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RESEARCH ARTICLE

Optimization Studies on Degradation of Congo Red Dye using *Nerium oleander*

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ABSTRACT:
Dyes are harmful for living organisms. *Nerium oleander* was used to degrade the synthetic Congo red dye. Optimization process was carried out for better degradation of synthetic dye using Placket-Burman design. Among the six parameters (Temperature, Incubation time, pH, Agitation speed, Biomass concentration, Dye concentration), pH (5), Temperature (35 ℃) and Incubation time (60 min) were found to increase the dye degradation (92.33 %). The global optimum levels of dye degradation were analyzed using Plackett-Burman design. The effects of pH, Temperature and Incubation time on degradation were further optimized by statistical analysis using Response Surface Methodology (RSM). RSM was used to optimize dye degradation by implementing the Central Composite Design (CCD). The optimal conditions for higher degradation were pH: 5, Temperature: 35℃, and Incubation time: 90 min. Under these conditions, the model predicted a dye degradation of 98.23 %. Verification of the optimization showed that dye degradation of 97.18 % was observed under the optimal conditions in the shake flasks experiment.

KEYWORDS: Degradation, *Nerium oleander*, Congo red dye, Plackett-Burman design, Response Surface Methodology.

INTRODUCTION:
In modern world the environmental pollution has been recognized one of the major problem. Textile influent includes dyes, detergent, grease, sulfates, solvents, heavy metals and other inorganic salts and fibers are causes of pollution. The textile dye effluent has a strong colour, high pH, high temperature, high cod and low bio degradability [1,2,3]. Synthetic dyes are mainly used in the cosmetics, paper, textile, color photo etc. [4]. Dye-colored wastewaters effluents get into the aquatic ecosystem represent both environmental and public health risks because of many toxicological effects and bioaccumulation in wildlife.

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Many dyes can decompose into aromatic amines which are classified as carcinogenic compounds under aerobic conditions which can cause serious effect to humans and animals. Dyes can cause allergy, dermatitis, skin irritation and cancer in humans [5]. Besides that, colored water especially in the surface prevents light penetration and it may damage photosynthesis and threat aquatic life [6]. There are many treatments technologies that can be used for treatment of dye pollution such as biological methods, coagulation, Nano filtration electrochemical process, activated carbon adsorption and catalytic oxidation processes. However, these methods were found to produce non-harmful small molecules like CO_2, H_2O and some other inorganic species [7]. In industry annually produced over 0.7 million tons of synthetic dyes are produced worldwide [8]. *Nerium oleander* is a shrub or little tree in the dogbane family Apocynaceae, poisonous in the entirety of its parts. It is the main species right now arranged in the family Nerium. It is most ordinarily known as Nerium or oleander, from its shallow likeness to the irrelevant olive Olea. Plackett-Burman design is used to optimize the parameters for degradation of the dye. For degradation of dye (Congo red) using various parameters like pH, temperature (C), time(minutes), dye concentration(mg/l), biomass concentration(mg/l) and agitation (RPM) by Plackett-Burman method [9,10]. The Response Surface Methodology is a statistical design through which we can determine the optimum concentration or conditions using Central Composite Design. This can be achieved by the use of commercial free software Minitab18 investigates the connections between a few informative factors and at least one reaction factors. The principle thought of RSM is to utilize an arrangement of planned examinations to acquire an ideal reaction. Box and Wilson propose utilizing a second-degree polynomial model [11,12].

MATERIALS AND METHOD:

Congo red:

Congo red is water soluble. Its solubility is greater in organic solvents. However, the use of congo red has long been abandoned, primarily because of its carcinogenic properties. Congo red has a propensity to aggregation aqueous and organic solution.

Nerium oleander:

Nerium oleander is a small tree in the family *Apocynaceae*, toxic in all its parts. Oleander has historically been considered a poisonous plant because some of its compounds may exhibit toxicity. The plant material does not eliminate toxins after drying.

CHEMICALS:

The chemicals used during the work are:

1. Formaldehyde
2. Hydrochloric acid
3. Sodium hydroxide

METHODS:

Collection and analysis of sample:

- *Nerium oleander* collected from koyambedu market.
- The flower was then dried (shade dry) for more than 7 days.

Preparation of stock solution:

The dye was brought from Tirupur dye industry and weighed about 1g was dissolved in 1000ml of water 10 to produce 1% stock solution. These stock solutions were used in the process optimization.

Preparation of adsorbent (biomass):

N. olender flower is washed several times and the clean algae is shade dried for 3 days for the removal of the moisture content. Algae is oven dried at 60°C for 24 hours. The resulting flower material is then crushed and blended using an electrical blender, then the biomass is now stored in air tight polyethylene bags and stored in room temperature at dry place [13].

The biomass was pretreated using 36% formaldehyde. Treat 20g of dry *N. oleander* flower in 36ml of 36% formaldehyde and 64ml of 0.1 N HCl solutions to make 100ml. The mixture is shaken in orbital shaker for cross linking. The biomass is washed using distilled water and again washed using 0.2 M Na_2CO_3 solution. It is dried over night at 60°C and 110°C for two hours [14,15].

Experimental Procedure:

The adsorption of Congo red dye using oleander powder**.** Firstly, the stock solution of 1000mg/L of dye was prepared by dissolving 1g of Congo red dye in 1000 ml water. Water has been used to dissolve Congo red particles effectively in the solution. The two dye solutions of concentration 20 ppm each were prepared from the stock solution and 1g of adsorbent i.e. biomass added to one of them. The solution with adsorbent was kept in the shaker for 120 minutes at 120rpm under normal conditions. After the passage of 2 hours, it was taken out and allowed to centrifuge at 2000rpm for 10minutes to separate all the undissolved particles. Then both the solutions-the solution with adsorbent and the one without the adsorbent were analyzed in a UV-Vis spectrophotometer at maximum absorbance. The results obtained from the spectroscopy confirmed that the considerable amount of dye was adsorbed on adding biomass powder. Thus, the further studies were feasible.

Plackett-Burman Design:

Plackett-Burman design is very efficient method of screening to identify the significant factors among large number of factors that influences a process using a few experimental runs. In this work the statistical technique using placket burman design was performed on oleander flower to determine the significant factor adsorption process.

Six parameters (Table: 1) were chosen to determine the most significant factor for adsorption process and the ranges were fixed accordingly. Placket Burman design matrix was developed for 6 parameters over 23 trials using the Minitab18 commercial software. It is a two factorial design and are very useful for screening the significant factors.

Table 1: Process parameters for Placket Burman design

Parameters	Low value	High value
pH	4	8
Temperature	25	35 °C
Biomass concentration	5	15 g/L
Dye concentration	100	500 ppm
Time	30	90minutes
Agitation speed	130	170 rpms

Placket Burman design is based on first order model.

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

All the trials were performed in the laboratory under the above-mentioned controlled parameters. Once the conditions are reached the degraded sample is filtered to remove the biomass and OD is measured. The % absorbance is calibrated using SHIMADZU 1800 UV Spectrophotometer using the formula:

$$Degradation\ (\%) = \left[\frac{(Initial\ OD - Final\ OD)}{Initial\ OD}\right] X\ 100 \qquad (2)$$

RESPONSE SURFACE METHODOLOGY:

Response surface methodology (RSM) is a collection of mathematical and statistical techniques through which we can determine the optimum concentration or conditions using central composite designs. RSM is a second order polynomial equation which works on the principle of relationships between diverse explanatory variables and response variables. The system was explained by the second order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 \quad (3)$$

Where Y is the response measured (dye degradation %) X_1, X_2, X_3 are the parameters. The optimum value is obtained by the regression equation, by analyzing the plots.

In the present work three significant parameters (Time, pH, dye concentration) were chosen (Table: 2) according to central composite design and the other parameters (biomass concentration, temperature, agitation) were fixed constant to determine the most significant factor for adsorption process. The three parameters are the Time: 60-120 mins, pH: 4-8 and Temperature: 25 - 45 °C.

Response surface methodology (CCD) was developed for 3 parameters over 20 trials using the Minitab18 commercial software.

Table 2: Process parameters for Response Surface Methodology (CCD)

Parameters	High value	Low value
pH	4	8
Temperature	25	45
Incubation time	60	120

RESULT AND DISCUSSION:

Plackett Burman design:

The influence of six parameters namely pH, temperature, dye concentration, biomass, agitation and time was investigated in 23 runs using plackett-burman design in Table (3).

Experimental value and predicted value for 23 trials in Plackett-Burman method:

The 23 runs and the corresponding experimental and predicted values were obtained from Minitab 18 commercial software. the maximum degradation of dye was found in the third run with 92.26%, minimal degradation is found in 14^{th} run with 78.21% which is showed in Table (3).

Table 3: Plackett-Burman design and experimental and predicted value

Run Order	Leaf Biomass	pH	Temp	Contact Time	Dye Concentration	Agitation Speed	Experimental value (%)	Predicted value (%)
1	5	3	25	30	100	130	84.89	85.09
2	5	3	35	90	100	170	85.25	85.24
3	5	7	35	30	500	170	81.10	80.86
4	15	3	35	90	100	130	83.45	83.23
5	10	5	30	60	300	150	92.33	92.27
6	5	3	25	30	500	130	86.43	86.00
7	5	7	35	90	500	130	79.79	80.22
8	15	7	35	30	100	170	78.89	79.66
9	15	3	35	90	500	170	86.13	85.87
10	15	7	25	30	500	170	83.02	83.25
11	5	3	35	30	500	130	82.98	83.33
12	15	3	25	30	100	170	86.55	86.53
13	5	3	25	90	100	170	88.24	87.91
14	10	5	30	60	300	150	92.16	92.27
15	15	7	25	30	100	130	81.18	80.62
16	15	3	25	90	500	130	85.87	86.82
17	5	7	25	90	500	170	85.29	84.62
18	15	7	35	90	100	130	79.55	79.03
19	5	7	35	30	100	130	78.29	78.22
20	10	5	30	60	300	150	92.31	92.27
21	15	3	35	30	500	170	85.01	84.78
22	5	7	25	90	100	170	82.95	83.71
23	15	7	25	90	500	130	82.75	82.62

Response surface methodology:
Response surface methodology (central composite design) is a statistical and experimental design to determine the significant parameter effective for the degradation process. the effect of parameters on dye degradation efficiency is investigated using central experimental design [16]. The result was obtained by central composite design to identify the significant factor among the Incubation time, pH and temperature are showed in Table (4).

Table 4: RSM design for optimizing significant variables for dye degradation

Run Order	pH	Temperature	Incubation Time	Experimental Degradation (%)	Predicted Value (%)
1	8.000	45.00	60.00	88.22	86.48
2	4.000	25.00	60.00	78.57	78.71
3	6.000	35.00	90.00	97.23	98.23
4	6.000	35.00	90.00	97.18	98.23
5	4.000	45.00	120.00	72.92	72.51
6	8.000	25.00	120.00	78.32	78.27
7	6.000	18.67	90.00	77.63	76.83
8	9.266	35.00	90.00	73.31	74.81
9	6.000	51.33	90.00	78.28	80.22
10	6.000	35.00	138.99	79.43	79.30
11	6.000	35.00	41.01	84.65	85.92
12	6.000	35.00	90.00	97.18	95.42
13	6.000	35.00	90.00	97.12	95.42
14	2.734	35.00	90.00	73.22	72.86
15	4.000	45.00	60.00	83.38	82.66
16	8.000	25.00	60.00	75.49	75.13
17	6.000	35.00	90.00	97.13	97.68
18	6.000	35.00	90.00	97.23	97.68
19	4.000	25.00	120.00	77.64	78.61
20	8.000	45.00	120.00	78.29	77.38

SURFACE PLOT

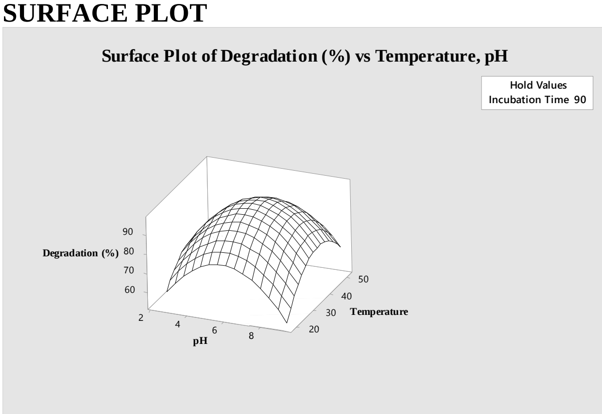

Fig 1: Surface plot representing the interaction between temperature and pH

The surface plot in the figure 1 shows the degradation of dye. The plot gives the interaction between the temperature and pH. From the plot we observed that doom shaped region had the maximum degradation when compared to other. Similarly for figure 2 and 3 explained the degradation profile for Incubation time and Temperature.

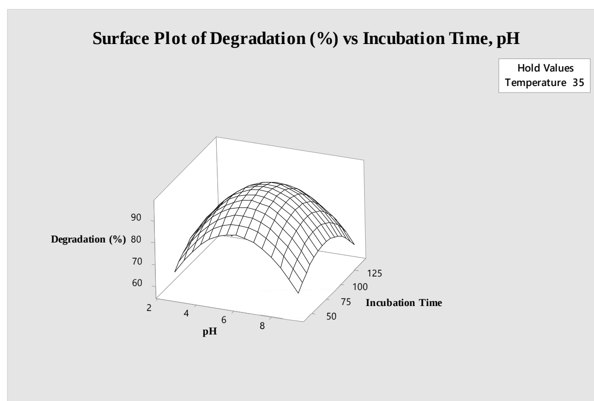

Fig 2: Surface plot representing the interaction between incubation time and pH

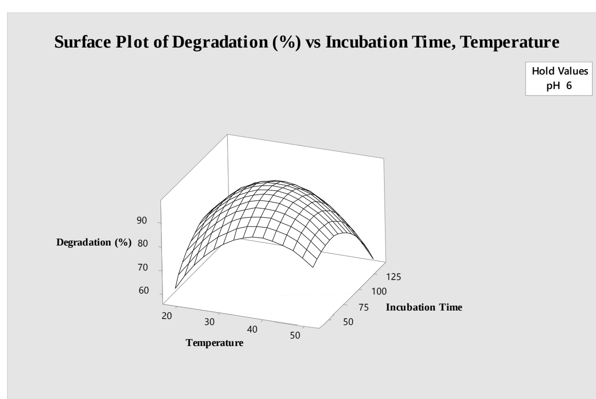

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Fig 3: Surface plot representing the interaction between incubation time and temperature

CONTOUR PLOT:

The contour plots (Figure: 4, 5 and 6) are the graphical representation for the dye degradation profile and maximum degradation percentage of the dye was found only in the inner circle of the plot for all the three parameters such as pH, Incubation time and Temperature while other circles in the contour plot shows the decreasing in degradation percentage. The contour plot of the second order polynomial equation is done by the interaction of two variables within the experimental ranges.

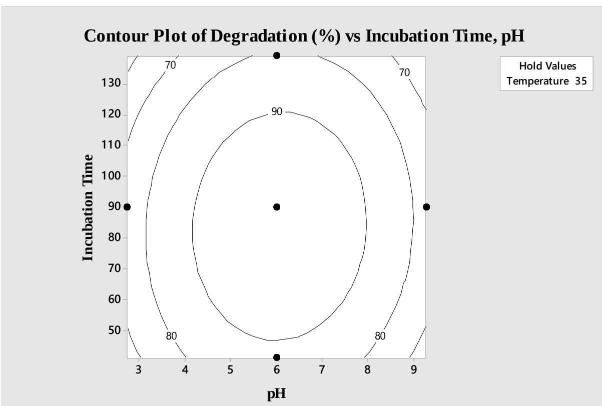

Fig 4: Contour plot representing the interaction of time and Ph

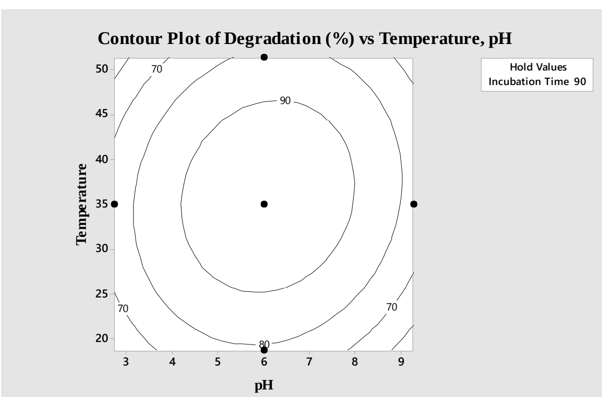

Fig 5: Contour plot representing the interaction of temperature and pH

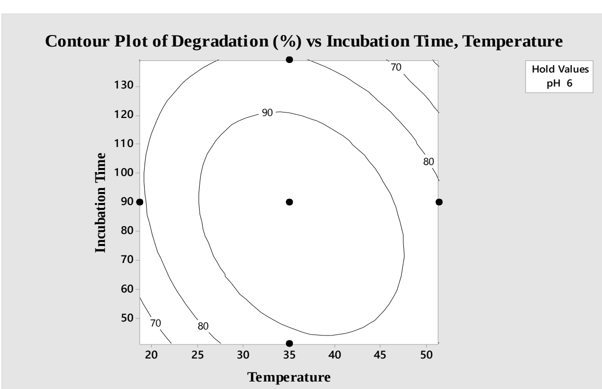

Fig 6: Contour plot representing the interaction of incubation time and temperature

CONCLUSION:

Nerium oleander flower was found to be a low-cost alternative for the biosorption of dye effluent when compared to other costly techniques such as osmosis, and electrocoagulation. The *oleander* flower was pretreated in order to enhance the process of biosorption of dyes, this was achieved using boiling water, formaldehyde, HCl and sodium carbonate. The statistical approach using Plackett-Burman design was used to screen the essential significant variables that enhance the biosorption process. In our study, out of six variables, only 4 variables had a significant impact on degradation ($P<0.05$) namely biomass concentration, pH, dye concentration and agitation speed which represents a strong evidence against the null hypothesis. The maximum decolorization of dye (92.33%) was obtained under optimized condition of pH (5), incubation time (60 min) and temperature (35 °C) using Plackett Burman design. The optimal conditions in RSM for higher degradation were pH: 5, Temperature: 35℃, and Incubation time: 90 min. Under these conditions, maximum dye degradation of 98.23 % was obtained. This bio-sorbent is economically effective towards the degradation of Congo Red dye.

CONFLICTS OF INTERESTS:

All authors conform that there is no Conflict of Interest.

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