ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 6 (2): 245-250, 2007
ISSN 1682-296X
© 2007 Asian Network for Scientific Information

Effect of Aeration on the Production of Endo-Pectinase from Coffee Pulp by a Novel Thermophilic Fungi *Mycotypha* sp. Strain No. AKM 1801

[1]C. Venugopal, [1]T. Jayachandra and [2]K.A. Anu Appaiah
[1]P.G. Department of Microbiology, Mangalore University, Cauvery Campus,
Madikeri-571 201, Karnataka, India
[2]Department of Food Microbiology, Central Food Technological Research Institute,
Mysore, Karnataka, India

Abstract: In the present research a novel thermophilic fungi-*Mycotypha* sp. strain No. AKM1801 was used to evaluate its efficiency for endo-pectinase production from coffee pulp, a waste produced during coffee processing. The culture was cultivated on coffee pulp through submerged fermentation at 45±2°C in aerated and stationary conditions. *Mycotypha* showed the maximum endo-pectinase activity of 5.4 U mL^{-1} at 96 h at pH 5.4 and 4.9 U mL^{-1} at 168 h at pH 5.6 in aerated and stationary conditions, respectively. The pectin content was reduced up to 85% in both conditions. Maximum decolorization was achieved in stationary condition. The present investigation was conducted to study the physiological behavior of *Mycotypha* during dimorphic structure i.e., yeast phase in aerated and mycelial phase in stationary for endo-pectinase production. This could be highly beneficial for the production of microbial enzymes from coffee pulp in food and beverage based biotechnological industries.

Key words: *Mycotypha*, coffee pulp, SmF, endo-pectinase, dimorphism

INTRODUCTION

Pectinases are the industrially important groups of microbial metabolites, which catalyze the breakdown of pectin containing substances. Endo-pectinase are the enzymes having potential application to reduce the viscosity of juices and facilitate extraction, maceration, liquefication, filtration and clarification processes in fruit, vegetable, paper, textile industries and wine industries. Today 75% of the estimated sale value of industrial enzyme is contributed by pectinases (Satyanarayana and Panda, 2002).

Endo-pectinase are produced by many species of microorganisms like *Bacillus*, *Clostridium*, *Pseudomonas*, *Xanthomonas*, *Erwinia* and fungi like *Pencillium*, *Aspergillus*, etc. (Rombouts and Pilnik, 1980).

Submerged fermentation (SmF) systems have been extensively used for the production of high priced materials and for the study of biochemical and physiological aspects during the synthesis of microbial metabolites. Characteristic differences in both type of fermentation viz SmF and SSF have been reviewed by Lonsane and Ramesh (1992).

Coffee pulp, the waste obtained after the processing of coffee by wet method which forms a major source of the pollution of rivers and lakes, located near the processing sites, as well as the environment (Martinez-Carrera, 1987). Coffee pulp is rich in carbohydrates especially high amounts of pectin, proteins, minerals, polyphenols and antinutritional or phytotoxic factors such as caffeine, tannin, polyphenols, etc. imparting a dark color to the waste. Hence its use in agriculture has been restricted to large extent (Bressani, 1979). The coffee pulp contains 23-27% fermentable sugars on dry weight basis (Zuluaga-Vasco, 1989) which is barely used for practical and economic avenues.

At present, majority of these commercial preparations of pectinases are obtained from fungi, since, they produce different extracellular enzymes with pectinolytic activity (Aguilar and Huitron, 1987).

The objective of the present research was to evaluate the effect of aeration and stationary condition on the production of endo-pectinase from coffee pulp by novel thermophilic fungi-*Mycotypha* sp. strain No. AKM1801 at different morphology. There are hardly few reports on the production of endo-pectinase in different conditions and sparce reports of fungi, which exhibit different morphology. There are no reports on the possible utilization of coffee pulp in biological system from this genus.

Corresponding Author: C. Venugopal, P.G. Department of Microbiology, Mangalore University, Cauvery Campus, Madikeri-571 201, Karnataka, India Tel: (+91) 9448108245

MATERIALS AND METHODS

Mycotypha sp. strain No. AKM-1801, isolated and preliminary investigated by Venugopal (2001) was used in this study. It was maintained on the coffee husk media (Brand *et al*., 2000) at 45±2°C for 4 days modified by Venugopal *et al.* (2004). Inoculum was prepared by suspending the spores in distilled water and counted in Neubauer chamber as explained by Aneja (1996). The spores were inoculated to coffee pulp at the rate of 10^4 spore mL^{-1}. The coffee pulp in both agitated and stationary condition was maintained at 45±2°C. The initial pH of the pulp was found to be 4.5. The fermentation in aerated condition was carried out in 500 mL Erlenmeyer flask on a rotary shaker at 200 rpm in duplicates at 45±2°C, for 216 h.

Enzyme extraction: Ten milliliter of the sample was withdrawn at regular intervals, filtrate was centrifuged at 3000 rpm for 10 min at 4°C and the supernatant was filtered using Millipore (0.45 μ) filter paper for aerated condition (yeast like phase) and Whatman No. 1 filter paper for stationary condition (mycelial phase). Filtrate was stored at 4°C for enzymatic assays and also to study the decolourization of the pulp at 660 nm. The amount of pectin and their percentage of reduction in the media were determined using viscosity studies. The filterant was analyzed to determine the biomass by dry weight method as explained by Wang and McNeil (1995).

Enzymatic assays: Endo-Polygalactouronase (endo-PG) action was assayed as described by Fellows and Worgan (1984) by determining the percentage decrease in apparent viscosity using fenske-Ostwald's viscometer. Mixtures of 2 mL enzyme extract with 18 mL of 2% pectin in 0.2 M citrate buffer, pH-4.5 at 45°C and incubated for 10 min. One unit of endo-PG activity was defined as the amount of enzyme, which reduced the initial viscosity of the pectin solution by 50% in 10 min.

RESULTS

In the present study, *Mycotypha* produced maximum endopectinase activity of 5.4 U mL^{-1} 96 h under in aerated condition, whereas in stationary condition, maximum endo-pectinase activity of 4.9 U mL^{-1} was observed at 168 h (Fig. 1). Endopectinase production followed the growth of the organism (Fig. 1 and 4). As the growth itself is retarded in stationary condition and the synthesis of enzyme was also delayed, but the initial production of the enzyme seems to trigger the growth. This initial increase of enzyme brought down the

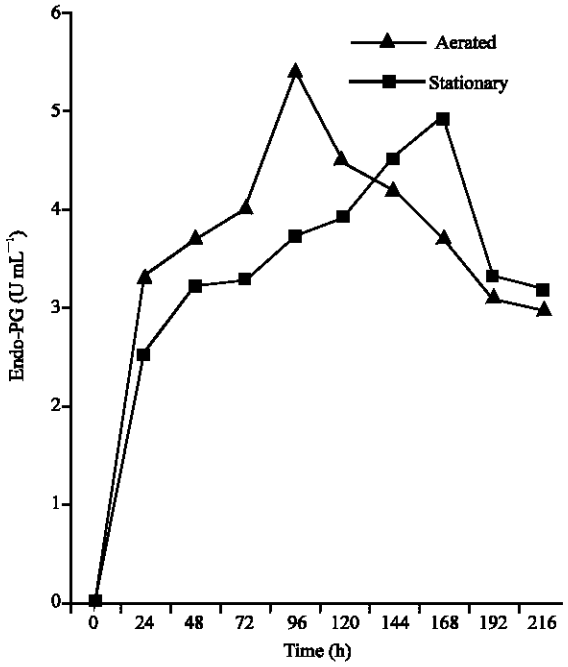

Fig. 1: Effect of aerated and stationary condition on the production of Endo-pectinase production by *Mycotypha* sp. on coffee pulp

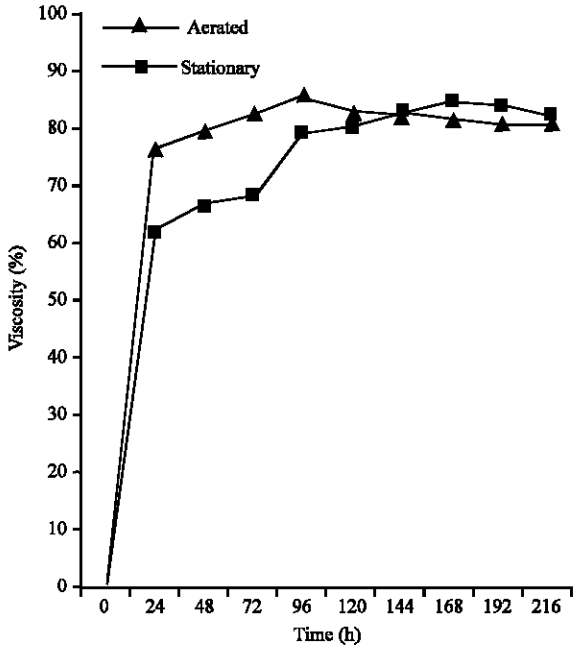

Fig. 2: Effect of aerated and stationary condition on the reduction in viscosity of coffee pulp by *Mycotypha* sp.

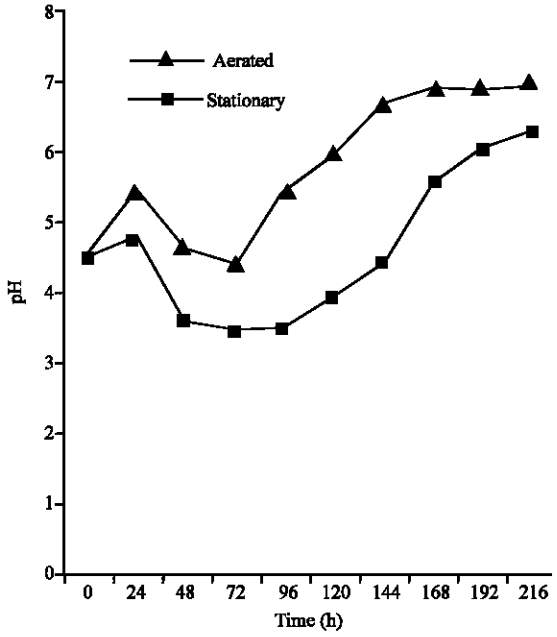

Fig. 3: Effect of aerated and stationary condition on pH of coffee pulp by *Mycotypha* sp.

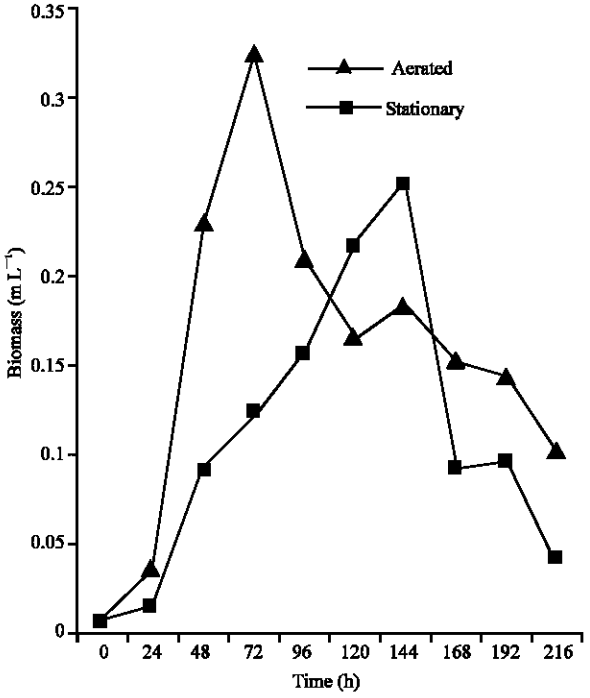

Fig. 4: Effect of aerated and stationary phase on the biomass produced by *Mycotypha* sp. on coffee pulp

viscosity by 60-70% within 12 h (Fig. 2), This coincided with the accelerated growth of the organism. Reduction in viscosity was more prounced aerated condition than

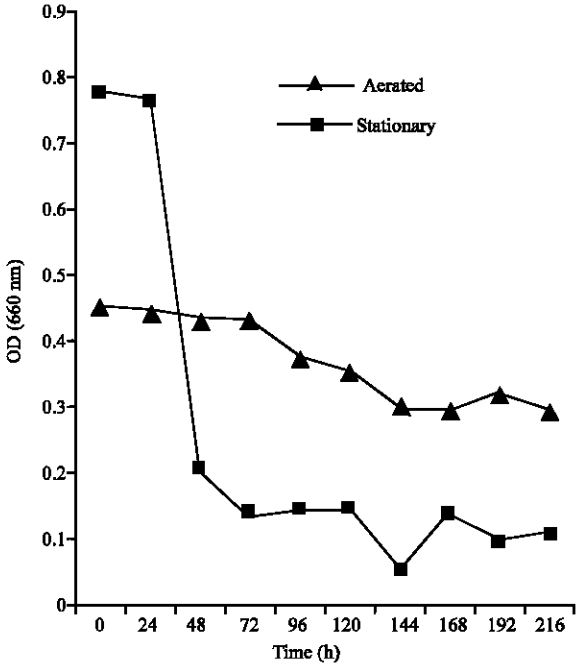

Fig. 5: Effect of aerated and stationary condition on color reduction by *Mycotypha* sp. on coffee pulp

in stationary condition (Fig. 2). The organism, which has a biphasic growth nature, grew as yeast under aerated condition and as mycelium under stationary condition.

Alkalization of growth medium was faster in aerated condition (Fig. 3) on the contrary color reduction was faster in stationary phase (Fig. 5). Degradation of polyphenols is an important requirement for the disposal of coffee pulp effluent. It is the polyphenolic acids, which import the color to the effluent. Mycelial phase of growth was found to be more efficient in color reduction.

The growth of the organism can be very effectively be controlled by aeration hence can be directed towards our requirements either for pectinase production or polyphenol reduction. The production of enzyme doesn't seem to be effected by sporulation of the culture as in observed in many other fungal forms.

DISCUSSION

Earlier studies (Venugopal, 2001) showed the production of pectinase by *Mycotypha* when pectin was used as the sole source of carbon. This may be due to the absence of any readily assumable carbon, thus forcing the organism to grown on pectin. Pectinases produced by mould can also be controlled by catabolic repression exerted by a readily assimiable carbon source (Siessere and Said, 1989). Preliminary investigation

(Venugopal *et al*., 2004) reveals the growing ability of *Mycotypha* sp. on coffee husk filtrate. Cultivation in Smf conditions is much easier to study and to control on the production of pectinolytic enzymes (Hermesdorfer *et al*., 1984).

Maximum endo-pectinase activity was achieved at different hours of growth under the conditions tested. This result is contrary to the work of Pereira *et al*. (1992) with regard to activity but correlated with regard to hours. They had observed maximum activity of 2.1 U mL^{-1} by 96 h with *Aspergillus* sp. The difference in enzyme activity and time of synthesis may be due to dimorphic growth of *Mycotypha* sp. i.e., exists as yeast like phase and mycelial phase. In aerated condition, organism continued the growth as yeast throughout the experiment whereas in stationary condition mycelial stage was maintained. The cells stop enzyme production when a certain ratio of enzyme concentration and cell number are reached (Schmidt *et al*., 1995).

The maximum endo-pectinase activity showed the variation with time but the pH was between 5.4 in aerated condition and 5.6 in stationary condition, which correlated with our previous work (Venugopal, 2001) where the maximum activity was achieved at pH 5.7 when grown on pectin. Similar results were observed by Alazard and Raimbault (1981) who observed the narrow range of pH for the production of endo-pectinase. It is generally agreed that the initial pH of the medium and the pH development in the growing culture plays an important role in both enzyme composition and the yield of the enzyme fractions (Alana *et al*., 1989). pH values proved to be valuable indicators of the initiation and end of the enzyme synthesis (Friedrich *et al*., 1989). The initial decrease of pH in both conditions correlates with the work of Daugulis and Wilke (1977) of cellulolytic fungi growing on carbohydrate substrates, where the pH drops significantly during the growth of the organism and then rises after active growth ceases. In both conditions pH increases towards basic at the end of the fermentation (Fig. 3), which correlates with our previous research (Venugopal, 2001) on coffee husk effluent. This change may probable be due to the exhaustion of reducing sugars forcing the organism to attacks the complex molecules leading to alkalization of coffee pulp.

During the enzyme production the variation in biomass depends on the morphology of the organism. Maximum biomass in yeast phase was achieved when compared to mycelial phase. A time course of the fermentation showed that the enzyme synthesis is not associated with growth (Friedrich *et al*., 1989). In both cases, the increase in biomass coincides with the type of growth phase maintained. Fang and Zhong (2002) claimed that the different morphology of the organism under different pH value was the critical factor in biomass accumulation. The final biomass of the cultures depended on the substrate and initial number of the germinating spores used as the inoculum (Alana *et al*., 1989) The increase in enzyme synthesis and the growth at different condition is not a growth bounded process but as a growth associated one (Schmidt *et al*., 1995).

Decolorization of coffee pulp was observed in both conditions but significant change was observed in case of stationary condition, which depends on morphological and physiological nature of the organism. To minimize the pollution caused by coffee pulp, BOD and COD has to be reduced, but in such stage also, decolorization was not efficient. *Pleurotus* sp. showed the decolourization was up to 60% on 4th day of cultivation (Gupta and Bhattacharya, 1985) but thereafter the color increased slightly. Efforts were also made to explore the possible utilization of enzyme system for decolorizing activity. Physical and chemical methods for removing the color are not attractive as it may lead to further pollution and also needs high input cost (Friedrich *et al*., 1989; Lundahl and Mansson, 1980). Efficiency of decolorization was linked with the amount of nitrogen present. Preliminary studies showed an higher increase in the Nitrogen content during stationary phase than in aerated condition. This correlates the work of Mehna *et al*. (1995) where the amount of decolorization increased with increase in nitrogen at the end of the fermentation. It is also noted that the efficiency of decolorization was more at the pH range of 4-5. *Trichoderma* sp. showed the optimum pH 4, which is essential for the growth as well as the decolorization process (Prasad and Joyce, 1991). Mittar *et al*. (1992) has reported that *Penicillium crysogenium* BKMF 1767 strain works well to decolorize at the pH range of 3.5-4.5, which is found to be optimum.

The major objective of the present study was to assess the oxygen requirement for the synthesis of the endo-pectinase. The results indicate that endopectinase production was more pronounced in yeast like phase of growth. Such observation has not been made earlier by workers to the best of our knowledge. On the contrary, the degradation of polyphenolic materials (color reduction) was faster in mycelial state of growth. This may be due to accumulation/degradation of polyphenolic acids on the mycelial mat, than on the walls of the yeast.

With regard to effect of temperature on decolorization, *Mycotypha* sp. showed maximum decolourization at 45±2°C, whereas Royer *et al*. (1985) described maximum color reduction was noted at 30°C.

So far, there are no reports on the production of endo-pectinases by genera *Mycotypha* sp. The present study showed the effect of aeration and stationary condition on production of endo-pectinase in SmF technique, thus having wide and immense potentiality during scale-up of endo-pectinase in food and beverage based biotechnological industries.

ACKNOWLEDGMENT

The authors are thankful to the Vice-Chancellor and Registrar of Mangalore University for their support and encouragement. We also thank the Chairman of Microbiology and Applied Botany Department, for their support.

REFERENCES

Aguilar, G. and C. Huitron, 1987. Stimulation of the production of extracellular pectinolytic activities of *Aspergillus* sp. by galactouronic acid and glucose addition. Enzyme Microb. Technol., 9: 690-696.

Alana, A., A. Gabilondo, F. Hernando, M.D. Moragues, J.B. Dominguez, L.J. Llama and J.L. Serra, 1989. Pectin lyase production by a *Pencillium italicum* Strain. Applied Environ. Microbiol., 55: 612-1616.

Alazard, D. and D. Raimbault, 1981. Comparative study of amyolytic enzyme production by *A. niger* in liquid and solid state fermentation. Eur. J. Microbiol. Biotechnol., 12: 113-117.

Aneja, K.R., 1996. Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Cultivation 2nd Edn., Vishwa Prakashana, New Delhi, pp: 380-404.

Brand, D., A. Pandey, S. Roussos and C.R. Soccol, 2000. Biological detoxification of coffee husk by filamentous fungi using a Solid State fermentation system. Enzyme Microb. Technol., 27: 127-133.

Bressani, R., 1979. Antiphysiological factors in coffee pulp. Coffee pulp; composition, technology and utilization. Int. Dev. Res. Cent. Publ., 108: 83-88.

Daugulis, C.J. and C.R. Wilke, 1977. Cell growth and catecholase production for *Polyporous versicolor* in submerged culture. Applied Environ. Microbiol., 33: 836.

Fang, Q.H. and J.J. Zhong, 2002. Effects of initial pH on production of ganodermicacid polysaccharide by submerged fermentation of *Ganoderma luciderm*. Proc. Biochem., 37: 769-774.

Fellows, P.J. and J.T. Worgan, 1984. An Investigation into the pectolytic activity of the Yeast *Saccharomyces fibuligens*. Enzyme Microb. Technol., 6: 405-410.

Friedrich, J., A. Cimerman and W. Steiner, 1989. Submerged production of pectinolytic enzymes by *Aspergillus niger:* Effect of different aeration/ agitation regimes. Applied Microbiol. Biotechnol., 31: 490-494.

Gupta, M.P. and P.K. Bhattacharya, 1985. Studies on color removal from bleach plant effluents of a Kraft pulp mill. J. Chem. Technol. Biotechnol., 35B: 23-32.

Hermesdorfer, H., E. Jelke, A. Leuchtenberger, C.H. Wardsack and H. Ruttloff, 1984. Gewinnung von pektinolytischen enzymen aus *Aspergillus niger* in Submerskultur. Z Allg Mikrobiol., 24: 413-424.

Lonsane, B.K. and M.V. Ramesh, 1992. Production of bacterial thermostable enzymes by solid-state fermentation: A potential tool for achieving economy in enzyme production, Adv. Applied Microbiol., 15: 1-48.

Lundahl, H. and I. Mansson, 1980. Ultra filtration for removing color from bleach plant effluent. Tappi., 63: 97-101.

Martinez-Carrera, D., 1987. Design of a Mushroom farm for growing *Pleurotus* on coffee pulp, Mushroom J. Trop., 7: 13-23.

Mehna, A., P. Bajpai and P.K. Bajpai, 1995. Studies on decolorization of effluent from a small pulp mill utilizing agriresidues with *Tremates versicolor* Enzyme Microb. Technol., 17: 18-22

Mittar, D., P.K. Khanna, S.S. Marwaha and J.F. Kennedy, 1992. Biobleaching of pulp and paper mill effluents by *Phanerochaete chrysosporium*. J. Chem. Technol. Biotechnol., 53: 81-92.

Pereira, S.S., E.T. Torres, G.V. Gonzalez and M.G. Rojas, 1992. Effect of different carbon sources on the synthesis of pectinase by *Aspergillus niger* in submerged and solid state fermentation. Applied Microbiol. Biotechnol., 39: 36-41.

Prasad, D.Y. and T.W. Joyce, 1991. color removal from Kraft bleach plant effluents by *Trichoderma* sp. Tappi., 74: 165-169.

Rombouts, F.M. and W. Pilnik, 1980. Microbial Enzymes and Bioconversions. In: Economic Microbiology by Rose, A.H. (Ed.), Academic Press, New York, 5: 227-282.

Royer, G., M. Desrochers, L. Jurasek, D. Rouleau and R. Mayer, 1985. Batch and continuous decolorization of bleached Kraft effluents by a white-rot fungus. J. Chem. Technol. Biotechnol., 35B: 14-22.

Satyanarayana, N.G. and T. Panda, 2002. Purification and biochemical proprieties of microbial Pectinases-a review. Proc. Biochem., 38: 987-996.

Schmidt, O., H. Angermann, T.I. Frommhold and K. Hoppe, 1995. Experimental and theoretical investigations of submerged fermentation and synthesis of pectinolytic enzymes by *Aspergillus niger*. Applied Microbiol. Technol., 43: 424-430.

Siessere, V. and S. Said, 1989. Pectic enzymes production in Solid-State fermentation's using citrus pulp pellets by *Thaloromyces flavus*, *Tubercularia vulgaris* and *Penicillium charlessi*. Biotechnol. Lett., 11: 343-344.

Venugopal, C., 2001. Biofertilizer from Coffee husk, M.Sc Thesis Mangalore University, Mangalore, India.

Venugopal, C., M. Ramya Rai and K.A. Anu Appaiah, 2004. *Mycotypha* sp. Strain No. AKM1801- a novel thermophilic fungi for alkalization of Coffee husk effluent. Asian J. Microbiol. Biotechnol. Environ. Sci., 6: 525-527.

Wang, Y.C. and B. McNeil, 1995. pH effects on exopolysaccharide and oxalic acid production in cultures of cultures of *Sclerotium glucauicum*. Enz. Microbial. Technol., 17: 124-130.

Zuluaga-Vasco, J., 1989. Utilization of Waste Products of Coffee. In: Roussas, S., R. Lincona Franco and M. Gutierrez Rojas (Eds.), Proceedings of 1 Seminario Internacional Sobre Biotecnologia En La Agro Industria Cafetalera (Sibac), Xalapa, Mexico, Orstom, Montpelliar, France, pp: 63-76.