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Biohydrogen production in the suspended and attached microbial growth systems from waste pastry hydrolysate

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HIGHLIGHTS

- This is the first study which reports biohydrogen production from waste pastry.
- Effects of HRT on hydrogen production in the CSTR and CMISR were compared.
- One gram pastry could be converted to 0.345 g glucose in the pastry hydrolysate.
- One gram waste pastry could produce 83.29 mL hydrogen in the CSTR.
- This study provides a novel way for biohydrogen production from organic solid wastes.

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ABSTRACT

Waste pastry was hydrolyzed by glucoamylase and protease which were obtained from solid state fermentation of *Aspergillus awamori* and *Aspergillus oryzae* to produce waste pastry hydrolysate. Then, the effects of hydraulic retention times (HRTs) (4–12 h) on hydrogen production rate (HPR) in the suspended microbial growth system (continuous stirred tank reactor, CSTR) and attached microbial growth system (continuous mixed immobilized sludge reactor, CMISR) from waste pastry hydrolysate were investigated. The maximum HPRs of CSTR (201.8 mL/(h·L)) and CMISR (255.3 mL/(h·L)) were obtained at HRT of 6 h and 4 h, respectively. The first-order reaction could be used to describe the enzymatic hydrolysis of waste pastry. The carbon content of the waste pastry remained 22.8% in the undigested waste pastry and consumed 77.2% for carbon dioxide and soluble microbial products. To our knowledge, this is the first study which reports biohydrogen production from waste pastry.

1. Introduction

Fossil fuels, which are responsible for fulfilling 80% of global energy demand, have played a crucial role in the development of industry (Nong et al., 2015; Konur, 2012). However, due to gradual depletion and environmental pollution, the international community has shown an increasing interest in sustainable and clean energy sources over recent years (Sivagurunathan et al., 2016; Ljunggren and Zacchi, 2010). Hydrogen is considered to be one of the most promising future energy carriers since it is renewable and produces only water when combusted (Clark et al., 2015; Ghanbarian and Kermani, 2016). Moreover, the energy yield of hydrogen is 122 kJ/g which is 2.75 times greater than that of hydrocarbon fuels (Tawfik et al., 2011).

Generally, the methods for hydrogen production could be categorized into physicochemical and biological processes (Panagiotopoulos et al., 2015). Conventional physicochemical processes (such as steam reforming of hydrocarbons and coal gasification) are neither sustainable nor environmental friendly because fossil fuels are used as substrate (Singh et al., 2015; Urbaniec and Grabarczyk, 2009). In contrast, biological processes seem to be more attractive because a wide variety of organic waste materials could be used as substrate and the processes could be operated under room temperature and pressure conditions (Hwang et al., 2011). In particular, dark fermentative hydrogen production is regarded as a commercial process since it could achieve high hydrogen production and ignore the limitation of light (Soltani et al., 2015; Hu and Chen, 2007).

Dark fermentation could utilize a wide range of organic waste or wastewater as substrate for hydrogen production (Urbaniec and Grabarczyk, 2014). Waste pastry could be a promising substrate for dark fermentative hydrogen production since it could

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reduce the unit hydrogen production cost and recycle the organic municipal solid waste (Ma et al., 2016). However, it is difficult to directly use waste pastry as substrate for biohydrogen production because the nutrients stored in the waste pastry are in the forms of macromolecules (starch and protein) which have to be converted into micromolecules (glucose and free amino nitrogen, FAN) before used by hydrogen-producing microorganisms (Zhou et al., 2013). Meanwhile, the hydrolysis is considered to be the rate-limiting step for biohydrogen production from waste pastry (Panda et al., 2016). So, the pretreatment is regarded as a crucial step for biohydrogen production from organic solid wastes. Simple physical pretreatment could increase the solubility and biodegradability of substrate, while a great amount of nutrients remained in the organic solid wastes. Chemical pretreatment could hydrolyze the macromolecules into micromolecules, but the inhibitors (such as furfural) for further biohydrogen production could be also produced (Panagiotopoulos et al., 2010). Enzymatic hydrolysis could be a promising way to release nutrients from waste pastry due to the high hydrolysis speed and conversion rate. However, there is little information for biohydrogen production from enzymatic hydrolysis of waste pastry.

Generally, continuous biohydrogen production could be achieved in the suspended microbial growth system and attached microbial growth system (Barros et al., 2011). Continuous stirred tank reactor (CSTR) is one of the most common suspended microbial growth systems for biohydrogen production. However, CTSR could encounter the problem of sludge wash-out at high substrate concentration or low hydraulic retention time (HRT), and therefore limit the hydrogen production efficiency (Peintner et al., 2010). It is reported that the attached microbial growth system could solve the above problem (Lin et al., 2006). In our previous study, we successfully developed a novel continuous mixed immobilized sludge reactor (CMISR) which could effectively keep high biomass concentration and hydrogen production at high organic loading rate (Han et al., 2012). However, study on comparison of hydrogen production in the CSTR and CMISR, especially using waste pastry hydrolysate as substrate, is limited.

Therefore, the objective of this study is to make a direct comparison of hydrogen production in the CSTR and CMISR from enzymatic hydrolysis of waste pastry. The waste pastry was first hydrolyzed by the glucoamylase and protease which were produced by fungi (*Aspergillus awamori* and *Aspergillus oryzae*) via solid state fermentation. The produced waste pastry hydrolysate was then used as substrate for biohydrogen production. The effects of hydraulic retention times (HRTs) on the performance of hydrogen production in the CSTR and CMISR from waste pastry hydrolysate were also investigated. It is hoped that the obtained results could provide basic information for continuous biohydrogen production from waste pastry in practical application.

2. Material and methods

2.1. Raw material and microorganisms

The waste pastry used in this study was provided by local Supermarket. The composition of waste pastry was analyzed according to the Standard Method (APHA, 2005) and summarized in Table 1. The collected waste pastry was first ground into smaller physical size and then stored at −4 °C until used for solid state fermentation and enzymatic hydrolysis.

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

Component	Value (g)	Component	Value (g)
Moisture	32.4 ± 1.6	Carbohydrate	30.2 ± 1.7
Starch (dry basis)	40.2 ± 2.3	Protein (N × 5.7) (dry basis)	6.8 ± 0.4
Phosphorous (dry basis)	1.3 ± 0.1	Ash (dry basis)	2.1 ± 0.3

Microorganisms of *A. awamori* and *A. oryzae* were utilized to produce glucoamylase and protease via solid state fermentation. The hydrogen-producing sludge was collected from a local municipal wastewater treatment plant and heat pretreated in a water bath at 100 °C for 6 h to inactivate the hydrogen consumers before inoculation. The volatile suspended solids (VSS) concentration of the seed inoculum was 6.3 g/L.

2.2. Solid state fermentation and enzymatic hydrolysis

Five gram pretreated waste pastry was added into the Petri dishes. Then, 1 mL of cryopreserved spores solution of *A. awamori* (4×10^6 spores/mL) or *A. oryzae* (1×10^6 spores/mL) was inoculated in the surface of the waste pastry. Solid state fermentation was performed at 30 °C for 96 h. The obtained solid mashes were rich in glucoamylase and protease.

Enzymatic hydrolysis of waste pastry was performed in a 3 L bioreactor which was operated at temperature of 55 °C and agitation speed of 500 rpm. The above conditions for solid state fermentation and enzymatic hydrolysis have been approved to be optimal by our previous publications (Han et al., 2015a). Waste pastry with a solid-to-liquid ratio of 10% (w/v) was added into bioreactor with the produced glucoamylase and protease. Hydrolysis samples were taken every hour for 24 h to measure the glucose and FAN productions.

The resultant broth was centrifuged at 10,000 rpm for 30 min and filtered by Whatman No. 1 filter paper to obtain the waste pastry hydrolysate which was used as substrate for subsequent biohydrogen production.

2.3. CSTR and CMISR

Biohydrogen production from waste pastry hydrolysate was performed in the CSTR and CMISR with the same working volume of 2.8 L. The CSTR and CMISR were constructed by transparent plexiglas with a gas-liquid-solid separating device. The detailed information for start-up of the CSTR and CMISR has been described in our previous studies (Han et al., 2015b, 2016). The produced waste pastry hydrolysate was diluted by distilled water to chemical oxygen demand (COD) of 6000 mg/L (Han et al., 2012). The produced biogas was collected with a waterlock and measured by a wet gas meter. In order to eliminate the negative effects on the hydrogen-producing sludge caused by low pH, fermentation pH in both systems was kept above 4 by using 5 M NaOH solution. The CSTR and CMISR were operated in batch mode until biogas was produced. Then, bioreactors were switched to continuous mode (COD = 6000 mg/L) with HRT of 12 h until steady state condition was obtained. The constant products with a variation of less than 10% were assumed to be steady state condition. The HRT was then decreased to the next level and the bioreactors were operated until steady state condition was achieved.

2.4. Analytical methods

The glucose concentration produced in the waste pastry hydrolysate was quantified using the high performance liquid chromatography. The nynhidrin reaction method was used to analyze the FAN production in the waste pastry hydrolysate. The detailed procedure of glucose and FAN analysis were described by the previous publication (Han et al., 2015). COD and biomass were determined according to Standard Methods (APHA, 2005). The composition of the produced biogas was determined by a gas chro-