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Conventional optimization of aqueous extraction of pullulan in solid-state fermentation of cassava bagasse and Asian palm kernel

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ABSTRACT

Solid-liquid extraction (leaching) is an important downstream processing step for the recovery of pullulan from solid state fermentation. In this study, pullulan was produced in a laboratory scale solid state fermentor (working volume 1.9 L) by *Aureobasidium pullulans* using cassava bagasse and Asian palm kernel as solid substrates. Pullulan was recovered from the substrate after fermentation by solid-liquid extraction. The effects of different factors, such as volume ratio of solvent and solid, pH of solvent, temperature of the system and agitation speed on the recovery of pullulan by *Aureobasidium pullulans* from solid state fermentation using cassava bagasse and Asian palm kernel as solid substrate. The leaching kinetics was followed and the first order rate constants for cassava bagasse and Asian palm kernel were found to be 0.167 min^{-1} and 0.144 min^{-1} respectively.

1. Introduction

Pullulan is a bio-degradable, water soluble microbial polysaccharide produced by *Aureobasidium pullulans*. It can form thin, transparent, oxygen impermeable, edible films (Yuen, 1974). It has been extensively used in food and pharmaceutical applications due to its high molecular weight and film forming capability (Oguzhan and Yangılar, 2013). The price of pullulan is three-fold greater than that of other polysaccharides such as xanthan gum and dextran. Pharmaceutical-grade pullulan costs $25 per kg while food grade pullulan costs $20 per kg (Singh et al., 2008).

Commercially, pullulan is produced from either sucrose or glucose in submerged fermentation (Smf) (Bender et al., 1959; Duan et al., 2008; Chen et al., 2012). Solid state fermentation (SSF) is widely employed for the production of food related enzymes and bio-polymers by filamentous microorganisms owing to less energy requirement, eco-friendly and economy of the process. It is well suitable for the attachment of fungi (or) yeast like fungi growth on moisturized solid substrates to produce value added bio-products with low volume fermentation and high volumetric productivity (Chen et al., 2011; Sugumaran et al., 2012; Sugumaran and Ponnusami, 2015). SSF conditions are similar to those of the natural habitats of many fungi and with abundance of industrial and agriculture residues it is gaining attention of the researchers in the recent past (Chen et al., 2011; Sugumaran et al., 2012; Sugumaran and Ponnusami, 2015). Recently, pullulan was produced from cassava bagasse and Asian palm kernel by *A.pullulans* by solid-state fermentation (SSF) with the aim of reducing the cost of production. The type of substrate, medium composition, moisture content and particle size strongly influence pullulan production in SSF (Sugumaran et al., 2013a, 2013b, 2014a, 2014b; Sugumaran and Ponnusami, 2015).

The fermentation cost and down stream processing covers the major portion of the product cost. Thus, both are equally important. In our previous works, the bioconversion of agricultural wastes like Asian palm kernel, cassava bagasse and jack fruit seed to produce pullulan were demonstrated in solid state fermentation. There are several literatures available on recovery of pullulan from submerged cultures by solvent precipitation technique (Singh et al., 2009; Wu et al., 2009; Durgalakshmi et al., 2014). The influence of different parameters such as screening of organic solvent, volume ratio of organic solvent and precipitation time on recovery of pullulan from Asian palmyra palm kernel was reported by solvent precipitation technique (Sugumaran and Ponnusami, 2014; Durgalakshmi et al., 2014). There are several reports focused the recovery of enzymes from solid matrix by leaching (Castilho et al., 1999, 2000; Gupta et al., 2008; Chen et al., 2011; Ikasari and Mitchell, 1996; Díaz et al., 2007; Bender et al., 2006; Pal and Khanum, 2010). However, to the best of our knowledge recovery of pullulan from solid state fermentation by leaching has not been reported earlier. Since recovery of a product from solid state fermentation requires totally different conditions a detailed study on this is required and is useful.

The objective of the present work is to recovery of pullulan from solid state fermentation by leaching technique. In this work, the effects of solvent volume ratio, solvent pH, extraction temperature, agitation speed and leaching time on the recovery of pullulan from Asian palm

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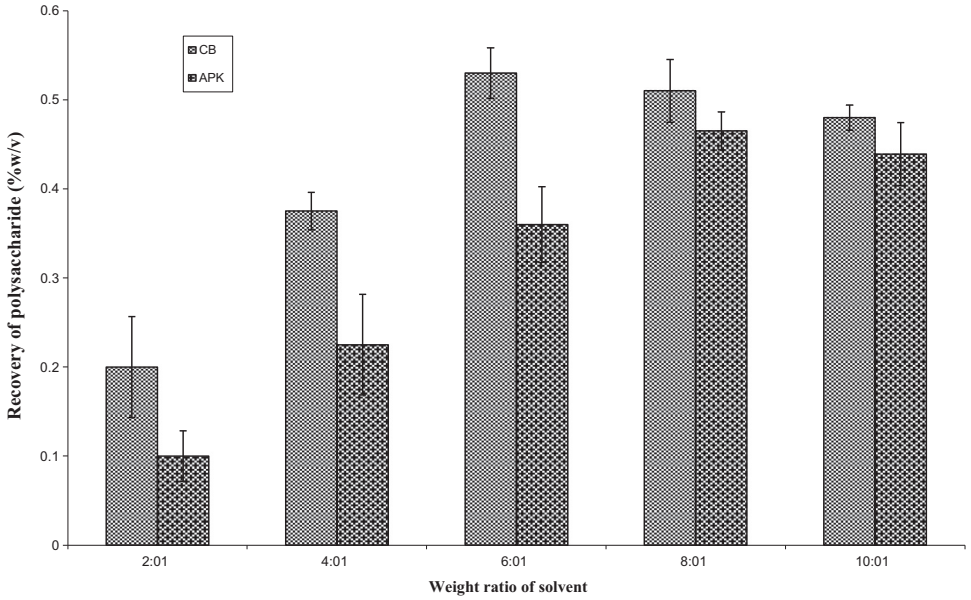

Fig. 1. Effect of volume ratio of solvent on recovery of polysaccharide (CB: Cassava bagasse; APK: Asian palm kernel: pH = 7; rpm = 100; Temperature = 30 °C).

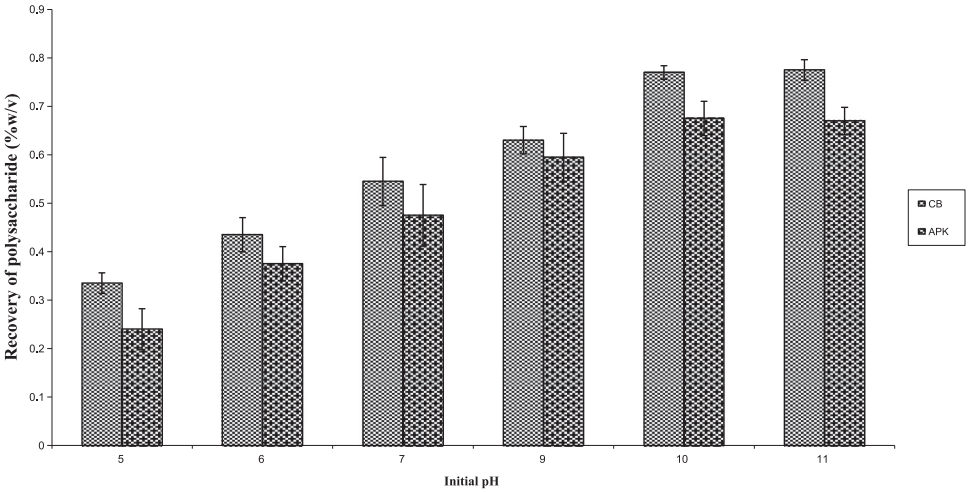

Fig. 2. Effect of initial pH of solvent on recovery of polysaccharide (CB: Cassava bagasse; APK: Asian palm kernel: rpm = 100; Temperature = 30 °C; 6:1 volume ratio for CB and 8:1 volume ratio for APK).

kernel and cassava bagasse by leaching are studied.

2. Materials and methods

2.1. Microorganism and substrate

A. pullulans MTCC 2670 was purchased from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Cassava bagasse (sago industry, salem district, Tamilnadu, India) and Asian palm kernel (local villages, Thanjavur, Tamilnadu, India) were used as solid substrates for pullulan production. Asian palm kernel (BSS # −12.5 + 10) and cassava bagasse (BSS # −5 + 7) were stored in air-tight container after sieving (Sugumaran et al., 2014a; Sugumaran and Ponnusami, 2015).

2.2. Solid state fermentation and recovery of pullulan

The biosynthesis of pullulan was investigated in a laboratory-scale solid-state bioreactor (Murhopye Scientific Company, Mysore, India) with working volume of 1.9 L using the optimized production medium of Sugumaran et al. (2014a); Sugumaran and Ponnusami (2015). Sterilized heterogeneous production medium was inoculated with 5% v/v of three-day old inoculum. The bed was manually agitated by a fork to mix the microorganism uniformly into the bed (Castro et al., 2015). During fermentation, samples were carefully taken from the bioreactor through a sample port. After fermentation, suitable volume of water was added to the fermented medium and it was incubated at 30 °C in a rotary shaker at 200 rpm for 2 h (Sugumaran et al., 2014a; Sugumaran and Ponnusami, 2015). The mixtures were filtered through muslin cloth (Chen et al., 2011) and then centrifuged at 10,000 rpm for 15 min. An equal volume of ethanol was added to the supernatant and the mixture was stored at 4 °C for 1 day for precipitation. The precipitate was re-suspended and then centrifuged again at 4400*g* for 15 min. The obtained pellet was hydrolyzed by commercial pullulanse solution (Sigma Aldrich, India; E. C. 3.2.1.41; aqueous solution, ≥400 units/ml) (Singh et al., 2010, 2011). The reaction mixture was incubated at 50 °C for 6 h. The incubated sample was added with equal volume of DNS solution and kept at 95 °C for 20 min. The reducing sugar present in the sample was assayed by Miller (1959) method. The concentration of pullulan (mg/ml) was estimated by calibration chart using commercial pullulan (TCI chemicals, Tokyo). The recovery of pullulan was expressed as percentage weight of pullulan per volume of reaction mixture (%w/v).