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STATISTICAL OPTIMIZATION OF BIOSORPTION OF BASIC BLUE 9 BY DEAD FUNGAL BIOMASS ISOLATED FROM ONION

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ABSTRACT

The textile industry consumes a substantial amount of water in its manufacturing processes used mainly in the dyeing and finishing operations of the textile plants. The wastewater from textile plants is classified as the most polluting of all industrial sector caused by discharged of untreated effluent into water bodies. In the present investigation dead fungal biomasses from onion isolate is evaluated as a potential biosorbent in the removal of basic blue 9 dye. The choice of onion isolate as a biosorbent for further work was based on its high growth rate as well as high rates of biosorption. Basic Blue 9 dye was chosen as a dye of interest considering its wide use in Indian textile industry and lack of any credible work on them. Experimental design based on sequential statistical approach consisting of Plackett–Burman Design (PBD) for screening critical factors followed by Central Composite Design (CCD) using Response Surface Methodology (RSM) was used for removal of basic blue 9 dye. The use of well-established statistical techniques, to build models, plots, to study the interactions between the variables and to select the optimum conditions of variables and minimizing the errors. Equilibrium and kinetic modeling of biosorption shows that the adsorption follows Langmuir isotherm. Kinetic studies were done using pseudo first order kinetics and pseudo second order kinetics. FTIR analyses indicated that the principal groups involved in sorption were CH and OH. Optimization of biosorption by RSM could achieve 94.16 % of dye removal.

KEYWORDS: Basic Blue 9 .Plackett – Burman design. Central Composite Design Response Surface Methodology. Isotherm .Kinetics.

1. INTRODUCTION

The start of rapid urbanization and industrialization, all driven by the exceptional population growth rate and increasing acceptance of an industrial– based lifestyle has inevitably led to an augmented anthropogenic impact on the biosphere. The dyeing units and the dyestuff industries as a whole are one of the sectors under the strong radar of the environmental agencies. The art of colour application to enhance the world around us has been known to man since the dawn of civilization. With the development of agriculture and fixed settlements around 7,000-2,000 B.C., man began to produce, use and colour textiles (Grierson, et. al 1989).

Dyes are coloured substance which are soluble or go into solution during application process and impart colour by selective absorption of light. The effluent of these industries are coloured and due to high stability of dyes, the colour is difficult to remove. At present more than 10000 dyes have been effectively commercialized. The dyes in industrial effluents reduce light penetration preventing the photosynthesis of aqueous flora. Some of the dyes many cause allergy, skin irritation and even cancer in humans. It is necessary to treat effluent before discharging them (D K singh et.al 2000).

Basic blue 9 is used as both the zinc-free product and the zinc chloride double salt. Some small amounts are used in leather dyeing and as a polymerization inhibitor in some monomeric organics. Smaller quantities of the zinc-free type are used medicinally and as an injectable solution to combat cyanosis. Losses of dyes (in general) to wastewater effluents during manufacture have been estimated to be 1-2%. For the organic dye industry in general, it has been estimated that as much as 10% of the dye is lost to wastewater effluents during dyeing operations.

Biosorption may be simply defined as the removal of substances from solution by biological material. Such substances can be organic and inorganic, and in gaseous, soluble or insoluble forms. Biosorption is a physico-chemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface

complexation and precipitation. Biosorption is a property of bothliving and dead organisms (and their components) and has been heralded as a promising biotechnology for pollutant removalfrom solution, and/or pollutant recovery, for a number of years, because of its efficiency, simplicity, analogous operation toconventional ion exchange technology, and availability of biomass (Geoffrey MichealGadd et.al 2008) Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents. It is consider an ideal alternative method for removing contaminates from effluents.

2. MATERIALS AND METHODS

2.1. Sample collection and growth media

Three soil samples were taken from different locations: Dead leaves soil, Root soil and garden soil. Onion and Bread were also taken as fourth and fifth sample. The organism to be isolated was a fungi; therefore Sabourauds Agar, Malt Extract Agar and Potato Dextrose Agar were used.

2.2. Isolation and selection

Serial dilutions of soil sample was done of each sample from 10^{-1} to 10^{-9}. Loopful suspension was taken from three dilutions such as 10^{-6}, 10^{-7}, 10^{-8} and was spreaded on the sterile media of Sabouraud's Agar, Malt Extract agar, Potato Dextrose Agar by spread plate technique. Spot inoculation of onion and bread sample was carried on Sabouraud's Agar, Malt Extract agar, Potato Dextrose Agar. Plated was incubated at room temperature for 5-7 days. Five samples were selected based on their maximum growth. The samples were checked for the maximum growth by inoculating it in Potato Dextrose Broth and incubated at room temperature. After four days, the biomass obtained in the broth was removed and washed twice with deionized weight. The biomass was then dried at 60°-70°C in hot air oven until constant weight was obtained. The biomass for biosorption was selected showing maximum weight in 4 days.

2.3. Identification

Wet mount for each of the sample obtained on Sabouraud's Agar, Malt Extract agar, Potato Dextrose Agar was performed. Lactophenol cotton blue staining was performed for selected strain. It was carried out by taking the culture in watch-glass and adding lactophenol cotton blue stain in it. The culture was transferred onto the grease free slide and was observed under the compound microscope at 40X.

2.4. Maintainence of culture

The selected culture was maintained on Potato Dextrose Agar Slants and preserved at 4°C with sub-culturing every week.

2.5. Preparation of stock solution

Basic Blue 9 was procured from Colourtex Pvt. Ltd. (Mumbai, India). Stock solution (1000 mg/L) of the dye was prepared by dissolving 100 mg of the dye in 100 mL distilled water. The stock solutions were then diluted to get the test solutions of the desired strength. The required pH was adjusted by 0.1 N HCl or 0.1 N NaOH using a pH meter.

2.6. Fungal biomass production

The fungal biomass was obtained by transferring mycelia (1 disk 5mm each) from the Potato Dextrose Agar inoculated culture plate into 100 ml of Potato Dextrose Broth in 250 ml Erlenmeyer flask under sterile conditions. The flask was incubated at room temperature, the biomass harvested after 4-5 days was washed thoroughly with deionized water and dried at 60°-70°C in the hot air oven for 12 hrs. The culture to be evaluated as biosorbent was initially chosen based on its growth rate giving high yield. The biomass was grined into fine powder with mixer grinder and sieved through a 150 mesh sieve to keep the particles size uniform. The powder was stored in a glass bottle and was used for further studies.

2.7. Batch biosorption studies

The biosorption experiments were carried out in 250 mL Erlenmeyer flasks with working volume of 50 mL of the reaction mixture consisting of desired concentration of dye prepared from stock solutions (1000 mg/L) and specified amount of biosorbent. The flasks were withdrawn from the rotating orbital shaker after shaking for the desired time of reaction. The residual dye concentration in the solution was determined after filtering the biomass from the system. The concentration of the dye/s in the solution was determined from the calibration curve prepared by measuring the absorbance of known concentrations of the dye at the maximum wavelength of sorption using a UV-Vis spectrophotometer. Blank without biosorbent was run simultaneously as a control. The concentration of the dye/s on the fungal biomass at the corresponding equilibrium conditions was determined using a mass balance equation expressed as specific uptake capacity (SUC) (Akar *et al*., 2007):

$$Q_e = \frac{(C_0 - C_e) * V}{M} \qquad (1)$$

Where, C_0 and C_e are the initial and final concentrations (mg/L), respectively, M is the biosorbent dosage (g) and V the volume of the solution (L) (Yang *et al*., 2012).

2.8. Application of statistical experimental design for the optimization of conditions for biosorption of basic blue 9

Batch biosorption experiments were conducted using sequential statistically designed experiments consisting of Plackett–Burman design (PBD) and Central Composite Design (CCD). Minitab 16 (State College, PA, USA) software was used for experimental design, construction of quadratic models and graphical analysis of the experimental data.

2.8.1. Plackett-Burman Design (PBD)

PBD, a fractional factorial design was used to identify the variable(s) having a significant effect on the sorption uptake capacity of the selected biosorbent. Six independent variables pH, concentration of dye (mg/L), biosorbent dosage (g/L), speed of agitation (rpm), contact time (min) and temperature (^oC)were screened at high (+) as well as low (-) levels. All trials were performed in duplicate and the average values of decolourization experiment were treated as responses. The main effect of each variable was determined with the following equation:

$$E(X_i) = \frac{2(\sum M_{+i} - \sum M_{-i})}{N} \quad (2)$$

Where, (X_i) is the effect of the tested variable (X_i) and M_{+i} and M_{-I} are responses (biosorption) of trials at which the variable is at its high or low level, respectively. N is the total number of trials. From the regression analysis, significant variables affecting biosorption were determined and the contribution of the variables towards the sorption of dyes was determined based on the *t*-value (main effect) (Plackett and Burman, 1946).

2.8.2. Optimization by response surface methodology using CCD

The optimal levels of the significant factors and the interactions of these variables on biosorption were analyzed by using CCD to locate the true optimum values of the significant factors obtained in PBD (Prakash *et al.*, 2008). The factors were coded according to the following Eq. 3:

$$X_i = \frac{Xi - X0}{\Delta X}, \quad i = 1,2,\dots,k \quad (3)$$

Where, X_i is the coded independent factor, X_i is the real independent factor and X_0 is the value of X_i at the center point; Δx is the step change value. For statistical calculations, the variables X_i are coded as x_i. A second-order polynomial response equation (Eq. 4) was proposed to correlate the dependent and independent variables:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_{i2} + \sum \beta_{ij} x_i x_j, \quad i = 1,2,3,\dots,k \quad (4)$$

Where, Y is the predicted response, b_0 is the intercept, x_i and x_j are the coded independent factors, b_i is the linear coefficient, b_{ii} is the quadratic coefficient and b_{ij} is the interaction coefficient.

2.9. Kinetic and isotherm modeling of biosorption basic blue 9

Kinetic study of the biosorption was carried out using conditions obtained in the CCD but by varying the time intervals. The samples were removed at defined time interval of 20 min over a period of 240 min. In order to understand the process of adsorption, two kinetic models namely Pseudo first order, Pseudo second order and two isotherm models, i.e. Langmiur and Freundlich were studied.

2.10. Characterization of the biosorbent

2.10.1. FTIR analysis

The FTIR spectral analysis was performed to identify the contribution of characteristic functional groups towards biosorption. The infrared spectrum of the biosorbent before and after biosorption was obtained by using spectrometer (Shimadzu 8400s, Japan) with a scan range of 400–4000 cm^1. The facilities at University of Pune were availed of for the studies.

2.11. Desorption studies

Desorption of Basic Blue 9 from the biosorbent was carried out by standardizing the molarity of the desorbing solution. Desorption was then carried out with solid/liquid ratio (S/L) of 10, by using optimized concentration of the eluent. The reusability of biosorbent was then determined using consecutive biosorption-desorption cycles at optimum S/L and repeated two times using the same biosorbent. The supernatant was analyzed for presence of the dye concentration after 200 min of shaking at 120 rpm. The eluted biosorbent was washed repeatedly with deionized water to remove any residual desorbing solution and placed into dye solution for the succeeding biosorption cycle (Akata *et al.*, 2011). Desorption efficiency was calculated by using following equation:

$$\text{Desorption efficiency} = \frac{\text{Amount of dye desorbed}}{\text{Amount of dye adsorbed}} \times 100 \quad (5)$$

3. RESULTS AND DISCUSSION

3.1. Selection of biosorbent

Selection of the biosorbent was done on the basis of the dry weight that was obtained after 4 days of growth. The results obtained were as follows.

Table: 1. Selection of biosorbent based on the maximum weight obtained in 8 days.

Sample	Weight of empty petriplate (g)	Weight of petriplate+ dry sample (g)	Weight of sample (g)
D_2	41.3	43.8	2.5
O_1	50.0	54.3	4.3
O_1 with twin 20	43.3	46.2	2.9
O_3	44.4	46.5	2.1
D_1	50.1	53.0	2.9
R_1	42.5	45.7	3.2
B_1	48.0	50.4	2.4

The maximum growth was obtained in sample O_1 that is 4.3 g whereas minimum growth obtained was of the sample D_2 that is 2.5g. Therefore, sample O_1 was selected as the biosorbent and further identification was done for the same

3.2 Identification

The colonies obtained on Potato Dextrose Agar consisted of a compact white felt covered by a dense layer of black conidial heads. Conidial heads were large, globose, dark brown, reverse colony colour was hyaline to light yellow/ uncoloured. Sterigmata were biseriate with the phialides ampuliform, often club shaped metulae. Morphological and microscopical characters of the selected sample were observed as follows-

Table 2: Microscopic characteristics of the isolate O_1.

Characteristics	Observation
Colony colour	Black
Reverse	Hyaline to light yellow/ uncoloured
Colony size (mm)	Full- plate
Vesicle	Globose
Sterigmata	Biseriate
Phialides	Ampuliform
Matulae	Club shaped
Conidia	Globose

Fig. 1: Lactophenol cotton blue staining for the selected biosorbent.

On observing the sample under microscope using lactophenol cotton blue, the above morphollogical features were seen (Fig. 1) i.e. the vesicles were globus and the conidia were arranged on the vesicles in straight lines one above the other forming a flower like pattern.

Based on the above morphological and microscopic characteristics the isolate obtained was thought to be *Aspergillus niger*. For further confirmation the sample was sent for 16s RNA determination.

3.3 Evaluation of operating conditions for biosorption of basic blue 9

3.3.1 Plackett- burman design

The Plackettt-Burman experimental design is used to identify the most important factors early in the experimentation phase, when complete knowledge about the system is usually unavailable.

In practical use, two-level full or fractional factorial designs, and Plackett-Burman designs are often used to screen for the important factors that influence process output measures or product quality. These designs are useful for fitting first-order models (which detect linear effects) and can provide information on the existence of second-order effects (curvature) when the design includes center points (www.isixsigma.com). The factors taken into consideration for biosorption of BB9 using the isolate are as follows:

Table 3: Levels of the variable tested in PBD for the biosorption of Basic Blue 9 by onion isolate.

Designation	Variable	Variable Values		
		-1	0	1
A	pH	2	4	6
B	Temperature(°C)	35	40	45
C	Concentration of dye(mg/L)	40	70	100
D	Biosorbent dosage(g/L)	0.8	1.4	2
E	Contact Time(min)	30	75	120
F	Speed of Agitation(rpm)	80	100	120

Table: 4. PBD of variables (In coded levels) with experimental values of biosorption of Basic Blue 9 by onion isolate.

	Coded Values						
Run order	A	B	C	D	E	F	%Biosorption
1	1	-1	-1	-1	1	1	41.2
2	1	-1	1	-1	-1	-1	32
3	0	0	0	0	0	0	71.5
4	1	1	-1	1	-1	-1	42.3
5	1	1	1	-1	1	1	39.98
6	-1	-1	-1	-1	-1	-1	42.3
7	0	0	0	0	0	0	60.12
8	-1	-1	-1	1	1	1	**91.16**
9	-1	1	1	-1	1	-1	42.24
10	-1	-1	1	1	1	-1	72.7
11	1	-1	1	1	-1	1	40.64
12	1	1	-1	1	1	-1	45.27
13	-1	1	-1	-1	-1	1	50.1
14	0	0	0	0	0	0	63.6
15	-1	1	1	1	-1	1	38.16

Keys: A- pH, B- Temperature, C- Dye concentration, D- Biosorbent dosage, E- Contact Time, F- Speed of Agitation.

According to the plackett- Burman design the maximum biosorption was obtained 91.16% under conditions such as pH- 8, Temperature 35°C, Concentration of Dye- 40(mg/L), Concentration of biosorbent 2.0(mg/L), Contact time-120(mins), speed of agitation 120(rpm). And minimum bisorption of was obtained 32% under conditions such as pH- 12, Temperature 35°C, Concentration of Dye- 40(mg/L), Concentration of biosorbent 0.82(mg/L), Contact time-120(mins), Speed of agitation 120(rpm). The first order polynomial equation showing the relationship between the variables and the regression coefficient is as follows:

Yield	=	48.17 - 7.94 pH - 5.16 Temperature - 3.88 Concentration of dye + 6.87 Concentration of Biosorbent + 7.25 Contact time + 2.04 Speed of Agitation + 16.90 Ct Pt

Analysis of the regression coefficients of six variables showed that initial concentration of biosorbent, contact time and speed of agitation had positive effect on biosorption, whereas pH, dye concentration and temperature had a negative effect on biosorption of the dye. The corresponding probability values (*P* values) indicate the significance of each of the coefficient. In general, the larger the magnitude of *t* and smaller the value of *P*, the more important is the corresponding coefficient term (Montgomery, 2005). It can be inferred from the regression analysis that pH, dye concentration and contact time play a significant role in the biosorption process.

Table: 5. Linear multiple regression analysis of Plackett-Bruman experiments for the biosorption of Basic Blue 9 by onion isolate.

Variables	Effect	Coef	SE Coef	T-Value	P-Value
Constant		48.17	2.81	17.14	0
Ph	-15.88	-7.94	2.81	-2.82	0.026
Temperature(°C)	-10.32	-5.16	2.81	-1.84	0.109
Concentration of dye(mg/L)	-7.77	-3.88	2.81	-1.38	0.21
Concentration of Biosorbent(g/L)	13.73	6.87	2.81	2.44	0.045
Contact time(min)	14.51	7.25	2.81	2.58	0.036
Speed of Agitation(rpm)	4.07	2.04	2.81	0.72	0.493
Ct Pt		16.9	6.28	2.69	0.031

R^2-82.78%, R^2 (Adj)-65.55%, R^2(Pred)-6.98%.

The regression coefficient (R^2) was found to be 82.78% which implies the statistical significance of the system.

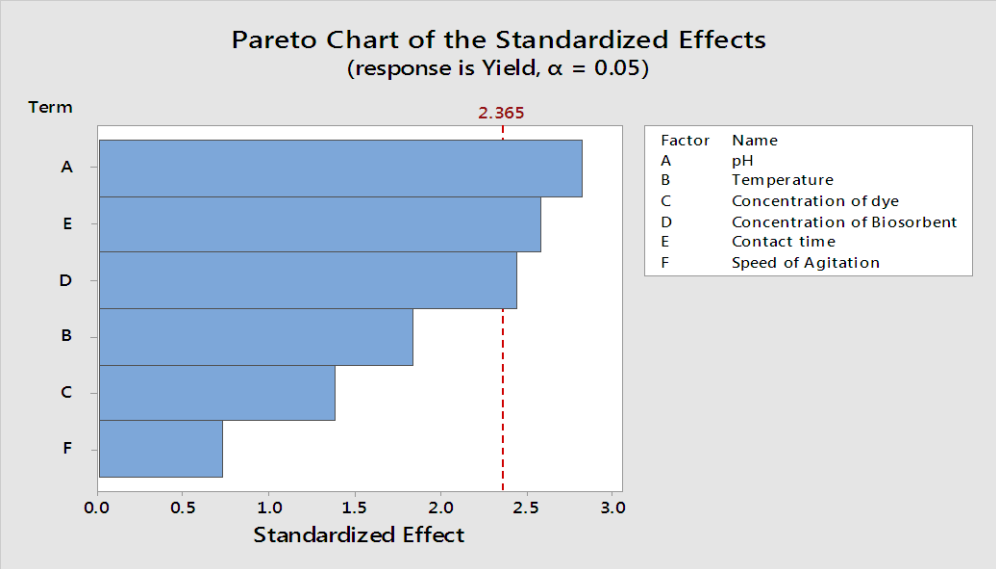

Fig. 2: Pareto Chart for biosorption of Basic Blue 9 by onion isolate.

The Pareto chart is to determine both bars and a line graph where as, it represented in descending order by bars, and the cumulative total is represented by the line graph. Plot shows the standardized effects of the selected terms i.e factors. From the above chart, three factors that is pH, Concentration of dye (mg/L) and biosorbent dosage (g/L) can be considered as significant for the biosorption as its show α-value less than 0.05.

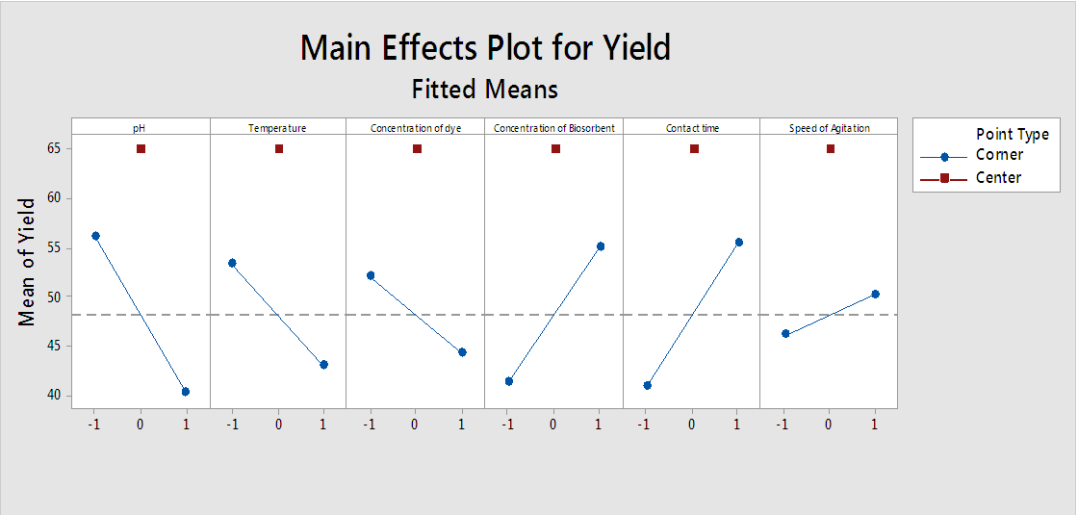

Fig. 3: Main effect plot of variable for biosorption of Basic Blue 9 by onion isolate.

The use of main effects plot is to examine differences between level means for one or more factors. A main effects plot graphs the response mean for each factor level connected by a line (support.minitab.com). When the line is horizontal (parallel to the x-axis), then there is no main effect present. Each level of the factor affects the response in the same way, and the response mean is the same across all factor levels. When the line is not horizontal, then a significant main effect may be present. Different levels of the factor affect the response differently. The greater the slope, the greater the likelihood that a main effect is statistically significant. Since the plot for the is pH, Concentration of dye (mg/L) and biosorbent dosage (g/L) shows the line is which not horizontal that means main effect is present and these factors has direct effect on the yield. However, pH, Concentration of Biosorbent and Contact time shows the maximum effect on the yield.

3.3.2 Central composite design(CCD)

A CCD spans a set of quantitative factors with fewer points than a standard Fractional Factorial multilevel design, without a large loss in efficiency. It uses central points, extreme (corner) points and either face points or extended points. Central composite designs with face points require three levels; with extended axial points, five levels are required.

These three-level designs are often used for response surface analysis to map out the shapes of the quadratic surfaces. The center and axial points allow estimates of quadratic terms. Repeat center points provide an estimate of pure error (http://qualityamerica.com/LSS-Knowledge Center/designedexperiments/central_composite_design.php). The factors that were found to be significant in PBD were used for further optimization of biosorption using CCD.

Table 6: Experimental ranges and levels of the independent process variable in CCD for biosorption of Basic Blue 9 by onion isolate.

Designation	Variable	Variable Values				
		-1.68	**-1**	**0**	**1**	**1.68**
A	pH	6.32	7	8	9	9.68
D	Biosorbent dosage(g/L)	1.16	1.5	2	2.5	2.84
E	Contact time(min)	69.6	90	120	150	169.6

The design matrix of tested variables in 20 experimental runs along with the experimental results and the results of theoretically predicted responses (using the model equations) are shown in table 5.

Table 7: CCD matrix for biosorption of Basic Blue 9 by onion isolate.

RunOrder	Coded Values			% Biosorption
	A	**D**	**E**	
1	0	-1.68	0	71.18
2	0	0	-1.68	75.55
3	**1.68**	**0**	**0**	**94.31**
4	0	0	0	91.61
5	0	0	0	92.5
6	0	0	0	91.08
7	1	-1	1	87.8
8	0	0	1.68	64.2
9	1	-1	-1	75.64
10	-1	-1	-1	70.13
11	-1	1	1	65.56
12	-1	1	-1	76.26
13	0	0	0	83.78
14	-1	-1	1	72.09
15	1	1	1	80.21
16	0	1.68	0	87.17
17	0	0	0	85.29
18	-1.68	0	0	88.43
19	0	0	0	82.56
20	1	1	-1	90.3

Keys: A- pH, D- Biosorbent dosage, E- Contact Time.

According to the CCD experimental design, Maximum biosorption was obtained to be 94.31% under the conditions such as pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min) whereas the minimum biosorption of 64.2% under the the conditions such as pH- 8, Concentration of biosorbent 0.1(g/L), Contact

time 169.6(min), and the other factors like temperature 35°C, speed of agitation, Concentration of dye 2.2(mg/L) were kept constant. The percent biosorption increased from 91.31% to 94.31% in CCD as only main factors are considered as variables in CCD.

The data were analyzed using multiple regression analysis in order to obtain empirical models for the best responses and to derive second-order polynomial equations for BB9 as follows:

Yield	=	87.88 + 4.38 pH + 2.46 Biosorbent dosage - 1.89 Contact time + 0.75 pH*pH - 3.56 Biosorbent dosage*Biosorbent dosage - 6.85 Contact time*Contact time + 0.93 pH*Biosorbent dosage+ 1.35 pH*Contact time-4.36 Biosorbent dosage*Contact time

Table 8: Estimated regression coefficient and corresponding *t* and *p*- values of the CCD for biosorption of Basic Blue 9 by onion isolate.

Term	Coef	SE Coef	T-Value	P-Value
Constant	87.88	2.11	41.73	0
pH	4.38	1.4	3.13	0.011
Biosorbent dosage	2.46	1.4	1.76	0.109
Contact time	-1.89	1.4	-1.35	0.207
pH*pH	0.75	1.36	0.55	0.594
Biosorbent dosage*Biosorbent dosage	-3.56	1.36	-2.62	0.026
Contact time*Contact time	-6.85	1.36	-5.04	0.001
pH*Biosorbent dosage	0.93	1.83	0.51	0.62
pH*Contact time	1.35	1.83	0.74	0.476
Biosorbent dosage*Contact time	-4.36	1.83	-2.39	0.038

The regression coefficients and the *F* and *P* values for all the linear, quadratic and interaction effects of the variables are given in Table 6.

Table 9: ANOVA for response surface quadratic model for biosorption of Basic Blue 9 by onion isolate.

Source	DF	Adj SS	Adj MS	F-Value
Model	9	1406.06	156.229	5.86
Linear	3	392.88	130.959	4.91
pH	1	261.84	261.84	9.82
Biosorbent dosage	1	82.48	82.479	3.09
Contact time	1	48.56	48.557	1.82
Square	3	839.26	279.754	10.49
pH*pH	1	8.09	8.088	0.3
Biosorbent dosage*Biosorbent dosage	1	182.89	182.894	6.86
Contact time*Contact time	1	676.46	676.456	25.37
2-Way Interaction	3	173.92	57.974	2.17

DF- Degree of freedom, SS- Sum of Squares, MS- Mean sum of square.

3.3.3 3-D Surface plots

A contour plot displays a two- dimensional view in which points that have the same response value are conneted to produce contour lines. Contour plot is mainly used to find the relationship between fitted response and two continuous variables (supportminitab.com).

The surface plot based on independent variables i.e. biosorbent dosage and pH, while the other independent variables were kept at zero level is shown in Fig. 9.An increase in SUC could be achieved when the biosorbent dosage was kept at 2g/L and beyond this, % sorption decreased sharply even for tiny increase in biosorbent dosage. The two-dimensional contour plot showed a clearly elongated line running diagonally on the plot, suggesting that the interaction between the contact time and biosorbent dosage(X3) was significant on SUC.

The relationship between various variables can be seen from the contour plots. The highest sorption was seen when pH approached the level of 1.68 and concentration of biosorbent was at the zero level i.e. pH 6.32 and concentration of biosorbent 2g/L. Similarly, percent biosorption increased when contact time and concentration of dye approached the zero level.

Footnote: Yield is maximum that is greater than 95 at pH 0.0 and biosorbent dosage 0.00.

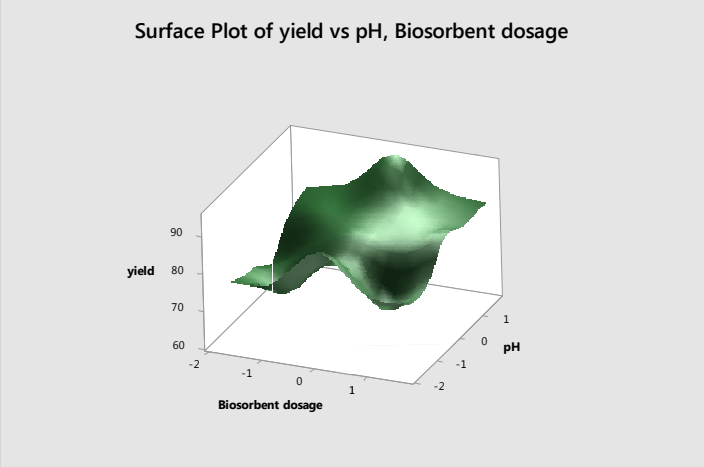

Fig. 4: a) pH and Biosorbent dosage.

Footnote: Yield is maximum that is greater than 90 at contact time 0.00 and biosorbent dosage 0.00.

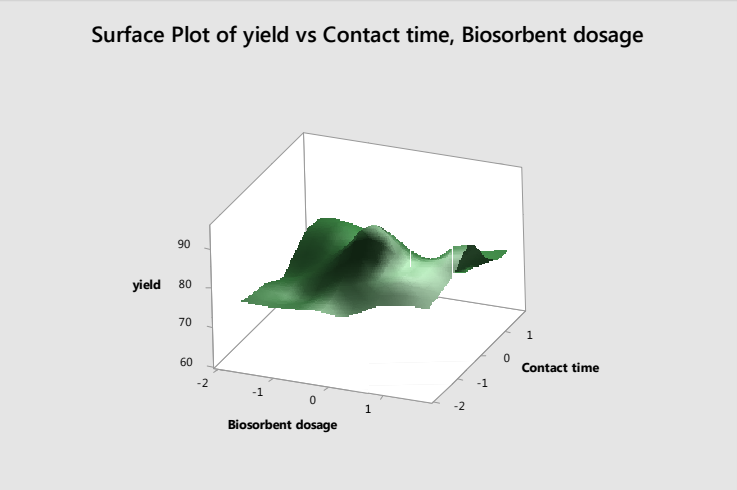

Fig. 4: b) Contact time and Biosorbent dosage.

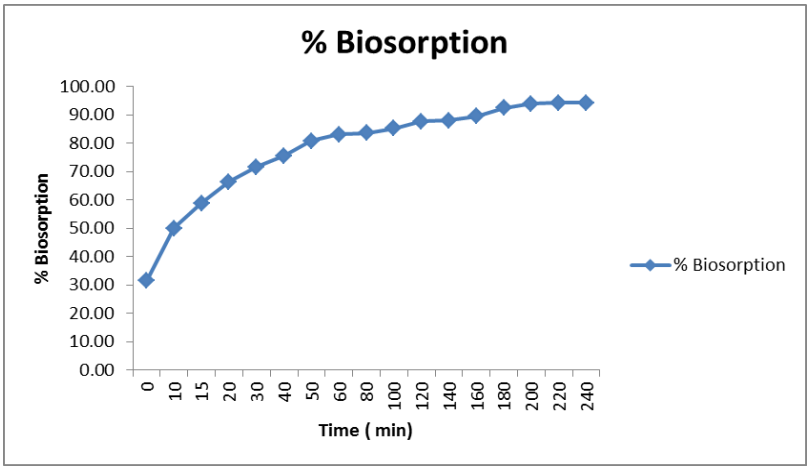

Fig. 4: Three-dimentional response surface plots of biosorption of Basic Blue 9 by onion isolate.

3.3 Reusability studies

The regeneration of the biosorbent and its reuse in several biosorption–desorption cycles is an important aspect from a point of view of its real implementation. The regeneration is usually carried out by using various eluting agents (acids or base) by different desorption mechanisms to release the adsorbed dye into the solution. Desorption of Basic Blue 9 dye was carried out from onion isolate biosorbent loaded with dyes. The reusability of the biosorbent were assessed by monitoring desorption cycles of 240 min each. Use of 0.01 N HCl at solid to liquid ratio (S: L) of 10 proved to be efficient for

desorption of Basic Blue 9. Biosorption Basic Blue 9 for dye was reversible process and the biosorbent went two successive adsorption–desorptionycles.

Table: 10 Biosorption and desorption of Basic Blue 9 by onion isolate.

	%Biosorption	% Desorption
Cycle1	94.31	87.31
Cycle 2	51.11	70.43

87.31% of Basic Blue 9 dye was desorbed in the first cycle and in the second cycle desorption was found to be 70.43%. Sorption percentage decreased from 94.31% to 51.11% after second cycle of desorption, therefore indirectly stating the decreased efficiency in biosorption. This could be due to masking of certain groups by 0.01 HCl.

3.4 Equilibrium and kinetic modelling

3.4.1 Isotherm studies

Fig. 5. Isotherm curve for biosorption of Basic Blue 9 by onion isolate biosorbent.

(Biosorbent conditions: pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min), Concentration of dye 2.2(mg/l), speed of agitation 120(rpm)).

The Langmuir isotherm is a well known linear model for monolayer adsorption and most frequently used to determine the adsorption parameters (Murali. et.al). Langmuir Equation which depicts a relationship between the number of active sites of the surface undergoing adsorption (i.e. extent of adsorption) and pressure. The Langmuir model assumes that the removal of dye occurs on an energetically homogeneous surface by monolayer sorption and there are no interaction between the adsorbate on adjacent sites (El.Haddad.et.al 2014). It has been successfully applied to adsorption processes of many pollutants and to quantify and contrast the performance of different sorbents.

The Freundlich isotherm is an empirical equation used to describe heterogeneous systems. It states that the adsorption process takes place on heterogeneous surface and the adsorption capacity is related to the concentration of methylene blue dye at equilibrium (El.Haddad.et.al 2014). This empirical model can be applied to multilayer adsorption, with non-uniform distribution of adsorption heat and affinities over the heterogeneous surface. This model assumes that the high affinity binding sites are occupied first, whereas those with low affinity are occupied later and at a slower rate. The Freundlich isotherm is based on the assumption that the adsorption process takes place by interaction of metal ions on a homogeneous surface (Murali. et.al).

(Biosorbent conditions pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min), Concentration of dye 2.2(mg/l), speed of agitation 120(rpm).

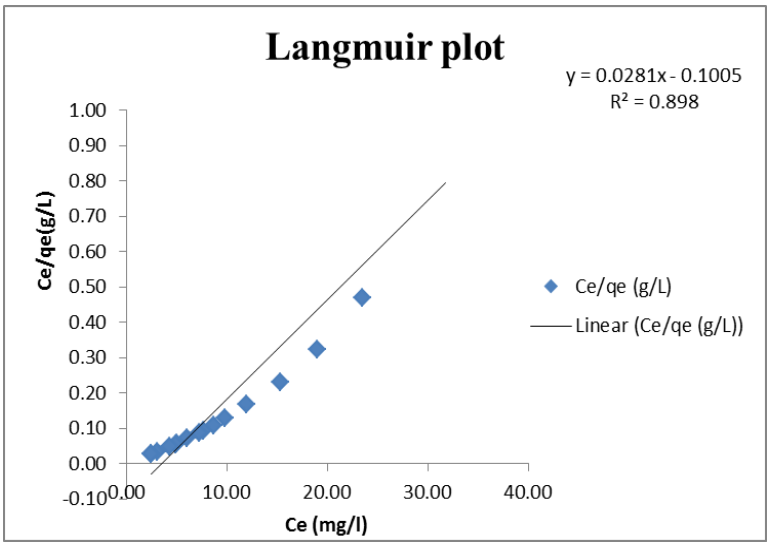

Fig: 6: Linearized plot of Langmuir isotherm for biosorption of Basic Blue 9 by onion isolate biosorbent.

Table: 11. Result of Linearized plot of Langmuir isotherm for biosorption of Basic Blue 9 by onion isolate biosorbent.

RL	KL	R2	Qm
	l/mg		
0.06674	0.2796	0.898	35.7142

(Biosorbent conditions: pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min), Concentration of dye 2.2(mg/l), speed of agitation 120(rpm)).

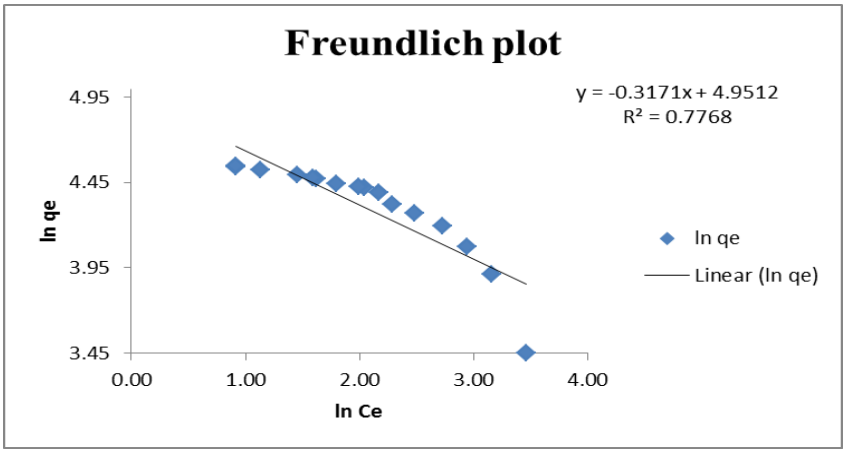

Fig: 7: Linearized plot of Freundlich isotherm for biosorption of Basic Blue 9 by onion isolate biosorbent.

Table: 12. Result of Linearized plot of Freundlich isotherm for biosorption of Basic Blue 9 by onion isolate biosorbent.

KF	N	R2
141.3445	3.15358	0.7768

The R^2 of the Langmuir model is more than fruendlich which shows that the biosorbent contains monolayer adsorption (the adsorbed layer is one molecule in thickness), with adsorption can only occur at a finite (fixed) number of definite localized sites, that are identical and equivalent, with no lateral interaction and streak hindrance between the adsorbed molecules, even on adjacent sites.

3.4.2 Kinetics studies

The Pseudo first order rate expression of Lagergren is a widely used kinetics model for adsorption data analysis. This kinetics model is used for reversible reaction with an equilibrium being established between liquid and solid phases (H.Radnia 2011).

(Biosorbent conditions pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min), Concentration of dye 2.2(mg/l), speed of agitation 120 (rpm).

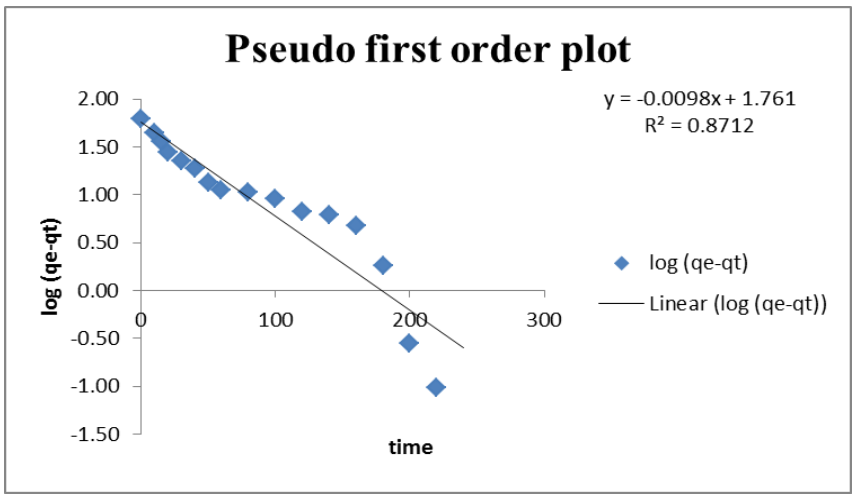

Fig. 8: Plot of pseudo first order kinetics for for biosorption of Basic Blue 9 by onion isolate biosorbent.

Table: 13. Results of Plot of pseudo first order kinetics for for biosorption of Basic Blue 9 by onion isolate biosorbent.

Sorption $_{exp}$	k1	Sorption $_{cal}$	R^2
%	1/min	%	
94.19	-0.0098	0.409088	0.8712

Difference in experimental and calculated %sorption was seen. The reason for these differences in the *%* sorption *value* is that there is a time lag at the beginning of the sorption process, possibly due to a boundary layer or external resistance control. Therefore, the adsorption of BB 9 on the biosorbent did not follow the pseudo-first order adsorption kinetics.

Most of the sorption systems followed a pseudo second order kinetic model which is based on the assumption that the rate limiting factor may be chemosorptions. In chemosorptions (chemical adorption) the metal ions stick to the adsorption surface by forming a chemical(usually covalent) bond and tend to find sites that maximize their coordination number with the surface. In other words chemosorption involves valence forces through sharing or exchange between the metal ions and the adsorbent(H.Radnia 2011).

(Biosorbent conditions pH- 9.68, Concentration of biosorbent 0.1(g/L), Contact time 120(min), Concentration of dye 2.2(mg/l), speed of agitation 120(rpm).

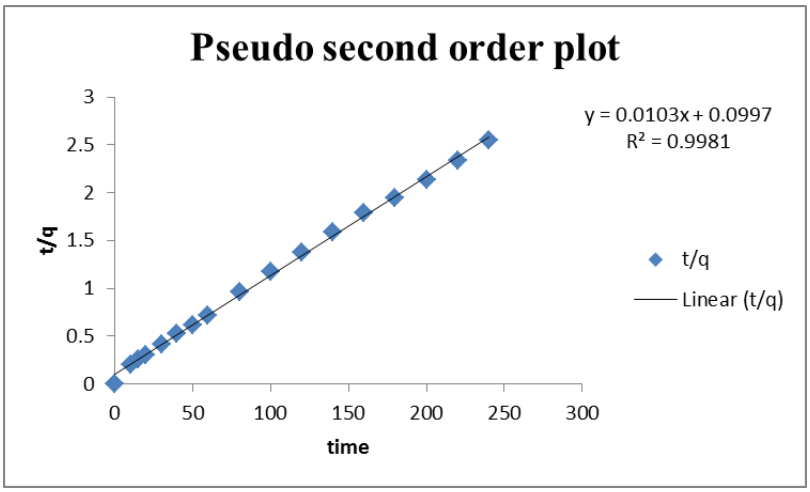

Fig: 9. Plot of pseudo second order kinetics for for biosorption of Basic Blue 9 by onion isolate biosorbent.

Table: 15. Result of Plot of pseudo second order kinetics for for biosorption of Basic Blue 9 by onion isolate biosorbent

% Sorption $_{exp}$	k2	% Sorption $_{cal}$	R2
94.19	0.388848	93.32348	0.9981

Correlation coefficient (*R2*) of 0.9981 and the proximity of the experimental % sorption estimated from the pseudo-second order kinetics with theoretical % sorption value for BB9 indicated a good compliance with the pseudo second-order equation. This suggests that the sorption system fits the pseudo- second order model, based on the assumption that the rate limiting step may be chemisorptions. Also R^2 for the pseudo second order kinetic model is more than that of pseudo first order kinetic model which shows that the rate-limiting step is the surface adsorption that involves chemisorptions.

3.5 Surface characterisation of onion isolate

FTIR analysis: The FTIR spectroscopy was applied to identified the functional groups responsible for Basic Blue 9 sorption(M.Angels.et.al). The spectrum of the dye displayed a peak 3418.11 cm-1 for –NH stretching. The stretching between C N was reported at 2271.30 cm-1 and amide, 5-membered ring peak at 1706.86 cm-1. The peak at 1637.21 cm-1 showed carbonyl stretching vibration. Peak at 1374.15 cm-1 showed unsaturated nitrogen compounds. Peak at 1229.06 cm-1 showed S=O stretching vibrations. The peak at 1106.62 cm-1 indicates the aromatic nature. The peak at 616.95 cm-1 showed hydrocarbon chromophore-C-H bending.

4. CONCLUSION

Most of dyes released during textile clothing, printing and dyeing processes are considered as hazardous and toxic to some organisms and may cause direct destruction of aquatic creatures (A.Regti.et.al 2014). So to overcome this problem, Biosorption can be one of best solution to clean up the dye contamination. The importance of biosorption in the environment and conventional biotreatment processes perhaps suggest further research should be directed in these areas (Gadd.G.M 29 July 2008).

For the removal of Basic Blue 9 dye, varieties of fungi were assessed for their biosorption capacity. Onion isolate proved to be the best biosorbent. As on the basis of high yield and ability to absorb dye, it was chosen for the further studies. For the removal of dyes,the experiment was carried out on the basis of parameters such as pH, temperature, biosorbent dosage, contact time and speed of agitation. For the optimization of the processes parameters Response Surface Methodology was successfully used to estimate the performance of Onion isolate as a biosorbent. The present study shows that the biosorption of Basic Blue 9 by Onion isolate was successfully archived by statistical experimental design experiment. The optimised conditions taken for the isolate were as follows: pH- 8, Temperature- 35^{o}C, Concentration of Dye- 40(mg/l), Concentration of

biosorbent- 2(g/l), Contact time-120 rpm, speed of Agitation- 120 rpm. Optimization of biosorption by RSM was achieved 91.16% dye removal from the effluent by the biosorbent. Desorption of Basic Blue 9 dye was carried out from biosorbent loaded with dyes. The resusability of the biosorbent were assessed by monitoring desorption cycles which showed that 87.31% dye was desorbed in the first cycle and in the second cycle 70.43% was desorbed. Desorption studies reveals that satisfactory desorption taking place confirming physisorptive nature of adsorption.

The kinetics parameters was also performed as they provide valuable information on the mechanism of the adsorption. Adsorption kinetics and isotherm was carried by well known parameters like Freundlich, Langmuir, Pseudo first order kinetic model, Pseudo second order kinetic model. The result showed that the, R^2 of Langmuir model is more than Freundlich which shows that the biosorbent contains monolayer adsorption. And the R^2 of Pseudo second order kinetic model is more than that of Pseudo first order kinetic model which shows that the rate limiting step is the surface adorption that involves chemisorptions.

Thus, from the studies, dead fungal biomass of onion isolate proved to the most effective for the biosorption of dyes.

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