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SOLID SUBSTRATE FERMENTATION OF CACTUS PULP BY ASPERGILLUS NIGER

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ABSTRACT: A new method of culture is described to study the growth of **Aspergillus niger** on cactus pulp in the solid state. After extraction of viscous polyelectrolytes, employed in water treatment, the pulp of **Cereus peruvianus** was used as substrate for solid -state fermentation by a wild strain of **A. niger** . The good conditions for **A. niger** growth were 60% moisture, initial pH 3.0, 35^{0}C, a nitrogen source made of 11% ammonium and 2% urea (on a nitrogen basis), 5% potassium phosphate, and 3.2 g spores/100 g substrate. Related to the dry pulp, maximum fungal growth was observed after 120 hours of fermentation with 14% of protein production.

Keywords: solid-state fermentation, protein, Aspergillus , agricultural wastes, cactus

RESUMO. Investigou-se um novo método de cultura para o crescimento do **Aspergillus niger** sobre a polpa do cactus no estado sólido. Após a extração do polieletrólito natural , uma substância viscosa utilizada em tratamento de águas, a polpa do cactus **Cereus peruvianus** foi utilizada como substrato na fermentação sólida por cepas naturais de **A.niger**. As condições ótimas para o crescimento do **A. niger** foram: umidade de 60%, pH inicial 3,0, 35^{0}C, fontes de nitrogênio constituído de 11% de amônea e 2% de urea (baseados no nitrogênio), 5% de fosfato de potássio, e 3,2 g de esporos por 100 g/substrato. Em relação à polpa seca, o crescimento máxmo do fungo foi observado após 120 horas de fermentação com produção de 14% de proteína.

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INTRODUCTION

Protein production by solid substrate fermentation (SSF) using renewable sources received worldwide attention. This is an important way , in developed countries ,of dietary changes for the partial substitution of animal protein with plant protein[1].

Fermentation with **Aspergillus niger**,a filamentous fungi, have been studied for citric acid[2] ,xylanolytic enzyme[3], pectolytic enzyme[4], β-glucosidase[5] , alpha and gluco-amylase[6] ,and nucleic acid-related substances production[7]. The study of fungal growth in SSF shows advantages over submerged cultures because liquid media are far from the natural environment of fungi[8].

Cereus peruvianus, a kind of cactus native in South America, is used as animal feed, despite its very low protein content, and is also used in the production of natural polyelectrolytes. The polyelectrolytes are used in water treatment as an auxiliary of flocculation and sedimentation[9]. Water, starch, cellulose, chlorophyll, and hemicellulose are the main constituents of cactus, with small amounts of pectin, and lignin[10]. After extraction of polyelectrolytes (soluble carbohydrates) there is the production of large amount of solid wastes constituted basically of fibers, starch, cellulose, and hemicellulose.It is the purpose of this research to use of cactus pulp, after extraction of natural polyelectrolytes, as animal and human feed. Wild strains of **A. niger** were used throughout this work,because of its high amylolytic and cellulolytic activities, for converting cactus pulp directly to feedable microbial protein by solid-state fermentation.

EXPERIMENTAL PROCEDURE

Organism

Wild strains of **Aspergillus niger** were isolated from decomposed wood pulps at different locations, and screened on agar plates containing 100g of dried cactus pulp, 10g $(NH_4)SO_4$, 5 g K_2HPO_4, 1.5 g $MgSO_4.7\ H_2O$, pH 3.6, for 5 days. Cactus pulp was used as the only carbon source, and those black colonies that grew well under such environment, were isolated and retained for second screening. Those isolated organisms from the previous screening were cultured in flasks at 35^0C , after autoclaving (110^0C for 20 min.) , at pH 3.0, containing 10 g of D-glucose and the same medium as the first screening .

Pulp preparation

After removing the external chlorophyll, the cactus was cut in small pieces (10 x 30 mm) . Approximately 132 g of cactus pieces and 750 mL of tap water were transferred to 2 liters flask with shaking for 30 min. The extraction of liquid and viscous natural polyelectrolytes (soluble sugars) was performed by maceration. The polyelectrolytes was kept at 4^0C until use in water treatment. The solid waste was dried, exposing directly to the sun for 6 hours, before using as substrate for SSF.

Solid-state fermentation

Five gram of KH_2PO_4 (Merck -Germany), $(NH_4)_2SO_4$ (Merck) (11.5 g) , and urea (33.7 g) were added to a (30 x 30 cm) tray containing 100 g of dried cactus pulp. After adjusting the moisture to 60 - 65 % with tap water and pH 3.0 with diluted solution of sulfuric acid , 0.5 g of inoculum was transferred to its surface. Three more trays were prepared in the same way and put inside of the greenhouse at 35^0C. The first tray was on the botton surface , and the vertical distance among the first , second , third , and fourth trays were 15 cm respectively. The aeration was performed with an air compressor using silicone tube (60 cm lenght and 5 mm i.d) from the top of the greenhouse.

Analytical methods

Protein and sugar determinations

Fermented mold was soaked with 100 mL of phosphate buffer (pH 6.9) and stirred for 30 min at 60^0 C. The extract was collected by filtration. The extraction process was repeated five times , all the extractants were transferred to flask, and the final volume was made up to 100 mL with distilled water. The method of Lowry et al was employed for protein determination[11] using bovine serum albumin as standard. Reducing sugars was determined using dinitrosalicylic acid (DNS) method[12] , and starch was measured by polarimetric method.

Sample preparation and starch determination by polarimetric method

Crushed sample (2.5 g) and 50 mL of 0.14 $mol.L^{-1}$ HCl (Merck-Germany) were transferred to a 100 mL volumetric flask in a thermostatic bath with shaking for 15 minutes. After cooling, clarifier solutions constituted by 10 mL of 1 $mol.L^{-1}$ zinc acetate dihydrate (Aldrich-USA) and 10 mL of 0.25 $mol.L^{-1}$ potassium ferrocyanide trihydrate (

Aldrich) were transferred to the flask, and the final volume was made up to 100 mL with distilled water. After one or two filtrations this solution was clear enough for polarimetric measurements.The sample solution was transferred to a 20 cm length polarimetric cell and the concentration was determined using the following relationship:

$$[\alpha]_{D(20)} = \frac{\text{observed rotation , degree x 100}}{\text{optical path length (dm) x concentration (g / 100 mL)}}$$

and $C = 100 \times \alpha / L \times \alpha_{D20}$

D = line of sodium (λ = 589.3 nm), L = optical path length (dm)

C = concentration (g / 100 mL) , α = observed rotation (degree)

The specific factor used for starch $[\alpha]_{D20}$ was 84.8 (20^0C).

RESULTS AND DISCUSSION

Table 1 shows the average composition of cactus **Cereus peruvianus.** The composition of cellulose, hemicellulose, chlorophyll , and lignin are variable and depends on cactus age, soil , time of collecting, etc. Table 2 shows the protein contents of cactus pulp, after extraction of natural polyelectrolytes, but before the solid-state fermentation.

Figure 1 shows the influence of moisture on mycelial growth and protein production by **Aspergillus niger** with optimal moisture observed at 60%. Higher moisture may increase the viscosity , decreasing the porosity and oxygen diffusion. The pH also play an important role in solid fermentation. The average pH should be low enough ,otherwise the system will have problems with bacterial contamination. Starting with pH 3.0 , after 140 hours of fermentation the pH increased to 6.0 (Figure 4), even keeping the moisture approximately at 60%. The increase of pH was due to the urea hydrolysis , and it should be controlled during the experiment. Starch consumption was not very related to the protein production, as shown in figure 2, because the substrate used was constituted mainly by cellulose and hemicellulose. This was a clear indication that **Aspergillus niger** was able to produce not only amylolytic, but also hemicellulolytic and cellulolytic enzymes. On the other hand, the glucose consumption was closely related to the protein production as shown in Figure 3 .

Table 1. Average composition % (m/m)* of cactus pulp[10]

Moisture	75-80
Sugar	12
Pectin	1
Protein	< 1

(*) Cactus **Cereus peruvianus**

Table 2. Protein content % (m/m) in cactus pulp (**Cereus peruvianus**)

Cactus pulp (powder)	% protein (*)
after extraction of chlorophyll (* *)	0.85
without extraction of chlorophyll (* *)	1.58
Protein determination by Lowry method	0.80

(*) Average of six determinations. Standard deviation of ± 0,05%.

(* *) Chlorophyll extracted using acetone and protein determination by Kjeldhal method .

Figure 1. Influence of moisture on mycelial growth of Aspergillus niger

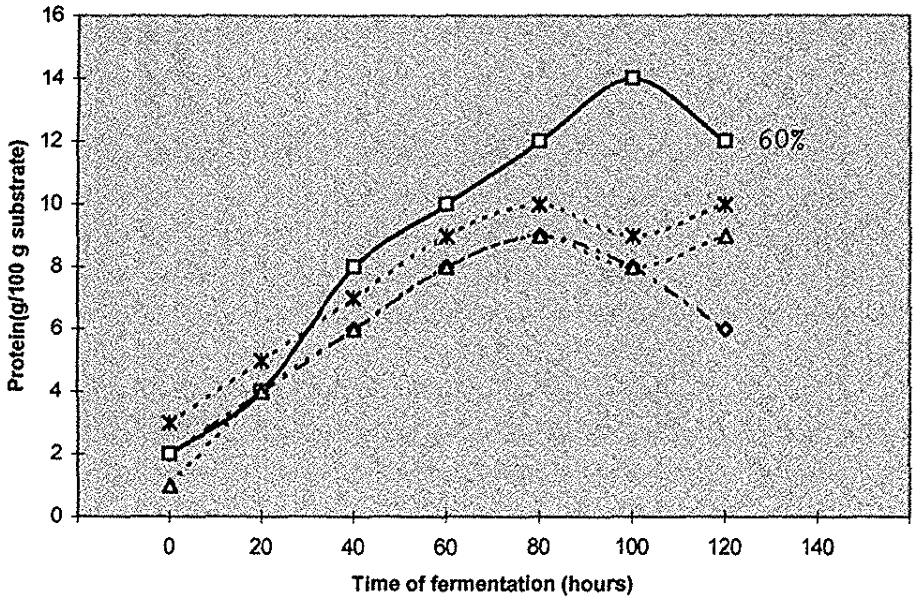
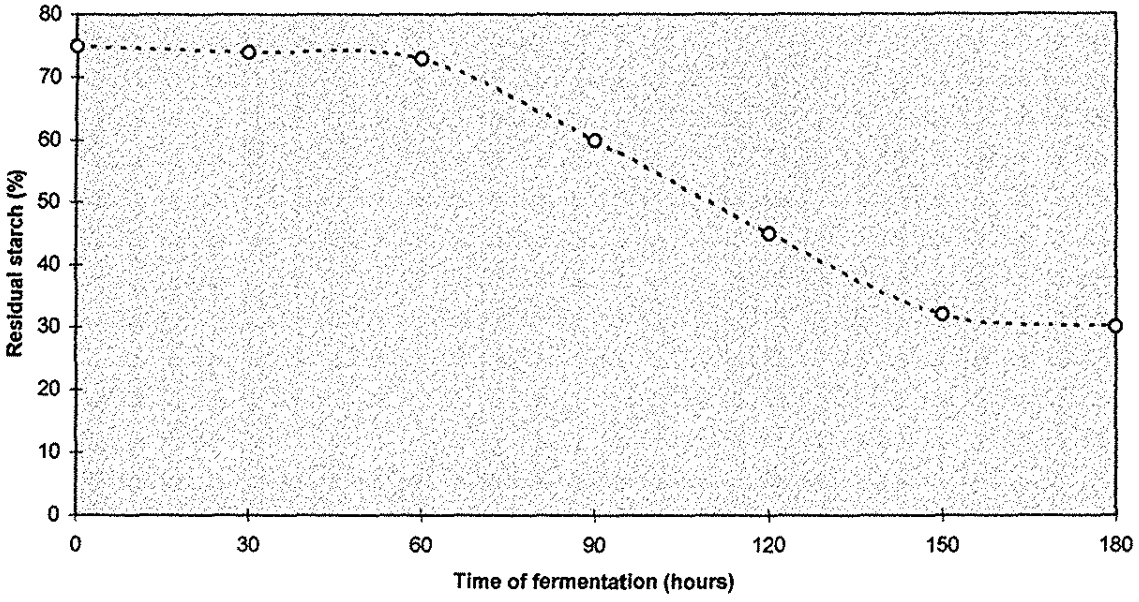


Figure 2. Starch consumption during fermentation



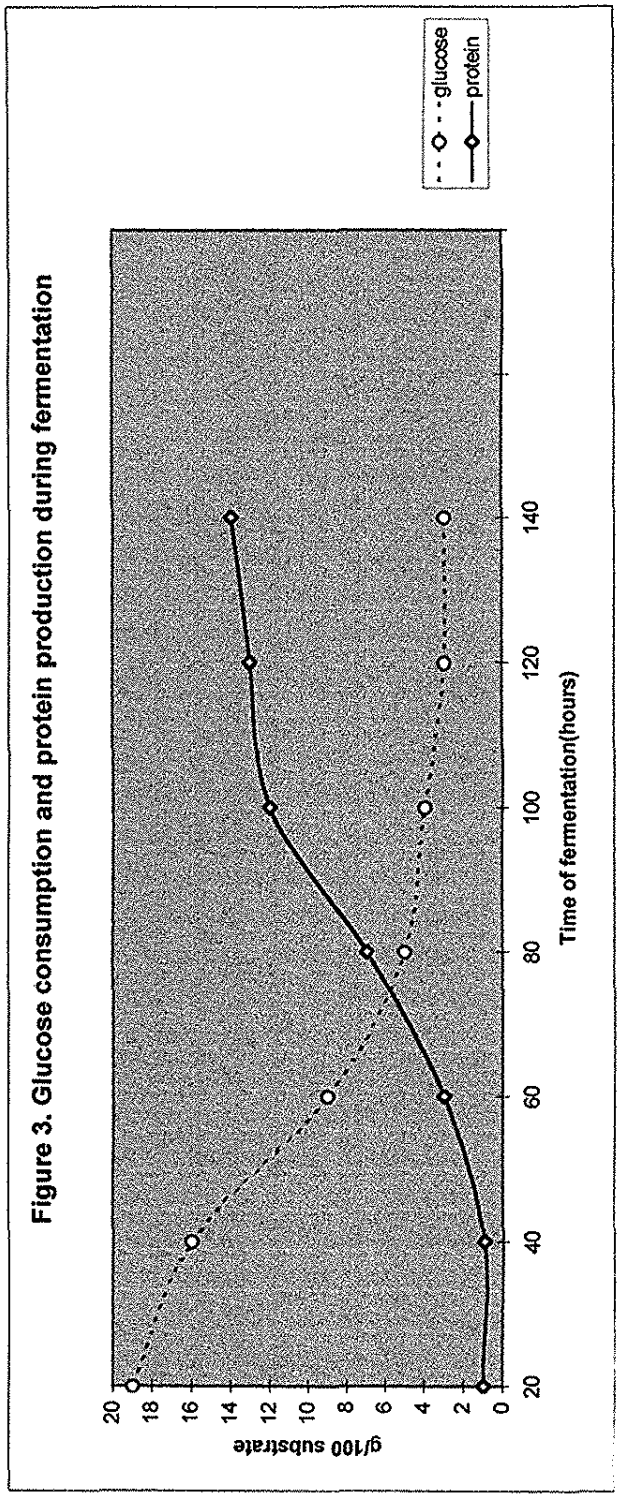


Figure 3. Glucose consumption and protein production during fermentation

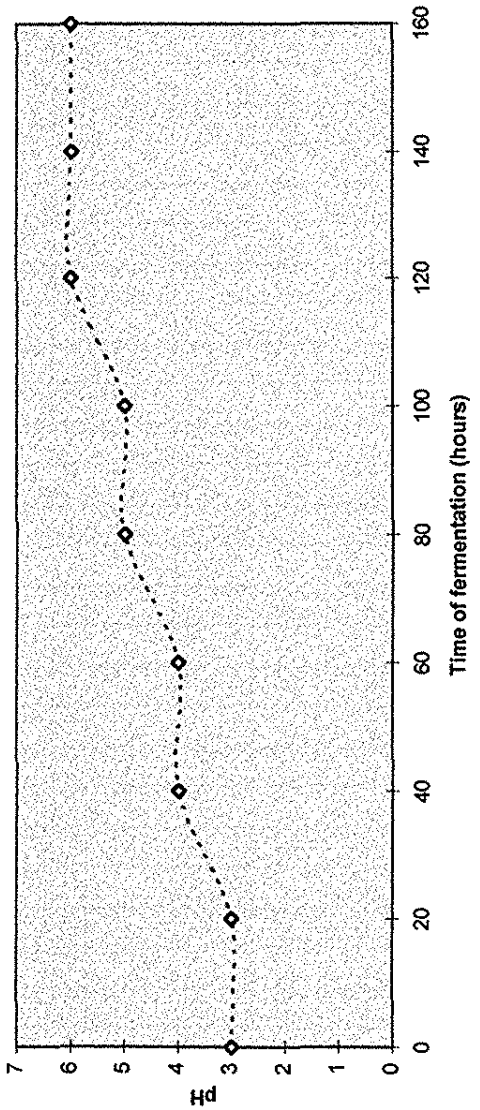


Figure 4. pH variation during fermentation

Conclusion

High efficiency of protein production, reduced energy requirement, low wastewater output, nonaseptic conditions, and low capital investment were the advantages of SSF with wild strain of **Aspergillus niger** using cactus pulp as substrate. High viscosity , due to the presence of natural polyelectrolytes, was a drawback for oxygen diffusion. The difficulty of oxygen diffusion was the main limitation of the process .

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