BIOSYNTHESIS OF PECTINOLYTIC ENZYMES BY ASPERGILLUS ON APPLE PULP

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Abstract: The aim of this work was to develop a low cost process for apple pulp utilization. The apple pulp combined with corn flour and simple mineral salts by submerged production of pectinolytic enzymes by the fungus *Aspergillus*. Different concentration on apple pulp and different pH initial on the bases were studied, and all other process parameters were same. Results of different concentration on apple pulp gived maximal endo- PG with 1% apple pulp, during from 96 h, and the growth of the microorganism showed maximum dry weight on initial pH on bases- 4, during from 48 h. From results can be see that the apple pulp can be used as inexpensive base for industrial production on pectinolytic enzymes by *Aspergillus*.

Key words: apple pulp, biosynthesis, pectinolytic enzymes, *Aspergillus*

Introduction

The pressed apple pulp is actual waste from food and agriculture industry and because the aim of this work was to develop a low cost process for apple pulp utilization. Accordingly this production of pectinolytic enzymes based on submerge state bioprocessing by Aspergillus niger of this actual waste, was developed.

Aspergillus niger has been recognized as an industrially important fungus since the U.S. Food and Drug Administration (FDA) approved the GRAS status for many substances which were produced with it. These fungi produce several commercial enzymes, amongst which is also the pectinasis, its commercial value being about 20% of a USD billion annual sales of all industrial enzymes (Kashyap et al., 2001).

The utilization of microbial enzymes has found broad technological application in different industrial processes. Fungal pectolytic enzymes are used in the food industry for the production of fruit juices, olive oil and wine to increase yields and in the clarification of juices and wines (Fogarty and Kelly, 1983; Pilknik and Voragen, 1993).

These enzymes are usually produced on solid or submerged fermentation (Schmidth et al., 1995). Submerged fermentations generally produce smaller quantities

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of secretory enzymes and solid fermentations are not susceptible to automation. For the industrial production of pectinolytic enzymes it is important to improve the fermentation conditions, for better production of extracellular enzymes on inexpensive carbon sources such as apple pomace, citric peels, pectin or other agricultural wastes which contain appreciable quantities of pectin (Alkorta et al., 1998; Larios et al., 1989). The most authors describe the use of an optimized medium composition to increase the enzyme content (Berovič and Ostroveršnik, 1997),

Material and methods

2.1 Micro-organism

The microorganism used in this work was the fungus *Aspergillus niger MK-10*, which was isolated from soil as a highly active producer of pectinolytic enzymes and was maintained on slant agar according to Czapek with 2% pectin. Spores from 3 days old agar slants were collected by adding sterile distilled water to each slant. The spores suspension was adjusted to a final concentration in the culture medium of 6**.** 10^6 spores ml-1.

2.2 Media and fermentation procedure

The medium for *Aspergillus niger MK-10* was prepared by adding different concentration of apple pulp (1%; 2%; and 3%, w/v) and different pH initial (2-8) to the basic medium of the following composition : corn flour- 0,5% (v/v); $(NH_4)_2HPO_4$- 0,7% (v/v); KH_2PO_4- 0,1% (v/v); $MgSO_4.7H_2O$- 0,05% (v/v); and KCl- 0,05% (v/v);. The base was previously sterilized by autoclaving at 121 ^oC for 30 min. The pressed apple pulp first are dry and after are mill to the ground apple pulp particles with the diameter under 0,315 mm.. The refuse apple pulp had the following content: moisture 10÷12 %, ashes 3÷5 %, proteins 6÷6,2 %, and pectin 9÷10 %.

The growth of the microorganism and synthesis of pectinolytic enzymes were performed in 500 ml flasks (100 ml base) with rotational shaking (200 rev min-1) on a rotational laboratory shaker, at 30 ^oC within 120 h.

2.3 Enzyme assay

Endo-pectinolytic activity (endo-PG), based on change in the viscosity of the reaction mixture (0.35% pectin solution, buffered at pH 4.5 in 0.1 mol l-1 citrate) at 30 ^oC, was determined using Ostwald viscometer. The degree of degraded pectin (A) under known amount of filtrate(enzyme) was calculated with the formula: $A= 100 \cdot (Ts-Tt)/(Ts-Tw)$ where Ts is the flow time of the substrate control. Tt is the flow time of the test and Tw is the flow time of water. 1 U was defined as the amount of enzyme which catalyses hydrolyse of 1 g pectin per 1 h at 40 ^oC. 1 IU is the amount of enzyme which catalysees hydrolyse of 1 μmol pectin per 1 min at 40 ^oC.

2.4 Biomass production measurements

Biomass production was measured as dry weight (DW). After filtering, the retained cell mass was dried at 100 ^{o}C to constant weight.

Results and discussion

Results of different concentration on apple pulp (Table 1), gived maximal endo- PG (480,5 U l^{-1}) with 1% apple pulp, compared with endo-PG (398,4 U l^{-1}) with 2% and endo-PG (190,5 U l^{-1}) with 3% apple pulp during of cultivation 96 h.

Results of different initial *pH* on bases (Table 2), gived maximal endo-pectinolytic activity (endo-PG) (496,4 U l^{-1}) on *pH*=4 compared with endo-PG (452,3 U l^{-1}) on *pH*=3, endo-PG (420,2 U l^{-1}) on *pH*=5 and endo-PG (353,9 U l^{-1}) on *pH*=6.

Tabela 1 Uticaj razlicitih concentracija jabukove pulpe na podloge na biosintezu pektinolitickih enzima

Table 1 The influence of different concentration apple pulp on bases on biosynthesis of pectinolytic enzymes.

Koncentraciju jabukove pulpe, %(w/v) *Concentration on apple pulp,%(w/v)*	Vreme kultivacije (h) *Duration of cultivation(h)*	pH krajno *pH final*	Endo-PG (U l^{-1}) *Endo-PG (U l^{-1})*	Biomasa (g l^{-1}) *Biomass (g l^{-1})*
1	0	4,0	0	0
1	24	3,25	53,5	24,5
1	48	2,58	289,4	32,3
1	72	2,64	455,2	21,3
1	96	2,96	480,5	15,6
1	120	3,10	398,2	11,9
2	0	4,0	0	0
2	24	3,24	44,2	15,8
2	48	2,43	232,4	24,5
2	72	2,46	304,7	13,2
2	96	2,83	398,4	12,4
2	120	2,84	334,8	11,2
3	0	4,0	0	0
3	24	3,31	23,3	12,9
3	48	2,49	54,6	21,2
3	72	2,43	98,2	12,2
3	96	2,57	190,5	11,3
3	120	2,66	176,4	10,9

Note: The values are average from 3 replicates

The growth of the microorganism (dry weight) (Table 1) showed maximum dry weight (32,3 g l^{-1}) on 1% concentration of apple pulp and initial *pH* on bases- 4, during of cultivation 48 h compared with maximum dry weight (24,5 g l^{-1}) on 2%, and (21,2 g l^{-1}) on 3% concentration of apple pulp. The growth of the microorganism (dry weight) (Table 2) showed maximum dry weight (31,2 g l^{-1}) on *pH*-4, (23,5 g l^{-1}) on *pH*-3, (25,4 g l^{-1}) on *pH*-5, (18,9 g l^{-1}) on *pH*-6.

The results gived that the concentration of apple pulp and *pH* on bases had a pronounced effect on the biosynthesis of pectinolytic enzymes and growt by *Aspergillus niger MK-10.* From results it became clear that on the biosynthesis of pectinolutic enzymes optimal concentration on apple pulp is 1% (w/v) and optimal *pH*=4 during of cultivation 96 h, and for the growth on microorganism optimal concentration on apple pulp is 1% (w/v) and pH=4 during of cultivation 48 h.

The results presented here as optimal concentration on apple pulp and *pH* on the medium with a inexpensive refuse apple pulp as a carbon source for maximal enzyme production by *Aspergillus niger MK-10* will be of commercial importance for using refuse apple pulp.

Tabela 2 Uticaj pocetne pH podloge na biosintezu pektinolitickih enzima
Table 2 The influence of initial pH of the medium on biosynthesis of pectinolytic enzymes

Pocetno pH *Inital pH*	Endo-PG (U l^{-1}) *Endo-PG (U l^{-1})*	Biomasa (g l^{-1}) *Biomass (g l^{-1})*
2	293,8	12,9
3	452,3	23,5
4	496,4	31,2
5	420,2	25,4
6	353,9	18,9
7	289,3	14,3
8	210,8	10,5

Note: The values are average from 3 replicates

Conclusion

Microbial enzymes are routinely used in many environmentally friendly and economic industrial sectors. Pectinolytic enzymes play an important role in food processing industries and alcoholic beverage industries. The production of food enzymes related to the degradation of different substrates. These enzymes degrade pectin and reduce the viscosity of the solution so that it can be handled easily. In this paper has been studied the effect of different concentration apple pulp on bases and initial pH on biosynthesis of pectinolytic enzymes in laboratories trials, and all other process parameters such as the type and concentration of nitrogen source, the growth

temperature, the aeration rate and the level of agitation were same and should be investigated in further research.

The best carbon source on base for better production of pectinolytic enzymes was the pressed apple pulp with optimal concentration of 10.0 g l^{-1}. As this residue is renewable and in an abundant supply, it represent a potential low cost material for microbial enzyme production. The results presented here will be of commercial importance for using pressed apple pulp as a carbon source for production of pectinolytic enzymes in submerged fermentation.

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