International Journal of Geology, Agriculture and Environmental Sciences
Volume – 2 Issue – 1 February 2014
Website: www.woarjournals.org/IJGAES *ISSN: 2348-0254*

Biodegradation of Dyes and Growth Kinetic Assay of Micro-Organisms

Ajay Kumar[1], Manisha[2], Shaktibala[3], Jahangeer[4]

[1, 4] Research Scholar, Department of Hydrology,
Indian Institute of Technology,
(Roorkee),Uttrakhand, India – 247667,

[2]Central Soil Salinity Research Institute (Karnal),
Haryana, India – 132001,

[3]Department of Civil Engineering, Poornima Group
of Institution (Jaipur), Rajasthan, India – 302022,

Abstract: ***The study rely on the bio-degradation of dyes for water quality management that having toxic and carcinogenic effect to abiotic and biotic environment; widely used in textiles industries and released as effluents. In present study species of algae and fungi used to degrade the Bromophenol blue and Coomassie blue R dyes viz. gram positive bacteria Rhodococcus rhodochrous MTCC 291 and white rot fungi Phanerochaete chrysosporiom MTCC 787. The bacteria has negatively oxidative, positively catalytic and have high nitrile hydratase activity properties. The fungus has unique extracellular oxidative enzymes activity with having lignin peroxidase biochemical intermediate and secretes array of peroxidases and oxidases. The cultured cell growth of Rhodococcus rhodochrous MTCC 291 and maintained in medium of yeast extract, peptones and NaCl while of Phanerochaete chrysosporiom MTCC 787 in the medium of malt extract, glucose and peptone in experiment. The λ_{max} for Coomassie blue R and Bromophenol blue observed at pH range 4.6±0.2 to 8.6±0.2 with concentration range 15µM to 240µM was 562 nm and 590 nm. Range of pH was selected by keeping in view the pH change in 48 hrs incubation period during which pH of cultured media changed from 5.5±0.2 to 8.4±0.2 in case of bacteria and 5.5±0.2 to 4.6±0.2. 0.2% to 1.4% and 1% to 2.4% variation of λ_{max} observed at different pH range for bromophenol blue and Coomassie blue R in different cultured media used for bacteria and fungi. Results are discussed after 48 hrs of microbial degradation***

Keywords: Dyes, Water Quality Management, Rhodocoocus sp., Phanerochaete sp., Toxicity.

1. Introduction

In human civilization importance of dyes to humans in both ancient and contemporary is well documented. Microbial degradation of these dyes an impressive step than conventional removal using adsorption onto activated carbon. Synthetic dyes mainly poly-aromatics from industrial wastewater gained concern due their carcinogenic health effects as well as environmental problems, persuaded environmental engineers to develop various techniques for the treatment of such hazardous compounds **[1][2][3].** Heterocyclic, multiple ring compounds and quaternary carbon atoms impart the increased resistance towards the biodegradability **[4].** Broadly use of synthetic dye in textiles industries are reported with diversified application **[5[[6].** The genus Rhodococcus is important for both environmental and biotechnological applications because of its extraordinary capacity for metabolizing recalcitrant organic compounds **[7][8].** *Phanerochaete sordid* Used for decolourisation azo and anthraquinone dyes from industrial effluents **[9].** A large number of enzymes from different plants and microorganisms have been reported to play an important role in an array of waste treatment applications **[10].** In recent years, a lot of research has been done to develop processes based on peroxidases from plants and fungi for the treatment of wastewater containing colored pollutants **[11].** This study finally emphasized that an enzymatic approach based on peroxidases activity has attracted much interest in the remediation/ decolourisation of various dyes present in wastewater/ industrial effluent **[12].** Peroxidase based dyes treatment will provide a reasonable basis for the development of biotechnological processes for continuous color and aromatic compounds removal from various industrial effluents at large scale **[10].** Decolourisation of was bromophenol blue and commassie blue R dyes was investigated by using three sources of fungal laccase with the origin of *Aspergillus oryzae, Trametes versicolor, and Paraconiothyrium variabile* **[13].** Selected microbial cultures for dyes degradation is based on having nitrile hydratase activity of bacteria and oxidases/ peroxidases activity for fungi **[14]**. In our study, nitrile hydratase activity of bacteria *Rhodococcus rhodochrous* and peroxidases, oxidases activity of white rot fungi *Phanerochaete chrysospori*om will be discussed for removal of bromophenol blue and commassie blue R dyes.

2. MATERIALS AND METHODS

The microorganism used in this study is bacterial strain *Rhodococcus rhodochrous MTCC 291* and fungus *Phanerochaete chrysosporiom MTCC 787*, was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh.

2.1 Procurement and Maintenance of microbial Cultures

Bacterial species culture are maintained by sub-culturing at regular intervals in freshly prepared autoclave medium prepared from yeast extract, peptones, NaCl and fungal species in presence of malt extract, glucose, peptones respectively. Cultures were incubated at 37^0C under stationary conditions. The broths were autoclaved at 15psi for 30 minutes.

2.2 Instrumentation

Among variable parameters pH and optical density were measured immediately by using portable pH meter (Eutech. pH tester 30) and UV-Visible double beam spectrophotometer (Hitachi-U2000; Japan). Centrifugation of sample to make the solution cell before taking the absorbance to determine the degradation phenomena by using centrifuge (TD5G Bench Lab 5000rpm). Maintenance of media culture done by autoclaving at different intervals of time in autoclave (Tuttanauer EL-D line model 28-160 liters).
liters).

3. RESULTS AND DICUSSION

Effect of concentration on maximum wavelength with different dyes in NaCl and glucose solution is studied. Calibration curves graphs of dyes are plotted in concentration vs. absorbance by taking different concentration of dyes (0.24mM, 0.12mM, 0.06mM, 0.03mM and 0.015mM) in the solutions of glucose and sodium chloride to check the effect of media on the optical density of concerned synthetic organic dye with λ_{max} 562 nm for Coomassie blue R and λ_{max} 590 nm Bromophenol blue. Decrease in the concentration leads to dectrease in the optical density of bromophenol blue and commasie blue R dyes with good corelatation in glucose media as shown in **Fig: 1, 2.** The scanning for λ_{max} were carried out by the adjusting the pH of the stock solution of the dyes with the help of 1M NaOH and 1M HCl. The scanning revealed no significant of pH on the dyes within pH range 4.6 to 8.5. The range was selected keeping in the view pH change in 48 hours incubation period during which pH of the culture medium changed from 5.2 to 8.7 in case of *Rhodococcus rhodochrous MTCC 291.* and from 4.9 to 8.1 in case of *Phanerochaete chrysosporiom MTCC 787.* No significant change was found in absorbance at different pH as shown in **Fig.3, 4.** Degradation of dyes by microorganisms determined by measuring optical density on spectrophotometer. Different concentration of two dyes bromophenol blue and commassie blue R of different concentration (0.24mM, 0.12mM, 0.06mM, 0.03mM and 0.015mM) were inoculated with cultures (10% v/v). The sample absorbance was measured at different time intervals (0h, 12h, 24h, 36h and 48h) in the supernatant sample of centrifuging at 5000 rpm for 20 minutes to make it cell free media. Decrease in the optical density with time is describing the growth kinetic of two microbial culture and degradation of two different dyes as shown by 3D-graphs in **Figure: 7, 8, 9, and 10.** Percentage degradation of different dyes at with different intervals and maximum and minimum degradation for both dyes bromophenol blue vs. commassie blue R in case of bacterial culture *Rhodococcus rhodochrous MTCC 291 vs.* fungal culture *Phanerochaete chrysosporiom MTCC 787* shown in **Figure: 5, 6.**

4. CONCLUSION

Maximum (75.1 %) and minimum (50 %) degradation of Bromophenol blue observed at concentration 120µM and 15 µM in case of *Rhodococcus rhodochrous MTCC 291* Maximum (68.1.0 %) and minimum (48.8 %) degradation of Commassie blue R observed at concentration 30 µM and 240/15 µM in case of *Rhodococcus rhodochrous MTCC 291.* Maximum (83.7 %) and minimum (64.5 %) degradation of Coomassie blue R observed at concentration 240µM and 120 µM in case of *Phanerochaete chrysosporiom MTCC 787.* Maximum (78.2 %) and minimum (68.5%) degradation of Bromophenol blue observed at concentration 15µM and 240 µM in case of *Phanerochaete chrysosporiom MTCC 787.* Maximum percentage degradation of bromophenol blue observed at higher concentration and at lower concentration for Coomassie blue R in case of *Rhodococcus rhodochrous MTCC 291* and vice versa for *Phanerochaete chrysosporiom MTCC 787.*

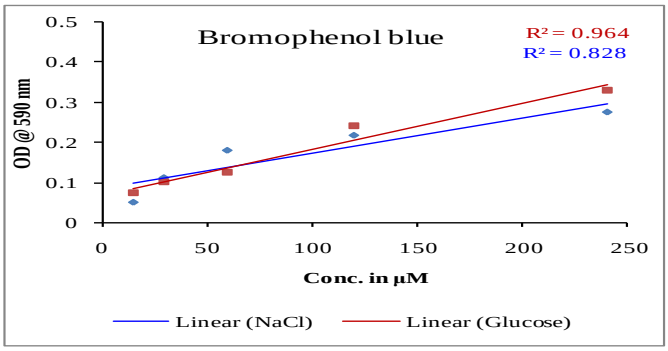

Figure: 1 λ max. Variation at different concentration

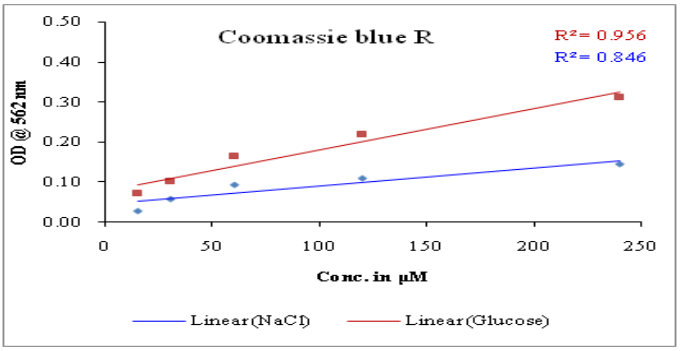

Figure: 2 λ max. Variation at different concentration

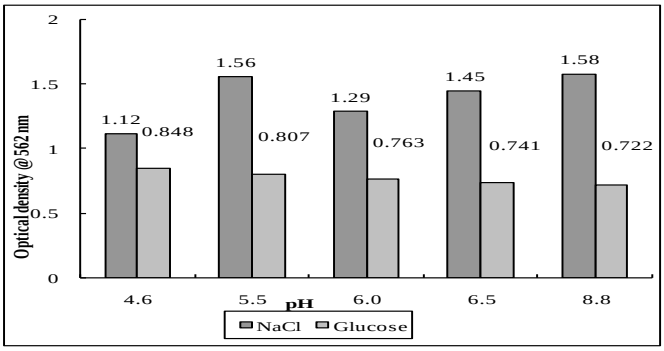

Figure: 3 λ max vs. pH effect for Commassie blue R

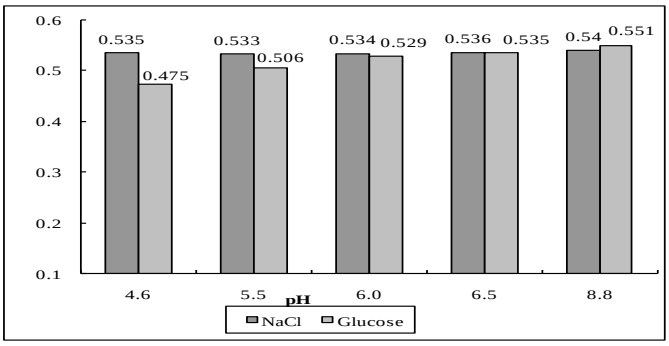

Figure: 4 λ max vs. pH effect for Bromophenol blue

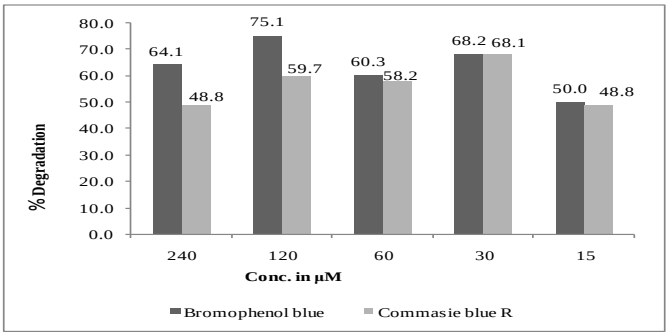

Figure: 5 Dyes degradation by *R. rhodochorous*

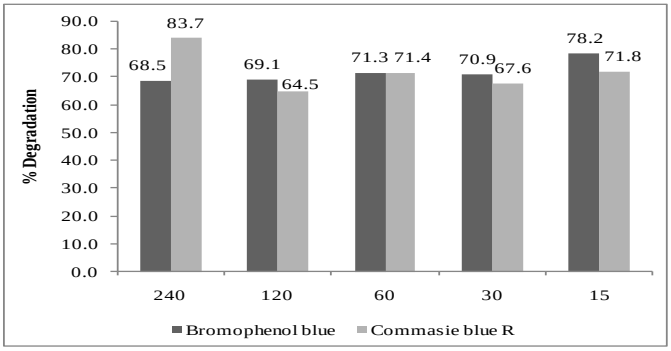

Figure: 6 Dyes degradation by *P. chrysosporium*

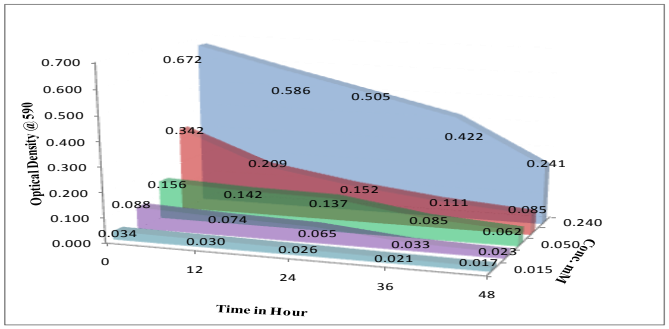

Figure: 7 Bromophenol degradation by *R. rhodochorous*

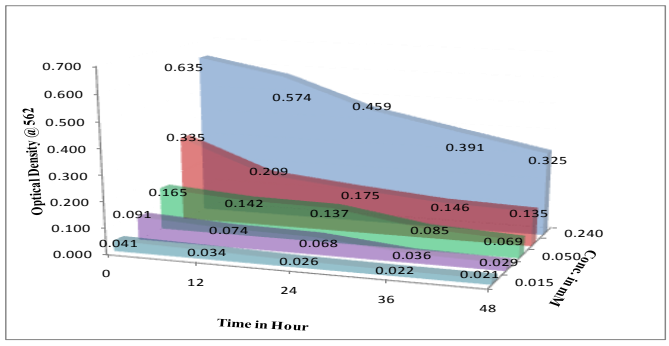

Figure: 8 Commasie B degradation by *R. rhodochorous*

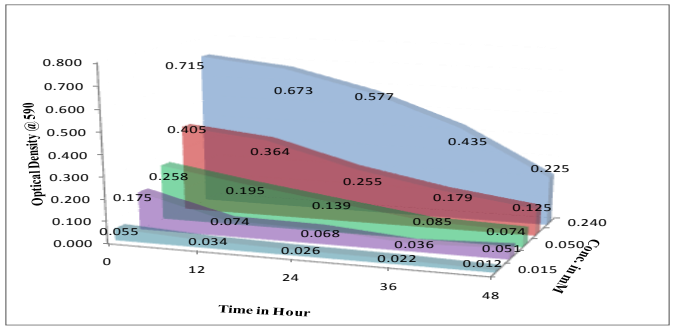

Figure: 9 Bromophenol degradation by *P. chrysosporium*

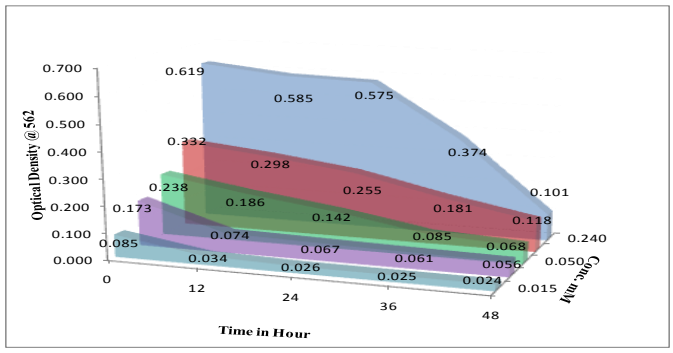

Figure: 10 Commasie B degradation by *P.chrysosporium*

5. REFERENCES

[1] *Gou M, Qu Y, Zhou J, Ma F, Tan L*: Azo. dye decolonization by a new fungal isolate. Penicillium sp. QQ and fungal-bacterial co-cultures. *J Hazard Mater* **2009**, 170:314–319.

[2] *Alimohammadi M*. Application of immobilized horseradish peroxidase for removal and detoxification of azo dye from aqueous solution. *Res J Chem Environ*. **2011**, 2:217–222.

[3] *Gholami-Borujeni F, Mahvi AH, Nasseri S, Faramarzi MA, Nabizadeh R, Alimohammadi*, Enzymatic treatment and detoxification of acid orange from textile wastewater. Appl Biochem Biotechnol. **2011**, 165:1274–1284.

[4] *J.D. Thompson, D.J. Higgins, T.J. Gibson, and CLUSTAL W:* Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22 , **1994**, pp. 4673–4680

[5] *Gupta GS, Shukla SP, Prasad G, Singh VN.* China clay as an adsorbent for dye house wastewaters. *Environ Technol* **1992**; 13:925 – 36.
[6] *Sokolowska-Gajda J, Freeman HS, Reife A.* Synthetic dyes based on environmental considerations: 2. Iron complexed formazan dyes. *Dyes Pigm* **1996**; 30:1 – 20.
[7] *Bell, K.S., Philp, J.C., Aw, D.W., Christofi, N*. The genus Rhodococcus. J. Appl. Microbiol. **1998**, 85, 195–210.
[8] *Larkin, M.J., Kulakov, L.A., Allen, C.C.* Biodegradation and Rhodococcus masters of catabolic versatility. *Curr. Opin. Biotechnol.* **2005**. 16, 282–290.
[9] *Harazono K, Nakamura K.* Decolonization of mixtures of different reactive textile dyes by the white-rot basidiomycete Phanerochaete sordidaand inhibitory effect of polyvinyl alcohol. *Chemosphere,* **2005**, 59:63–68.
[10] *Husain Q.* Potential applications of the oxidoreductive enzymes in the decolonization and detoxification of textile and other synthetic dyes from polluted water: a review. *Crit Rev Biotechnol,* **2006**. 60:201–22.
[11] *Husain M, Husain Q.* Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: a review. *Crit Rev Environ Sci Technol,* **2008**. 38:1–41.
[12] *Qayyum Husain.* Peroxidase mediated decolonization and remediation of wastewater containing industrial dyes: a review. *Rev Environ Sci Biotechnol* **2010**. 9:117–140.
[13] ***Hamid Forootanfar, Atefeh Moezzi, Marzieh Aghaie-Khozani, Yasaman Mahmoudjanlou, Alieh Ameri, Farhad Niknejad*** *and* ***Mohammad Ali Faramarzi.*** Synthetic dye decolonization by three sources of fungal laccase. Forootanfaret al. Iranian *Journal of Environmental Health Sciences & Engineering* **2012**, 9:27.
[14] *Saratale RG, Saratale GD, Chang JS, and Govindwar SP:* Bacterial decolonization and degradation of azo dyes: *A review.J Taiwan Inst Chem Eng* **2011**, 42:138–157.

Author Profile

Presently**,** Ajay Kumar is working as Research Schloar in Department of Hydrology, Indian Institute of Technology, Roorkee, India. He completed his masters in Environmental Sciences from Panjab University, Chandigarh. He worked as Scientific Assistant in Thapar University, R&D Center, India and as Senior Research Fellow in Division of Irrigation and Drainage Engineering, Central Soil Salinity Research Institute, Karnal, India. He has qualified Union Grant Commission (UGC), National eligibility test for fellowship and lectureship. He has 8 publications includings research papers,abstracts and poster presntations in journals and conferences with field applications of water and soil quality manangement.

Presently, Manisha is working as technical officer in Central Soil Salinity Research Institute, Karnal, India. She completed his masters in Environmental Sciences from CDLU,Sirsa, Haryana. She has qualified Union Grant Commission (UGC), National eligibility test for fellowship and lectureship. She has 16 publications includings research papers, book chapters, abstracts, articles and radio talks in journals and conferences with field applications of water, soil and forestry manangement.

Shaktibala have completed B.E. from Vinayaka Mission University, Tamilnadu and M. Tech dissertation is under review in Environmental Engineering from Rajasthan Technical University, Rajasthan, India. She had published more than 3 research paper in International Journals and presented research work in many conferences. She had attained many National workshops. Presently she is working at Poornima Group of Institution, Department of Civil Engineering at Jaipur as Assistant Professor with more than one year of working experience.

Presently**,** Jahangeer is working as Research Schloar in Department of Hydrology, Indian Institute of Technology, Roorkee, India. He completed his masters in Environmental Engineering from National Institute of Foundry & Forge Technology Ranchi, India. He has qualified Gradauate Aptitude Test (GATE) for National Fellowship. He is a life time member if Indian Assosiation of Hydrologist (IAH). He has 3 publications includings research papers and conferences with field applications of environmental engineering and bioremediation.