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COMPARITIVE STUDY ON OPTIMISATION OF CELLULASE ENZYME FROM VEGETABLE WASTE BY USING TRICHODERMA ATROVIRIDE

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ABSTRACT

It's a study about the production of cellulose enzyme from vegetable waste using Trichoderma atroviride, through solid state fermentation process. The cellulose enzyme production was studied, by optimising the physical parameters such as incubation time, temperature and pH. By optimization of these three parameters, the maximum activities of cellulose produces by Trichoderma atroviride were observed after 5.5 days of incubation at a pH of 5.50 and 32.5°C temperature. The high activity of cellulase produced by the fungus had high activity, which inturn proves its potential for commercial scale production and thereby having various industrial applications.

KEYWORDS: Cellulase, Optimization, Solid State Fermentation, Trichoderma Atroviride, Incubation Time, Temperature & Ph

INTRODUCTION

The attention on cellulose has increased to meet the future demand for fuel as it is a renewable resource. The primary cell wall of plant biomass is cellulose, which is a polymer of β-1, 4 linked glucose units. This is an abundantly available waste from agriculture and is a rich carbon source that is renewable. The big challenge for enzymatic hydrolysis is due to its crystalline structure and insoluble nature. Enzyme celluloses which is a multi complex structured, degrade cellulose. Cellulase, that belongs to the class of enzyme plays an important role in natural biodegradation. Priority of these in industry is due to its major role, in the production of fermentable sugars and ethanol, organic acids and chemicals. The further claims of cellulose are in the bio-stoning, bio-polishing and paper recycling. This can be used as animal feed additives, due to its efficiency in refining the nutritional quality and digestibility for the animals. Paper industry uses it for de-inking purposes and textile industry, to enhance pulp drainage. Cellulase has its importance in food sciences for food processing, drying of beans in coffee and some other application. Certain processes in food industry like extraction, clarification and stabilization of fruit juices and vegetables requires cellulase enzyme.

Production of cellulase from fungi is highly useful for the enzyme production when compared to its production from other microorganisms. Fungis like *Trichoderma sp., Aspergillus sp. and Penicillium sp.* belong to the class of most generally used fungi for cellulase production. Lignocellulose degrading fungi, particularly *Trichoderma sp.* is the most commonly and widely used fungi to produce cellulose. The growth parameters and extracellular enzyme production circumstances will vary among the individual isolates. Fermentation conditions that critically affect the yield and those that play vital role are incubation period, carbon source, temperature, pH etc. In this comparative study three parameters such as temperature, pH, time period were analysed using two different methods like statistical analysis where data is

represented on the basis of average of three replicates and response surface method where data is represented based.

METHODOLOGY

Cellulase Production

A250 ml Erlenmeyer flasks containing mandel and weber media is used to produce the cellulase by solid state fermentation. This is supplemented with 5 g of vegetable waste which act like a substrate. Autoclave for 20 min at 121°C is then carried out. After cooling for a period of 5 days, each flask are inoculated with 2 ml of the spore suspension of *Trichoderma atroviride*. Crude enzyme has to be extracted from fermented substrate by adding of 0.05 M citrate buffer. This is then mixed for 1 hr at 180 rpm in an orbital shaker. Filtration need to be done in which a muslin cloth is used and it's squeezed to get the enzyme out. Centrifugation of the extract at 10,000 rpm for 10 min is done and the supernatant collected to determine the activity of enzyme.

Statistical Analysis

Data was presented as the average of three replicates (±SE) obtained from their independent experiments.

Response Surface Method

Here parameters like temperature, time and pH in the following range was analysed.

Parameter	Range
Temperature	20-45 ^{0}C
Incubation Time	2-9 days
pH	2.0-9.0

RESULT AND DISCUSSIONS

Optimization by Statistical Analysis: Production of cellulase enzyme is affected by the parameters like temperature, pH, incubation time and they have a significant impact on the enzyme activity. Based on statistical analysis the following parameters were optimised:

Parameter	Range
Temperature	30^{0}C
Time	5 days
pH	6

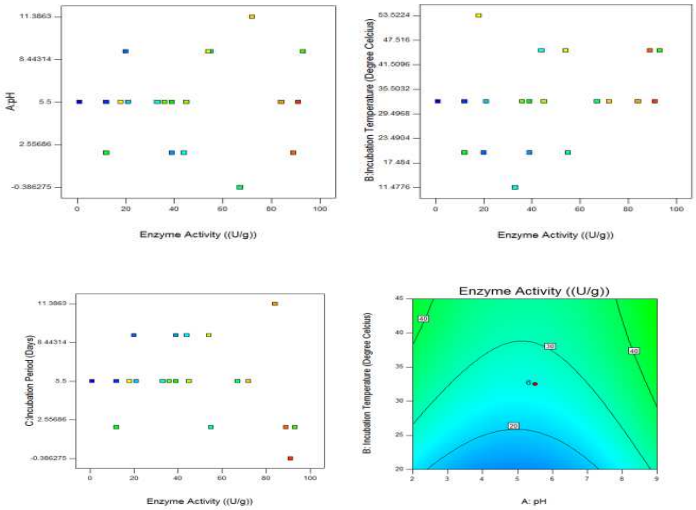
Optimization by Response Surface Method: Based on response surface method, the same parameters analysed in the previous study was optimised.

Effect of Ph: pH is a physical parameter that plays an important role by inducing morphological changes in microbes and in enzyme secretion. *Trichoderma atroviride*was allowed growing in the media of different pH ranging from 2.0 to 9.0 and least enzyme activity was observed at the lowest and highest pH, whereas maximum activity was observed for a pH of 5.50

Effect of Temperature: Temperature is another physical factor that impacts the cellulase production. Even slight changes in temperature can affect enzyme production. The optimised temperature for maximum cellulase production was studied as 32.5°C. Temperature ranging from 20-45°C was selected for optimisation.

Effect of Incubation Time: Incubation time have a major influence on the product formation. Enzyme

production is directly correlated to the incubation time. In this the time course required for the production of cellulase enzyme is examined and fermentation for a period will be carried out for duration of 2 to 9 days. It was observed that an incubation period of 5.5 days give the maximum yield and highest enzyme activity.



CONCLUSIONS

The cost-effective production of cellulases using vegetable waste as substrate requires cost effective technologies. Multiple factors like incubation time, pH, temperature and etc affect the cellulase. Among this only the prime physical factors that affect the fermentation process were studied and optimised. There are several other factors like presence of carbon, nitrogen and detergent sources which also need to be optimised. This was a work on the fungi *Trichoderma atroviride*that resulted in the production of cellulase under recommended conditions and further study on this will be convenient for its production in industrial level as well as in industrial application.

REFERENCES

1. Dhillon, Gurpreet Singh, et al. "Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray fermentation employing mixed-culture of fungi." Industrial Crops and Products 34.1 (2011): 1160-1167.
2. dos Santos, TamiresCarvalho, et al. "Optimisation of solid state fermentation of potato peel for the production of cellulolytic enzymes." Food Chemistry 133.4 (2012): 1299-1304.
3. Graminha, E. B. N., et al. "Enzyme production by solid-state fermentation: Application to animal nutrition." Animal Feed Science and Technology 144.1 (2008): 1-22.
4. G. L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analytical Chemistry, 31(3), 1959, 426–428.
5. Latifian, Maryam, ZohrehHamidi-Esfahani, and Mohsen Barzegar. "Evaluation of culture conditions for cellulase production by two Trichoderma reesei mutants under solid-state fermentation conditions." Bioresource Technology 98.18 (2007): 3634-3637.

6. Oberoi, Harinder Singh, et al. "Production of cellulases through solid state fermentation using kinnow pulp as a major substrate." Food and Bioprocess Technology 3.4 (2010): 528-536.
7. S. P. Gautam, P. S. Bundela, A. K. Pandey, K. Jamaluddin, M. K. Awasthi, and S. Sarsaiya, Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi, Biotechnology Research International, 1-8, 2011.
8. M. B. Baig, M. S. Zia, N. Sombathova, and A. Zaujec, Rehabilitation of problem soils through environmental friendly technologies: Role of sesbania (Sesbaniaaculeata) and farm yard manure, AgriculturaTropicaEtSubtropica, 38(1), 2005, 12–17.
9. R. Rajendran, R. Radhai, S. S. Karthik, and V. Rajalakshmi, Utilization of cellulosic biomass as a substrate for the production of bioethanol, International Journal of Environmental Sciences, 5(4), 2015, 743-753
10. Z. Jaradat, A. Dawagreh, Q. Ababneh, and I. Saadoun, Influence of culture conditions on cellulase production by Streptomyces sp. (strain J2), Jordan Journal of Biological Sciences, 1(4), 2008, 141–146.