Production and Partial Purification of Cellulase Complex by Aspergillus niger and A. nidulans Grown on Water Hyacinth Blend

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Abstract: The production of cellulases' by two local fungal isolates; Aspergillus niger and A. nidulans when grown on water hyacinth blend fortified with Czapek-Dox medium (4 : 1), was studied and was found to reach its maximum activity at 35 °C, pH 7.0, sodium nitrate as nitrogen source and 7 & 3 days under static and shaked condition respectively for A. niger and at 30 °C, pH 7.0, sodium nitrate as nitrogen source and 7 & 4 days under static and shaked condition respectively for A. nidulans. β-glucosidase was purified using ammonium sulphate precipitation (70%), Sephadex G100 and DEAE cellulose chromatography to 3.75 folds for Aspergillus niger and 8.0 folds for Aspergillus nidulans. Carboxy methyl cellulase was purified using Sephadex G100 and DEAE cellulose chromatography to 18.48 folds for Aspergillus nidulans and 17.78 folds for Aspergillus niger. Avicelase was purified using Sephadex G100 and DEAE cellulose chromatography to 16.6 folds for Aspergillus niger and 19.88 folds for Aspergillus nidulans.

Key words: Production, Cellulases', Aspergilli, Purification, water hyacinth

INTRODUCTION

Lignocellulosic wastes are considered to be the cheapest source for the production of different utilisable products throughout the world[12,11]. In Egypt the river Nile suffers from the huge amounts of water hyacinth (Eichhornia crassipes (Mart.) Solms) spread over large surface areas. This water plant comprises considerable amount of α-cellulose (~ 60%) Abd-El-Naby[11]. It was thought to be used as a cheap source of cellulose for the production of cellulases' by local fungal isolates. The term “lignocellulose” implies such materials are comprised, in the main, of cellulose, hemicelullose and lignin. Biological conversion of these major polymers to simpler constituents is preferred over chemical conversion. Cellulase complex is used for commercial food processing in coffee. It performs hydrolysis of cellulose during drying of beans. It has also been used in the pulp and paper industry for various purposes, and they are even used for pharmaceutical applications. Cellulase is used in the fermentation of biomass into bio-fuels, although this process is relatively experimental at present. Cellulase is used as a treatment for Phytobezoars, a form of cellulose bezoar found in the human stomach. Cellulase enzymes are widely used for textile and detergent applications to improve the properties of cellulose-based textiles. The improved colour brightness and depilling effects are the most successful applications of these enzymes[8,14]. The benefits of the effects delivered by cellulases' are the result of controlled action of the enzymes on the cellulose fibers. Aspergilli have been reported as potential cellulase producers[12,13,14,1].

MATERIALS AND METHODS

Organisms: Aspergillus niger (van Tieghem) and A. nidulans (Eidam) Winter were isolated as wild types from heaps of Eichhornia crassipes on the banks of water ponds located at the rural areas around Cairo, Egypt. The fungi were isolated on Czapek – Dox Agar supplemented with 5 g L⁻¹ yeast extract (CDYA). Pure cultures were isolated, identified according to Moubasher[17] and maintained at 5°C on CDYA slants.

Substrate: Water hyacinth (Eichhornia crassipes (Mart.) Solms) plants were gathered from local ponds, washed and a known weight of fresh leaves were blended by a mixer with equal volumes of water. The whole blend was used as a carbon source throughout.

Cultivation of the Fungi and Crude Enzyme Preparation: The basal medium (without carbon source) for cellulase production had the following composition (g L⁻¹): NaNO₃, 2.0; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.5 and (mg L⁻¹) FeSO₄, 10.0. The fungi were grown in 250 ml Erlenmeyer flasks containing 50 ml of the basal medium supplemented with different

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Enzyme Assay: Cellulase activities were determined by estimation of the reducing sugar liberated by the action of endoglucanase (CMC-ase) on carboxymethyl cellulose (CMC), exoglucanase or avicellase on crystalline cellulose (avicol) and β-glucosidase acting on celllobiose according to the method adopted from Reese and Mandel[10].

One unit of the enzyme was defined as one micromole of glucose equivalent released per minute under the assay conditions.

Estimation of Glucose: Glucose was measured as glucose units liberated from the cellulosic substrate in the reaction mixture using glucose oxidase / peroxidase reagent[13]. This reagent was supplied as an enzyme kit from bio-Merieux Co. (France). The reaction mixture (1.0 ml) consists of 0.1 ml enzyme solution, 0.5 ml reagent and 0.4 ml of citrate phosphate buffer (CP, pH 5.0) containing 1% of the appropriate substrate i.e. CMC for endoglucanase, avicel for avicellase and celllobiose for β-glucosidase, respectively. The reaction mixture was left to stand at room temperature for 15 min. and the optical density was measured at 505 nm using a Karl Zeiss Spectrocolorimeter (Spaekol-K11).

Estimation of Protein: Protein was estimated by UV absorption at 280 nm[13] using bovine serum albumin as standard.

Purification of Cellulases: Cellulases’ were purified from the fungal filtrates after concentration by freeze drying and storing at 4°C. The enzyme protein was bulk precipitated by (NH₄)₂SO₄ (70%) and dissolved in a minimum volume (100 ml) of 0.1 M citrate phosphate buffer (pH 5.0). The enzyme was dialyzed against the same buffer for 24 h at 4°C. 50 ml of each enzyme protein was loaded onto a column chromogram of Sephadex G₁₀₀ (18 x 2 cm) pre-equilibrated with 50 ml buffer. Each column was eluted with the same buffer at 20 ml h⁻¹ and 5 ml fractions were collected. Fractions were analyzed for protein and activity of β-glucosidase, endoglucanase and exoglucanase. The most active fractions were pooled, concentrated by freeze-drying and dialyzed as before. The pooled fractions were loaded separately (25 ml) onto column chromatograms of DEAE-cellulose (Diethylaminoethyl-cellulose). Each column was eluted with gradient of 0 - 0.8 M NaCl at a flow rate of 10 ml/h⁻¹ and 5 ml fractions were collected and dialyzed once again to remove Na⁺ and Cl⁻. This enzyme was lyophilized and stored at 0°C.

RESULTS AND DISCUSSION

Results:

Effect of Different Ratios of Hyacinth Blend / Synthetic Medium (HBM) on the Production of Cellulases’ by Aspergillus niger and A. nidulans: Mixtures of synthetic medium with hyacinth blend with different ratios (1:1, 1:2, 1:3, 1:4 & 1:5) were prepared, inoculated with spore suspension (10⁶ spores ml⁻¹) of the experimental fungi and incubated at 30°C for 7 days then the cellulolytic activities were determined. The results Fig. (1) show that the best ratio was 1:4 for the three cellulolytic enzymes. So this ratio was used as a control for the subsequent experiments.

Effect of Different Incubation Temperature on the Production of Cellulases’ by Aspergillus niger and A. nidulans Grown on 1: 4 HBM: Flasks containing 1:4 ratio of synthetic medium to hyacinth blend, inoculated with the experimental fungi were incubated at temperatures 25, 30, 35, 40 and 45°C for seven days then the filtrates were assayed for cellulases’ the results were represented in Fig. (2), which shows that the optimum incubation temperature for the three cellulases’ was 35°C for A. niger and 30°C for A. nidulans.

Effect of Different Nitrogen Sources on the Production of Cellulases’ by Aspergillus niger and A. nidulans Grown on 1: 4 HBM: Equimolecular amounts of three nitrogen sources namely; sodium nitrate, ammonium nitrate and peptone were investigated as to affect the production of cellulases’ by the two experimental fungi using the best conditions of incubation. The results obtained were presented in Fig. (3) show that sodium nitrate was the best nitrogen for the cellulolytic activities of both the experimental fungi.

Effect of Ph Value on the Production of Cellulases’ by Aspergillus niger and A. nidulans Grown on 1: 4 HBM: The effect of pH value on the production of cellulases’ was studied using best conditions of incubation. Citrate phosphate buffer (0.1 M) was used for adjustment of pH of HBM from pH 3-8. The results Fig. (4) show that the best pH value for the production of cellulases’ was 7.0 for both of the experimental fungi.
Effect of different concentrations of hyacinth blend / synthetic medium on the production of cellulases by *Aspergillus niger* and *A. nidulans*.

**Fig. 1:** Effect of synthetic medium / hyacinth blend ratio on the cellulases' activities of *Aspergillus niger* and *A. nidulans*. 
Effect of incubation temperature on the production of cellulases' by *Aspergillus niger* and *A. nidulans*.

Fig. 2: Effect of different incubation temperatures on cellulases' activities of *Aspergillus niger* and *A. nidulans*. 
Effect of different nitrogen sources on the production of cellulases' by *Aspergillus niger* and *A. nidulans*.

**Fig. 3:** Effect of different nitrogen sources on cellulases' activities of *Aspergillus niger* and *A. nidulans.*
Effect of different pH values on the production of cellulases' by *Aspergillus niger* and *A. nidulans*.

**Fig. 4:** Effect of different pH values on cellulases' activities of *Aspergillus niger* and *A. nidulans*.
Effect of different static incubation period on the production of cellulases' by Aspergillus niger

Fig. 5: Effect of different incubation periods on cellulases' activities of Aspergillus niger and A. nidulans under static conditions.

Effect of different static incubation period on the production of cellulases' by Aspergillus nidulans
Effect of different shaked incubation period on the production of cellulases by Aspergillus niger and A. nidulans under shaking conditions.

Fig. 6: Effect of different incubation periods on cellulases' activities of Aspergillus niger and A. nidulans under shaking conditions.
Fig. 7: First purification pattern of β-glucosidase produced by *Aspergillus niger* by gel filtration through Sephadex G_{100}.

Fig. 8: Second purification pattern of β-glucosidase produced by *Aspergillus niger* by ion exchange through DEAE-Cellulose.

Effect of Static Incubation on the Production of Cellulases by *Aspergillus niger* and *A. nidulans* Grown on 1:4 HBM: Triplicate flasks were inoculated with each of the experimental fungi using the best conditions of incubation and incubated statically for different incubation periods (5 – 11 days) so as study the effect of static incubation on the cellulolytic activities of the experimental fungi. The results in Fig. (5) show that seven days of static incubation was the best for the cellulolytic activities of both of the experimental fungi.
### Table 1: A summary of steps used in the purification of β-glucosidase produced by *Aspergillus niger*.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-Free Filtrate</td>
<td>100</td>
<td>2.791</td>
<td>116.01</td>
<td>41.57</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate ppt (70%)</td>
<td>100</td>
<td>2.077</td>
<td>91.96</td>
<td>44.28</td>
<td>79.269</td>
<td>1.065</td>
</tr>
<tr>
<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.747</td>
<td>109.03</td>
<td>39.69</td>
<td>93.983</td>
<td>0.954</td>
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<td>1st Purification on Sephadex G50</td>
<td>50</td>
<td>0.986</td>
<td>77.21</td>
<td>78.30</td>
<td>133.108</td>
<td>3.767</td>
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<tr>
<td>2nd Purification on DEAE-cellulose</td>
<td>25</td>
<td>1.353</td>
<td>52.77</td>
<td>39.0</td>
<td>181.948</td>
<td>3.752</td>
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### Table 2: A summary of steps used in the purification of β-glucosidase produced by *Aspergillus nidulans*.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
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<td>100</td>
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<tr>
<td>Ammonium sulphate ppt (70%)</td>
<td>100</td>
<td>1.404</td>
<td>117.95</td>
<td>84.0</td>
<td>76</td>
<td>1.29</td>
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<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.336</td>
<td>140.07</td>
<td>59.96</td>
<td>90</td>
<td>0.92</td>
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<tr>
<td>1st Purification on Sephadex G50</td>
<td>50</td>
<td>0.704</td>
<td>107.07</td>
<td>152.08</td>
<td>68</td>
<td>4.66</td>
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<td>2nd Purification on DEAE-cellulose</td>
<td>25</td>
<td>0.546</td>
<td>71.39</td>
<td>130.75</td>
<td>46</td>
<td>8.0</td>
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### Table 3: A summary of steps used in the purification of carboxy-methyl cellulase by *Aspergillus niger*.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
</tr>
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<tbody>
<tr>
<td>Cell-Free Filtrate</td>
<td>100</td>
<td>2.791</td>
<td>33.37</td>
<td>11.96</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate ppt (70%)</td>
<td>100</td>
<td>2.077</td>
<td>26.38</td>
<td>12.70</td>
<td>79</td>
<td>1.06</td>
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<tr>
<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.747</td>
<td>28.71</td>
<td>10.45</td>
<td>86</td>
<td>0.87</td>
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<td>1st Purification on Sephadex G50</td>
<td>50</td>
<td>0.598</td>
<td>42.83</td>
<td>41.52</td>
<td>128</td>
<td>6.94</td>
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<tr>
<td>2nd Purification on DEAE-cellulose</td>
<td>25</td>
<td>0.323</td>
<td>17.86</td>
<td>55.29</td>
<td>53</td>
<td>18.48</td>
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### Table 4: A summary of steps used in the purification of carboxy methyl cellulase produced by *Aspergillus nidulans*.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
</tr>
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<tbody>
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<td>Cell-Free Filtrate</td>
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<td>2.39</td>
<td>35.31</td>
<td>14.77</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate ppt (70%)</td>
<td>100</td>
<td>1.404</td>
<td>26.77</td>
<td>19.07</td>
<td>76</td>
<td>0.92</td>
</tr>
<tr>
<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.336</td>
<td>31.82</td>
<td>13.62</td>
<td>89</td>
<td>1.33</td>
</tr>
<tr>
<td>1st Purification on Sephadex G50</td>
<td>50</td>
<td>0.428</td>
<td>30.26</td>
<td>70.70</td>
<td>85</td>
<td>9.57</td>
</tr>
<tr>
<td>2nd Purification on DEAE-cellulose</td>
<td>25</td>
<td>0.325</td>
<td>21.34</td>
<td>65.66</td>
<td>60</td>
<td>17.78</td>
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</table>

### Table 5: A summary of steps used in the purification of avicellase produced by *Aspergillus niger*.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
</tr>
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<td>Cell-Free Filtrate</td>
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<td>2.791</td>
<td>28.32</td>
<td>10.15</td>
<td>100</td>
<td>1.0</td>
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<tr>
<td>Ammonium sulphate ppt (70%)</td>
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<td>2.077</td>
<td>20.95</td>
<td>10.08</td>
<td>74</td>
<td>0.99</td>
</tr>
<tr>
<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.747</td>
<td>23.67</td>
<td>8.61</td>
<td>84</td>
<td>0.84</td>
</tr>
<tr>
<td>1st Purification on Sephadex G50</td>
<td>50</td>
<td>0.629</td>
<td>21.72</td>
<td>34.53</td>
<td>76</td>
<td>6.8</td>
</tr>
<tr>
<td>2nd Purification on DEAE-cellulose</td>
<td>25</td>
<td>0.432</td>
<td>18.24</td>
<td>42.22</td>
<td>64</td>
<td>16.6</td>
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Table 6: A summary of steps used in the purification of avicelase produced by Aspergillus nidulans.

<table>
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<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Protein (mg/ml)</th>
<th>Total activity (U/ml)</th>
<th>Specific activity (U/mg. Protein)</th>
<th>Recovery %</th>
<th>Purification fold</th>
</tr>
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<td>Cell-Free Filtrate</td>
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<td>2.39</td>
<td>28.23</td>
<td>11.81</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulphate ppt (70%)</td>
<td>100</td>
<td>1.404</td>
<td>22.12</td>
<td>15.75</td>
<td>78</td>
<td>1.33</td>
</tr>
<tr>
<td>Cell-Free dialysate</td>
<td>100</td>
<td>2.336</td>
<td>25.61</td>
<td>10.96</td>
<td>90</td>
<td>0.92</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Purification on Sephadex G&lt;sub&gt;100&lt;/sub&gt;</td>
<td>50</td>
<td>0.462</td>
<td>22.46</td>
<td>48.61</td>
<td>79</td>
<td>8.23</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Purification on DEAE-cellulose</td>
<td>25</td>
<td>0.271</td>
<td>15.91</td>
<td>58.71</td>
<td>56</td>
<td>19.88</td>
</tr>
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</table>

Fig. 9: First purification pattern of β-glucosidase produced by Aspergillus nidulans by gel filtration through Sephadex G<sub>100</sub>.

Fig. 10: Second purification pattern of β-glucosidase produced by Aspergillus nidulans by ion exchange through DEAE-Cellulose.
Fig. 11: First purification pattern of carboxy-methyl cellulase produced by *Aspergillus niger* by gel filtration through Sephadex G_{100}.

Effect of Shaked Incubation on the Production of Cellulases' by *Aspergillus niger* and *A. nidulans*

*Grown on 1:4 HBM*: The same previous experiment was performed using shaking conditions (150 rpm). The results in Fig. (6) show that the best incubation period in the shaked cultures was 3 days for *Aspergillus niger* and 4 days for *Aspergillus nidulans*.

Purification of β-glucosidase Produced by *A.niger* and *A. nidulans*: The results of purification steps of β-glucosidase of *Aspergillus niger* are presented in Table (1). The initial step of purification was the
precipitation of proteins with 70% ammonium sulphate, which gave 1.065 folds with the yield 79.269 % of the original activity.

The second step of purification of β-glucosidase was made by chromatography on a Sephadex G_100 column. The elution profile was shown in Figs. 7 & 8. All β-glucosidase activities were located in one peak and reached 3.767 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of β-glucosidase was located in one peak with 3.752 fold of purification.
Fig. 15: First purification pattern of avicellase produced by *Aspergillus niger* by gel filtration through Sephadex G_{100}.

Fig. 16: Second purification pattern of avicellase produced by *Aspergillus niger* by ion exchange through DEAE-Cellulose.

The results of purification steps of β-glucosidase of *Aspergillus nidulans* are presented in Table (2). The initial step of purification was the precipitation of proteins with 70% ammonium sulphate, which gave 1.404 folds with the yield 76% of the original activity (Units /ml).
The second step of purification of β-glucosidase was made by chromatography on a Sephadex G_{100} column. The elution profile was shown in Figs. 9 & 10. All β-glucosidase activities were located in two peaks and reached 4.66 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of β-glucosidase was located in one peak with 8.0 folds of purification.

**Purification of Carboxymethyl Cellulase Produced by A. niger & A. nidulans:** The results of purification steps of Carboxymethyl cellulase of Aspergillus niger are presented in Table (3). The initial step of purification was the precipitation of proteins with 70% ammonium sulphate, which gave 1.06 folds with the yield 79.0 % of the original activity.

The second step of purification of Carboxymethyl cellulase was made by chromatography on a Sephadex G_{100} column. The elution profile was shown in Figs. (11 and 12). All Carboxymethyl cellulase activities were located in one peak and reached 6.94 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of Carboxymethyl cellulase was located in one peak with 18.48 folds of purification.

The results of purification steps of Carboxymethyl cellulase of Aspergillus nidulans are presented in Table (4). The initial step of purification was the precipitation of proteins with 70% ammonium sulphate, which gave 0.92 folds with the yield 76 % of the original activity (Units /ml).

The second step of purification of Carboxymethyl cellulase was made by chromatography on a Sephadex G_{100} column. The elution profile was shown in Figs. (13 and 14). All Carboxymethyl cellulase were located in two peaks and reached 8.23 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of Carboxymethyl cellulase was located in one peak with 19.88 fold of purification.

**Purification of Avicellase produced by A. niger and A. nidulans:** The results of purification steps of Avicellase of Aspergillus niger are presented in Table (5). The initial step of purification was the precipitation of proteins with 70% ammonium sulphate, which gave 0.99 folds with the yield 74 % of the original activity.

The second step of purification of Avicellase was made by chromatography on a Sephadex G_{100} column. The elution profile was shown in Figs. (15 and 16). All Avicellase activities were located in one peak and reached 6.8 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of Avicellase was located in one peak with 16.6 fold of purification.

The results of purification steps of Avicellase of Aspergillus nidulans are presented in Table (6). The initial step of purification was the precipitation of proteins with 70% ammonium sulphate, which gave 1.33 folds with the yield 78 % of the original activity (Units /ml).

The second step of purification of Avicellase was made by chromatography on a Sephadex G_{100} column. The elution profile was shown in Figs. (17 and 18). All Avicellase activities were located in one peak and reached 8.23 folds of purification.

The third step of purification was performed by passage through an ion exchange chromatography of DEAE-cellulose. The potent activity of Avicellase was located in one peak with 19.88 fold of purification.

**Discussion:** Local isolates of A. niger & A. nidulans grown on water hyacinth blend fortified with NaO and mineral solution (HBM) produced potent cellulases'. Alternative approaches for production of low-cost cellulases' from fungi using inexpensive growth media[22,24,21,5,2,11,1]. Water hyacinth was used as substrate for production of cellulase[5,12].

The optimum temperature for cellulolytic activities of A. niger was 30 °C and 35 °C for A. nidulans. Ali et al.[5] stated that maximum yield of cellulases' from A. terreus QTC 828 was 40 °C. Temperature is a cardinal factor affecting the amount and rate of growth of an organism[9] and the increasing temperature has the general effect of increasing enzyme activity[11], but the enzyme begins to suffer thermal inactivation at higher temperatures.

Maximum yield of cellulases' from A. niger & A. nidulans grown on HBM was at pH 7.0. Ali et al.[5] reported that maximum yield of cellulase from A. terreus QTC 828 was at pH 6.0. this may be attributed to pH tolerance of our isolates. It is well known that pH of the culture medium affects the availability of certain metabolic ions and permeability of fungal cell membranes.

Sodium nitrate enhanced the cellulolytic activities of A. niger & A. nidulans. Similar results were obtained by Tweddle et al.[23] in case of Stachybotrys elegans. Inorganic source of nitrogen were reported as the more suitable cellulase production by some authors[2,14].

The maximum yield of cellulases' from A. niger was found after 7 and 3 days incubation under static
and shaked conditions respectively. While \textit{A. nidulans} produced its maximum yield of cellulases' after 7 and 4 days under static and shaked conditions respectively. Abu-Shady et al.\cite{2} found that cellulases' of \textit{Trichoderma longibrachiata} grown on corn stalks attained their maximal accumulation after 7 days of static cultivation. Reduction of the incubation period under shaking condition may be attributed to the stirring of the medium and allowing enzyme / substrate mixing as well as increasing aeration.

REFERENCES


