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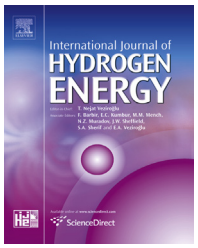

Fermentative hydrogen production using wheat flour hydrolysate by mixed culture

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ABSTRACT

Wheat bran was first used to produce glucoamylase by *Aspergillus awamori* from solid-state fermentation (SSF). Wheat flour with different mass ratios of 2%–8% (w/v) were hydrolyzed by glucoamylase to generate the wheat flour hydrolysates (containing glucose concentrations of 10.69–35.14 g/L) which were then utilized as substrate for fermentative hydrogen production by heat pretreated sludge. The cumulative hydrogen production increased from 1181.3 ml to 2379.6 ml as glucose concentration increased from 10.69 g/L to 35.14 g/L. The modified Gompertz model was used to describe the cumulative hydrogen production for different glucose concentrations. However, the maximum hydrogen yield of 1.9 mol H_2/mol glucose was observed at glucose concentration of 10.69 g/L probably due to the products inhibition and oxidization/reduction of NADH. The wheat flour hydrolysate could be used to replace commercial glucose for fermentative hydrogen production and therefore reduce the cost of hydrogen production for large scale.

Introduction

Concerns about the excessive dependence on fossil fuels, as well as environmental pollution, have led to extensive research on the alternative fuels [1,2]. Hydrogen is considered to be a promising and sustainable energy source since it is clean and renewable. Furthermore, it has a high energy yield of 122 kJ/g which is 2.75 times higher than hydrocarbon fuels [3]. Currently, hydrogen is mainly produced by steam reforming of hydrocarbons or coal gasification which entails the use of fossil fuels and emits greenhouse gas [4]. However, biological hydrogen production is an exciting biotechnology that could use a wide variety of low cost renewable materials or waste/wastewater as feedstock [5,6]. Among the biological approaches for hydrogen production, dark fermentation is an attractive option because it could produce clean energy and dispose waste/wastewater simultaneously without the limitation of light [7,8].

However, conventional fermentative hydrogen production using commercial glucose as substrate is quite expensive [9]. Utilizing renewable raw materials could overcome this problem for large scale of fermentative hydrogen production [10,11]. Wheat is considered to be one of the most suitable raw materials for fermentative hydrogen production because of

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high carbohydrate content and low cost [12]. However, nutrients stored in wheat are in the form of macromolecules which have to be broken down into available form, such as sugars, before utilized by microorganisms for fermentative hydrogen production. Therefore, the hydrolysis rate is regarded as the limited step for fermentative hydrogen production [13]. There are many pretreatments (such as physical, chemical and thermal) could convert carbohydrate polymers (wheat starch) into monosaccharides [14,15]. However, these pretreatments could also produce undesirable byproducts such as aliphatic acid and phenol compounds which inhibit the enzyme activity and affect the performance of hydrogen production [16]. Enzymatic hydrolysis could convert the starch contained in the wheat flour into glucose with advantages of lower costs, higher monosacchride conversion rate and lower release of inhibitory compounds. However, there are a limited number of studies on dark fermentation of enzyme hydrolyzed wheat flour for fermentative hydrogen production.

Therefore, this study investigates the feasibility of fermentative hydrogen production using wheat flour hydrolysate as substrate by mixed culture. Wheat bran was first utilized by *Aspergillus awamori* in solid-state fermentation (SSF) to produce glucoamylase which was used to hydrolyze wheat flour to obtain the generic feedstock. The wheat flour hydrolysate was then used as substrate for fermentative hydrogen production by heat pretreated sludge. The effect of substrate concentration on the performance of hydrogen production using glucose solution derived from enzymatic hydrolysis of wheat flour was also investigated. This substitution of using enzyme hydrolyzed wheat flour to replace commercial glucose as substrate for fermentative hydrogen production could effectively decrease hydrogen production cost and create an economically feasible bioprocess.

Materials and methods

Microorganisms and raw material

Microorganism strain of *A. awamori* CICC2254 was purchased from Shanghai Beinuo Biotechnology Co., Lid. and utilized in solid-state fermentation (SSF) to produce glucoamylase. Prior to experimental work, *A. awamori* spores were prepared according to Koutinas [17] and stored at −20 °C. The anaerobic sludge was obtained from a local municipal wastewater treatment plant and screened by a sieve (diameter: 2 mm) to eliminate large particulate materials. Heat treatment was carried out in a water bath at temperature of 100 °C for 6 h to inhibit methanogenic activity. The heat pretreated sludge was used as inoculum for fermentative hydrogen production.

The wheat used in this study was purchased from local agricultural market and milled to produce wheat flour and bran. Table 1 shows the compositions of the wheat flour and bran which were analyzed according to procedures described in Koutinas et al. [18].

Table 1 – Compositions of wheat flour and bran used in this study.

Components	Wheat flour (%)	Bran (%)
Starch	73.5	15.6
Total organic nitrogen	1.83	0.54
Protein	10.7	1.76
Phosphorous	0.158	0.089

Solid-state fermentation (SSF) and enzymatic hydrolysis

Appropriate 10 g wheat bran was distributed into a Petri dish and 1 ml *A. awamori* solution (5.4×10^6 spores/ml) was then spread evenly on the surface of wheat bran. The SSF was carried out in an incubator at 30 °C for 96 h to produce the solid enzyme (glucoamylase).

Enzymatic hydrolysis using solid enzyme obtained from SSF was carried out in a 3 L fermentor which was equipped with automatic temperature controller and magnetic stirrer. Various wheat flour mass ratios (2–8%, w/v) were blended with 1 L water for 15 min and then transferred into the bioreactor at 55 °C. Enzymatic bran solid was added into the fermentor to hydrolyze the wheat flour suspension which was agitated at 500 rpm without pH control. Samples were taken every hour for 24 h to analyze glucose production. The resultant broth was centrifuged at 10,000 rpm for 30 min and filtrated by vacuum filtration using Whatman No. 1 filter paper to obtain wheat flour hydrolysate which was then used as the feedstock for fermentative hydrogen production.

Hydrogen production

Fermentative hydrogen production by heat pretreated sludge was performed in another 3 L fermentor with working volume of 500 ml using wheat flour hydrolysate. The initial volatile suspended solid (heat pretreated sludge) concentration of the seed inoculum was 2.8 g/L. The pH during the fermentation was automatically controlled within 4.0–4.5 by the addition of 10 M $NaHCO_3$ and 0.05 M H_2SO_4. The fermentor was agitated at 500 rpm and sparged with 0.5 vvm N_2 for 30 min to achieve the anaerobic condition for fermentative hydrogen production. Fermentations were considered to be done when glucose concentration was stable. Samples were collected at the end of the fermentation and analyzed for the concentration of total volatile fatty acid (TVFA).

The proposed schematic diagram of fermentative hydrogen production from wheat flour hydrolysate by mixed culture is presented in Fig. 1.

Analytical methods

Glucose concentration in the wheat flour hydrolysate was quantified by a high performance liquid chromatography (HPLC) which was equipped with a BIO-RAD column (HPX-87H), a refractive index detector (RID) and a photodiode array analyzer (PDA). The procedures were described by our previous study [19].

The amount of produced biogas was collected with a waterlock and measured by a wet gas meter. The composition of biogas 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