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Bacterial consortia for effective decolorization and bio degradation of Anthraquinone dyes

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Abstract

In the present study an attempt was made to examine the potential of bacterial consortia for effective decolourization and degradation of Anthraquinone dyes (Acid blue 25, Reactive blue 19). The effect of culture media composition, pH, temperature and initial concentration of dyes was studied with an aim to determine the optimal conditions required for maximum decolourization and degradation. The Bacterial consortia were used mainly by *Klebsiella* sps, *Acinetobacter* sps, *Citrobacter* sps, *Bacillus cereus* and *Bacillus subtilis*. The most potential combination was tested and selected for further studies. The selected consortium showed maximum decolourization at static conditions as compared to shaking conditions. The optimum pH for decolourization was pH 8 in both the dyes. It shows good decolourization even in pH 7.The optimum temperature was 37^{o}C.The optimum decolourization showed by Acid blue 25 (97%) with in 48 hrs and Reactive blue 19 showed (89%) with in 72 hrs. The optimum conditions are stabilized like static, pH 8, 37^{o}C and 100 ppm initial dye concentration. The results showed that the selected bacterial consortium has good potential in removal of Anthraquinone dyes from effluents under static conditions.

Keywords: Bacterial consortia, textile effluents, Decolourization, Biodegradation, and Anthraquinone dye.

Introduction

Removal of color from dye bearing which was advancement in industrialization spoiling a lot causes pollutants in soil and water environment. Many contaminants present in wastewater, such as acids, bases, toxic organic and inorganic**,** Dissolved solids, and colors. Among them, colors are considered the most undesirable and are mainly caused by dyes. Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade. The textile industry utilizes about 10000 different dyes and pigments the effluents of these industries are highly colored and the disposal of these wastes into receiving waters causes damage to the Environment. Synthetic dyes have many structural varieties such as acid dyes, basic, reactive dyes, dispersive, Azo, Anthraquinone dyes. Anthraquinone based dyes are the most resistant to degrade due to their fused aromatic ring structure. The chromophores in anionic and non-ionic dye are mostly Anthraquinone dyes and azo dyes. Several physical and chemical methods have been used to eliminate the colored effluents in waste water. They are usually expensive and produced large amount of sludge ultimately lead to the formation of unusual products effects the environment. The interest is therefore now focused on the microbial degradation of dyes is a better alternative process. The microorganisms including Bacteria, Fungi and also Algae can degrade and adsorb the wide range of dyes. The biological mode of treatment of dye effluents offers distinct advantages over the conventional modes of treatment. Most importantly biological treatment of dye effluents is eco friendly and causes mineralization

of dyes to simple components which are not lethal. In view of these problems the most potent bacterial cultures was selected in this study for maximum decolourization of Anthraquinone dyes.

Bacterial biodegradation has observed that several bacteria can degrade Anthraquinone dyes. The isolation of potential species and there by degradation is one of the interest in biological aspect of effluents treatment. During the past decade the use of the use of microbiological degradation methods have been under active development in textile and dye stuff industries. Among the most, bacteria are the commonly used for various bioremediation of textile dyes from environment. In the present study an attempt has been made to utilize the common soil bacteria isolated indigenously from textile industry for decolourization of dyes.

Materials and Methods

The textile effluents samples were collected from the discharges of Siera Silk Mills Ltd, located at Bangalore, India, where the effluents were discharged from the industries.

The Anthraquinone textile dyes used was Acid blue 25, Reactive blue 19 supplied by sigma Aldrich chemicals limited. These are water soluble dyes and used in textile industries.

Isolation and screening of dye degrading bacteria:

Isolation of bacterial strains were carried out from textile effluent by enrichment culture technique. 1000ml of Zimmermann medium with 100mg/l of Acid blue 25 & Reactive blue 19 dye was inoculated with 2ml of effluent sample. After 48 hrs of incubation, a loopful of sample from the decolorized broth was streaked on Zimmermann agar plate containing 100mg/l of Acid blue 25 & Reactive blue 19 dyes and colonies showing decolorization zone were selected. Morphological distinct bacterial strains were selected for screening of dye decolorization. Dye degrading bacterial isolates were identified on the basis of morphological and biochemical according to Bergey's manual of systematic Bacteriology. The isolates showing more decolorization of the respective dyes were selected for further studies.

Development of Bacterial consortium:

The isolates obtained from textile effluents are screened and selected for their maximum degradation and decolourization potential of the textile dyes and further by using the different combinations of above isolates were used for the bacterial consortia construction.

Decolourization studies:

The Decolourization experiments were carried out in triplicates and the decolorization activity was performed in 100ml of ZZ medium containing 0.01g of Acid blue 25 & Reactive blue 19 dyes in separate flasks and 24 hrs old cultures of all five bacterial isolates were inoculated in to the medium. Without inoculums served as control. All the decolorization studies were carried at 37^0 C and at pH 7 and were incubated for six days under static conditions and 2ml of the test samples were withdrawn at 24 hours time intervals aseptically from experiment and control media and was further centrifuged at 8,000 rpm for 20 minutes. Decolorization potential was assessed by measuring the absorbance of supernatant with the help of UV-Vis spectrophotometer at wavelengths maxima of respective dyes (Acid blue 25 – 600nm & Reactive blue 19-595nm). Percentage of decolorization was calculated using the following formula.

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

.

Results

Present study was mainly focused on to screen out the potential bacterial strains for their decolorization and biodegradation efficiency on Acid blue 25 and Reactive blue 19 of Anthraquinone dyes. We selected five bacteria cultures isolated from the textile dye effluents for the decolourization studies. Five different bacterial strains were selected and identified based on the morphology, physiology & biochemical characterization (**Table-1)** as *klebsiella* sps, *Acinetobacter* sps, *Citrobacter* sps, *Bacillus cereus & Bacillus subtilis*. Interestingly all five bacterial isolates were able to degrade the textile anthraquinone dyes. All the bacterial isolates showed confluent growth on selective medium containing dye concentration of 100 ppm of both Acid blue 25 and Reactive blue 19 respectively. So far the decolourization ability of five bacterial isolates were investigated by using Acid blue 25 and Reactive blue 19 anthraquinone dyes. With Acid blue 25 the maximum decolourization extent was observed with *Klebsiella* sps (92.11%) within 48hrs of incubation followed by *Acinetobacter*

Table 1: Biochemical characteristics of bacterial isolates from textile effluents

Test	Isolate-B1	Isolate-B2	Isolate-B3	Isolate-B4	Isolate-B5
Gram' nature	-	-	-	+	+
Shape	Rod	Rod	Rod	Rod	Rod
Indole production	-	-	+	-	-
Methyl red	-	-	+	-	-
Vogues- prausker	+	-	-	+	+
Citrate utilization	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	-
Isolate Identification	*Klebsiella* sps	*Acinetobacter* sps	*Citrobacter* sps	*Bacillus cereus*	*Bacillus subtilis*

+ = positive; - = Negative

(84.48%), *Citrobacter* (89.31%), *Bacillus cereus* (65.05%) and *Bacillus subtilis* (60.13%) within 96 hrs of incubation. Similarly with Reactive blue 19 the decolourization efficacy of the above five bacterial isolates analyzed and the maximum extent of decolorization potential was observed again with *Klebsiella* sps (80.98%), followed by *Acinetobacter* sps (66.14%), *Citrobacter* sps (48.77%), *Bacillus cereus* (76.47%) and *Bacillus subtilis* (59.54%) within 96 hrs of incubation. The results were showed in (**Table 2 & Fig 1)** of both the dyes. From the present investigation the *Klebsiella* sps was proved to be highly potential bacteria for its decolorization efficiency showed with Acid blue 25 and Reactive blue 19. The decolourization efficiency of five bacterial isolates was measured by optical density of both dyes after 24, 48, 72 and 96 hrs time intervals of incubation and the results were recorded in the (**Tables 3, 4 & Fig 2, 3**). Further, it was observed that there was a tremendous decrease in optical density and at the same time high range of decolourization was recorded at 48 hrs with Acid blue and & 72 hrs in Reactive blue 19. In this study, a total of six consortia were developed using combinations of 3 to 5 isolates. Bacterial consortium was constructed by mixing of various combinations of five bacterial isolates. All the six bacterial combinations and their maximum decolorization potential of each combination with their respective decolorization percentages were shown in (**Table 5 & Fig 4).** The bacterial consortium-1 showed 97.29 % of decolourization with Acid blue 25 and 89.31 % with Reactive blue19. Similarly, the consortium-2 showed 90.11% & 84.05% of decolourization the consortium-3 showed 81.74% & 80.57%. The consortium- 4 and 5 was recorded with 66.63% and 67.70% & 64.92% and & 70.37% of decolourization. Since the consortium- 6 showed 55.64% & 68.75% of decolourization for both anthraquinone dyes. While comparing both the individual bacterial isolates and bacterial consortium, it was observed that the different combinations of bacterial consortia showed high decolourization efficacy than the individual bacteria. The maximum decolourization was obtained for the Anthraquinone dyes by bacterial consortium-1 (97% & 89%) and followed by consortium-2, 3 & 5 respectively, which is leading to an enhanced decolourization potential depends on relevant and effective bacterial combinations (**Fig-5).**

Table 2: Percentage of dye decolourization by bacterial isolates

Isolated Bacterial strains	Percentage of Decolourization	
	Acid blue 25	Reactive blue 19
B1	92.11	80.98
B2	84.48	66.14
B3	89.31	48.77
B4	65.05	76.47
B5	60.13	59.54

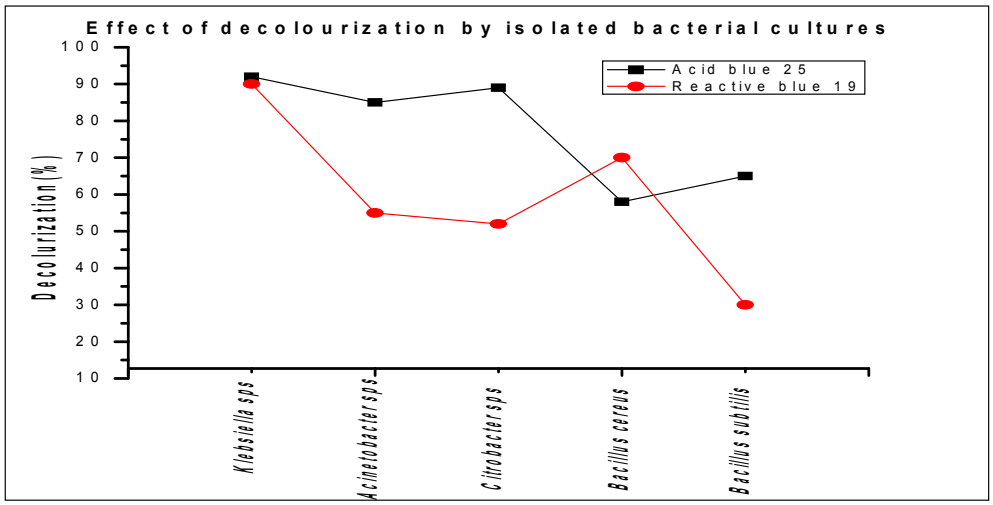

Figure 1: Decolourization of Acid blue 25 & Reactive blue 19 dyes by the bacterial isolates

Table 3: Decolourization of Acid blue 25 dyes by isolated bacterial strains at different incubation period

Isolated Bacteria	Time(hrs) taken for decolourization			
	24	48	72	96
B1	29.47	91.48	92.11	92.11
B2	23.87	49.02	83.98	84.48
B3	36.81	86.92	87.42	89.37
B4	20.71	54.87	64.26	65.05
B5	24.29	58.40	58.92	60.13

Table 4: Decolourization of Reactive blue 19 dyes by isolated bacterial strains at different incubation period

Isolated Bacteria	Time(hrs) taken for decolourization			
	24	48	72	96
B1	21.40	66.34	80.85	80.98
B2	17.96	56.54	58.05	58.97
B3	17.54	43.73	44.52	48.77
B4	9.34	68.31	74.45	76.47
B5	31.47	41.78	54.87	59.54

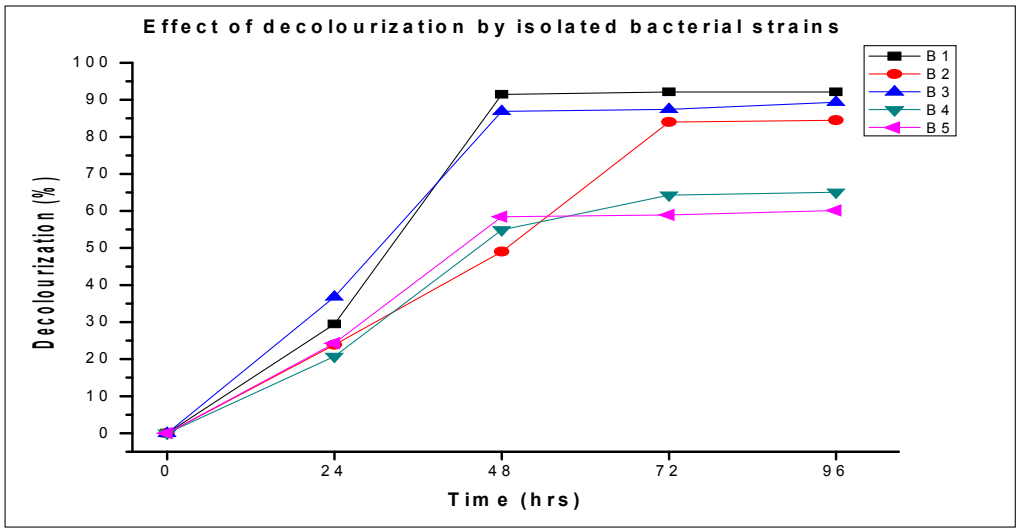

Figure 2: Decolourization of Acid blue 25 dyes by isolated bacterial strains at different time periods

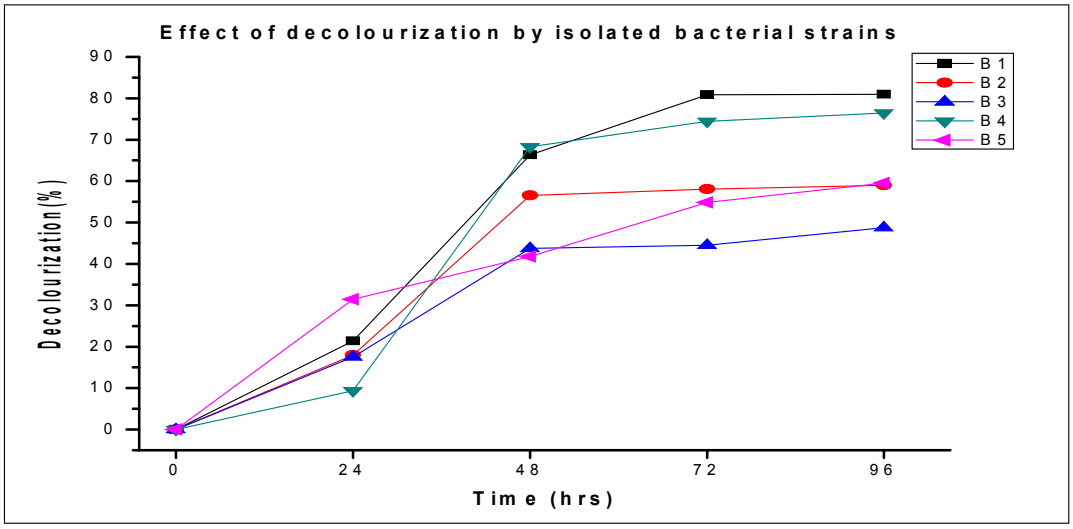

Figure 3: Decolourization of Reactive blue 19 dyes by isolated bacterial strains at different time periods

Table 5: Construction of Bacterial Consortia using isolates and their decolorization percentages

Bacterial Consortium	Bacterial Isolates	Acid blue 25 Decolorization (%)	Reactive blue 19 Decolorization (%)
BC-1	1,2,3,4,5	97.29	89.31
BC-2	1,2,3,4	90.11	84.05
BC-3	2,3,4,5	81.74	80.57
BC-4	2,3,4	66.67	67.92
BC-5	3,4,5	64.92	70.37
BC-6	2,4,5	55.64	68.75

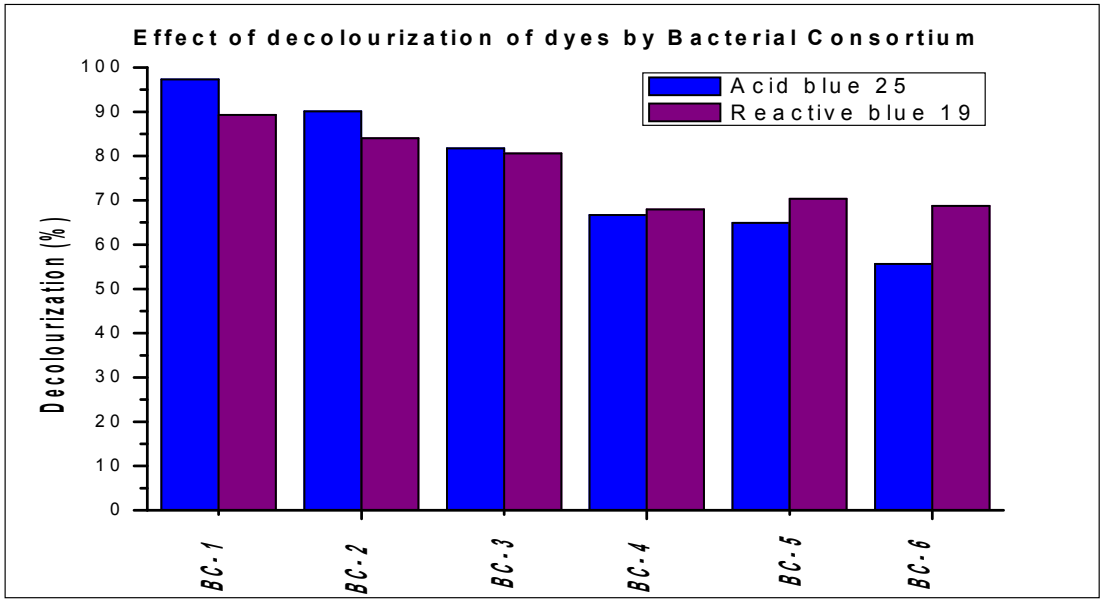

Figure 4: Decolourization of Acid blue 25 & Reactive blue 19 dyes byBacterial Consortium

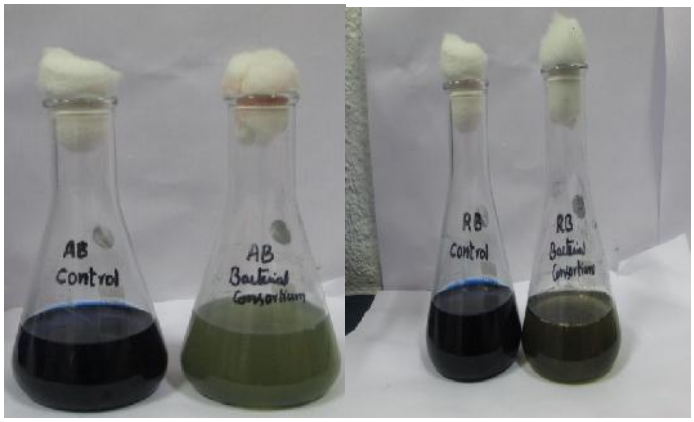

Figure5: Maximum decolourization showed by Bacterial consortium-1(1, 2, 3, 4, 5) by two Anthraquinone dyes

Discussion

The effluents released from the textile industries causes serious threat to ground water & soil, which also affects the human beings where there is a poor sanitation (Jiunkins et al., 1982). The effluents collected from the textile industries consists of toxic chemicals, dyes and also acclimatized with microbial population such as Bacteria, Fungi, Algae & Yeast etc.,. More frequently encountered population was the bacteria can be isolated from the dye amended effluents, sludge & soil was demonstrated by Madamwar D.et al., (2006) & Rashid Mahmood et al., (2012). Five dominated bacterial species were isolated and characterized from the textile effluents and therefore coded them as B1, B2, B3, B4, B5. After though analysis of these species with morphological and biochemical tests with reference to the Bergey's manual (Edition - VIII) and molecular characterization at NCCS, PUNE confirmed these sps (*Klebsiella* sps, *Acinetobacter* sps, *Citrobacter* sps, *Bacillus subtilis*, *Bacillus cereus* respectively).These Five isolates showed maximum decolourization potential of Anthraquinone dyes Acid blue 25, Reactive blue 19 with different combinations). Similarly Kalyani et al., (2009); Wong P, Yuen P (1998); Khalid et al., (2008) were also analyzed the textile dyes for their maximum decolorization and degradation showed very interesting results for azo dyes by *bacillus sps*, *Klebsiella sps* & *Pseudomonas sps*. And further the bacterial consortia was made by different sps and extensively used them for the biotreatment of textile effluents & all the consortia groups showed good results for decolourization. A similar observation was reported by Moosvi et al. (2007) where maximum decolourization of 93% was observed under static condition. Khadijah et al., (2009) developed bacterial consortia for decolourization of Congo red. Rashid Mahmood et al., (2012) & Sivaraj et al., (2011) constructed the bacterial consortium for biotreatment with different sps of *Bacillus* showed optimum decolourization with Azo dyes. Valli Nachiyar et al. (2012) were studied on *Citro bacter* sps it has also been reported maximum decolorizing ability against both Azo & Anthraquinone dyes. Saranjal et al (2010)., isolated five bacterial sps *viz Bacillus subtilis*, *pseudomonas aeurozinosa*, *E.coli*, *Proteus mirabilis* & *Klebsiella pneumoniae* from textile effluents and the extent of colour removal capability varied depending on the dye complexity, nitrogen availability and ligninolytic activity of the bacterial culture used for the study. Deepti Gulati et al., (2014) isolated *Bacillus cereus* & *Klebsiella* sps from the effluents amended by Reactive blue 19. The *Bacillus subtilis* showed 70% decolorization of Anthraquinone dyes and 90% of Azo dyes like Crystal violet (Sapna Kouhu et al., (2011) & Khehra et al (2010). *Acinetobacter* sps showed 84% decolourization of Anthraquinone dyes like Acid blue25, but in similar way other study 85% decolourization showed with Acid red dye (Mohandas Ramya et al; & G. Godakhae et al. (2009). In the optimization at known nitrogen concentration, 90% of the colour was removed within 6 hours, while when excess nitrogen was provided up to 5 days and was required to achieve 63- 93% decolourization of Anthraquinone dyes (Cripps et al (1990)). Haug et al (1991) also described a bacterial consortium capable of mineralizing the sulfonated azo dye mordant yellow. However an alteration from anaerobic to aerobic conditions facilitated to require for complete degradation. There is a need for different members of the consortium required different conditions for optimization & bond cleavage ensured by the reductase enzymes which are mainly functional under anaerobic conditions, A *Rhodococcus* capable of effectively decolorize copper based azo dyes & also by *Actinomycetes* strain (Zhou & Zimmermann (1993). Recently an isolated new bacterial strain,

strain, *Pseudomonas luteola* has the ability to remove colour of reactive dyes such as Red G, RBB, and similarly, the microorganisms like Rp2B & V_2RP has been isolated from the treated sludge of dyeing industry. Hence forth the isolated bacterial strains used for decolourization should have capability to decolorize some of the structurally different dyes. So, Consortium (group) played an important role over single bacterial isolate because of collective effect to degrade and decolorize the dyes (Watanabe & Baker (2000), so that it is essential to remove the textile dye toxicants which are only possible by developing consortia. Therefore the aim of the present work was the isolation of single bacteria and developing different bacterial consortia, and they were formulated for high efficiency of decolourization and degradation under static conditions.

Conclusion

The textile dyes are harm full, toxic and carcinogenic to the environment & living beings. Among the most economically viable choices available for the effluent treatment/decolourization, and the most practical systems are adopted & developed, the biological systems are appear to be the known capable of dealing BOD & COD reduction or removal through conventional aerobic biodegradation. Although decolourization is a challenging process to both the textile industries and Waste water treatment. Present study leads to conclude that the isolates had adequate potential to decolorize the Anthraquinone dyes. Thus the isolates could be more exploited or its bioremediation ability, there is much significant and concerted efforts were still required to establish biological systems for its colour removal. By the development of new and prompted techniques by which the decolourization efficiency was improved and it could be occurs vary and among isolates of their corresponding adsorption capacity that seems to be of great significant choice for future development in bio-removal or bio-recovery of dye substances.

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