Biosorption of Textile Dye Using Immobilized Bacterial (Pseudomonas aeruginosa) and Fungal (Phanerochate chrysosporium) Cells

Natarajan Saravanan, Tiruvenkadam Kannadasan, Chiya Ahmed Basha and Veerasamy Manivasagan

1Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635 109, Tamil Nadu, India
2Department of Chemical Engineering, Coimbatore Institute of Technology, Coimbatore-641 014, Tamil Nadu, India
3Department of Chemical Engineering, Adhiyamaan College of Engineering, Hosur-635 109, Tamil Nadu, India

Received 2012-08-24, Revised 2013-01-04; Accepted 2013-09-16

ABSTRACT

Wastewater containing dyes presents a serious problem due to its high toxicity which leads to creating enormous environmental pollution and ecological hazards. Therefore the removal of the high stable dyes from the textile effluents is of major importance. The purpose of this study is to remove the reactive dye Procion Blue 2G from textile dye solution by biosorption process using immobilized cells of Pseudomonas aeruginosa and Phanerochate chrysosporium. It was found that maximum dye uptake is 1.648 mg g\(^{-1}\) of bead for P. aeruginosa and it is 1.242 mg g\(^{-1}\) of bead for P. chrysosporium. Both the results are derived from higher initial dye concentration (100 mg L\(^{-1}\)) and high cell concentration (in terms of volume of inoculum 20 mL) and at low mass of biosorbent (5 g of bead). Comparatively better results are produced by the beads having the cells of P. aeruginosa than P. chrysosporium. Further, due to the cell immobilization, both the cell beads can be utilized repeatedly in continuous reactors by selecting suitable eluent in industrial scale with the advantage of avoiding wash out of cells.

Keywords: Biosorbent, Microorganism, Immobilization

1. INTRODUCTION

Textile industries consume large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition, ranging from inorganic compounds to polymers and organic products (Mishra and Tripathy, 1993; Banat et al., 1996; Juang et al., 1996). The presence of very low concentrations of dyes in effluent is highly visible and undesirable (Nigam et al., 2000). Synthetic dyes have increasingly been used in the textile and dyeing industries because of their ease and cost-effectiveness in synthesis, firmness, high stability to light, temperature, detergent and microbial attack and variety in color compared with natural dyes. This has resulted in the discharge of highly polluted effluents. Normally colour is noticeable at a dye concentration higher than 1 mg L\(^{-1}\) and an average concentration of 300 mg L\(^{-1}\) has been reported in effluents from textile manufacturing processes (Goncalves et al., 2000; O’Neill et al., 1999). Over \(7 \times 10^5\) ton and approximately 10,000 different dyes and pigments are produced annually world-wide, about 10% of which may be found in wastewater (Deveci et al., 2004). Color interferes with penetration of sunlight into waters, retards photosynthesis, inhibits the growth of aquatic biota and interferes with gas solubility in water bodies (Banat et al., 1996). In addition, many dyes are believed to be toxic carcinogenic or to be prepared from known carcinogens such as benzidine or other aromatic compounds that might be formed as a result of microbial metabolism (Novotny et al., 2006; Karimniaae-Hamedaani et al., 2007). Hence, removal of...
these dyes from the effluents is necessary. The structural diversity of dyes comes from the use of different chromophoric groups (e.g., azo, anthraquinone, triarylmethane and phthalocyanine groups) and different application technologies (e.g., reactive, direct, disperse and vat dyeing) (Heinfling et al., 1998).

Various techniques have been employed for the treatment of dye bearing industrial effluents, which usually come under two broad divisions: Abiotic and biotic methods. Abiotic methods include precipitation, adsorption, ion exchange, membrane and electrochemical technologies. Much has been discussed about their downside aspects in recent years (Crini, 2006), which can be summarized as expensive, not environment friendly and usually dependent on the concentration of the waste. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated. In recent years, research attention has been focused on biological methods for the treatment of effluents, some of which are in the process of commercialization. There are three principle advantages of biological technologies for the removal of pollutants; first, biological processes can be carried out in situ at the contaminated site; Second, bioprocess technologies are usually environmentally benign (no secondary pollution) and third, they are cost effective.

Of the different biological methods, bioaccumulation and Biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of dyes/metals (Malik, 2004). Some confusion has prevailed in the literature regarding the use of the terms “bioaccumulation” and “Biosorption” based on the state of the biomass. Herein, therefore, bioaccumulation is defined as the phenomenon of living cells; whereas, Biosorption mechanisms are based on the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. The toxicant can transport into the cell, accumulate intracellularly, across the cell membrane and through the cell metabolic cycle. Conversely, Biosorption can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials derived from biological sources. Biosorption is due to a number of metabolism-independent processes that essentially take place in the cell wall, where the mechanisms responsible for the pollutant uptake will differ according to the biomass type.

Biosorption possesses certain inherent advantages over bioaccumulation processes, which are based up on the cost, pH, Temperature, maintenance, selectivity, versatility, degree of uptake, rate of uptake, regeneration and reuse and toxicant recovery (Vijayaraghavan and Yun, 2008a). In general, the use of living organisms may not be an option for the continuous treatment of highly toxic organic/inorganic contaminants. Once the toxicant concentration becomes too high or the process operated for a long time, the amount of toxicant accumulated will reach saturation (Eccles, 1995). Beyond this point, an organism’s metabolism may be interrupted, resulting in death of the organism. This scenario can be avoided in the case of dead biomass, which is flexible to environmental conditions and toxicant concentrations. Thus, owing to its favorable characteristics, biosorption has, not surprisingly, received much attention in recent years.

Biosorbents for the removal of dyes mainly come under the following categories: bacteria, fungi, algae, industrial wastes, agricultural wastes and other polysaccharide materials. With respect to dye biosorption, microbial biomass (bacteria, fungi, microalgae) outperformed macroscopic materials (seaweeds, crab shell). The reason for this discrepancy is due to the nature of the cell wall constituents and functional groups involved in dye binding. Many bacteria, fungi and microalgae have been found to bind a variety of dye classes.

Microbial biosorbents are basically small particles, with low density, poor mechanical strength and little rigidity. Even though they have merits, such as high biosorption capacity, rapid steady state attainment, less process cost and good particle mass transfer, they often suffer several drawbacks. The most important include solid-liquid separation problems, possible biomass swelling, inability to regenerate/reuse and development of high pressure drop in the column mode (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2007). Several established techniques are available to make biosorbents suitable for process applications. Among these, immobilization techniques such as entrapment and cross linking have been found to be practical for biosorption (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2008b; Volesky, 2001). Immobilization of microorganisms within a polymeric matrix has exhibited greater potential, especially in packed or fluidized bed reactors, with benefits including the control of particle size, regeneration and reuse of the biomass, easy separation of biomass and effluent, high biomass loading and minimal clogging under continuous-flow conditions (Hu and Reeves, 1997). Very few efforts have been made to utilize the immobilization concept for dye (Fu and Viraraghavan, 2003; Vijayaraghavan et al., 2008a) compared to metal.
biosorption (Hu and Reeves, 1997; Prakasham et al., 1999; Yan and Viraraghavan, 2001; Khoo and Ting, 2001; Beolchini et al., 2003; Bai and Abraham, 2003). Important immobilization matrices used in biosorbent immobilization include sodium alginate (Bai and Abraham, 2003; Xiangliang et al., 2005), polysulfone (Vijayaraghavan et al., 2008a; Beolchini et al., 2003), polyacrylamide (Bai and Abraham, 2003) and polyurethane (Hu and Reeves, 1997). The choice of immobilization matrix is a key factor in the environmental application of immobilized biomass. The polymeric matrix determines the mechanical strength and chemical resistance of the final biosorbent particle to be utilized for successive sorption-desorption cycles (Bai and Abraham, 2003). Batch experiments usually focus on the study of factors influencing biosorption, which are important in the evaluation of the full biosorption potential of any biomaterial. The important factors are solution pH, temperature, ionic strength, biosorbent dosage, biosorbent size, initial solute concentration and agitation rate. Biosorption kinetics can be adjusted to several models, such as the pseudo-first-order model, the pseudo-second-order model and the intra particle diffusion model and elovich model. If dye uptake is only controlled by diffusion through a boundary layer the kinetics generally adjusts to the pseudo-first order model. However, biosorption involves several processes: Electrostatic forces and chemical reactions between binding sites and dye.

In this study, the potential of immobilized cells of bacterial strain P. aeruginosa and fungal strain P. chrysosporium are tested against the reactive textile dye Procion blue 2G removal by biosorption technique by varying biosorbent dosage, size of the biosorbent, initial dye concentration and cell concentration.

2. MATERIALS AND METHODS

All the chemicals used for the study are AR grade. The dye Procion blue 2G obtained from a local supplier. The bacterial strain, P. aeruginosa was obtained from Central Electrochemical Research Institute (CECRI) Karaikudi, TamilNadu, India. The bacterial cultures were grown in 250 mL Erlenmeyer flasks containing 100 mL of nutrient broth medium, containing (g L\(^{-1}\)) peptone 5, NaCl 5, beef extract 3 and incubated for 24 h at 30°C at static condition. The fungal strain P. chrysosporium obtained as lyophilized form from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The strain P. chrysosporium was initially grown on malt agar and incubated at 35°C until extensive spore growth occurred. Then the fungal culture was inoculated into 250 mL Erlenmeyer flasks having 100 mL of Potato-Dextrose Broth (PDB) medium containing (g L\(^{-1}\)) peeled potatoes 200, glucose 20, yeast extract 0.1 and incubated for 4 d at 30°C at static condition.

2.1. Preparation of Immobilized Cells (Biobeads)

Sodium alginate and polyvinyl alcohol with a concentration range of 0.5-10% was used for cell immobilization. In the current study 0.5 g of sodium alginate and 0.5 g of Poly Vinyl Alcohol (PVA) were added to 35 mL of boiling water and autoclaved at 121°C for 15 min and then allowed to cool at room temperature. Known quantity (10 mL) of liquid culture (P. aeruginosa) at mid logarithmic growth phase were mixed thoroughly in it and extruded drop by drop into a cold, sterile 5% calcium chloride solution with the help of a sterile 5 mL syringe. Gel beads of approximately 2 mm diameter were thus obtained. The beads were hardened by resuspending it into a fresh calcium chloride solution for 24 h at 4°C with gentle agitation. Finally these beads were washed with distilled water to remove excess calcium ions and unentrapped cells, then the beads were autoclaved at 121°C and then cooled to room temperature and used for biosorption process. Similarly, another set of bio-beads were prepared using 20 mL bacterial cell suspension. The similar procedure adapted to prepare the bio-beads for the fungal strain P. chrysosporium.

2.2. Experimental Procedure

Batch biosorption studies were conducted for the immobilized cells of P. aeruginosa at room temperature for two different mass (5 g and 10 g) of bio-beads and two different dye concentrations such as 50 and 100 mg L\(^{-1}\). 5 g and 10 g of pre weighed biobeads containing 10 mL of fungal cell suspension were transferred and suspended into two 250 mL Erlenmeyer flasks, each containing 200 mL of effluent with the Procion blue 2G concentration 50 mg L\(^{-1}\). Another 5 g and 10 g of bio-beads containing 10 mL of fungal cell suspension were transferred and suspended into another two 250 mL Erlenmeyer flasks, each containing 200 mL of effluent containing dye concentration 100 mg L\(^{-1}\). The same experiments were repeated for the bio-beads containing 20 mL of cell suspension. The flasks were kept in
shaking condition (130 rpm) for 5 h in an orbital shaker at room temperature. Samples were withdrawn at 1 h intervals and centrifuged at 5000 rpm in cooling centrifuge for 20 min to sediment the suspended particles. The similar procedure repeated for the immobilized cells of *P. chrysosporium*.

2.3. Spectrophotometer Analysis

During biosorption studies, samples were collected for every hour from all the experiments and subjected to dye adsorption analysis using UV-Visible Spectrophotometer (Spectroquant NOVA 60, Merk at $\lambda_{\text{max}} = 605$ nm). Extent of dye adsorption and amount of dye adsorption by bio-beads were calculated by the following formula Equation 1:

$$X_t = \frac{C_o - C_t}{C_o}$$

Where:
- $X_t$ = Extent of adsorption
- $C_o$ = Initial absorbance
- $C_t$ = Absorbance with respect to time

Equation 2:

$$Q = \frac{C_t X_t V}{m_b}$$

Where:
- $Q$ = Amount of dye adsorption per g of bio-beads (mg g$^{-1}$)
- $C_t$ = Initial concentration of dye (mg L$^{-1}$)
- $V$ = Throughput volume of the textile effluent (L)
- $m_b$ = Mass of bio-beads (g)
- $X_t$ = Extent of adsorption

2.4. Kinetics Experiments

Kinetic studies were carried out in order to determine the contact time required to reach the equilibrium. About 200 mL samples of various concentrations of dye solutions were adjusted to desired pH and then mixed with 10 g of each sorbent. Sorption processes were carried out in flasks placed in a thermostatic water-bath shaker at fixed temperature, until the equilibrium was reached. About 3 mL samples were collected at specified intervals (1 h) for the analysis of dye concentration.

2.5. Scanning Electron Microscopic Analysis

The bio-beads were rinsed with distilled water and fixed with 2.5% (w/v) glutaraldehyde solution for 12 h to allow the glutaraldehyde molecules to have complete penetration into the gel. The fixed gel beads were then dehydrated by sequential immersion in increasing concentrations of acetone to remove the final traces of water. The dehydrated beads were then dried in a Carbon Dioxide (CO$_2$) atmosphere under critical conditions. The subsequent samples were cut into halves with a sterile scalpel and fixed on carbon paper placed on stub and coated with gold for increasing the conductivity and examined using a Scanning Electron Microscope (HITACHI S-3000 H, Japan).

3. RESULTS AND DISCUSSION

The microorganisms *P. aeruginosa* and *P. chrysosporium* cells were immobilized in a sol-gel matrix of sodium alginate and PVA. The beads were analyzed using SEM. This was performed on the basis of the results proposed by Vijayaraghavan *et al.* (2008b) in which they emphasized the biosorption abilities of bacterial biomasses towards dyes and metal ions and also they demonstrated the technical feasibility of biosorption process for industrial applications, using the dye Reactive Black 5 by *Corynebacterium glutamicum* biomass immobilized in alginate and Polysulfone matrices.

In our study, Biosorption experiments were performed for two different dye concentrations (50 and 100 mg L$^{-1}$) using the biobeads having two different concentration of cell mass (10 and 20 mL of inoculum) and using two different mass of beads (5 and 10 g). The experiments were carried out for 4 h for all combinations separately. For every 1 h the absorbance value was measured for each combination.

3.1. Effect of Initial dye Concentration on Dye Removal

The amount of dye adsorbed on the surface of bio-beads is increased when time proceeds in all the experiments for the bio-beads containing bacterial strain *P. aeruginosa* and fungal strain *P. chrysosporium*. Further, the amount of dye adsorbed is more when the initial concentration of dye is increased from 50 mg L$^{-1}$ to 100 mg L$^{-1}$ for both the beads having cells of *P. aeruginosa* and cells of *P. chrysosporium*. The maximum dye uptake is 1.648 mg g$^{-1}$ of bead for *P. aeruginosa* and it is 1.242 mg g$^{-1}$ of bead for *P. chrysosporium*. Both the results are derived from higher initial dye concentration (100 mg L$^{-1}$) and high cell
concentration (in terms of volume of inoculum 20 mL) and at low mass of biosorbent (5g of bead). Comparatively better results are produced by the beads having the cells of *P. aeruginosa* than *P. chrysosporium*. These results are shown Fig. 1a and b.

3.2. Effect of Cell Concentration on Dye Removal

In all combination of experiments, if the cell concentration is increased then the amount of dye adsorption is also increased for both initial dye concentrations (50 and 100 mg L\(^{-1}\)) and both mass of biosorbents(5 and 10 g). In this case also, the beads having the cells of *P. aeruginosa* shows superior results than *P. chrysosporium* for both cell concentrations in terms of volume of inoculum which is used to prepare the beads.

Effect of absorbent dosage on dye removal: when the mass of biosorbent (bio-beads) is increased with the same cell concentration, the amount of dye adsorbed per gram of beads is decreased for both initial dye concentrations and both cell concentrations for the same period of adsorption. These results clearly revealed that there is no need of large quantity of biosorbent required for the adsorption. Further optimization studies are required in this aspect to confirm the optimum adsorbent requirement. These results are shown Fig. 2a-d.

3.3. Kinetic Studies

The prediction of adsorption rate gives important information for designing batch adsorption systems. Information on the kinetics of solute uptake is required for selecting optimum operating conditions for full-scale batch process. The kinetics of the adsorption data was analyzed using pseudo-first order, pseudo-second order, Elovich kinetic model and intra-particle diffusion. These models correlate solute uptake, which are important in predicting the reactor volume. These models are explained as follows.

3.4. Pseudo-First Order Model

The possibility of adsorption data following Lagergren pseudo-first order kinetics is given by:

\[
\frac{dq}{dt} = k_1(q_e - q)
\]  

Integrating Equation 3 with respect to integration conditions q = 0 to q = q at t = 0 to t = t, the kinetic rate expression becomes Equation 4:

\[
\log(q_e - q) = \log(q_e) - \frac{k_1}{2.303} t
\]  

The pseudo first order rate constant \(K_1\) can be obtained from the slope of plot between log (q_e-q) versus time, t (not shown). The calculated \(K_1\) values and their corresponding linear regression correlation coefficient values are shown in Table 1. The linear regression correlation coefficient values \(R^2_1\) found for adsorption of dye by bio-beads for the *P. aeruginosa* and *P. chrysosporium* cultures were in the range of 0.944-0.951 and 0.875-0.939 respectively, which shows that this model can be applied to predict the adsorption kinetic model.

Pseudo-second order model: A pseudo-second order model can be used to explain the adsorption kinetics. This model is based on the assumption the adsorption follows second order chemisorption. The pseudo-second order model can be expressed as:

\[
\frac{dq}{dt} = k_2(q_e - q)^2
\]  

Separating the variables in Equation 5 gives:

\[
\frac{dq}{(q_e - q)^2} = k_2 dt
\]

Integrating Equation 6 for the boundary conditions q=0 to q = q at t = 0 to t = t, simplifies to Equation 7:

\[
\frac{t}{q_t} = \frac{1}{k_2q_e} + \frac{1}{q_e} t
\]

Where:

\(t' = tq_e = q\) (min) and \(q_t = q\) (mg g\(^{-1}\)) = The contact time (min) 

\(q_e = q\) (mg g\(^{-1}\)) and \(q_t = q\) (mg g\(^{-1}\)) = The amount of the solute adsorbed at equilibrium and at any time t

A plot between t/q_e versus t (not shown) gives the value of the constant \(K_2\) (g(mg min\(^{-1}\))\(^{-1}\)) and also q_e (mg g\(^{-1}\)) can be calculated. The pseudo-second order rate constant \(K_2\) the calculated q_e value and the corresponding linear regression correlation coefficient values \(R^2_2\) are given in Table 1. All the linear regression correlation coefficient values \(R^2_2\) found for adsorption of dye by biobeads for the *P. aeruginosa* and *P. chrysosporium* cultures were in the range of 0.358-0.936 and 0.431-0.977 respectively.
Fig. 1. (a) Effect of Initial dye concentration (10mL culture) on dye removal, (b) Effect of Initial dye concentration (20 mL culture) on dye removal.
Fig. 2. (a) Effect of adsorbent dosage on dye removal for the initial dye concentration (50 mg L\(^{-1}\)) and for the biomass concentration (10 mL) (b) Effect of adsorbent dosage on dye removal for the initial dye concentration (50 mg L\(^{-1}\)) and for the biomass concentration (20 mL) (c) Effect of adsorbent dosage on dye removal for the initial dye concentration (100 mg L\(^{-1}\)) and for the biomass concentration (10 mL) (d) Effect of adsorbent dosage on dye removal for the initial dye concentration (100 mg L\(^{-1}\)) and for the biomass concentration (20 mL)
Table 1. Pseudo first order and Pseudo second Kinetic constants for dye biosorption

| C₀ (mg L⁻¹) | K₁ g (mg min)⁻¹ | qₑ (mg g⁻¹) | R²₁ | K₁I g (mg min)⁻¹ | qₑ (mg g⁻¹) | R²₁ |
|-------------|----------------|-------------|-----|----------------|-------------|-----|
| 50          | 0.576          | 1.010       | 0.944 | 0.682          | 0.863       | 0.875 |
| 100         | 0.370          | 1.563       | 0.951 | 0.850          | 1.131       | 0.939 |

Pseudo Second order kinetics

| C₀ (mg L⁻¹) | K₁I g (mg min)⁻¹ | qₑ (mg g⁻¹) | R²₁ |
|-------------|----------------|-------------|-----|
| 50          | 1.963          | 1.719       | 0.358 |
| 100         | 0.574          | 2.711       | 0.936 |

3.5. The Intra-Particle Diffusion (Weber and Morris) Model

The intra-particle diffusion model is characterized by a linear relationship between the amount of metal adsorbed (qₜ) and the square root of the time and is expressed as Equation 8:

\[ qₜ = (Kₐd) t^{0.5} \]  

(8)

Where:

- qₜ (mg g⁻¹) = The amount of dye adsorbed at time t (h)
- Kₐd (mg g⁻¹ h⁻⁰.⁵) = The initial rate of the intra-particle diffusion

The rate constant of intra-particle diffusion Kₐd was determined by plotting qₜ as a function of the square root of the time. The graph of qₜ Vs t⁻⁰.⁵ shows a non-linear distribution of points, with two distinct portions (Figure not shown). The calculated intra-particle diffusion coefficient for dye at different concentrations is listed in Table 2. The intra-particle diffusion rate constants were found to increase for dye with different concentrations. It gives the conclusion that the intra-particle diffusion is the rate determining step in the adsorption of the dye on biobeads. Kinetic data obtained in this work were analyzed by applying simplified form of Vermeulen’s approximation of the model of Boyd et al. (1972) for the calculation of effective particle diffusivity of dye onto adsorbent using following Equation 9:

\[ F²(t) = 1 - \exp \left( -\frac{\pi²D_e}{R_a²} t \right) \]  

(9)

3.6. Elovich Model

One of the most useful models for the studies of activated chemisorption is the Elovich equation. It is generally expressed as Equation 10:

\[ \frac{dqₜ}{dt} = aₑ(-βqₜ) \]  

(10)

Where:

- qₜ (mg g⁻¹) = The amount of solute adsorbed at time t
- aₑ (mg (g min⁻¹)) and β (g mg⁻¹) during any one experiment

The Elovich constants aₑ and β can be obtained from the plot of qₜ Vs ln(t) as shown in Table 2.
Table 2. Intra-particle diffusion and Elovich kinetic constants for dye biosorption

| Intra-particle Diffusion model |          |          |          |          |          |          |
|-------------------------------|----------|----------|----------|----------|----------|----------|
|                               | P. aeruginosa |          | P. chrysosporium |          |          |          |
|                               | C₀ (mg L⁻¹) | Kᵣ₀ mg (g min 0.5⁻¹) | Dₑ×10⁸ (m²s⁻¹) | R² | Kᵣ₀ mg (g min 0.5⁻¹) | Dₑ×10⁸ (m²s⁻¹) | R² |
| 50                            | 1.113     | 4.06E-06 | 0.840 |          | 1.079     | 4.37E-07 | 0.887 |
| 100                           | 1.133     | 4.13E-06 | 0.962 |          | 0.497     | 2.02E-07 | 0.933 |
| Elovich model                 | C₀ (mg L⁻¹) | β (g mg⁻¹) | aₑ mg (g min⁻¹) | R² | β (g mg⁻¹) | aₑ mg (g min⁻¹) | R² |
| 50                            | 1.984     | 0.678    | 0.999 |          | 2.499     | 0.535    | 0.983 |
| 100                           | 1.238     | 0.996    | 0.932 |          | 2.643     | 1.254    | 0.978 |

4. CONCLUSION

From the studies, the inactivated cells of microorganisms *P. aeruginosa* and *P. chrysosporium* immobilized in a sol-gel matrix of sodium alginate and PVA shows its potential in adsorbing the reactive dye Procion blue 2G at room temperature and constant pH. From the results, the maximum dye uptake is 1.648 mg g⁻¹ of bead for *P. aeruginosa* and it is 1.242 mg g⁻¹ of bead for *P. chrysosporium*. Both the results are derived
from higher initial dye concentration (100 mg L\(^{-1}\)) and high cell concentration (in terms of volume of inoculum 20 mL) and at low mass of biosorbent (5g of bead). Comparatively better results are produced by the beads having the cells of \(P. \text{aeruginosa}\) than \(P. \text{chrysosporium}\). Further, due to the cell immobilization, both the cell beads can be utilized repeatedly in continuous reactors by selecting suitable ‘eluent’ in industrial scale with the advantage of avoiding wash out of cells. The minimum amount of biosorbent with same cell concentration produce better adsorption capacity than larger amount of biosorbent. Further optimization studies are required in this aspect to confirm the optimum adsorbent requirement.

5. REFERENCES

Bai, R.S. and T.E. Abraham, 2003. Studies on chromium (VI) adsorption-desorption using immobilized fungal biomass. Bioresour. Technol., 87: 17-26. DOI: 10.1016/S0960-8524(02)00222-5

Banat, I.M., P. Nigam, D. Singh and R. Marchant, 1996. Microbial decolorization of textile-dye-containing effluents: A review. Bioresour. Technol., 58: 217-227. DOI: 10.1016/S0960-8524(96)00113-7

Beolchini, F., F. Pagnanelli, L. Toro and F. Veglio, 2003. Biosorption of copper by \(Sphaerotilus \text{natans}\) immobilised in polysulfone matrix: Equilibrium and kinetic analysis. Hydrometallurgy, 70: 101-112. DOI: 10.1016/S0304-386X(03)00049-5

Boyd, G.D., E. Buehler, F. Storz and J. Wernick, 1972. Linear and nonlinear optical properties of ternary \(A^\text{II}B^\text{IV}C^2\text{V}\) chalcopyrite semiconductors. IEEE J. Quantum Electron., 8: 419-426. DOI: 10.1109/JQE.1972.1076982

Crini, G., 2006. Non-conventional low-cost adsorbents for dye removal: A review. Bioresour. Technol., 97: 1061-1085. DOI: 10.1016/j.biortech.2005.05.001

Deveci, T., A. Unayayr and M.A. Mazmanci, 2004. Production of Remazol Brilliant Blue R decolourising oxygenase from the culture filtrate of \(Fumaria \text{trogii}\) ATCC 200800. J. Mol. Catal B: Enzyme, 30: 25-32. DOI: 10.1016/j.molcatb.2004.03.002

Eccles, H., 1995. Removal of heavy metals from effluent streams-why select a biological process? Int. Biodeterior. Biodegrad., 35: 5-16. DOI: 10.1016/0964-8305(95)00044-6

Fu, Y. and T. Viraraghavan, 2003. Column studies for biosorption of dyes from aqueous solutions on immobilized \(Aspergillus \text{niger}\) fungal biomass. Water SA., 29: 465-472.

Goncalves, I., A. Gomes, R. Bras, M.I.A. Ferra and M.T.P. Amorim \textit{et al}, 2000. Biological treatment of effluent containing textile dyes. J. Coloration Technol., 116: 393-397. DOI: 10.1111/j.1478-4408.2000.tb00016.x

Heinfling, A., M.J. Martinez, A.T. Martinez, M. Bergbauer and U. Szewzyk, 1998. Transformation of industrial dyes by manganese peroxidases from \(Bjerkandera \text{adusta}\) and \(Pleurotus \text{eryngii}\) in a manganese independent reaction. Applied Environ. Microbiol., 64: 2788-2793.

Hu, M.Z.C. and M. Reeves, 1997. Biosorption of uranium by \(Pseudomonas \text{aeruginosa}\) strain CSU immobilized in a novel matrix. Biotechnol. Prog., 13: 60-70. DOI: 10.1021/bp9600849

Juang, R.S., R.L. Tseng, F.C. Wu and S.J. Lin, 1996. Use of chitin and chitosan in lobster shell wastes for colour removal from aqueous solutions. J. Environ. Sci. Health A, 31: 325-338. DOI: 10.1080/10934529609376360

Kariminciaae-Hamedaaei, H.R., A. Sakurai and M. Sakakibara, 2007. Decolorization of synthetic dyes by a new manganese peroxidase producing white rot fungus, \(Dyes \text{Pigm.}\), 72: 157-162. DOI: 10.1016/j.dypeig.2005.08.010

Khoo, K.M. and Y.P. Ting, 2001. Polyvinyl alcohol as an immobilization matrix-a case of gold biosorption, Water Sci. Technol., 43: 17-23. PMID: 11443959

Malik, A., 2004. Metal bioremediation through growing cells. Environ. Int., 30: 261-278. DOI: 10.1016/j.envint.2003.08.001

Mishra, G. and M. Tripathy, 1993. A critical review of the treatments for decolourization of textile effluent. Colourage, 40: 35-38.

Nigam, P., G. Armour, I.M. Banat, D. Singh and R. Marchant, 2000. Physical removal of textile dyes and solid state fermentation of dye adsorbed agricultural residues. Bioresour. Technol., 72: 219-226. DOI: 10.1016/S0960-8524(99)00123-6

Novotny, C., N. Dias, A. Kapanen, K. Malachova and M. Vandrovcova \textit{et al}, 2006. Comparative use of bacterial, algal and protozoan tests to study toxicity of azo- and anthraquinone dyes. Chemosphere, 63: 1436-1442. DOI: 10.1016/j.chemosphere.2005.10.002
O’Neill, C., R.H. Freda, L.H. Dennis, D.L. Nidia and M.P. Helena et al., 1999. Colour in textile effluents—sources, measurements, discharge contents and simulation: A review. J. Chem. Technol. Biotechnol., 74: 1009-1018. DOI: 10.1002/(SICI)1097-4660(199911)74:11<1009::AID-JCTB153>3.0.CO;2-N

Prakasham, R.S., J.S. Merrie, R. Sheela, N. Saswathi and S.V. Ramakrishna, 1999. Biosorption of chromium VI by free and immobilized Rhizopus arrhizus. Environ. Pollut., 104: 421-427. DOI: 10.1016/S0269-7491(98)00174-2

Veglio, F. and F. Beolchini, 1997 Removal of metals by biosorption: A review. Hydrometallurgy, 44: 301-316. DOI: 10.1016/S0304-386X(96)00059-X

Vijayaraghavan, K. and Y.S. Yun, 2007. Chemical modification and immobilization of Corynebacterium glutamicum for biosorption of Reactive black 5 from aqueous solution. Ind. Eng. Chem. Res., 46: 608-617. DOI: 10.1021/ie061158g

Vijayaraghavan, K. and Y.S. Yun, 2008a. Bacterial biosorbents and biosorption. Biotechnol. Adv., 26: 266-291. DOI: 10.1016/j.biotechadv.2008.02.002

Vijayaraghavan, K. and Y.S. Yun, 2008b. Biosorption of C.I. Reactive Black 5 from aqueous solution using acid-treated biomass of brown seaweed Laminaria sp. Dyes. Pigm., 76: 726-732. DOI: 10.1016/j.dyeppig.2007.01.013

Vijayaraghavan, K., J. Mao and Y.S. Yun, 2008a. Biosorption of methylene blue from aqueous solution using free and polysulfone-immobilized Corynebacterium glutamicum: Batch and column studies. Bioresour. Technol., 99: 2864-2871. DOI: 10.1016/j.biortech.2007.06.008

Vijayaraghavan, K., M.W. Lee and Y.S. Yun, 2008b. A new approach to study the decolorization of complex Reactive bath effluent by biosorption technique. Bioresour Technol., 99: 5778-5785. DOI: 10.1016/j.biortech.2007.10.012

Volesky, B., 2001. Detoxification of metal-bearing effluents: Biosorption for the next century. Hydrometallurgy, 59: 203-216. DOI: 10.1016/S0304-386X(00)00160-2

Xiangliang, P., W. Jianlong and Z. Daoyong, 2005. Biosorption of Pb (II) by Pleurotus ostreatus immobilized in calcium alginate gel. Process. Biochem., 40: 2799-2803. DOI: 10.1016/j.procbio.2004.12.007

Yan, G. and T. Viraraghavan, 2001. Heavy metal removal in a biosorption column by immobilized M. rouxii biomass. Bioresour. Technol., 78: 243-249. DOI: 10.1016/S0960-8524(01)00020-7