SYNOPSIS

The thesis deals with the biosynthesis of cellulase enzyme from coconut coir pith in solid state fermentation (SSF) by the mesophilic fungi *Trichoderma viride* NCIM 1051 and *Aspergillus niger* NCIM 1005 in monocultures. It also deals with the modelling of a) fungal growth and b) the heat transfer aspects in a static solid substrate bed.

Enzymatic conversion of waste cellulosic material to glucose which can serve as a raw material for the production of fuel and other chemicals, is a very promising process because cellulose is the most abundant renewable resource. For the overall cellulose conversion technology to be economical, it is necessary to produce an active enzyme complex inexpensively (Wilke *et al.*, 1976). Keeping this in view, considerable work has been done on the biosynthesis of cellulase, employing various cellulosic substrates and cellulolytic organisms by several investigators. Most of the work was carried out using the submerged fermentation technique, while the simple and less expensive solid state fermentation was employed only to a limited extent. A cheaper substrate, fermented with a good cellulase producing organism in a well designed SSF reactor will help in reducing the enzyme production cost.

Coconut pith is a waste product obtained abundantly from the coir industry and has a good cellulose content (Dan, 1992). It has no other significant commercial end uses and also poses disposal problems causing environmental pollution. The present investigation is an endeavour to find the possibility of harnessing this waste biomass.

In literature, modelling of fungal growth in solid state fermentation are few. Still fewer are the attempts to model the interaction between heat transfer and bioreaction. In this thesis, models are proposed to describe the growth and heat transfer in SSF beds.

Ten fungi, known for their cellulolytic activity, were screened for their ability to grow on hydrogen peroxide pretreated coconut coir pith solid culture and
synthesise the cellulase enzyme complex. Two fungal strains *Trichoderma viride* NCIM 1051 and *Aspergillus niger* NCIM 1005 were identified among them, as good cellulase producers and chosen for further study.

The effect of some significant fermentation conditions such as temperature, initial culture pH, substrate particle size, nutrient level and inoculum size on cellulase synthesis by each of both the organisms were studied in static conical flasks and the most favourable conditions were identified. Filter paper degrading activity (FPA) and carboxy methyl cellulase activity (CMCase) were estimated according to the procedures of Mandeles *et al.* (1976). Cellobiase activity was measured utilising cellobiose as the substrate as per the procedure recommended by IUPAC, (1986).

*T. viride* produced maximum activities of 4.48 IU/gm FPA, and 12.25 IU/gm CMCase on the 7th day and 1.47 IU/gm cellobiase on the 8th day. Initial culture pH of 6.5, temperature of 28°C, average particle size of 375 microns, and substrate to nutrient solution ratio of 1:10 (W:V) were found to be optimal for enzyme production by the fungus. *A. niger* produced maximum activities of 4.13 IU/gm FPA, 12.21 IU/gm CMCase and 7.16 IU/gm cellobiase on the 7th day. Optimum fermentation conditions were the same as those for *T. viride*, except the initial culture pH which was 5.0. With both organisms the inoculum size had little effect on enzyme production.

Growth of *T. viride* NCIM 1051 on coconut pith was studied in order to elucidate the relationship between growth and cellulase production. The protein assay (Raimbault and Alezard, 1980) used, characterised the growth in the lag and exponential phases with reasonable accuracy, but failed to represent the stationary and decay phases of the culture. The study provided some qualitative answers to the mechanism of enzyme synthesis by the fungus in SSF of coconut pith.

Fermentation was also carried out on a larger laboratory scale, in a tray...
fermentor, for improving the enzyme yield and to study the effect of type of inoculum, initial moisture content, aeration and water activity. The cellulase activity obtained per unit weight of initial dry substrate taken for fermentation was higher in the tray fermentor than in conical flasks. Liquid inoculum resulted in more homogenous growth and better enzyme production in a shorter time compared to solid inoculum. Neither the initial moisture content of the culture, nor the aeration significantly affected the enzyme synthesis. An interesting inference made was that the most emphasised water activity of the substrate necessarily be considered in the fermentation of most lignocellulosic substrates, need not be paid much attention with coconut pith as substrate, as the pith acts as its own reservoir of water. Another economically significant observation was that the expensive procedure of aeration with sterilised and saturated air can be dispensed with in the biosynthesis of cellulase from coconut pith.

Separate experiments on a model substrate were carried out to obtain growth kinetic data of \textit{T.viride} NCIM 1051. The logistic model for cell growth

\[
\frac{dx}{dt} = \mu X\left(1 - \frac{X}{X_{\text{max}}}ight)
\]

where \(X\) is biomass concentration, \(X_{\text{max}}\) is maximum biomass concentration and \(\mu\) is the specific growth rate during exponential growth, was found to fit the experimental data satisfactorily. Specific growth rate and the biomass yield coefficient were also estimated as 0.0081 \(\text{hr}^{-1}\) and 1.286 mg cells per mg substrate.

A model equation in dimensionless form, for describing the temperature gradients, coupling the bioreaction occurring to the heat transport in a static solid substrate bed supported on a tray, has been developed. An exact solution to the model was obtained by the method of Laplace transforms. The model proposed is;
\[ T(Z, \theta) = 1 + \beta' e^{\theta} \left[ 1 - \frac{N_{Bi} \text{Cosh}(\sqrt{q}Z)}{\sqrt{q} \text{Sinh}(\sqrt{q}) + N_{Bi} \text{Cosh}(\sqrt{q})} \right] \]

where \( T \) is dimensionless temperature, \( Z \) is dimensionless bed depth, \( \theta \) is dimensionless time, \( N_{Bi} \) is the Biot number and \( \beta' \) and \( q \) are model parameters, comprised of growth kinetic parameters, physical properties of the bed and heat of combustion of cells and cellulose. Experimental data of variation of temperature with fermentation time and bed depth, enabled the evaluation of model parameters.

Model verification was accomplished using experimental data obtained. The model was found to represent the temperature distribution in the SSF bed reasonably well.

From the model equation, based on the maximum allowable temperature at any location in the bed, an equation for determining the 'critical bed depth' is deduced, which is essential in the design of tray fermentors. Further, the model provides an indirect method for determining the kinetic parameters of specific growth rate and biomass yield coefficient which are difficult to determine in SSF.

**Summary of findings:**

1. The cheap and abundantly available agro industrial lignocellulosic waste, coconut coir pith, can be used for the production of cellulase enzyme complex.

2. The microorganisms *A. niger* NCIM 1005 and *T. viride* NCIM 1051 are able to grow well and synthesize the enzyme in coconut coir pith solid culture.

3. Some significant fermentation conditions such as initial culture of pH, temperature, nutrient level, substrate particle size and inoculum size, favourable for cellulase synthesis by *T. viride* NCIM 1051 and *A. niger* NCIM 1005 are identified.

4. The lag and the exponential growth phases of *T. viride* NCIM 1051 grown on coconut pith is characterised by the cell protein.
5. Cellulase production is higher in the specially designed and fabricated tray type bioreactor. Liquid inoculum is better than solid starter culture. The initial moisture content above the minimum level does not influence the enzyme production. Substrate water activity and aeration of culture are insignificant in the fermentation.

6. Mathematical models proposed for a) growth and b) heat transfer, in SSF beds represent the experimental data satisfactorily.