THE APPLE PULP WASTE USED AS A NOURISHING BASE BY ASPERGILLUS NIGER

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Abstract: The aim of this work was to develop a low cost process for apple pulp utilization. This actual waste from food and agriculture industry was used as a nourishing base by Aspergillus niger MK-15. The apple pulp combined with corn flour and simple mineral salts was utilized as a nourishing base by submerged production of pectinolytic enzymes by the fungus Aspergillus niger MK-15. Different concentration on apple pulp (1%; 2%; and 3%, w/v) and different pH initial on the bases (4; 5 and 6) were studied. All other process parameters such as inoculation, mixing, aeration, temperature, fermentation time were same. 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 ^{o}C within 120 h. Concentration of inoculum: suspension of 2**.** 10^6 spores ml-1 up to 3 days old (added 3 ml, or 6**.** 10^6 spores). 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 mol l-1 citrate), was determined using Ostwald viscometer.. Biomass production was measured as dry weight (DW), (mg ml-1).
Results of different concentration on apple pulp gived maximal endo- PcAc (31,50 IU ml-1) with 1% apple pulp, and the growth of the microorganism (dry weight) showed maximum dry weight (18,2 mg ml-1) on initial pH on bases- 4, during from 48 h.
From results can be see that the apple pulp waste from food and agriculture industry can be used as inexpensive base (carbon source) for industrial production on pectinolytic enzymes by Aspergillus niger. The best concentration on apple pulp was 1 % (w/v) and initial pH on base 4. 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.
Key words: apple pulp, fermentation, pectinolytic enzymes, Aspergillus niger

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 pectolytic enzymes based on submerge state bioprocessing by Aspergillus niger of this actual waste, was developed.
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 [5], [11].
These enzymes are usually produced on solid or submerged fermentation [3], [6], [12]. 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 [1], [2], [7], [8], [9]. The most authors describe the use of an optimized medium composition to increase the enzyme content [4], [6], [10].

MATERIAL AND METHODS

Micro-organism

The microorganism used in this work was 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 in the culture medium of 6**.** 10^6 spores ml-1.

Media and fermentation procedure

The medium for Aspergillus niger MK-15 was prepared by adding different concentration of apple pulp (1%; 2%; and 3%, w/v) and different pH initial (4; 5 and 6) 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.

Enzyme assay

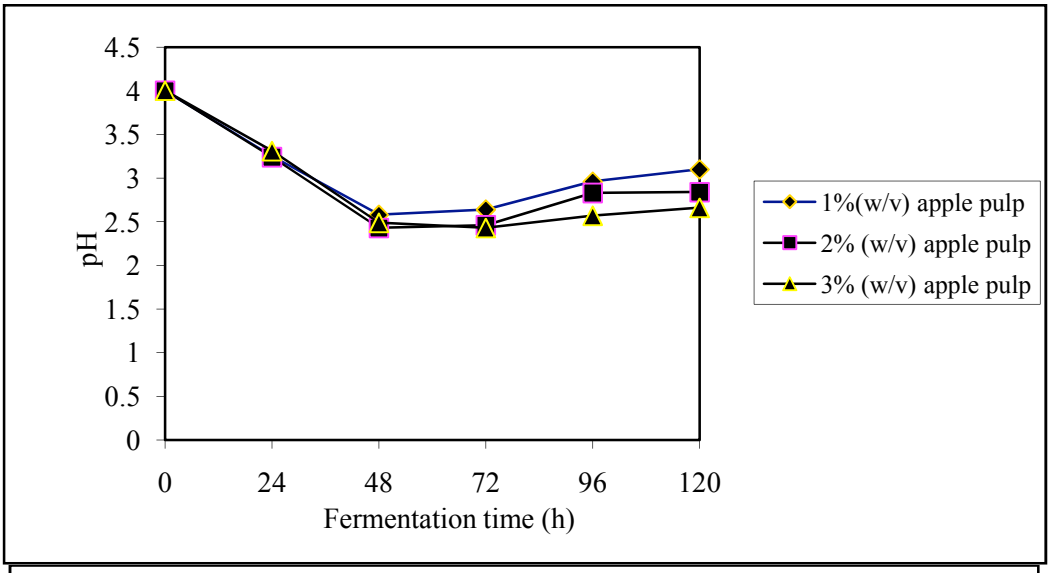
Endo-pectinolytic activity, based on change in the viscosity of the reaction mixture (0.35% pectin solution, buffered at pH 4.5 in 0.1 mol l-1 citrate) at 30 ^oC, was determined using Ostwald viscometer. The degree of degraded pectin (A) under known amount of filtrate(enzyme) was calculated with the 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. $1\ U= (1 \cdot 10^6)/(176 \cdot 60) = 94{,}696$ IU where 10^6 –transfer of (g) into (μg); 176- stands for the conditional meaning of the pectin molecular mass; 60- calculation of the enzyme activity per min.

Biomass production measurements

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

RESULTS AND DISCUSSION

Results of different concentration on apple pulp (Fig.1), gived maximal endo- PcAc (31,50 IU ml-1) with 1% apple pulp, compared with endo-PcAc (28,24 IU ml-1) with 2% and endo-PcAc (24,50 IU ml-1) with 3% apple pulp.
Results of different initial *pH* on bases (Fig.2), gived maximal endo-pectinolytic activity (endo-PcAc) (31,50 IU ml-1) on *pH=4* compared with endo-PcAc (29,00 IU ml-1) on *pH=5* and endo-PcAc (25,74 IU ml-1) on *pH=6.*
The growth of the microorganism (dry weight) (Fig. 3 and Fig.4) showed maximum dry weight (18,2 mg ml-1) on 1% concentration of apple pulp and initial *pH* on bases- 4, during from 48 h compared with maximum dry weight (14,5 mg ml-1) on 2%, and (14,0 mg ml-1) on 3% concentration of apple pulp, during by 72 to 96 h. Dry weight (15,2 mg ml-1) on *pH*-5 and (13,9 mg ml-1) on *pH*-6, during by 72 to 96 h.
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-15. 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 96 h, and for the growth on microorganism optimal concentration on apple pulp is 1% (w/v) and pH=4 during of 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-15* will be of commercial importance for using refuse apple pulp.



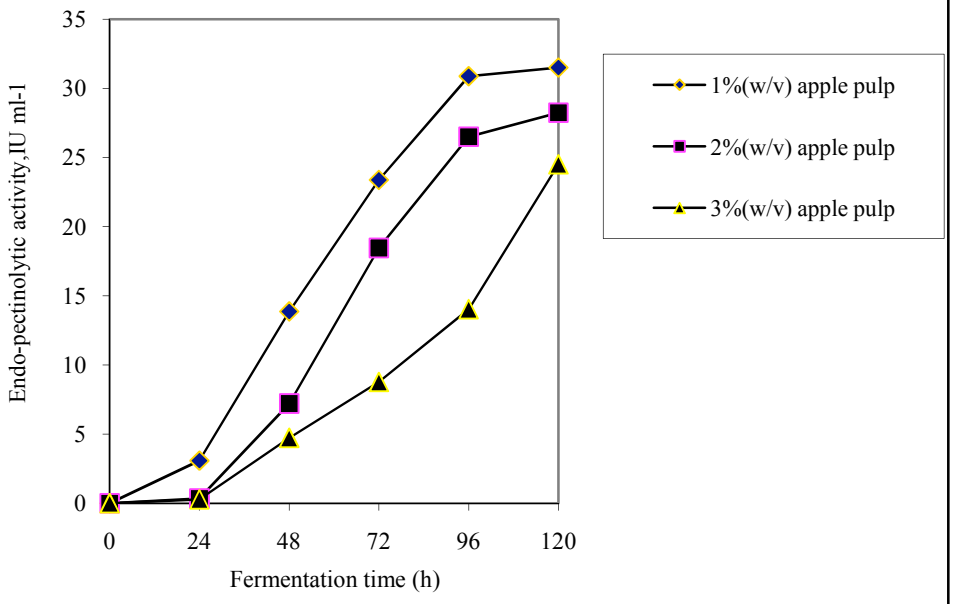


Figure 1. The influence of different concentration apple pulp on bases on biosynthesis of endo-pectinolityc enzymes by Aspergillus niger MK-15. pH starting-4

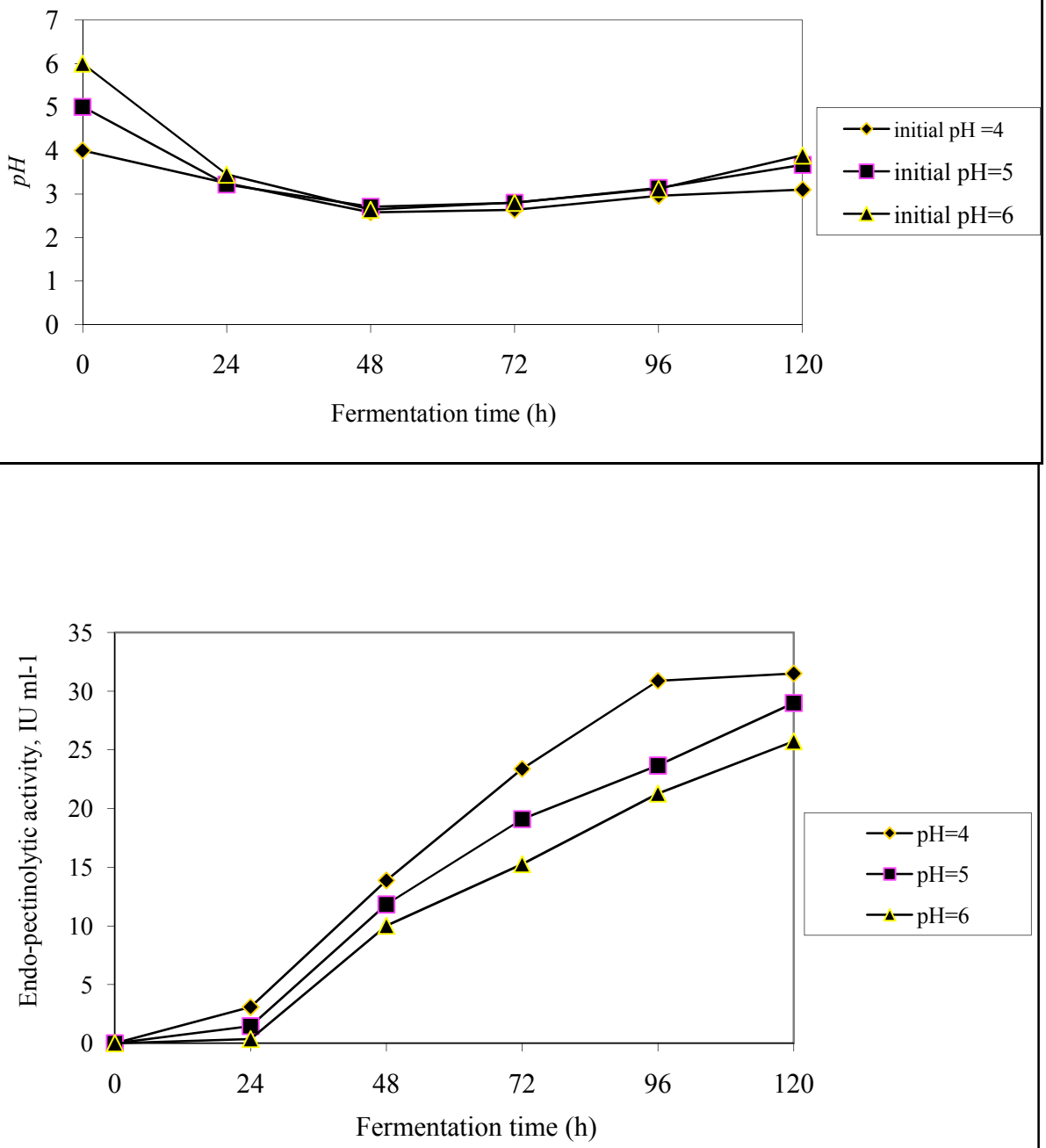


Figure 2. The influence of different pH on bases on biosinthesis of endo-pectinolytic enzymes by Aspergillus niger MK-15

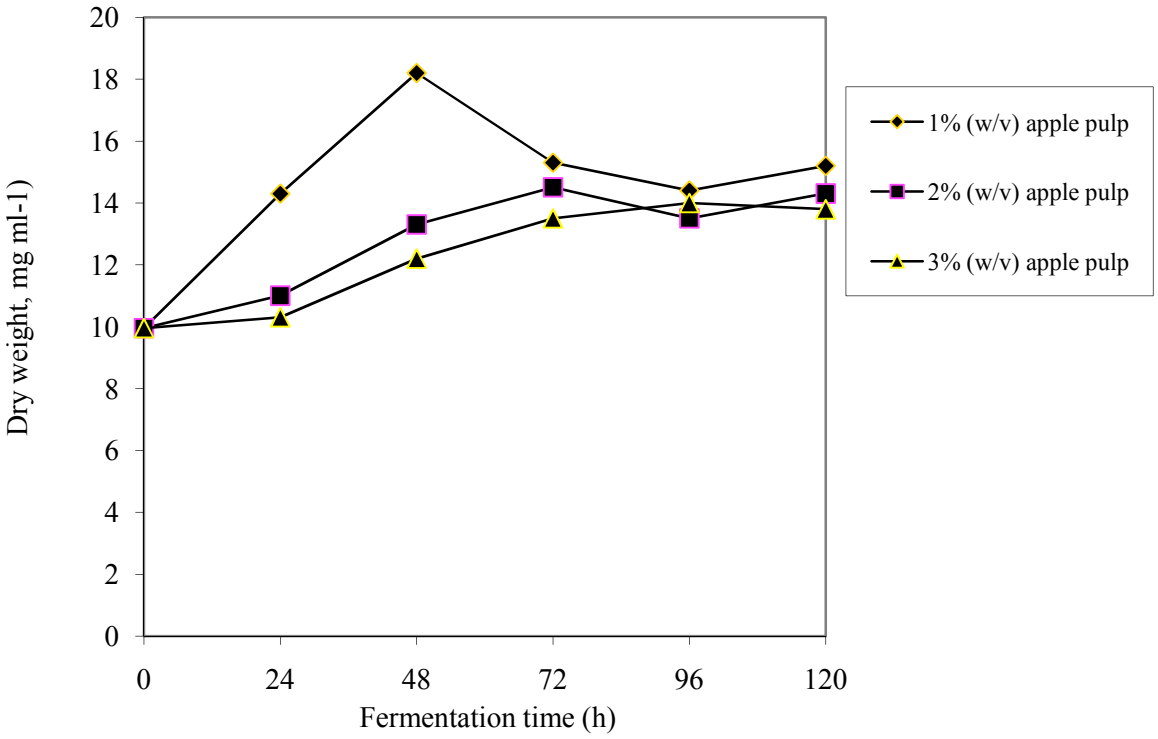


Figure 3. The influence of different concentration apple pulp on growth by Aspergillus niger MK-15

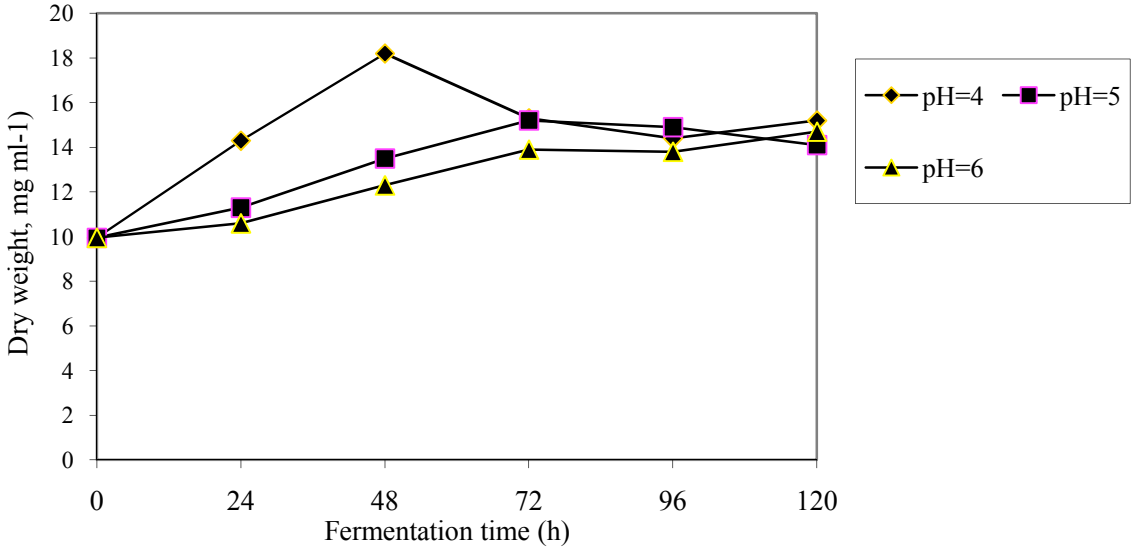


Figure 4. The influence of different initial pH on bases on growth by Aspergillus niger MK-15

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. As this residue is renewable and in an abundant supply, it represent a potential low cost material for microbial enzyme production. The significance of this agro-industrial residue as material for pectinolytic enzyme production is highlighted in this article. 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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