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# Production of extracellular amylase by *Aspergillus niger* under submerged fermentation using jack fruit rag as the carbon source

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Production of extracellular amylase by *Aspergillus niger* was studied under submerged fermentation using jackfruit rag as the carbon source. Different parameters, such as incubation period, pH of the culture broth and level of substrate were changed to optimise the conditions for amylase production. Maximum enzyme production ~ 8400 units/g was obtained in 5 days old cultures, grown at pH, 6.5 and 30°C with substrate level 20 $gL^{-1}$. As nitrogen sources $NH_4Cl$, $KNO_3$ casein, peptone and beef extract were tested. Except $NH_4Cl$ all other sources enhanced the amylase production. Study on the kinetics of extracellular and intracellular amylase production revealed that extracellular amylase production was always higher than that in intracellular. Crude amylase obtained from culture broth was partially purified by ammonium sulphate fractionation followed by DEAE Cellulose chromatography. Partially purified enzyme exhibited optimum pH and incubation temperature at pH 6 and 60°C respectively and higher thermal and pH stability at 50-60°C and pH 5-7 respectively and enhanced activity with $Ca^{2+}$. These unique features of the enzyme indicates its suitability for various industrial applications. Shorter incubation period and lower substrate cost offer the potential for inexpensive production of amylase, making the process industrially and economically feasible.

**Keywords:** Amylase, *Aspergillus niger,* Jackfruit rag, Submerged fermentation
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Amylase in one of the important and well known industrial enzyme that can use to breakdown starch and glycogen. Though it can be derived from several sources such as plants, animals, and microorganisms, the microbial sources generally meet the industrial demand owing to their rapid growth rates that lead to short fermentation cycles and bulk production capacity. Apart from that, microbes are easy to manipulate to obtain enzymes of desired characteristics[1].

To date a large number of microbial amylases are available commercially and most commonly used organisms are *Bacillus licheniformis, Bacillus amyloliquifaciens* and *Aspergillus niger*. Fungal amylases particularly from *Aspergillus species* find various application in food industry as an anti-staling agent in baking industry, for haze clarification in fruit juices, alcoholic beverages, glucose and maltose syrup production and other food products. Several methods such as submerged fermentation and solid-state fermentation have been successfully used for amylase production from various microorganism. Since, the contents of synthetic medium used for amylase production are very expensive and uneconomical, they need to be replaced with more economically available agricultural and industrial by products. Agro industrial residues such as wheat bran, spent brewing grain, maize bran, rice bran, rice husk coconut oil cake, mustard oil cake, corn bran *etc*. have been used as substrates for amylase production[2,3,4]. Present study deals with use of inexpensive highly abundant jackfruit rag powder as the substrate for amylase production by *A. niger* under submerged conditions. In this work, growth conditions will be optimised to achieve maximum amylase production. Amylase, thus produced will be partially purified and characterised.

## Materials and methods

All the chemicals used were obtained from Fluka, Chemi new Ulm, Switzerland. *Aspergillus niger* strain was obtained from culture collection of Department of Microbiology, University of Kelaniya, Sri Lanka. The culture was maintained by sub-culturing fortnightly on Potato Dextrose Agar (PDA) slants and stored at 4°C in a refrigerator.

### *Culture conditions and growth*

The growth medium for the fungal strain consisted of $KH_2PO_4$ (1.40 g), $(NH_4)_2SO_4$ (1.00 g),

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