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Utilization of agricultural wastes for production of pectinmethylesterase

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Utilization of agricultural wastes is important not only to reduce the cost of production, but also to provide useful economic products to solve disposal problem as well as to minimize the environmental pollution hazards. The production of pectinmethylesterase by filamentous fungi were made by utilizing agricultural wastes. Studies were made to optimize fermentation conditions to improve yield of enzyme. Pectinase enzyme production was carried out by wheat bran alone and in combination with orange peel under SSF condition. The maximum production of pectinmethylesterase was obtained at 30°C , for 120 hr fermentation under static condition using wheat bran and orange peel in the ratio of (1:1 w/w) with hydration (1:1 w/v) using *Penicillium notatum.*

Key words: Solid-state fermentation, pectinmethylesterase, agricultural wastes, hazardous gases, filamentous fungi.

INTRODUCTION

Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source. In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformations involving organic solvent media, mainly for bioactive compounds. This system offers numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipments, etc. as reported by Pandey (1991, 1992, 1994), Nigam and Singh (1994) Doelle *et al.* (1992). A large number of microorganisms, including bacteria, yeast and fungi produce different groups of enzymes. Selection of a particular strain, however, remains a tedious task, especially when commercially competent enzyme yields are to be achieved. For example, it has been reported that while a strain of *Aspergillus niger* produced 19 types of enzymes, a -amylase was being produced by as many as 28 microbial cultures as reported by Pandey (1992) . Agro-industrial residues are generally considered the best substrates for the SSF processes, and use of SSF for the production of enzymes is no exception to that. House hold agro-wastes,which are considered as one of the major pollutants due to unfavourable gas production via natural fermentation beside creating disposal problem were used as fermentation medium for production of alpha amylase from *A oryzae* as reported by Bose *et al.* (2006).Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc. as reported by Mitra *et al.* (1994), Babu and Satyanarayan (1994), Nigam (1994), Pandey and Radhakrishnan (1993), Pandey *et al.* (1995), Selvakumar *et al.*(1998). Wheat bran, however, holds the key, and has most

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commonly been used, in various processes. Microbial pectinesterase catalyzing the degra ation of pectin polysaccharides as reported by Rombouts and Pilink (1980), have an important role in the invasion of plant tissues by phytogens and spoilage of fruits and vegetables. These enzymes are widely used for the disintegration of plant tissues in the fruit and vegetable processing industries, increasing the extraction of juice, decreasing the viscosity of concentrates and solubilizing pectic complexes to complete the clarification of juice as reported by Kawano *et al.* (1999).Other industrial uses of pectin enzymes include the extraction of oils, flavors, pigments from plant materials and maceration of vegetables and fruits as reported by Gupta *et al.*(1997) The enzyme may be produced from different organisms such as bacteria, fungi and yeast also. Our present investigation deals with the production of pectinase enzyme from *Penicillium notatum* by solid state fermentation utilizing solid wastes.

MATERIALS AND METHODS

Microorganisms used

The cultures of *Aspergillus niger* (wild type) and *Penicillium notatum* (NCIM No.923) collected from National collection of Industrial Microorganisms, National Chemical Laboratory, Pune(India) are maintained on Czapek Dox medium at 4°C by monthly subcultured.

Materials

Wheat bran, orange peel.

Fermentation

10 g of solid substrate was taken in 500 ml Erlenmeyer flasks and distilled water was added to the substrate to obtain final moisture content 50%. Then the substrates were autoclaved at 15 psig for 15 min. The sterile medium was inoculated with spores suspension (10^7 /ml) and inoculated at 30° C for 5 days under stationary condition.

Crude enzyme extraction

After fermentation was over , the substrate was mixed with distilled water in the ratio of 1:10(w:v) and mixed thoroughly then centrifuged at 10,000 rpm for 30 min. The centrifugate was used as crude enzyme.

Enzyme assay

For enzyme assay of pectinase, pectin, water and NaOH were required. The amount of acid produced was neutralized by 0.02(N) NaOH soln.

Optimization of enzyme production

Effect of incubation time

10 g of wheat bran was inoculated with the organism *Aspergillus niger* at 30° C for 5 days and assay was done at 24 hr intervals.

Effect of organisms

Wheat bran was inoculated with *Aspergillus niger* and *Penicillium notetum* separately at 30°C for 5 days and assay was done at 24 hr intervals.

Effect of time

Mixture of wheat bran and orange peel (1:1) was inoculated with *Penicillium notatum* at 30° C for 5 days and at 24 hr interval assay was done.

Effect of temperature

The fermentation process was carried out at three different temperatures (20° C,30° C,37° C) under the same environmental reaction conditions.

Effect of variety of the substrates

10 g wheat bran(w), 10 g orange peel(o) and mixture of both (w+o) 1:1 were inoculated separately with *Penicillium notatum* at 30° C for 5 days.

Effect of hydration

Substrate was hydrated with water of different ratios(1:1,1:2,1:3,1:4,1:5,1:6,1:7,1:8)

RESULTS AND DISCUSSION

Incubation time optima

Incase of wheat bran substrate maximum enzyme production was obtained at 48 hr for *Aspergillus niger* .(Fig. 1)

Organism optima

Aspergillus niger showed maximum enzyme activity at 48 hr. whereas *Penicillium notatum* showed maximum enzyme production at 120 hr. For both the cases wheat bran was used as the substrate.(Fig.2)

Time optima

The combination of orange peel and wheat bran showed maximum activity at 120 hr for *Penicillium notatum.*(Fig.3)

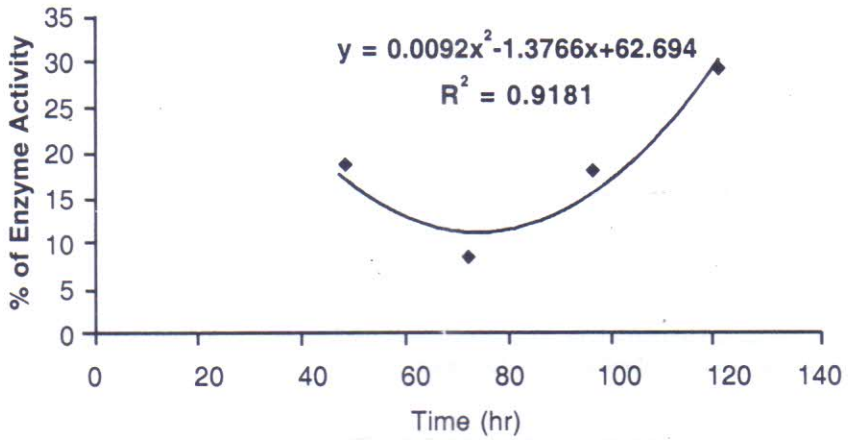

Fig.1 : Optimization of incubation time for fermentation for production of pectinmethylesterase using *Aspergillus niger* by SSF from wheat bran.

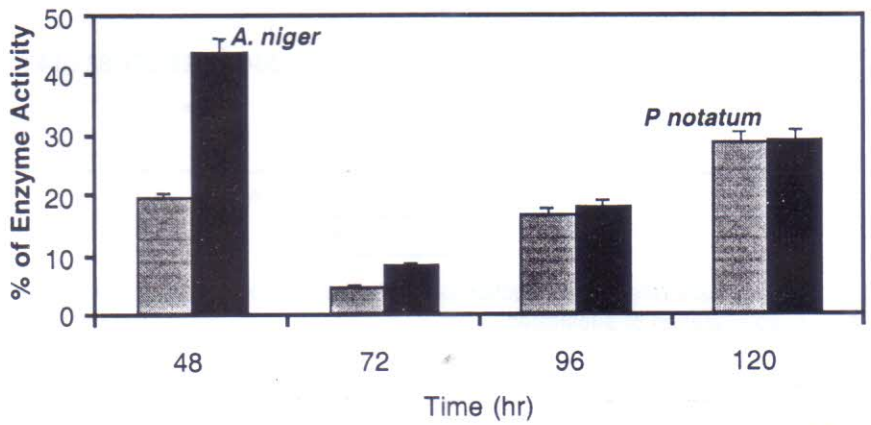

Fig. 2: Optimization of organisms for fermentation for production of pectinmethylesterase by SSF from wheat bran.

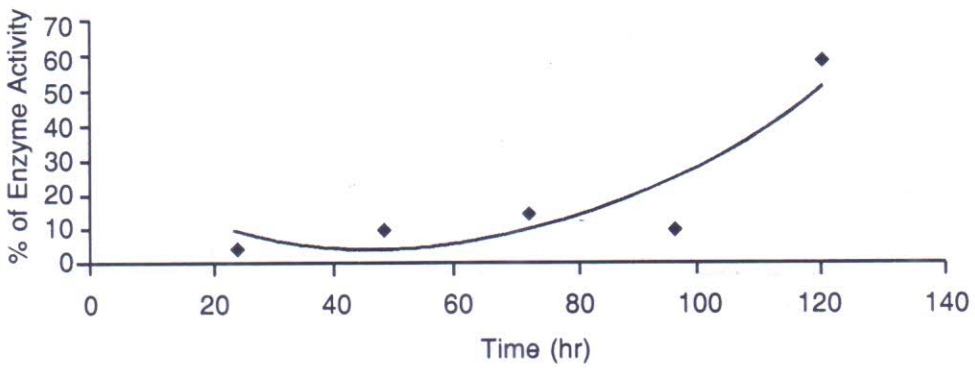

Fig. 3: Optimization of time for fermentation for production of pectinmethylesterase using *Penicillium notetum* by SSF from combination of substrates

Temperature optima

Optimum temperature for the enzyme production was found at 30°C than the other two temperatures 20° C and 37° C (Fig.4)

Substrates optima

Maximum enzyme activity was obtained with combination of wheat bran and orange peel (1:1) for *Penicillium notatum* under assay of 120 hr.(Fig.5)

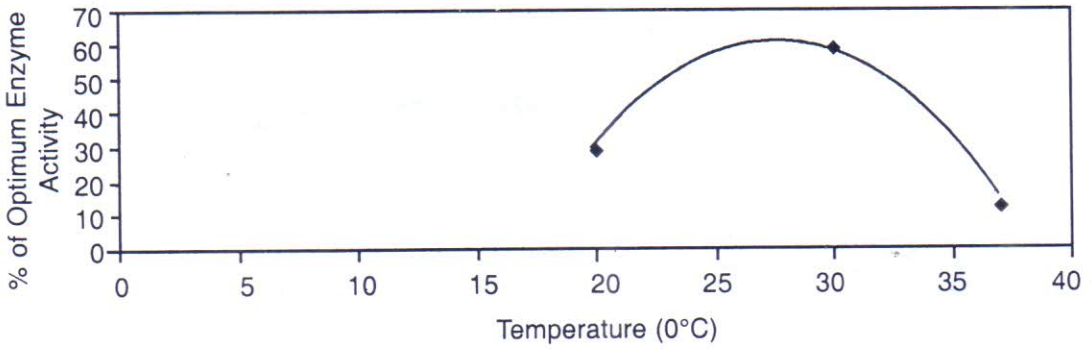

Fig.4 : Effect of fermentation temperature on production of pectinmethylesterase using *Penicillium notatum* produced by SSF from combination of substrates.

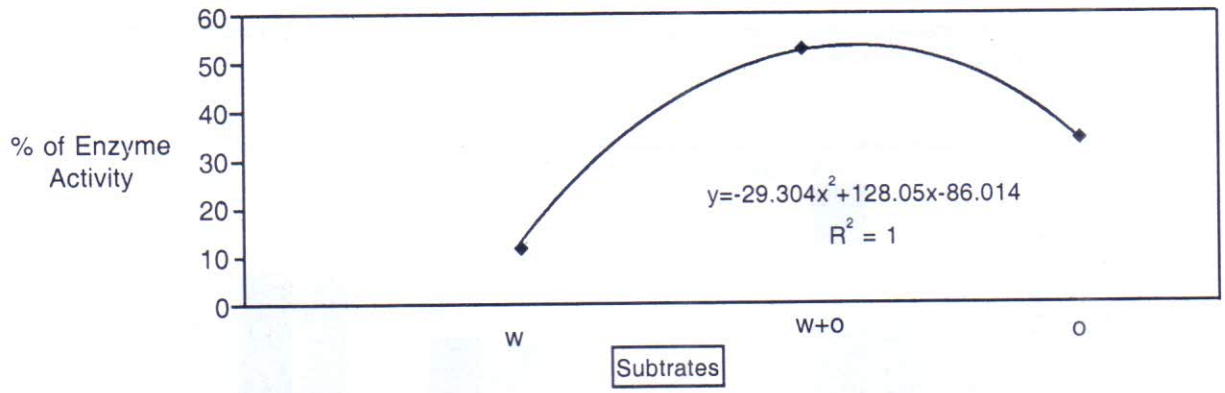

Fig.5: Effect of varity of substrates on production of pectinmethylesterase using *Penicillium notatum* produced by SSF from combination of substrates.

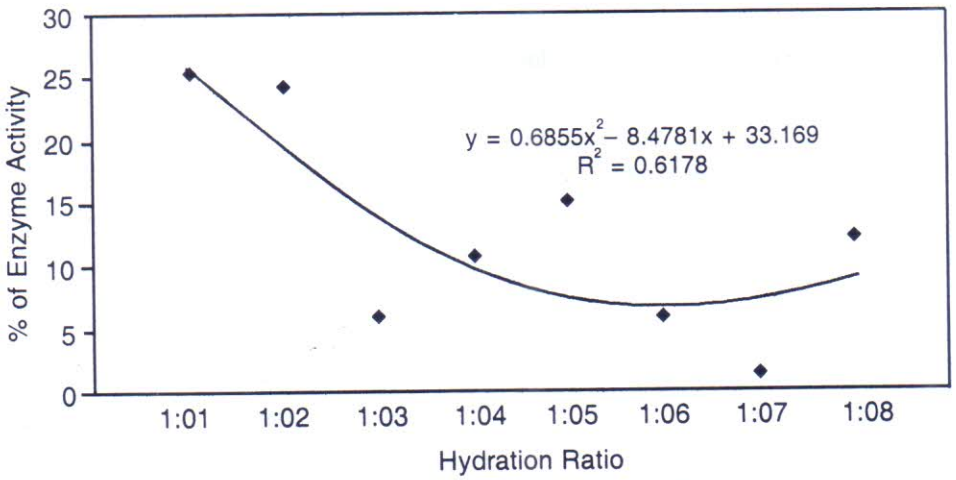

Fig. 6 : Effect of Hydration on production of pectinmethylesterase using *Penicillium notatum* produced by SSF from combination of substrates.

Hydration ratio

1:1 and 1:2 hydration ratio produced maximum yield for *Penicillium notatum* (Fig.6)

Critical analysis of the literatures have showed that production of industrial enzymes by SSF offers several advantages. It has been well established that enzyme titres produced in SSF systems are many-fold more than in SmF systems. It is hoped that enzyme production of pectinmethylesterase by *Penicillium notatum* and *Aspergillus niger* processes based on SSF systems will be the technologies of the future from agro wastes.

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