Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Stepwise optimisation of enzyme production in solid state fermentation of waste bread pieces

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

ABSTRACT

When it is not consumed, bread presents a major source of food waste, both in terms of the amount and its economic value. However, bread also possesses the characteristics of an ideal substrate for solid state fermentation. Yet nearly all wasted bread ends up in landfill sites, where it is converted into methane by anaerobic digestion. Governments are finally taking action and, according to the EU Landfill Directive, for example, biodegradable municipal waste disposed into landfills must be decreased to 35% of 1995 levels, by 2020. Solid state fermentation of waste bread for the production of value added products is a novel idea, which could help with the achievement of this target. In this study, glucoamylase and protease production from waste bread pieces, via solid state fermentation, was investigated in detail. The optimum fermentation conditions for enzyme production were evaluated as, 20 mm particle size, 1.8 (w/w, db) initial moisture ratio, and duration of 144 h. Under these conditions, glucoamylase and protease activities reached up to 114.0 and 83.2 U/g bread (db), respectively. This study confirms that waste bread could be successfully utilised as a primary raw material in cereal based biorefineries.

Keywords: *Aspergillus awamori*; Glucoamylase; Protease; Solid state fermentation; Waste bread; Cereal based biorefineries

1. Introduction

Solid surfaces are susceptible to microbial attachment and consequent growth of microorganisms. This phenomenon is considered as one of the most important fields in microbiological studies (Fujikawa and Morozumi, 2005). In the last two decades, solid state fermentation has attracted interest in western countries due to its advantages in the production of secondary metabolites, and production of novel foods (Barrios-Gonzalez, 2012). In addition, via solid state fermentation, solid wastes can be utilised as commercially desirable substrates. Various raw materials are used for solid state fermentations. Due to their high nutrient composition and availability, cereals such as corn, wheat, and rice are the most common. However, utilisation of such food grade raw materials carries many economical (Pimentel, 2003) and ethical problems (Mercer-Blcakman et al., 2007). Instead, utilisation of food waste can be a synergistic solution to these problems. Bread is a major food waste in many countries around the world and in most European countries. In the United Kingdom, it is estimated that the amount of bread wasted could be up to 1.2 million tonnes per annum (Melikoglu, 2008). Moreover, bread possesses the characteristics of an ideal substrate for solid state fermentations (Sakurai et al., 1985). More than 95% of the bread that is wasted ends in landfill sites, where converted into methane by anaerobic digestion. Methane has 21 times more global warming potential than carbon dioxide over a time span of 100 years (Einola et al., 2008). According to

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Received 24 November 2012; Received in revised form 12 February 2013; Accepted 26 April 2013

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http://dx.doi.org/10.1016/j.fbp.2013.04.008

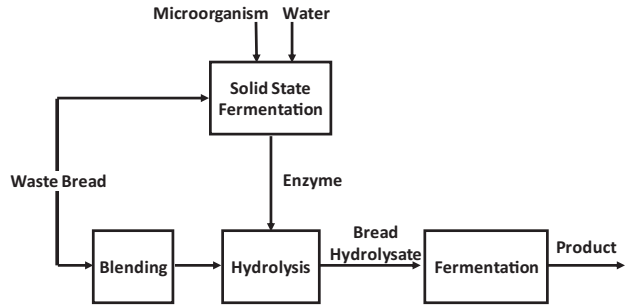

Fig. 1 – Novel bioprocess for the production of value added products from waste bread.

the EU Landfill Directive, biodegradable municipal waste disposed in landfills should be decreased to 35% of 1995 levels, by 2020 (The European Commission, 2009).

A novel process, based on the production of hydrolytic enzymes from a small portion of waste bread, via solid state fermentation, and the subsequent use of these enzymes to hydrolyse the remaining portion for the production of a nutrient rich hydrolysate, has been developed at the University of Manchester in the United Kingdom (Melikoglu, 2008). The nutrient rich hydrolysate then can be converted into desired product(s) by proper subsequent fermentations as shown in Fig. 1.

In this study, production of a multi-enzyme solution, rich in glucoamylase and protease, from waste bread pieces using the fungus *Aspergillus awamori*, was optimised. Experiments were carried out in petri dishes to enable multiple conditions to be assessed simultaneously. Medium temperature, inoculum size, pH, particle size, initial moisture content, and duration were the key process parameters targeted for optimisation. For the optimal growth of *A. awamori*, operating temperature and inoculum size are reported elsewhere (Wang et al., 2007, 2009). These were selected as 30 °C and 1.0 million spores/g dry substrate, respectively. Medium pH was uncontrolled.

Glucoamylases and proteases are the most widely used industrial enzymes with applications in various industries. These enzymes can also be utilised in the production of nutrient rich hydrolysates which can then be fermented to produce value-added chemical products such as ethanol, lactic acid, monascus pigments, glycerol, succinic acid and polyhydroxybutyrate (PHB) by subsequent fermentations (Botella et al., 2009).

The main goal of this study was to find the optimum conditions for fungal growth and enzyme production. The use of a biorefinery concept for the utilisation of food waste was adopted, as part of the strategy for tackling the food waste issue and for the environmentally friendly production of alternative platform chemicals. In the case of many waste materials, the process would start with the production of hydrolytic enzymes.

2. Materials and methods

The objective of this study was to optimise the remaining process parameters, particle size, initial moisture content, and fermentation time, all of which affect enzyme production in solid state fermentations of waste bread pieces. These parameters were evaluated by stepwise/single parameter optimisation. First, initial estimates for the three parameters were taken from the literature, then one parameter was optimised in each step while keeping the remaining two constant. After each step, the best value found for the selected parameter was used as the set value for the next optimisation.

Table 1 – Average dimensions and weight of a slice of white waste bread.

Component	Measurement
Height	135 ± 10 mm
Width	105 ± 10 mm
Thickness	13 ± 2 mm
Weight	42.5 ± 1.0 g

2.1. Microorganism

All experiments were conducted using *A. awamori* 2B. 361 U2/1. Detailed information about sporulation, storage and inoculum preparation are given in our previous publication (Wang et al., 2007).

2.2. Waste bread

The only source of nutrients during fermentation was provided by sliced white bread waste obtained from an on-site Refectory at The University of Manchester, UK. Details about the physical properties and composition are presented in Tables 1 and 2. Waste white bread slices were cut into small pieces of designated size before solid state fermentations.

2.3. Solid state fermentation

Waste bread pieces were sterilised at 120 °C for 30 min and fungal spore suspension of 3×10^9 spores mL^{-1} was mixed into the required volume of sterile water to form a constant inoculum size of 10^6 spores g^{-1} on wet basis (wb). Fermentations were carried out batchwise at 30 °C in 9 cm petri dishes. At the beginning of the optimisation studies, it was decided to adjust the initial moisture ratio of the solid samples to 2.1 on a dry basis (w/w, db) i.e. approximately 67% moisture content, and to carry out the fermentations for 168 h. All the fermentations were carried out at least in triplicate. Temperature of the solids during the fermentations was monitored by using type K online thermocouples, aseptically placed in the petri dishes.

2.4. Enzyme extraction

At the end of the fermentations the fermented solids were suspended in distilled water at room temperature using a kitchen type blender to form a suspension of 50 g/L (wb). After blending for 30 min, the suspension was centrifuged at 2500 × *g* for 10 min, and filtered using Whatman No. 1 filter paper to collect the liquid phase as enzyme solution.

Table 2 – Composition of a slice of white waste bread.

Component	Weight (g)
Water	28.67
Starch	45.34
Nitrogen (N)	1.61
Protein (N × 5.7)	9.18
Phosphorous	0.10
Ash	2.26