TECHNICAL ASPECTS OF THE USE OF WASTEWATERS, TO GET USEFUL PRODUCTS THOUGH FERMENTATIVE PROCESSES

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SUMMARY

The use of wastewaters, or by-products from agro, food and beverage industry, presents a great deal of interest, because their chemical composition, rich in carbohydrates, nitrogen, vitamins, aminoacids is useful to promote growth and production of a lot of products using fermentation techniques. In Brasil, our research group is studying different wastewaters, to get some products, using different types of fermenters and different processes, though projects that are sponsored by FAPESP (FUNDAÇÃO DE AMPARO A PESQUISA NO ESTADO DE SAO PAULO) and OEA (ORGANIZATION OF AMERICAN STATES)

INTRODUCTION

Several microorganisms have been studied to get useful products by fermentation processes using defined culture media.(1) However in Brazil, there is a lot of wastes and wastewaters which are riches as sources of carbon, nitrogen and so on, useful to growth of various types of microorganisms. (4).

Sugar cane molasses, corn steep liquor, cocoa water, alcohol stillage, some types of meals and brans (rice, soya, wheat, sorghun) and residues from pulp and paper industry, where used to get a bacterial insecticide and a type of protease, in this study.(2,3,4).

Bacterial insecticide using *Bacillus thuringiensis*, are very important, because they are toxics only to insect pests, not to vertebrates, fishes, beneficial insects, humans, and so on.

Two patents of these two type of toxins, one for Lepidopters and another against Diptera, were get in Brazil.(2,4).

This paper deals with some aspects, relatively to the microorganisms, processes used, types of wastes and wastewaters, parameters of fermentation processes.

Submerged and semi solid fermentation were studied (1,2,3,4).

MATERIALS AND METHODS

Twenty strains of molds were examined in order to obtain the one that produces proteolitic enzymatic extract of better performance in acidic medium by a semi solid fermentation process.

Proteases were obtained as an extracelular raw extract, in 250 mL erlenmeyers, at 37° C with 2,0 g of soybean flour enriched with 10% of sucrose or 5% of yeast extract, moistened with buffer acetate solution pH 7,8, at a rate of 1:1 (w/v).

Under these conditions the enzymatic extract was obtained in 32 hours.

To get the bacterial insecticide, the microorganism used was *Bacillus thuringiensis* and was studied both the submerged and semi solid fermentation. The culture media ,in submerged process was composed by sugar cane molasses and corn steep liquor.

In semi solid fermentation it was used some types of meals , residues from pulp and paper industry, from malt fermentation (beer production), and cocoa water, to maintain moisture level.

Fermentors from 1000 mL to 20 L , besides studies in shakers with erlenmeyers of 500 mL , were used in submerged fermentation. Important parameters and variables involved in the submerged process of fermentation, were pH, sugar consumption and dipicolinic acid formation (DPA). The kinetics of growth, pH variation, sugar consumption and DPA formation were determined.Aeration and agitation importance were verified in *Bacillus thuringiensis* fermentation.

In semi solid fermentation several thicknesses of the fermentation layer were studied and the moisture level was observed.

RESULTS AND DISCUSSION

Table I presents the results of the semi solid fermentation of *Bacillus thuringiensis* (Bt) using culture media composed by residues from the industry of pulp and paper and from beer industry (malt bran). The moisture level was adjusted with cocoa water.

Table I : Sporulation of Bt in two substrates

time	pulp and paper residue			malt bran		
		humidity	sporulation		humidity	sporulation
hs	pH	%	s/g	pH	%	s/g
0	8.0	50.0	-	5.0	52.5	
48	6.9	-	$7.9\ 10^{8}$	5.2	50.4	$3.4\ 10^{8}$
120	8.5	50.2	$5.6\ 10^{8}$	6.3	54.8	$5.9\ 10^{10}$
168	8.3	54.2	$5.1\ 10^{11}$	6.3	64.5	$1.8\ 10^{12}$
220	8.2	55.7	$3.0\ 10^{13}$	8.1	62.8	$3.0\ 10^{15}$

Table II presents the results of Bt sporulation (measured as DPA concentration) in submerged fermentation (1 L fermentor), varying the aeration rate from 0.6 vvm to 1.6 vvm (air volume by culture media volume by minute). The culture medium used was composed by 10 g/L sugar cane molasses and 25 g/L corn steep liquor.

Table II DPA and Biomass formation during submerged fermentation of Bt varying the aeration rate (10 mg/g DPA corresponds to $30\ 10^{8}$ spores)

Aeration rate	time of fermentation	Biomass	DPA/Biomass
vvm	hs	g	mg/g
0.6	25	0.050	18.06
0.8	8	0.033	39.06
0.8	30	0.081	23.89
1.2	8	0.039	29.76
1.2	30	0.039	19.85
1.6	8	0.033	29.32
1.6	30	0.045	21.50

Table III presents the relative activity of the proteolitic extract obtained with *Aspergillus awamori* NRRL 312 in different carbon sources. The semi solid fermentation was performed in 250 ml erlenmeyers with 2 g of culture media. 2 ml of the spores suspension fermented at 37° C, 48 hs.

Table III : Relative activity of the enzymatic extract

Substrates	Relative activity %				
wheat meal	69.0	53.7	75.5	50.6	72.1
soybeam extract	73.6	79.1	98.9	74.0	70.1
cassava meal	0.0	0.0	0.0	0.0	5.0
peanut bran	0.0	0.0	0.0	0.0	0.0
corn meal	3.2	0.0	7.7	2.2	7.6
cotton bran	10.2	3.9	12.6	11.6	8.1
paper/pulp	0.0	4.4	11.6	10.0	0.0
time hs	30	32	34	36	38

CONCLUSIONS

Agroindustrial wastes and wastewaters showed to be important sources of carbon, nitrogen ,vitamins and aminoacids, useful to the growth of *Bacillus thuringiensis* and *Aspergillus awamori* , to produce bacterial insecticide and proteases , respectivelly

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