

**THE EFFECTS OF INORGANIC SALTS ON  
BIOSYNTHESIS OF PECTINOLYTIC ENZYMES  
BY *ASPERGILLUS NIGER***

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**Key words:** Apple pulp, fermentation, inorganic salts, pectinolytic enzymes, *Aspergillus niger*

**Abstract:** The paper examines effects of different inorganic salts in an apple pulp base on the production of pectinolytic enzymes with the aim of optimizing the medium for maximal enzyme production. The apple pulp combined with corn flour and simple mineral salts was used as a nourishing base in submerged production of pectinolytic enzymes by the fungus *Aspergillus niger* MK-15. The growth of the microorganism (dry weight) on different sources of nitrogen showed maximum dry weight with  $(\text{NH}_4)_2\text{HPO}_4$ . The growth of the microorganism (dry weight) on different concentration of  $(\text{NH}_4)_2\text{HPO}_4$  (by 0.2% to 0.8%) provided maximal dry weight with 0.7%  $(\text{NH}_4)_2\text{HPO}_4$ . The different inorganic salts (sources of nitrogen) on base stimulated the production of pectinolytic enzymes and enhanced by up to twofold the growth of *Aspergillus niger* in submerged fermentation. The best source of nitrogen on base was  $(\text{NH}_4)_2\text{HPO}_4$  with optimal concentration of 0.7%. The obtained results represent practical importance for using apple pulp as a carbon source for production of pectinolytic enzymes in submerged fermentation.

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## Introduction

Pectinolytic enzymes are usually produced on solid or submerged fermentation (Friedrich et al., 1989; Bailey and Pessa., 1990; Schmdth et al., 1995). Submerged fermentations generally produce smaller quantities 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; Maldonado et al., 1989; Leuchtenberger et al., 1989; Aguilar and Huitron, 1986; Lariou et al., 1989). The most authors describe the use of an optimized medium composition to increase the enzyme content (Pericin et al., 1992; Berovic and Ostroversnik, 1997).

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 Kell, 1983; Pilknik and Voragen, 1993).

Extracellular enzyme production can be enhanced by the presence of some inorganic salts in the fermentation medium (Kleinman et al., 1988; Archer et al., 1990). In this work was to investigate the effects of different inorganic salts in an apple pulp base on the production of pectinolytic enzymes with the aim of optimizing the medium for maximal enzyme production.

## Materials and methods

**Symbols and abbreviations.** The following symbols are used in analysis:  $d_p$  - particle diameter, mm;  $n_s$  - stirring speed,  $\text{min}^{-1}$ ;  $t$  - time, h;  $V$  - volume, L;  $\nu$  - temperature,  $^{\circ}\text{C}$ ;  $s$  - moisture, %;  $a$  - ashes, %;  $p_R$  - proteins, %;  $p_E$  - pectin, %.

The abbreviations used are: endo-PG - endo-pectinolytic activity;  $\text{U L}^{-1}$  - Unit per liter.

**Micro-organism.** The microorganism used in this work is the fungus *Aspergillus niger* MK-15, 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 ( $2.10^6$  spores  $\text{mL}^{-1}$ ). In the fermentation medium was added 3mL ( $6.10^6$  spores).

**Media and fermentation procedure.** The medium for *Aspergillus niger* MK-15 was prepared by adding different inorganic salts (sources of nitrogen) as  $(\text{NH}_4)_2\text{HPO}_4$ ;  $(\text{NH}_4)_2\text{SO}_4$ ;  $\text{NH}_4\text{NO}_3$ ;  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_3$  in an apple pulp base. The base was previously sterilized by autoclaving at  $\nu=121^{\circ}\text{C}$  for  $t=30$  min. The pressed apple pulp was air dried and then dry milled to  $d_p=0.315$  mm size. The refuse apple pulp had the following content: moisture  $s=10\div 12$  %,  $a$ =ashes  $3\div 5$  %,  $p_R$ =proteins  $6\div 6,2$  %, and  $p_E$  = pectin  $9\div 10$  %.

The growth of the microorganism and synthesis of pectinolytic enzymes were performed in  $V=500$  mL flasks (100 mL base) with rotational shaking ( $n_s=200$   $\text{min}^{-1}$ ) on a rotational laboratory shaker, at  $\nu=30^{\circ}\text{C}$  within  $t=120$  h.

**Enzyme assay.** Endo-pectinolytic activity, based on change in the viscosity of the reaction mixture (0.35% pectin solution, buffered at pH 4.5 in 0.1  $\text{mol L}^{-1}$  citrate) at  $30^{\circ}\text{C}$ , was determined using Ostwald viscometer. The degree of degraded pectin ( $A$ ) under known amount of filtrate (enzyme) was calculated with the formula:  $A=100*(T_s-T_t)/(T_s-T_w)$  where  $T_s$  is the flow time of the substrate control.  $T_t$  is the flow time of the test and  $T_w$  is the flow time of water. One unit ( $U$ ) was defined as the amount of enzyme which catalyses hydrolyse of 1 g pectin per 1 h at  $40^{\circ}\text{C}$ .

FIGURE 1. THE INFLUENCE OF DIFFERENT SOURCES OF NITROGEN ON BIOSYNTHESIS OF PECTINOLYTIC ENZYMES BY *ASPERGILLUS NIGER* MK-15

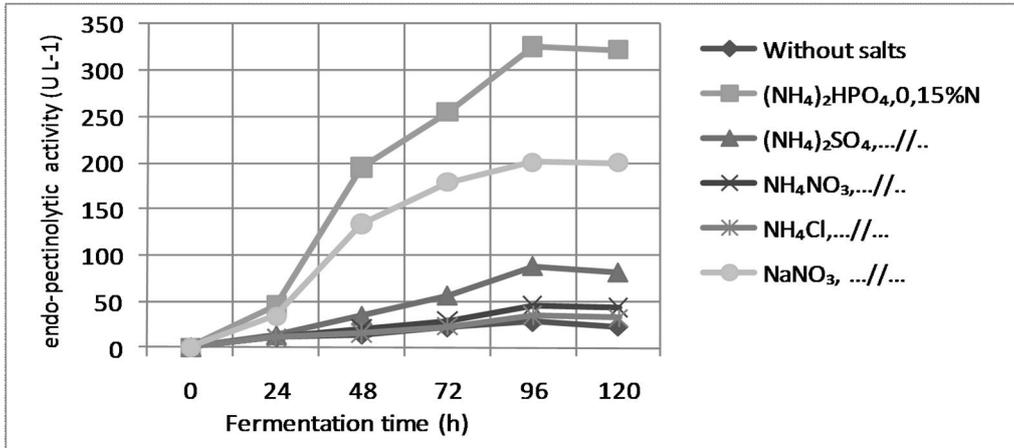


FIGURE 2. THE INFLUENCE OF DIFFERENT SOURCES OF NITROGEN ON BIOMASS PRODUCTION BY *ASPERGILLUS NIGER* MK-15

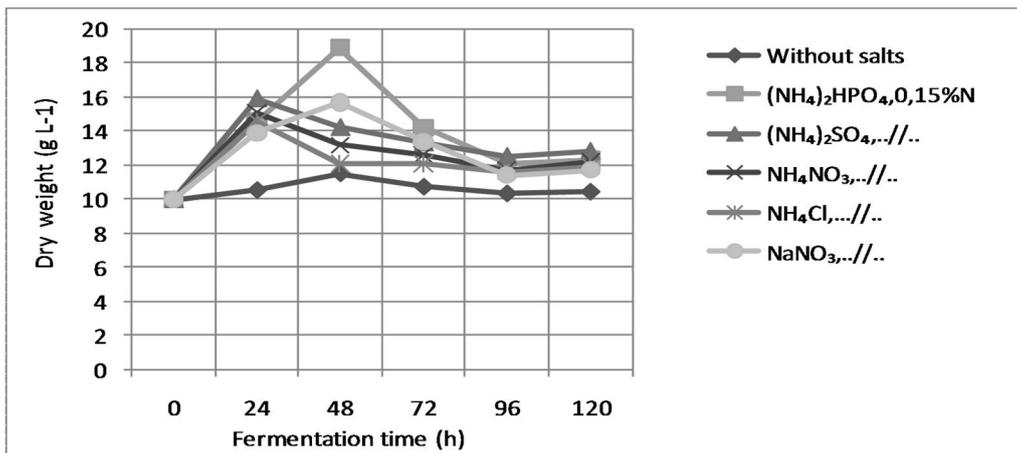


FIGURE 3. THE INFLUENCE OF DIFFERENT CONCENTRATION (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> ON BIOSYNTHESIS OF PECTINOLYTIC ENZYMES BY *ASPERGILLUS NIGER* MK-15

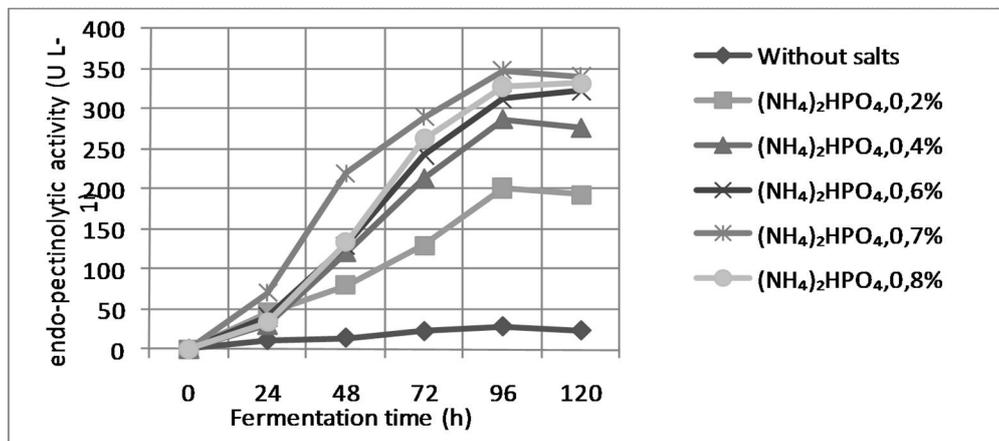
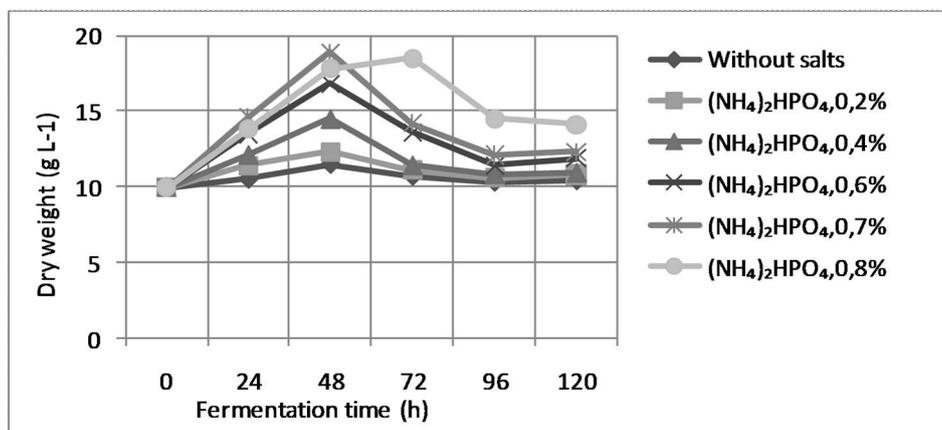


FIGURE 4 . THE INFLUENCE OF DIFFERENT CONCENTRATION  $(\text{NH}_4)_2\text{HPO}_4$  ON BIOMASS PRODUCTION BY *ASPERGILLUS NIGER* MK-15



**Biomass production measurements.** Biomass production was measured as dry weight . After filtering, the retained cell mass was dried at 100°C to constant weight.

### Results

Applying different sources of nitrogen(0.15%N) on base (Figure 1) has resulted maximal endo-pectinolytic activity (endo-PG) (328 U L<sup>-1</sup>) with  $(\text{NH}_4)_2\text{HPO}_4$ , compared with endo-PG (201 U L<sup>-1</sup>) with NaNO<sub>3</sub>, endo-PG (88 U L<sup>-1</sup>) with  $(\text{NH}_4)_2\text{SO}_4$ , endo-PG (45 U L<sup>-1</sup>) with NH<sub>4</sub>NO<sub>3</sub>, endo-PG (35 U L<sup>-1</sup>) with NH<sub>4</sub>Cl and endo-PG (28 U L<sup>-1</sup>) without salt. Maximal results were achieved 96h after inoculation.

Results of different concentrations of  $(\text{NH}_4)_2\text{HPO}_4$  (by 0.2% to 0.8 %) (Figure 3) have demonstrated maximal endo-pectinolytic activity (348 U L<sup>-1</sup>) with 0.7%  $(\text{NH}_4)_2\text{HPO}_4$ , 96 h after inoculation.

The growth of the microorganism (dry weight) on different sources of nitrogen (0.15%N) has showed (Figure 2) maximum dry weight (18.9g L<sup>-1</sup>) with  $(\text{NH}_4)_2\text{HPO}_4$ , compared with dry weight (15.7g L<sup>-1</sup>) with NaNO<sub>3</sub>, (14.2g L<sup>-1</sup>) with  $(\text{NH}_4)_2\text{SO}_4$ , (13.2g L<sup>-1</sup>) with NH<sub>4</sub>NO<sub>3</sub>, (12.1g L<sup>-1</sup>) with NH<sub>4</sub>Cl, and (11.5g L<sup>-1</sup>) without salt. Maximal results were obtained 48 h after inoculation.

The growth of the microorganism (dry weight) on different concentration of  $(\text{NH}_4)_2\text{HPO}_4$  (from 0.2% to 0.8%) (Figure 4) has provided maximal dry weight (18.9g L<sup>-1</sup>) with 0.7%  $(\text{NH}_4)_2\text{HPO}_4$ , 48h after inoculation. The highest phosphate concentration (0.8%) has also stimulated fungal growth though the maximum biomass production was reached later (72h after inoculation).

From results can be see that inorganic salts (sources of nitrogen) as  $(\text{NH}_4)_2\text{HPO}_4$ ;  $(\text{NH}_4)_2\text{SO}_4$ ; NH<sub>4</sub>NO<sub>3</sub>; NH<sub>4</sub>Cl and NaNO<sub>3</sub> ) stimulated the production of pectinolytic enzymes (maximal results were 96 h after inoculation) and enhanced by up to twofold the growth of *Aspergillus niger* in submerged fermentation (maximal results were 48 h after inoculation).

The phosphate concentration (0.2-0.7%) has stimulated maximal production of pectinolytic enzymes (96h after inoculation) and fungal growth (48h after inoculation).

### Conclusions

Pectinolytic enzymes play an important role in food processing industries and alcoholic beverage industries. These enzymes degrade pectin and reduce the viscosity of the solution so that it can be handled easily. The different inorganic salts (sources of nitrogen) on base as  $(\text{NH}_4)_2\text{HPO}_4$ ;  $(\text{NH}_4)_2\text{SO}_4$ ; NH<sub>4</sub>NO<sub>3</sub>; NH<sub>4</sub>Cl and NaNO<sub>3</sub> stimulated the production of pectinolytic enzymes and enhanced by up to twofold the growth of *Aspergillus niger* in submerged fermentation. The best source of nitrogen on base was  $(\text{NH}_4)_2\text{HPO}_4$  with optimal concentration of 0,7%. The results presented here will be of commercial importance for using apple pulp as a carbon source for production of pectinolytic enzymes in submerged fermentation.

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