Gallic Acid Production by Immobilization of *Aspergillus orzae.*

Dr.N.Lokeswari

Abstract— Gallic acid (3,4,5 trihydrixybenzoic acid) is an organinc substance occuring in many plants either as a free molecule or as part of tannic acid molecule. Production of gallic acid using the immobilized cells of *Aspergillus oryzae* has been studied. It was observed that 12% tannic acid concentration, 200 numbers of calcium alginate beads of spore concentration 2 x 105/ml and initial pH 5.5 gave the maximum gallic acid production. The % of tannin conversion was 78.5% whereas in free cell culture, the % conversion was 73.5% in 4 days of incubation period. The beads were used for 3 times successfully. A drastic fall in the hydrolysis process observed when the beads were treated with glutaraldehyde.

Index Terms— Gallic acid, Immobilization, Tannins, Tanase, Trimethoprim,

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1.Introduction

Gallic acid, an important raw material, has its specific use in the pharmaceutical industry, tea industry and also used as photosensitive resin in semiconductor production [1].The enzyme tannase hydrolyse the ester bonds of tannin to produce gallic acid.In our previous papers [2, 3] it was reported that the fungus *Aspergillus oryzae* could secrete the enzyme tannase (tannin acyl hydrolase). In this paper the author report on the production of gallic acid from tannic acid by the fungus *Aspergillus oryzae* under immobilized condition.

2. Material and Methods

Microorganism: *Aspergillus oryzae* was isolated from MTCC and was maintained in 2% malt-extract agar slant.

Preparation of pre-induced inoculum.

Tannase being an inducible enzyme, the pre-induced inoculum was prepared as advised by chen [4] by taking 2% (w/v) tannic acid in Czapek dox medium. The pH of the media was adjusted to 5.5 and was sterilized. The solution was inoculated with 2 ml spore suspension of *Aspergillus oryzae* having 2 x 10^6/ml concentration and kept in the shaker for four days. The culture broth known as pre induced inoculum was preserved at 4°C.

Preparation of media:

A solution of tannic acid of the necessary concentration (w/v) was prepared to which the salts like NaNO3 0.5 g/l, MgSO4 7HzO 0.5 *g/l,* KCl 05 g/1 were added. Adjusting the pH to an appropriate value using liquid ammonia, the solution was autoclaved at 121°C for 20 min. The sterilized solution after cooling was inoculated with the definite numbers of immobilized beads and incubated under different environmental conditions.

Immobilization of whole cell

The induced inoculum in appropriate proportion was aseptically added to the 2% (w/v) of sterile sodium alginate solution to achieve the requisite spore concentration. This solution was dropped into 0.5 M calcium' chloride solution using a pump to obtain equal size polymeric beads of calcium alginate thereby immobilizing the spores of the microbial cells. The entire process was done aseptically in the laminar air flow chamber with proper care.

Assay The enzyme activity was determined by a method reported by Iibuchi [5]. *0.5* ml broth containing the enzyme was added to 2 ml of 0.35% *(w/v)* purified acid from tannic acid solution in a test tube. 2o µl of this solution was added to 2 ml of 95% ethanol solution to stop the enzyme activity. The absorbance o this was noted at 310 nm. *After* certain time, 20 µl of reaction mixture was again taken and added to 2 ml of 95% ethanol and the absorbance readings the enzyme activity was calculated.

One unit of enzyme activity is defined as the amount of enzyme required to hydrolyze 1 µmole of ester bond in min.

Isolation of gallic acid after fermentation the broth was separated from the immobilized cells and was concentrated approximately to one tenth of its initial volume by boiling. The concentrated broth was then subjected to diethyl ether in 1:1 ratio to extract gallic acid in the organic phase which forms immiscible layer in water. After separation the diethyl ether was removed by evaporation. The residue containing gallic acid was further studied its characterization was done by IR and NMR.

In the present paper the parameters studied are:

1. Effect of pH i.e. pH 3-6
2. Effect of substrate concentration
3. Effect of incubatior; period
4. Isolation of gallic acid from the broth
5. Characterisation and purification of gallic acid

3. Results

3.1 Effect of initial pH

To evaluate the effect of initial pH on tannase secretion by immobilized *Aspergillus oryzae* , the immobilized beads (200 nos of. 10^6/ml spore cone.) were inoculated in 100 ml media (modified czapek *dox)* of different pH and incubated at 30°C for 4 days in shaking condition. The pH of the media was adjusted from pH 3 to 6. It is observed that at pH 5.5 the enzyme tannase activity was found to be maximum beyond which the secretion of enzyme stopped (Fig.I). The optimum tannase and gallic acid produced was at pH 5.5.

3.2 Effect of substrate concentration

The product concentration has increased with increase in substrate concentration and an optimal gallic acid concentration of 17.79 mg/ml was obtained at 12% substrate concentration. The optimum substrate concentration required for maximum tannase production was 6%.

From Fig.II it can be concluded that 12% substrate concentration was found to be optimum though at lower concentration of substrate the conversion efficiency was slightly higher but 12% concentration was considered to be the optimum considering the commercial production of gallic acid.

3.3 Effect of incubatior period

The production of tannase has increased with increase in incubation time up to 48 hrs, with further increase in the incubation time, decrease in the tannase production was observed.This may be due to the starting of the declining phase of the organism after 48 hrs. Fig.III shows that, an incubation period of 48 hrs was optimum for gallic acid production .

3.4 Gallic acid characterization

The gallic acid was extracted from the broth using di-ethyl ether and the recrystallised gallic acid was characterised by its melting point of 253°c. IR. Spectra and PMR spectra. The IR spectra was analysed for the functional groups which matched with the gallic acid molecule and the peaks of the PMR spectra were verified by comparing standard gallic acid peaks from Aldrich PMR catalogue.

3.5 Effect of glutaraldehyde on whole cell immobilization

While designing the experiment it has been found that the normal immobilised spore of *A..oryzae* gets germinated and ultimately it looses the immobility to overcome this problem the immobilized beads were treated with different concentration of glutaraldehyde with different time period. It was found that with the lower concentration of glutaraldehyde of 4 minutes treatment the conversion was only 37% (data not shown) beyond which the conversion was totally stopped.

4. Discussion

The whole cell immobilization of Aspergillus *oryzae* though slows down the fermentation process due to limited in time. The interest towards searching a new microbial strain for the production of gallic acid is the main thrust for this research work. The microorganism commonly employed for the fermentation process is fungi. This newly isolated Aspergillus *oryzae* is very suitable for gallic acid production because it can grow easily and produce a huge amount of tannase within a short period. The novelty of this enzyme is that it is salt – tolerant as well as stable over a wide pH range. These features make the strain promising for industrial exploitation in various fields. More than 75% of enzymes require the presence of metal in activators to express their full catalytic activity metal ion activation of enzyme reactions is important industrially in achieving maximum catalytic efficiency.

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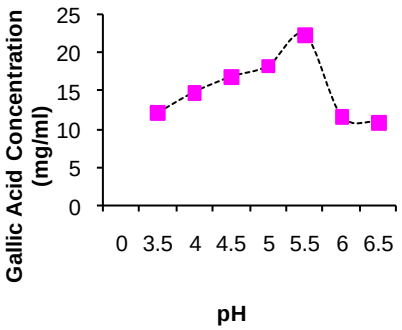

Fig. 1. Effect of initial PH

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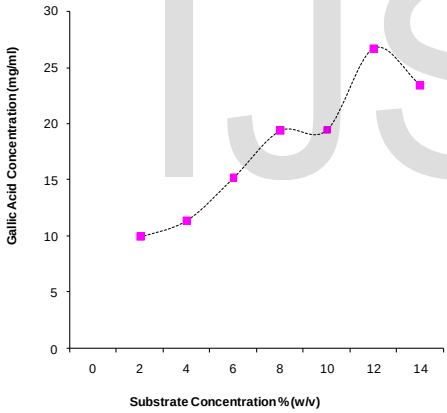

Fig. 2. Effect of substrate concentration

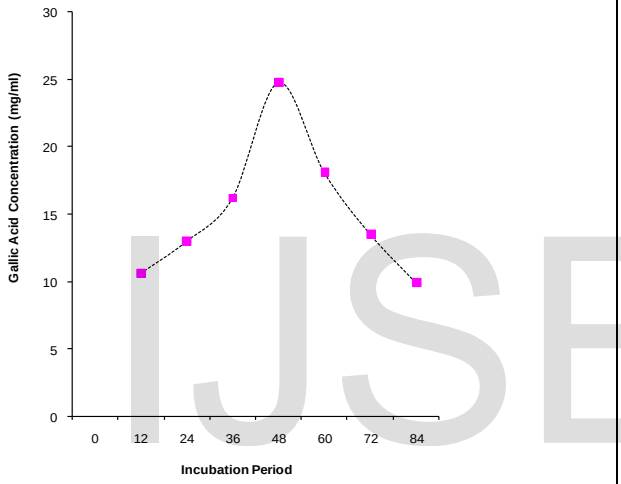

Fig. 3. .Effect of Incubation period

Author name: Dr.N.Lokeswari, Assistant Professor,
Department of Biotechnology,
Dr.B.R.Ambedkhar University, Srikakulam,
Andhra Pradesh, INDIA. Phone No: 9052525370,
Email: lokeswarin@hotmail.com