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BIO ADSORPTION OF ALIZARIN RED DYE USING IMMOBILIZED *SACCHAROMYCES CEREVISIAE*

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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, Immobilization, Alizarin red, Ca-Alginate, *Saccharomyces cerevisiae*.

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 color, 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, bio absorption, aerobic and anaerobic digestion. In the present study, bio adsorption 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 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 are 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 *Saccharomyces cerevisiae* was selected as the organism to be immobilized because it is cost-effective and easily available commercially. The dye to be de-colorized 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 pH 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 these 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 these 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:

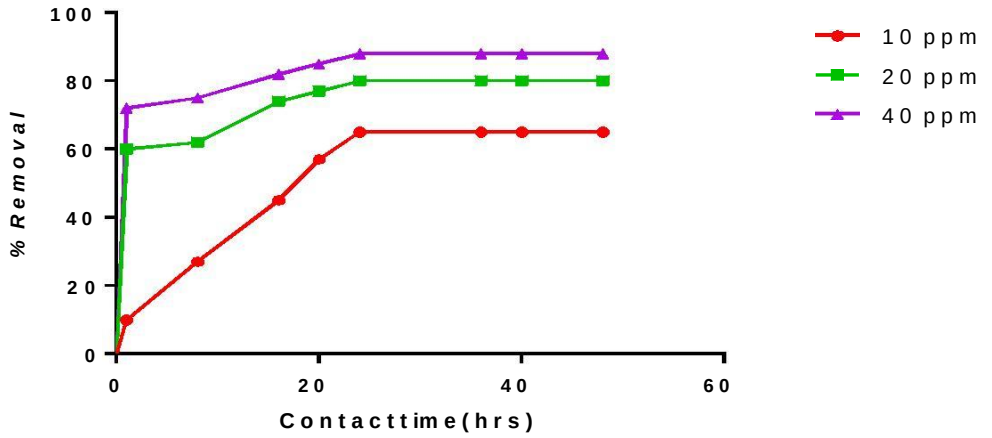

Figure-1: Effect of contact time by empty (Ca-Alginate) beads

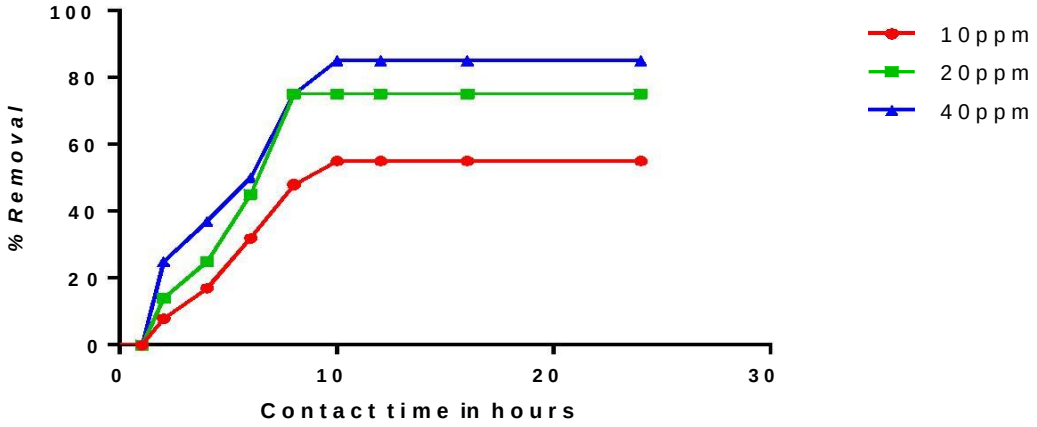

Figure-2: Effect of Contact time by immobilized *Saccharomyces cerevisiae* beads

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 up to 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

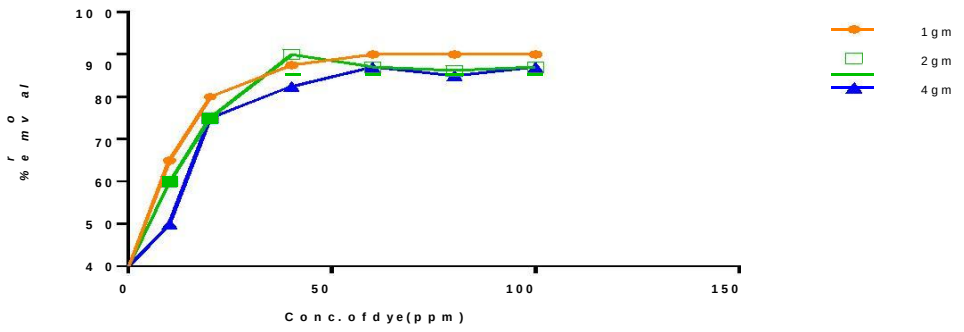

Figure-3: Effect of dye concentration on empty (Ca-Alginate) beads

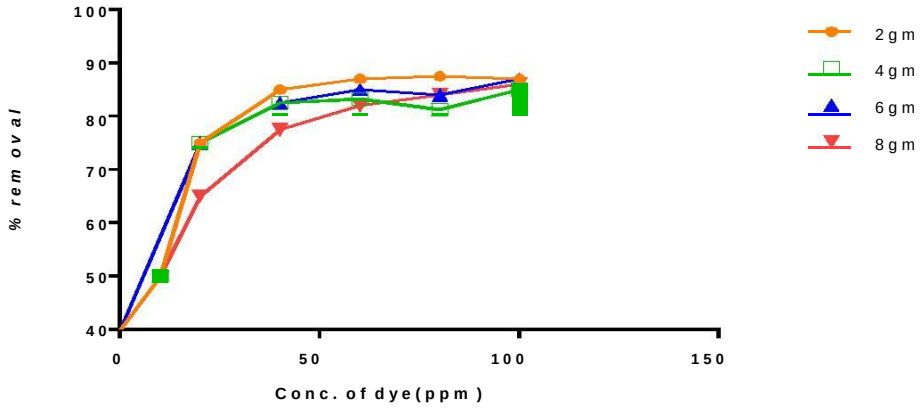

Figure-4: Effect of dye concentration on by immobilized *Saccharomyces cerevisiae* beads

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

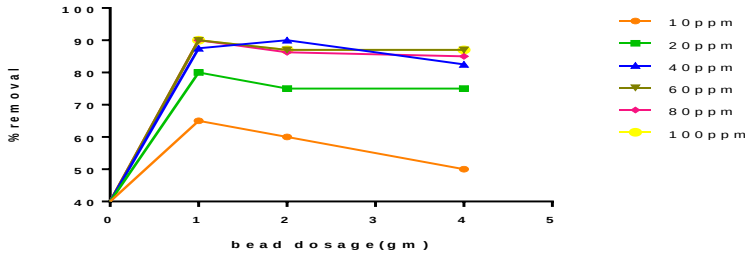

Figure-5: Effect of empty bead (Ca-Alginate) dosages

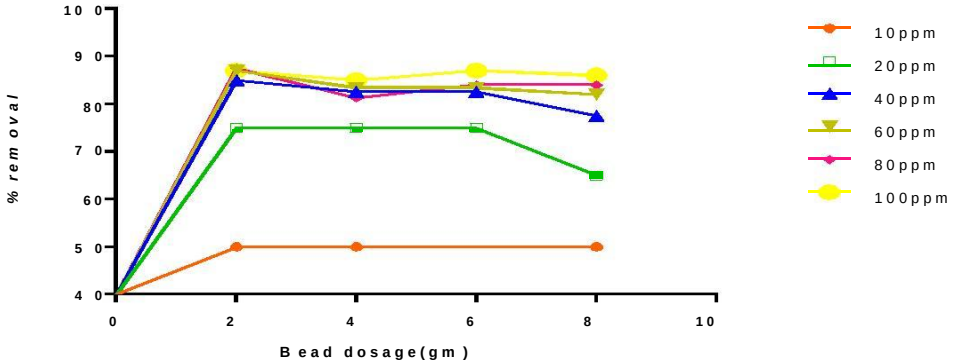

Figure-6: Effect of by immobilized *Saccharomyces cerevisiae* bead dosages

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 *Saccharomyces cerevisiae* beads. Beyond that there is no significant improvement has observed. The results of both Ca- alginate and immobilized *Saccharomyces cerevisiae* beads, shown in figure 5 & 6 respectively [23].

Kinetic Models:

Kinetic models allow the estimation of sorption rates and leads to the suitable rate expressions for characterization 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 order kinetic models and Elovich, Intraparticle diffusion models.

Pseudo First Oder kinetic model:

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

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

Where *q*e and *q*t 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 (*q*e–*q*t) versus *t* should give a straight line (Figure- 7 & 8) from which rate constant k_1 and *q*e 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 both the beads (Ca-Alginate beads and Immobilized *Saccharomyces cerevisiae* beads) do not fits into pseudo first order kinetic model. The correlation coefficient values are very low compare to pseudo second order kinetic model of both beads. The R^2, absolute some of the square value and k1 values were represented in table-1 and table-2.

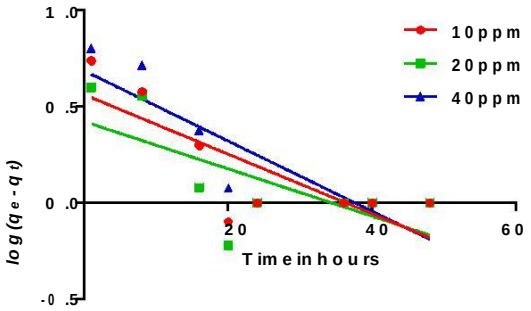

Figure-7: Pseudo first order kinetic model for Adsorption of alizarin red dye by immobilized *Saccharomyces cerevisiae* beads

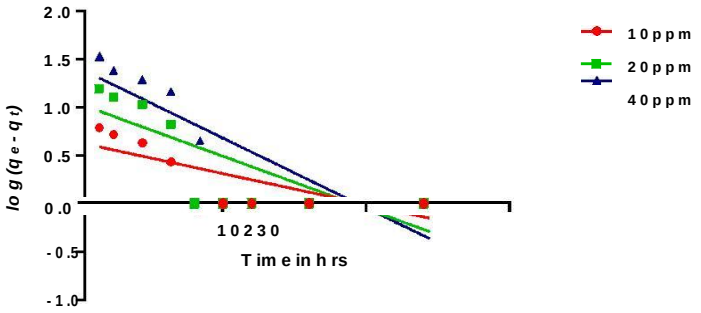

Figure-8: Pseudo first order kinetic model for Adsorption of alizarin red dye by Ca-alginate beads

Pseudo Second Order Kinetic 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 *Saccharomyces cerevisiae* beads is showing both physical and chemical reactions. The interaction between adsorabate molecules and adsorbent taking place in two phases. The correlation coefficient values of adsorption of alizarin red dye from aqueous solution by Immobilized *Saccharomyces cerevisiae* beads were higher compared to pseudo first order kinetic model. It was indicating that the adsorptive removal of alizarin red dye from aqueous solution by Immobilized *Saccharomyces cerevisiae* beads is following pseudo second order kinetic model. There is no significant change in correlation coefficient 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 *Saccharomyces cerevisiae* showing chemical adsorption process by catalytic enzymes. The statistical data, which includes k_2, ASS, and R^2 values of pseudo second order kinetic model of both adsorbents (Ca-Alginate beads and Immobilized *Saccharomyces cerevisiae* beads) were shown in table-1 and 2.

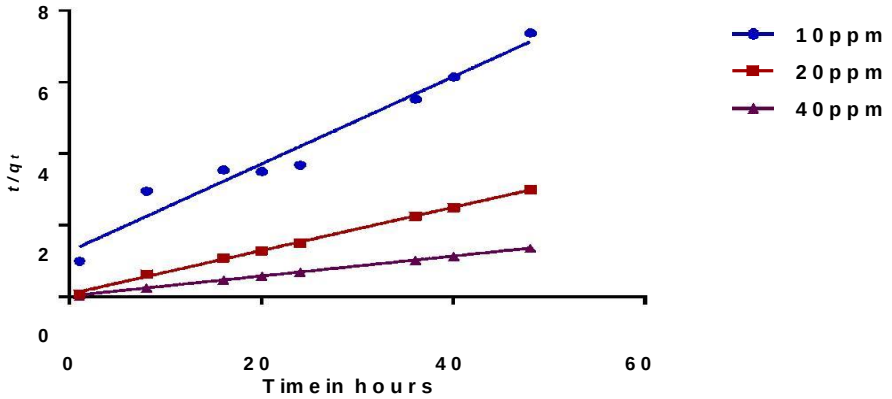

Figure-9: Pseudo Second order kinetic model for Adsorption of alizarin red dye by immobilized *Saccharomyces cerevisiae* beads

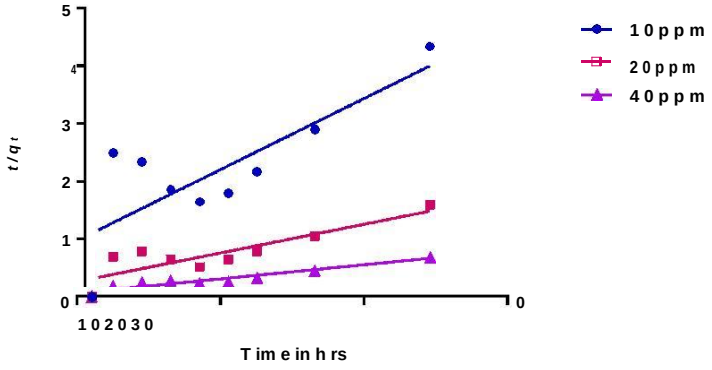

Figure-10: Pseudo Second order kinetic model for Adsorption of alizarin red dye by Ca-alginate beads

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.

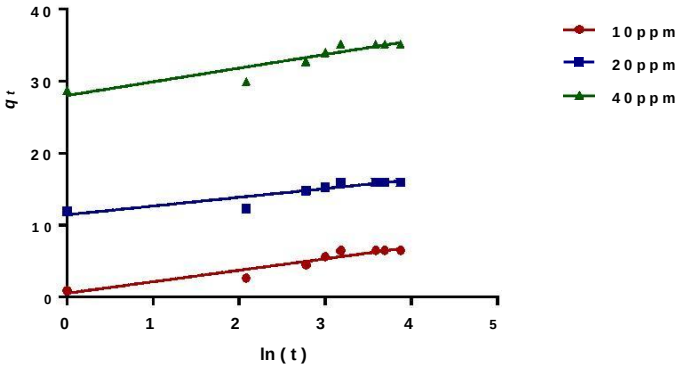

Figure-11: Elovich Model for Adsorption of alizarin red dye by immobilized *Saccharomyces cerevisiae* beads

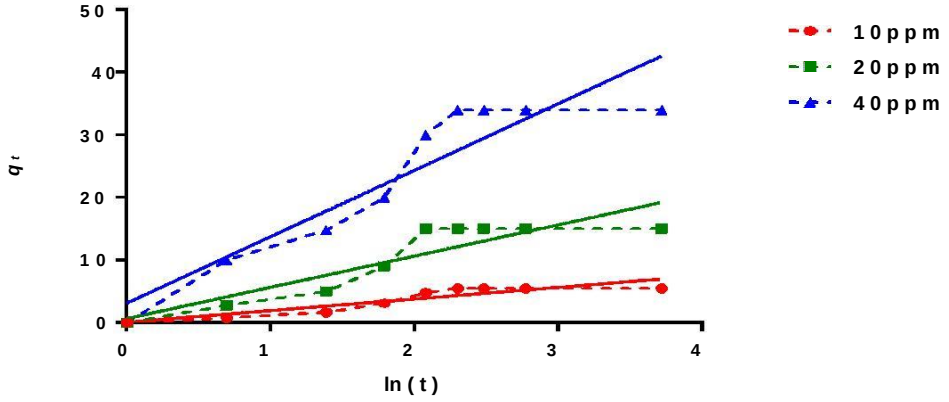

Figure-12: Elovich Model for Adsorption of alizarin red dye by by Ca-alginate beads

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 intraparticle 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 both the adsorbents were partially fits into intraparticle diffusion model.

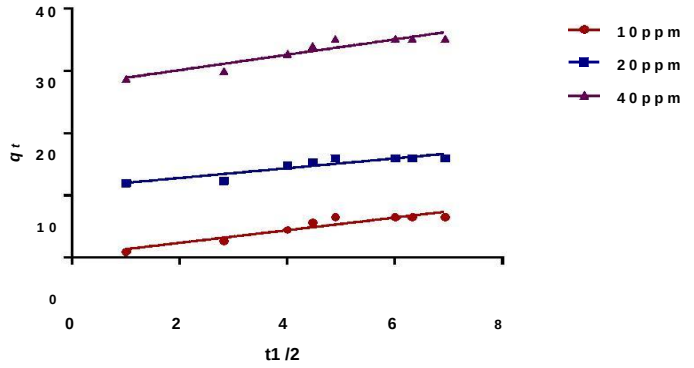

Figure-13: Intraparticle Diffusion model for Adsorption of alizarin red dye by immobilized *Saccharomyces cerevisiae* beads

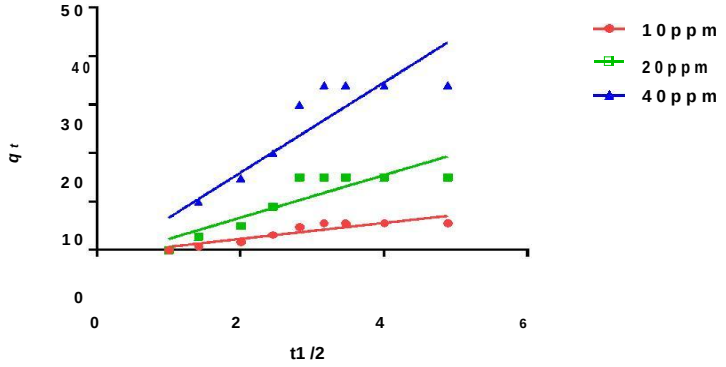

Table-1: Kinetic parameters for Adsorption of alizarin red dye by immobilized *Saccharomyces cerevisiae* beads

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 *Saccharomyces cerevisiae* beads and Ca-alginate beads were tested for efficiency of adsorption of alizarin red dye from aqueous solution. The results showed the
- Immobilized *Saccharomyces cerevisiae* beads offered more porous surface than Ca-alginate beads.
- Kinetic studies demonstrated the addition of *Saccharomyces cerevisiae* increase the contact time but increased the rate of adsorption and efficiency of percentage removal.
- The suitability of fitting of the kinetic models was 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 *Saccharomyces cerevisiae* beads showed more correlation with pseudo second order compared to 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 *Saccharomyces cerevisiae* beads concluding that availability of more surface area in Immobilized *Saccharomyces cerevisiae* 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.

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