Agricultural, farm and agro-industrial residues are significant biomass resources which can be converted biochemically to many useful and value added products. The most prominent raw material in this respect is the lignocellulosic wastes. The cellulose present in these can be converted to produce glucose and/or ethanol, which will form an alternative and attractive proposition for the generation of vast chemical and fuel feed stocks. The cellulose present in the lignocellulosic can be hydrolyzed to glucose either by acids or by enzymes. Enzymatic hydrolysis, having more advantages over acid hydrolysis, requires the use of cellulase enzyme. In the overall bioconversion of cellulose to glucose, the cost of production of this cellulase enzyme complex is the most expensive part. The process will be an attractive and economical proposal only if cellulase of desirable quality is produced inexpensively.

One way of reducing the enzyme production cost is to use a cheaper and easily available lignocellulosic as the substrate. Coconut coir pith, a waste product obtained from coir industries has a cellulose content of nearly 35% and has no significant industrial uses. Further it causes environmental pollution and disposal problems to the coir industries. The pith, abundantly available in all major coconut producing countries including India (Table 1.6) is chosen as the substrate.

Solid state fermentation has numerous advantages compared to the submerged fermentation technique and has a high potential for economic exploitation. With its simplicity and record of success with other enzymes production, it is a
viable mode for cellulase production and is chosen as the fermentation technique. The pretreatment studies of the lignocellulosic to liberate the available cellulose have shown that the $H_2O_2$ treatment process is most effective.

Apart from the selection of a cheap and easily available substrate and a simple mode of operation, selection of a good cellulase producing microorganism is necessary for improving the economics of the enzyme production. After an exhaustive survey of the various microorganisms employed for cellulase production in SSF (Table 2.1), ten cellulolytic fungal strains were chosen for a screening study. When cultivated on hydrogen peroxide pretreated coconut coir pith, the strains *T. viride* NCIM 1051, and *A. niger* NCIM 1005 were found to produce the maximum cellulase activities of 3.46 IU/gm and 3.53 IU/g respectively in 8 days of fermentation at temperature 28°C and initial culture pH 6.5. This study confirmed that coconut coir pith can be used as a substrate for cellulase synthesis in SSF and the organisms *T. viride* NCIM 1051 and *A. niger* NCIM 1005 are able to grow and synthesise the enzyme well.

Suitable conditions for fermentation by the organisms were found by conducting a number of pure culture experiments in static conical flasks. This study provided many significant results. Both the strains produced the cellulase enzyme complex, consisting of all the three major components, with *A. niger* producing more cellobiase activity and *T. viride* producing more filter paper activity and carboxy methyl cellulase activity. The suitable values of fermentation conditions, initial culture pH, fermentation temperature, substrate particle size, nutrient level, inoculum size and the time of occurrence of maximum enzyme activity were determined and are given in Table 9.1. Variation of the pH with the activity was also studied.

The growth characteristics of the organism *T. viride*, on coconut coir pith solid culture was studied, using the protein content of the culture to characterise
growth. The study revealed that the protein content adequately represented the exponential growth phase and failed to represent the stationary and decay phases. The reasons and implications were also discussed in detail. Absence of cellulase activity during lag phase, cellulase production was associated with growth and sugar formed catabolitely repress cellulose production are some of the findings made in the study.

Fermentation was carried out in a specially designed tray type fermentor (scale up ratio 1:0.1) with a view to study the enzyme yield. The effect of more fermentation conditions such as inoculum type, moisture content, water activity and aeration in the tray type fermentor was studied. The results have shed light on many interesting and significant finding. The cellulase productivity per gram of dry substrate initially taken, was found to be approximately 3 times more in the tray fermentor than in the conical flasks. Liquid inoculum produced more homogeneous growth, better production and achieved maximum production of about 10, 31 and 2.1 IU of FPA, CMCase and cellobiase respectively per gram of dry substrate in time of 144 hours compared to solid inoculum which produced 3.3, 6.5 and 0.7 FPA, CMCase and cellobiase in 216 hours. The initial moisture content of the substrate

<table>
<thead>
<tr>
<th>Fermentation condition</th>
<th>T. viride NCIM 1051</th>
<th>A. niger NCIM 1005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial culture pH</td>
<td>6.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Temperature</td>
<td>28°C</td>
<td>28°C</td>
</tr>
<tr>
<td>Time of fermentation</td>
<td>168 hrs.</td>
<td>168 hrs.</td>
</tr>
<tr>
<td>Average particle size</td>
<td>375μ</td>
<td>375μ</td>
</tr>
<tr>
<td>Nutrient level</td>
<td>10 cc/g</td>
<td>10 cc/g</td>
</tr>
<tr>
<td>Inoculum size</td>
<td>2-5 cc/g</td>
<td>2-5 cc/g</td>
</tr>
</tbody>
</table>
does not affect the enzyme production and the most emphasised water activity of the substrate need not be paid much attention in the case of coconut pith as the pith has a high water retention capacity and acts as its own reservoir of water during the entire fermentation. As aeration of the culture during fermentation with saturated air made no noticeable difference in the activity, aeration with saturated sterilised air can be dispensed with, resulting in a more economical process.

Growth kinetic data obtained employing a model substrate was used to obtain the model parameters of a logistic equation, proposed to represent the biomass growth in SSF. The equation fitted with experimental data was found to represent the growth adequately, showing good agreement. The specific growth rate, biomass yield coefficient and the maximum biomass concentration were determined as 0.0081 hr$^{-1}$, 1.286 mg/mg and 0.52 mg/ml respectively.

A mathematical model in dimensionless form coupling the interaction of heat transfer with the bioreaction has been developed.

\[
\bar{T}(\bar{Z}, \theta) = 1 + \beta e^{\theta} \left[ 1 - \left( \frac{N_{R_t}\cosh(\sqrt{\bar{q}}\bar{Z})}{(\sqrt{\bar{q}_t}\sinh(\sqrt{\bar{q}}) + N_{R_t}\cosh(\sqrt{\bar{q}}))} \right) \right]
\]

The equation describes the variation of temperature in an SSF bed with time of fermentation and depth. Model parameters have been successfully estimated using experimental data of temperature variation in a SSF bed. The model predictions were compared with experimental data and found to well represent the temperature distribution in the bed. A concept of critical depth based on the highest temperature permissible in the bed is evolved, and an equation for determining the same from the model equation is deduced as:

\[
l = \sqrt{\frac{\alpha}{\mu}} \ln \left[ \frac{b + \sqrt{b^2 + 4a^2}}{2a} - 1 \right]
\]
Critical bed depth calculated for the biosynthesis of cellulase in SSF coconut pith was found to be 23.29 cms for a 240 hours fermentation and 40.88 cms for 168 hours fermentation. The concept will enable better design of the tray type fermentors. Further, the model equation can be employed for determining the kinetic parameters of an SSF system from the temperature variation data, which are generally difficult to determine.

In conclusion, the contribution of this work can be summarised as follows.

1. The cheap and abundantly available lignocellulosic agro-industrial waste, coconut coir pith can be employed as a substrate for the biosynthesis of cellulase enzyme complex.

2. The organisms \textit{T. viride} NCIM 1051 and \textit{A. niger} NCIM 1005 are able to grow well in the coconut coir pith solid culture and produce the enzyme appreciably.

3. Favourable fermentation conditions for the synthesis of the enzyme by the two strains are established at the laboratory level.

4. The study of growth of the organism \textit{T. viride} on the coconut pith solid culture, shed some light on the kinetics of the system.

5. Enhanced cellulase production was obtained in a scaled up tray bioreactor. Studies in the reactor revealed that maintenance of external water content, water activity and aeration of culture are not necessary in the fermentation as coconut pith acts as its own reservoir of water.

6. Mathematical models have been proposed for growth kinetics and heat transfer aspects. The heat transfer model presents explicit equations for prediction of temperature in a SSF bed. Further it provides a means for determining the kinetic parameters of SSF system.
7. A method for determining the critical depth of the bed, which will help in the optimum design of tray type bioreactors is developed.