BIO ADSORPTION OF ALIZARIN RED DYE USING IMMOBILIZED SACCHAROMYCEUS SERVICE

N. Gandhi[1], D. Sirisha[1], and Smita Asthana[2]

1. Center for Environment and Climate Change, Jawaharlal Nehru Institute of Advanced Studies (JNIAS), Hyderabad, Telangana.

2. Department of Chemistry, St.Ann's College for Women, Mehdipatnam, Hyderabad, Telangana.

ABSTRACT

One of the main sources with severe pollution problems worldwide is the textile industry and its dye-containing wastewaters (10-25% of textile dyes are lost during the dyeing process, and 2-20% is directly discharged as aqueous effluents in different environmental components. Without adequate treatment, these dyes can remain in the environment for a long period. Some of the toxins in industrial waste may only have a mild effect whereas others can be fatal. They can cause immune suppression, reproductive failure or acute poisoning. The dye chosen for the present study was alizarin red ($C_{14}H_6Na_2O_7S$ M.W 364.24). Traditional batch adsorption processes carried out for present studies. Experiments were conducted with respect to dosage of the beads, dye concentration and contact time. It was found that by empty beds, the optimum time of adsorption was 24 hrs and by immobilized yeast, it was only 2 hrs.

Key words: Adsorption, Immobilisation, Alizarin red, Ca-Alginate, *saccharomyceus service.*

INTRODUCTION:

Safe, clean and adequate fresh water is vital to the survival of all living organisms and proper functioning of the ecosystem and communities. Water pollution destroys the natural ecosystem that supports health, food production and biodiversity. Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Water pollution affects drinking water, rivers, lakes and oceans all over the world. The residual dyes from different sources (e.g., textile industries, paper and pulp industries, dye and dye intermediates industries, pharmaceutical industries, tannery, and Kraft bleaching industries, etc.) are considered a wide variety of organic pollutants introduced into the natural water resources. Water pollution affects plants and organisms living in these bodies of water. In almost all cases, the effect is damaging not only to individual species and populations, but also to the natural biological communities [1-3]. An estimated 700 million Indians have no access to a proper toilet, and 1,000 Indian children die of diarrheal sickness every day [4]. Some 90% of China's cities suffer from some degree of water pollution, [5] and nearly 500 million people lack access to safe drinking water [6]. In addition to the acute problems of water pollution in developing countries, developed countries continue to struggle with pollution problems as well. In the most recent national report on water quality in the United States, 45 percent of assessed stream miles, 47 percent of assessed lake acres, and 32 percent of assessed bays and estuarine square miles were classified as polluted [7].

In particular, the discharge of dye-containing effluents into the water environment is undesirable, not only because of their colour, but also because many of dyes released and their breakdown products are toxic, carcinogenic or mutagenic to life forms mainly because of carcinogens, such as benzidine, naphthalene and other aromatic compounds [8] .Without adequate treatment these dyes can remain in the environment for a long period of time. Some of the toxins in industrial waste may only have a mild effect whereas others can be fatal. They can cause immune suppression, reproductive failure or acute poisoning. Organic matter and nutrients causes an increase in aerobic algae and depletes oxygen from the water column. This causes the suffocation of fish and other aquatic organisms.

Many processes can be used to remove dye from waste water like flocculation, adsorption, filtration, ozonation, sedimentation, bioabsorbtion, aerobic and anaerobic digestion. In the present study, bioadsorbtion technique was selected because it is simple, cost-effective and eco-friendly.

MATERIALS AND METHODS:

Selection of the immobilization matrix

The most important aspect is selecting the most effective matrix that can be used to immobilize the organism. There are many substances that are commercially available that can be used for immobilization like like alginate, Chitosan, chitin, Collagen, Carrageenan, Gelatin, Cellulose, Starch, Pectin etc. Synthetic polymers include amberlite and DEAE cellulose and Inorganic materials like Silica, Charcoal, etc. However, the aim was to select a low cost, easily available and natural matrix. The substance selected for immobilization was calcium alginate- which is obtained when aqueous sodium alginate is added to aqueous calcium chloride (calcium alginate is formed having a fibrous texture which doesn't dissolve in water) [9-21].

Selection of the organism to be immobilized

Bacteria are very well known for their decomposing activity and certain fungi are used for breaking down phenobiotic and xenobiotic compounds, especially treating effluents in paper industries. Recently, certain algae is also being used for bioremediation. However, yeast is easier to grow, culture and there are less chances of contamination compared to bacteria. In the present study, the yeast *saccharomyceus service* was selected as the organism to be immobilized because it is cost-effective and easily available commercially. The dye to be de-colorised was alizarin red, which is used commercially for dyeing textiles and many other purposes. It is also used in laboratories as a coloring agent and as a p^H indicator.

Preparation of alizarin red solution

A stock solution of 100 ppm (0.01g/100ml) was prepared by dissolving appropriate quantity of alizarin red 100 ml of distilled water from the Millipore purification unit. The stock solution was further diluted to desired working solutions. Final concentrations of the dye after adsorption was directly measured using Lambda scientific UV- visible spectrophotometer.

Preparation of the calcium alginate beads

An aqueous solution of sodium alginate was prepared by adding 1gm of sodium alginate to 100 ml of distilled water and 0.1M (1.1gm/100ml) of aqueous calcium chloride was prepared. When the sodium alginate was added drop wise to the calcium chloride solution, round cream-colored calcium alginate beads were formed. Both empty beads as well as beads containing yeast cells were prepared. (Yeast cell concentration was 4.8gm wet wt per 100ml sodium alginate).

Effect of contact time

Different solutions of alizarin red were prepared from the stock solution(10, 20, 40, 60, 80 and 100 ppm) and to this different dosages of both empty beads and beads containing yeast (2, 4, 6, and 8gm) were added separately. The initial (before adsorption) and final (after adsorption) concentrations were determined at regular time intervals *i.e.* 1, 2, 4, 6, 24, and 48hrs. The results are given in fig-1 and 2.

Effect of dye concentration

Different solutions of alizarin red were prepared from the stock solution (10, 20, 40, 60, 80 and 100 ppm) and to this different dosages of both empty beads and beads containing yeast (2, 4, 6, and 8gm) were added. Optimum time was taken depending on contact time experiments, results are given in fig -3 and 4.

Effect of bead dosages

To the different dye concentration solutions different dosages of both empty beads and yeast beads were added separately and optimum dosage was determined at optimum time taken from contact time experiments results are given in fig- 5 and 6.

RESULTS AND DISCUSSION:

Effect of Contact time:

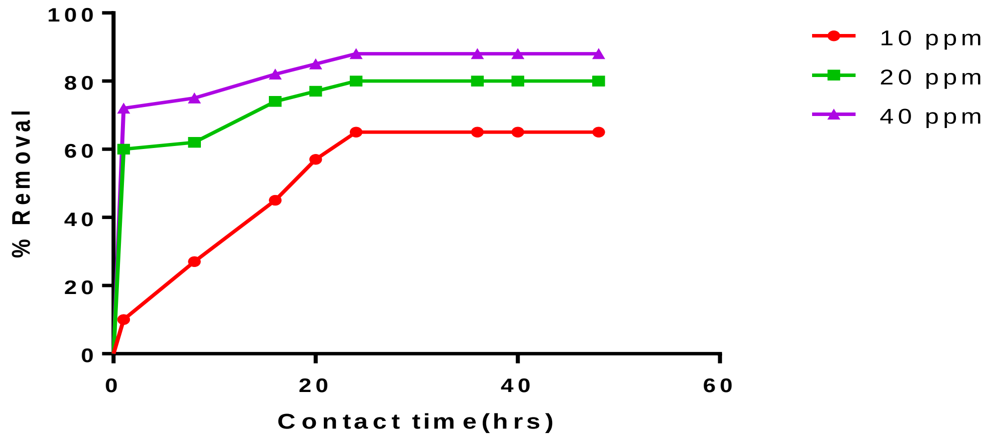

Figure-1: Effect of contact time by empty (Ca-Alginate) beads

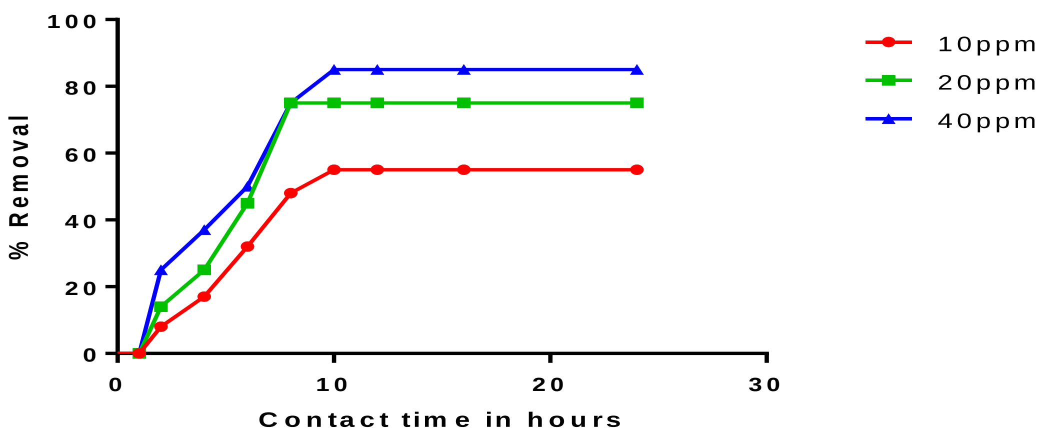

Figure-2: Effect of Contact time by immobilized *saccharomyceus service* beads

In the case of empty beads (Fig-1), the percentage removal increases with increase in contact time. Beyond 24 hrs, there is no change in the percentage removal which indicates that the optimum contact time is 24hrs. After 24 hrs the line is becoming parallel to the x-axis indicating a saturation point in empty beads.

In case of yeast beads (Fig-2), it is clear that percentage removal increases upto 10 hrs and reaches a maximum point. Since there is not much change in the percentage removal after 10 hrs the optimum time is 10 hrs [22].

Effect of initial Dye concentration

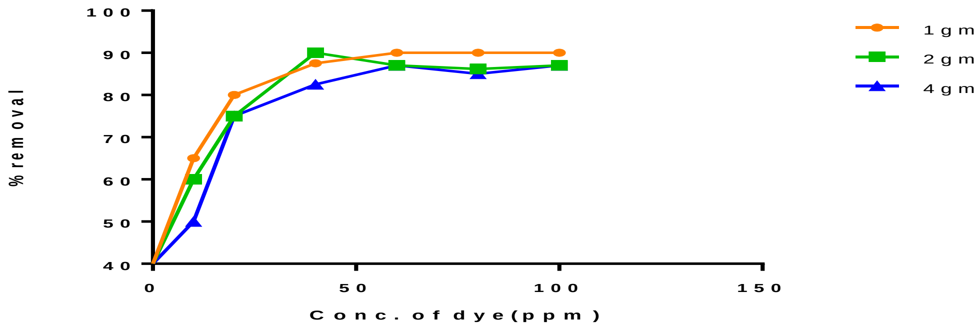

Figure-3: Effect of dye concentration on empty (Ca-Alginate) beads

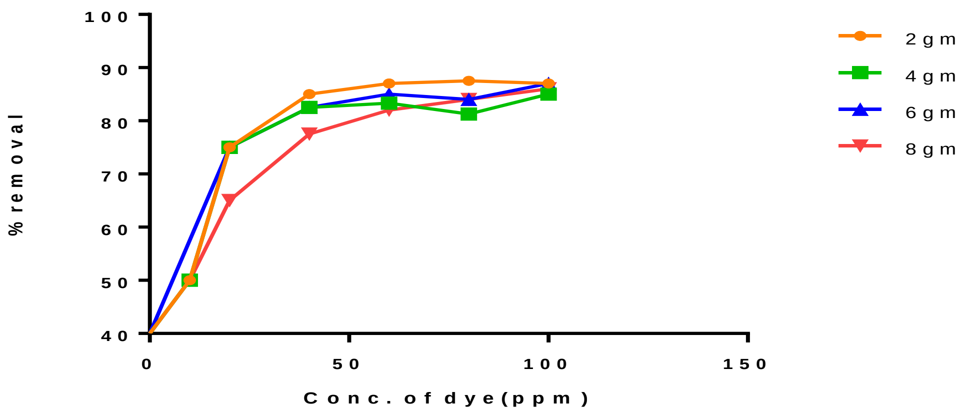

Figure-4: Effect of dye concentration on by immobilized *saccharomyceus service* beads

In case of both empty and yeast beads (Fig-3 & 4), percentage removal is more at higher concentrations compared to lower concentrations. As concentration increases, the noumber of dye molecules which are in contact with the surface of adsorbent also increases indicating the increase in dye removal. From figures 3 and 4, the line increases along the x-axis proving that percentage removal is high at higher dye concentrations.

Effect of Adsorbent dosage on dye removal:

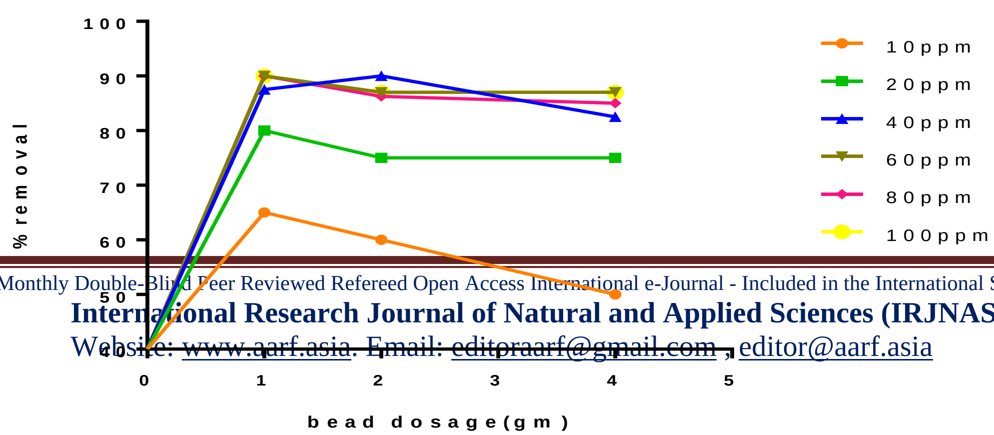

Figure-5: Effect of empty bead (Ca-Alginate) dosages

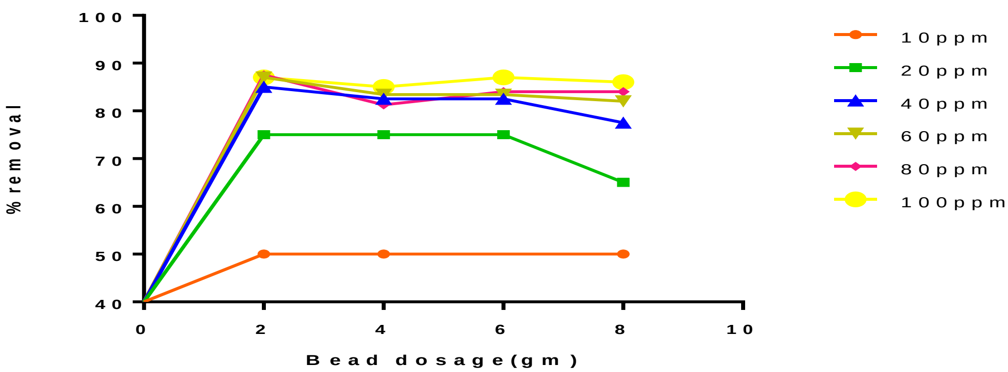

Figure-6: Effect of by immobilized *saccharomyceus service* bead dosages

In case of empty beads the there is no increase in the percentage removal with increase in calcium alginate beads, where as the percentage removal is maximum at 2gm of immobilized *saccharomyceus service* beads. Beyond that there is no significant improvement has observed. The results of both Ca- alginate and immobilized *saccharomyceus service* beads, shown in figure 5 & 6 respectively [23].

Kinetic Models:

Kinetic models allows the estimation of sorption rates and leads to the suitable rate expressions for charecterisation and determination of reaction mechanism. Several kinetic models were proposed to explain the mechanism of adsorption. In the present study the experimental data were tested with Pseudo first order, pseudo second oreder kinetic models and Elovich, Intraparticle diffusion models.

Pseudo First Oder kinetic model:

The linear pseudo first order kinecti model equation is given as follows [24]

$$\log\left(q_e - q_t\right) = \log q_e - \frac{K_1}{2.303} X\, t$$

Where qe and qt are the amount of Alizarin red dye adsorbed at equilibrium (mg/g) and the amount of Alizarin red adsorbed time t (mg/g), respectively;

k_1 (min-1) is the rate constant of pseudo-first order adsorption reaction.

The plot of log (qe–qt) versus t should give a straight line (Figure- 7 & 8) from which rate constant k_1 and qe can be calculated from the slope and intercept of the plot, respectively. If the plot was found to be linear with good correlation coefficient, it indicates that Lagergren's equation is appropriate to Alizarin red sorption. From the figure- 7 and 8, it is observed that boath the beads (Ca-Alginate beads and Immobilized *saccharomyceus service* beads) does not fits into pseudo first order kinetic model. The correlation coeeficient values are very low compare to pseudo second order kinectic model of both beads. The R^2, absolute some of the square value and k1 values were represented in table-1 and table-2.

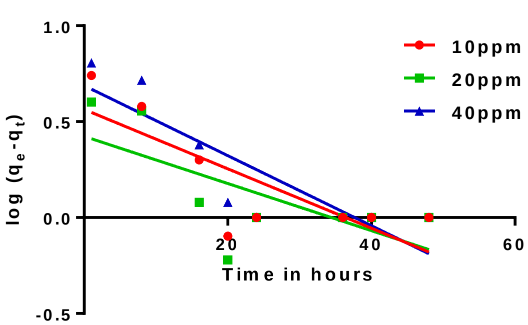

Figure-7: Pseudo first order kinetic model for Adsorption of alizarin red dye by immobilized *saccharomyceus service* beads

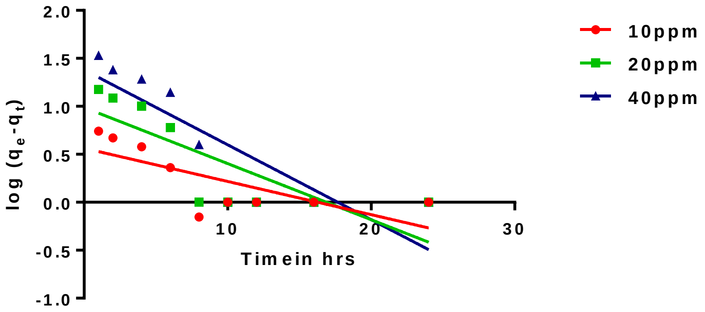

Figure-8: Pseudo first order kinetic model for Adsorption of alizarin red dye by Ca-alginate beads

Pseudo Second Order Kinectic Model:

The pseudo second order kinetic order equation expressed as [25]

$$\frac{t}{q_t} = \frac{1}{k_2\, q_e^2} + \frac{t}{q_e}$$

Where k_2 is the rate constant of pseudo second order adsorption (g/mg min) and q_e is the equilibrium adsorption capacity (mg/gm). Pseudo second order kinetic plot of (*t/qt*) versus (*t*) gave the perfect straight line for the adsorption of Alizarin red. In this model, the rate limiting step is the chemisorptions. The experimental data were shown in figure- 9 & 10. From the figures it is concluded that removal of alizarin red by the Ca- alginate beads and Immobilized *saccharomyceus service* beads is showing boath physical and chemical reactions. The interaction between adsorabate molecules and adsorbent taking place in two phases. The correlation coeeficent values of adsorption of alizarin red dye from aqueous solution by Immobilized *saccharomyceus service* beads were higher compared to pseudo first order kinectic model. It indicating that the adsorptive removal of alizarin red dye from aqueous solution by Immobilized *saccharomyceus service* beads is following pseudo second order kinetic model. There is no siginificant change in correlation coeeficent values of adsorption of alizarin red dye from aqueous solution by Ca- alginate for both pseudo first order and pseudo second order kinetic models. This result indicating that *saccharomyceus service* showing chemical adsorption process by realsing catalytic enzymes into mediaum. The statistical data, which includes k_2, ASS, and R^2

values of pseudeo second order kinetic model of both adsorbents (Ca-Alginate beads and Immobilized *saccharomyceus service* beads) were shown in table-1 and 2.

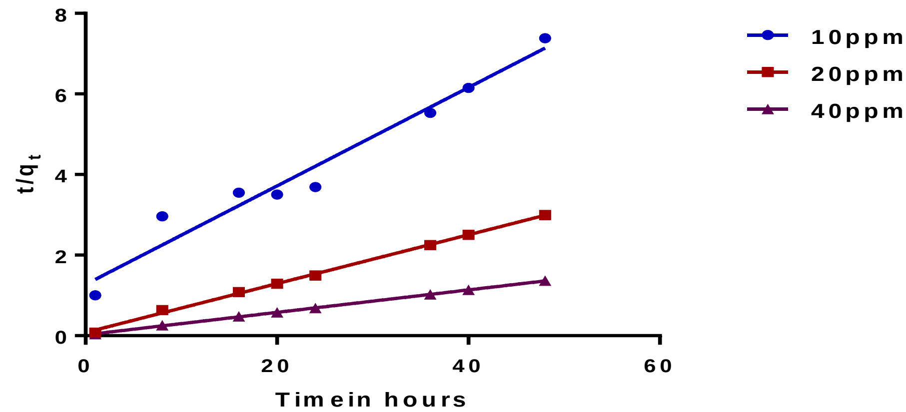

Figure-9: Pseudo Second order kinetic model for Adsorption of alizarin red dye by immobilized *saccharomyceus service* beads

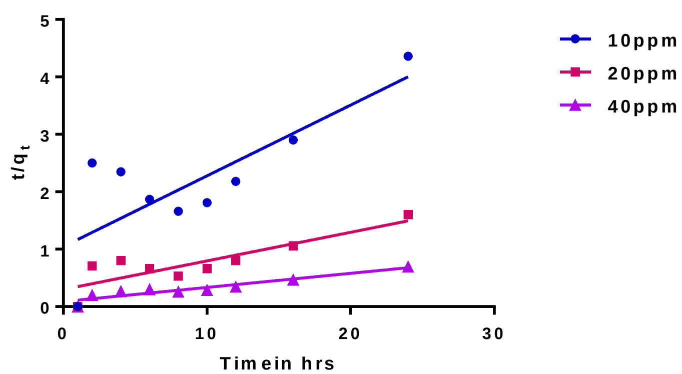

Figure-10: Pseudo Second order kinetic model for Adsorption of alizarin red dye by Ca-alginate beads

Elovich Model:

The Elovich model equation is generally expressed as [26]

$$\frac{dq_t}{d_t} = \alpha \exp(-\beta \, q_t)$$

Where:

α is the initial adsorption rate ($mg \cdot g^{-1} \cdot min^{-1}$)

β is the desorption constant ($g \cdot mg^{-1}$).

If the adsorption of aqueous alizarin red solution by both adsorbents fits to the Elovich model, a plot of q_t versus ln (t) should give a linear relationship with a slope of (1/β) and an intercept of 1/β In (αβ). The results of Elovich plot for the adsorption of alizarin red solution by both adsorbents at various initial concentrations are given in figure- 11 and 12. From the results it is concluded that adsorptive removal of alizarin red solution by both adsorbents fits to the Elovich model at lower concentration only.

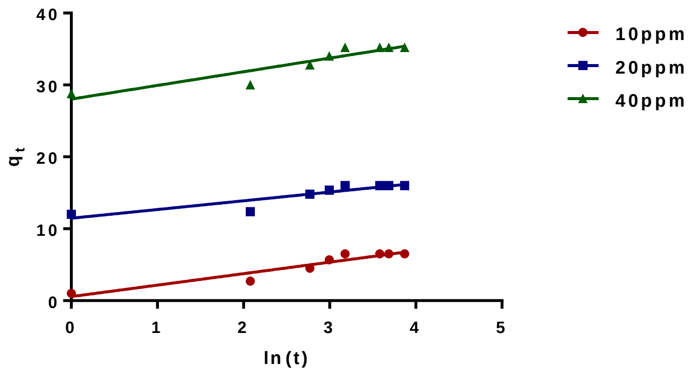

Figure-11: Elovich Model for Adsorption of alizarin red dye by immobilized *saccharomyceus service* beads

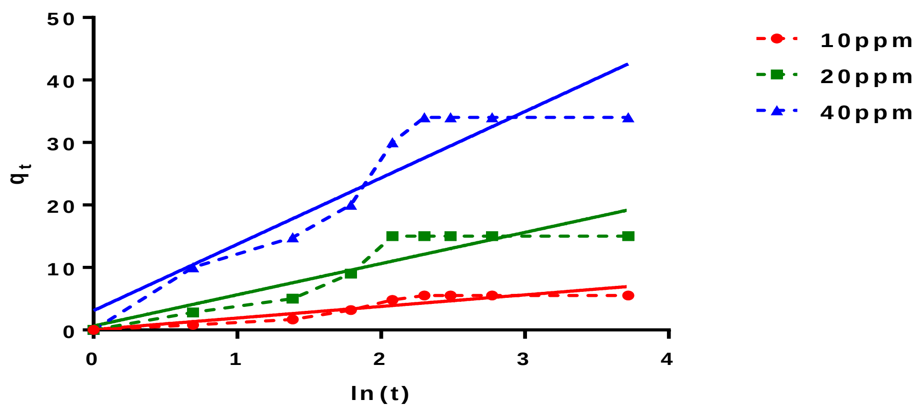

Figure-12: Elovich Model for Adsorption of alizarin red dye by by Ca-alginate beads

Intraparticle diffusion model:the intraparticle diffusion model describes adsorption process, where the rate of adsorption depends on the capacity and speed of adsorbate diffusion on adsorbent. The interaparticle diffusion model is represented by following equation [27]

$$q_t = K_{id}\, t^{1/2} + I$$

Where q_t is the amount of alizarin red adsorbed (mg/g) at time t (min), and I is the intercept (mg/g). k_{id} and I values are obtained from the slopes and intercept of the linear plot. The double nature of these plots may be explained as the initial curve portion is attributed to boundary layer diffusion effect while the final linear portion is due to intraparticle diffusion effect. The results are represented in figure 13 and 14. From the figures it is observed that boath the adsorbents were partially fits into intraparticle diffusion model.

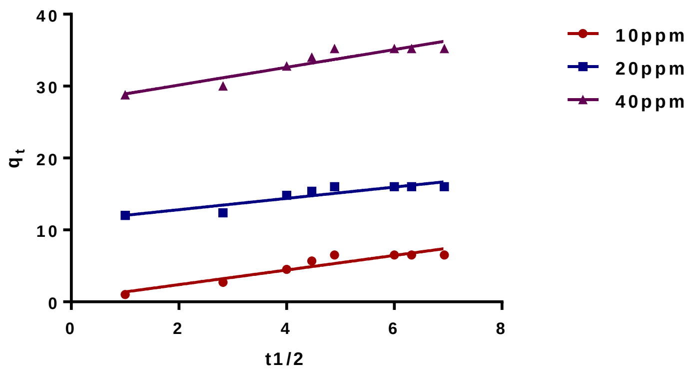

Figure-13: Intraparticle Diffusion model for Adsorption of alizarin red dye by immobilized *saccharomyceus service* beads

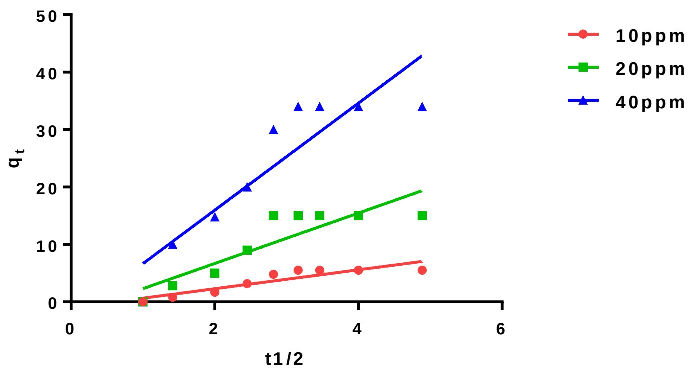

Figure-14: Intraparticle Diffusion model for Adsorption of alizarin red dye by Ca-alginate beads

Table-1: Kinetic parameters for Adsorption of alizarin red dye by immobilized *saccharomyceus service* beads

S.No	Parameters	Dye concentration (10 ppm)	Dye concentration (20 ppm)	Dye concentration (40 ppm)
	Pseudo first order kinetic model			
01	R^2	0.636	0.465	0.745
	ASS	0.251	0.319	0.209
	K_1	0.067	0.186	0.238
	Pseudo Second order kinetic model			
02	R^2	0.959	0.998	0.999
	ASS	1.155	0.011	0.000
	K_2	0.122	0.060	0.027
	Elovich model			
03	R^2	0.909	0.826	0.863
	ASS	2.807	3.424	6.311
	α	-2.525	2.121	2.663
	β	0.626	0.825	0.526
	Intraparticle diffusion model			
04	R^2	0.892	0.844	0.883
	ASS	3.326	3.065	5.401
	k_{id}	1.014	0.786	1.233
	I	0.368	11.24	27.69

Table-2: Kinetic parameters for Adsorption of alizarin red dye by empty Ca-Alginate beads

S.No	Parameters	Dye concentration (10 ppm)	Dye concentration (20 ppm)	Dye concentration (40 ppm)
	Pseudo first order kinetic model			
01	R^2	0.543	0.627	0.720
	ASS	0.434	0.877	1.018
	K_1	0.078	0.103	0.173
	Pseudo Second order kinetic model			
02	R^2	0.614	0.736	0.890
	ASS	4.108	0.382	0.032
	K_2	0.123	0.049	0.024
	Elovich model			
03	R^2	0.845	0.818	0.858
	ASS	6.254	54.82	184.6

	α	-1.032	0.390	0.960
	β	0.539	0.200	0.094
	Intraparticle diffusion model			
04	R^2	0.823	0.783	0.817
	ASS	7.156	65.52	238.0
	k_{id}	1.645	4.390	9.318
	I	-0.994	-2.088	-2.661

Conclusions:

- Immobilized *saccharomyceus service* beads and Ca-alginate beads were tested for efficiency of adsorption of alizarin red dye from aqueous solution. The results showed the Immobilized *saccharomyceus service* beads offered more porous surface than Ca-alginate beads.

- Kinetic stuies demonstrated the addition of *saccharomyceus service* increase the contact time but increased the rate of adsorption and efficiency of percentage removal.

- The suitability of fitting of the kinetic models were determined by introducing correlation coefficient values. The closer values of R2 ~ 1 the more applicable the model was, adsorption rates fitted well for pseudo second order kinetic model compared to pseudo first order kinetic model. This result indicating that physicochemical adsorption taking place at the interface or at adsorbent surface. The Immobilized *saccharomyceus service* beads showed more correlation with pseudo second order compared tho Ca-alginate beads.

- Ca-alginate beads were thick sealed gel structure and that suppressed the penetration of alizarin red molecules deeply into the internally trapped support. The higher rate constant of pseudo second order indicated that affinity is for physicochemical adsorption with increase in concentration for both adsorbents

- The rate constant for Ca-alginate beads were less compare to Immobilized *saccharomyceus service* beads concluding that availability of more surface area in Immobilized *saccharomyceus service* beads.

- Finally this study successfully provided an alternative low cost technology for treatment of dye containing wastewater in the field of textile, paper and pulp industries.

REFERENCES

1. A. Baban, A. Yediler and N.K. CilizIntegrated water management and CP implementation for wool and textile blend processes. *Clean*, .38(1), 2010, 84-90.
2. 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. *Bioresource Technology*, 77, 2001, 247-255.
3. P.A. Soloman, C.A. Basha, V. Ramamurthi, K. Koteeswaran and N. Balasubramanian. Electrochemical degradation of Remazol Black B dye effluent. *Clean,* 37(11), 2009, 889-900.
4. "A special report on India: Creaking, groaning: Infrastructure is India's biggest handicap". *The Economist*. December 11, 2008.
5. "China says water pollution so severe that cities could lack safe supplies". Chinadaily.com.cn. June 7, 2005.
6. "As China Roars, Pollution Reaches Deadly Extremes". The New York Times. August 26, 2007.
7. United States Environmental Protection Agency (EPA). Washington, DC. "The National Water Quality Inventory: Report to Congress for the 2002 Reporting Cycle – A Profile." October 2007. Fact Sheet No. EPA 841-F-07-003.
8. D. Suteu, C. Zaharia, D. Bilba, A. Muresan, R. Muresan and A. Popescu. Decolorization wastewaters from the textile industry – physical methods, chemical methods. *Industria Textila*, 60(5), 2009, 254-263.
9. Encapsulation of urease enzyme in xanthan-alginate spheres. Elçin YM Biomaterials. 1995 Oct; 16(15), 1157-61.
10. A. Flores-Maltos, L.V. Rodríguez-Durán, J. Renovato, J.C. Contreras, R. Rodríguez, and C.N. Aguilar. Catalytical properties of free and immobilized Aspergillus niger tannase. *Enzyme Res.* 2011 [PMC free article]

11. S. Chen, N. Song, X. Liao and B. Shi. Immobilization of catalase on ferric modified collagen fibers. *Chin J Biotechnol*. 27, 2011, 1076–1081.
12. K. Girigowda and V.H. Mulimani. Hydrolysis of galacto-oligosaccharides in soymilk by κ-carrageenan-entrapped α-galactosidase from *Aspergillus oryzae*. *World J Microbiol Biotechnol*. 22, 2006, 437–442.
13. N.A. Dung, Huyen, N.D. Hang and T.T. Canh. Immobilization of urease on grafted starch by radiation method. *Radiat Phys Chem*. 46, 1995, 1037–1042.
14. M.P. Klein, C.W. Scheeren, A.S.G. Lorenzoni, J. Dupont, Frazzon and P.F. Hertz. Ionic liquid-cellulose film for enzyme immobilization. *Process Biochem*. 46, 2011, 1375–1379.
15. A.I. Raafat, E. Araby and S. Lotfy. Enhancement of fibrinolytic enzyme production from *Bacillus subtilis* via immobilization process onto radiation synthesized starch/dimethylaminoethyl methacrylate hydrogel. *Carbohyd Polym*. 87, 2011,1369–1374.
16. Q. Shen, R. Yang, X. Hua, F. Ye, W. Zhang and W Zhao. Gelatin-templated biomimetic calcification for β-galactosidase immobilization. *Process Biochem*. 46, 2011, 1565–1571.
17. S. Ateş and N.S. Doğan. Properties of immobilized phenylalanine ammonia lyase and investigation of its use for the prediagnosis of phenylketonuria. *Turk J Biochem*. 35, 2010, 58–62.
18. R. Satar, M. Matto and Q. Husain. Studies on calcium alginate–pectin gel entrapped concanavalin A–bitter gourd (Momordica charantia) peroxidase complex. *J Sci Ind Res India*. 67, 2008, 609–615.
19. A. Kumari and A.M. Kayastha. Immobilization of soybean (Glycine max) α-amylase onto chitosan and amberlite MB-150 beads: optimization and characterization. *J Mol Catal B-Enzym*. 69, 2011, 8–14.
20. A.S. Rani, M.L.M. Das and S. Satyanarayana. Preparation and characterization of amyloglucosidase adsorbed on activated charcoal. *J Mol Catal B-Enzym*. 10, 2000, 471–476.
21. R.P. Pogorilyi, E.Y. Siletskaya, V.P. Goncharik, L.I. Kozhara, Y.L. Zub. Immobilization of urease on the silica gel surface by sol–gel method. *Russ J Appl Chem*. 80, 2007, 330–334.

22. Y.B. Lin, B. Fugltsu, N. Terai and S. Tanaka. Removal of Organic compounds by aliginate gel beads with entrapped activated carbon, *Journal of Hazard. Mater*. 120 (1-3), 2005, 231-241.
23. T.W. Tzu, T. Tsuritani and K. Sato. Sorption of Pb(II), Cd(II), and Ni(II) Toxic Metal Ions by Alginate-Bentonite, *Journal of Environmental projection*, 4(1B), 2013, 51-55.
24. N. Gandhi, D. Sirisha and K.B. Chandra Sekar. Bioremediation of wastewater by using *Strychnos potatorum* seeds (clearing nuts) as bio adsorbent and natural coagulant for removal of Fluoride and Chromium, *journal of international academic research for multidisciplinary*, 2(1), 2014, 253-272.
25. N. Gandhi, D. Sirisha and K.B. Chandra Sekar. Adsorption of fluoride from aqueous solution by using chalk powder. World journal of Pharmacy and Pharmaceutical Sciences, 2(5), 2013, 3897-3914.
26. N. Gandhi, D. Sirisha and K.B. Chandra Sekar. Adsorption of Chromium (VI), from aqueous solution by using multani mitti. International journal of Research in Pharmacy and Chemistry, 4(1), 2014, 168-180.
27. N. Gandhi, D. Sirisha and Vikas Chandra Sharma. Microwave-Mediated Green Synthesis of Silver Nanoparticles Using *Ficus Elastica* Leaf Extract and Application in Air pollution Controlling Studies. *International Journal of Engineering Research and Applications.* 4(1), 2014, 01-12.