ABSTRACT

Prednisolone is an important steroid used as an effective drug for rheumatoid arthritis. It is produced by microbial method from Reichstein's compound S in two steps. The first step is the transformation of Reichstein's compound S to hydrocortisone by the fungus *Curvularia lunata* and the latter is then 1-dehydrogeneted to prednisolone. Both the compounds are useful as anti-inflammatory drugs, but prednisolone is 6 times more effective than the other. So the demand for prednisolone is much higher than hydrocortisone and the microbial method of production has drastically reduced the price compared to chemical synthesis. As a result, lot of interest has been created to develop the microbial method of prednisolone production economically and commercially. Many researchers have undertaken investigations on the above process to find out the optimal process parameters along with the basic kinetics and reactor systems.

The prime objective of the present investigation is to isolate, purify and characterize the effective strain - *Arthrobacter simplex* which can bring about the desired transformation, from the indigenous soil, following the classical method of strain isolation using the "Enrichment culture technique". The free microbial cells has been used for studying the optimum values for maximum cell mass growth and fermentation of hydrocortisone under various environmental conditions like pH, temperature, substrate concentration, culture age, surfactants etc. The optimum conditions for maximum cell mass growth has been found at an initial hydrocortisone concentration of

0.75 g/l, pH 7, temperature 37°C, 150 rpm speed and at an inoculum concentration of 7.5 ml and inoculum age of 20 hrs.

For prednisolone formation, optimum conditions have been found to be similar except for the optimum inoculum age and concentration which were 16 hours and 5 ml respectively with the agitator speed of 150 rpm. It was found that presence of surfactants had negligible effect on product formation.

There are some inherent problems with free cell fermentation, like product separation, deactivation, structural deformation, pollution and maintaining aseptic conditions. So cells have been immobilized in calcium alginate solid matrix and microbial transformations have been carried out as enzymatic systems. Immobilized cells have the advantages of easy separation, stability of the enzymes and less pollution problems. Immobilization studies have been done with *A. simplex* entrapped in calcium alginate gel and the beads formed were used for the fermentation of hydrocortisone under the same experimental conditions as the free cells. Optimal parameters studied in the immobilized cell system remained almost unchanged, though the pH and temperature tolerance were better. The optimum values for cell and solid loading and bead diameter were 6.8 g/l and 7.792×10^4 cells/bead and 2mm respectively.

The kinetics of the cell mass growth has been described by a logistic curve model and the parameters x_s, k & b have been evaluated and the values show satisfactory simulation results.

For prediction of product formation the non-growth associated model has been used, given by $dp/dt = \beta x\ (t)$, where β has been evaluated

from the plot of dp/dt vs. x and the linearity of the plot establishes the validity of the model.

Attempts have been made to fit the data on immobilized systems in the Michaelis-Menten equation and the kinetic parameters of the model have been determined from the Lineweaver-Burk plot and the values of k_m and v_{max} are 2.332 g/l and 0.0833 g/l hr respectively for the present system.

Simulation studies carried out with the above model show close correspondence of the experimental results and the calculated ones.

Diffusion effect has been evaluated by finding the Thiele parameter, ϕ for different immobilized particle sizes using the kinetic parameters, v_{max}, k_m and $D_{es}$and the values of ϕ have been found to be much less than 3, which is the limiting value of ϕ above which diffusion becomes predominant, indicating that diffusion had no effect on the system.