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Valorization of bakery waste for biocolorant and enzyme production by *Monascus purpureus*

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ABSTRACT

A concept of utilizing bakery waste as a nutrient source for the fermentative production of bio-colorant by *Monasucus purpureus* has been developed. The proposed ideas provide an innovative approach to establish a system or method to reduce the bakery waste problem encountered by Hong Kong or other countries. Bakery waste collected from bakery store was used in submerged and solid-state fermentation of *Monascus purpureus* to produce bio-colorant, which could potentially be applied in food and textile industries. The feasibility of utilizing bakery waste hydrolysate deriving from hydrolytic reactions by *Aspergillus awamori* and *Aspergillus oryzae* for fermentative pigment production was investigated. Preliminary data from bakery waste hydrolysate experiment presented that the highest pigment yield (about 24 AU/g glucose) was obtained with bakery waste hydrolysate containing 5 g/L initial glucose. Results from the solid state fermentation studies presented that the highest activity of glucoamylase and protease achieved was 8 U/g and 117 U/g respectively, at an initial moisture content of 55% and 65% respectively at 30 °C incubation temperature. The outcome from this study demonstrated that *Monascus purpureus* constitutes a promising host for bio-colorant and enzyme production using recovered sugars and amino acids from bakery waste.

1. Introduction

Species of the genus *Monascus* have been used for many years in the manufacture of traditional foods (red wines, tofu, meats, sausages, hams and other products) in East Asian countries as a colorant (Jung et al., 2003). Bio-colorants are coloring agents obtained from biological sources (Chattopadhyay et al., 2008). Pigments are defined as water insoluble substances that are used to color articles like ink, paper, textiles and many more (Chattopadhyay et al., 2008). In this study, pigments deriving from the fungus *Monascus purpureus* referred as both pigment and bio-colorant. *M. purpureus* can produce yellow (ankaflavin—$C_{23}H_{30}O_5$, or monascin—$C_{21}H_{26}O_5$), orange (monascorubrin—$C_{23}H_{26}O_5$ or rubropunctatin—$C_{21}H_{22}O_5$) and red pigments (monascorubramine—$C_{23}H_{27}NO_4$ and rubropunctamine—$C_{21}H_{23}NO_4$) (Hajjaj et al., 2000; Bühler et al., 2013). Among the various pigment producing microorganisms, *Monascus purpureus* pigment has been reported no adverse effect, which can be used as food colorant (Gheith et al., 2008; Kumari et al., 2009; Wang et al., 2007). The pigment of *Monascus* improves the coloring appearance of foods and their sensory characters (Vidyalakshmi et al., 2009).

Production of numerous metabolic products such as alcohols, organic acids (c-aminobutyric acid-also known as GABA, possesses anti-hypertensive in human), antimicrobial agents Monascidin A inhibits bacterial growth of the genera of *Bacillus*, *Streptococcus* and *Pseudomonas* and substances with therapeutic activity (example Cholesterol inhibiting enzyme, lovastatin in preventing cancer) have been investigated (Chen and Johns, 1994; Su et al., 2003; Wong and Bau, 1977; Heber et al., 1999; Dimitroulakos et al., 2001). However, little information about enzymes from *M. purpureus* is available (Liang et al., 2006). Glucoamylase and protease are two widely used hydrolytic enzymes. The amylolytic enzyme glucoamylase catalyzes the cleavage of α-(1, 4) glycosidic bonds in starch and breaks down starch into glucose as end product (Liu et al., 2004; Dufossé et al., 2005). Glucose is commonly used as the major carbon source in the bio-based production of platform chemicals, materials and fuel, such as succinic acid, polyhydroxyalkanoates (PHAs) and ethanol. Major portion of food waste from restaurants composes of starch and small amount of proteins (Liu et al., 2004; Liang et al., 2006; Gustavsson et al., 2011). Utilization of bulk volume of bakery waste as growth substrate in biotechnological process could represent an added value feedstock to the

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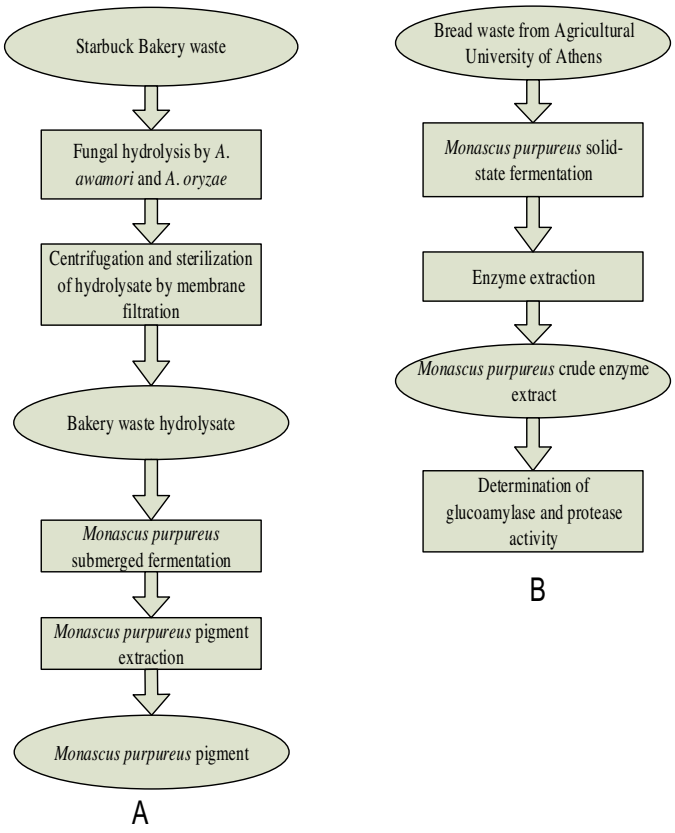

Fig. 1. Fungal bioconversion of bakery waste hydrolysate for pigment and enzyme production.

industry and meets the increasing consciousness of nutrient recovery. This innovative approach for nutrient recovery from bakery waste could provide an insight in reducing the enormous amount of bakery waste disposed around the world (Lin et al., 2013). Recent research conducted by our groups has succeeded in generating nutrient-rich bakery waste hydrolysate using *A. awamori* and *A. oryzae*, which contains glucose, free amino nitrogen (FAN), and phosphate (Pleissner et al., 2014; Pleissner and Lin, 2013; Zhang et al., 2013; Leung et al., 2012; Lin et al., 2014). This hydrolysate could serve as a fermentation feedstock for a wide range of product formation by microbial entities depending on their metabolic capabilities (Pleissner and Lin, 2013).

Filamentous fungus *Monascus* has long been used in Asia as a natural source of pigment in many food products. This genus is subjected to constant studies, mainly due to the growing interest for natural pigments to be used in the food industry. Nevertheless, many researchers have successfully produced *Monascus* pigments under laboratory scale utilizing defined medium containing glucose, maltose, peptone and monosodium glutamate. However, the industrial application of *Monascus* pigments is very limited due to high cost of the defined nutrients. Many researchers have studied low cost substrates such as jackfruit and wheat flour for *Monascus* pigments production (Domínguez-Espinosa and Webb, 2003; Babitha et al., 2006). Rice grain is editable food for human and has also been employed as substrate for pigment production for years. However, microbial pigment production using bakery waste as substrate has not been reported in the literature.

In this study, the nutrient-rich bakery waste derived hydrolysate was investigated to produce bio-colorant from *M. purpureus*. Apart from bio-colorant production using bakery waste hydrolysate, enzymes such as glucoamylase and protease production via solid state fermentation using *M. purpureus* were also evaluated. Fig. 1 illustrates the overall concept for valorization of bakery waste for bio-colorant and enzyme production via fungal bioconversion using *Aspergillus awamori*, *Aspergillus oryzae* and *M. purpureus*.

2. Materials and methods

2.1. Microorganism

Monascus purpureus ATCC 16365 was purchased from the American Type Culture Collection (Rockville, MD, USA) and stored in cryopreservation vials at −80 °C until use in fermentation. *A. oryzae* was isolated from a soy sauce starter provided by the Amoy Food Ltd., Hong Kong (Leung et al., 2012). *A. awamori* ATCC 14331 was purchased from the American Type Culture Collection (Rockville, MD, USA). Spore solutions of both fungi were produced as described earlier (Lam et al., 2013).

2.2. Culture media

Potato dextrose agar (PDA) from Becton, Dickinson and Company (BD), USA was used as a substrate for spore production by *M. purpureus*. After autoclaving PDA media (3.9% w/v) at 121 °C for 30 min, 15–20 mL and 100 mL were used in a petri dish and 250 mL shake flask culture, respectively. The agar plates were inoculated with 100 μL of fungal stock and subsequently spread over the petri dish using glass beads. All the glass beads were removed afterwards to facilitate the growth of fungal spores. The plates were incubated for 3 days in the incubator at 30 °C. Spores were then collected by scratching the surface of the culture with sterile deionized water. Spore counting was conducted using a haemetocytometer and stored in a cryovial in freezer at −80 °C for future use.

Seed culture medium was prepared by dissolving 30 g nutrient broth, into 1.0-L of distilled water. Nutrient agar (Becton, Dickinson and Company (BD), USA) was used for stock culture preparation. An amount of 30 g agar was mixed with nutrient broth (Becton, Dickinson and Company (BD), USA) to prepare 1-L nutrient agar medium using deionised water. The spores were added into the nutrient agar plate and incubated at 30 °C for 7 days.

2.3. Feedstock preparation

Bakery waste collected from Starbucks, Sha Tin New Town Plaza, Hong Kong was hydrolyzed *in-situ* by fungi *A. awamori* and *A. oryzae*. Fungal hydrolysis and solid state fermentation were followed as described by Pleissner et al. (2014). Prior to *in-situ* hydrolytic reactions of bakery waste in the bioreactor, solid state fermentation of the bakery waste was performed in order to produce the fungal solid mass and to facilitate the hydrolysis process.

In the case of *M. purpureus* as enzyme secretor for hydrolysate production, batch hydrolyses of bread waste under different solid concentration and temperature were performed to optimize the hydrolysis conditions. Bakery waste was collected from a bakery store in Agricultural University of Athens, Greece. The collected bakery waste was grinded manually and homogenization was ensured by blending the bakery waste. The well homogenized blended bakery waste was used as the medium for solid state fermentation and the hydrolysis experiments. Two sets of experiments were performed to evaluate the optimum hydrolysis conditions. In order to hydrolyze the bread waste, *M. purpureus* was grown aseptically in a 250 mL shake flask that contained 5 g of bread waste with an initial moisture content of 60% and an incubation temperature of 30 °C. Fungal mashes of *M. purpureus* after 48 h cultivation were then blended aseptically and were transferred in a 1-L Duran bottle for hydrolysis (solid-liquid 10:100) using sterilized deionized water.