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Bioethanol production from mixed food waste by an effective enzymatic pretreatment

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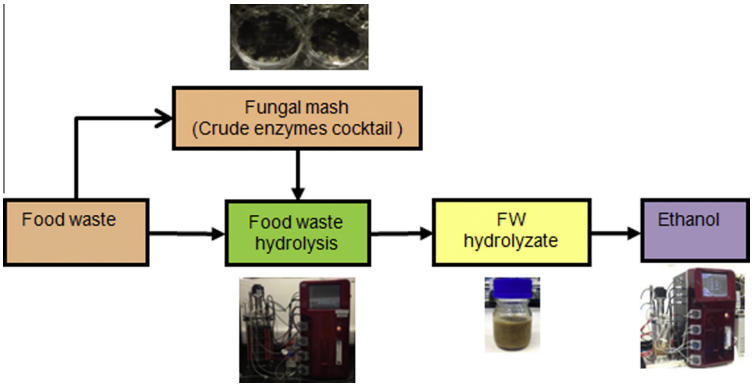
HIGHLIGHTS

- A fungal mash rich in hydrolytic enzymes was *in-situ* produced from waste cake.
- The enzymatic pretreatment of FW with this fungal mash was investigated.
- A hydrolyzate with 127 g/L glucose and 1.8 g/L FAN was obtained.
- Ethanol (58 g/L) was produced with 0.5 g/g glucose yield using the hydrolyzate.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a fungal mash rich in hydrolytic enzymes was *in-situ* produced from the waste cake and was further applied for the hydrolysis of mixed food wastes. A well balanced nutrient stream containing a 127 g/L glucose and 1.8 g/L free amino nitrogen was produced from the enzymatic pretreatment of food wastes using this fungal mash at 24 h. Using this solution as sole fermentation feedstock, 58 g/L of ethanol corresponding to an ethanol yield of 0.5 g/g glucose was obtained within 32 h. It was demonstrated that the pretreatment of mixed food wastes with the fungal mash produced in this study is an effective option for food waste saccharification and bioethanol production.

1. Introduction

Recently, global demand for ethanol has been increasing due to its wide industrial applications. Ethanol is mainly used as a chemical feedstock to produce ethylene with a market demand of more than 140 million tons per year, which is a key material for further production of polyethylene and other plastics [1]. Traditionally,

Abbreviations: FW, Food waste; SSF, Solid state fermentation; *A. awamori*, *Aspergillus awamori*; *A. niger*, *Aspergillus niger*; *S. cerevisiae*, *Saccharomyces cerevisiae*; GA, Glucoamylase; FAN, Free amino nitrogen; DNSA, Dinitrosalicylic acid; µmol, micromole.

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bioethanol is produced mainly from corn and sugar cane [2], while the use of crops as a fuel source may comprise global food supply. As such, waste organic materials such as lignocellulosic biomass and food waste; have been explored as alternative substrates for ethanol production.

Food waste (FW) is an organic waste discharged from various sources, e.g. households, cafeterias, restaurants. Nearly 1.3 billion tons of food is lost or wasted throughout the food supply chain [3]. Given its organic-rich nature, FW should be considered as a useful resource for producing bioethanol. However, microorganisms generally cannot directly assimilate the nutrients in FW without proper pre-treatment. Therefore, different commercial enzymes have been used to improve the saccharification of FW [4], among which the pretreatment of FW with consortium of commercial enzymes was found to be more efficient than that with a single commercial enzyme in terms of glucose production. Obviously, high-concentration of glucose favors the subsequent bioethanol production. It should be realized that commercial enzymes are costly and generally available in a single-type form [5]. In order to make the enzymatic hydrolysis of FW more cost-effective, the enzymes should be produced *in situ* from a cheap feedstock without complex and costly downstream separation and purification.

So far, various kinds of FW have been explored for the production of enzymes including proteases, cellulases, amylases, lipases and pectinases, particularly through solid state fermentation (SSF) [6–10]. Higher enzyme yields can be obtained via SSF as it provides a similar environment to the natural living conditions of microorganisms for their growth and enzymes production [11]. Melikoglu [12] developed a multi-enzyme solution of glucoamylase and protease from SSF of waste bread with *Aspergillus awamori*, which was then used for the hydrolysis of waste bread and wheat flour. Similar idea was also applied for the enzymatic hydrolysis of mixed FW to produce a fermentation medium [13], which was further used as a nutrient-complete feedstock for the cultivation of microalgae [14,15] and the production of succinic acid [16]. Therefore, the specific objectives of this study are to *in-situ* produce fungal mash rich in hydrolytic enzymes with FW as feedstock for high-efficiency hydrolysis of FW with the aim to produce ethanol.

2. Material and methods

2.1. Materials

In our previous work, bakery wastes, particularly waste cake, were found to be a good substrate for glucoamylase (GA) production [17]. In this study, *A. awamori* obtained from ABM Chemicals Ltd (Cheshire, England) was used to produce GA with waste cake collected from local catering as substrate through SSF. The waste cake was first ground, sieved and then stored in zipped plastic bags at −20 °C for further experiments. Mixed FW used in this study was collected from a cafeteria at Nanyang Technological University. The mixed FW homogenized in a blender immediately after the collection and then stored in zipped plastic bags at −20 °C for further use. The compositions of waste cake and mixed FW are presented in Table 1.

Table 1
Composition of food wastes per gram of dry mass.

FW	Total carbohydrates (mg)	Starch (mg)	Protein (mg)	Lipid (mg)	Ash (mg)
Cake waste	643 ± 12	458 ± 30	141 ± 8	161 ± 8	39 ± 2
Mixed FW	768 ± 52	603 ± 38	86 ± 4	146 ± 31	29 ± 2

A commercial dry baker's yeast *Saccharomyces cerevisiae* was purchased from a local store and dispersed in sterile water at room temperature at a concentration of 10 g/L (g dry baker's yeast/liter of distilled water). The yeast was incubated on agar plates containing 20 g/L agar, 20 g/L glucose, 10 g/L yeast extract and 20 g/L peptone for 2 days at 30 °C. One loop from the agar plate was transferred into liquid YPD medium where it was precultivated for 24 h at 30 °C and 150 rpm in a shaking incubator. Fermentation medium contained: 20 g/L glucose, 10 g/L yeast extract, 10 g/L peptone, 3 g/L $Na_2HPO_4 \cdot 7H_2O$, 3 g/L KH_2PO_4, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0.08 g/L$CaCl_2 \cdot 2H_2O$. The precultivated cultures were finally transferred to the fermentation media at an inoculum size of 10% (v/v) for ethanol production.

2.2. Production of fungal mash

Waste cakes with a particle size of 1.2 to 2.0 mm were used as sole carbon source for producing an enzyme cocktail using solid state fermentation in which the initial moisture content was adjusted to 70% (wb) with 0.1 M phosphate buffer (pH 7.9). After sterilization by autoclaving at 120 °C for 20 min, the flasks were cooled down and then inoculated with *A. awamori* to obtain a spore concentration of 10^6/g substrate and the contents were mixed thoroughly with a sterile spatula. Such mixture (10 g) was distributed into Petri dishes and incubated at 30 °C for 6 days under stationary conditions. Fungal mash, i.e. the GA-rich fermentation solids were obtained at the end of the fermentation. This fungal mash indeed was a cocktail of various crude enzymes, and was directly employed to hydrolyze mixed FW without further separation of produced enzymes.

2.3. Food waste hydrolysis

The hydrolysis was carried out in a 3L bioreactor with 1L working volume at 60 °C, 500 rpm. Blended domestic FW (180 g dry) was inoculated with the fungal mash (7.7 g dry). Then, the total volume of the blend was adjusted to 1L by adding distilled water. The reaction was conducted at 60 °C with 500 rpm mixing speed. The pH was not controlled as it was in the range of 4.0–4.5 during the hydrolysis. Hydrolysis samples were taken at several time intervals and centrifuged at 10,000 rpm for 10 min for the analyses. At the end of the hydrolysis, the medium was centrifuged at 10,000 rpm for 20 min and then filtered by vacuum filtration using Whatman No. 1 filter paper. The filtrate was kept at −20 °C and was subsequently used as the sole fermentation feedstock in the subsequent ethanol fermentation. All experiments were carried out in duplicate.

2.4. Ethanol fermentation

Fermentation experiments were conducted in 250 mL Erlenmeyer flasks with a working volume of 100 mL. The yeast was added at a ratio of 10% (v/v) to the fermentation mixture under aseptic conditions. The initial glucose concentrations were adjusted to 40, 60, 80, 100 and 116 g/L by the addition of distilled water. Before inoculation, the flasks and medium were sterilized by autoclaving. The temperature and agitation speed were maintained constant throughout the experiment at 30 °C and 100 rpm, respectively. The fermentation lasted for 72 h.

For bioreactor experiments, the fermentation medium (1 L) was prepared using 900 mL hydrolyzate, and then the inoculum (100 mL) was transferred aseptically. The fermentation was performed at 30 °C with 100 rpm mixing speed for 48 h without supplementing oxygen. Samples were taken at regular intervals to follow the yeast growth, glucose and ethanol concentrations.