Infra-red spectroscopic analyses of banana waste degraded by oyster mushroom

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Carbon, hydrogen and nitrogen analyses of banana leaf and pseudostem biomass revealed their potentiality as substrates for microorganisms. Infra-red (IR) spectra of both biomasses showed presence of cellulose, xylan and lignin. IR spectra of leaf and pseudostem biomass degraded in solid state fermentation (SSF) by two Pleurotus species (P. sajor-caju and P. ostreatus) for 40 days showed the utilization of cellulose, xylan and lignin by these microbes. Dynamics of various lignocellulolytic enzymes of Pleurotus species and analyses of carbon, hydrogen and nitrogen contents of degraded biomass supported the same. Both the Pleurotus species exhibited lignin consumption ability on both the substrates.

Solid state fermentation (SSF) has important industrial application including manufacture of selected high value microbial products. SSF is advantageous to obtain concentrated metabolites and subsequent purification procedures are economical. Infra-red (IR) spectroscopy provides evaluation of fungal growth, monitoring of respiratory quotient (as an indicator of physiology of fungi through CO₂ determination) and cell components estimation in SSF process.

Banana, one of the prominent crops in India, generates 7.77 x 10⁶ tonnes of agro-waste with 3.86 x 10⁵ hectares under cultivation. This can be used for industrial fermentation processes such as myco-protein production, mushroom cultivation, animal feed production, lignocellulolytic enzymes production, etc. employing SSF. Agro-industrial residues are generally considered to be the best substrates for SSF processes. Microbial conversion of an abundant supply of agricultural wastes has become a subject of considerable interest as a renewable source of raw material for food and pharmaceutical sectors.

Pleurotus sps. are efficient degrader of lignocellulosic complexes of agricultural wastes. They are the most suitable fungi for producing protein rich food from various agro-wastes due to their lignocellulolytic activities. They produceexo-1,4-β-D-glucanase (celllobiohydrolase, EC 3.2.1.91), endo-1,4-β-D-glucanase (carboxymethylcellulase, CMCase, EC 3.2.1.4) and β-D-glucosidase (cellbioase, EC 3.2.1.21) to utilize cellulose, xylanase (EC 3.2.1.8) for hemicellulose, and laccase (EC 1.14.18.1) for lignin utilization.

In the present investigation degradation of banana waste by two Pleurotus species (P. sajor-caju and P. ostreatus) has been studied through IR spectroscopy. Enzyme dynamics of the process have been investigated and carbon, hydrogen, nitrogen analyses have also been done to corroborate the results obtained from IR spectroscopy.

Materials and Methods

Banana leaves and pseudostems collected after harvesting fruits were washed with sterile water, dried in oven at 100°C and powdered to 25-mesh size. Cellulose, hemicellulose and lignin contents were estimated. Amounts of carbon, hydrogen and nitrogen were obtained using C, H, N analyzer (Perkin-Elmer series II). Infra-red spectra of powdered biomass of banana leaf (L) and pseudostem (P) were recorded on FT-IR impact 400D using KBr pellet which is extensively used for IR spectra of cellulose and related compounds. IR spectra of pure cellulose, xylan and lignin were recorded and used as control.

P. sajor-caju and P. ostreatus procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India were maintained on PDA slants at 25°C and subcultured after every 30 days. Inoculum for these organisms on wheat grains was prepared as described by Kumar and Chandra and was used for inoculating SSF substrate.

Substrate for SSF was prepared by mixing 25g leaf/pseudostem biomass with 75 ml of distilled water in one litre conical flask. Each flask was sterilized by autoclaving at 15 lbs for 2 hr, and inoculated with wheat grains based inoculum (3 g) of P. sajor-caju and P. ostreatus. Fermentations was incubated at 25°C.
and the degraded samples were collected after 40 days, dried in oven at 100°C and powdered to 25-mesh. IR spectra of degraded leaf and pseudostem biomass were recorded and carbon, hydrogen and nitrogen contents were estimated.

Degraded sample (5g) was collected at the interval of 10 days and homogenized in 20 ml of cold 0.05 M acetate buffer (pH at 6.5). The homogenate was filtered through Whatman No. 1 filter paper and filtrate was used for the assay of celllobiohydrolase \( l^3 \), CMCase \( l^3 \), xylanase \( l^3 \), and laccase \( l^4 \). Total protein contents in the enzyme was estimated according to Lowry et al. \( l^5 \). One unit of celllobiohydrolase, CMCase and xylanase activity has been expressed as 1μmole of glucose and xylose equivalents liberate per min respectively. Single unit of laccase activity has been defined as the amount catalyzing 0.1 absorbance change in guaiacol per min.

**Results and Discussion**

Results of carbon, hydrogen and nitrogen analyses in undegraded leaf and pseudostem have been shown in Table 1. It is apparent from these results that both these biomass were potent source of carbon and nitrogen which could support growth of microorganisms.

IR spectrum of pure cellulose (Fig. 1A) showed four well-defined bands at 1035, 1065, 1112 and 1172 cm\(^{-1}\). Absorption bands in this region can be attributed\(^{11}\) mainly to stretching vibrations of C-O. Xylan (hemicellulose) which has structural resemblance with cellulose, also exhibited absorption in this region (1000-1200 cm\(^{-1}\)), but the peak positions were slightly different and it showed a strong absorption at 890 cm\(^{-1}\) (Fig. 1A). The banding vibrations of C-OH and CH groups were found in the region 1300-1400 cm\(^{-1}\) in both cellulose and xylan spectra. IR spectra of pure cellulose and pure xylan had absorption at 1609 and 1629 cm\(^{-1}\).

On careful consideration of the spectrum of the undegraded banana leaf (Fig. 1B) biomass in the region 1000-1200 cm\(^{-1}\) and around 1600 cm\(^{-1}\), the presence of both cellulose and xylan (hemicellulose) was inferred. This was in conformity with biochemical estimation of banana leaf for cellulose (28.92%) and hemicellulose (25.23%). A band at 1735 cm\(^{-1}\) found in IR spectrum of pure cellulose (Fig. 1A) was also seen in IR spectrum of banana leaf.

Pure lignin had characteristic bands at 1602 and 1515 cm\(^{-1}\), these were probably associated with benzene ring. Two prominent peaks were also observed at 1220 and 1280 cm\(^{-1}\) in IR spectrum of pure lignin (Fig. 1A). In undegraded banana leaf biomass there were peaks at 1260, 1520 and around 1600 cm\(^{-1}\), indicating the presence of lignin in the leaf biomass. Since the relative intensity of peaks at 1260 and 1520 cm\(^{-1}\) were quite low compared to the same in the characteristic peaks of cellulose and xylan, it appeared that the amount of lignin in leaf biomass was less than that of cellulose and xylan. Biochemical estimation showed 10.56% of lignin in leaf biomass.

IR spectra of banana leaf biomass degraded by two Pleurotus species (\( P. \) sajor-caju and \( P. \) ostreatus) showed significant change from that of undegraded biomass in the region discussed above and absorption around 1100 cm\(^{-1}\) became very broad (Fig. 1B). Also, the well-defined two band of undegraded banana biomass at around 1600 cm\(^{-1}\) appeared as a single band in degraded biomass. It was likely that cellulose and xylan in the banana biomass were being utilized in the fermentation. From the pattern of this spectra it appeared that \( P. \) ostreatus degraded leaf biomass more efficiently than \( P. \) sajor-caju. This was in conformity

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
<th>Carbon reduction</th>
<th>Hydrogen reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf (L)</td>
<td>42.03 ± 0.25</td>
<td>5.74 ± 0.14</td>
<td>1.10 ± 0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pseudostem (P)</td>
<td>31.45 ± 0.47</td>
<td>4.79 ± 0.18</td>
<td>0.81 ± 0.01</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>L + S</td>
<td>36.96 ± 0.44</td>
<td>5.52 ± 0.11</td>
<td>1.14 ± 0.01</td>
<td>12.07</td>
<td>3.84</td>
</tr>
<tr>
<td>L + O</td>
<td>36.39 ± 0.21</td>
<td>5.34 ± 0.01</td>
<td>1.51 ± 0.01</td>
<td>13.42</td>
<td>6.97</td>
</tr>
<tr>
<td>P + S</td>
<td>28.38 ± 0.54</td>
<td>4.21 ± 0.07</td>
<td>0.66 ± 0.02</td>
<td>9.77</td>
<td>12.11</td>
</tr>
<tr>
<td>P + O</td>
<td>30.51 ± 0.37</td>
<td>4.37 ± 0.10</td>
<td>0.53 ± 0.02</td>
<td>2.99</td>
<td>8.77</td>
</tr>
</tbody>
</table>

L + S and L + O: Leaf biomass degraded by \( P. \) sajor-caju and \( P. \) ostreatus respectively;
P + S and P + O: Pseudostem biomass degraded by \( P. \) sajor-caju and \( P. \) ostreatus respectively.
Fig. 1—IR spectra of (A) cellulose, xylan and lignin; (B) degraded and undegraded leaf biomass; and (C) IR spectra of degraded and undegraded pseudostem biomass
with the exhibition of cellulases and xylanase activities during degradation (Table 2).

IR spectra (Fig. 1B) of banana leaf biomass degraded by two Pleurotus species (P. sajor-caju and P. ostreatus) did not show any significant presence of lignin. Peaks at 1220, 1280 and 1520 cm\(^{-1}\) were characteristic peaks of lignin. These peaks were observed as shoulders in the degraded leaf biomass. The band around 1600 cm\(^{-1}\) however, could not be used to infer anything regarding lignin consumption because cellulose and xylan also had absorption around this region. Presence of characteristic peaks of lignin as shoulder indicated the maximum utilization of lignin during the degradation and the high activity of laccase supported this observation (Table 2).

Considering the regions 1000-1200 cm\(^{-1}\) and the 1600-1635 cm\(^{-1}\), it appeared that cellulose and xylan (hemicellulose) were present in the pseudostem biomass (Fig. 1C), which was supported by biochemical estimation of cellulose (36.40%) and hemicellulose (33.30%). The weak absorption at around 1260 cm\(^{-1}\) might be taken to indicate the presence of comparatively less amount of lignin in the pseudostem. Biochemical estimation showed 16.36% lignin was present in pseudostem. In pseudostem biomass, degraded by two Pleurotus species (P. sajor-caju and P. ostreatus) (Fig. 1C), there was change in the pattern of absorption in the range 1000-1200 cm\(^{-1}\). No absorption was observed around 1260 cm\(^{-1}\) and only one peak was observed at around 1640 cm\(^{-1}\) in the region 1600-1640 cm\(^{-1}\). All these changes led to conclusion that cellulose, xylan and lignin were utilized in degradation process and cellulase, xylanases and laccase activities of P. sajor-caju and P. ostreatus in degraded pseudostem biomass further confirmed the same (Table 2). The absence of absorption at 1510 and 1260 cm\(^{-1}\) probably indicated maximum utilization of lignin during degradation.

An overall reduction in carbon and hydrogen contents was observed (Table 1) in both the substrates after degradation by two Pleurotus species supporting utilization of cellulose, xylan and lignin as carbon sources during process of degradation. Loss of these components in the form of carbon dioxide produced during fermentation has been reported\(^{16,17}\).

In mushroom cultivation, lignin content of the substrate regulates the activity of laccase\(^{16,17}\), and it changes during morphogenesis of fruiting bodies. So, lignin content in the substrate may be used as morphogenetic landmark and its decline indicates maximum vegetative growth on substrate\(^{16}\).
Present study can be useful to monitor degradation in SSF of banana waste by *Pleurotus* species, which can be utilized as an aid in mushroom cultivation on banana substrate.

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**References**