Removal of some reactive dyes by untreated and pretreated *Saccharomyces cerevisiae*, an alcohol fermentation waste

E S Bireller1, P Aytar1, S Gedikli1 and A Cabuk2*

1Graduate School of Natural and Applied Science, Eskisehir Osmangazi University, Eskisehir, Turkey
2Department of Biology, Faculty of Arts and Science, Eskisehir Osmangazi University, Eskisehir, Turkey

Received 09 May 2012; revised 08 August 2012; accepted 09 August 2012

This study presents *Saccharomyces cerevisiae*, an alcohol fermentation waste, for removal of acidic dyes (Red 3:1 & Orange 13) even after chemical and physical modification. Biosorption performance was found 96.29% through untreated biomass and 97.31% through pretreated biomass with hydrogen peroxide solution for Red 3:1, whereas 93.49% through untreated biomass and 94.71% through pretreated biomass with dimethyl sulfoxide and phosphoric acid solution for Orange 13. Good results were obtained when modified biomass was used to treat dye wastewater. FTIR analyses, before and after treatment, suggest that increase in dye adsorption was due to hydrolysis of yeast. Besides, Freundlich and Langmuir adsorption models were found suitable for biosorptions of both dyestuffs.

**Keywords**: Biosorption, Dye removal, Modified biomass, *Saccharomyces cerevisiae*, Toxicity

**Introduction**

Because of toxicity to aquatic life and reducing light penetration, even in low concentrations, dye effluents have adversely affected environment and lives1-3. Among various methods for treatment of reactive dye containing wastewater, ozone, H2O2 treatment, Fenton’s oxidation may produce more toxic by-products than parent compound4. Activated carbon, which is hard to regenerate, and ion resins, which is more easily regenerated, both of these effective methods are expensive5. Thereby, low-cost, more easily regenerated biosorbents such as fermentation waste yeast biomasses have been recently investigated6. Cell wall of yeast contains functional groups including chitin, chitosan, β-1,3-D-glucans, β-1,6-D-glucans, and mannoproteins which are abundant sources of different functional groups such as carboxyl, amine and hydroxyl7. Most widely applied pretreatment techniques include freeze-drying, boiling, autoclaving, contacting with some chemicals (NaOH, H2SO4, NaHCO3, formaldehyde, acetic acid, dimethyl sulfoxide, H2O2, and CaCl2 etc.)8,9. Usage of waste beer yeast for biosorption has been reported10,11. This study presents *Saccharomyces cerevisiae* as a biosorbent to remove reactive dyes (Red 3:1 & Orange 13).

**Experimental Section**

**Dyes and Microorganism**

Reactive dyestuffs [Reactive Orange 13 (RO13) & Reactive Red 3:1 (RR3:1)] in commercial purity were kindly provided by Sarar Textile Co., Eskisehir, Turkey. Their maximum absorbance peak wavelengths were determined prior to use. *S. cerevisiae*, provided from industrial waste of Eskisehir alcohol plant, Turkey, was incubated in 1000 ml of malt broth medium (Merck) at 30°C on an orbital shaker at 150 rpm for 48 h and then dried at 60°C for 12 h before use.

**Optimization Studies**

Batch biosorption studies were conducted by using dye solution in 100 ml Erlenmeyer flasks. All experiments were performed in triplicate. Effects of initial pH (1.0-7.0) on biosorption of dyestuffs (initial conc., 25 mg/l) were examined. Solution pH was adjusted using concentrated HCl and NaOH. Effects of different amounts of dried biomass (0.5-10 g/l) on biosorption were examined using dyestuffs (initial conc., 25 mg/l; and pH, 2.0). Effects of initial concentrations (10-150 mg/l) of dyes on biosorption were tested using...
optimized biosorbent dosage and pH. Effects of agitation rate (static to 500 rpm), temperature (20-40°C) and contact time (5-1440 min) on adsorption process were also investigated. At the end of experiments, biosorbent and dye solutions were centrifuged at 4650 x g for 10 min and supernatants were analyzed to determine residual dye concentration. Absorbances were measured by using an UV/VIS spectrometer (Schimadzu-UV2550).

Toxicity Experiments
Acute toxicity was investigated by determining luminescence inhibition of Vibrio fischeri NRRL B–11177. Toxicity test was performed before and after biosorption using a Microtox® 500 analyzer (AZUR Environmental, Carlsbad, CA, USA) following basic test protocol, with measurements taken 15 min after exposure. Toxicity device and also model is equipped with a 30-well incubator block controlled by an internal incubation unit. Luminescence was recorded at 490 nm. Data were processed using Microtox Omni software. Sample concentration that produced 50% decrease in luminescence following 15-min exposure was designated as effective concentration (EC$_{50}$).

Batch Biosorption using Synthetic Textile Wastewater
Synthetic textile wastewater$^{12}$ was prepared under laboratory conditions. This composition included optimized each dye concentration and mix dye solution, which was prepared using both RR3:1 and RO13. Simulated textile wastewater was used with raw biosorbent at optimized pH (2.0).

Biosorbent Pretreatment
Using physical methods, autoclaving, and chemical methods including treatment with formaldehyde, acetic acid, dimethyl sulfoxide, hydrogen peroxide, and phosphoric acid plus dimethyl sulfoxide, biosorbent was modified. All chemicals were obtained from Merck. Wet biomass (WB, 30 g) was then pretreated in different ways as follows: i) WB was autoclaved for 15 min at 121°C, 15 psi; ii) WB was boiled for 15 min in 500 ml of 15% (v/v) formaldehyde solution; iii) WB was boiled for 15 min in 200 ml of 10% (v/v) acetic acid solution; iv) WB boiled for 15 min in 300 ml of 10% (v/v) H$_2$O$_2$ solution; v) WB boiled for 15 min in 200 ml of 50% (v/v) dimethyl sulfoxide solution; and vi) boiled for 15 min in 200 ml of 10% (v/v) o-phosphoric acid solution and 50% (v/v) dimethyl sulfoxide solution. After each pretreatment with chemicals, biomasses were washed with deionized water and then dried at 60°C for 12 h. Prepared biosorbents were studied by using optimum conditions that were found at the end of batch biosorption studies with natural biomass.

Analytical Methods
Mechanism of biosorption process was studied using zeta potential and FTIR spectroscopy. FTIR spectra of biosorbent were recorded using a Bruker Tensor 27 spectrophotometer in the region of 400-4000 cm$^{-1}$. FTIR spectra were measured on KBr pellets prepared by pressing mixtures of 1 mg dried powdered sample and 100 mg spectrometry grade KBr under vacuum.

Results and Discussion
Effects of Biosorption Parameters
Reactive dyes releasing colored dye anions in aqueous solution are characterized by azo-based chromophores combined with different types of reactive groups$^6$. Biosorption performance of raw biosorbent for 2 dyes was tested over different pH (1-7). Maximum uptake was observed when solution pH was decreased to pH 1 for RR3:1 and RO13 (Fig. 1a). However, it is costly and difficult to decrease pH of wastewater to such a low level. Therefore, pH 2 was selected for 2 dyes. Biosorption capacities of biosorbent were recorded at pH 2 as: RR3:1, 50.88; and RO13, 59.33%. Biosorption capacities decreased with increasing pH, may be due to attractive forces between dye anion molecules and dye binding sites of positively charged biosorbent surface. As pH increased, negative charge density on biomass surface enhanced, therefore biosorption performance decreased$^{13}$.

Uptake of two dyes increased with increasing initial dye concentration (Fig. 1b). Increase in driving force of concentration gradient become when initial dye concentration enhanced. Amounts of two dyes adsorbed (q) increased from 8.01 to 45.12 and from 8.34 to 66.45, respectively, as concentrations were increased from 10 to 150 mg/l. An initial dye concentration (25 mg/l) was selected for both dyes. Above this value, percentage of dye removal decreased with increase in initial dye concentration, may be owing to saturation of sorption sites on biosorbent as concentration of dye increases. According to a reported study$^{14}$, when initial dye concentrations were increased from 100 to 250 mg dm$^{-3}$, adsorption capacity of dye increased from 252.8 to 505.3 mg/g at 20°C, from 234.7 to 480.4 mg/g at 30°C and from 209.5 to 472.4 mg/g at 40°C.

Stirring rate (150 rpm) was selected for each dye (Fig. 1c). Chu & Chen tested effect of shaking rate on
biosorption of Basic Yellow 24 using dried activated sludge biomass and observed that uptake capacity of biomass increased with increasing shaking rate from 40 to 160 rpm. A boundary layer surrounding biomass particles and a decrease in its effect with increasing shaking rate have been observed\textsuperscript{15,16}. Effects of contact time on biosorption efficiency of reactive dyes indicates (Fig. 1d) that sorption of both dyes increase with increasing contact time to 120 min. Biosorption increased with increase in reaction temperature from 20 to 30°C, and then decreased with further increase in temperature (Fig. 1e). Biosorption of both dyes are relatively high when reaction temperature is 25°C.

Highest dye removal was attained for adsorbent masses of 1 g/l for RR3:1 and 0.5 g/l for RO13 (Fig. 1f). For adsorbent masses higher than these values, dye
removal remained constant or decreased. Increase in dye removal% with adsorbent mass can be attributed to increase in adsorbent surface, augmenting number of adsorption sites available for adsorption. In contrast, increase in adsorbent mass promotes a negligible decrease in the amount of dye uptake per g of adsorbent (Fig. 1f).

**Toxicity Analysis**

Acute toxicity experiments with *V. fischeri* indicated that toxicity decreased because of bacterial EC_{50} value increased after biosorption with *S. cerevisiae* (Table 1). Detoxification was achieved simultaneously after biosorption. Prigione *et al* \(^{18}\) also performed Lemna minor toxicity test before and after biosorption experiments and showed a significant reduction of toxicity after biosorption treatments, indicating that decolorization corresponds to detoxification after treatment of wastewaters \(^{18}\). Tigini *et al* \(^{19}\) carried out toxicity test with Pseudokirchneriella subcapitata before and after treatment and observed reduction in effluent toxicity.

**Treatment for Synthetic Wastewater**

Biosorption capacity of raw biomass in treating synthetic dye wastewater was studied. According to obtained results, 95.76% of RR3:1 biosorption efficiency under optimum conditions decreased to 88.63%, when synthetic dye wastewater based on RR3:1 was prepared. Also, 92.73% of RO13 biosorption performance reduced up to 78.50% with treatment of simulated wastewater based on this dye. Also, biosorption capacity of a synthetic wastewater prepared using two dyes at determined initial concentrations was found as 41.32%. A reason of decrease biosorption performance may be the complicated content of wastewater. Therefore, it contained various types of organic and inorganic compounds. However, results with this wastewater indicate that biosorbent has good performance. This biomass can be used in practical applications for removal of dyes from wastewater.

### Table 1—Toxicity measurements of dyes before and after biosorption

<table>
<thead>
<tr>
<th>Dyes</th>
<th>EC_{50} (5th min) Before biosorp.</th>
<th>EC_{50} (5th min) After biosorp.</th>
<th>EC_{50} (15th min) Before biosorp.</th>
<th>EC_{50} (15th min) After biosorp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>RR 3:1</td>
<td>5</td>
<td>55</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>RO13</td>
<td>64</td>
<td>86</td>
<td>60</td>
<td>86</td>
</tr>
</tbody>
</table>

### Table 2—Biosorption of reactive dyes after biomass pretreatment (pH, 2.0; initial dye conc., 75 mg/l; biosorbent dosage, 0.5 g/l; temp., 20°C; agitation rate, 200 rpm; time, 60 min).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RR3:1%</th>
<th>RO13%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified biomass</td>
<td>96.29</td>
<td>93.49</td>
</tr>
<tr>
<td>Autoclave treatment</td>
<td>94.49</td>
<td>90.96</td>
</tr>
<tr>
<td>Acetic acid treatment</td>
<td>40.52</td>
<td>24.78</td>
</tr>
<tr>
<td>Formaldehyde treatment</td>
<td>38.13</td>
<td>21.44</td>
</tr>
<tr>
<td>H_{2}O treatment</td>
<td>97.31</td>
<td>92.81</td>
</tr>
<tr>
<td>DMSO treatment</td>
<td>87.41</td>
<td>72.14</td>
</tr>
<tr>
<td>DMSO+phosphoric acid treatment</td>
<td>93.46</td>
<td>94.71</td>
</tr>
</tbody>
</table>

**Pretreatment of Biosorbent**

Biosorption of dyes by pretreated *S. cerevisiae* either increased or decreased depending on pretreatment method in comparison with biosorption using natural biomass. Pretreatment with H_{2}O_{2} had higher biosorption capacity in comparison to raw biomass for RR3:1 (Table 2). An increase in biosorption of this dye as a result of pretreatment could be owing to an exposure of active dye binding sites embedded in cell wall or chemical modifications of cell wall components. Acetic acid and formaldehyde pretreatment significantly reduced biosorption of all dyes even inhibited while other pretreatment methods did not change adsorption performance. Decrease in dye adsorption may result from masking of some of the cellular groups, which can participate in sorption process. Huang & Huang \(^{20}\) also suggested that when biomass was pretreated with formaldehyde, methylation of amino groups present in cell wall significantly reduced biosorption capacity.

**FTIR Analysis**

Functional groups on biosorbent surface are carboxyl, amino, hydroxyl, and phosphate groups, originated from chitin, chitosan, glucan, and phosphomannan, which are the constituent components of cell wall \(^{21}\). To confirm existence of amine, carboxyl, and phosphonate groups in *S. cerevisiae* biomass, FTIR spectrum (Figs 2 & 3) displays a number of absorption peaks, indicating complex nature of the biomass examined. For FTIR spectra of after RR3:1 biosorption, absorption peaks around 1546 and 1544, 1382 cm\(^{-1}\) are indicative of existence of amine groups, respectively, unmodified biomass, after modified biomass. Spectrum also displays absorption peaks at 3415 and 3400, 1645 and 1654, and 1228 and 1222 cm\(^{-1}\), corresponding to carboxyl groups, respectively.
Fig. 2—FT-IR spectra: a) *S. cerevisiae* biomass; b) After RR3:1 biosorption with *S. cerevisiae*; c) *S. cerevisiae* after pretreatment with H₂O₂; and d) After RR3:1 biosorption with *S. cerevisiae* pretreated by H₂O₂.
Fig. 3—FT-IR spectra: a) S. cerevisiae biomass; b) After biosorption of RO13 with S. cerevisiae; c) S. cerevisiae pretreated by dimethyl sulfoxide and phosphoric acid; and d) After RO13 biosorption with S. cerevisiae pretreated by dimethyl sulfoxide and phosphoric acid
unmodified biomass, after modified biomass. Phosphonate group shows a characteristic absorption peak around 1062 cm\(^{-1}\) (P–OH stretching). Peaks showed presence of hydroxyl, amide and phosphonate groups on biomass surface. Similar band profiles indicated also after biosorptions of other dyes (Fig. 2). Increase in adsorption capacity of modified yeast with H\(_2\)O\(_2\) (Fig. 2) may be due to hydrolysis of certain biomolecules. Appearance of a peak in the region of 1546 cm\(^{-1}\) can be seen in FTIR of modified sample. This was not present in FTIR of untreated sample. This suggests that there could be formation of a new \(-\text{COOH}\) group as a result of hydrolysis. Observation of this peak at FTIR of untreated biosorbent after dye biosorption is probably because RR3:1 has acidic character. This is consistent with findings of Pratibha et al\(^{22}\). Other peaks did not show appreciable change\(^{22}\).

Because of electrostatic repulsive force between sulfoxide group of DMSO, used for modification of biosorbent \(-\text{COO}\) and \(-\text{PO}_4^2-\) groups of biomass surface, bioadsorption would be hindered. Therefore, for RO13, unmodified biosorption capacity was higher while modified biosorbent by DMSO was lower (Fig. 3). Binding of reactive dyes to biomass of \textit{C. glutamicum} takes place via electrostatic interactions between sulfonate groups of anionic dyes and positively charged amine sites present in biomass, and that negatively charged groups (carboxyl and phosphonate) can inhibit binding of reactive dyes via repulsive interactions\(^{23}\). However, addition of phosphoric acid with DMSO as modification agent enhanced significantly dye adsorption performance. Repulsive force between modified biosorbent and negatively charged dye molecules decreased, which facilitated absorption of anionic dyes (RR3:1 & RO13). Hence, adsorption capacities of DMSO plus phosphoric acid-modified biomass samples, especially for RO13 showed a significant increase (Fig. 3).

**Biosorption Isotherms**

Equilibrium sorption isotherms are fundamental in describing interactive behavior between sorbates and sorbent and are important in design and analysis of sorption systems\(^{24-26}\). Plots of Langmuir and Freundlich (Fig. 4) shows dyestuff biosorption isotherm constants for studied dyestuffs in terms of Langmuir and Freundlich models. \(R_L\) values for RO13 (0.0716) and RR3:1 (0.0066) indicated that biosorption process is favorable. Langmuir and Freundlich models fitted well with experimental data. Therefore, surfaces of dried \textit{S. cerevisiae} cells were thought to contain homogeneous and heterogeneous biosorption patches, hence interaction between RO13, RR3:1 and biosorbent might exhibit both homogenous and heterogeneous adsorption because of higher correlation coefficients. Similar results are also reported\(^{27}\).

**Conclusions**

Biomass of \textit{S. cerevisiae}, alcohol fermentation byproduct, has a significant potential for dyes biosorption. To improve manipulation with this biosorbent, simple modifications performed include with H\(_2\)O\(_2\) or phosphoric acid and dimethyl sulfoxide that led to formation of biocomposite material, which could be used as an efficient adsorbent for removal of various dyes. Modified yeast cells can thus be a promising adsorbent, which
may be used for removal of dyes. Other types of xenobiotics (heavy metal ions) can specifically interact with this new composite material.

Acknowledgements
Authors thank O Z Yesilel, Dept of Chemistry, Eskisehir Osmangazi University for FTIR spectra of biomass. This study is partially based on M Sc thesis of one of the authors (E S Bireller).

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