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Effect of organic loading rate on dark fermentative hydrogen production in the continuous stirred tank reactor and continuous mixed immobilized sludge reactor from waste pastry hydrolysate

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ABSTRACT

Waste pastry (6%, w/v) was hydrolyzed by the produced glucoamylase and protease to obtain the glucose (19.8 g/L) and free amino nitrogen (179 mg/L) solution. Then, the effect of organic loading rate (OLR) (8–40 kgCOD/(m^3 d)) on dark fermentative hydrogen production in the continuous stirred tank reactor (CSTR) and continuous mixed immobilized sludge reactor (CMISR) from waste pastry hydrolysate was investigated and compared. The maximum hydrogen production rate of CSTR (277.76 mL/(h L)) and CMISR (320.2 mL/(h L)) were achieved at OLR of 24 kgCOD/(m^3 d) and 32 kgCOD/(m^3 d), respectively. Carbon recovery ranged from 75.2–84.1% in the CSTR and CMISR with the balance assumed to be converted to biomass. One gram waste pastry could produce 0.33 g (1.83 mmol) glucose which could be further converted to 79.24 mL (3.54 mmol) hydrogen in the CMISR or 91.66 mL (4.09 mmol) hydrogen in the CSTR. This is the first study which reports dark fermentative hydrogen production from waste pastry.

1. Introduction

Because of the shortage of fossil fuels and environmental problems, the international community has shown an increasing interest in clean and renewable energies which do not have negative effects on environment (Ma et al., 2016; Soltani et al., 2015). Hydrogen is being focused on as a promising alternative to fossil fuels because it does not discharge contaminants (Panda et al., 2016). Besides the energy density of hydrogen is 10.9 kJ/L which is 2.75 times higher than that of conventional fossil fuels (Singh et al., 2015).

Hydrogen production can be divided into physiochemical and biological methods (Nong et al., 2015). Physiochemical hydrogen production (such as electrolysis and carbohydrate pyrolysis) requires not only enormous production costs, but also greater consumption of the fossil fuels (Clark et al., 2015). Biological hydrogen production seems to be more attractive because organic waste materials can be used as substrate and the hydrogen-producing system can be operated under low temperature and pressure conditions (Sivagurunathan et al., 2016a). Among the biological hydrogen production, dark fermentation is an effective way in terms of the absence of light and rapid hydrogen production rate (Ghanbarian and Kermani, 2016).

A wide variety of raw materials can be used for dark fermentative hydrogen production, including organic waste or wastewater (Redondas et al., 2012). Waste pastry, which is one of the most common bakery wastes, is reported to be 1.3 million tons per year in China (Han et al., 2016). Waste pastry could be a promising substrate for dark fermentative hydrogen production because it could not only lower the cost of the feedstock, but also deal with the waste recycling. However, the nutrients stored in the waste pastry are in the form of macromolecules (such as starch and protein) which have to be hydrolyzed into micromolecules (such as glucose and free amino nitrogen, FAN) before utilized by microorganisms for hydrogen production (Alibardi and Cossu, 2016). So, the hydrolysis is considered to be limiting step for dark fermentative hydrogen production from waste pastry (De Gioannis et al., 2013). Furthermore, the nutrients of the waste pastry are in the solid phase which inhibits the nutrient conversion efficiency for dark fermentative hydrogen production (Tawfik et al., 2011; Zhou et al., 2013). For these reasons, it is difficult to directly use the cost-effective waste pastry as substrate for dark fermentative hydrogen production. It is reported that physiochemical treatment could accelerate the liquification and hydrolysis of organic solid wastes, while the inhibitory (e.g. furfural) for the subsequent dark fermentative hydrogen production could also be generated. Enzymatic hydrolysis, which could effectively release the

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micromolecules from the organic solid wastes, could be a promising pretreatment for dark fermentative hydrogen production from waste pastry. However, information about dark fermentative hydrogen production from waste pastry hydrolysate was limited.

Dark fermentative hydrogen production has been performed in a variety of bioreactors, including suspended and attached microbial growth systems (Sivagurunathan and Lin, 2016). The conventional continuous stirred tank reactor (CSTR) is the most common suspended microbial growth system and has been widely used to produce hydrogen (Fang and Liu, 2002). In a conventional CSTR, the solid retention time is the same as the hydraulic retention time (HRT) which would limit the biomass concentration in the mixed liquor and therefore reduce the substrate utilization and hydrogen production (Wu et al., 2008; Zhu et al., 2010). However, the addition of immobilized sludge in the bioreactor (attached microbial growth system) could prevent the biomass from being washed out at high dilution rate (Barros et al., 2011; Lin et al., 2006). Recently, we have successfully developed a novel continuous mixed immobilized sludge reactor (CMISR) which could significantly enhance hydrogen production rate by increasing the retention of hydrogen-producing sludge (Han et al., 2012). However, direct comparison of attached microbial growth system (CMISR) with conventional suspended microbial growth system (CSTR) for hydrogen production, especially using waste pastry hydrolysate as substrate, is not yet available in the literature.

Therefore, the objective of this study was to examine the effect of organic loading rate (OLR) on the performance of hydrogen production in a novel CMISR and a conventional CSTR from waste pastry hydrolysate. It aimed at identifying preferable operation condition for suspended and attached microbial growth systems to achieve better hydrogen production performance, thereby promoting dark fermentative hydrogen production for commercial application. The proposed enzymatic hydrolysis pretreatment could also provide a potential way for industrial hydrogen production from high-starch containing raw materials.

2. Material and methods

2.1. Microorganisms and enzymatic hydrolysis

The waste pastry used in this study was provided by the local Wumei Supermarket. Table 1 showed the composition of the waste pastry which was analyzed according to the Standard Method (APHA, 2005).

The glucoamylase and protease used in this study were produced by *Aspergillus awamori* and *Aspergillus oryzae* via solid state fermentation. An amount of 15 g waste pastry was added to a Petri dish with 1 mL of *A. awamori* (4.2×10^5 spores/mL) or *A. oryzae* (1.6×10^5 spores/mL) inoculating on the surface of the waste pastry. The mixed samples were then cultured in the incubator at 30 °C for 5 days to get the solid mashes of glucoamylase and protease.

The sludge used in this study was collected from a local municipal wastewater treatment plant and screened by a sieve (diameter: 2 mm) to eliminate large particulate materials. The sludge was heat pretreated (30 min at 100 °C) to inhibit the hydrogen consumers and harvest the spore-forming microorganisms.

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

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

The enzymatic hydrolysis of waste pastry was carried out in two 2.5 L bioreactors which were equipped with automatic temperature controller and stirrers. The produced enzymes (glucoamylase and protease) were used to hydrolyze waste pastry (6%, w/v) to generate waste pastry hydrolysate which was rich in glucose and FAN. The enzymatic hydrolysis was operated at 55 °C throughout the experiment with agitation speed of 500 rpm. Samples were taken every hour to analyze the glucose and 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 pastry hydrolysate which was used as substrate for subsequent dark fermentative hydrogen production.

2.2. Experimental set-up of CSTR and CMISR

Dark fermentative hydrogen productions from waste pastry hydrolysate were carried out in the CSTR and CMISR with the same working volume of 5.6 L. Nitrogen gas was purged into the bioreactors for 20 min to attain anaerobic condition. Operating temperature and agitation speed of the bioreactors were 35 °C and 250 rpm, respectively. Fermentation pH was controlled above 4 by using 5 M $NaHCO_3$ solution. The influent flow rate was controlled by a feed pump to regulate the HRT at 6 h. The biomass activity of the CSTR and CMISR was 73% (VSS/SS). The generated biogas was collected by the water displacement method through the port designed at the top of the reactors.

In order to investigate the effect of OLR on dark fermentative hydrogen production in the CSTR and CMISR, the waste pastry hydrolysate was diluted by distilled water to certain OLR (8–40 kgCOD/(m^3 d)). The amount of inorganic nutrients was proportional to the influent OLR (e.g., if the influent OLR was doubled, the nutrient concentration was also doubled).

The reactor performance (biogas production, composition in hydrogen and soluble microbial products, SMPs) was monitored daily throughout the experimental period. Complete characterization of the reactor performance took place at each steady state. Steady-state condition was considered to be achieved when the biogas production and the proportion of SMPs were consistent within 5% for three consecutive days.

2.3. Analytical methods

The glucose and FAN, which were produced in the waste pastry hydrolysate, were measured according to our previous study (Han et al., 2015). Determinations of dissolved chemical oxygen demand, suspended solid (SS) and volatile suspended solid (VSS) were carried out according to Standard Methods (2005). The mixed liquor samples were centrifuged at 10,000*g* for 15 min at 4 °C and the supernatant was filtered through a 0.45 μm membrane filter. The hydrogen production rate (HPR) could be calculated according to Eq. (1).

$$\text{HPR mL/(h L)} = \text{Hydrogen yield (mL)/(Running time (h)} \times \text{Working volume (L))} \quad (1)$$

Biogas was quantified with a gas chromatography (SC-7, Shandong Lunan Instrument Factory) 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. Biogas samples were compared to five standard levels of pure gas injections. Based on the percentage of hydrogen in biogas, the hydrogen yield could be calculated.

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