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Utilization of copra waste for the solid state fermentatative production of inulinase in batch and packed bed reactors

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ABSTRACT

In this study, screening and optimization of nutrients for inulinase production using copra waste has been studied. Plackett–Burman Design (PBD) was employed to screen the significant nutrients for inulinase production. Response surface methodology (RSM) was used to evaluate the effects of nutrient components in the medium. The second order regression equation provides the inulinase activity as the function of K_2HPO_4, $ZnSO_4{\cdot}7H_2O$ and soya bean cake. The optimum conditions are: K_2HPO_4 – 0.0047 g/gds, $ZnSO_4{\cdot}7H_2O$ – 0.02677 g/gds and soya bean cake – 0.06288 g/gds. At these optimized conditions, experiments were performed in packed bed bioreactor to optimize the process variables like air flow rate, packing density, particle size and moisture content. The optimum conditions were: air flow rate – 0.76 L/min, packing density – 38 g/L, particle size – 10/14 mesh and moisture content – 60%. At the optimized conditions, a maximum inulinase production of 239 U/gds was achieved.

1. Introduction

Industries like dairy, food and pharmaceuticals employ fats such as fructooligosaccharides and fructose as vital ingredients. Low calorific value and dietary fiber content make fructooligosaccharides to possess good nutritional properties. They have used as a replacement for sugars in many industries like confectionery and dairy. In addition to this, fructose is also a potential sweetening agent finding more utilization in the place of sucrose. This is attributed to the fact that sucrose is reported to cause atherosclerosis and cancer (Singh, Sooch, & Puri, 2007). Moreover, fructose is reported to enhance iron absorption in children and has a comparatively higher sweetening capacity. Interestingly, the production of both fructose and fructooligosaccharides are produced enzymatic hydrolysis (single step) of inulin using inulinase as the catalyst (Dilipkumar, Rajasimman, & Rajamohan, 2011a). Production of inulinase using different strains of microorganism like *Streptomyces* sp., *Kluyveromyces marxianus*, *Aspergillus niger*, *Bacillus smithi* have been reported (Laowklom, Chantanaphan, & Pinphanichakarn, 2012). Inulin is a polyfructan found in plant consisting of linear chains–β-(2,1)–linked fructose residues attached to a terminal sucrose molecule. Inulin is also found as a carbohydrate reserve in tuberous roots like chicory, dandelion and dahlia. It can be acid hydrolyzed at 80 °C and at low pH values leads to the destruction of fructose, a highly unfavored reaction. The complete hydrolysis of inulin by inulinase can result in 95% yield of pure fructose. With ultra-high fructose syrup productions strongly depending on the availability of inulinase, the requirement for high activity inulinase is very essential.

Solid-state fermentation (SSF) is generally defined as that in which microbial growth and product formation take place on a solid substrate in the absence of free water (Pandey, 2003). In the last two decades, SSF has received increasing interest partly because, as fermentation technology, it has lower energy requirements and produces less wastewater than submerged fermentation (Makino, Treichel, Mazutti, Maugeria, & Rodrigues, 2009). Utilization of cheaper, locally identified agro industrial residues is an attractive alternative for inulinase production, since the activity should be improved over, or at least remain the same as that obtained using a synthetic medium. The choice of the suitable microorganism will be more important for the successful production of this enzyme using agro waste as a substrate. In this research study, a cheap substrate, Copra waste, which is locally available, has been tried as a source for the production of inulinase using the Fungi, *Penicillium rugulosum.* Placket–Burman approach was implemented to screen the nutrients in the medium and a central composite design (CCD) was utilized for the optimization.

In solid state fermentation (SSF), packed bed reactors (PBR) are widely used for enzyme production (Cavalcanti, Gutarra, Freire, Castilho, & Sant'Anna Junior, 2005; Dilipkumar, Rajamohan, & Rajasimman, 2013; Mazutti et al., 2010a, 2010b, 2010c). The advantages of PBR are simplicity of construction and operation and the

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limitations are the difficulty in their control and optimization, owing to the limitations in mass and heat transfer. The objective of this work is to investigate the feasibility of producing inulinase in PBR. The performance of a PBR was investigated by manipulation variables such as air flow rate, packing density, particle size of the substrate and initial moisture content.

2. Materials and methods

2.1. Microorganism

Fungi, *P. rugulosum* (MTCC-3487) was purchased from Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. It was well preserved in the laboratory on solid medium at 5 °C. The medium composition was: Czapek concentrate 10.0 ml, K_2HPO_4 1.0 g, yeast extract 5.0 g, sucrose 30.0 g, and agar 15.0 g, distilled water 1.0 L. Composition of Czapek concentrate was: $NaNO_3$ – 30.0 g, KCl – 5.0 g, $MgSO_4{\cdot}7H_2O$ – 5.0 g, $FeSO_4{\cdot}7H_2O$ – 0.1 g and distilled water – 100.0 ml. Cells were harvested from slants and used to inoculate liquid medium.

2.2. Solid state fermentation

The substrate, Copra waste, was obtained from the Oil mill, Chidambaram, Tamilnadu, India. It was sundried for 48 h and then used as substrate. 10 g of substrate was taken in 250 ml Erlenmeyer flasks and the flask was supplemented with nutrients based on the experimental design. The moisture content of the media was adjusted to 60%. All the flasks were covered with hydrophobic cotton and autoclaved at 121 °C for 20 min. After cooling, each flask was inoculated with 2 ml of the suspension previously prepared and incubated for 120 h in a chamber with temperature and humidity control (Lark, India). In the preliminary screening process, the experiments were carried out for 5 days and it was found that at the 48th hour, the maximum production occurs. Hence experiments were carried out for 48 h.

2.3. Enzyme extraction procedure

After fermentation, distilled water was added (about 5 times) to the fermented matter and the contents were agitated for 30 min at 200 rpm on a rotary shaker (REMI, India) at 28 °C. Then the sample was centrifuged at 15 000 rpm for 20 min and the supernatant were analyzed by DNS method.

Table 1
Nutrient supplements for screening using Plackett–Burman design for inulinase production.

Variable		Levels, g/10 gds	
Nutrient code	Nutrient	Low (−1)	High (+1)
A	Yeast extract	0.01	0.05
B	Beef extract	0.05	0.15
C	$MnSO_4{\cdot}7H_2O$	0.10	0.50
D	K_2HPO_4	0.02	0.07
E	Soya bean cake	0.40	0.80
F	$MgSO_4{\cdot}7H_2O$	0.002	0.012
G	NH_4Cl	0.01	0.03
H	KCl	0.005	0.015
J	$(NH_4)_2HPO_4$	0.05	0.30
K	NH_4NO_3	0.05	0.10
L	$ZnSO_4{\cdot}7H_2O$	0.10	0.50
M	$(NH_4)_2SO_4$	0.06	0.10
N	Corn steep liquor	0.40	0.80
O	Peptone	0.05	0.15
P	Dextrose	0.10	0.30
Q	$FeSO_4{\cdot}7H_2O$	0.0005	0.002
R	KH_2PO_4	0.10	0.60
S	Urea	0.10	0.30

2.4. Experimental methods by response surface methodology (RSM)

RSM consist of a group of empirical techniques used for evaluation of relationship between cluster of controlled experimental variables and measured response. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach. To determine which variables significantly affect inulinase production by *P. rugulosum*, Plackett–Burman design was used. Eighteen nutrients (Table 1) were screened in 20 experimental runs (Table 2) and insignificant ones were eliminated. The low level (−1) and high level (+1) of each factor are listed in Table 1. The statistical software package 'Design Expert 7.1.5' was used for analyzing the experimental data.

After the screening of nutrients, central composite design (CCD) was used to optimize the selected nutrients and to obtain a quadratic model. The selected nutrients were studied at five different levels (Table 3) and experiments were performed according to CCD shown in Table 3. The statistical software package 'Design Expert 7.1.5 was used to analyze the experimental data. Experiments were carried out in triplicate and the average inulinase activity was reported. A second order polynomial equation, which

Table 2
PBD for screening of important variables for inulinase production using *Penicillium rugulosum*.

Run no.	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	Inulinase, U/gds
1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	47.65
2	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	41.67
3	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	66.34
4	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	56.68
5	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	146.94
6	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	82.34
7	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	137.63
8	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	128.65
9	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	71.43
10	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	61.74
11	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	78.76
12	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	65.33
13	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	51.32
14	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	45.22
15	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	57.02
16	1	1	1	1	−1	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	132.56
17	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	−1	1	1	−1	34.23
18	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	1	78.73
19	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	−1	−1	1	62.76
20	−1	1	1	−1	1	1	−1	−1	−1	−1	1	−1	1	−1	1	1	1	1	67.34

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