Decoloration of textile dyes by alginate-immobilized 
*Trametes versicolor*

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Abstract

Alginate-immobilized *Trametes versicolor* decolorized Amaranth at similar rates in repeated batch culture when the dye was present in either (i) modified Kirk’s medium containing 0.22 g l⁻¹ ammonium tartrate, (ii) the same buffer, thiamine, trace elements and glucose concentrations as in the modified Kirk’s medium, or (iii) glucose alone at either 1, 5 or 10 g l⁻¹. With glucose alone (0.5 g l⁻¹), Amaranth, Reactive Black 5, Reactive Blue 19 and Direct Black 22 had first-order decoloration rate constants of 0.56, 0.76, 0.52, and 0.15 h⁻¹, respectively. Mixtures of these dyes were also completely decolorized. After four successive decolorations, beads were kept in storage solutions for 48 d at 6 °C. CaCl₂ (1 g l⁻¹) was the best storage solution as the beads were easier to handle and had the fastest decoloration rates after storage.

Decoloration rates were faster with lower viscosity (less than 2000 cps) alginates and with softer beads which had a lower resistance to compression. Fungal colonization of the beads resulted in higher biomass concentrations with a corresponding higher decoloration rate but the beads became larger, had a lower resistance to compression and a higher percentage of bead breakage in a stirred tank reactor. Biomass, recovered from beads in which there was no growth, could be dispersed while the biomass from colonized beads formed a hollow, spherical shell due to growth on and near the bead surface and no growth in the bead interior. If alginate-immobilized *T. versicolor* is to be used in a stirred tank reactor, a high biomass loading during the immobilization phase and no fungal growth in the beads is recommended to have high decoloration rates and low bead breakage.

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1. Introduction

White rot fungi such as *Phanerochaete chrysosporium* (Kirby et al., 1995; Swamy and Ramsay, 1999a), *Trametes hispida* (Rodriguez et al., 1999), *Pleurotus ostreatus* (Rodriguez et al., 1999), *Bjerkandera adusta* (Heinfling et al., 1998), and *Pycnoporus cinnabarinus* (Schliephake and Lonergan, 1993) have been shown to decolorize textile dyes or colored effluent. While most of these evaluations have been done with suspension culture, cultures immobilized on a variety of supports such as polyurethane, stone, wood particles,
jute twine, and porous foam have also been successful in dye decoloration (Yang and Yu, 1996; Kapdan et al., 2000; Shin et al., 2002).

Imobilized cultures tend to have higher activity and are more resilient to environmental perturbations such as pH, or exposure to toxic chemical concentrations than suspension culture. Immobilization by encapsulation in a matrix such as alginate has been shown to decolorize Orange II (an azo dye) (Zhang et al., 1999), bleach plant (Livernoche et al., 1983; Pallerla and Chambers, 1997) and molasses wash (Fahy et al., 1997) effluents. For example, T. versicolor immobilized in calcium alginate reduced 80% of the bleach plant effluent color in 3 d compared to 60% color removal in 6 d with a suspension culture (Livernoche et al., 1983). A few studies have evaluated alginate-immobilized fungi for dye decoloration of a single dye (Zhang et al., 1999) and none have related decoloration rates to the mechanical properties of the beads or whether these beads could be stored and re-used. The mechanical properties help to determine the choice of reactor and operating conditions as well as the practicality of using this type of immobilization technique. The structural properties of alginate beads are frequently determined by measuring the compression strength of the bead itself (i.e., bead hardness), and do not usually take into account the presence of growing cells (Martinsen et al., 1989; Yamagiwa et al., 1999; Edwards-Levy and Levy, 1999).

In zebra fish fry toxicity assays (unpublished data) and MICROTOX studies (Ramsay and Nguyen, 2002), the components of the fungal growth medium have been shown to be more toxic than the dye or its decolorized products. Hence, in a treatment process, it may be necessary to separate fungal growth from decoloration and to determine the minimal nutrient requirements for decoloration. The objectives of this study were to determine (i) the minimum nutrients required for decoloration of single dyes and dye mixtures, (ii) whether the immobilized culture could decolorize after storage, and (iii) the effect of bead properties on their integrity and on textile dye decoloration by alginate-immobilized T. versicolor.

2. Materials and method

2.1. Culture maintenance and growth medium

T. versicolor ATCC 20869, maintained on 2% (w/v) malt agar plates at 4°C, was used to inoculate 100 ml of malt extract broth (20 g l−1) or 200 ml of modified Kirk’s medium in 500 ml Erlenmeyer shake flasks. The contents of the flasks were incubated for 7–10 d at 30°C and 200 rpm on a New Brunswick rotary shaker. The composition of the modified Kirk’s medium per liter was 10.1 g glucose, 2.9 g 2,2-dimethyl succinate (DMS), 2.2 g ammonium tartrate, 0.2 g KH2PO4, 0.05 g MgSO4·7H2O, 0.01 g CaCl2·2H2O, 1 μg thiamine, and 1.0 ml trace element solution (TES). The TES per liter consisted of: 30 g MgSO4·7H2O, 10 g NaCl, 5 g MnSO4·H2O, 1 g CoSO4, 1 g FeSO4·7H2O, 1 g ZnSO4·7H2O, 0.82 g CaCl2, 100 mg CuSO4·5H2O, 100 mg NaMoO4·2H2O, 100 mg H3BO3 and 1 g EDTA.

2.2. Immobilization of T. versicolor

Biomass from 7 to 10 d old cultures was recovered by gravity filtration and blended with 2% (w/v) sodium alginate (Sigma) for 5 s at the “Mash” setting of an Osterizer blender. The mixture was kept at 4°C until all air bubbles had disappeared. Using a 10 or 50 cc syringe, the biomass–alginate mixture was added drop-wise into a solution of 0.2 M CaCl2 with constant stirring for 1 h then washed with sterile, deionized, distilled water. T. versicolor was allowed to colonize the beads by incubating in modified Kirk’s medium for 0–7 d. Free fungal biomass was removed and the mechanical properties of the beads and dye decoloration by the immobilized culture were evaluated. Sodium alginites of different viscosities (Manugel GMB, GHB and DMB) were obtained from Monsanto, Surrey, UK. All steps were performed aseptically with sterile media.

2.3. Decoloration in minimal media

To evaluate the minimal medium components required for decoloration, a packed volume of 20 ml of beads was added to duplicate flasks containing about 50 mg l−1 Amaranth in one of three media: (A) modified Kirk’s medium containing 0.22 g l−1 ammonium tartrate, (B) medium B: DMS, thiamine, TES and glucose at the same concentrations as in modified Kirk’s medium, and (C) medium C: 10.1 g l−1 glucose adjusted to pH 3.6–3.7 with HCl. Decoloration was done at 30°C and 200 rpm in 500 ml Erlenmeyer shake flasks containing an initial volume of 200 ml. For all other decoloration assays, the decoloration medium contained 1 g l−1 glucose at pH 3.6–3.7 and 50–60 mg l−1 of dye. Triplicate experiments are shown as averages with standard deviations and duplicate experiments are shown as results from experiments A and B.

2.4. Storage and subsequent decoloration

At the end of the experiment reported in Table 3, all the beads were pooled, washed with sterile distilled water then divided into five equal volumes. Each volume was added to one of the following sterilized storage media: modified Kirk’s medium, 1 g l−1 CaCl2, 9 g l−1 NaCl, 0.5 g l−1 glucose, or a mixture of 9 g l−1 NaCl and 0.02 g l−1 NaNO3. The pH of all solutions were adjusted to 3.8. After 48 d at 6°C, the beads were
recovered by filtration and washed again with sterile distilled water. Free biomass on the bead surface was removed before adding them to a decoloration medium containing 0.5 g of glucose and 50 mg of Reactive Blue 19 per liter.

2.5. **Bead hardness**

The hardness of individual beads was determined as the amount of force (mN) applied to achieve 35% compression of the beads. Values reported are the average measurements of 10 different beads.

2.6. **Biomass determination in beads**

Alginate was solubilized by stirring the beads in 0.2 M citrate buffer and the biomass was recovered by centrifuging at 23 500 g for 10 min, rinsed once with citrate buffer then twice with distilled water. The biomass dry weight was determined gravimetrically.

2.7. **Bead fragility in stirred tank reactor**

At the end of the decoloration experiments, beads from replicate flasks were pooled, counted and added to a 2-l Multigen F-2000 stirred tank reactor (New Brunswick Scientific, Edison, NJ) containing 1.5 l of water. After 3 d at 0.5 VVM (i.e., 0.75 l of air per min) and 400 rpm, the percentage of intact beads was determined.

2.8. **Other analyses**

Dye decoloration was measured spectrophotometrically at the maximum wavelength of absorbance ($\lambda_{\text{max}}$) for each dye using a calibration curve. The $\lambda_{\text{max}}$ for Amaranth, Reactive Black 5, Reactive Blue 19 and Direct Black 22 were 523, 597, 591 and 478 nm, respectively. Glucose concentration was determined spectrophotometrically at 600 nm using 3,5-dinitrosalicylic acid (Miller, 1959). Samples before dye addition and at the end of decoloration were chosen for glucose analysis as the dye was found to interfere with the glucose assay.

3. **Results and discussion**

Decoloration with a suspension culture has been shown to follow first-order kinetics with respect to dye concentration (Swamy and Ramsay, 1999b). Since the biomass recovered from colonized beads grew only at or near the surface of the beads (as discussed later), the effect of mass transfer on decoloration may be considered to be constant with alginate immobilized *T. versicolor* and since the biomass concentration within each experiment was similar, a pseudo-first-order decoloration rate constant ($k$, with units of h$^{-1}$) was used as a basis of comparison within each experiment.

### 3.1. Minimal medium composition for Amaranth decoloration

Since toxicity reduction is an important aspect of effluent treatment and the growth medium components have been shown to be more toxic than some dyes (Ramsay and Nguyen, 2002), it is useful to know the minimum nutrients required for decoloration to occur. This would not only minimize toxic effects but would also reduce the number and/or quantity of components which would be needed in a treatment process and, consequently, the cost.

*T. versicolor* colonized beads were evaluated for Amaranth decoloration in (a) complete medium (i.e., modified Kirk’s medium) as a point of comparison (Fig. 1a), (b) medium B containing the buffer, all the trace elements, thiamine and glucose as in the complete medium (Fig. 1b), and (c) 10 g l$^{-1}$ of glucose alone (medium C, Fig. 1c) for six successive decolorations. After the first, third and fifth decolorations were completed, the immobilized culture was incubated in modified Kirk’s medium for about 18 h. At the end of each incubation period, and after the third and fifth decolorations, the spent medium was replaced with a fresh volume of the decoloration medium containing about 50 mg l$^{-1}$ of Amaranth. For medium A, there are no data for the fourth decoloration as the sample vials were accidentally broken. For medium B and C, the nitrogen source was omitted but not glucose, as dye decoloration is known to occur in an excess of carbon under nitrogen limitation by this culture (Fenn and Kirk, 1978; Swamy and Ramsay, 1999b). When the complete medium was used, there was no observable lag in decoloration even though decoloration is not expected to occur in the presence of excess nitrogen. It is highly likely that the higher level of biomass in the alginate beads (compared to typical suspension cultures) consumed the low amount of ammonium tartrate (0.22 g l$^{-1}$) so rapidly that nitrogen-limited conditions were created almost immediately resulting in no observable decoloration lag.

The decoloration kinetics in the three media were similar for all decolorations, indicating that it is possible to achieve the same Amaranth decoloration rates with glucose alone (Fig. 1 and Table 1). Furthermore, similar decoloration rates were obtained whether 1, 5 (data not shown) or 10 g l$^{-1}$ of glucose was used. Regardless of the composition of the decoloration medium, about 0.5 g l$^{-1}$ of glucose was consumed when 50 mg l$^{-1}$ of Amaranth was decolorized (Table 1). In all subsequent experiments, colonization of immobilized *T. versicolor* was done in modified Kirk’s medium.
followed by decoloration in fresh medium C (i.e., 0.5 g l\(^{-1}\) glucose in distilled water). It is unlikely that growth would have occurred during decoloration as nitrogen was limiting.

Little change in pH was observed during decoloration in this (Table 1) and all other experiments even though no buffer was used.

### 3.2. Decoloration of different dyes in minimal medium

Alginate-immobilized *T. versicolor* also decolorized other dyes and a mixture of Amaranth and Reactive Blue 19 in minimal medium C (i.e., 0.5 g l\(^{-1}\) glucose in distilled water) (Table 2). There was little to no lag phase when Amaranth, Reactive Black 5, Reactive Blue 19 or

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**Table 1**

<table>
<thead>
<tr>
<th>Decoloration medium</th>
<th>pH Initial</th>
<th>pH Final</th>
<th>First-order decoloration rate constant (h(^{-1}))</th>
<th>(t_{75%}) (h)</th>
<th>Glucose consumed (mg l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Kirk’s medium</td>
<td>3.32 ± 0.07</td>
<td>3.39 ± 0.01</td>
<td>0.76 ± 0.02</td>
<td>1.4 ± 0.1</td>
<td>0.49 ± 0.17</td>
</tr>
<tr>
<td>Medium B</td>
<td>3.46 ± 0.05</td>
<td>3.47 ± 0.05</td>
<td>0.59 ± 0.05</td>
<td>2.3 ± 0.1</td>
<td>0.55 ± 0.13</td>
</tr>
<tr>
<td>Medium C</td>
<td>3.92 ± 0.02</td>
<td>3.86 ± 0.03</td>
<td>0.79 ± 0.02</td>
<td>1.7 ± 0.0</td>
<td>0.54 ± 0.08</td>
</tr>
</tbody>
</table>

Results are for the second decoloration shown in Fig. 1.

\(t_{75\%}\) is the time for 75% of the Amaranth to decolorize.

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Fig. 1. Successive batch decolorations of Amaranth by alginate-immobilized *T. versicolor* in three decoloration media: (a) modified Kirk’s medium containing 0.22 g l\(^{-1}\) ammonium tartrate; (b) medium B: DMS, thiamine, TES and glucose at the same concentrations as in modified Kirk’s medium; and (c) medium C: 10.1 g l\(^{-1}\) glucose adjusted to pH 3.6–3.7 with HCl. At the end of the first, third and fifth decolorations, the immobilized culture was incubated in fresh growth medium for 12–18 h.
Direct Blue 22 were decolorized. Reactive Black 5 decolorized the fastest with a $k$ of about 0.76 h$^{-1}$ and a $t_{75\%}$ of 2.0 h in duplicate flasks while Direct Black 22 had the slowest decoloration ($k = 0.14$ h$^{-1}$) and the longest $t_{75\%}$ (10.2 h). Since there was no visible dye sorption to alginate beads with or without \textit{T. versicolor}, all loss of color is attributed to fungal activity.

When a 1:1 mixture of Amaranth and Reactive Blue 19 was decolorized, the decoloration rate constant for each dye was 0.65 and 1.48 h$^{-1}$/C$_0^{1}$, respectively, measured at the maximum wavelength of absorbance for each dye. The presence of Amaranth enhanced the decoloration rate of Reactive Blue 19, as Amaranth or Reactive Blue 19 alone had decoloration rate constants of about 0.7 and 0.5 h$^{-1}$/C$_0^{1}$, respectively. Amaranth decoloration was slightly enhanced by the presence of Reactive Blue 19. Although, other paired combinations of the four dyes and a mixture of the four appeared to be degraded faster than the individual dyes, it was difficult to monitor the disappearance of each dye by spectroscopic analysis when they were present as a mixture. The only exception was a 1:1 mixture of Amaranth and Reactive Blue 19 which did not appear to interfere with each other.

After an individual dye was decolorized in two successive treatments, the fungus was challenged with a different dye. While the rates for the first two decolorations were generally comparable (Table 3), the third decoloration of a different dye was usually slower than when it was decolorized as a first dye. For example, when two successive decolorations of Reactive Blue 19 was followed by an Amaranth decoloration, $k$ and $t_{75\%}$ were similar for both decolorations of Reactive Blue 19. However, Amaranth was decolorized about 50% more slowly in the 3rd decoloration compared to its decoloration as the first dye (Tables 2 and 3). Similar results were obtained with two decolorations of Direct Black 22 followed by Reactive Blue 19. However, the second decoloration of Reactive Black 5 was notably slower than the first (Table 3). These results may be due to decreasing enzyme activity.

After the third decoloration, the decolorized medium was replaced with fresh growth medium and the immobilized culture was incubated for 5 d, then challenged with the same dye as in the third decoloration. In all cases, the decoloration rate constant, $k$, increased by a factor of 3 and $t_{75\%}$ decreased by 80%. This enhanced rate of decoloration was most likely due to an increase in biomass and/or the production of enzymes involved in decoloration. The most likely decolorizing enzymes are laccase and/or manganese peroxidase as their activities are detected in suspension culture under similar conditions but lignin peroxidase, cellobiose dehydrogenase or manganese independent peroxidase were not.

While previous studies have shown a need for a carbon source (Swamy and Ramsay, 1999b), this study is the first to demonstrate that alginate-immobilized \textit{T. versicolor} can decolorize individual dyes or dye mixtures in the presence of glucose alone. Although, the exact role of glucose is not known, it may play a role in decoloration by acting as the substrate for glucose oxidase (Daniel et al., 1994; Machida and Nakanashi, 1994) to generate H$_2$O$_2$ needed for peroxidase activity or it may produce small organic acids which complex with Mn$^{3+}$ generated by manganese peroxidase (Roy and Archibald, 1993).

### Table 2

<table>
<thead>
<tr>
<th>Dyes</th>
<th>$\lambda_{max}$ (nm)</th>
<th>Lag phase (h)</th>
<th>$k$ (h$^{-1}$/C$_0^{1}$)</th>
<th>$t_{75%}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Amaranth</td>
<td>523</td>
<td>1</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>Reactive Black 5</td>
<td>597</td>
<td>0</td>
<td>0</td>
<td>0.77</td>
</tr>
<tr>
<td>Reactive Blue 19</td>
<td>591</td>
<td>1</td>
<td>1</td>
<td>0.63</td>
</tr>
<tr>
<td>Direct Black 22</td>
<td>478</td>
<td>0</td>
<td>0</td>
<td>0.13</td>
</tr>
<tr>
<td>Mixture of 50% Amaranth and 50% Reactive Blue 19</td>
<td>523</td>
<td>0.4</td>
<td>nd</td>
<td>0.65</td>
</tr>
<tr>
<td>50% Reactive Blue 19</td>
<td>591</td>
<td>0.0</td>
<td>nd</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Columns A and B are data from duplicate flasks where $k$ is the first-order decoloration rate constant, $t_{75\%}$ is the time for 75% of the dye to decolorize and nd indicates that no experiment was done.
amounts were added to different storage media (i.e., modified Kirk’s medium, 1 g l⁻¹ CaCl₂, 9 g l⁻¹ NaCl, 0.5 g l⁻¹ glucose, or a mixture of 9 g l⁻¹ NaCl and 0.02 g l⁻¹ NaNO₃). During storage for 48 d at 6 °C, most growth occurred in modified Kirk’s medium where a thick fungal mat covered the beads (Table 4). This created significant problems in handling the beads. About half of them were lost when attempts were made to remove the excess biomass. When stored in either a CaCl₂ or NaCl solution, there was much less growth and the beads were easier to handle. Subsequent decoloration of Reactive Blue 19 was fastest when the beads were stored in CaCl₂ than in modified Kirk’s medium. Even slower decoloration occurred with the beads which had been stored in NaCl with most of them breaking during a second decoloration. The beads probably became more fragile as a result of the exchange of Ca²⁺ ions in the alginate matrix with Na⁺ in the storage medium. There was little to no visible growth or subsequent decoloration of Reactive Blue 19 when the immobilized culture was stored in either a glucose solution or a mixture of NaCl and NaNO₃. CaCl₂ (1 g l⁻¹) was the best choice of all the storage media evaluated, since the beads were easy to handle and the best decoloration rates were obtained after storage.

### 3.4. Effects of bead properties on decoloration

While bead properties such as bead hardness could affect the growth of *T. versicolor* within the alginate beads, and consequently the rate of decoloration, the growth of *T. versicolor* inside the beads could in turn affect bead properties and hence the integrity of the beads. Hence, the relationships between bead properties with fungal growth, bead integrity and decoloration were investigated.

Since bead properties can be affected by the immobilization process, the effects of alginate viscosity, length of time in a CaCl₂ bath and CaCl₂ concentration in the bath on bead properties and decoloration were investigated. When *T. versicolor* was immobilized in alginites of different viscosities, the rate of decoloration was faster when the viscosity was 2000 cps or less (Table 5) probably because fungal growth in higher viscosity alginates was reduced.

The length of time that the beads were in the CaCl₂ bath and the CaCl₂ concentration may affect bead hardness and, perhaps, decoloration. When droplets of the alginate mixture were “cured” in 0.2 M CaCl₂ bath for 1–3 h, bead hardness was similar after 1 and 2 h of “curing”, but after 3 h, the bead hardness increased when there was no biomass in the bead (Table 6). After 7 d of colonization (i.e., incubation in modified Kirk’s medium) of beads containing *T. versicolor*, bead hardness decreased compared to beads without biomass as time of curing increased. However, there was no apparent
In a separate experiment, drops of the T. versicolor–alginate mixture were gelled in either 0.05, 0.2 or 0.3 M CaCl₂ bath for 1 h, then evaluated for bead properties and Amaranth decoloration without an additional growth phase for the fungus. The beads from the 0.05 M CaCl₂ solution had the lowest resistance to compression (i.e., were softer) than the beads gelled at higher CaCl₂ concentrations (Table 7). The bead hardness increased as the CaCl₂ concentration increased from 0.05 to 0.2 M and seemed to be unaffected above 0.2 M. The softer beads had a higher decoloration rate probably because lower CaCl₂ concentrations resulted in fewer cross-links at the guluronic acid binding sites leading to weaker cohesion in the alginate matrix to allow better diffusion through the bead. There was no colonization period prior to decoloration in this experiment.

The mean bead diameter was unaffected if there was no biomass in the beads (Table 6), or if there was no colonization phase (Table 7). However, when T. versicolor was allowed to grow within the beads, the average bead diameter increased by 15% (Table 6) to 30% (Table 7) when compared to beads prepared without biomass or before colonization, respectively. These differences are most likely due to growth of the mycelium within the beads thus increasing the bead volume. Similar observations were reported by Zhang et al. (1999) when P. chrysosporium was immobilized in alginate.

### Table 4
Growth from beads of alginate-immobilized T. versicolor after 48 d at 6 °C in different storage media and decoloration of Reactive Blue 19 after storage

<table>
<thead>
<tr>
<th>Storage medium</th>
<th>Growth after 48 d</th>
<th>Decoloration after storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified Kirk’s medium</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Glucose (5 g l⁻¹)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Saline (9 g l⁻¹)</td>
<td>++</td>
<td>+ (beads broke during 2nd decoloration)</td>
</tr>
<tr>
<td>Saline (9 g l⁻¹) and NaNO₃ (0.02 g l⁻¹)</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>CaCl₂ (1 g l⁻¹)</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

After immobilization, the culture was incubated in Kirk’s medium for 7 d before bead properties and Amaranth decoloration were evaluated.

### Table 5
Effect of alginate viscosity on Amaranth decoloration by immobilized T. versicolor

<table>
<thead>
<tr>
<th>Alginate</th>
<th>Viscosity of 2% solution (cps)</th>
<th>First-order decoloration rate constant (h⁻¹)</th>
<th>t_{75%}a (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manugel GHB</td>
<td>550</td>
<td>0.42 ± 0.03</td>
<td>3.85 ± 0.07</td>
</tr>
<tr>
<td>Manugel GMB</td>
<td>2000</td>
<td>0.51 ± 0.01</td>
<td>4.28 ± 0.11</td>
</tr>
<tr>
<td>Manugel DMB</td>
<td>3000</td>
<td>0.14 ± 0.01</td>
<td>7.15 ± 0.35</td>
</tr>
</tbody>
</table>

* a_{75%} is the time for 75% of the Amaranth to decolorize.

### Table 6
Length of time in 0.5 M CaCl₂ bath on bead hardness and decoloration in duplicate flasks A and B

<table>
<thead>
<tr>
<th>Time in CaCl₂ (h)</th>
<th>No Biomass</th>
<th>Biomass</th>
<th>No Biomass</th>
<th>Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance at 35% compression (mN)</td>
<td>Mean bead diameter (mm)</td>
<td>Resistance at 35% compression (mN)</td>
<td>Mean bead diameter (mm)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>240</td>
<td>3.86 ± 0.07</td>
<td>104</td>
<td>4.67</td>
</tr>
<tr>
<td>2</td>
<td>230</td>
<td>3.73 ± 0.09</td>
<td>103</td>
<td>4.55</td>
</tr>
<tr>
<td>3</td>
<td>275</td>
<td>3.80 ± 0.09</td>
<td>86</td>
<td>4.12</td>
</tr>
</tbody>
</table>

3.5. Mycelial growth in alginate beads on bead mechanical strength and Amaranth decoloration

The average bead diameter was smaller and the beads were harder when there was no colonization after T. versicolor was immobilized in alginate (Table 7). These
beads could be compressed indefinitely with the beads becoming flatter and extruding water but not breaking. After fungal growth, the beads could not be compressed beyond 40% of their original diameter, and many of them split in two at around 35% compression. The overall hardness of the colonized beads had decreased by 60% and the bead diameter had increased by 30%. Amaranth decoloration rate of the colonized beads was much faster (0.76 h\(^{-1}\)) than the control (0.14 h\(^{-1}\)), more likely due to the higher biomass level in the beads rather than the change in bead hardness (Table 7). Biomass recovered from beads in which the fungus had been immobilized without colonization had similar characteristics to the biomass before immobilization, consisting of small fragments in suspension. However, the biomass recovered from the colonized beads was in the form of hollow, spherical shells which retained the shape of the beads. During incubation, the mycelium must have grown predominantly near or on the surface of the beads where the nutrient concentration was highest with very little growth in the interior of the bead. This observation is consistent with reports in which immobilized bacteria (Mater et al., 1999) and yeast (Wada et al., 1979) form colonies preferentially in a peripheric ring.

3.6. Effect of bead hardness on its fragility

Although the colonized beads gave higher decoloration rates, they were more fragile, with almost all the beads damaged after they had been in a stirred tank reactor for 3 d (Table 7). Whenever the resistance at 35% compression was 200 mN or more, the beads were sufficiently tough that few were damaged (less than 1%) (Tables 3 and 5). However, when the resistance to 35% compression was reduced due to mycelial growth, there was significant damage to the beads (about 94% destroyed). Although the non-colonized beads were tougher, the rate of decoloration was five times slower. It may be possible to obtain high decoloration rates with reasonable mechanical properties if there was a very high biomass loading in the alginate beads and no mycelial growth. This may achieve a compromise between high decoloration rates and bead toughness to enable use in a stirred tank reactor. Alternately, the colonized beads may not be damaged in a fluidized reactor so that higher decoloration rates may be obtained with more fragile beads.

4. Conclusions

Alginate-immobilized *T. versicolor* was shown to decolorize Amaranth, Reactive Black 5, Reactive Blue 19 and Direct Black 22 and mixtures of these dyes in the presence of glucose alone. After 48 d at 6 °C in five
storage solutions, 1 g l⁻¹ CaCl₂ was found to be the best as the beads were easy to handle and the fastest decoloration rates were obtained after storage. After immobilization, fungal growth within the beads made them fragile resulting in a high percentage of bead breakage in a stirred tank reactor compared to beads in which the fungus was immobilized but not colonized.

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References


