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# Optimization of Various Parameters Used in Immobilizing *Acremonium Chrysogenum* 1391 for Cephalosporinproduction

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***Abstract:***
*The present study deals with the examination of cephalosporin production by Acremonium chrysogenum 1391 would be improved with sodium alginate immobilization in submerged fermentation when compared with free cells. Results showed that in free cells produced 145 to 150µg/mL of cephalosporin whereas immobilized cells produced 360-380 µg/mL. Immobilization of cells retards the growth rate of Acremonium chrysogenum but increased the length of growth period and improved the cephalosporin production. The optimum immobilized conditions were alginate 3%, Inoculum size 3mL, the culture temperature at 28°C and bead diameter 2.50mm. Cephalosporin production increased with increasing Inoculum density, but decreased with increasing bead diameter.*

***Keywords****: Sodium alginate, cell immobilization, acremonium chrysogenum, cephalosporin, free cells*

## 1. Introduction

*Acremonium chrysogenum* was first isolated by Brotzu [1] from sea water in 1945. Later Abraham and coworker in 1955-56 isolated cephalosporin C, cephalosporin P and penicillin N from culture of *C. acremonium*[2]. The beta-lactam antibiotics particularly penicillin and Cephalosporins share approximately 65% of worldwide market of antibiotics [3]. A number of reports are available on the various aspects of cephalosporin production such as production in batch as well in continuous mode using immobilized cells and free cells [4][5][6][7][8][9]. The optimization of immobilization conditions for cephalosporin biosynthesis has been reported by few authors [10][11] and different hypothesis has been proposed. Immobilization of Cephalosporium, Aspergillus, Pencillium, Sacharomyces, Streptomyces, and Cladonia has been used for the production of ethanol, organic acids, antibiotics, enzymes and alkaloids. Agar, alginate, carrageenan, collagen, gelatin, polyelectrolyte, polyurethane , and polyacrylamide have been used as supporting materials for gel entrapping carrier binding and cross linking immobilization. This study compared the production of cephalosporin by immobilized *Acremonium chrysogenum* in submerged cultivation with free cells.

## 2. Materials & Methods

### *2.1. Micro-Organisms*

*Acremonium chrysogenum* MTCC 1391 was procured from Microbial Type Cell Culture Chandigarh. Escherichia coli was obtained from NCIM 5051.

### *2.2. Culture Media and Growth Conditions*

*Acremonium chrysogenum* was cultivated in YPSS medium containing soluble starch 15g/L, yeast extract 4g/L, di-potassium hydrogen phosphate 1g/L, magnesium sulphate 1g/L, at pH 7±0.1 at 28°c for 4 days. The mycelia was then transferred to a synthetic medium comprised of peptone 20 g/L, malt extract 20g/L, cornsteep liquor 5g/L, magnesiumsulphate 0.25 g/L, $K_2HPO_4$ 0.5 g/L, potassium di hydrogen phosphate 1.0 g/L,$Cacl_2$ 0.1 g/L in a 250mL Erlenmeyer flask at 28°C and 150 rev/min for 3 day.Spores were then harvested by centrifugation at 1000×g for 10 min to production medium.

### *2.3. Immobilization*

The optimization of parameters like size of the bead, culture temperature, inoculum density, and concentration of sodium alginate used for immobilization of spore suspension were studied. The optimized immobilization support material was gently mixed with spore solution and passed through a sterile syringe into 2% $CaCl_2$ solution to immobilize the spores. The beads were washed several times

with sterilized water for cephalosporin production. The beads were then inoculated into production media and incubated for 12 days at 28°C and bioassay was performed.

*2.4. Submerged Fermentation*

Spore solution, 3% (v/v) was transferred to a production medium containing peanut meal 60g/L, starch 40g/L, dextrose 7g/L, sucrose 5g/L, calcium carbonate 10g/L, DL-Methionine 10g/L, methyl acetate 25g/L in a 250 mL Erlenmeyer flask and cultivated at 28°C, pH 6.5 and rotated at 150 rev/min shaker for 10 days. All experiments were carried out in triplicate and the mean values and standard deviation were calculated. Biological assay was performed to estimate the cephalosporin production of free cells (spore suspension).

*2.5. Biological Assay Method (Diffusion Method)*

The plates for the assay were prepared with a base of 20mL of antibiotic test medium no: 1, which is overlaid in petri plate of even thickness seeded with a diluted stock suspension of the organism (E.coli), OD of 0.01 at 610nm. After the medium was solidified, wells were prepared with the help of stainless steel cork borer employing a well of diameter 5mm. Diluted solutions of standard cephalosporin as well as diluted samples of unknown concentrations were added to the wells. After incubation at 35 - 37° C for generally 18 – 20hrs, antibiotic diffuse through the agar, growth of the susceptible bacteria is suppressed and no growth is observed. No growth is observed within a circular zone around the well. The diameters of the zone of inhibition were measured and average for each standard and test dilution was taken. The potency of the unknown samples can be determined from the standard graph of cephalosporin.

**3. Results**

*3.1. Optimization of Immobilization Parameters*

3.1.1. Size of Beads

The effect of size of immobilized beads on cephalosporin production is illustrated in Figure1, when beads with smaller size were used, the time period for maximal cephalosporin was short, but the yield was same at different bead size. Each 100 milliliter of culture broth could support 310µg/mL of cephalosporin production with bead size of 1.25mm diameter on the day 5 whereas 2.50mm diameter on day 8 and 3.20 mm diameter on day 10.

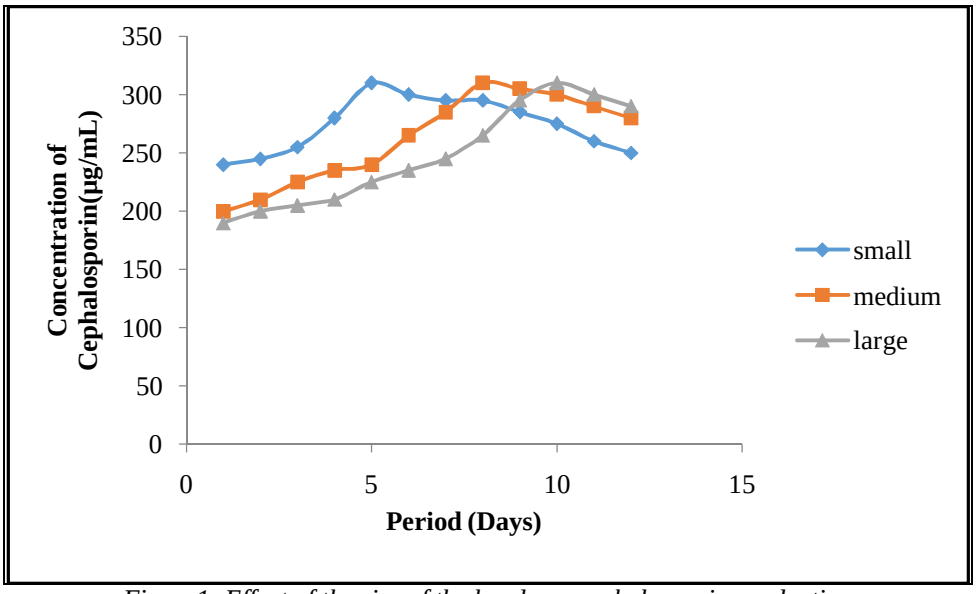


*Figure1: Effect of the size of the beads on cephalosporin production*

*3.2. Concentration of Sodium Alginate*

Effect of Concentration of sodium alginate on cephalosporin production is shown in Figure 2. The best concentration of sodium alginate for sporesimmobilization was 3%. Each 100µl of culture broth yielded 300µg/mL, 320µg/mL, 330µg/mL, 290µg/mL of cephalosporin on day 8 of incubation with sodium alginate 1%,2%, 3%, and 4%.respectively.Sodium alginate 3% has shown maximal production on eighth day.

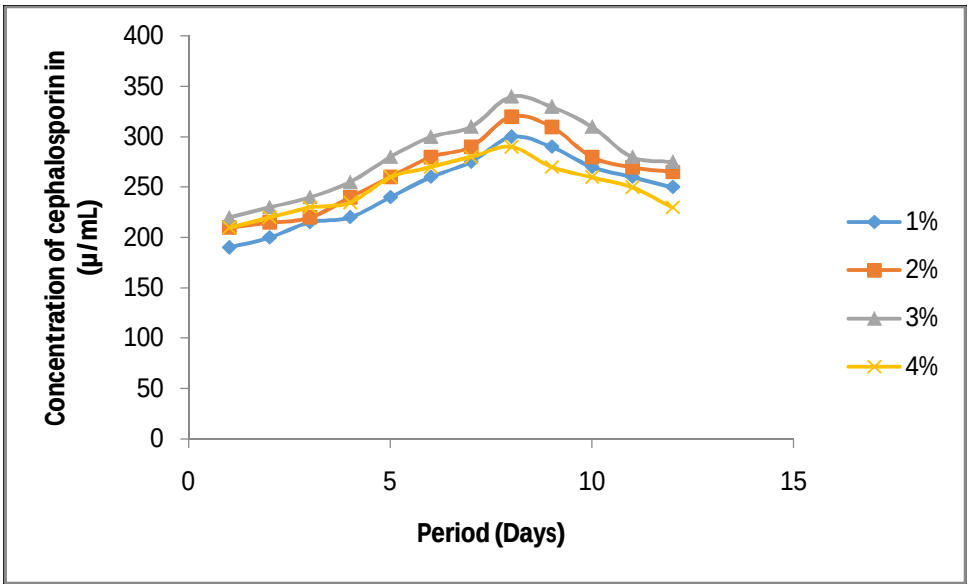


*Figure 2: Effect of Concentration of sodium alginate on cephalosporin production*

*3.3. Inoculum Density*

The effect of cell density in immobilized beads on cephalosporin production is shown in Figure 3. The effect of inoculum density with 1L, 2mL, 3mL and 4mL of spore solution in 3% of sodium alginate on cephalosporin production was not significant at the early cultivation stage; however, high inoculum density resulted in high cephalosporin productivity on day 8 of incubation. Each 100 µl of culture broth produced 280µg/mL, 290µg/mL, 300 µg/mL, 285 µg/mL respectively.

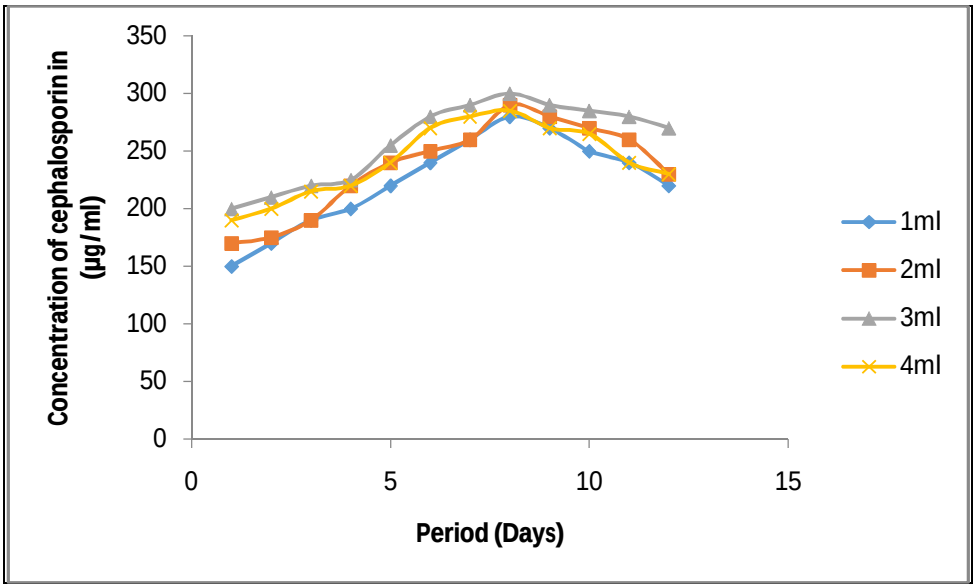


*Figure 3: Effect of Inoculum density (spore concentration) on cephalosporin production*

*3.4. Culture Temperature*

The effect of culture temperature on cephalosporin production is illustrated in Figure 4. Both free and immobilized cells had maximal Cephalosporin potency when grown at 28˚C,followed by 25˚C and produced the lowest amount of Cephalosporin at 33 ˚C. Each 100 µl of culture broth produced 340µg/mL, 290µg/mL, 190µg/mL of cephalosporin in free and immobilized cells at 28˚C.

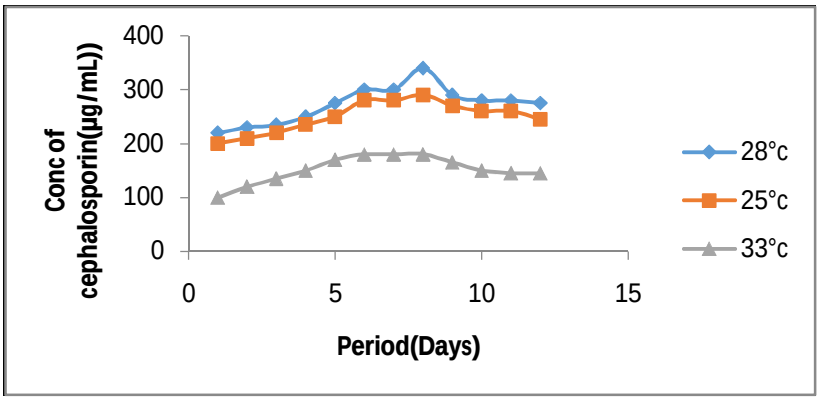


*Figure 4: Effect of temperature on cephalosporin production*

*3.5. Comparison of Cephalosporin Production from Free Cells and Immobilized Cells*

The production of cephalosporin from free and immobilized cells is illustrated in Figure 5. The immobilized 3mL spore solutionin 3% sodium alginate of 2.50 mm diameter beads at 28°C has shown the maximal cephalosporin production of 380µg/mL on day 8 when compared with optimized free cell growth conditions which were done earlier.

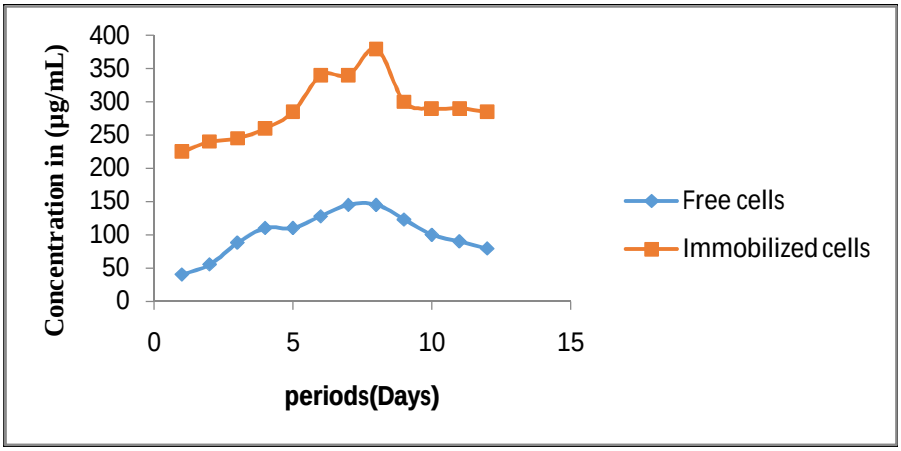


*Figure 5: Antibiotic production in free and immobilized cells of A.chrysogenum*

**4. Discussion**

The antibiotic production decreased with increase in bead diameter, because the gel conformation prevented the exchange of oxygen and substrate between the cell and the environment,[12] beads with the diameter of 1.5mm have a 3 fold higher penicillin production in *P.chrysogenum* than beads with a diameter of 3.5mm.Concentration of alginate effect not only the structure of immobilized beads and the growth of the cells but also the production of antibiotic i.e., by high concentration of alginate results tight cross linking between cells and alginate which reduces nutrient transport and microbial activity vice versa.[13]High inoculum density enhance microbial growth and antibiotic production but the specific productivity of unit immobilized spores decreased,[12]showed that chlortetracycline production with immobilized spores of *S.aureofaciens* increased with spore concentrations. During incubation period antibiotic that is released from the beads gets diffused into the medium and formed a gradient concentration. This study demonstrated that spore immobilization of *A.chrysogenum* initially inhibited the growth period but enhanced the antibiotic production by 2 folds higher than that of the production by free cells. Therefore, large scale production of metabolite by industries can adhere to cell immobilization technique.

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