
RESEARCH NOTE

BIOCONVERSION AND ENZYMATIC ACTIVITIES OF *NEUROSPORA SITOPHILA* GROWN UNDER SOLID STATE AND SUBMERGED FERMENTATION ON SAGO HAMPAS

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Abstract *N.sitophila* was grown under controlled conditions of solid state and submerged fermentation on Sago hampas. The optimum conditions of protein enrichment previously established for sugar beet pulp was used for this study. Under this condition the protein content of Sago hampas under solid state increased from 1.4 to 14.45% (W/W) whereas for Sago hampas and Sago starch, the protein content under submerged condition increased from 1.4%(W/W) and 0.7%(W/W) to 18.56%(W/W) and 43/16%(W/W) based on dry weight of product respectively. The cellulase, α -amylase and glucoamylase activities of *N.sitophila* under solid state condition on Sago hampas were 9.0, 0.6 and 11.8 U/g of wet fermented solid respectively. The enzymatic activities were also measured under submerged fermentation using both Sago hampas and Sago starch as substrate.

Key Words Bioconversion, Enzymatic Activities, *Neurospora sitophila*, Sago Hampas

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18.56 3M p k a r j a 7 ° 1.4 p A k 10 P M C 1A T W 2 ° t B p u C 1A S 1A C O A k r 3x t o 7 h U k ° n
S d C r B S 1A M J o k P A 1C ± E p A 1C B S p ± W ° B a k C S 1B S S B y 1A } p k a r j 43.16 °
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INTRODUCTION

Protein enrichment of various cellulosic residues such as creal milling by-product [1], sugar cane bagasse [2], pulp sludges from sugar industry [3] and starchy lignocellulosic by product [4] are potentially useful in reducing

the environmental impact of those residues and enhancing animal and human food supplies.

Sago hampas is a starchy lignocellulosic by-product generated from Sago palm (Metroxylan sagut) starch processing. It is a starchy fibrous pith residue left over after starch

extraction and it is abundantly and cheaply available in Malaysia. It has been reported to have about 60% starch and 14% fibre on a dry weight basis of which about 25% is made of lignin [6]. Several authors have pointed out the feasibility and advantages of the protein enrichment of Sago hampas by fermentation method using various types of microorganism [4-7]. Although many cellulosic degrading microorganisms mostly fungi are known, few would qualify as food or feed grade [8]. The microfungus, *N.sitophila* which has a long history of use as food in oriental preparations such as Ontigon [9-10], is particularly suited for such a process.

The aim of the present study was to determine the potential of *N.sitophila* for the production of food and enzyme using untreated Sago hampas by solid state and submerged fermentation.

MATERIALS AND METHODS

Inoculum Preparation The inoculum was prepared in 250 ml conical flasks containing 100 ml medium of the following composition (per liter): glucose, 10.0 g; yeast extract (Difco), 2.0 g; $(\text{NH}_4)_2\text{SO}_4$, 0.47 g; Urea, 0.8 g; KH_2PO_4 , 0.714 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; CaCl_2 , 0.2 g; FeCl_3 , 3.2 mg; $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$, 0.78 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.144 mg. The pH was adjusted to 5.5 after sterilization at 121°C for 30 minutes. The fermentation condition used, explained elsewhere, was optimal for *N.sitophila* culture [11].

Medium for Solid State and Submerged Fermentation The production medium contained neither yeast extract nor glucose. Ground dry untreated Sago hampas and Sago starch were used as carbon source. The submerged fermentation was carried out in 1 liter flasks with 100 ml of medium containing

95% (W/W) of ground Sago hampas. The solid state fermentation was conducted in 500 ml flasks with medium of untreated ground Sago hampas and nutrient salts. The inoculum size was based on 10% (v/v) of submerged fermentation.

Substrate Sago hampas, kindly provided by Hup Guan Sago Factory in Johor Darul Takzim, Malaysia, was air-dried and sieved through a 2.0 mm sieve and hammer-milled and stored in plastic bags at room temperature.

Microorganism *N.sitophila* (ATCC 36935) was maintained at 4°C on potato dextrose agar (PDA) slants.

ANALYTICAL TECHNIQUES

Amylase Assay The crude enzyme was extracted from fermented medium by addition of water (1:10 solid medium: water) The suspension was mixed on a rotatory shaker for 1 hour, and centrifuged at 6000 rpm for 15 mins. A reaction mixture of 3.0 ml of final volume containing 2 ml of 0.5% Sago starch in 0.1 M citrate phosphate buffer at pH 4.5 and 1 ml crude enzyme extract was used. The reaction mixture was incubated for 1 hour in a rotary shaker at 250 rpm. The reaction was terminated with 2 ml of 0.12 N NaOH. The released sugar was measured using 3,5-dinitrosalicylic acid (DNS) method of Miller [12]. One unit of enzyme was taken as 1 mmole of glucose released per hour.

Cellulase Assay Mendel method [13] was used in the cellulase assay using 1% carboxymethyl cellulose (CMC) in citric buffer as substrate. The released sugar was measured by DNS method. The same was carried out for glucoamylase assay using Sago starch as substrate.

Determination of total nitrogen and crude

TABLE 1. Results of Solid State and Submerged Fermentation of Sago Hampas and Sago Strach with *N.sitophila* (temp. = 35^aC, pH = 5.5)

Sample	Condition		Duration (h)	Protein (%)	Enzyme Activity (U/g)		
	SSF	SF			a-A	CEL	GA
SH	X		48	14.45	0.60	9.00	15.80
SH		X	36	18.56	0.52	0.56	15.00
SS		X	36	13.16	0.20	1.04	20.50

Average standard deviation = 3% , n = 9

SSF = Solid Stale Fermentation

SF = Submerged Fermentation

a-A = a-amylase

GA = Glucoamylase

SH = Sago Hampas

SS = Sago Starch

CEL = Cellulase

protein. The total nitrogen content was determined by the semi-micro Kjeldahl method (AOAC 1990) using a Tecator Kjeldahl Auto 1030 Analyser System. Crude protein in the biomass was expressed as N x 6.25.

RESULTS AND DISCUSSION

The results for a typical solid state and submerged fermentation of *N.sitophila* on untreated Sago hampas and Sago starch are presented in Figure 1 and Table 1. According to these results the crude protein content of Sago hampas under solid state and submerged increase from 1.4% (W/W) to 14.45% (W/W) and from 1.4%(W/W) to 18/56% (W/W) based on dry weight of product respectively. The bioconversion of cooked Sago starch to protein give rise to biomass containing 43.16% (W/W) crude protein, since the original crude protein content of Sago hampas is low, and it's bioconversion by *N.sitophila* is fast compare to other microorganism, therefore Sago hampas is very promising for large scale production of animal food. The protein production rate for solid state fermentation of Sago hampas in flask was compared with submerged fermentation of it (Table 1). Although the rate of protein production for solid-state fermetation is lower,

but there are numerous advantages for the process of solid state fermentation of Sago hampas wich could lead to industrial scale, such as: nonaseptic conditions, high productivity per unit volume of reactor, reduced energy requirment, low waste water output and low capital cost. Further study on the production yield and productivity for economic evaluation will be necessary. Enzymatic activities that were measured after fungal bioconversion of Sago hampas are presented in Table 1 and Figure 1. Maximal enzymatic activities were observed after 2-3 days of fermentation for glucoamylase.

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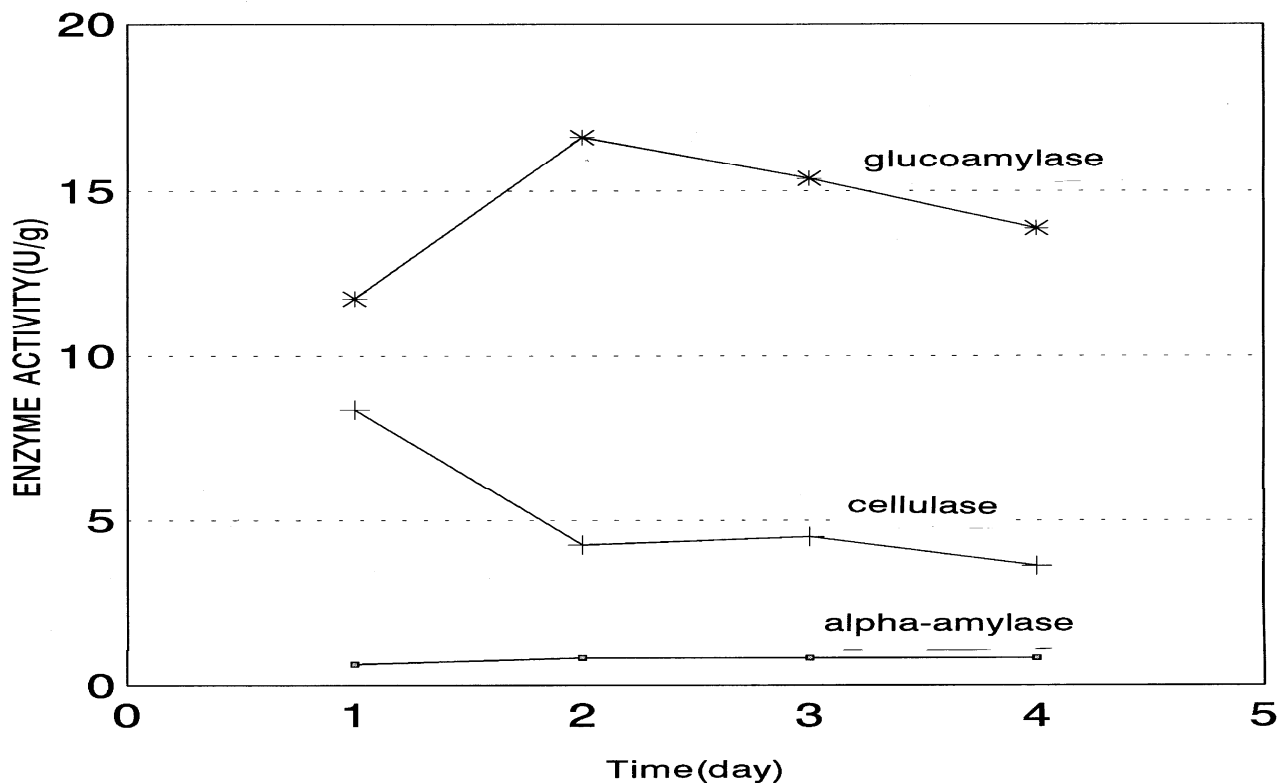


Figure 1. Enzymatic activities of *N. sitophila* during solid state fermentation on Sago hampas (Temperature = 35°C, Moisture = 75% , pH = 5.5).

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