Biochemical Engineering Journal 80 (2013) 76–82

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular Article

Kinetic studies on the multi-enzyme solution produced via solid state fermentation of waste bread by *Aspergillus awamori*☆

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ARTICLE INFO

Article history:
Received 14 March 2013
Received in revised form
15 September 2013
Accepted 25 September 2013
Available online 4 October 2013

Keywords:
Enzyme kinetics
Glucoamylase
Thermal deactivation
Protease
Waste bread

ABSTRACT

The aim of this study was kinetic analysis of the multi-enzyme solution produced from waste bread via solid state fermentation by *Aspergillus awamori*. It was found that at normal temperature for hydrolysis reactions, 60 °C, the activation energies for denaturation of *A. awamori* glucoamylase, 176.2 kJ/mol, and protease, 149.9 kJ/mol, are much higher than those for catalysis of bread starch, 46.3 kJ/mol, and protein, 36.8 kJ/mol. Kinetic studies showed that glucoamylase and protease in the multi-enzyme solution should have at least two conformations under the two temperature ranges: 30–55 °C and 60–70 °C. Thermodynamic analysis showed that, deactivation of glucoamylase and protease in the multi-enzyme solution can be reversible between 30 °C and 55 °C, since ΔS is negative and ΔH is positive. On the other hand, for glucoamylase and protease, both ΔS and ΔH are positive between 60 °C and 70 °C. This means that the deactivation of both enzymes in the multi-enzyme solution is spontaneous in this temperature range. It was also found that the glucoamylase produced in the solid state fermentation of waste bread is more thermally stable than the protease in the mixture. Consequently, the protease had little or no effect on the stability of the glucoamylase. Furthermore, the half-life of the glucoamylase produced from waste bread pieces was much higher than that produced from wheat flour. This is an important finding because the mode of production, via solid state fermentation, appears to have increased the thermostability of the enzyme significantly.

1. Introduction

Biorefineries are facilities where biofuels, biochemicals and other bioproducts are produced from biomass [1,2]. Currently, bioethanol is the most widely produced biofuel [3] and amongst the various types of biomass used for its production, cereals and sugar crops are the most common [4,5]. Corn in the United States, wheat in Europe, and sugar cane in Brazil are the main crops used. However, these are generally food crops and their use is difficult to justify when hunger is such a major problem around the world. Accordingly, production of bioethanol from crops creates ethical dilemmas and triggers agflation. Making a bad situation worse, millions of tonnes of food are wasted annually, particularly in developed countries. However, a synergistic solution can be created to alleviate both problems; namely, the production of bioethanol directly from food wastes.

Amongst the many types of food that are wasted, bread is the most common in Europe and many Asian countries [6]. The novel idea: "Production of Sustainable Alternatives to Petrochemicals and Fuels from Waste Bread" was published by us elsewhere [7]. There are also some other recent studies related to the bioconversion of waste bread into fuels and chemicals [8–13]. In order to produce bioethanol from waste bread first the macromolecules: starch, lipids and proteins must be converted into monomers: glucose, fatty acids and amino acids [7]. This is similar to bioethanol production from wheat, the main ingredient of bread [14]. Today, conversion of macromolecules to monomers is predominantly carried by enzymatic hydrolysis [15]. Although commercial enzymes could be used it would inevitably increase the operation costs [16]. As an alternative, a hydrolytic multi-enzyme solution could be produced from a portion of bread that is wasted, which can then be used for the production of a monomer rich hydrolysate from the

☆ This article is dedicated to the memory of Dr. Ruohang Wang, our collaborator and beloved friend, who passed away in July 2010.

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http://dx.doi.org/10.1016/j.bej.2013.09.016

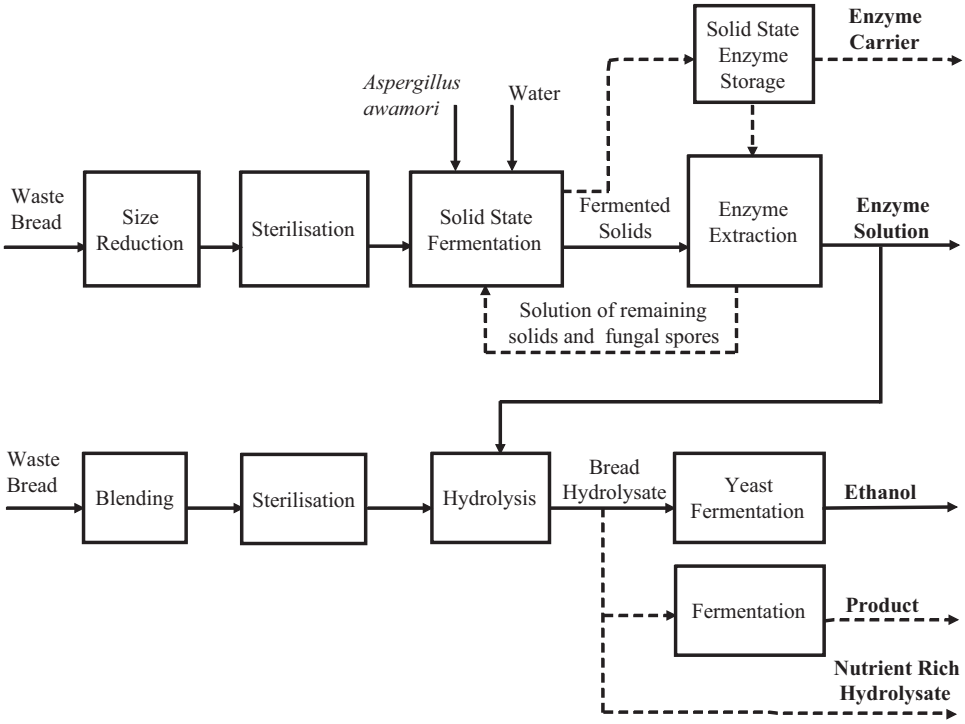

Fig. 1. Proposed bioprocess for the production of bioethanol and other biocommodities from waste bread by *Aspergillus awamori* in solid-state fermentation. Dotted lines indicate optional steps in the process.

other segment of waste bread. Finally, the nutrient rich hydrolysate can be converted into bioethanol or any other desired product as shown in Fig. 1.

The current practice of enzyme production is principally based on submerged fermentation [17]. However, it has been reported that solid state fermentation, SSF, could provide higher enzymatic activities at higher yields with minimal water consumption [18–20]. Bread can be considered as an ideal raw material for solid state fermentation due to its porous structure and nutrient composition [7].

Glucoamylase is an enzyme, which is extensively used for the hydrolysis of starch in cereal based biorefineries [21]. Fungi from the genus *Aspergillus* could produce this enzyme in large quantities with high activity [22]. In its natural habitat *Aspergillus* also secretes protease enzymes in relatively high quantities to assimilate and consume proteinaceous substrates [23]. Solid state fermentation is considered very similar to the natural habitat of fungi and coupling this with the availability of an ideal substrate, waste bread, simultaneous glucoamylase and protease production could provide significant bioprocess enhancements. The purpose of the research report here was to study the kinetic of the multi-enzyme solutions produced using this strategy.

As biological catalysts the most important problem in the utilisation of enzymes is their deactivation in the processes in which they are used [24–27]. Enzyme activities are generally measured at a single standard temperature and therefore do not indicate temperature sensitivity; however, temperature is one of the primary controls on enzyme activities [28]. Enzymatic activity generally increases with temperature to a certain point, after the enzyme will be irreversibly deactivated. Therefore, thermal deactivation of the multi-enzyme solution produced from waste bread should be characterised in detail before using it in other bioprocesses and/or for subsequent commercialisation.

2. Materials and methods

2.1. Microorganism

The fungal strain *Aspergillus awamori* 2B. 361 U2/1 was used in all experiments. Detailed information about storage, sporulation, and inoculum preparation have been given in a previous publication [29].

2.2. Waste bread

Sliced white bread waste was obtained from an on-site Refectory at The University of Manchester, UK. Details of its composition are given in Table S1. The waste bread was stored in chest freezers at −18 °C in airproof containers and defrosted as required. Waste bread slices were cut into 20 mm squares, based on previous studies [7], prior to solid state fermentations.

2.3. Solid state fermentation

Waste bread pieces were sterilised at 120 °C for 30 min before the solid state fermentations. *A. awamori* spore suspension of 3×10^9 spores/mL was mixed into the required volume of sterile water to form an inoculum size of 10^6 spores/g on wet basis (wb). Batchwise fermentations were carried at 30 °C in 9 cm petri dishes. Initial moisture ratio was adjusted to 1.8 dry basis (db) and fermentations were carried out for exactly 168 h, as optimised in a previous study [7]. All the fermentations were carried out at least in triplicate.

2.4. Enzyme extraction

As soon as the fermentations were terminated the solids were suspended in distilled water at room temperature using a kitchen