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Decolorization of Reactive Dyes by Immobilized Bacterial Cells from Textile Effluents

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A B S T R A C T

Textile effluent is said to be one of the major sources of water pollution which contains various chemicals and hazardous wastes that contaminate the receiving water bodies. This study was conducted to study the efficiency of immobilized *P.putida* and *B.licheniformis* on decolorization of reactive dyes (RR 195, RO 72, RY 17, RB 36). Immobilization was done using sodium alginate and polyacrylamide gel beads both at static and shaken conditions. Sodium alginate immobilized bacteria exhibited maximum decolorization than that of polyacrylamide gel bead method.

Introduction

The quality of life on earth is directly or indirectly linked to the overall quality of the environment. Due to rapid increase in population, there is vast increase in textile industries which has shown a significant use of synthetic complex organic dyes as the coloring material. It has been found that these industries discharge around 28,000 tons of dyes worldwide every year into the environment (Tom Sinoy *et al.*, 2011) Out of which a large number of dyes are azo compounds (-N=N-) linked by an azo bridge and are used by a number of industries because of their cost effectiveness in synthesis compared to natural dyes (Saratale *et al.*, 2010).

Azo dyes are widely used in food, pharmaceutical, paper, printing, leather and cosmetics (Asamudo *et al.*, 2005). These represent the largest and most versatile class of dyes which accounts for more than 50% of the dyes produced annually. Taking into account the volume and composition of effluents, the textile wastewater is rated as the most polluting among all in industrial sectors (Sen & Demirer, 2003). The wastewater released from textile industry is a complex mixture of many polluting substances comprising a wide range of heavy metals and organochlorides based waste pesticides (Correia *et al.*, 1994).

The effluents from textiles significantly affect the photosynthetic activity of aquatic life by impeding light penetration, damages the quality of the receiving streams thus disturbing the food chain of aquatic ecosystem (Aksu *et al*., 2007). In recent times, government legislation is becoming more stringent especially in developed/developing countries regarding the removal of dyes from industrial effluents. There are many physico-chemical techniques in removal of color from wastewater such as coagulation, flocculation, membrane filtration, precipitation, adsorption etc. But these methods have their own drawbacks of being economically unfeasible, unable to remove the recalcitrant azo dyes inturn generating a significant amount of sludge that may cause secondary pollution problems (Anjaneyulu *et al*., 2005). The biological decolorization and degradation of synthetic dyes has been of considerable interest since it is eco-friendly, cheap and produces less amount of sludge (Moosvi *et al*., 2005; Kalyani *et al.*, 2009). The ubiquitous nature of microorganisms makes them an invaluable tool in treatment of wastewater from industries (Olukanni *et al.*, 2006)

Recently the application of immobilized cells for biosorption of dyes has been gaining attention in the field of wastewater decolorization. Many researchers have studied the effect of immobilized whole cells and enzymes on decolorization characteristics since immobilization provides distinct stability over free cells (Ha *et al*., 2009). Immobilization of the microbial cells offers a great potential in various bioremediation processes. Intensive research has been carried out in the field of immobilization of cells since last two decades. Therefore the present study is directed to investigate the decolorization ability of free and immobilized cells of *B.licheniformis* and *P.putida* on reactive azo dyes.

Materials and Methods

Sample Site

The samples were collected from textile effluent water from Erode effluent discharge points located in Kaveri river, Karunkalpalayam, India.

Sample Collection

Samples were collected in screw capped sterilized bottles aseptically and transported to the laboratory in an ice bucket.

Dyes and Media

To see the degrading efficiency of isolated bacteria, four different commercially available dyes namely Reactive Red 195, Reactive Yellow 17, Reactive Orange 72, and Reactive Blue 36 were used in this study. All chemicals and dyes were of highest purity and of analytical grade.

Isolation of Bacteria

Bacillus licheniformis and *Pseudomonas putida* were isolated from the textile effluent samples. Bacteria were isolated through serial dilution and streak plate method on Nutrient Agar. Identification of bacteria was done by colony, cellular morphological characters and performing biochemical tests following Bergey's manual of Systematic Bacteriology.

Decolorization by Immobilized Whole Bacterial Cells

In the present study, the efficiency of isolated bacteria to decolorize the reactive dyes RR 195, RO 72, RY 17, and RB 36

immobilized on polyacrylamide gel beads and sodium alginate were evaluated. Initially, percentage of degradation of bacterial isolates against the four reactive dyes was studied in liquid media. *P.putida* against RR 195 and RO 72 showed efficient decolorization. *B.licheniformis* produced significant color reduction with RY 17 and RB 36.

Hence immobilization studies were carried out with *P.putida* (RR 195 and RO 72) and *B.licheniformis* (RY 17, RB 36) The dye decolorizing potential was determined at both static (0 rpm) and agitated conditions (50 rpm, 100 rpm). Immobilization of the isolate was carried out using 4% sodium alginate adopting the method of Bettman & Rehm (1984). Polyacrylamide gel entrapment of cells was performed according to the method of Jonathan, 1988.

Results and Discussion

P.putida and *B.licheniformis* were identified and isolated from textile effluent sample. The efficiency of immobilized bacterial cells to decolorize the reactive dyes was investigated at static and agitated conditions.

Table 1 shows that at static conditions *P.putida* against RR 195 showed 91% degradation. Sodium alginate embedded cells gave maximum decolorization (96.59%) than polyacrylamide immobilization (93.58%). At 50 and 100 rpm the decolorizing activity gradually decreased.

Table 2 shows the percentage of decolorization of RO 72 by immobilized *P.putida*. Above 90% degradation of RO 72 was recorded at static conditions by free cells. Under agitated conditions of 50 rpm the rate of decolorization decreased gradually. It was noted that sodium alginate immobilized cells gave maximum color reduction compared to polyacrylamide. There was a decrease in decolorizing activity when immobilization was done at 100 rpm.

Table.1 Decolorization of RR 195 by Immobilized *P.Putida*

Immobilization types	Relative specific decolorization rate (%)		
	0 rpm (static)*	50 rpm*	100 rpm*
Free cells	91.80±0.66	29.66±1.73	25.00±1.20
PAA immobilized cells	93.58±0.70	67.33±1.76	61.66±4.72
SA immobilized cells	96.59±0.53	84.33±2.33	62.00±2.33

***** Values represent mean of triplicate experiments along with standard error (Mean ± SE)

Table.2 Decolorization of RO 72 by Immobilized *P.Putida*

Types	Relative specific decolorization rate (%)		
	0 rpm (static)*	50 rpm*	100 rpm*
Free cells	92.07±0.81	30.00±4.04	30.33±1.45
PAA immobilized cells	93.01±0.46	66.00±6.02	42.00±4.35
SA immobilized cells	96.40±0.95	87.66±2.40	51.00±7.93

* Values represent mean of triplicate experiments along with standard error (Mean ± SE)

Table.3 Decolorization of RY 17 by Immobilized *B.Licheniformis*

Types	Relative specific decolorization rate (%)		
	0 rpm (static)*	50 rpm*	100 rpm*
Free cells	85.03±0.84	34.33±0.66	32.66±2.02
PAA immobilized cells	88.63±0.50	71.66±0.88	48.66±3.17
SA immobilized cells	91.66±0.58	87.00±2.00	55.66±5.17

***** Values represent mean of triplicate experiments along with standard error (Mean ± SE)

Table.4 Decolorization of RB 36 by Immobilized *B.Licheniformis*

Type of immobilization	Relative specific decolorization rate (%)		
	0 rpm (static)*	50 rpm*	100 rpm*
Free cells	90.77±0.53	39.33±1.76	36.66±1.20
PAA immobilized cells	92.12±0.76	78.33±2.40	55.33±1.85
SA immobilized cells	96.99±1.43	84.66±1.66	62.33±2.96

***** Values represent mean of triplicate experiments along with standard error (Mean ± SE)

In recent times, the application of immobilized cell has been receiving increased attention in the field of wastewater decolorization since this method not only simplifies separation and recovery of immobilized bacteria and the binding agent but also makes the application reusable, which reduces the overall cost. On the whole, immobilized cells are more tolerant to local perturbations like changes in temperature, pH and presence of inhibitor compounds (Tallur *et al.*, 2009). It has been stated that sodium alginate is a suitable matrix material because it is non-toxic and the method used for its gelation is mild towards the microorganisms (Sriamornsak, 1998).

Similar trend was observed with immobilized *B.licheniformis* against RY 17 and RB 36 (Tables 3 and 4). In all cases, sodium alginate immobilized cells showed maximum color reduction and static conditions were found suitable for decolorization than that of 50 and 100 rpm. This was similar to the results obtained by Satheesh *et al.*, 2013 in which significance of static conditions for better decolorization has been highlighted.

To conclude, the present study reveals that *P.putida* and *B.licheniformis* immobilized in sodium alginate at static condition is promising for its application in biodegradation of the reactive dyes in textile effluents.

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