Biochemical Engineering Journal 65 (2012) 90–95

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

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

Short communication

Pectinase production from lemon peel pomace as support and carbon source in solid-state fermentation column-tray bioreactor

Héctor A. Ruiz[a,*], Rosa M. Rodríguez-Jasso[a], Raúl Rodríguez[b], Juan C. Contreras-Esquivel[b], Cristóbal N. Aguilar[b,**]

[a] IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] Food Research Department. School of Chemistry, Universidad Autónoma de Coahuila, Unidad Saltillo, Blvd. V. Carranza e Ing. José Cárdenas Valdés, Saltillo, Coahuila C.P. 25001, Mexico

ARTICLE INFO

Article history:
Received 8 March 2012
Received in revised form 19 March 2012
Accepted 22 March 2012
Available online 30 March 2012

Keywords:
Solid-state fermentation
Bioreactor design
Pomace
A. niger
Pectinase

ABSTRACT

Pectinase is an important enzyme that finds application in many food processing industries and solid-state fermentation (SSF) is an attractive technology for enzyme production. In this work, a SSF process is described for the production of pectinase by *Aspergillus niger* Aa-20 and lemon peel pomace (LPP) as support and carbon source in a solid-state bioreactor. The process consists of three steps. (1) Selection of microorganism for SSF. Eight different fungal strains from the genus *Aspergillus* and *Penicillium* were screened for invasion ability on LPP; (2) Selection of particle size. Invasion ability of selected fungal strain was analyzed on four particle sizes of LPP; (3) SSF process was operated in a column-tray bioreactor at 30 °C and 70% moisture content, 194 mL/min of air flow rate and substrate particle size (2–0.7 mm) of LPP for 96 h. Results showed, that high levels of pectinase activities were obtained. The maximum pectinase activity obtained was 2181 U/L. Maximum biomass and maximum specific growth rate of *A. niger* Aa-20 were X_{max} = 8 mg glucosamine/g of LPP and μ_{max} = 0.127 1/h. The LPP and the use of *A. niger* Aa-20 in SSF suggest as a very promising process for pectinase production.

1. Introduction

Pectinase or pectinolytic are enzymes, which degrade pectin substances and are of great importance to the food industry. It has been reported that microbial pectinases account for 25% of the global food enzymes sales. These enzymes have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater [1]. An alternative for the production of these enzymes is solid-state fermentation (SSF). SSF is a complex heterogeneous three-phase (gas–liquid–solid) and generally defined as the growth of microorganisms, often fungi, on the surface of a porous and moist solid substrate particle in which enough moisture is present to maintain microbial growth and metabolism. This process is carried out in absence or near-absence of visible liquid water between the particles. This condition favors the development of filamentous fungi, given their unique capacity to colonize the interparticular spaces of solid matrices [2]. The spaces between the particles contain a continuous gas phase. SSF generally employ a natural raw material as carbon and energy source, moreover SSF can employ an inert material as solid matrix, which requires supplementing a nutrient solution, containing necessary nutrients as well as a carbon source [3]. SSF plays an important role, and has a great perspective for the use and bioconversion of different agro-industrial residues such as lemon peel pomace (LPP), this material is rich in pectin that acts as the inducer and support, thus it can be used as a substrate for the production of pectinolytic enzymes by microorganisms [2].

Utilization of agro-industrial residues for enzymes production using SSF minimizes the pollution and allows obtaining high added-value products using an economical technology. LPP is the main solid by-product resulting from processing industry lemon and constitute about 19.8% of the dry mass of lemon [4]. As per FAO statistics, in Mexico, the lime production in 2009 was about 2 million tons. It gives an estimation of about 396,000 tons of lemon peel produced per year. The term “pomace” is referring the product remain from agro-industrial residues, principally to citrus peel, apple bagasse, lemon skin, obtained after a thermal pretreatment followed by several washes with water to lower the concentration of soluble sugars and acids before dry [4]. SSF substrates require characteristics (e.g. carbohydrates, nitrogen source, mineral salts) for a good invasion capacity of filamentous fungus over the culture

* Corresponding author at: IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel.: +351 253 604 400; fax: +351 253 678 986.
** Corresponding author at: Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila, Blvd. V. Carranza and González Lobo, ZIP 25280 Saltillo, Coahuila, Mexico. Tel.: +52 844 4161238; fax: +52 844 4159534.
E-mail addresses: hector_ruiz@deb.uminho.pt (H.A. Ruiz), cristobal.aguilar@uadec.edu.mx (C.N. Aguilar).

1369-703X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bej.2012.03.007

matrix. Other important characteristic of substrate is the particle size, may affect the flow pattern and porosity in the substrate. Natural solid substrates generally need some kind of physical pretreatment such as chopping or grinding to reduce particle size to make their chemical constituents more accessible and their physical structure more susceptible to mycelial penetration. The small particles would have reduced porosity, leading to lowering of gas diffusion, while the big particles would absorb less moisture, swell less and thus by drying rapidly support only a sub optimal growth of fungi. The available surface area will decrease with an increase in particle size of the substrate [2,5,6]. For this reason a particle size distribution that has these characteristics of nutrients, specific area and porosity will be a potential substrate for SFF process as support and carbon source.

In order to develop bioreactors for large-scale SSF, a quantitative analysis of kinetics and stoichiometry of the reaction is needed. This analysis is hampered in most natural SSF systems by experimental difficulties, because the substrate/support is structurally and nutrionally heterogeneous and measurement of biomass dry matter is impossible. In every fermentation process, the bioreactor provides the environment for growth and activity for the microorganisms, which cause the biological reaction. SSF employs a great variety of matrices, which vary in composition, mechanical resistance, porosity and water holding capacity. All these factors also affect the reactor design and the control strategy for the parameters. In addition, the morphology of the fungus and its resistance to mechanical agitation and the necessity or not to have a sterile process should be considered in the election of the bioreactor configuration. The most important advances in SSF bioreactors for laboratory, bench and industrial scale have carried out in the last decade being tray, packed bed and rotary drum have being the most useful models [7]. However the majority of the studies have occurred slowly, due to the operational, transport phenomenon (heat and oxygen transfer) and scale-up complications. For that reason, it is necessary to develop and improve bioreactors designed for SFF in order to cover the microorganism growth metabolic necessities for obtaining high concentration of enzymes [8]. The main aim of this work was development a process for the production of pectinase enzyme by a selected *Aspergillus niger* and particle size of LPP through SSF technology. The design and performance of a column-tray bioreactor also was considered.

2. Materials and methods

2.1. Preparation of lemon peel pomace (LPP)

Mature limes (*Citrus aurantifolia*) were obtained in the local market (Saltillo, Coahuila, Mexico). LPP preparation was realized following the procedure reported by Rodríguez-Jasso [4]. Subsequently the LPP was milled using a laboratory knife mill and separated into fractions >2.0 mm (mesh 10); 2.0–0.7 mm (mesh 25); 0.7–0.3 mm (mesh 50) and 0.3–0.17 mm (mesh 180) using a vibratory sieve shaker. A homogeneous blend of this particle size was used for the selection of the microorganism (see below).

2.2. Chemical characterization of LPP

The different particle size distribution of LPP was analyzed for their chemical composition. Physicochemical analysis included the evaluation of total solids, moisture, crude fiber, protein and fat were carried out using the methods described by AOAC methods [9]. Total sugars were determined using colorimetric method (phenol–sulfuric).

Table 1
Filamentous fungi isolated from northeast Mexican desert.

Source	Species	Symbology
Quercus spp	*Penicillium pinophilum*	EH_2
Quercus spp	*Penicillium pinophilum*	EH_3
Larrea tridentate	*Aspergillus niger*	GH_1
Larrea tridentate	*Penicillium purpurogenum*	GH_2
Larrea tridentate	*Aspergillus fumigatus*	GS
Pinus cembroides	*Aspergillus niger*	PSH
Pinus cembroides	*Aspergillus ustus*	PSS

2.3. Screening for selection of microorganism and particle size of LPP

2.3.1. Microorganism and culture medium

Eight filamentous microorganisms of *Aspergillus* and *Penicillium* genus were used in radial growth kinetic. Seven were isolated from Mexico semi-desert plants (Table 1) and obtained from DIA/UAdeC (Food Research Department/University of Coahuila) collection and one fungus strain *A. niger* Aa-20 was obtained from IRD, France-UAMI, México (Institut de Recherche pour le Développement – Universidad Autónoma Metropolitana-Iztapalapa) collection [10,11]. For inoculum preparation the culture was grown in potato dextrose agar (PDA) on flask at 30 °C for 5 days. The growth medium used was Czapek-Dox modified (g/L): $NaNO_3$ (7.66); KH_2PO_4 (3.04); $MgSO_4$ (1.52); KCl (1.52); KH_2PO_4 (2.47); $(NH_4)_2SO_4$ (6.60); $CaCl_2$ (0.48). The initial pH of the medium was adjusted to 5.0. The selected microorganism was used in solid-state fermentation for pectinase production.

2.3.2. Radial growth kinetic for microorganism selection

Fungal strains screening was performed for determination of the microorganism growth rate in Petri dishes measuring radial growth on a homogenous blend particle size of LPP. The moisture (70%) was adjusted with Czapek-Dox modified (g/L): $NaNO_3$ (7.66); KH_2PO_4 (3.04); $MgSO_4$ (1.52); KCl (1.52) and 30% of a homogenous blend of particle size distribution were added to Petri dishes. The LPP was used as sole energy and carbon source. Spores suspension containing around 2×10^7 spores/mL was inoculated at the center over dishes with LPP, monitored the support invasion capacity every 8 h during 4 days. In general, the radial growth can be described and fitted using the exponential model. In this model, reported by Mitchell et al. [12], X is the radial growth (cm), μ is the maximum specific growth rate constant (1/h) and t is time (h).

2.3.3. Radial growth kinetic for LPP particle size selection

Fungal strain selected with highest μ (see above) was used for the selection of LPP particle size. Four particle size distribution of LPP were used >2.0 mm (mesh 10); 2.0–0.7 mm (mesh 25); 0.7–0.3 mm (mesh 50) and 0.3–0.17 mm (mesh 180). The microorganism growth rate was performed in Petri dishes measuring radial growth on each particle size distribution. For the determination of invasion capacity was used the same method for radial growth kinetic in the microorganism selection evaluating maximum specific growth rate μ. The selected particle size was used in solid-state fermentation for pectinase production.

2.4. Solid-state fermentation for pectinase production

2.4.1. Column-tray bioreactor design

The schematic of the column-tray bioreactor, which consisted of a vertical cylindrical, was constructed of acrylic with diameter Ø (25 cm) and height L (38 cm). As shown in Fig. 1A, the bioreactor containing eight perforated base trays (Fig. 1B) that supported the solid substrate (LPP) and through which forced aeration is applied using an aerator pump to increase the accessibility to O_2. The