

# Immobilization of *Aspergillus awamori* 1-8 for Subsequent Pectinase Production

Zh. B. Suleimenova, Zh. K. Rakhmetova, R. K. Blieva, and A. E. Nurlybayeva

**Abstract**—The overall objective of this research is a strain improvement technology for efficient pectinase production. A novel cells cultivation technology by immobilization of fungal cells has been studied in long time continuous fermentations. Immobilization was achieved by using of new material for absorption of stores of immobilized cultures which was for the first time used for immobilization of microorganisms. Effects of various conditions of nitrogen and carbon nutrition on the biosynthesis of pectolytic enzymes in *Aspergillus awamori* 1-8 strain were studied. Proposed cultivation technology along with optimization of media components for pectinase overproduction led to increased pectinase productivity in *Aspergillus awamori* 1-8 from 7 to 8 times. Proposed technology can be applied successfully for production of major industrial enzymes such as  $\alpha$ -amylase, protease, collagenase etc.

**Keywords**—*Aspergillus awamori*, immobilization, pectolytic enzymes.

## I. INTRODUCTION

THERE is a serious problem of low yield wine stock tons of raw materials resulting from the presence of fruit and berry production of pectin substances that impede the processes of mash-impact, juice clarification and filtration [1]. One way to solve this important problem is addition of pectinolytic enzymes to fruit and vegetable mash. With the addition of pectinases the viscosity of the fruit juice drops, the press ability of the pulp improves, the jelly structure disintegrates and the fruit juice is easily obtained with higher yields [2]. Moreover commercially produced enzymes benefit a number of aspects of the wine industry, including maturation, extraction, clarification, and filtration [3]. Nevertheless, there are still limited sources of pectinases which suitable to be used in all juices and wine production. Thus, screening for ideal pectinolytic enzymes producer with more approving properties and engineering of pectinases in order to optimize their catalytic and stability features still are enduring research interest [4]. Industrial production of enzymes requires highly productive strains [5]. However, production levels of industrial enzymes are often disappointing low. In the present work a pectinase production using immobilized *A. awamori* 1-8 strain was studied. Immobilization has been achieved by using a new carrier for absorption of immobilized cultures stores called belting, which is inexpensive and biodegradable and for the first time

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has been used for immobilization of microorganisms. Effect of different carbon and nitrogen sources on pectinase production in *A. awamori* 1-8 was considered.

## II. MATERIALS AND METHODS

### A. Microorganisms

*Aspergillus awamori* 1-8 (own collection) was used. This culture was maintained on Czapek medium. For inoculum preparation, 25mL of sterile distilled water was added to the 5-day-old slant grown on Czapek agar plate and scraped aseptically with inoculating loop. This suspension having spore concentration of approx.  $1.3 \times 10^7$  cells/ml was used as inoculum for cultivation.

CZAPEK (%):  $\text{NaNO}_3$  - 0,7; sucrose - 2,0;  $\text{KH}_2\text{PO}_4$  - 0,1;  $\text{MgSO}_4$  - 0,05; KCL - 0,05; Fe  $\text{SO}_4$  - 0,001.

### B. Enzyme Production

Production of pectinase in periodic cultivation was made by growing the fungi in liquid CZAPEK medium on orbital shaker for 3 days.

For enzyme production in long period cultivation, 750-ml Erlenmeyer flasks containing 100ml sterilized liquid Czapek medium with round - shaped carrier for fungal immobilization were inoculated with 2ml mycelium/spore suspension made from a 7-day-old culture on basal agar plates. They were incubated at 26-28°C on a rotary shaker (180rpm) for 24 days. The immobilization medium was exchanged at 3-day intervals.

### C. Pectinase Assay

Polygalacturonase (PG) and polymethylgalacturonase (PMG) activities were assayed by viscometric method as viscosity loss % after 2 minutes. The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. 5ml of pectin (PMG) and pectic acid (PG) in 0.5ml of 0.1M acetate buffer (pH 4.6) and 0.5ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at 25°C temperature. The efflux time of the mixture at 0, 2, 4 and 6 minutes was recorded with the help of stop watch.

## III. RESULTS AND DISCUSSION

### A. Effect of Carbon and Nitrogen Sources on Pectinase Activity of *A. awamori* 1-8

Various carbon and nitrogen sources were supplemented in the production medium to study their effect on PG and PMG

production in *A. awamori* 1-8 fungus via periodic cultivation. Basal medium containing sucrose served as the control. In order to study the effect of carbon sources on PG and PMG activities in *A. awamori* 1-8 fungus different carbon sources were supplemented individually (in concentration 2%) and combined with pectin (in concentration 0.5%) as inductor in the basal medium. All carbon sources belong to monosaccharide, disaccharides and polysaccharides. The effect of various carbon sources on PG and PMG production after 3 days is summarized in Table I. Enzyme production was maximal when glucose plus pectin were used as a carbon sources (21.36% in PMG and 20.23% in PG). All other carbon sources used had a little effect on PG and PMG activities.

TABLE I  
EFFECT OF VARIOUS CARBON SOURCES ON POLYGALACTURONASE AND POLYMETHYLGALACTURONASE PRODUCTION IN *A. AWAMORI* 1-8

Carbon sources	pH	Biomass, g/100 ml	PMG activity, %	PG activity, %
Lactose	6,63	0,01	10,8	8,2
Lactose + Pectin	6,55	0,02	10,7	9,3
Glucose	2,32	0,31	4,96	9,8
Glucose + Pectin	2,06	0,87	21,36	20,23
Sucrose	2,29	0,37	7,37	6,91
Sucrose + Pectin	2,28	0,48	11,19	12,8
Maltose	2,63	0,17	6,23	9,81
Maltose + Pectin	2,10	0,62	16,3	17,72
Pectin	3,34	0,33	19,83	17,42

Inorganic and organic nitrogen sources were incorporated separately in the basal medium. Effect of various nitrogen sources on production in *A. awamori* 1-8 and is shown in Table II.

TABLE II  
EFFECT OF VARIOUS NITROGEN SOURCES ON POLYGALACTURONASE AND POLYMETHYLGALACTURONASE PRODUCTION IN *A. AWAMORI* 1-8

Nitrogen sources	pH	Biomass, g/100 ml	PMG activity, %	PG activity, %
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3,0	2,34	25,79	24,86
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	3,8	1,19	17,71	15,75
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	3,5	1,68	21,15	19,56
NaNO <sub>3</sub>	4,3	1,14	20,48	18,38
NH <sub>4</sub> NO <sub>3</sub>	3,2	0,73	18,39	14,36
Peptone	3,6	0,73	10,57	9,62
Casein	3,7	1,02	3,98	4,67

It was found that ammonium sulfate supported moderate growth PMG and PG production in *A. awamori* 1-8 (25.79% in PMG and 24.86% in PG, respectively) whereas organic nitrogen sources like peptone and casein reduced fungal enzymatic activity.

#### B. Immobilization of *A. awamori* 1-8

The most significant outcome of the present study was the maximum PG and PMG production from *A. awamori* 1-8 obtained through novel cells cultivation technology by immobilization of fungal cells using the cheapest and most easily available new material for absorption of stores of

immobilized cultures, which has been for the first time used for immobilization of microorganisms [6]. The cultivation process was carried out by growing the fungus in liquid medium for long period of time with optimal carbon and nitrogen sources containing: pectin – 0.5%; glucose – 2%; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> – 0.7%; KCl – 0.05%; MgSO<sub>4</sub> – 0.05%; KH<sub>2</sub>PO<sub>4</sub> – 0.1%; FeSO<sub>4</sub> – 0,001%. Fungal morphology immobilized on carrier presented on Fig. 1.

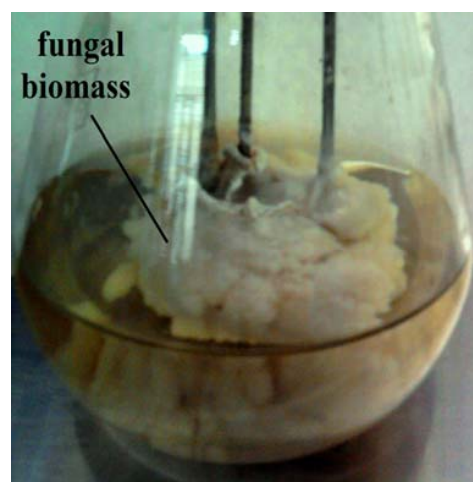


Fig. 1 Immobilized *A. awamori* 1-8

*A. awamori* 1-8 was immobilized perfectly twisting around carrier. The immobilization medium was exchanged at 3-day intervals. All data obtained are presented in Fig. 2. It was concluded that enzymatic activity was enhanced significantly after 6 days of cultivation of immobilized cells and was 54.09% for PMG and 55.71% for PG.

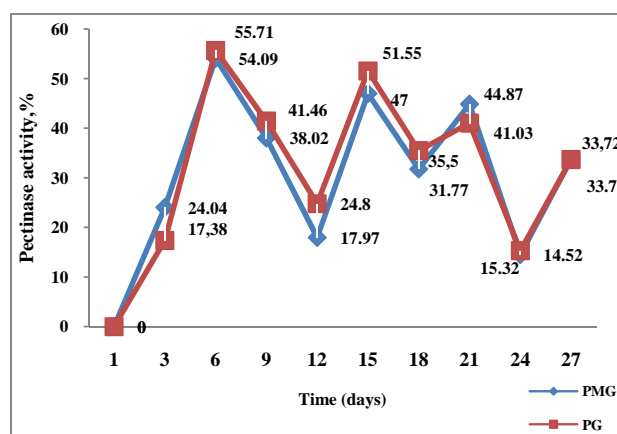


Fig. 2 PG and PMG activities from immobilized *A. awamori* 1-8

A presented method may be used for storage of vegetative mycelia in immobilized state, which provides stabilization and activation of the same cultures for a long period of time.

#### IV. CONCLUSION

In conclusion, presented immobilization procedure along with optimal carbon and nitrogen sources allow increase PMG and PG activities from 7.37% and 6.91% to 54.09% and 55.71%, respectively. Furthermore, presented strain improvement technology allows maintain high activity of the fungus for efficient enzyme production. Proposed technology can be applied successfully for production of any industrial enzymes such as collagenase, protease, amylase etc.

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