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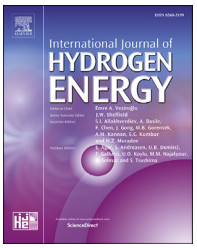

BioH$_2$ production from waste bread using a two-stage process of enzymatic hydrolysis and dark fermentation

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ABSTRACT

The potential of bioH$_2$ production using waste bread via a two-stage process was examined. In the first stage, the waste bread was utilized by *Aspergillus awamori* and *Aspergillus oryzae* through solid state fermentation to generate glucoamylase and protease which were then utilized to hydrolyze the waste bread to produce the waste bread hydrolysate. The highest starch conversion of 96.6% and glucose yield of 0.521 g glucose/g waste bread were achieved within 24 h. In the second stage, the waste bread hydrolysate was utilized for bioH$_2$ production by *Biohydrogenbacterium* R3. The maximum H$_2$ yield of 103 mL H$_2$/g waste bread could be obtained. The proposed two-stage bioprocess, which was able to increase the nutrient conversion efficiency and H$_2$ production from organic solid wastes, should be a promising way for bioH$_2$ production in industrial application.

Introduction

Because of depletion of fossil fuel and environmental pollution, it is urgent to search an alternative energy to replace fossil fuel [1]. Hydrogen is widely regarded as a clean energy carrier in the future since non-pollutant could be released after combustion [2]. H$_2$ could be produced by various strategies, such as electrochemical, thermochemical and biological processes. Biological H$_2$ production, which is more environmental friendly than non-biological processes, is regarded as an attractive way [3]. However, the high production cost is one of the largest challenges of bioH$_2$ production for industrial application [4]. Using organic wastes (such as food waste) for bioH$_2$ production, which is able to reduce the H$_2$ production cost, is considered to be an efficient solution [5].

Food waste, one of the most important parts of the municipal solid waste (MSW), is a crucial global issue [6]. Generally, there are three basic ways for MSW treatment, including landfill, incineration and composting.

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Unfortunately, these traditional technologies are not proper to treat food waste due to the limited space and toxic gas emission [7]. Using food waste as substrate for biofuels production (such as bioH_2 production) seems to be an attractive biotechnology since food waste contains high percentage contents of starch and protein. However, these nutrients are in the form of macromolecules which have to be hydrolyzed into micromolecules before used by microorganism for bioH_2 production. The food waste hydrolysis is regarded as the limited step for the overall bioH_2 production process [8]. Furthermore, the nutrients stored in the food waste are in the form of solid status. The substrate conversion efficiency is lower than using organic liquid substrate [9].

In order to accelerate the hydrolysis of macromolecules and increase the substrate conversion efficiency, a two-stage process of enzymatic hydrolysis and dark fermentation for bioH_2 production using waste bread was developed in this study. In the first stage, the waste bread was utilized by *Aspergillus awamori* and *Aspergillus oryzae* to produce glucoamylase and protease via solid state fermentation (SSF). The produced enzymes were then used to hydrolyze the macromolecules nutrients (starch and protein) contained in the waste bread to achieve the waste bread hydrolysate. In the second stage, the waste bread hydrolysate (glucose and free amino nitrogen, FAN) was utilized for bioH_2 production by *Biohydrogenbacterium* R3. The effects of the waste bread solid to liquid ratios (5%, 7% and 10%) on the performances of waste bread hydrolysis and bioH_2 production were also investigated. The proposed two-stage process should be applied for bioH_2 production from waste bread in industrial scale.

Materials and methods

Fungi and H_2-producing bacteria

The fungi of *A. awamori* and *A. oryzae*, which were provided by Shanghai Beinuo Biotechnology Co., Ltd, were used for glucoamylase and protease production. The H_2-producing bacteria used in this study were *Biohydrogenbacterium* R3 whose 16S rRNA accession number was AY363375. The *Biohydrogenbacterium* R3 was prepared and cultured according to the previous study [10].

Detailed description of two-stage process for bioH_2 production from waste bread

Fig. 1 showed the detailed description of bioH_2 production from waste bread in the two-stage process of enzymatic hydrolysis and dark fermentation. Waste bread was collected from the local Supermarket. The composition of the waste bread was analyzed according to the Standard Method [11] and summarized in Table 1.

Waste bread was grounded (E1) into physical size smaller than 1 cm. SSF was carried out in two Petri dishes (E2) with 5 g grounded waste bread and 1 mL of spore solution of *A. awamori* (4×10^6 spores per mL) or *A. oryzae* (1×10^6 spores per mL). The Petri dishes were then incubated at 30 °C for 4 days, which has been approved to be optimal for enzymes activity [12], for glucoamylase and protease production.

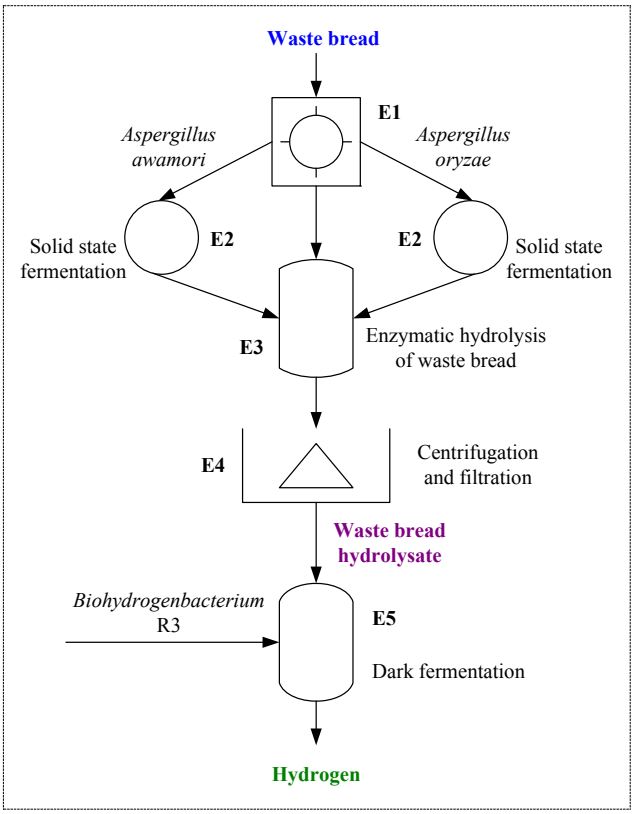

Fig. 1 – Detailed description of bioH_2 production from waste bread using two-stage process of enzymatic hydrolysis and dark fermentation.

Table 1 – Composition of the collected waste bread (per 100 g).

Component	Value (g)	Component	Value (g)
Moisture	24.3 ± 0.8 g	Starch	48.6 ± 1.5 g
Protein (N × 5.7)	9 ± 0.3 g	Phosphorous	0.08 ± 0.01 g
Total organic nitrogen	1.5 ± 0.1 g	Ash	2.3 ± 0.2 g

The produced enzymes were then used to hydrolyze waste bread with solid to liquid ratios of 5%, 7% and 10% (w/v) in a 3 L bioreactor (E3). The demineralized water was added into the bioreactor until the volume of the blend reached 1 L. The agitation speed and temperature of waste bread hydrolysis were set to be 500 rpm and 55 °C, respectively. Then, the mixture was centrifuged (10,000 rpm for 25 min) and filtered by vacuum filtration (Whatman No. 1 filter paper) in the E4.

BioH_2 production from waste bread hydrolysate was conducted in a 3 L bioreactor (E5) with a working volume of 1 L. The *Biohydrogenbacterium* R3 with liquid-to-liquid ratio of 2% (v/v) was inoculated to the bioreactor. The N_2 was sparged into the bioreactor to ensure the anaerobic condition. The agitation speed and temperature of dark fermentation were kept constant at 300 rpm and 37 °C, respectively. The pH was maintained in the range of 4–4.6 by adding 4 M $NaHCO_3$ and 0.01 M H_2SO_4 automatically.

The SSF for enzymes production, enzymatic hydrolysis of waste bread and bioH_2 production from waste bread hydrolysate were performed in triplicate.

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