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Biodegradation of Novacron Turqueiose (Reactive Blue 21) by *Pseudomonas aeruginosa*

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Summary: In the present study four bacterial strains *Escherichia Coli, Salmonella typhi, Shiegella* and *Pseudomonas aeruginosa* were used to evaluate their dye decolorization/degradation ability. Out of these bacterial strains *Pseudomonas aeruginosa* exhibited high potential for selected dye decolorization and hence it was used in subsequent experiments. The effect of dye concentration, pH, temperature, time, glucose and sodium chloride concentrations on decolorization were also studied to determine the optimal conditions required for maximum decolorization/degradation of selected dye by *Pseudomonas aeruginosa*. Maximum decolorization was observed at: 0.01 mg/L dye concentration, pH 10, temperature 45°C, 0.1 mg/L glucose concentration, 0.1 mg/L sodium chloride concentration and 3 days incubation period at 37°C. The metabolites formed after degradation by selected bacteria at optimum conditions were isolated and characterized by FTIR and mass spectrometry. In the mass spectra molecular ion peak was not observed and it was difficult to draw a conclusion from it. However, FTIR spectra provided some valuable information. The peaks at 1301.8 cm^{-1} for C-N and 1231.5 cm^{-1} for O-H stretch observed for original dye were completely absent in the decolorized products. The disappearance of C-N (part of the porphyrin ring system) peak in FTIR spectra shows that the porphyrin ring has been destroyed by bacteria. The extensive fragmentations in the mass spectra also confirm the degradation of the dye parental structure.

Keywords: Biodegradation; Bacteria; Dye; Metabolites.

Introduction

Dyes are chemical substances used for coloring different materials. The dyes by means of physical adsorption or by the process of mechanical retention can attach to the surface when applied in form of solution/emulsion [1]. They are generally used in paper, food, textile, pharmaceutical, cosmetics, plastics and photographic industries [2-4]. At present more than 10,000 different types of pigments and dyes are used in different industries. Worldwide more than 7,000,000 tons of the synthetic dyes are produced annually [5, 6]. Dyes and pigments are different from each other; dyes are carbon based organic compounds while pigments are normally inorganic compounds which often contain heavy metals [7]. The effluents with high concentration of dyes have the ability to change biological cycles when discharged into aquatic environments by affecting the process of photosynthesis, the oxygenation of the water, high toxicity and carcinogenic and mutagenic potentials [5, 6, 8-10].

Reactive dyes like Novacron Turqueiose are preferably used in textile industries for dyeing of cotton fiber because of their extra ordinary characteristics like better dyeing processing, bright colors and reduction in the energy consumption. These dyes are adsorbed on cellulose and attached to surface of the fibers through covalent bonding. Unfortunately, 15% of the reactive dyes are discharged in the effluents. The difficulty in biodegradation of these synthetic dyes is due to their complex aromatic molecular structures [11]. The presence of textile dyes in high concentrations in water bodies interfere with the re-oxygenation process of the receiving water and cut-off sunlight which upsets the biological activities of aquatic organisms and also the photosynthesis process of aquatic plants or algae [12]. Additionally, a number of dyes or their metabolites are carcinogenic and mutagenic in nature. The adverse effects of dyes on human health (lungs, respiratory and skin disorders) have been reported from different parts of the world [13].

A number of techniques are used to remove dyes from water. Broadly these methods are classified in: physical, chemical and biological methods [5, 13-15]. Amongst them the biological methods are preferably used as they are environment friendly and compatible to nature. Biodegradation of the synthetic dyes and other intractable components by means of bacteria forms the basis of the decolorization of dyes containing effluents. The decolorization of aryl methane, azo and anthraquinone dyes by anaerobic and aerobic bacteria have already been reported [17-24].

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As mentioned above the biological methods are compatible to the natural environment, the present study was aimed to determine the capability of *Pseudomonas aeruginosa* to decolorize Novacron turquoise. The effect of different physico chemical parameters on dye decolorization was also determined and the metabolites formed after decolorization/degradation was characterized by FTIR and Mass spectrometry.

Experimental

Bacterial strains

Four bacterial strains *Escherichia Coli* (ATCC25922), *Salmonella typhi* (ATCC0650), *Shiegella* (ATCC13147) and *Pseudomonas aeruginosa* (ATCC27853) were used in this study. These bacterial strains were obtained from the Department of Biotechnology, University of Malakand, Khyber Pakhtunkhwa, Pakistan. The average viable count of the selected bacteria/mL of the stock suspension was determined by means of the surface viable counting technique. About 10^8 -10^9 colony forming units/mL were used. A fresh stock suspension was used in all experiments.

Dye and Chemicals

Novacron turquoise (Reactive blue 21) was obtained from textile industry situated in Karachi, Pakistan. Nutrient broth (Oxoid, UK), sugar, sodium chloride, sodium hydroxide, hydrochloric acid and all other chemicals available were analytical grade chemicals. The dye manufacturer was Toronto Research Chemicals. The rest of chemicals were purchased from Dae jung Korea. The dye structure is shown in Fig. 1.

Preparation of dye stock solution

Textile effluents were collected from industrial estate of Karachi (Liberty Textile Mill Karachi) and checked for the concentration of the selected dye. The minimum limits were 0.005 mg/L and maximum was 25 mg/L monitored from 9 Am to 4 Pm. Taking the maximum limit, 25 mg/L dye working stock solution was prepared by dissolving 25 mg of Novacron turquoise (Reactive blue 21) in 1000 mL of distilled water (25 mg/L) with continuous shaking for 5 minutes.

Culture media

Growth or culture media is needed for proper bacterial growth. Nutrient broth (Oxoid, UK) was used as growth media for the bacterial strains. Distilled water was taken in conical flask and then nutrient broth was added into the conical flask. Distilled water (1000 mL) and nutrient broth powder (13 gram) were mixed in conical flask under continuous shaking for 5 minutes.

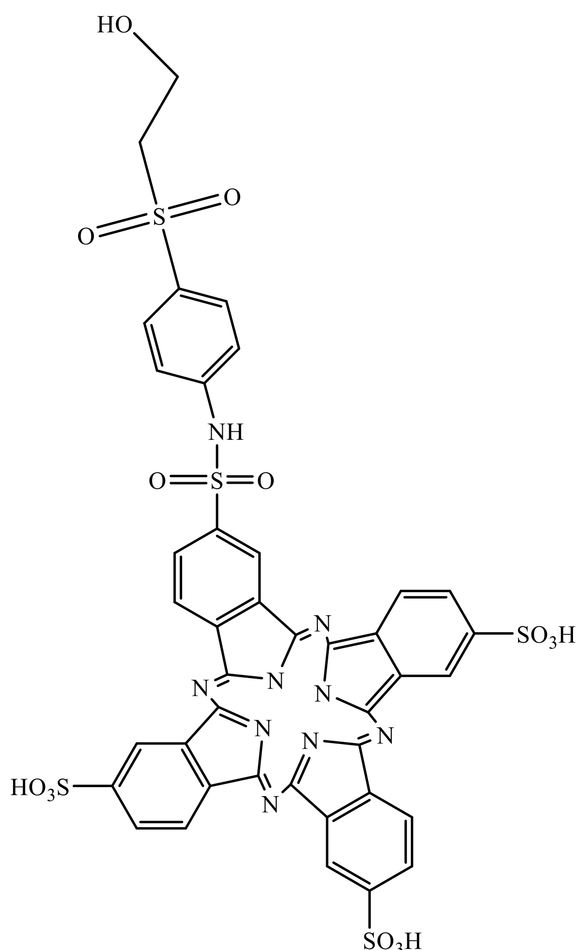

Fig 1: Chemical structure of the dye.

Decolorization activity

The bacterial cultures were transferred to sterilized nutrient broth medium (10 mL) containing 5 ml solution of Novacron turquoise (Reactive blue 21) (25 mg/L) in test tubes and incubated at 37°C, under static condition for 3 days. After 3 day, aliquots (5 mL) of the culture media was withdrawn and centrifuged at 10,000 rpm for 10 minutes in a centrifuge at room temperature to separate the bacterial cell mass. The supernatant were analyzed for the remaining un-degraded dye using UV-Visible spectrophotometer. A solution of 5 mL dye from stock solution (25 mg/L) and 10 mL distilled water was used as control solution. The following formula was used to determine the percent decolonization of the dye:

$$\%\ Decolorization = \frac{Initialabsorbance\ at\ 665\ nm - final\ absorbance\ at\ 665\ nm}{Initialabsorbance\ at\ 665\ nm}\ 100$$

The bacterial strain *Pseudomonas aeruginosa* gave maximum decolorization values and hence was selected and used for further decolorization (degradation) experiments.

Effect of various physiochemical parameters on dye decolorization

To determine the effect of various physiochemical parameters initial dye concentration, pH, temperature, time, glucose and sodium chloride concentrations on decolorization activity of selected bacteria a number of experiments were carried out. Their details are given as follow:

To determine the effect of initial dye concentration on decolorization/growth of bacteria a number of test tubes containing 10 mL nutrient broth were mixed with 5 mL dye from working dilutions (0.08, 0.06, 0.04, 0.025, 0.02 and 0.01 mg/L). Six control solutions of working dilutions were prepared by mixing 5 mL from each dilution with 10 mL distilled water. All these test tubes were already inoculated with selected bacterial strains and incubated at 37°C for 3 days.

To determine the effect of pH on decolorization, a number of test tubes containing 10 mL sterile nutrient broth. The pH of the test tubes containing 10 mL sterile nutrient broth were adjusted using 1 N NaOH and 1 N HCL to reach pH in the range 1-13. A solution (15 mL) where instead of nutrient broth 10 mL distilled water were mixed with 5 mL of dye stock solution (25 mg/L) having final concentration of 8.3 mg/L was used as control. The tubes were inoculated with bacterial strain and incubated at 37°C under static condition for 3 days.

To determine the effect of incubation temperature on dye decolorization, sterile nutrient broth 10 mL were taken in test tubes and were inoculated with selected bacterial strain. Each test tube was spiked with 5 mL from dye stock solution (25 mg/L). One control solution (for each temperature) of dye was also taken containing 10 mL distilled water and 5 mL dye from stock solution. The tubes were then incubated at 25, 37, 45 and 50°C for three days.

In order to determine the effect glucose supplementation on selected dye decolorization, five test tubes containing 10 mL sterile nutrient broth was inoculated with selected bacterial strain. To each tube; 0.1, 0.2, 0.3, 0.4 and 0.5 mg of glucose and 5 mL dye solution from stock solution (25 mg/L) were added and incubated at 37°C under static condition for three days. One control solution of dye was also taken containing 10 mL distilled water and 5mL dye from stock solution (25 mg/L).

In order to determine the effect of NaCl supplementation on decolorization, in five test tubes10 mL sterile nutrient broth were taken and inoculated with selected bacterial strain. Additionally 0.1, 0.2, 0.3, 0.4 and 0.5 mg of sodium chloride and 5 mL dye solution from stock solution (25 mg/L) were also added to each test tube and incubated at 37°C for three days. Control solution was prepared by mixing 10 mL distilled water and 5 mL dye from stock solution (25 mg/L).

To study the effect of the time on the decolorization % decolonization was measured at various time intervals starting after 3 days till 24 days as mentioned above.

Determination of optimum conditions

The effect of various physicochemical parameters like pH, initial dye concentration, temperature, incubation time, glucose and sodium chloride concentrations were determined in different sets of experiments as discussed above. Out of these physicochemical parameters, only optimum conditions favorable to growth of microorganism and dye decolorization were selected and combined in a single experiment in a single test tube and inoculated with selected bacteria. The inoculated test tube was incubated for optimum time at 37°C.

Spectroscopic analysis of metabolites formed after decolorization

The cellular mass along with aliquots was crushed using mechanical blinder and the resulting mixtures were then centrifuged at 10,000 rpm to collect the metabolites. The supernatants were extracted with dichloromethane, dried over anhydrous sodium sulfate and then mixed with KBr to form pellet which were then subjected to FTIR analysis using IR Prestige-21, Shimadzu Japan. Mass spectrophotometric analyses were carried out to identify the various products formed after the de-colorization of the given dye. FAB (Fast atom bombardment) spectra were obtained using JEOL-600H-2 mass spectrometer. Ions were produced by a primary beam of xenon atoms of 8 keV, extracted and accelerated with an 8 kV potential.

Results and discussions

Selection of best decolorizer bacterial strain

Initially, four different bacterial strains; *Escherichia Coli, Salmonella typhi*, *Shiegella* and

Pseudomonas aeruginosa, were tested for their dye decolorization/degradation potentials (Fig 2a). Out of these four strains, *Pseudomonas aeruginosa* was found the best decolorizer (62.28%), followed by *Salmonella typhi*, *Shiegella* and *Escherichia coli* with an efficiency of 57.45%, 52.75% and 27.75% respectively (Fig 2b). Thus in the subsequent experiments *Pseudomonas aeruginosa* was used. In literature, this bacteria has also been used by other authors. For example, Prasad [25], studied the degradation of textile dyes by different bacterial strains. *Pseudomonas aeruginosa* was amongst them. Saranraj *et al.* [26] investigated the decolorization and degradation of azo dyes using bacteria isolated from textile effluent. Amongst the isolated bacteria, *Pseudomonas aeruginosa* was identified as the best decolorizer of Congo red with 97.33% decolorization ability.

The effect of various physiochemical parameters such as dye concentration, temperature, pH, glucose concentration, sodium chloride concentration and time on dye decolorization were determined to find out optimum conditions for the better decolorization of the selected dye. The metabolites formed from dye degradation were characterized by FTIR and mass spectrometry.

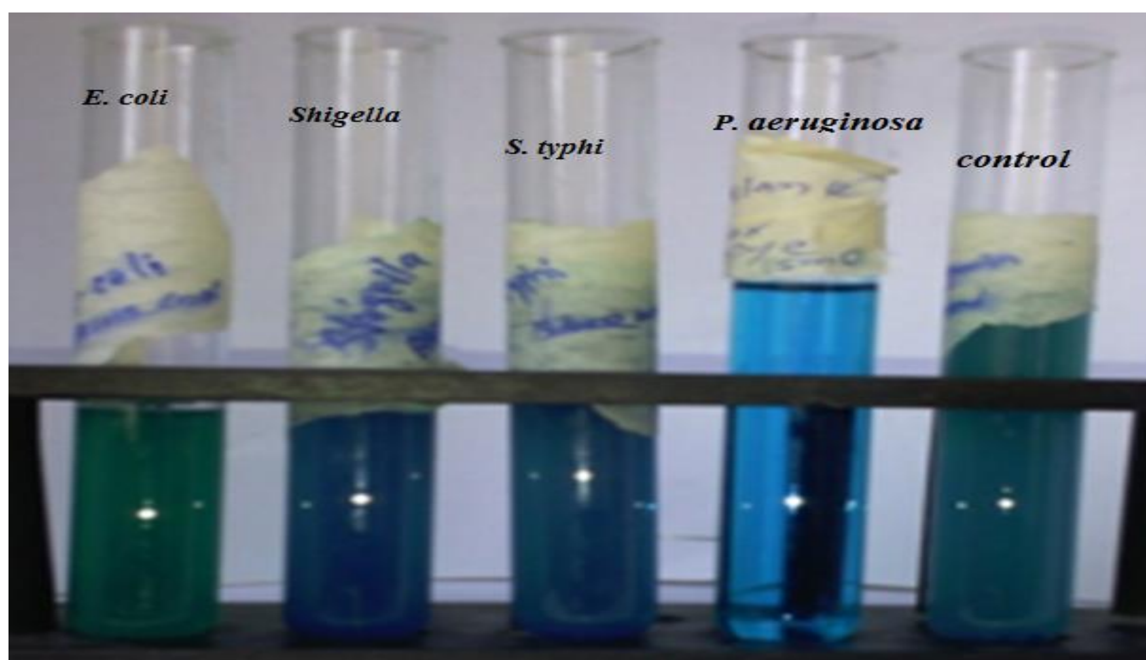

Fig. 2: (a) Decolorization of the selected dye by bacterial strains in test tubes while control solution is also there which has retained original color of the dye Novacron turquoise (Reactive blue 21).

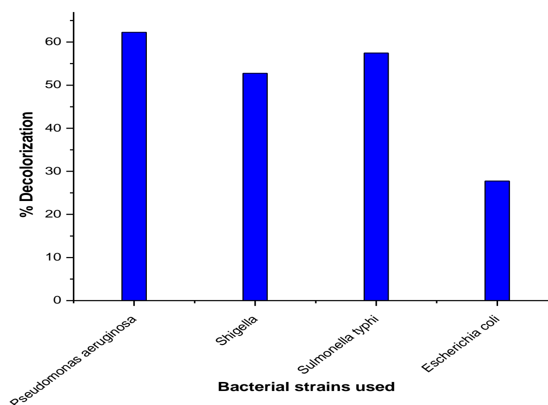

Fig. 2b: Percent decolorization of Novacron turquoise (Reactive Blue 21) by selected bacterial cultures.

Effect of different physicochemical parameters on decolorization and determination of optimum conditions

A number of experiments were carried out in order to determine the optimum conditions under which the dye degradation would be maximum. High concentration of the dye have toxic effects on bacteria, therefore determination of optimum dye concentration tolerable to bacteria is very important. For *Pseudomonas aeruginosa,* the optimum dye concentration was found to be 0.01 mg/L (Fig. 3). The concentration limit of a particular chemical substance tolerable for a microorganism is a very important factor in decolorization studies. It is clear from the Fig that decolorization is maximum at lower concentration of dye whereas at high concentration the rate of decolorization/degradation decreases which may be due to the toxic effect of dye. As in living organisms the biological reactions are catalyzed by different enzymes. At high substrate concentrations the active sites all the available enzyme molecules form complexes with substrate and no free enzyme are available for further substrate molecules. Thus for higher concentrations, the complete decolorization would require longer time intervals [27]. Anjaneya *et al.* [28] found that when the dye concentration is high the rate of decolorization decreases which was probably due to the inhibitory effects of dyes molecules at high concentration.

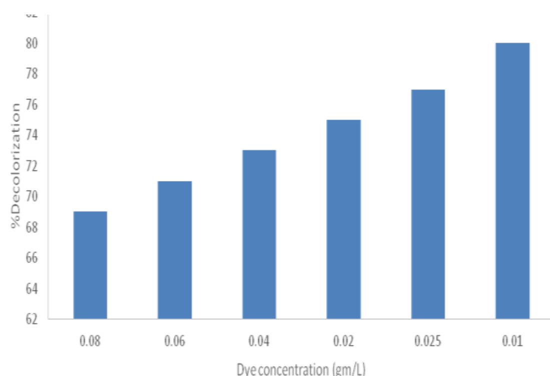

Fig. 3: Percentage decolorization at different concentrations of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa.*

As stated above all biological reaction are catalyzed by enzymes. For each enzyme there is optimum pH at which the enzymatic activities are maximum. Thus pH is an important factor and as a whole all microorganisms requires an optimum pH at which their growth is maximum. Fig. 4 shows the extent decolorization of Novacron turquoise by *Pseudomonas aeruginosa* at different pH. Maximum decolorization (99.4%) occurred at pH 10 (alkaline pH). Generally high pH are needed for the decolorization of reactive azo dyes. According to Chen *et al.* [29] pH plays an important role in dyes decolorization and in studies involving bacteria the optimum pH is usually in between 6 to 10.

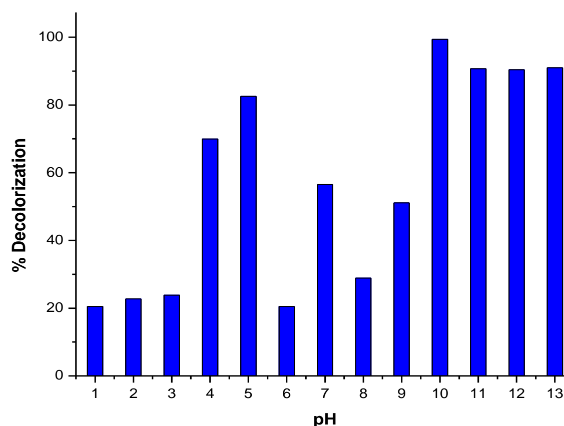

Fig. 4: Percentage decolorization at different pH of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa.*

The decolorization of the dye were also studied at different temperatures; 25, 37, 45 and 50°C. The optimum temperature was found to be 45°C (Fig. 5). The rate of chemical reaction depends on temperature. Generally for each 10°C in temperature the rate of reaction doubles. Bacteria being a living organism require an optimum temperature for their growth. Fig 5 shows that the maximum decolorization took place at 45°C and then at high temperature 50°C there was a decline in decolorization activity. According to Pearce et al. [30] for reactive azo dyes decolorization increases with increase in temperature till a maximum is reached. Above optimum temperature a steady decrease in the decolorization occurs which probably may be due to the denaturation of the enzyme at high temperature. According to them optimum growth temperature range was from 35 to 45°C. According to Anjaneya *et al* [28] the inactivation of the bacterial enzymes are responsible for low rate of decolorization at higher temperatures and the number of viable bacteria considerably decreases.

Supplementation of the growth media with food normal constituents like glucose and sodium chloride were also studied. The supplementation of growth media with 0.1 mg each of glucose and sodium chloride were found best (for optimum

decolorization; Fig. 6 and 7). Glucose acts as a source of carbon and energy in almost all living organisms. For maximum bacterial growth high amount of glucose is required which in turn would favor the decolorization process. However maximum (82.17 %) decolorization was observed with low glucose supplementation rather than high amount showing that supplementation carbon source (glucose) is less effective to promote the decolorization activity probably due to the preferences of the bacterial cells to assimilate the added carbon sources rather than using dye molecules as the carbon source [31]. Thus minimal concentration of glucose supplementation is required to achieve maximum decolorization activity.

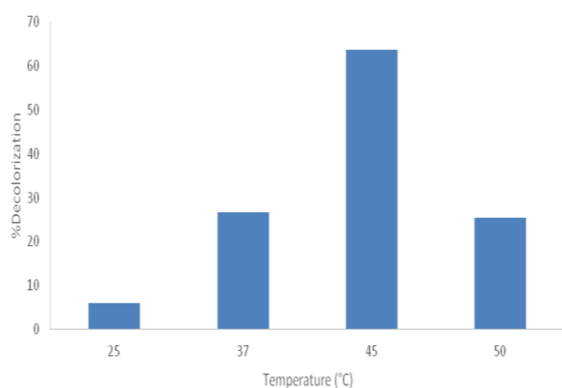

Fig. 5: Percentage decolorization at different range of temperature of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa*.

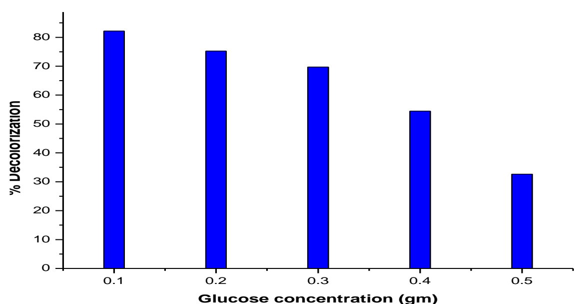

Fig. 6: Percentage decolorization at different glucose concentrations of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa.*

Fig. 7 shows that with increase in sodium chloride concentration there is a decrease in decolorization ability of the selected bacteria. Maximum activity was observed at lower concentration of sodium chloride. At high concentration the decrease may be attributed to the toxic effect of the sodium chloride which inhibits bacterial enzymes and may lead to death as well. This feature could be of great value in case when the decolorization activities are to be performed in saline textile effluents.

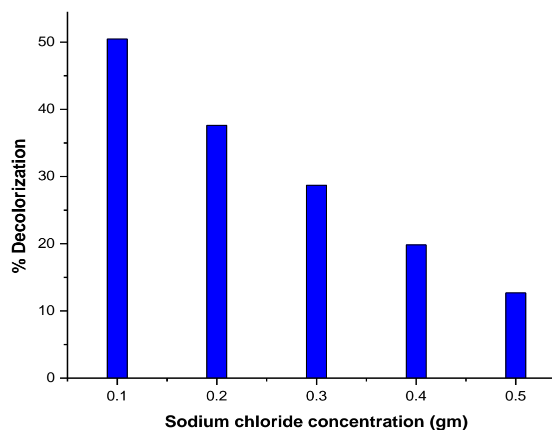

Fig. 7: Percentage decolorization at different sodium chloride concentrations of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa.*

With passage of time when the bacterial growth promotes in a particular media, the nutrient supplies vanishes while the toxic products gradually accumulated in the media as well leading to suffocation of the microorganisms. Therefore, time is an important factor in such types of studies. For *Pseudomonas aeruginosa,* the optimum time was three days at which high decolorization activities were observed (Fig. 8) while after 18 days there were no further decolorization activities that may be due to accumulation toxic metabolites. Thus it was concluded that extended/prolong time of incubation leads to decrease in decolorization activities. Different bacterial strains shows different responses to incubation time periods which might be due to the differences in bacterial enzymatic systems and affinity of a particular bacterial strain to particular dye as different dyes have different chemical structures [32]

Optimum conditions for bacteria were determined for the better decolorization of the selected dye. As mentioned in material and method section, the optimum conditions determined above were combined in a single experiment and the decolorization at all these optimum conditions were also determined. Better decolorization activity was observed when culture was grown under combined optimum conditions.

Table-1: FTIR peaks patterns of the original dye and after degradation under different physiological conditions.

Peak at (cm^{-1})	Possible component	Control	Dye opt. Conc	glucose	NaCl	pH	temp	time	opt
3449.7	N-H/O-H stretch	P	P	p	p	a	p	p	p
2928.1	C-H stretch	p	p	a	p	a	p	p	p
1660	Bending Vib. C=N and C=C (aromatic)	p	p	a	p	p	p	p	p
1597.06	–C-C- stretch	p	p	p	p	p	p	p	p
1301.8	C-N stretch	p	a	a	a	a	a	a	a
1231.5	O-H stretch	p	a	a	a	a	a	a	a
1136.02	C=N	p	p	p	p	p	p	p	p

P=present, a= absent

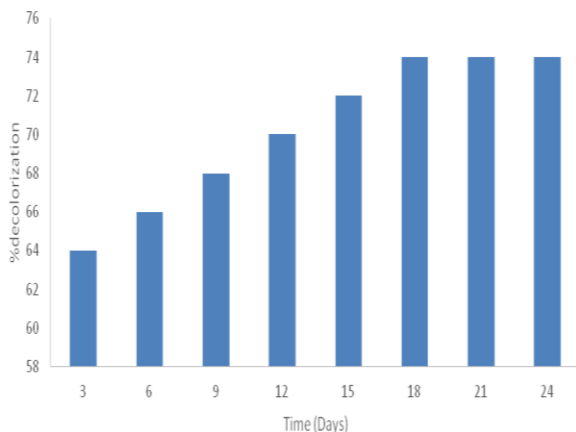

Fig. 8: Percentage decolorization at different time intervals of Novacron turquoise (Reactive Blue 21) by *Pseudomonas aeruginosa*.

FTIR analysis

The metabolites were collected for each set of experiments (optimum samples only) and were characterized by FTIR and mass spectrometry to identify various degradation products. FTIR spectrum of control dye was compared with that of metabolites extracted after each set of experiments. FTIR spectra indicated that certain specific functional groups present in the parental dye molecule were totally absent in the degraded products. However, a conclusion from FTIR analysis alone is not adequate; it must be combined with other analytical techniques such as mass spectrometry for the determination of the actual chemical structures of metabolites.

A peak at 3782 cm^{-1} is because of moisture contents in the sample. Peak at 3449.7 cm^{-1} represent OH/NH stretch, while peak at 2928.1 cm^{-1} is for C-H stretch. The peak at 1630.0 is executed by bending vibration of C=N or C=C (aromatic) functional groups. Peak at 1597.06 cm^{-1}represents --C-C- stretch. The peak at 1301.8 cm^{-1} is for C-N and 1231.5 cm^{-1} is for O-H stretch. At 1136.02 there is a peak for C=N. The FTIR spectra of the control dye are clear from the Fig 1S. The FTIR spectra of the dye degraded products are shown in Figs 2S to 8S. Table-1 shows comparison of the FTIR spectra of the parent dye with that obtained for the degradation experiments under different circumstances. The peaks at 1301.8 cm^{-1} for C-N and 1231.5 cm^{-1} for O-H stretch has been disappeared which means that dye has degraded by bacteria. The C-N group is a part of the porphyrin ring system its disappearance shows that the porphyrin ring system has completely destroyed by bacteria.

FAB-MS analysis

The molecular mass of the selected dye is 1079. Fast atom bombardment technique was used for ionization of the molecule. The results so obtained revealed that the same compound does not give molecular ion peak in the range of its molecular mass. It indicates that the dye has been degraded completely. The overall inspection of the dye does not give any clue regarding disintegration of the corresponding molecule. Several fragments were obtained from various experiments but unfortunately their structure cannot be proposed on the bases of parent structure of the dye. Samples collected for dye degraded under various experimental conditions showed different behavior. The degraded dye under optimum pH (Fig 9S) gave m/z (%) at 115(97), 185(100), 207(60), 277(8), 299(8). Experiment at optimum time (Fig 10S) gave fractions with the same m/z but different relative abundance (percentage) I.e., 115(65), 185(100), 207(40), 277(12), 299(5). The data obtained in experiment at all optimum condition (combined in single experiment; Fig 11S) was quite different and 115(100), 185 (40), 207(60) and many more fragments with relative abundance less than 8 % were obtained. In presence of NaCl in the medium very few fragments (Fig 12S) were of considerable abundance such as 115(100), 137(10), 173(9), 185(16), 207(40), 165(8). In experiment under optimum dye concentration (Fig 13S), base peak was observed at 183 and all other signals were of less abundance, 127(16), 151(10), 205(8), 219(8), 275(16). An overview of the data obtained in above mentioned experiments reveals that these experiments cannot be correlated with each other and scientific justification is therefore not possible for disintegration or decolorization of the dye. It can only

be concluded that extensive dissociation of dye has been occurred and further studies are needed to isolate various fractions for better understanding of the disintegration pathway.

Conclusion

This study was aimed to evaluate the ability of *Pseudomonas aeruginosa* to decolorize Novacron turquoise (Reactive blue 21). The selected bacteria showed better and potent decolorization ability. The metabolites from each set of experiment were collected and characterized by FTIR and mass spectrometer. In FTIR spectra, disappearance of C-N peak which is part of the porphyrin ring system in the dye structure completely disappeared. Its disappearance clearly indicates the destruction of the porphyrin ring system which is an integral part of the dye structure.

References

1. A. Bafana, S. S. Devi and T. Chakrabarti, Azo dyes: past, present and the future, *Environ. Rev.,* **19**, 350 (2011).
2. E. P. Chagas and L. R. Durrant, Decolourization of azo dyes by *Phanerochaete chrysosporium*, *Enzyme Microb. Technol.,* **1,** 473 (2001).
3. P. K. Malik, Use of activated carbon prepared from sawdust and rice husk for adsorption of acid dyes: a case study of acid yellow 36, *Dyes Pigm.*, **56**, 239 (2003).
4. P. A. Carneiro, R. F. P. Nogueira and M. V. B. Zanoni, Homogeneous photodegradation of C.I. Reactive Blue 4 using a photo-Fenton process under artificial and solar irradiation, *Dyes Pigm.*, **74,** 127 (2007).
5. T. Robinson, G. McMullan, R. Marchant and P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, *Biores. Tech.,* **77**, 247 (2001).
6. C. J. Ogugbue and T. Sawidis, Bioremediation and detoxification of synthetic wastewater containing triarylmethane dyes by *Aeromonas hydrophila* isolated from industrial effluent, *Biotech. Res. Int.,* **102,** 1 (2011).
7. M. S. Balakrishnan, S. Arul Antony, R. K. Gunasegaram and Natarajan, Impact of dyeing industrial effluents on the ground water quality in Kancheepuram (India), *Indian J. Sci. Tech.,* **1,** 117 (2008).
8. A. Abdel-Aty, M. B. Hamed, A. S. Fahmy and S. A Mohamed, Comparison of the potential of Ficus sycomorus latex and horseradish peroxidases in the decolorization of synthetic and natural dyes, *Genet. Eng. Biotechnol. J.,* **11**, 95 (2013).
9. J. Paul, A. A. Kadam, S. P. Govindwar, P. Kumar and L. Varshney, An insight into the influence of low dose irradiation pretreatment on the microbial decolouration and degradation of Reactive Red-120 dye. *Chemosphere,* **90,** 1348 (2013).
10. Q. Saeed, I. A. Batthi, M. Zuber, S. Nosheen, M. A. Zia and M. Abbas, Study of application of mono azo reactive dyes on cotton by exhaust method and printing properties, *J.* Basic *Appl. Sci.,* **12**, 191 (2012).
11. P. Nigam, G. Armour, I. Banat, M. D. Singh and R. Merchant, Physical removal of textile dyes from effluents and solid-state fermentation of dye adsorbed agriculture residues. *Biores. Technol.,* **72**, 219 (2000).
12. C. Namasivayam, R. Radhika and S. Suba, Uptake of dyes by a promising locally available agricultural solid waste: coir pith, *Waste Manag.,* **21**, 381 (2001).
13. M. S. Tsuboy, J. P. E. Anjeli, M. S. Mantovani, S. Knasmiiller, G. A. Umbuzeiro, and L. R. Ribeiro, Genotoxic, mutagenic and cytotoxic effects of the commercial dye CI Disperse Blue 291 in the human hepatic cell line HepG2, *Toxicol. In vitro,* **21**, 1650 (2007).
14. O. J. Hao, H. Kim and P. C. Chiang, Decolourisation of waste water, *Crit. Rev. Environ. Sci. Tech.,* **30**, 449 (2000).
15. M. M. Hassan M. Z. Alam and M. N. Anwar, Biodegradation of Textile Azo Dyes by Bacteria Isolated from Dyeing Industry Effluent. *Int. Res. J. Biological. Sci*., **2**, 27 (2013).
16. E. Forgacs, T. Cserhtli, and G. Oros, Removal of synthetic dyes from waste waters: A Review, *Environ. Int.,* **30**, 953 (2004).
17. M. Husain and Q. Husain, Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: A review, Crit. Rev. *Environ. Sci. Technol*., **38**, 1 (2008).
18. Q. Husain, Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: A review, Rev. *Environ. Sci. Biotechnol.,* **9**, 117 (2010).
19. P. Kaushik and A. Malik, Fungal dye decolourization: Recent advances and future potential. *Environ. Int.,* **35**, 127 (2009).
20. F. P. Van der Zee and F. J. Cervantes, Impact and application of electron shuttles on the redox biotransformation of contaminants: A review, *Biotechnol. Adv.,* **27**, 256 (2009).
21. V. K. Gupta and Suhas, Application of low cost adsorbents for dye removal: A review, *J. Environ. Manag.*, **90**, 2313 (2009).

22. A. Srinivasan and T. Viraraghavan, Decolorization of dye wastewaters by biosorbents: A review, *J. Environ. Manag.*, **91**, 1915 (2010).
23. P. Ramachandran, R. Sundharam, J. Palaniyappan and A. P. Munusamy, Potential process implicated in bioremediation of textile effluents: A review, *Adv. Appl. Sci. Res.,* **4**, 131 (2013).
24. J. Plácido, X. Chanagá, S. Ortiz-Monsalve, M. Yepes and A. Mora, Degradation and detoxification of synthetic dyes and textile industry effluents by newly isolated Leptosphaerulina sp. from Colombia, *Biores. Bioprocess,* **3**, 1 (2016).
25. M. P. Prasad, Studies on the degradation of textile dye by *Pseudomonas aeruginosa*, Res. *J. Recent Sci.,* **3**, 59 (2014).
26. P. Saranraj, V. Sumathi, D. Reetha and D. Stella, Decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent, *J. Ecobiotechnol.,* **2**, 7 (2010).
27. K. Wuhrmann, K. Mechsner and T. Kappeler, Investigation on rate-determining factors in the microbial reduction of azo dyes, *Eur. J. Appl. Microbiol.*, **9**, 325 (1980).
28. O. Anjaneya and S. Yogesh, Decolorization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus* sp. Strain AK1 and *Lysinibacillus* sp.strain AK2, *J. Hazard. Mat.,* **190**, 351 (2011).
29. C. H. Chen, C. F. Chang, C. H. Ho, T. L. Tsai and S. M. Liu, Biodegradation of crystal violet by a *Shewanella sp*. NTOU, *Chemosphere*, **72**, 1712 (2008).
30. C. I. Pearce, J. R. Lloyd and J. T. Guthrie, The removal of color from textile waste water using whole bacterial cells: A review, *Dyes and Pigments*, **58**, 179 (2003).
31. R. G. Saratale, G. D. Saratale, J. S. Chang and S. P. Govindwar, Ecofriendly degradation of sulfonated diazo dye C.I.Reactive Green 19A using *Micrococcus glutamicus* NCIM-2168, *Biores. Technol.,* **100**, 3897 (2009).
32. S. D. Kalme, G. K. Parshetti, S. U. Jadhav and S. P. Govindwar, Biodegradation of benzidine based dye Direct Blue-6 by *Pseudomonas desmolyticum* NICIM 2112, *Bires. Technol.,* **98**, 1405 (2007).