Biosorption of Baftkar textile effluent

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Decolourization of wastewater from a textile plant by a marine Aspergillus niger was studied. The fungus was previously isolated from Gorgan Bay in the Caspian Sea. The kinetics of decolourization was studied by varying energy sources. The best decolourization was achieved when sucrose was used as source of carbon and energy. NH₄⁺ ion was demonstrated to be the best nitrogen source. Color reduction was found to increase from 80-97% as inoculum concentration increased from 0.04-1.0g/L. A minimum inoculum of 0.2g/l is necessary to achieve decolourization. The optimal temperature for the growth of A. niger on Baftkar wastewater is found to be 30°C. 90-96% color reduction is achieved in 19-20 hr of contact of mycelium cell with the wastewater. Colour reduction in a continuous column reactor of 70% was obtained using treated mycelium (NaOH, 90°C) after 1 hr.

Keywords: Continuous column reactor, Decolourization, Marine A. niger, Textile dyes

Synthetic dyestuffs are widely used in a number of industrial processes such as textile dyeing, paper printing, and colour photography. At present over 100,000 dyes are commercially available. 111 million kg of synthetic organic dyestuffs were produced in the US in 1980. Large quantities of these compounds are released to the environment each year. Generally from hygiene point of view, dyes and chemical products in waste effluent is harmful to both aquatic and terrestrial life. Some textile dyes are carcinogenic or mutagenic to human and many synthetic dyes such as the azo dyes found in textile mill wastewater create a pollution problem and are recalcitrant to aerobic biological treatment. However, under anaerobic conditions, the azo linkage can be reduced to form aromatic amines which are colourless but which can be both toxic and carcinogenic. Very little is known about decolourization of textile effluent by marine fungi. Biological decolourization has been attributed to biodegradation and to adsorption. Azo reductase and lignin peroxidase have been implicated in biodegradation. Considerable decolourization (50-60%) of dye solutions by fungi occurs in the first 1-6 hr of and is probably primarily a result of adsorption to fungal biomass. In our laboratory a fungus (Aspergillus niger) was previously isolated that showed high adsorption capacity toward textile dyes and dyes wastewater. This paper describes the capability of this microorganism for decolourization of various dyes, which are present in Baftkar textile wastewater.

Materials and Methods

Collection and chemical characterization of effluent—The waste effluent, obtained from the Baftkar textile company (Tehran, Iran) in 10 L. Black plastic carboys stored at 5°C and used for all subsequent examination. The types of dyes and their concentration in the effluent were measured. The dye types of effluent and their concentration in the effluent are listed in Table 1. Azo, sulfur, reactive, and pigment dyes are present in addition to a complex mixture of other waste substances from the textile dyeing process.

Chemical characterization of the effluent was carried out to determine pH, biological oxygen demand (BOD), total nitrogen, using standard methods, are reported in (Table 1).

Microorganism—The fungus was isolated from Gorgan Bay in the Caspian Sea. Growth and colony
characteristics were compared with A. niger PTCC 5011. Fungus was stored on potato dextrose agar (4°C) with periodic sub-culturing.

Culture conditions — The fungus was tested for colour removal ability on medium containing: glucose 10 g; KH₂PO₄ 1 g; MgSO₄·7H₂O 0.05 g; NH₄Cl 0.22 g; in one liter of effluent, (pH 4.5-5.5)⁸. After inoculation (10⁸ spores in 100 ml medium) cultures were incubated on a rotary shaker (120-150 rpm) at 30°C for 45 hr., prior to effluent treatment. Pellets were formed which were further used as culture inoculum. The experiments in all cases performed in triplicate cultures. Pellet quantification was determined by placing a known volume (5 ml) of culture fluid in a Petri-dish and counting the number of pellet according to Assadi and Jahangiri.¹²

Optimization of decolourization — The influence of co-substrate was tested using glucose, sucrose, fructose, carboxy methyl cellulose (CMC), microcrystalline cellulose (MCC), and starch to optimize the decolourization process. Additionally, sucrose was tested at concentrations ranging from 0-15 g/L. NH₄Cl urea, yeast extract, (NH₄)₂SO₄, NaNO₃, NH₄NO₃ were tested to examine the influence of available nitrogen sources. NH₄Cl was tested at concentrations ranging from 0 to 12.5 g/L. Effect of temperature (20-40°C) and pH (3-10) on decolourization was examined using cultures with mycelium concentrations ranging from 0.04-1.0 g/L.¹³

The kinetics of decolourization was studied using the optimized medium/conditions in batch and continuous culture. Batch studies were performed in optimized media (sucrose, 5 g; KH₂PO₄, 0.5 g; and NH₄Cl 1 g) in one liter of effluent, inoculated with 1 g mycelium/L. Incubation was carried out at 30°C/24 hr at 150 rpm. Continuous culture studies were carried out in a packed column glass reactor (20 ml capacity, L/D = 17/2.5 cm) with a flow rate of 1 ml/min wastewater. From the column wastewater was constantly removed after passing through the death mycelium cell and analyzed. The column was packed with 0.1 g of dry mycelium which corresponds to 5 g of wet mycelium pellet.¹⁵

Cell fractionation — In this experiment mycelia collected by centrifugation at 800 rpm for 15 min were homogenized in distilled water (1:2 cell mass: distilled water) and sonicated (3-5 min). The samples were then centrifuged (10000 rpm, 20 min) and the absorbance of the supernatant measured. This fraction is referred to as the distilled water-fraction (fraction 2). The disrupted mycelia were then resuspended in, 1.0% triton×100 (1:2 cell mass: distilled water), resolicited and centrifuged (10000 rpm, 20 min) and absorbance measured as above (referred to as fraction 3). The remaining mycelia were then extracted with methanol (1:8 w/v) and the absorbance of the resulting supernatant measured (fraction 4). Dye recovery refers to the amount of dye recovered from fungal biomass, compared to the control dye solution incubated without cells (i.e. sum of dye in all fractions/dye in no. cell control×100)⁴.

Analytical methods — Sample was centrifuged at 1000-2000 rpm, 15 min. The degree of decolourization was quantified by measuring reduction of optical density (O.D.) at 584 nm by a Unicam 8620 uv/vis spectrophotometer.

Results

Optimization of decolourization was examined by varying carbon and energy source (Fig. 1) and concentration of sucrose (Table 2). Decolourization was achieved when glucose, starch, fructose and sucrose were the energy source (Fig. 1). When sucrose was used as energy source colour removal was substantially higher. The used sucrose was not a reagent grade (local brand name) and it was cheaper as compare to other energy sources hence we used it throughout these studies. A continuous increase in colour reduction was observed with increases in sucrose concentration to a level of 5 g/L.

Depicted results showed (Table 2) the differences at concentration above 1 g/L are marginal but since at 5 g/L of sucrose, colour removal exceeded by 0.72%,
Effect of sucrose and nitrogen concentration on decolorization in continuous column reactor (NaOH, 90°C) after 1 hr (Fig. 6).

Table 2 — Effect of sucrose and nitrogen concentration on decolorization on effluent

<table>
<thead>
<tr>
<th>Concentration (g/L)</th>
<th>Colour reduction (%) using sucrose</th>
<th>Colour reduction (%) using NH₄Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>90.20</td>
<td>86.4</td>
</tr>
<tr>
<td>0.5</td>
<td>93.60</td>
<td>89.2</td>
</tr>
<tr>
<td>1.0</td>
<td>95.48</td>
<td>88.8</td>
</tr>
<tr>
<td>2.0</td>
<td>95.77</td>
<td>88.4</td>
</tr>
<tr>
<td>5.0</td>
<td>96.20</td>
<td>87.9</td>
</tr>
<tr>
<td>10</td>
<td>96.00</td>
<td>88.7</td>
</tr>
<tr>
<td>12.5</td>
<td>96.20</td>
<td>97.3</td>
</tr>
<tr>
<td>15</td>
<td>95.23</td>
<td></td>
</tr>
</tbody>
</table>

hence, in subsequent experiments 5 g/l of sucrose was added to the effluent. (NH₄)₂SO₄ and NH₄Cl were demonstrated to be the best nitrogen source (Fig. 2). Comparing these two different nitrogen sources NH₄Cl showed slightly better results as compare to (NH₄)₂SO₄. Hence Variation concentration of NH₄Cl were used for further studies. Increase in NH₄Cl concentration resulted in higher colour removal up to 97.3% at 12.5g/l (Fig. 2, Table 2). The maximum amount of decolourization occurred at pH 7.0-8.0 (Fig. 3), although in our previous investigation using the same fungi but different textile wastewaters, higher decolourization occurred between pH 3-7. Decolourization at higher pH (8.5-10) was between 88-92% (Fig. 3).

The degree of decolourization depended on the inoculum size. The reduction in colour intensity was found to increase from 80-97.2% as inoculums concentration increased from 0.04 to 1.0 g/L (Fig. 5). A change in temperature did not affect decolouri-

Fig. 1 — Influence of carbon source on decolorization of baftkar textile wastewater by marine A. niger. The influence of 1% co-

substrate was tested using glucose, sucrose, fructose, carboxy-
methyl cellulose (CMC), microcrystalline cellulose (MCC), and
starch CMC=Carboxyl Methyl Cellulose, MCC=microcrystalline cellulose.

Fig. 2 — Influence of nitrogen source on decolorization of baftkar textile wastewater by marine. 1% of nitrogen sources such as
NH₄Cl urea, yeast extract, (NH₄)₂SO₄, NaNO₃, NH₄NO₃ were tested to examine the influence of available nitrogen sources.

Fig. 3 — Influence of pH on decolorization of textile wastewater by marine A. niger.

zation (Fig. 4). Recovery percentage in cell fractionation results (Table 3) revealed that the sonication for more than 30 sec. would effect the mycelium breakage. The color is distributed between fractions 2, 3, and 4, indicating external binding (fraction 2) plus internalization (fractions 2 and 4) (Table 4). By deionised sonicated sample would be expected to contain the intracellular compartment with 32.68% recovery and Triton-X-100 sonicated sample to contain the membrane fraction with 65.14% recovery. The results also showed that membrane and intracellular compartment play an important role in dye binding and not degradation. Furthermore the investigation showed that 1g (dry weight) of treated mycelial cells were estimated to have a removal capacity of over 90% colour reduction in the first 10 hr of contact (Fig. 5).

Colour reduction of 70% was obtained with treated mycelium in a continuous column reactor (NaOH, 90°C) after 1 hr (Fig. 6).
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Fig. 4 — Effect of temperature on decolourization of Baftkar wastewater by marine A. niger

Table 3 — Effect of inoculum size on decolourization

<table>
<thead>
<tr>
<th>Inoculum (g/l)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>80.0</td>
</tr>
<tr>
<td>0.20</td>
<td>94.1</td>
</tr>
<tr>
<td>0.40</td>
<td>97.2</td>
</tr>
<tr>
<td>0.60</td>
<td>96.6</td>
</tr>
<tr>
<td>0.80</td>
<td>97.0</td>
</tr>
<tr>
<td>1.00</td>
<td>97.2</td>
</tr>
</tbody>
</table>

Table 4 — Cellular localization of dyestuff

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-absorbed</td>
<td>2.52</td>
</tr>
<tr>
<td>DH2O-sonicated</td>
<td>32.68</td>
</tr>
<tr>
<td>Triton X100-sonicated</td>
<td>65.14</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>1.004</td>
</tr>
</tbody>
</table>

Discussion

The decolourization of the textile wastewater by microorganism depends on the fungal strains used. In this investigation rate of colour removal was studied after the tested fungi were cultured in different carbon and nitrogen sources. Among the tested carbon sources, sucrose was taken as energy source. In our previous studies the same fungus demonstrated 90.2% decolourization of textile effluent without a carbon source in 5 hr.

Surprisingly the decolourization without addition of NH4Cl is much higher than seen without urea as an energy source. This may be due to the fact, that A. niger can utilize ammonia (NH4 ion), which reflects its central role in nitrogen metabolisms as the form in which nitrogen is incorporated into organic cell components. These findings are similar to the observation summarized by Dun. Another comment on using NH4 as comparing to urea is may be due to production of urease by A. niger which degrade the urea completely. Effect of nutritional factor studied by too. Using different textile effluent, Kirby et al. reported Phanerochaete chrysosporium decolourized 3 textile dyes and 9 synthetic textile dyes in the absence of a primary carbon source. Decolourization was complete after 7 days. There was no effect of nitrogen source on decolourization.

Our observation is in contrast to the findings of Cripps et al., they showed that the decolourization of dyes with P. chrysosporium was enhanced when the microorganism was precultured in nitrogen limited medium this is may be due to strain differences. The A. niger used here is likely to be by dye adsorption. By cell fractionation method of Marie and Horn we proved that, the isolated marine A. niger does not produce a biodegradative enzyme in the process. The ability of A. niger in batch flasks showed that decolourization increased 90% within 10 hr using 1g (dry weight) of treated mycelial cell mass it was earlier shown. In continuous bed column colour reduction increased up to 70% as the function of time and death mycelium pellet.
Marie and Horn, reported that 1 kg of wet mycelia cells of *Mycrothecium verrucaria* was estimated to have a removal capacity of 4 g reactive dye molecules when saturated by repeated exposure to dye solutions in 5 hr. Presented studies indicate that the fungal biomass of *A. niger* may be employed an efficient manner to remove a mixture of textile dyes (Azo, sulfur, reactive blue, reactive yellow, pigment green and pigment yellow) from plant effluent and aqueous solutions. Generally from hygiene point of view, dyes and chemical products in waste effluent is harmful to both aquatic and terrestrial life. Some textile dyes are carcinogenic or mutagenic to human and many synthetic dyes such as the azo dyes found in textile mill wastewater create a pollution problem and are recalcitrant to aerobic biological treatment, here almost 97% of all kinds of dyes in wastewater were removed by this marine *A. niger*. We expect the remaining 3% is negligible and will not cause any harm to human beings and from hygiene point of view is safe although this claim need further future investigation.

References