INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

POLYURETHANE FOAM AND AGAR-AGAR AS PROMISING MATRICES FOR DEGRADATION OF REACTIVE BLACK 5 DYE

MS. Usha*, L. Anitha and LN. Ella

Department of Microbiology, CPGS, Jayanager, Jain University, Bangalore-560 011, Karnataka, India.

ABSTRACT

Presence of *Aeromonas punctata* in polyurethane foam and Agar-Agar matrices was confirmed by scanning electron microscopy after immobilization process. Free cells of *A. punctata* was able to show 100% degradation of Reactive Black 5 dye upto 100 ppm of dye concentration, whereas both PUF and Agar-Agar immobilized cells of *A. punctata* showed 100% degradation of Reactive Black 5 dye upto 200 ppm of dye concentration. On repeated use of PUF and Agar-Agar immobilized cells of *A. punctata* 100% degradation of Reactive Black 5 dye was recorded upto 10 cycles at 200 ppm of dye concentration. On continuous treatment, PUF immobilized cells of *A. punctata* showed 100% degradation of Reactive Black 5 dye upto 1600 ppm of dye concentration at 10 ml/hr flow rate and Agar-Agar immobilized cells of *A. punctata* could show 100% degradation of Reactive Black 5 dye upto 1800 ppm of dye concentration at 10 ml/hr flow rate.

Keywords: Polyurethane foam, Agar-Agar, immobilization, Reactive Black 5 dye, degradation.

INTRODUCTION

Dyes are widely used in the textile, leather, paper, printing inks, plastics, cosmetics, paints, pharmaceutical, and food industries. While 1, 00,000 commercially available dyes exist; over $7x10^5$ metric tons of dye stuff is produced annually.[1] The major users of dyes in India are textiles, paper, plastics, printing ink and foodstuffs. The textiles sector consumes around 80% of the total production due to high demand for polyester and cotton, globally. Dyes are one of the most hazardous chemical compound classes found in industrial effluents and need to be treated since their presence in water bodies reduces light penetration, precluding the photosynthesis of aqueous flora.[2,3] They are also aesthetically objectionable for drinking and other purposes[4] and can cause allergy, dermatitis, skin irritation[5] and also provoke cancer[6] and mutation in humans.[7] Textile industry activities lead to large quantities of textile wastewaters, highly colored and containing a variety of compounds such as dyes, organic chemicals, inorganic salts and others.

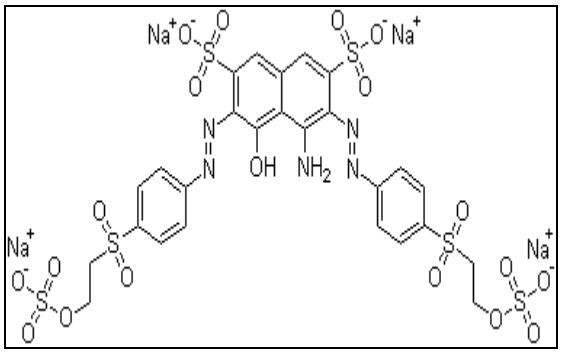
Reactive dyes are a class of dyes which have essentially a functional group having ability to make a bond (essentially a covalent bond) with fabric. It is estimated that 15% of Reactive dyes is lost in the synthesis, processing of colorants, dyeing, printing and finishing.[8] This invariably corresponds to a release of about 615 tons per day into the environment and ecological system. Due to their toxicity and recalcitrance, these dyes are hazardous to the environment and even when they are present in very low concentrations, can present serious carcinogenic effects.[9] Reactive black 5 dye is widely used in textile dyeing industry. It has been commercially important and commonly used in textile industries for the dying of cotton, woolen and nylon fabrics worldwide. It is suitable for exhaust dyeing, printing, continuous dyeing or discharge printing of a blended or interwoven fabric of cellulose fibers or cellulose nylon blends. It is reported to be toxic[10,11] and cause allergic reactions of respiratory tract.

Degradation of Reactive Black 5 dye has been carried out by few investigators using fungi,[12,13,14,15] yeast[16] and bacteria.[17,18,19,20,21] Zille *et al.*[22] have used immobilized laccase for the degradation of Reactive Black 5 dye. Calcium alginate immobilized *Aeromonas punctata* has been used for degradation of Reactive Black 5 dye by Usha *et al.*[17] However there are no reports on use of polyurethane foam and Agar-Agar immobilized cells for the degradation of

Reactive Black 5 dye. Hence the present investigation has been taken up to study the efficiency of cells immobilized in the above mentioned matrices.

MATERIALS AND METHODS

Reactive Black 5 dye was procured from Himedia Pvt. Ltd., Bombay, India. All chemicals and reagents used were of analytical grade.



REACTIVE BLACK 5 DYE

Bacterial culture

Aeromonas punctata isolated from textile industrial effluent, identified by nucleotide sequencing and deposited in gene bank with the accession numbers JN561149 was used for degradation studies. Culture was maintained on mineral salts agar medium[23] containing 50 ppm of Reactive Black 5 dye.

Preparations of inoculum

A loopfull of culture from mineral salts agar medium was inoculated into mineral salts broth medium containing 50 mg/l of Reactive Black 5 dye. Flask was incubated till the culture reach log phase. This culture containing an average of 6 X 10^5 CFU/ml was used as inoculum for further studies.

Growth of *Aeromonas punctata* under optimized condition

Growth of *Aeromonas punctata* and *Pseudomonas aeruginosa* under optimized condition was carried out under optimized condition. Flasks containing 100 ml of mineral salts medium with pH 7, 50 mg/l of dye, 100 mg/l of Glucose, 100 mg/l of yeast extract and 5 ml of inoculums were incubated for 24 hrs at 37°C under shaking speed 100 rpm. After incubation the bacterial cells were harvested by centrifugation at 10,000 rpm for 10 minutes. These cells after washing with 0.01M PO_4 buffer were used for the immobilization experiments.

Immobilization with Polyurethane foam

Aeromonas punctata was immobilized in polyurethane foam (PUF) according to the procedure given by Lingappa *et al.*[24] The polyurethane foam material used for the immobilization studies had a porosity of 100-500 µm. It was cut into 1cm^2 pieces and washed with distilled water. One gram of the foam pieces was submerged in a 250 ml Erlenmeyer flask containing 50 ml of the fermentation medium and then sterilized at 121ºC for 15 min.[25] One gram of cell pellet was then mixed in one gram of PUF. The contents of the flasks were agitated properly and allowed to stabilize. Cell-free foam was made for use as a control by substituting carbon free growth medium for the cell suspensions. The reaction vessel was kept on ice for 2 h while the polyurethane foam hardened. The foam was removed from the reaction vessel, rinsed with buffer to remove free cells and stored at 4°C in carbon free growth media. At the beginning of each experiment the foam was rinsed three times with buffer to remove any free cells.[26]

Immobilization with Agar-Agar

Agar-Agar entrapment of *Aeromonas punctata* was performed according to procedure given by Buitelaar *et al.*[27] One gram of cell pellet was added to 2 % molten agar dissolved in 0.9% NaCl, maintained at 40°C and poured into sterile flat bottom petriplates and allowed to solidify. The solidified agar blocks were cut into equal size cubes under asceptic condition, added to sterile 0.1 M phosphate buffer (pH 7.0) and kept at 4°C for 1 h. Phosphate buffer was decanted and cubes were washed with sterile distilled water 3-4 times and were stored at 4°C in phosphate buffer.

Scanning Electron Micrograph

A. punctata immobilized PUF cubes and Agar-Agar blocks were fixed in phosphate buffer (pH 7.3) containing glutaraldehyde (2% w/v) for two hours at 4° C. After fixation, the specimens were washed with saline and dehydrated gradually in a series of ethanol gradients (30%, 50%, 70%, 80%, 94% and 96%, 1 hour for each gradient). Then the samples were washed three times in 100% ethanol. The specimens were dried to a critical point of drying in a sputter coating process. The particles were coated with a gold layer in an argon medium and attached on to the stubs with silver glue. The scanning electron microscopy (SEM) observations were made in Philips Scanning Electron Microscope (Model 515). The SEM photographs were outsourced from Metallurgical Department, Indian Institute of Science, Bangalore and the arrangement and concentration of *A. punctata* embedded in the two matrices were observed.

Batch degradation of Reactive Black 5 dye using free cells

To carry out biodegradation studies with free cells, 5 ml of inoculums (8 x 10^{10} CFU/ml) of *Aeromonas punctata* was added to flask containing 100 ml of medium and were incubated under optimized conditions. The degradation efficiencies of the two cultures were checked by increasing concentration of Reactive Black 5 dye from 50 mg/l to 250 mg/l with interval of 50 mg/l.

Estimation of percent degradation

After the incubation period, growth medium was subjected to centrifugation at 10, 000 rpm for 10 min. Cell free supernatant was subjected to estimate the amount of dye degradation by UV-Vis spectrophotometer reading at 597 nm for Reactive Black 5 dye. Percent degradation of the dyes was calculated using the formula

Percent degradation = [(Initial absorbency – Observed absorbancy) / Initial absorbency] x 100

Batch degradation of Reactive Black 5 dye Polyurethane foam immobilised cells

The batch degradation of Reactive Black 5 dye using PUF immobilized cells was performed by taking 5 g of PUF cubes with immobilized cells in 250 ml conical flask containing 100 ml of mineral salts medium[23] with 100 mg/ml of Yeast extract and glucose with 100 mg/l of dye. The degradation process was carried out in shaker incubator at 100 rpm at 37°C for 24 hrs by varying the Reactive Black 5 dye concentration from 50 to 500 ppm with a difference of 50 ppm.

Batch degradation of Reactive Black 5 dye using Agar-Agar immobilized cells

The batch degradation using Agar-Agar immobilized cells was performed by taking 5 g of Agar-Agar blocks with immobilized cells in 250 ml conical flask containing 100 ml of mineral salts medium[23] with 100 mg/ml of Yeast extract and glucose with 100 mg/l of Reactive Black 5 dye. The degradation process was carried out in shaker incubator at 100 rpm at 37°C for 24 hrs by varying the dye concentration from 50 to 500 ppm with a difference of 50 ppm.

Repeated batch degradation of Reactive Black 5 dye

To observe the long term stability of dye degradation by immobilized cells the PUF cubes and Agar-Agar blocks were used for repeated batch degradation. After each cycle of incubation (24 hr) the PUF cubes and Agar-Agar blocks were washed in buffer and transferred to fresh flask containing media. Spent media were used for analysis of degradation. To check the cell leakage of PUF cubes and Agar-Agar blocks one ml of degradation samples after each cycle was taken and plated onto nutrient agar media. After overnight incubation visible colonies were counted and cell leakage values were given as CFU/ml.

Continuous degradation of Reactive Black 5 dye

The continuous treatments of Reactive Black 5 dye was carried out in a continuous flow reactor. The reactor was filled with PUF and Agar-Agar immobilized cells separately. The degradation process was carried out by continuous supply of sterile media containing Reactive Black 5 dye with the help of peristaltic pump (Miclins PP10-4C, India). To check the efficiency of *Aeromonas punctata* under immobilized condition continuous treatment of respective dyes was carried out by varying the dye concentration from 200 ppm to 2000 ppm as well as flow rate from 10 ml/hr to 100 ml/hr. The Detention time and degradation rates were calculated by the following formula.

Detention time

void volume/ flow rate (ml/h).

Degradation rate (R) = (Ci-Ce) x D,

Where Ci and Ce are concentrations of respective dyes in influent and effluent respectively

D (dilution rate) = flow rate (ml/h) / void volume of reactor (ml)

UV-Vis spectrophotometer scanning

In order to confirm the degradation of Reactive Black 5 dye by free and immobilized cells of *A. punctata* overnight incubated samples were subjected to UV-Vis scanning. Culture media were subjected to centrifugation at 10,000 rpm for 15 minutes. Cell free growth media were scanned between 190 nm and 1100 nm.

RESULTS AND DISCUSSION

Scanning Electron Micrograph

Scanning electron micrograph of PUF and Agar-Agar control samples showed absence of bacterial cells (Plate 1 and 2). Presence of immobilized cells of *Aeromonas punctata* was demonstrated in both the matrices (Plate 3 and 4). Andleeb *et al.*[28]showed the efficient attachment of *Aspergillus niger* on sand particles as well as in sodium alginate beads.

Batch degradation of Reactive Black 5 dye by free and immobilized cells

When batch degradation was carried out using free cells and immobilized cells, free cells of *Aeromonas punctata* was able to show 100% degradation upto 100 ppm of dye concentration (Plate 5), whereas 100% degradation was seen upto 200 ppm of dye concentration with both PUF (Plate 6) and Agar-Agar immobilized cells of *Aeromonas punctata* (Plate 7).

Further increase in dye concentration resulted in decreased efficiencies of both free and immobilized cells of *Aeromonas punctata*. At 500 ppm of dye concentration, free cells of *Aeromonas punctata* showed 30% degradation of Reactive Black 5 dye. PUF immobilized cells of *Aeromonas punctata* showed 76% degradation and Agar-Agar immobilized cells showed 79% degradation of Reactive Black 5 dye at 500 ppm of dye concentration (Fig. 1). Similar results in higher efficiencies of immobilized cells as compared to free cells have been reported by Usha *et al*.[26] and Lingappa *et al*.[24] According to Usha *et al*.[26] *Alcaligenes latus* cells immobilized in PUF showed higher degradation rate of H-acid than the cells immobilized in calcium alginate cells. Immobilization studies by Lingappa *et al*.[24] using PUF as a support material for *Streptomyces gulbargensis* showed enhanced L-Asparaginase production compared to free cells. Kuhad *et al*.[25] have reported increase in pectinase production by *Streptomyces* sp. RCK-SC after employing PUF as an inert support matrix.

Repeated batch degradation of Reactive Black 5 dye by immobilized cells

On repeated use of PUF and Agar-Agar immobilized cells of *Aeromonas punctata* 100% degradation was obtained at 200 ppm concentration of Reactive Black 5 dye upto 10 cycles. Further use of immobilized cells resulted in decreased efficiency of *Aeromonas punctata*. By the end of 25 cycles PUF immobilized cells of *Aeromonas punctata* showed 79% degradation of Reactive Black 5 dye and 85% degradation of the dye was observed with Agar-Agar immobilized cells of *Aeromonas punctata* (Fig. 2).

Stability of PUF and Agar-Agar blocks were checked by plating one ml of degradation medium onto nutrient agar medium and then counting the number of visible colonies after overnight incubation. By the end of 90 days PUF immobilized cells of *Aeromonas punctata* showed a cell leakage of 12 x 10^5 CFU/ml and Agar-Agar immobilized cells of *Aeromonas punctata* showed a cell leakage of 15 x 10^6 CFU/ml. According to Usha *et al*.[26] calcium alginate immobilized *A. latus* could show 100% degradation of H-acid upto 5 cycles, further use resulted in decreased efficiency. A cell leakage of 2x10^1 CFU/ml was recorded in PUF immobilized cells after 25 cycles. Lingappa *et al*.[24] checked the stability of PUF immobilized *S. gulbargensis* for L-Asparaginase production under repeated batch cultivation. Maximum enzyme titre was reached during third cycle, gradual cell leakage from the matrix was observed from first to seventeen cycles.

Continuous degradation of Reactive Black 5 dye by immobilized cells

Efficiencies of PUF and Agar-Agar during continuous treatment were checked by varying the flow rate from 10 ml/hr to 100 ml/hr as well as dye concentration from 200 to 2000 ppm. On continuous treatment, PUF immobilized cells of *Aeromonas punctata* showed 100% degradation of Reactive Black 5 dye upto 1600 ppm of dye concentration at 10 ml/hr flow rate (detention time of 360 min). With 200 ppm of dye concentration PUF immobilized cells of *Aeromonas punctata* showed 100% degradation upto 90 ml/hr flow rate (detention time 72 min). At 100 ml/hr flow rate (detention time of 36 min) 96% of degradation of dye was recorded with 200 ppm of Reactive Black 5 dye concentration (Plate 8, Fig. 3).

Agar-Agar immobilized cells of *Aeromonas punctata* showed 100% degradation of Reactive Black 5 dye upto 1800 ppm of dye concentration at 10 ml/hr flow rate. At 100 ml/hr flow rate 100% degradation of dye was recorded with 200 ppm of dye concentration. At 10 ml/hr flow rate with 2000 ppm of dye concentration Agar-Agar immobilized cells of *Aeromonas punctata* could show 94% degradation of Reactive Black 5 dye where as at 100 ml/hr flow rate with 2000 ppm of dye concentration only 40% degradation of Reactive Black 5 dye was recorded (Plate 9, Fig. 4). Similar result has been shown by Usha *et al*.[17] where ca-alginate immobilized cells of *A. punctata* was used for continuous degradation of Reactive Black 5 dye. Ajao *et al*.[29] have demonstrated that a bioreactor packed with Agar-Agar immobilized *Pseudomonas aeruginosa* and *Bacillus subtilis* could be efficiently used for bioremediation of textile industrial effluent.

UV-Vis spectrophotometer scanning

On UV-Vis spectrophotometer scanning between 190 nm and 1100 nm Reactive Black 5 dye control sample showed the presence of a peak at 597 nm (Plate 10). The same peak was not seen in media with PUF and Agar-Agar immobilized cells of *Aeromonas punctata*, but peaks appeared at different wavelengths indicating degradation

of Reactive Black 5 dye by immobilized cells and formation of degradation products. Media with PUF immobilized cells of *Aeromonas punctata* showed peaks at 229 nm, 277 nm, 291 nm and 324 nm (Plate 11) whereas media with Agar-Agar immobilized cells of *Aeromonas punctata* showed peaks at 221 nm and 261 nm (Plate 12). UV-Vis scanning of samples to confirm decolorization and degradation has been carried out by Enayatizamir *et al.*,[13] Kariyajjinavar *et al.*[30] and Kalyani *et al.*[31]

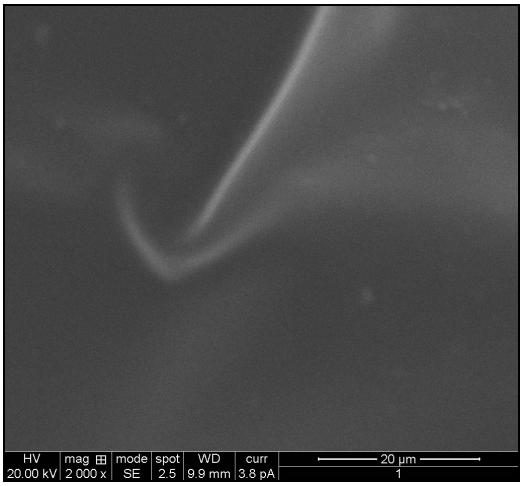


Plate. 1: Scanning electron micrograph of PUF control

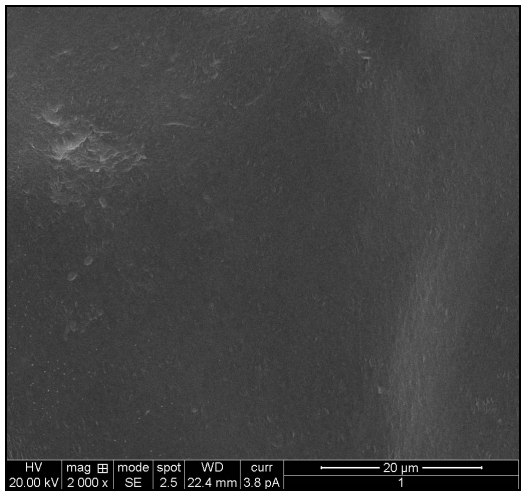


Plate. 2: Scanning electron micrograph of Agar-Agar control

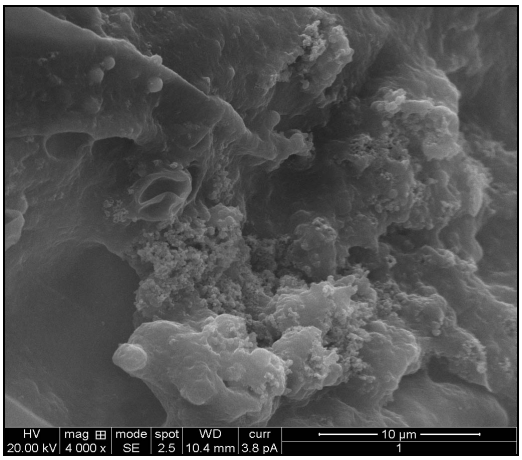


Plate. 3: Scanning electron micrograph of PUF with immobilized cells of *Aeromonas punctata*

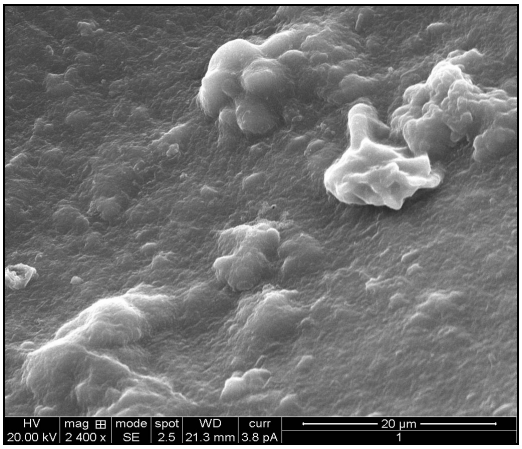


Plate. 4: Scanning electron micrograph of Agar-Agar with immobilized cell of *Aeromonas punctata*

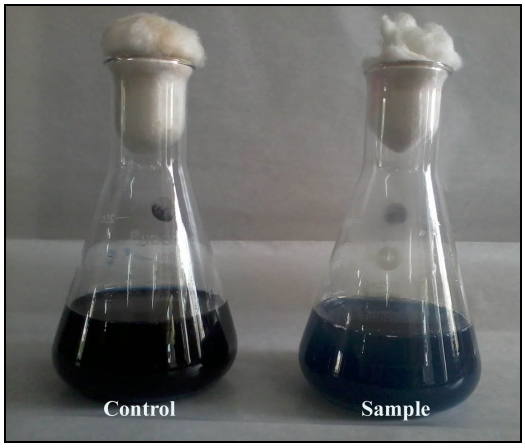


Plate. 5: Batch degradation of Reactive Black 5 dye by free cells of *Aeromonas punctata*

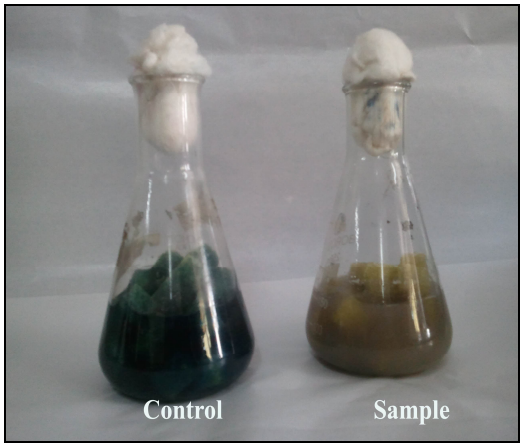


Plate. 6: Batch degradation of Reactive Black 5 dye by PUF immobilized cells of *Aeromonas punctata*

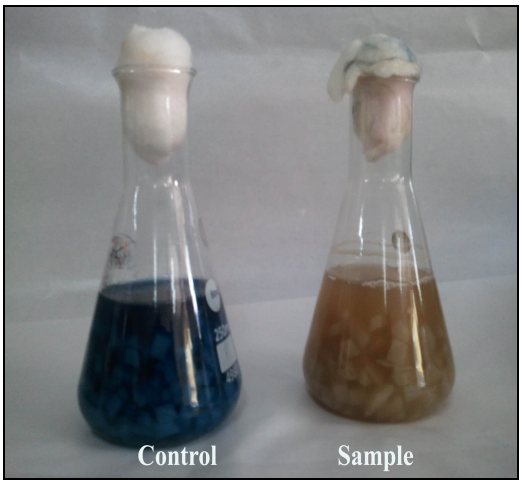


Plate. 7: Batch degradation of Reactive Black 5 dye by Agar-Agar immobilized cells of *Aeromonas punctata*

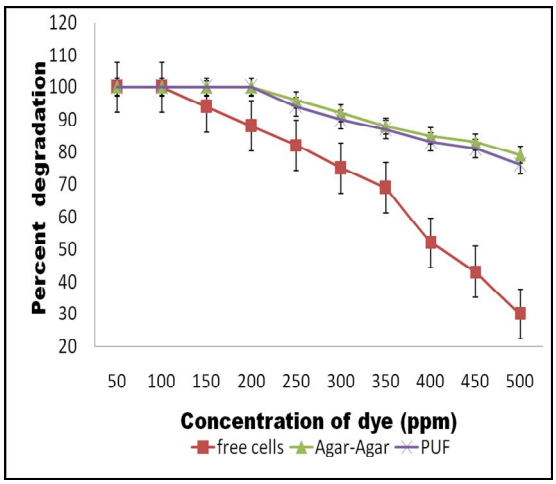


Fig. 1: Batch degradation of Reactive Black 5 dye by free and immobilized cells of *Aeromonas punctata*

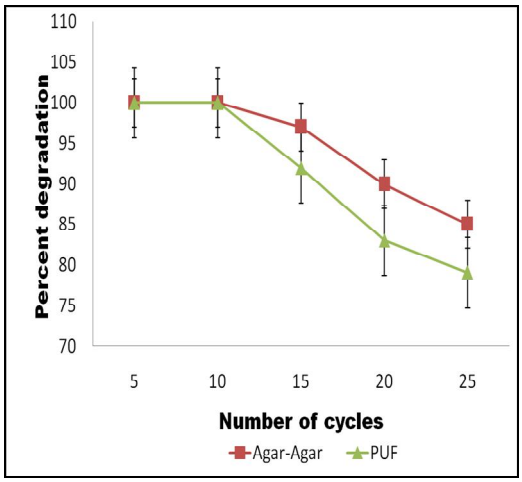


Fig. 2: Repeated batch degradation of Reactive Black 5 dye by immobilized cells of *Aeromonas punctata*

Plate. 8: Continuous degradation of Reactive Black 5 dye using PUF immobilized cells of *Aeromonas punctata*

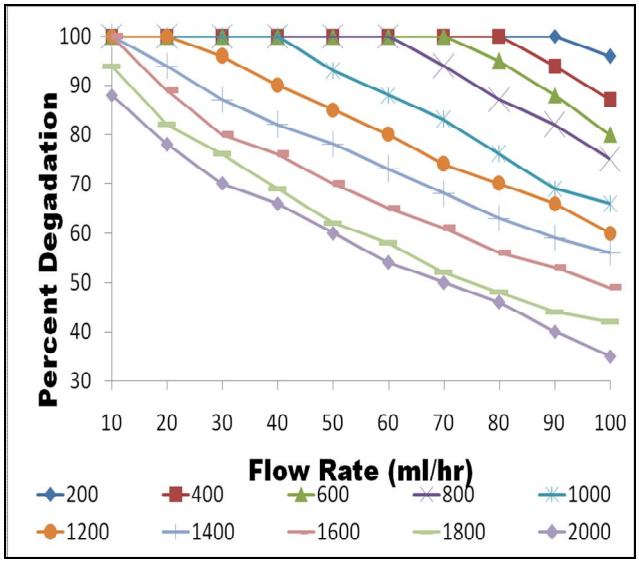


Fig. 3: Continuous degradation of Reactive Black 5 dye using PUF immobilized cells of *Aeromonas punctata*

Plate. 9: Continuous degradation of Reactive Black 5 dye using Agar-Agar immobilized cells of *Aeromonas punctata*

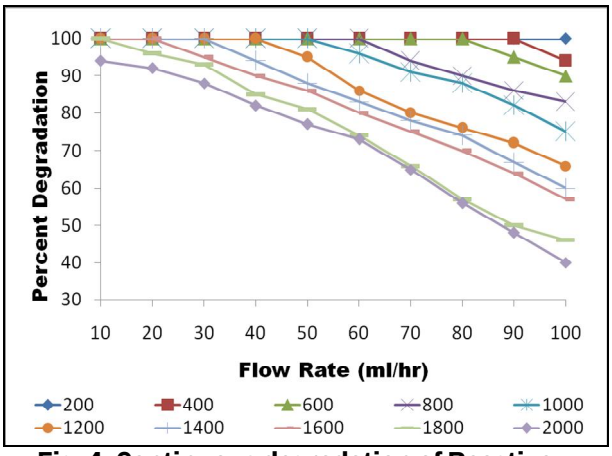


Fig. 4: Continuous degradation of Reactive Reactive Black 5 dye using Agar-Agar immobilized cells of *Aeromonas punctata*

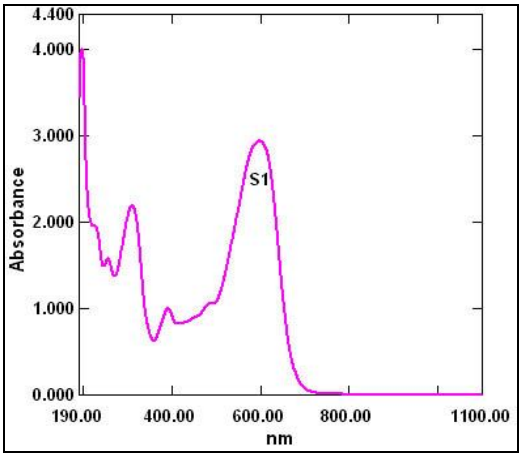


Plate. 10: UV-Vis spectra of Black 5 dye control

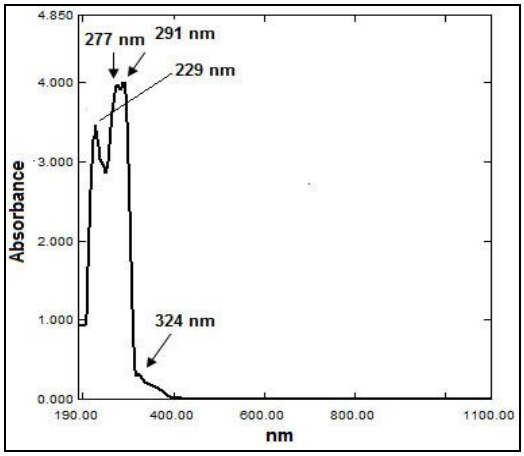


Plate. 11: UV-Vis spectra of media with PUF immobilized cells of *Aeromonas punctata*

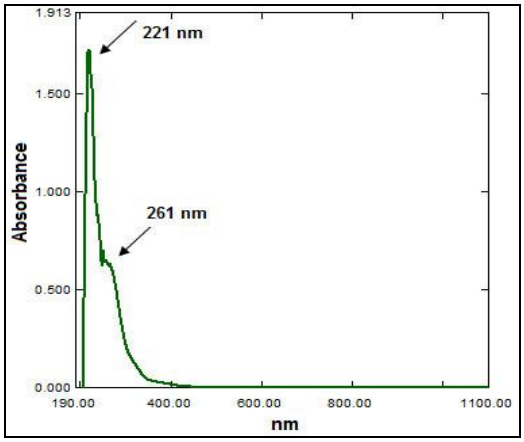


Plate. 12: UV-Vis spectra of media with Agar-Agar immobilized cells of *Aeromonas punctata*

REFERENCES

1. Zollinger H. Colour Chemistry-Synthesis, Properties and Applications of Organic Dyes and Pigments. VCH Publishers, New York, 1987;92-100.
2. Royer B, Cardoso NF, Lima EC, Vaghetti JC, Simon NM, Calvete T and Veses RC. Applications of Brazilian pine-fruit shell in natural and carbonized forms as adsorbents to removal of methylene blue from aqueous solutions-Kinetic and equilibrium study. J Haz Mat. 2009;164(2-3):1213–1222.
3. Lima EC, Royer B, Vaghetti JCP, Nathalia MS, Bruna MC, Flavio AP, Edilson VB, Renato CV and Claudio A. Application of Brazilian pine-fruit shell as a biosorbent to removal of reactive red 194 textile dye from aqueous solution. Kinetics and equilibrium study. J Haz Mat. 2008;155(3):536–550.
4. Royer B, Cardoso NF, Lima EC, Macedo TR and Airoldi C. A useful organofunctionalized layered silicate for textile dye removal. J Haz Mat. 2010;181(1–3):366–374.
5. Brookstein DS. Factors associated with textile pattern dermatitis caused by contact allergy to dyes, finishes, foams, and preservatives. Dermat Clin. 2009;27(3):309–322.
6. Alves LRO, Bazo AP, Salvadori DMF, Rech CM, Oliveira DP and Umbuzeiro GA. Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source. Mut Res. 2007;626(1-2):53–60.
7. Carneiro PA, Umbuzeiro GA, Oliveira DP and Zanoni MVB. Assessment of water contamination caused by a mutagenic textile effluent/dyehouse effluent bearing disperse dyes. J Haz Mat. 2010;174(1–3):694–699.
8. Aye T, Anderson WA and Mehrvar M. Photocatalytic treatment of Cibacron Brilliant Yellow 3G-P. J Environ Sci Heal. 2003;38:1903-1914.
9. Brown MA and DeVito SC. Predicting azo dye toxicity. Crit Rev Environ Sci Technol. 1993;23:249-324.
10. Lars BR and Mallika I. Photocatalytic decolourization of reactive azo dyes; a comparision between TiO2 and CdS photolysis. Chemos. 1997;35:585-596.
11. Rafi F, Franklin W and Cerniglia CE. Azoreductase activity of anaerobic bacteria isolated from human intestine microflora. Appl Environ Microbiol. 1990;7:2146-51.
12. Hadibarata T, Abdull RMY, Azmi A, Risky AK, Topic H and Adhi Y. Effects of Glucose on the Reactive Black 5 (RB5) Decolorization of two white basidiomycets. ITB J Sci. 2011;43(3):179-186.
13. Enayatizamir N, Fatemeh T, Susana RC, Bagher Y, Hossein AA and Leila M. Biodegradation pathway and detoxifiacation of the diazo dye Reactive Black 5 by Phanerochaete chrysosporium. Biores Technol. 2011;102:10359-10362.
14. Erdal S and Taskin M. Uptake of textile dye reactive black 5 by Penicillium chrysogenum MT-6 isolated from cement-contaminated soil. Afric J Microbiol. 2010;4(8):618-625.
15. Patel R and Suresh S. Kinetic and equilibrium studies on the biosorption of reactive black 5 dye by Aspergillus foetidus. Biores Technol. 2008;99:51-58.
16. Qingxiang Y, Tao L, Yang M and Zhang H. Effect of glucose on the decolorization of reactive black 5 by yeast isolates. J Environ Sci. 2008;20:105-108.
17. Usha MS, Sasirekha B, Devi S, Kamalini C, Manasa G and Neha P. Batch, repeated batch and continous degradation of Reactive black 5 and Reactive red 120 dyes by immobilized bacteria. J Sci Ind Res. 2012;71:504-510.
18. Usha MS, Sasirekha B, Devi S, Kalmani C, Manasa G and Neha P. Optimization of Reactive black 5 and Reactive red 120 dye degradation. J chem Pharam Res. 2011;3(6):450-457.
19. Xingzu W, Cheng X, Sun D and Hong Q. Biodecolorization and partial mineralization of reactive black 5 by a strain of Rhodopseudomonas palustris. J Env Sci. 2008;20:1218-1225.
20. Karatas M, Sukru D and Mehmet EA. The decolorization of azo dye reactive black 5 in a sequential anaerobic-aerobic system. Ekol. 19. 2010;74:15-23.
21. Sagarika M, Nishant D and Nageswara NR. Microbial decolorization of reactive black 5 in a two-stage anaerobic-aerobic reactor using acclimatized activated textile sludge. Biodegr. 2006;17:403-413.

22. Zille A, Tzanko T, Georg MG and Artur CP. Immobilized laccase for decolourization of Reactive Black 5 dyeing effluent. Biotechnol lett. 2003;25:1473-1477.
23. Brilon CW, Beckmann and Hellwig M. Enrichment and isolation of naphthalene sulphonic acid-utilizing Pseudomonas. Appl Environ Microbiol. 1981;42:39-43.
24. Lingappa K, Syeda A, Vishalakshi N and Prabhakar M. Immobilization of Streptomyces gulbargensis in polyurethane foam: A promising technique for L-asparaginase. Iran J Biotechnol. 2009;7(4):199-204.
25. Kuhad RC, Kapoor M and Rustagi R. Enhanced production of an alkaline pectinase by Streptomyces sp. RCK-SC by wholecell immobilization and solid-state cultivation. W J Microbiol Biotechnol. 2004;20:257-263.
26. Usha MS, Sanjay MK, Gaddad SM and Shivannavar CT. Degradation of H-acid by free and immobilized cells of Alcaligenes latus. Braz J Microbiol. 2010;41:931-945.
27. Buitelaar RM, Bucke C, Tramper J and Wijffels RH. Immobilized Cells: Basics and Applications: Basics and Applications. In: Screening of immobilization materials for anaerobic wastewater treatment, Hwu CS and Tsend SK. 1996;98-105.
28. Andleeb S, Atiq N, Ali MI, Ur-Rehman F and Ahmad S. Biodegradation of Antraquinone dye by Aspergillus niger in self designed fluidized bed bioreactor. Iran J. Environ Health Sci. 2010;7:371-376.
29. Ajao A, Adebayo GB and Yakubu SE. Bioremediation of textile Industrial effluent using mixed culture of Pseudomonas aeruginosa and Bacillus subtlis immobilized on agar agar in bioreactor. J Microbiol Biotech Res. 2011;1(3):50-56.
30. Kariyajjanavar P, Narayana J, Nayaka YA and Umaril M. Electrochemical degradation and cyclic voltametric studies of textile Reactive Azo dye Cibacron Navy WB. Port Electochim Acta. 2008;28:265-277.
31. Kalyani DC, Patil PS, Jadhav JP and Govindwar SP. Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1. Biores Technol. 2008;99:4635-4841.