ASIA-PACIFIC JOURNAL OF CHEMICAL ENGINEERING
Asia-Pac. J. Chem. Eng. 2010; **5**: 396–400
Published online 25 June 2009 in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/apj.296

Curtin University of Technology

Short Communication

Studies on the effect of pH, temperature and metal ions on the production of pectinase from tamarind kernel powder by submerged fermentation using *Aspergillus foetidus* (NCIM 505)

P. E. JagadeeshBabu,[1]* and Renuka Viswanathan*[2]

[1] *Department of Chemical Engineering, National Institute of Technology-Karnataka, Surathkal, Mangalore, India*
[2] *Department of Chemical and Biotechnology, St.Joseph's College of Engineering, Chennai, India*

Received 17 July 2008; Revised 13 February 2009; Accepted 15 February 2009

ABSTRACT: Filamentous fungi *Aspergillus foetidus* NCIM 505 was studied for its capacity to produce exo-pectinase in submerged fermentation (SMF) from a new substrate of tamarind kernel powder (TKP). The process was further studied to optimize the initial operating variables like pH, time and temperature. Maximum pectinolytic activity was reached at 72 h of growth and the best fungal strain was found to be *A. foetidus* NCIM 505. Further, to increase the production rate of pectinase, the effects of metal ions were studied. Metal ions like Cu^{++}, Mg^{++}, Fe^{++}, Co^{++} and Zn^{++} at different concentrations were used. © 2009 Curtin University of Technology and John Wiley & Sons, Ltd.

KEYWORDS: *Aspergillus*; Pectinase; tamarind; submerged fermentation

INTRODUCTION

Pectinase is one of the best fungal enzymes associated with food industries, particularly in fruit ripping and degradation of pectic substance in the vegetable cell wall. The degradation process plays a vital role in food technology, due to reduction in the time of filtration and juice clarification, which leads to more stable and value-added products.[1–5]

The main sources for the pectinolytic complex enzymes are yeast, bacteria and a large variety of filamentous fungi, although for commercial production penicillium or aspergillus species are utilized.[6–8] Pectinase production in these fungi is stimulated by the presence of pectin or pectin-containing compounds in the fermentation medium. As pectinase is partly retained in the cells and partly excreted into the medium, the enzyme is recovered from both the sources.[1,2,4–6,8]

The tamarind (*Tamarindus indica*) is the only species of the genus *Tamarindus* in the family Febaceae. It is a tropical tree, native to eastern Africa, including parts of the dry deciduous forests of Madagascar. India is a semiarid tropical region, well known for the production of pulses (red gram, bengal gram, and green gram), oil seeds (sunflower), and tamarind. Tamarind seeds have been used in a limited way as emergency food. They are roasted, soaked to remove the seed coat, and then boiled, fried, or ground to a flour or starch. Roasted seeds are ground and used as a substitute for, or adulterant of, coffee. It can be used in fruit preserving with or without acids and gelatinizes with sugar concentrates even in cold water or milk. It is recommended as a stabilizer in ice cream, mayonnaise, and cheese and as an ingredient or agent in a number of pharmaceutical products.[9,10]

Pectin is a generic name for high-molecular-weight polysaccharides present in the cell wall of higher plants. Their common property is the 1–4 glycosidic linkage of D-galacturonic acid unit.[11] Pectinase enzymes belong to the group of hydrolytic enzymes and are utilized to eliminate pectin and pectin-like colloids in fruit juices, thus facilitating clarification of the juice and as a means of preventing the gelling of the juice during the concentration step of processing. Pectinases catalyze the breakdown of pectin-containing substances and can be produced by various fermentation methods like solid-state fermentation (SSF)[12,13] or by submerged fermentation (SMF)[14,15] methods.

In light of its importance at the industrial-level regulation study of the enzyme synthesis of the pectinolytic complex, the influence of different types of carbon sources, pectin, and polygalacturonic acid on

**Correspondence to*: P. E. JagadeeshBabu, Department of Chemical Engineering, National Institute of Technology-Karnataka, Surathkal, Mangalore, India, 575025.
E-mail: jagadeesh_78@yahoo.com

the production of pectinase using *Aspergillus foetidus* (NCIM 505) were already studied. In this present study, some of the major controlling variables of the process like temperature and pH were optimized for a submerged fermentation process using *A. foetidus* (NCIM 505). Further, the effect of metal ions (Cu^{++}, Mg^{++}, Fe^{++}, Co^{++}, and Zn^{++}) on the production of pectinase was studied.

MATERIALS AND METHODS

A. foetidus NCIM 505, a fungal strain was obtained from National Chemical Laboratory, Pune, India. The strain was maintained on potato dextrose agar slant which was maintained at 4 °C by proper refrigeration and periodic subculture. The inoculum was prepared by growing the organism (*A. foetidus* NCIM 505) in potato dextrose agar slants for about 72 h at 30 °C. The conidia were dispersed in 10 ml of dilute Tween 80 solution. Fresh tamarind seeds with testa were properly soaked in water for about 8 h and then the testa were removed from the raw source. Testa-free tamarind seeds were ground to a fine powder and then used as substrate for submerged fermentation.[16,17]

Fermentation medium

The optimized medium (using Design Expert) consisted of 17.5 (g/l) tamarind kernel powder (TKP), 32 (g/l) glucose, and 6 (g/l) ammonium sulfate. In all the fermentations 50 ml of medium was dispersed into 250-ml Erlenmeyer flasks. An acidic range of pH has been reported to be more favorable for the synthesis of hydrolases by Aspergillus species[18,19] Further, the favorable temperature of fermentation reported was 30 °C. Studies were made to optimize the temperature and pH for the production of pectinase from TKP using *A. foetidus* NCIM 505. The addition of metal ions and their effect on the synthesis of pectinase (Cu^{++}, Mg^{++}, Fe^{++}, Co^{++} and Zn^{++}) was studied by adding 5–25 mM of metal ions, individually.

Enzyme assay

The method of Miller[20] has been employed by several research scientists using galacturonic acid as the Refs [21,22] A known suitably diluted quantity of enzyme was added to 0.42 ml (9 g/l) of substrate solution (pectin or polygalacturonic acid) mixture with 0.7 ml of 0.1 M acetate buffer, pH 5.2, incubating at 45 °C for 30 min. After incubation, the reaction was completed by adding alkaline sodium potassium tartarate reagent. The sample was estimated spectrophotometrically at 540 nm. Various range of temperatures (25, 30, 35, and 40 °C) and pH (3.5, 4.0, 4.5, 5.0, 5.5, and 6.0) of the fermentation medium were performed to optimize the process to obtain high yields of pectinase from TKP.

RESULTS AND DISCUSSIONS

Pectinase is a commercial enzyme which has wide application in many fields of food and other industries. Though we have few sources for the production of pectinase, a cheap and easily available source is TKP. A fungal species from *Aspergillus* family (*A. foetidus* NCIM 505) was used in this study. The optimal value of carbon source (glucose, fructose, and sucrose), pectin, polygalacturonic acid was used from our preliminary study.[23] The experiments were repeated twice to check their consistency with the results given below, which showed good agreement with other trials.

Evaluation of age of slant

Erlenmeyer flask containing 50 ml sterilized medium [TKP-20 (g/l), ammonium sulfate-10 (g/l), potassium dihydrogen orthophosphate-2 (g/l)] was inoculated asceptically with 1 ml spores of 2–day-, 3-day-, 4-day-, and 5-day-old slants of *A. foetidus* NCIM 505 for a period of 4 days.[24] From the experimental analysis it was observed that the 3-day-old slant of *A. foetidus* NCIM 505 produced a maximum pectinase level of 1.207 U after 72 h of fermentation. Experiments were conducted to evaluate the inoculum level of the selected organism. It was observed that out of four chosen inoculum levels of (0.5, 1.0, 1.5, 2.0 ml), 1.0 ml which contains 10^5–10^7 spores/ml gave a maximum level of pectinase for the entire selected organism (Figs. 1 and 2).

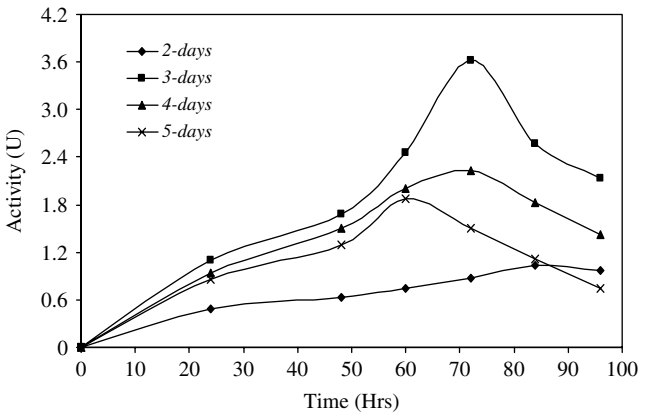

Figure 1. Determination of slant of *A. foetidus* NCIM505.

DOI: 10.1002/apj

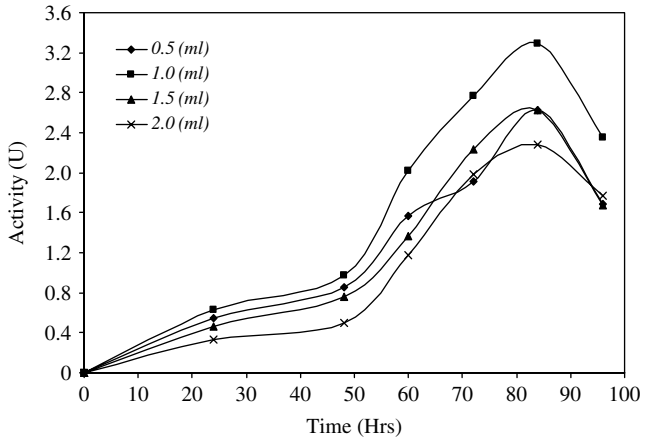

Figure 2. Determination of inoculum level of *A. foetidus* NCIM505.

Table 1. Concentration of metal ions and their maximum activity.

Metal ions	At optimum condition (30 °C, pH 4.5)	
	Concentration (mM)	Activity (U)
Magnesium (Mg^{++})	5	7.62
Copper (Cu^{++})	5	8.91
Ferrous (Fe^{++})	5	6.43
Cobalt (Co^{++})	2	9.18
Zinc (Zn^{++})	3	4.50

Evaluation of fermentation period

Production of pectinase was evaluated up to 96 h. From the analysis it was observed that a gradual increase in the production of pectinase was observed over a period of 24–72 h after which the activity declined.

Effect of temperature and pH

Initially, the synthesis of pectinase at different values of pH (3.5, 4.0, 4.5, 5.0, 5.5,and 6.0) at 25 °C was studied, which is shown in Fig. 3. Maximum level of pectinase was obtained at 4.5 pH after 72 h of fermentation after which the death phase was reached. Though the optimal value of pH was found, the same procedure was repeated for different temperatures to have an optimized temperature range. Figure 4 shows the synthesis of pectinase at 30 °C for different pH values. Figures 5 and 6 show the profile of pectinase synthesis when the fermentation was carried out at 35 and 40 °C respectively.[25] At 30 °C, the maximum level of pectinase was found to be at 4.5 pH and the same trend of fermentation was observed in all other ranges, i.e. at 35 and 40 °C.

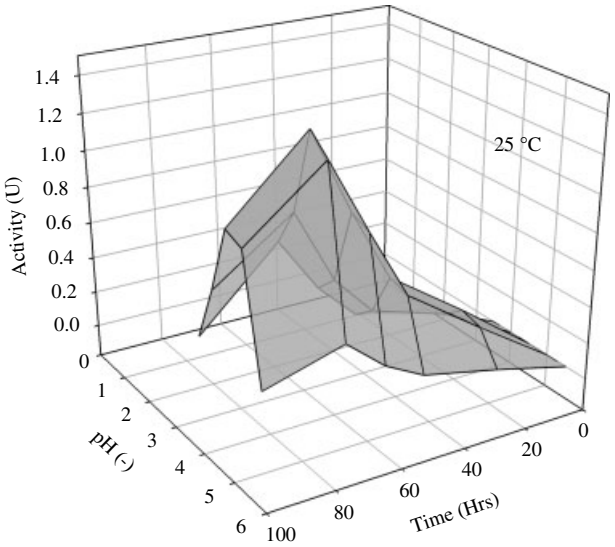

Figure 3. Effect of pH on the synthesis of pectinase at 25 °C.

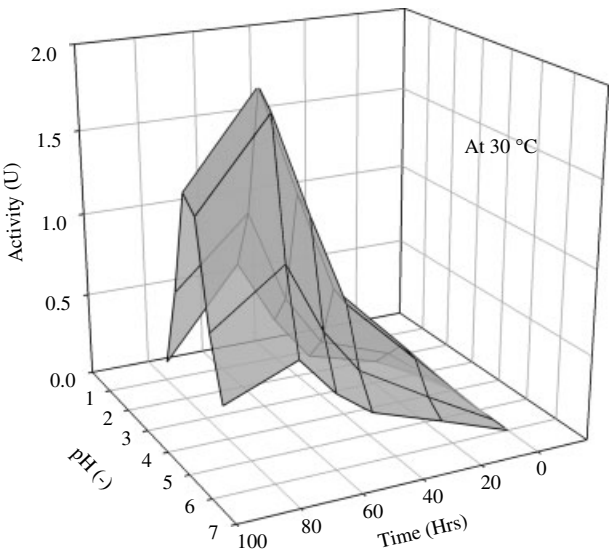

Figure 4. Effect of pH on the synthesis of pectinase at 30 °C.

But the overall synthesis of pectinase at 35 °C was lower than that obtained with temperatures at 25, 30, and 40 °C.[26,27] From the analysis it was observed that the maximum yield of pectinase was obtained at pH 4.5 irrespective of the fermentation temperature maintained, whereas higher level of pH leads to enzyme intolerance, and the optimum temperature was found to be 30 °C, after which the activity decreased due to the denaturation of the enzyme because of high temperature.

Effect of metal ions

Experiments were carried out to study the effect of metal ions on the production of pectinase using

DOI: 10.1002/apj

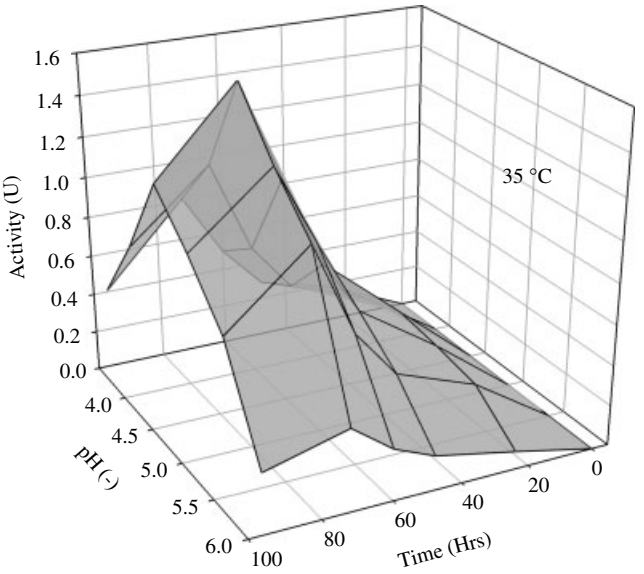

Figure 5. Effect of pH on the synthesis of pectinase at 35 °C.

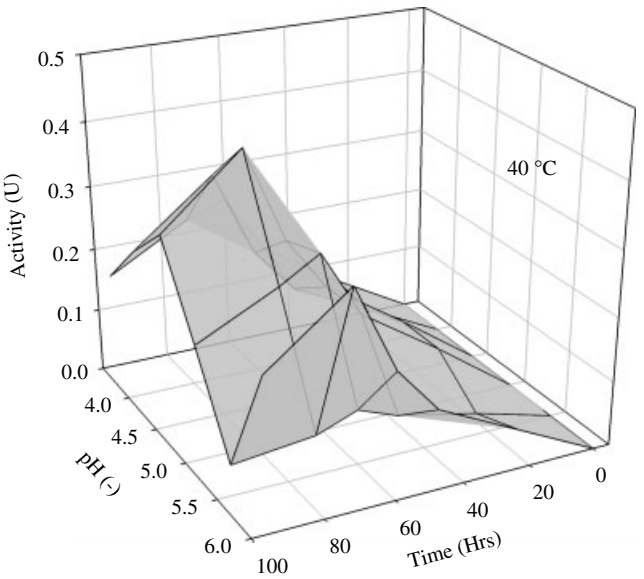

Figure 6. Effect of pH on the synthesis of pectinase at 40 °C.

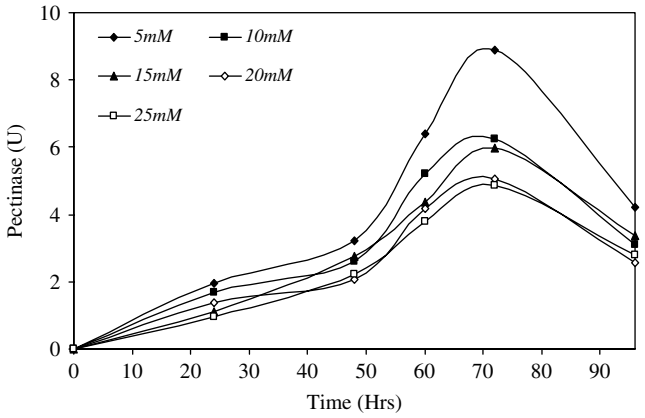

Figure 7. Effect of pH on the synthesis of pectinase.

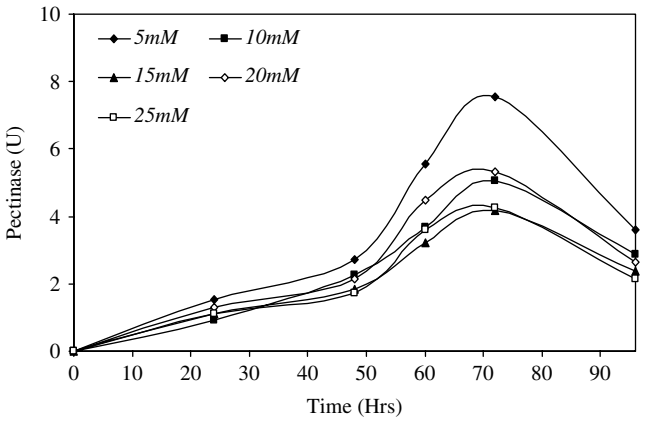

Figure 8. Effect of pH on the synthesis of pectinase.

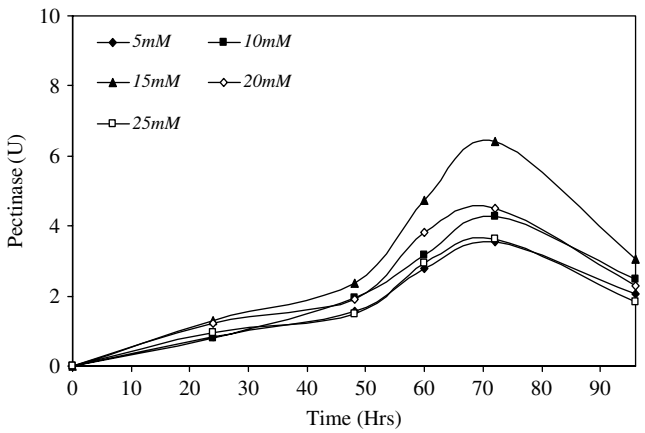

Figure 9. Effect of pH on the synthesis of pectinase.

A. foetidus NCIM 505 which was found to be the best organism. The optimal value of the medium along with the glucose source was taken from our previous work.[23] Further, to enhance the production of pectinase from TKP, the addition of metal ions (Cu^{++}, Mg^{++}, Fe^{++}, Co^{++} and Zn^{++}) was studied by adding 5–25 mM of metal ions, individually. Figures 7–11 show the individual effect of metal ions on the production of pectinase. Figure 7 shows the effect of addition of Cu^{++} to the medium along with the optimized medium [TKP (17.5 g/l), ammonium sulfate (6g/l), and glucose (32g/l)] and Fig. 8 for Mg^{++}. Significant increase in the level of pectinase (8.91 U) in the case of Cu^{++} was observed. But for Mg++, the level of pectinase synthesized was minimum when compared with the level of pectinase obtained in the normal optimized condition (5.5 U) (using Design Expert).[23]

DOI: 10.1002/apj

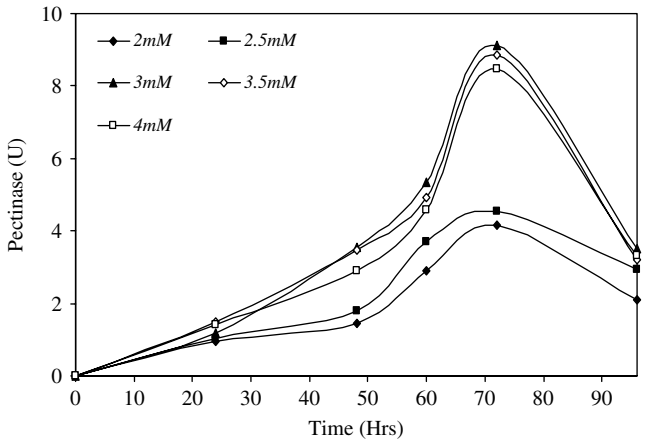

Figure 10. Effect of pH on the synthesis of pectinase.

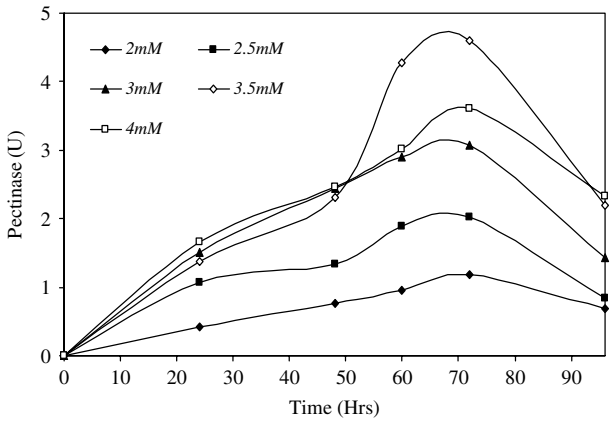

Figure 11. Effect of pH on the synthesis of pectinase.

Figures 9 and 10 show the effect of Fe^{++} and Co^{++}. The presence of Co^{++} shows a higher level of synthesis of pectinase 9.18 U more than that obtained using the optimal medium. But the presence of Fe^{++} does not have any influence on the synthesis of the enzyme, whereas (as shown in Fig. 11) the addition of Zn^{++} inhibits the synthesis of the enzyme and the quantity of pectinase obtained was very minimum. Thus it is possible to increase the production of pectinase by the addition of metal ions like Cu^{++} or Co^{++}.

CONCLUSION

In this study metal ions were used to enhance the production of pectinase by using *A. foetidus* NCIM 505 and operating variables like temperature and pH were optimized. Based on the previous work, the initial operating variables (inoculum level, age of slant, carbon and nitrogen source, medium level) were used for the current work and it was found that the production of pectinase increased by 70%. In conclusion, pectinase can be synthesized using a cheap and easily available source (TKP) under optimized temperature of 30 °C and optimized pH of 4.5. Further, production can be increased by the addition of metal ions like Cu^{++} and Co^{++}.

Acknowledgement

The authors are thankful to CSIR New Delhi, India for the financial support to carry out this research work.

REFERENCES

[1] G. Aguilar, C. Huitron. *Enzyme Microb. Technol.,* **1987;** *9*, 690–696.
[2] A. Hesham Essa, F. Manal Salama. *Mol. Nutr. Food Res.,* **2002;** *46*(4), 245–250.
[3] C. Dinella, A. Stagni, G. Lanzarini, M. Laus. *Prog. Biotechnol.,* **1996;** *14*, 971–978.
[4] C. Grassin, P. Fauquembergue. *Prog. Biotechnol.,* **1996;** *14*, 453–462.
[5] H.P. Heldt-Hansen, L.V. Kofod, G. Budolfsen, P.M. Nielsen, S. Huttel, T. Bladt. *Prog. Biotechnol.,* **1996;** *14*, 463–474.
[6] S.R. Nair, S.K. Rakshit, T. Panda. *Bioprocess Eng.,* **1995;** *13*, 37–40.
[7] X. Kazuhiro Nakaho, C. Allen. *J. Phytopathol.,* **2008;** *157*, 219–227, DOI:10.1111/j.1439-0434.2008.01467.
[8] L. Perenirova, J.A.E. Benen, J. Visser. *Eur. J. Biochem.,* **1998;** *251*, 72–78.
[9] P. Goyal, V. Kumar, P. Sharma. *Starch – Stärke,* **2008;** *60*(1), 41–47.
[10] B. Cheirsilp, K. Umsakul. *J. Food Process Eng.,* **2008;** *31*(1), 78–90.
[11] J. Friedrich, A. Cimerman, W. Steiner. *Enzyme Microb. Technol.,* **1994;** *16*, 703–707.
[12] C.G. Garzon, R.A. Hours. *Bioresour. Technol.,* **1992;** *39*, 93–96.
[13] M.E. Acuna–Argüelles, M. Gutierrez-Rojas, G. Viniegra-Gonzalez, E. Favela-Torres. *Appl. Microbiol. Biotechnol.,* **1995;** *48*, 808–814.
[14] D. Pericin, D. Karlovic, M. Jarak. *Mikrobiologia.,* **1992;** *2*, 57–63.
[15] M.C. Maldonado, A.M.S. Saad, D.A.S. Callieri. *Sci. Aliments,* **1989;** *9*, 101–110.
[16] A.F. Perley, O.T. Page. *Can. J. Microbiol.,* **1971;** *17*, 415–420.
[17] A. Rajendran, V. Selvaraj, V. Thangavelu. *Asia Pac. J. Chem. Eng.,* **2008;** *3*(3), 347–353.
[18] K. Zetelaki-Horvath. *Acta Alimentaria,* **1980;** *10*, 371–378.
[19] W. Shroeder. *Mycologia,* **1982;** *74*, 440–466.
[20] G.L. Miller. *Anal. Chem.,* **1959;** *31*, 426–428.
[21] M.C. Wang, M.T. Keen. *Arch. Biochem. Biophys.,* **1970;** *414*, 749–757.
[22] S. Solis, M.E. Flores, C. Huitron. *Lett. Appl. Microbiol.,* **1997;** *24*, 77–81.
[23] R. Viswanathan, P.E. JagadeeshBabu. *Chem. BioChem. Eng. Q.,* **2008;** *22*(4), 481–489.
[24] S. Couri, G. Adolfo, S. Pinto, L. Ferreira de Senna, H.L. Martelli. *Br. J. Microbiol.,* **2003;** *34*, 16–21.
[25] G. Najafpour, H. Younesi, K.S.K. Ismail, A.R. Mohamed, A.H. Kamaruddin. *Asia Pac. J. Chem. Eng.,* **2005;** *13*(5–6), 549–562.
[26] T.E. Wong, R.M. Illias, R.A. Rahman, A.F. Ismail, M.M. Salleh, O. Hassan, K. Kamaruddin. *Asia Pac. J. Chem. Eng.,* **2005;** *13*(5–6), 541–547.
[27] J. Liu, Y.-Y. Huang, J. Liu, l.-P. weng, L.N. Ji. *Lett. Appl. Microbiol.,* **2001;** *32*, 16–19.