermentation pH was controlled above 4 by using 5 M NaOH solution. Each CMISR was operated in batch mode until gas was produced. Reactors were then switched to continuous mode (HRT = 6 h) with OLR of 8 kg/m^3/d until steady state conditions were obtained. Steady state conditions were based on the constant products with a variation of less than 10%. Each CMISR was sampled at a fixed OLR over at least 5 days. The OLR was then increased to the next level and the reactor 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 food waste hydrolysate was quantified using the high performance liquid