Chemical and Biochemical Degradation of Waste Cellulosic Materials

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Some of the agricultural wastes such as, pith bagasse, rice husk and corn maize are subjected to both chemical and biochemical degradation. Different acid concentrations as well as cellulase-producing microorganisms are tested. Comparative studies between the chemical hydrolysis and enzymatic degradation, for the selected cellulosic materials, are discussed. Cellulase(s) enzyme activity, 50.25 µg/mL is obtained (pH 5.5 using 2g/L carbon source) in shaking culture. Results indicate that the optimum conc. of HCl, which gives the highest yield of reducing sugars from the used cellulose materials is 5 per cent. Byproducts formed by acid degradation and enzymatic hydrolysis reveal that the residue of chemical hydrolysis is the most appropriate substrate for enzyme degradation rather than the raw materials. Screening of cellulase producing-organisms in the soil, show that actinomycetes and fungi are predominant. The most efficient fungal isolate is identified as Aspergillus ochraceus. Occurrence of free reducing sugars in the growth medium as well as prolonged incubation times, both drastically affect cellulase(s) yield. Reduction in sugar content and cellulose remaining are inversely proportional.

Introduction

The hydrolysis of cellulose and lignocellulosic materials can be carried out using chemical and/or biochemical methods. The cellulose molecules are hydrolyzed under acidic conditions with rapid depolymerization in strong mineral acids. The β-D-glycopyranosidic bonds are susceptible to hydrolysis as they are in simple gluco-ides. Hydrolysis proceeds by protonation of the glucosidic oxygen, followed by a displacement on carbon either through attack by hydroxyl ion or by simple cleavage to the protonated glucoside bond to give a carbonium ion at C1. The carbonium ion takes up a hydroxyl ion from the aqueous medium to become a reducing end of the chain fragment. Heterogeneous acid hydrolysis results in rapid reduction in the tensile strength and viscosity. This is because of preferential attacks on those cellulose chains and more accessible amorphous regions. Following this initial rapid reaction the chain length approaches a "leveling off" or "limiting" value, depending on the history of the cellulosic materials, this value is approximately 250 D-glucose units per chain.

The bioconversion of cellulose to simple soluble reducing sugars is catalyzed by group of enzymes called cellulase(s). Cellulase(s) enzymes are widely used in the biconversion of waste cellulose to compounds of economic importance such as, glucose and celllobiose. The cellulase(s) systems of fungi consist of endo-1,4-β-D-glucanase [1,4-β-D-glucan glucanohydrolase], exo-1,4-β-D-glucanase [1,4-β-D-glucan cellubiohydrolase] and β-glucosidase [cellubiose or β-glucoside, gluco-hydrolase], either separately or in a complex.

Cellulases enzymes are the most important enzymes that degrade cellulose materials are have potential applications in bioconversion of agricultural waste materials to more useful products such as, reducing sugars, single cell protein, fuels, and chemical feed stocks.

In this study, we focus our attention towards the acid hydrolysis of some agricultural wastes by using different concentrations of hydrochloric acid. The products of the acidic hydrolysis were compared with those obtained by the microbial degradation of the selected materials.
Table 1 — Chemical analysis of raw materials

<table>
<thead>
<tr>
<th>Substrate</th>
<th>α-cellulose</th>
<th>Hemicellulose per cent</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pith bagasse</td>
<td>28.60</td>
<td>24.80</td>
<td>4.12</td>
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<tr>
<td>Corn maize</td>
<td>30.55</td>
<td>24.90</td>
<td>3.90</td>
</tr>
<tr>
<td>Rice husk</td>
<td>35.34</td>
<td>26.56</td>
<td>4.56</td>
</tr>
</tbody>
</table>

Material and Methods

Cellulosic Raw Materials

The raw agricultural wastes were pith bagasse, rice husk, and corn maize which collected from Sharkia governorate, Egypt. These materials were treated, physically by grinding and crushing before subjecting them to chemical and biochemical treatment.

Analysis of Raw Materials

α-cellulose, lignin and pentosan estimation for these raw cellulosic materials was determined according standard methods. Table 1 show the chemical analysis of raw materials.

Microorganisms

Soil samples collected from different locations in Sharkia Governorate were used for screening of cellulases producing microorganisms. The growth medium employed was amended by cellulose as sole carbon source. Microbial colonies showed clear zone on the agar plates and were considered as cellulase producers. Selected individuals of such organisms were picked up and purified by frequent streaking on the same medium. The pure isolates were insured microscopically and the pure organisms were stored on agar slants at 4°C for further studies.

Chemical Hydrolysis

The hydrolysis process was carried out using different concentrations of HCl (being 3.5 and 7 per cent) with liquor ratio 1:25 solid to liquid at 100°C for inter-

val extended up to (15 up to 120) min. The treated cellulosic fibres were filtered, neutralised by washing with dilute aqueous sodium hydroxide, before washing with water until the filtrate had a pH 7 which considered to be suitable for enzymatic hydrolysis. The resulting products of chemical hydrolysis were treated enzymatically by *Aspergillus ochraceous* in fermentation medium the reducing sugars were estimated by Somogyi method.

Fermentation Medium

Modified Czapek’s cellulose medium used for the growth and production of cellulase(s) enzymes has the following composition (g/L): cellulose, 10.0; NaNO₃, 3.0; K₂HPO₄, 1.0; KCl, 0.5; MgSO₄·7H₂O, 0.5; FeSO₄·5H₂O, 0.01 and distilled water 1000 mL. Pith bagasse, rice husk, and corn maize used as waste cellulosic material were replaced by cellulose. Aliquots (49 mL) of the medium were autoclaved in 250 mL Erlenmeyer flasks. Inoculated cultures were incubated at 30°C for 10 d either in static state or shaked at 150 rpm in an incubator shaker. At the end of the incubation period the whole cultures were centrifuged the supernatants were decanted and filtered through seitz filter for determination of reducing sugars in the filtrate.

Enzyme Assay

In the cell, free filtrate of each culture the extracellular cellulase activity was determined as follows:

One mL of the cell free filtrate was incubated with 9 mL of 1 per cent carboxy methylcellulose in 55 mM citrate buffer (pH 5.0) for 30 min at 40°C. At the end of the reaction time the reducing sugar liberated was determined.

One unit of cellulase enzyme is defined as the amount of enzyme that liberates 1 μg of glucose from carboxy methyl-cellulose/1 mL of total solution.

Enzyme Solubilization

The saccharification experiments were carried out in 50 mL flasks containing 8.0 mL citrate buffer (pH 5.0); a certain amount of cellulose substrates or cellulose derivatives were added to 1.0 mL of enzyme solution. The flasks were incubated at 40°C on rotatory shaker at 150 rpm for different hydrolysis time 1, 3, 6, 24 and 48h. Samples were centrifuged to remove solids. The supernatant obtained is used for analysis determination of liberated reducing sugars.
The degree of enzymatic solubilization was calculated according to the following formula:

\[
\text{Solubilization per cent} = \frac{\text{Amount of reducing sugars produced (mg/mL)}}{\text{Amount of substrate (mg/mL)}} \times 0.9 \times 100,
\]

where 0.9: means polysaccharide : monomer molecular weight ratio value.

**Protein Estimation**

Protein was determined colorimetrically by Folin-ciocalteu reagent (BDH) at 750 nm.

**Results and Discussion**

**Acidic Hydrolysis**

The three agricultural wastes were subjected to acid degradation using different conc. of HCl (3 per cent, 5 per cent, and 7 per cent) for different intervals in liquor ratio 1 : 25 at 100°C. The decomposition process was expressed in terms of glucose content, liberated per cent age of both remaining cellulose and conversion rates as shown in Table 2.

It is evident from the Table 2 that 5 per cent HCl gives the highest degradation rate of all the treated raw materials. The highest glucose figures, conversion per cent age, and lowest cellulose remaining (per cent) were observed at this concentration.

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Concentration of HCl (per cent)</th>
<th>Reducing sugar (µg/mL)</th>
<th>Cellulose remaining (per cent)</th>
<th>Conversion (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pith bagasse</td>
<td>3</td>
<td>58.10</td>
<td>60.59</td>
<td>14.52</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>145.25</td>
<td>20.56</td>
<td>36.31</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Corn maize</td>
<td>3</td>
<td>62.79</td>
<td>65.25</td>
<td>15.69</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>128.37</td>
<td>32.77</td>
<td>32.09</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>105.45</td>
<td>42.63</td>
<td>26.36</td>
</tr>
<tr>
<td>Rice husk</td>
<td>3</td>
<td>82.50</td>
<td>71.75</td>
<td>20.62</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>206.25</td>
<td>30.42</td>
<td>51.56</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>145.25</td>
<td>44.25</td>
<td>36.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Hydrolysis time (min)</th>
<th>Reducing sugar (µg/mL)</th>
<th>Cellulose remaining (per cent)</th>
<th>Conversion (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pith bagasse</td>
<td>15</td>
<td>145.25</td>
<td>20.56</td>
<td>36.31</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>139.50</td>
<td>24.24</td>
<td>34.87</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>136.50</td>
<td>28.97</td>
<td>34.12</td>
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<td></td>
<td>90</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Corn maize</td>
<td>15</td>
<td>124.50</td>
<td>38.98</td>
<td>31.12</td>
</tr>
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<td></td>
<td>30</td>
<td>128.37</td>
<td>32.77</td>
<td>32.09</td>
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<td></td>
<td>60</td>
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<td></td>
<td>90</td>
<td>92.50</td>
<td>50.00</td>
<td>23.12</td>
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<td></td>
<td>120</td>
<td>62.50</td>
<td>66.64</td>
<td>15.62</td>
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<tr>
<td>Rice husk</td>
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<td>118.12</td>
<td>53.09</td>
<td>29.53</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>162.50</td>
<td>40.82</td>
<td>40.62</td>
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<td></td>
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<td>90</td>
<td>206.25</td>
<td>30.42</td>
<td>51.56</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>199.75</td>
<td>31.93</td>
<td>49.93</td>
</tr>
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</table>
Increasing acid concentrations over 5 per cent, showed greater loss in the liberated reducing sugar as well as in remaining glucose and consequently in the conversion rate. In a preliminary experiment, the optimum hydrolysis time was found to be 15 min. The physico-chemical nature of the experimented raw material has also its effect on the degradation performance, depending on acid concentration. Table 2 illustrates complete combustion of pith bagasse at 7 per cent, this could be due to the lower α-cellulose and higher hemicellulose content of this raw material. It is evident from Table 2 that corn maize and rice husk are less susceptible to acid hydrolysis compared to pith bagasse.

Effect of Hydrolysis Time

In the subsequent experiments the hydrolysis was carried out using 5 per cent HCl for different times, with liquor ratio 1.25 solid to liquid at 100°C.

It is evident from Table 3 that the maximum decomposition rate of pith bagasse was noticed during hydrolysis 15 min, in which the highest glucose amount (reducing sugars), conversion percentage and lowest cellulose remaining per cent were observed. By increasing the time the hydrolysis process was impaired. The attacks of the acid to the pith fibres at short intervals may be due to the physical-chemical and mechanical nature of the pith bagasse fibres which were characterized by weaker fibre bonds compared with other cellulosic materials. Increase in hydrolysis time of pith bagasse to 60 min showed loss in cellulose content by complete combustion of the cellulosic fibre.

Corn maize as another agricultural waste is the dry corn itself, after the crushing and grinding. It contains (30.55 per cent) α-cellulose, (24.90 per cent) hemicellulose, and (3.90 per cent) lignin content.

It is evident from Table 3 that the rigidity state of the corn maize particles needs about 30 min in order to decompose since the largest amount of monosaccharides (128.37 μg/mL), have been recorded after that the amount of glucose tends to disappear.

Rice husk which represent the external fibres of rice grain, contains large amount of silica and lignin, and a suitable amount of α-cellulase (35.34 per cent). The effect of hydrolysis time on the decomposition of rice husk by 5 per cent HCl (Table 3) showed that rice husk needs 90 min to attain maximum rate of decomposition to produce reducing sugars (206.25 μg/mL). The prolonged hydrolysis time compared to pith bagasse and corn maize, may be due to the physico-chemical nature of the rice husk.

Table 4—Assay of cellulase activity of different isolates in a shaking cultures

<table>
<thead>
<tr>
<th>Organism No.</th>
<th>Actinomyces species</th>
<th>Concentration, μg/mL</th>
<th>Reducing sugars</th>
<th>Cellulase activity</th>
<th>Soluble protein</th>
<th>Specific activity</th>
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<tr>
<td>1</td>
<td>16.75</td>
<td>14.62</td>
<td>20.56</td>
<td>0.71</td>
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<td>2</td>
<td>13.56</td>
<td>15.23</td>
<td>24.56</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29.06</td>
<td>24.68</td>
<td>23.89</td>
<td>1.03</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>25.06</td>
<td>22.43</td>
<td>25.22</td>
<td>0.88</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>08.60</td>
<td>07.67</td>
<td>14.66</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>19.87</td>
<td>14.38</td>
<td>19.12</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>22.62</td>
<td>19.93</td>
<td>24.06</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>22.31</td>
<td>15.03</td>
<td>24.66</td>
<td>0.62</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>20.18</td>
<td>17.71</td>
<td>24.13</td>
<td>0.73</td>
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<tr>
<td>10</td>
<td>21.93</td>
<td>17.23</td>
<td>24.50</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi species</td>
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<tr>
<td>11</td>
<td>57.50</td>
<td>31.56</td>
<td>24.06</td>
<td>1.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>60.18</td>
<td>50.25</td>
<td>22.98</td>
<td>2.18</td>
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<td>13</td>
<td>50.12</td>
<td>38.06</td>
<td>23.87</td>
<td>1.59</td>
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<td></td>
</tr>
<tr>
<td>14</td>
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<td>45.51</td>
<td>23.55</td>
<td>1.93</td>
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<tr>
<td>15</td>
<td>40.87</td>
<td>29.70</td>
<td>25.43</td>
<td>1.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>25.75</td>
<td>21.54</td>
<td>26.60</td>
<td>0.80</td>
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</tr>
<tr>
<td>17</td>
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<td>14.04</td>
<td>27.12</td>
<td>0.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Note: Specific activity = Cellulase activity μg/mL.
Application of the optimum acid concentration (5 per cent) and increase hydrolysis time illustrated complete combustion of pith bagasse as there was no reducing sugars or remaining cellulose could be removed after hydrolysing for 90-120 min. However in the case of corn maize and rice husk prolonged hydrolysis for 120 min in the presence of 5 per cent HCl, reducing sugars and remaining cellulose residues were still present. These results can be explained on the basis of nature and chemical composition of the treated materials.

Isolation and Screening of Cellulase-producing Microorganisms

In the screening for cellulose(s) producing micro-organism it was found that, actinomycetes and fungus are the most dominant organisms in this respect. On the basis of the production efficiency, 12 isolates from each were selected as shown in Table 4 and 5. There are great variations between the selected organisms regarding cellulase(s), reducing sugars and conversion rates either in shaking or static cultures. Under the two condition of growth the most active actinomycetes isolate was strain # 3 while for fungi it was isolate # 12. Regarding the growth conditions and their effect on cellulase production, it is evident from Tables (4 and 5) that the production capacity of both actinomycetes and fungi has been greatly enhanced under shaking compared to static condition. Among all the tested isolates the highly efficient cellulose(s) producer (fungal isolate # 12) has been identified as Aspergillus ochraceous. The degradation of waste cellulosic material by cellulase-producing microorganism compared with chemical hydrolysis by 5 per cent HCl.

Enzymatic Hydrolysis

In this study, degradation byproducts of the waste cellulosic materials (pith bagasse, rice husk, and corn maize) were evaluated by acid hydrolysis and enzymatic hydrolysis.

Residue left after filtration may be used as substrates for fermentation which incubated at 30 °C for 10 d with Aspergillus ochraceus in a shaking incubator at 150 rpm.

The results given in Table 6 show that the highest yields of cellulase enzyme are obtained on residue of treated pith bagasse (40.00 µg/mL) than other substrates either filtrate (18.62 µg/mL) or raw pith bagasse without acid treatment (25.00 µg/mL). These results are in agreement with that obtained by Enari et al16, who de-
Table 6 — Cellulase degradation of acid and non-acid treated pith bagasse, corn maize and rice husk with *Aspergillus ochraceous*

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Acid treatment byproduct</th>
<th>Concentration, µg/mL</th>
<th>Specific activity</th>
<th>Conversion activity per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reducing sugar</td>
<td>Cellulase activity</td>
<td>Soluble protein</td>
<td></td>
</tr>
<tr>
<td>Pith bagasse</td>
<td>Filtrate</td>
<td>95.75</td>
<td>18.62</td>
<td>36.90</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>56.87</td>
<td>40.00</td>
<td>49.16</td>
</tr>
<tr>
<td></td>
<td>Raw material</td>
<td>27.37</td>
<td>25.00</td>
<td>24.90</td>
</tr>
<tr>
<td>Corn maize</td>
<td>Filtrate</td>
<td>86.00</td>
<td>19.25</td>
<td>36.00</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>58.25</td>
<td>41.37</td>
<td>48.38</td>
</tr>
<tr>
<td></td>
<td>Raw material</td>
<td>25.62</td>
<td>25.75</td>
<td>24.51</td>
</tr>
<tr>
<td>Rice husk</td>
<td>Filtrate</td>
<td>106.87</td>
<td>24.50</td>
<td>30.06</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>67.62</td>
<td>47.50</td>
<td>42.32</td>
</tr>
<tr>
<td></td>
<td>Raw material</td>
<td>36.00</td>
<td>27.25</td>
<td>24.90</td>
</tr>
</tbody>
</table>

Produced that monosaccharides are not inducers of cellulase production and as an end product it may behave as suppressor for this enzyme. The yield of soluble protein produced in residue of pith bagasse acid hydrolyzed is higher compare to filtrate liquor (36.90 µg/mL) and raw pith bagasse without acid hydrolysis (24.90 µg/mL).

From Table 6, it is evident that the highest yield of crude enzyme in treated rice husk as (47.50 µg/mL) compared to filtrate liquor 24.50 µg/mL and untreated rice husk 27.25 µg/mL.

In Table 6 the crude enzyme yield was found to be 41.37, 19.25 and 25.75 µg/mL for acid hydrolysed corn maize residue, filtrate liquor and raw material respectively, and the per cent age of conversion reached 58.25 per cent and 25.62 per cent respectively, in treated residue and raw material (non-treated). Pretreatment before enzymatic hydrolysis increases the surface area of the cellulose by reducing particle size, opening up pores by hydrolysis.

**Solubilization of Waste Cellulosic Materials Using Cellulase(s) Enzymes Produced by Aspergillus ochraceous**

The results in Table 7 show that the highest yields of enzyme activity were obtained after 1 h (16.32 µg/mL). By increasing the solubilization time the cellulase activity expressed as reducing sugar syrup in medium was reduced and this may be due to the physical nature of granules of pith bagasse.

Table 7 — Pith bagasse solubilization using cellulase enzyme produced by *Aspergillus ochraceous* at different solubilization times

<table>
<thead>
<tr>
<th>Solubilization time h</th>
<th>Concentration, µg/mL</th>
<th>Solubilization, per cent</th>
<th>Conversion, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reducing sugars</td>
<td>Cellulase activity</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25.01</td>
<td>16.32</td>
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</tr>
<tr>
<td>3</td>
<td>23.70</td>
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<td>22.36</td>
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</tr>
<tr>
<td>48</td>
<td>22.36</td>
<td>13.53</td>
<td></td>
</tr>
</tbody>
</table>

Table 8 show that increase in solubilization time is associated with increase in cellulase activity reducing sugars content up to 48 h.

In Table 9 the increase in the reaction time between cellulase enzyme and rice husk show increase in rate of reducing sugars produced, cellulase activity and solubilization and conversion per cent ages in 24 h. Time expansion after that showed insignificant increase.

**References**

Table 8 — Corn maize solubilization by using cellulase enzyme produced by Aspergillus ochraceus at different solubilization time

<table>
<thead>
<tr>
<th>Solubilization</th>
<th>Reducing sugars</th>
<th>Cellulase activity</th>
<th>Solubilization, per cent</th>
<th>Conversion, per cent</th>
</tr>
</thead>
<tbody>
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<td>6.35</td>
<td>7.05</td>
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<td>6.13</td>
<td>7.31</td>
<td>8.12</td>
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<td>18.31</td>
<td>9.55</td>
<td>8.23</td>
<td>9.14</td>
</tr>
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<td>18.35</td>
<td>9.60</td>
<td>10.84</td>
<td>12.04</td>
</tr>
<tr>
<td>48</td>
<td>18.35</td>
<td>9.61</td>
<td>10.85</td>
<td>12.05</td>
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</tbody>
</table>

Table 9 — Rice husk solubilization by using cellulase enzyme produced by Aspergillus ochraceus at different solubilization time

<table>
<thead>
<tr>
<th>Solubilization</th>
<th>Concentration, μg/mL</th>
<th>Reducing sugars</th>
<th>Cellulase activity</th>
<th>Solubilization, per cent</th>
<th>Conversion, per cent</th>
</tr>
</thead>
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<td>27.14</td>
<td>17.84</td>
<td>13.74</td>
<td>15.24</td>
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</tr>
</tbody>
</table>

17 Kassim E A, Cellulolytic activity of some soil fungi, J Coll Agric, King Saud University, (1983) 169-177.
18 Enari T M, Markkanen P & Korhonen E In symposium on enzymatic hydrolysis of cellulase, Aulanko, Finland, 12-14 Situa, Helsinki, (1975) 171.