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Decolorization of Ranocid Fast Blue Dye by Bacterial Consortium SV5

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Abstract

Synthetic dyes are not uniformly susceptible to degradation in conventional wastewater treatment processes. A number of biotechnological processes have been suggested as of potential interest in combating these pollutants in an ecofriendly manner. We determined the optimal parameters necessary for the bacterial consortium SV5 to decolorize Ranocid Fast Blue dye. The best results were obtained with a 0.1% (w/v) concentration of both starch and yeast extract supplemented in Bushnell Hass Medium under static conditions at a temperature of 37°C in less than 24 h with an initial dye concentration of 100 ppm.

Index Entries: Ranocid Fast Blue dye; decolorization; bacterial consortium; biomass; microorganisms.

Introduction

A great variety of synthetic dyes are used for textile dyeing and other industrial applications. More than 7×10^5 t and approx 10,000 dyes are produced annually worldwide, of which 10–15% is lost in industrial effluents *(1)*. This discharge causes environmental problems. These dyes are poorly biodegradable because of their structures *(2)*. A number of physicochemical methods, such as adsorption, coagulation, precipitation, filtration, and oxidation, have been used to treat dyestuff effluents, but these methods have many limitations. It is, therefore, important to develop efficient and cost-effective methods for the decolorization and degradation of dyes present in industrial effluents and contaminated soil *(3)*.

Several dye-degrading microorganisms have been reviewed *(4–8)*. Remediation of dye-containing wastewaters by different microorganisms

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appears to be highly attractive. In recent years, a number of studies have focused on microorganisms, which are able to biodegrade and absorb dyes in wastewaters. A wide variety of microorganisms capable of decolorizing a wide range of dyes includes bacteria, fungi, and algae *(9)*.

In this article, we summarize our studies on the bacterial decolorization of Ranocid Fast Blue (RFB), a diazo dye that is heterogeneously soluble in water, is acidic in nature, and has a λ_{max} of 544 nm. We also report the optimization parameters required for the bacterial consortium SV5 to decolorize the dyes efficiently in a short period.

Materials and Methods

Isolation of RFB Dye-Decolorizing Bacteria

Dye-containing soil was collected from the waste-dumping site of an industrial area in Vatva, Gujarat, India where the effluent from the dyeing industry was the major source of wastewater. A soil suspension was made and the mixture was serially diluted. Aliquots of 0.1 mL were spread onto Bushnell Hass Medium (BHM) agar plates supplemented with 0.1% (w/v) each of glucose and yeast extract and 100-ppm of the dye RFB. The BHM comprised 0.2 g/L of $MgSO_4$, 0.02 g/L of $CaCl_2$, 1.0 g/L of KH_2PO_4, 1.0 g/L of $(NH_4)_2NO_3$ and 0.05 g/L of $FeCl_3$ with a pH of 7.0. All the plates were incubated at 37°C for 3 d. The plates were, however, sealed with Parafilm to create a microaerophilic condition. Thirty bacterial isolates, which showed zone of decolorization, were selected for further studies.

Decolorization of RFB Dye by Selected Bacteria in Liquid Medium

Among the 30 isolates, seven different bacterial strains showing a higher zone of RFB dye decolorization were selected. Stock cultures of these bacterial isolates were prepared by growing them in 250-mL Erlenmeyer flasks containing 100 mL of BHM supplemented with 0.1% (w/v) each of glucose and yeast extract. Aliquots of 10% (v/v) of these isolates were inoculated in 250-mL Erlenmeyer flasks containing 100 mL of medium with 100 ppm of RFB dye. The concentration of the dye was determined by measuring the absorbance of the culture supernatant at absorbance maxima (λ_{max} = 544 nm) of RFB dye using a UV Visible Diode Array Scanning Spectrophotometer (Helwett Packard 8452A). The structure of the RFB dye is shown in Fig. 1. Growth was determined by measuring the absorbance of the culture at 660 nm using a Spectronic 20D+ (Milton Roy) spectrophotometer. The bacterial isolates SV5A, SV5B(a), and SV5B(b) when combined formed a bacterial consortium, SV5, that showed a faster rate of dye decolorization and was thus chosen for further studies. All the experiments were performed in triplicate, and mean values of the results were used to interpret the data. Gram staining of the bacterial isolates was carried out to determine the nature of these microorganisms.

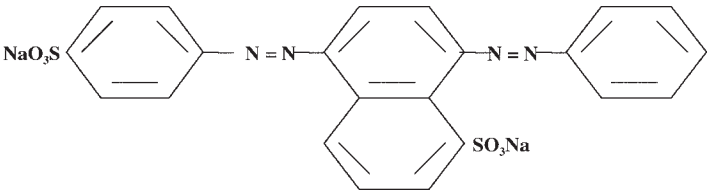

Fig. 1. Structure of RFB dye.

Effect of Static and Shaking Conditions

Stock cultures of the selected bacterial consortium were prepared by growing them in 250-mL Erlenmeyer flasks containing 100 mL of medium. Ten percent (v/v) of the overnight grown culture was inoculated in 250-mL Erlenmeyer flasks containing 100 mL of medium with a dye concentration of 100 ppm. The flasks were incubated under static and shaking conditions at 37°C. Aliquots were removed at different time intervals and analyzed to determine the effect on growth and decolorization.

Effect of Carbon and Nitrogen Sources

Different carbon sources—glucose, galactose, glycerol, sucrose, starch, sorbitol, maltose, mannitol, carboxymethylcellulose (CMC), and lactose—were supplemented in BHM at a concentration of 0.1% along with 100 ppm of dye and 0.1% (w/v) yeast extract to determine their effect on dye decolorization. The different nitrogen sources tested were yeast extract, beef extract, peptone, proteose peptone, ammonium sulfate, and ammonium chloride.

Effect of Temperature and pH

Procedures to determine the effects of temperature and pH on RFB decolorization and cell mass of culture of the bacterial consortium were similar to those described above. The cultures were incubated at 25, 30, 37, 40, and 50°C to determine the effect of temperature. Decolorization was also studied at different pH values. The pH of the medium was adjusted in the range of 3.0–10.0 with an increment of 1.0.

Effect of Dye Concentration

The concentration of the RFB dye was varied from 100 to 500 ppm with a difference of 50 ppm to determine the amount of dye that could be decolorized by the bacterial consortium SV5.

Effect of Different Dyes

The bacterial consortium SV5 was tested for its ability to decolorize various other dyes: Acid Red 119, Reactive Blue 4, Reactive Blue 19, Acid Blue 45, Acid Red 5b, Acid Orange 3, Acid Black 10 BX, Acid Black 210, and Acid Red 97.

Effect of NaCl Concentration

The bacterial consortium SV5 was tested for its salt tolerance capacity in the decolorization of the RFB dye. The salt concentration was varied from 0.5 to 5.0% (w/v).

Effect of Inoculum Concentration

Experiments were conducted to determine the best inoculum concentration for decolorizing the RFB dye at a faster rate and within a short period of time. Concentrations of 5, 10, 15, and 20% (v/v) of the bacterial consortium SV5 were inoculated in the BHM supplemented with 0.1% (w/v) each of starch and yeast extract.

Results and Discussion

Decolorization on Solid Medium

The present study focused on the isolation and optimization of conditions of a bacterial consortium capable of decolorization of an azo dye, RFB. Soil samples collected from various sites in the vicinities of dyestuff-manufacturing units located in Vatva, Gujarat, India were used to isolate dye-decolorizing bacteria by applying a soil suspension on BHM agar containing 100 ppm of RFB dye and supplemented with glucose and yeast extract. Thirty bacterial isolates capable of decolorizing RFB on the BHM agar dye plates were obtained. Among these, seven bacterial strains showed a higher zone of decolorization.

Decolorization of RFB in Liquid Medium

The seven bacterial cultures were subjected to further screening for their ability to decolorize the dye in liquid medium. Three cultures, SV5A, SV5B(a), and SV5B(b), exhibited dye decolorization at a faster rate when incubated under static conditions. No decolorization was noticed under shaking conditions, indicating a microaerophilic or facultative anaerobic nature of the microorganisms. Therefore, only these bacterial isolates were selected for further studies. Various combination studies among these bacterial isolates showed that when all three isolates were combined, they formed a consortium, SV5, that exhibited maximum dye-decolorizing ability (Fig. 2). It has been suggested that a mixed microbial community is required to mineralize azo dyes completely through a combined metabolism *(10–12)*.

Visible sorption of the dye by the biomass was not observed, and no dye was recovered on homogenization of biomass resuspended in various solvents (data not shown), thus indicating that the decolorization was owing to biotransformation. Similar results were also observed in earlier studies carried out in our laboratory *(13)*. Gram-staining results showed that the cultures were all Gram-positive and exhibited sugar fermentation.

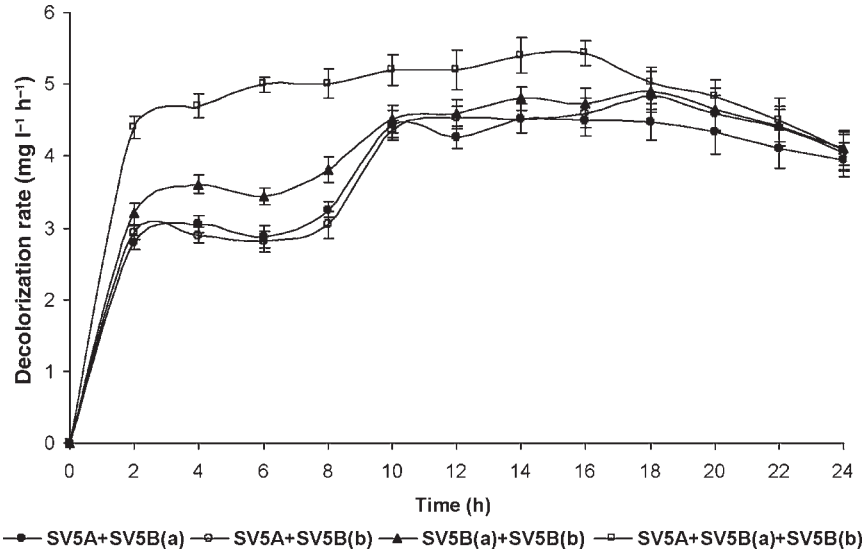

Fig. 2. RFB dye decolorization profile of various combinations of bacterial isolates at 37°C.

Effect of Static and Shaking Conditions

When the bacterial consortium was tested for its dye-decolorizing ability under static and shaking conditions, it was found that under shaking conditions these isolates were incapable of decolorizing the dye, whereas under static conditions (facultative anaerobic condition), there was full decolorization of RFB dye (Fig. 3). Azoreductase-driven bacterial decolorization of azo dyes is inhibited by the presence of oxygen primarily owing to the competition in the oxidation of reduced electron carriers (e.g., NADH) with either oxygen or azo groups as the electron acceptor *(14)*. In static incubation, only a trace amount of oxygen was transferred, probably onto the broth surface, and, thus, the cells mostly sedimented to the bottom of the flasks were likely to undertake decolorization under anaerobic conditions. It should also be pointed out that decolorization of azo dyes cannot take place in extremely anaerobic conditions under oxygen-free nitrogen sparging *(10)*. This indicates that bacteria may still need a small amount of oxygen to maintain basic cellular activity for decolorization.

Effect of Carbon and Nitrogen Sources

Of all the carbon sources tested for the optimal conditions for maximum dye decolorization, it was found that a 0.1% (w/v) concentration of soluble starch gave maximum decolorization at a faster rate, followed by glucose (Fig. 4). Fu et al. *(15)* suggest that glucose is a readily biodegradable carbon and energy source and that sufficient glucose promotes microbial proliferation, thus producing more bacterial cells capable of removing

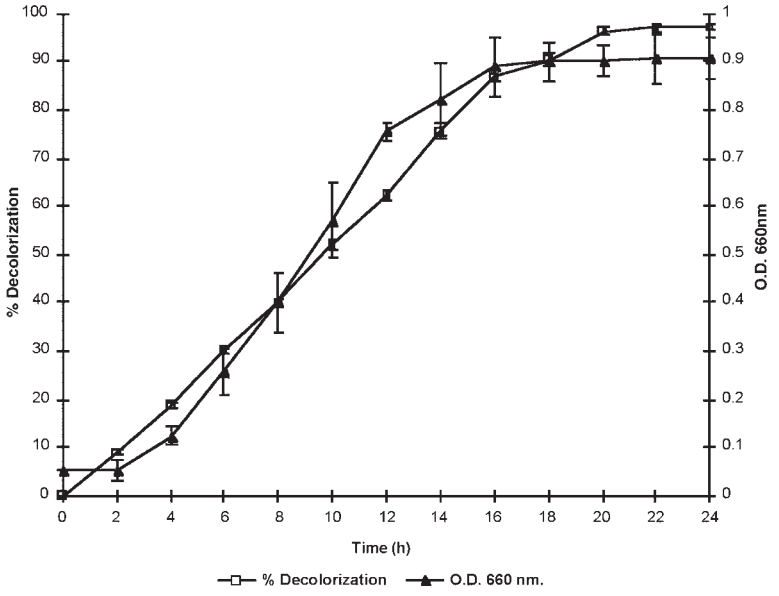

Fig. 3. Decolorization profiles and growth pattern of bacterial consortium SV5 at 37°C.

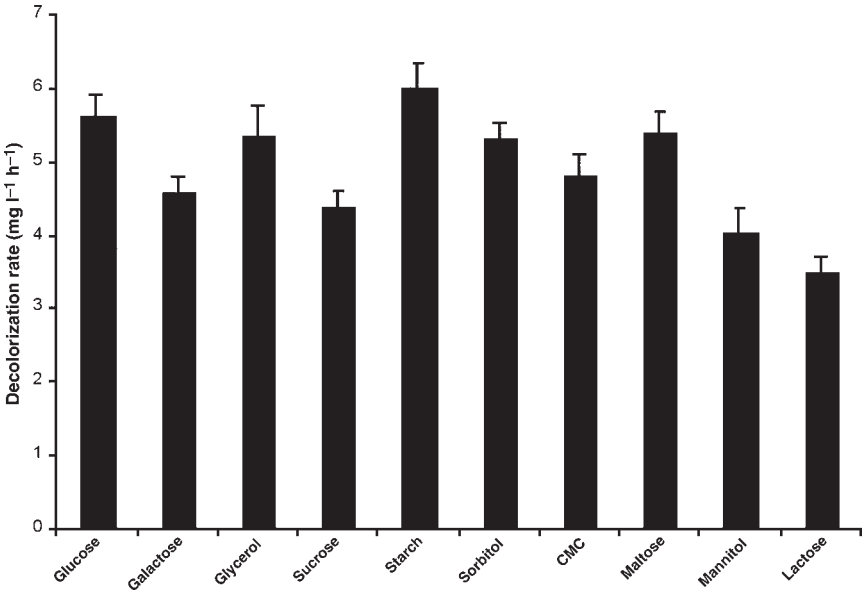

Fig. 4. Effect of different carbon sources on decolorization of RFB dye by bacterial consortium SV5.

Reactive Blue 21 (RTB). Another possible effect of glucose was that it could enhance microbial activity including biosorption and even utilization of RTB. However, our results showed that when starch was supplemented as

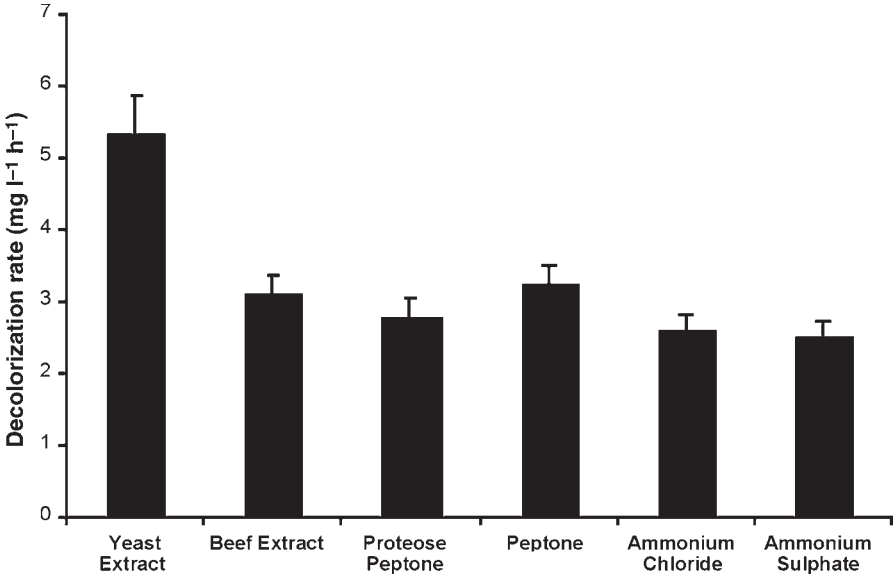

Fig. 5. Effect of different nitrogen sources on decolorization of RFB dye by bacterial consortium SV5.

a carbon source in BHM, the bacterial consortium showed a faster removal of RFB. Reports have also shown that decolorization of synthetic textile dye effluent was best in carbon- and nitrogen-sufficient medium along with a twofold increase in biomass *(16)*. In our studies, of the various organic and inorganic nitrogen sources used, yeast extract was the best nitrogen source for efficient decolorization of RFB by the SV5 bacterial consortium (Fig. 5). This may be owing to the metabolism of yeast extract, which is considered essential for the regeneration of NADH *(17)*.

Effect of Temperature and pH

Various microorganisms showed their survival at various temperatures ranging from 25 to 50°C. At 37°C the bacterial consortium showed maximum decolorization of RFB, followed by at 30°C (Fig. 6). This may be owing to a greater production of enzymes and optimal growth conditions of the consortium for its dye-decolorizing ability. The decolorization at this optimal temperature may be owing to higher respiration and substrate metabolism. This also demonstrates that decolorization of the dye was through the microbial reaction, which relies on optimal temperature, and not by adsorption, where the temperature effect was not great *(18)*. A comparison of decolorization of RFB at various pH levels by the SV5 bacterial consortium is presented in Fig. 7. A faster and efficient decolorization of RFB occurred at an optimal pH of 7.0. It has been indicated that neutral pH would be more favorable for decolorization of the azo dye *(19)*.

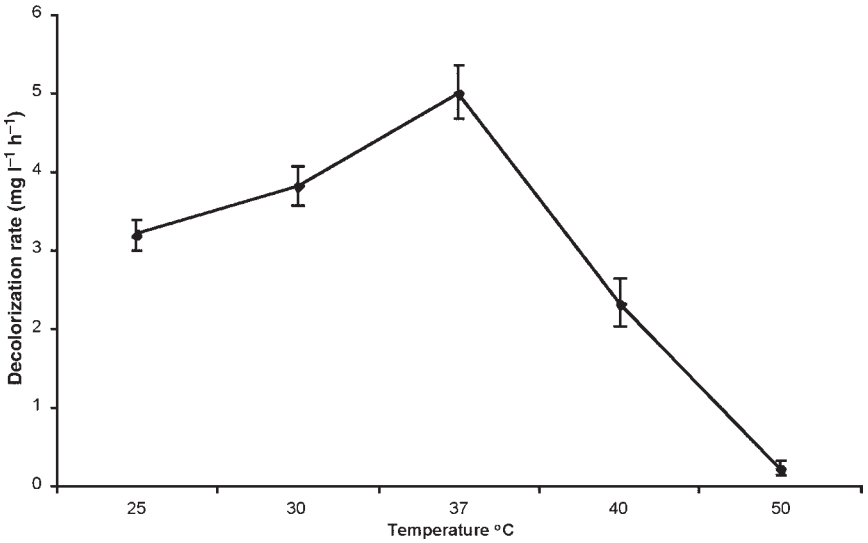

Fig. 6. Effect of temperature on decolorization of RFB dye by bacterial consortium SV5.

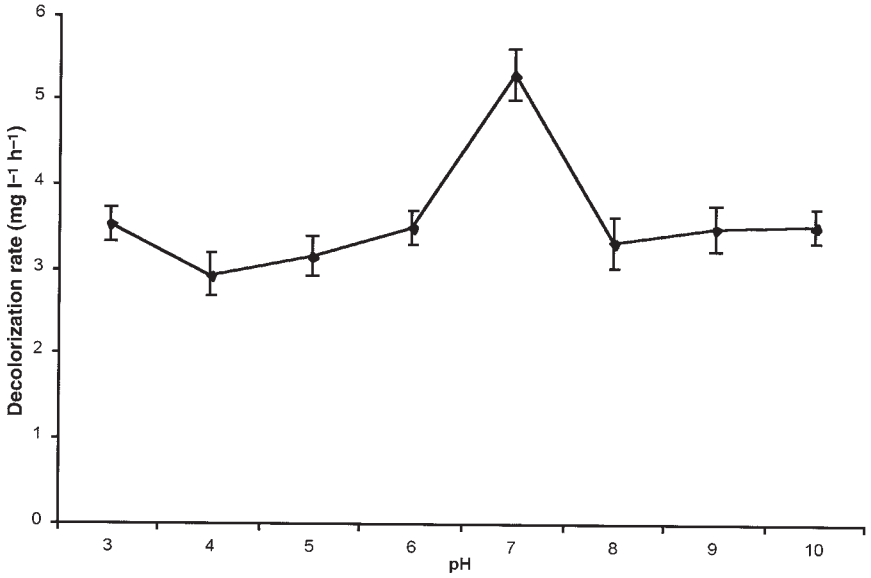

Fig. 7. Effect of pH on decolorization of RFB dye by bacterial consortium SV5.

Effect of Dye Concentration

Although the bacterial consortium under study was capable of decolorizing RFB up to an initial concentration of 500 ppm, it was found that the optimal initial dye concentration for faster decolorization was 100 ppm (Fig. 8).

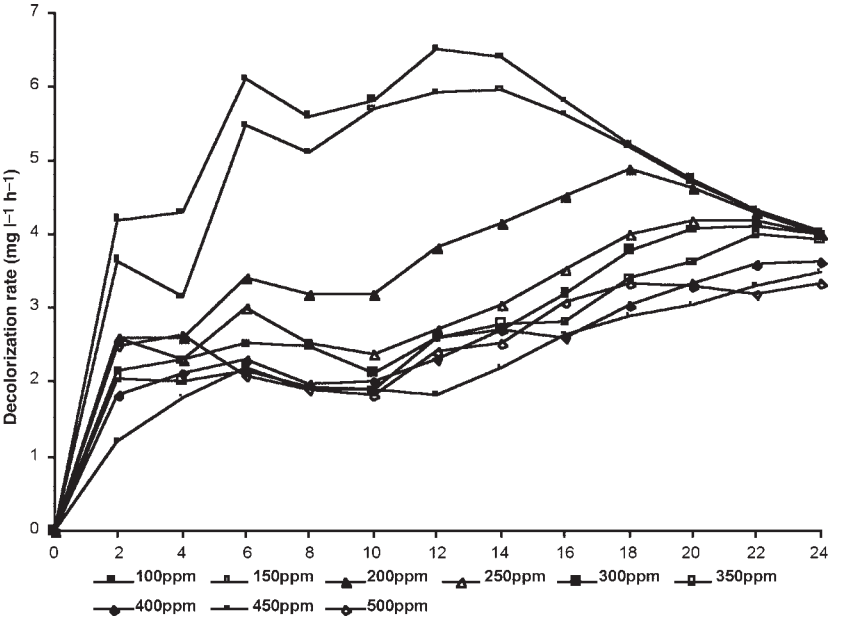

Fig. 8. Decolorization of RFB dye at different initial dye concentrations by bacterial consortium SV5.

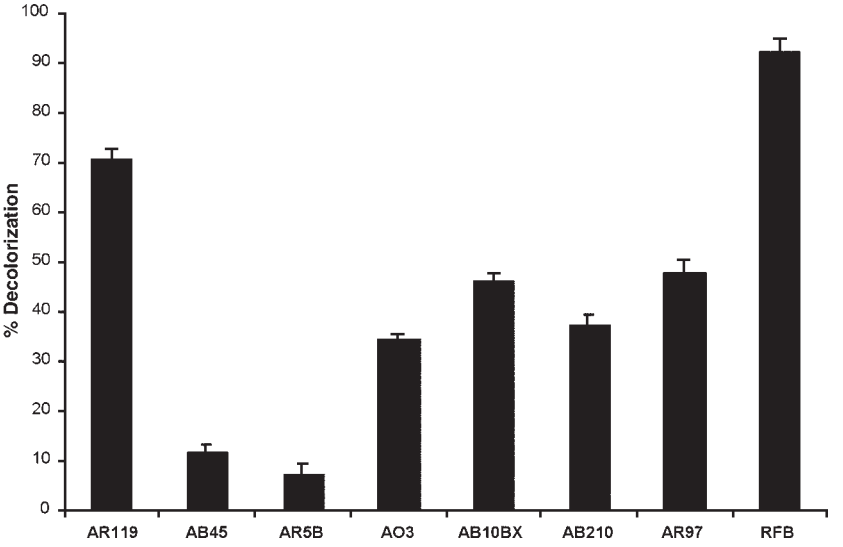

Fig. 9. Decolorization of different dyes by bacterial consortium SV5.

Effect of Different Dyes

The bacterial consortium SV5 could effectively decolorize the dyes RFB and Acid Red 119. The dyes Acid Black 10BX, Acid Black 210, and Acid Red 97 could also be decolorized by the bacterial consortium SV5 but with little efficacy (Fig. 9). The bacterial consortium SV5 could not decolorize the other dyes under study.

Effect of NaCl Concentration

Textile dye effluents have a high salt concentration as their constituent. When the effect of salt tolerance on the bacterial consortium for dye decolorization was tested up to a salt concentration of 5.0% (w/v), there was a steady increase in the rate of decolorization, but excess salt in the medium could also inhibit the microorganism's ability to decolorize the dye. A high salt concentration may also affect the normal metabolic activity of the organism.

Effect of Inoculum Concentration

In order to find out the optimum inoculum needed for faster and higher percentage of decolorization by our consortium, decolorizing ability was tested at different inoculum concentrations ranging from 5 to 20% (v/v). A trend of increase in decolorization was noticed with increase in inoculum concentration. However, beyond 10% (v/v) inoculum size, rate of increase in decolorization was not very significant.

Conclusion

Our study demonstrates that the bacterial consortium SV5 has a very efficient azo dye-decolorizing capability. Studies of the identification and characterization of the bacterial consortium SV5 and the mode of action of decolorization of RFB by these microorganisms are under way.

Acknowledgment

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