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Response Surface Methodology for Rapid Removal of an Azo Dye Methyl Orange by Indigenous Bacterial Stain (*Bacillus Cereus J4*)

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KEYWORDS
Methyl orange, Isolation, Identification, Optimization, Bacterial strain.

ABSTRACT:

Sulfonated azo dyes such as Methyl Orange (MO) are broadly popular in textiles, paper, food, and printing industries and are mostly discharged through industrial wastewater posing a serious threat to aquatic flora and fauna. As this dye is difficult to degrade by conventional physicochemical methods, it attracts eco-friendly bioremediation methods as an alternative. In the present study, eleven bacterial strains (named J1 to J11), capable of partial/full removal of Methyl Orange (MO) were isolated from the sample collected from the soil and effluents of the industrial area, Panipat, Haryana, India using the standard plate count method. Of these eleven, J4 showed encouraging results and was subjected to further studies. Initial morphological and biochemical characteristics showed the strain to be gram-positive, rod-shaped, non-motile, MR+ve, and VP-ve reaction. The J4 strain is unable to utilize citrate, urea, and indole. Additionally, it showed an oxidase +ve and catalase -ve reaction during the study. 16SrRNA sequence revealed the isolated J4 strain as *Bacillus cereus* and a maximum decolorization efficiency at 50 mg/L concentration of Methyl Orange (MO) dye within 40h at pH 7 and 370C. The optimization and statistical optimization studies of important Physico-chemical parameters of the strain (*Bacillus cereusJ4OQ392442*) have been done. Under optimal conditions, the bacterial strain was able to decolorize completely (>89%) the dye within 40 h. Further study may establish the *Bacillus cereusJ4 (OQ392442)* bacterial strain as a promising candidate for dye-containing effluent treatment in an eco-friendly modus.

Introduction

The Industrial revolution enabled mass production and transformed human history. Industrialization had its benefits, but it also came at a cost. While economic growth comforted humans, it also led to environmental pollution in water, land, and air[1]. With the rise of global warming and climate change, there is an increasing need to modify environmental pollution.

Our water bodies have been contaminated, and the quality of the water has declined, due to shifting economic patterns and increasing industrialization. The health of the populace deteriorated as a result of the rivers' degrading appearance and the declining quality of their waters [2]. Water bodies have become contaminated as a result of many human activities. Effluents discharged by industries are one of the major causes of this contamination. Examining factors contributing to environmental degradation has garnered more attention in recent years, one of which is the use of dyes by various industries. Dyes damage water because of their inherently harmful nature. The Central Pollution Control Board has designated the dyeing business as a highly polluting industry [3].

The earliest indication of water pollution is colored effluent [4]. Water bodies that get colored wastewater are contaminated. The usage of this water can have

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adverse impacts on the environment and the health of individuals [5]. The unprocessed wastes create a major threat to agricultural land as well as human health [6]. The release of this polluted wastewater decreases the ability of light to penetrate the water and disturbs the photosynthetic activity of aquatic plants, thereby affecting the source of food for aquatic ecosystems [7].

Recently,[8] It has been reported that The textile and leather industries reportedly lose around 84,000 tons of dye in water annually, contributing to approximately 20% of industrial water pollution [9]. About 80% of all organic dyes are azo dyes [10]. During dyeing processes, up to 50% of the dyestuff is wasted and enters wastewater due to a lower fixation rate[11]. Azo dyes and their intermediate amines are environmentally hazardous due to their toxicity and potential carcinogenicity, particularly at high concentrations [12]. An azo dye is a kind of dye that has both sulfonic groups (SO^{-3}) and one or more azo groups [13, 14], linking naphthyl and phenyl rings generally substituted with chloral, triazine amine hydroxyl, methyl, sulfonate, and nitro [15]. Discharging dye-containing wastewater into wetlands, agricultural fields, and rivers has several drawbacks: (1) Causes health issues in humans, (2) Affects crop quality, and soil fertility and (3) Decreases the ability of aquatic plants to photosynthesize, harming biodiversity. (4) lowering gas solubility and dissolved oxygen while raising the levels of Total Organic Carbon, Chemical Oxygen Demand, and Biological Oxygen Demand in the water [16-19]. Methyl Orange (MO) is a sulfonated azo dye with the molecular name Dimethyl amino azobenzene sulfonate. It is employed as a pH indicator in biochemical applications and used in textile dyeing facilities. Azo dye metabolites, including Methyl Orange (MO), are extremely resistant and have been linked to mutagenic, carcinogenic, and teratogenic effects. [20]. Before being discharged into the environment, wastewater containing Methyl Orange (MO) should be detoxified and decolorized.

Physicochemical techniques are employed to remove the color and eliminate the toxicity of Methyl Orange present in industrial effluent. [21, 22]. There are several physicochemical methods used for the remediation of dyes in water, including filtration, adsorption, precipitation, chemical oxidation, coagulation, electrolysis, and photodegradation. However, these techniques have a number of disadvantages, including reagent requirements, insufficient decolorization, and high costs, high energy and sludge generation. Furthermore, these techniques might produce dangerous byproducts. The Advanced Oxidization Processes decolorize and detoxify Methyl Orange (MO) dye by using oxidizing chemicals and/or catalysts. [23].This process is highly cost-effective and energy-consuming. Significant advantages come from using microbes to remove synthetic dyes from industrial wastewater, including minimal processing costs, non-toxic products, and total mineralization.

Dye removal is mostly accomplished by two procedures: (a)degradation of dyes by microbial cells and (b) adsorption of dyes by microbial biomass. Dyes do not fragment into smaller pieces after adsorption, and preserving their original structure. However, in the degradation process, the original structure is broken down into smaller fragments, and the parental structure is ultimately destroyed. In some cases, it transformed into carbon dioxide, water, and inorganic salts. [24]. Many different types of microorganisms, including fungi, bacteria, yeast, and algae, can decolorize and fully mineralize numerous azo dyes. [25]. These include *Candida krusei*, *Gloeocapsa*, *Micrococcus*, *Pleurocapsoides*, *Aspergillus niger*, *Citrobacter sp.*, *Enterobacter sp., and Aeromonas hydrophila*. [4]. In general, bacterial decolorization proceeds more quickly than fungal decolorization. It is hence more favoured. There are very few studies on the dye removal process that caused by gram-+ve bacteria. [4].

Material and Methods

Chemicals and Dye

The chemicals used of prescribed analytical grade. Methyl Orange was procured from Sigma Aldrich in Bangalore, India. Nutrient media (Nutrient Agar and Nutrient Broth) and Nitrogen sources and Carbon sources were obtained from HI Media (Mumbai, India).

Isolation of Bacteria from collected samples

The bacterial colonies were isolated from the industrial dye effluents, wet sludge, dry sludge and soil sample by following serial dilution method. For the isolation of bacterial colonies, 1ml (v/v) liquid or 1gm (w/v) of solid was put into the sterile water and serial dilution was carried out up to the 10^{-1} to 10^{-10}. Then 100µl of the diluted sample was transferred onto Nutrient Agar medium, followed by incubation at 37 ºC for 24 hours. To obtain pure colonies, a loopful of selected colonies was streaked on fresh nutrient agar plates. The process is repeated till pure cultures are obtained.

Culture conditions

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The pure bacterial cultures were preserved on slants of Nutrient Agar Medium(NA) and stored in refrigerator (4^0C) and glycerol stock solution at -20^0C for long-term preservation according to [26].

Inoculum preparation

A colony of the isolated strain (*Bacillus cereus* J4) was added to the nutrient broth and incubated at 37°C under shaking conditions (120 rpm) until the optical density $(OD)_{600}$ reached 0.6 to 0.8. Next, the inoculum was transferred aseptically into the dye solutions for further experimentation i.e., screening of dye-removing bacterium.

Prepared the 50 ml nutrient broth in 250 ml borosilicate glass flask containing dye solution concentration 10mg/ml for each pure isolate, then inoculated the media with the 24hrs old inoculum and incubated at 37°C for 24h. For successive experiments, ambient culture conditions were used. Aliquots (2.5ml) were taken at regular time of intervals and centrifugated at 6000rpm for 20 minutes to segregate the bacterial biomass, which interfered with measurement. The optical density of the supernatant was noted. The maximum wavelength of the tested dye was calculated by using a spectrophotometer at wavelength 465nm. The standard curve of (MO) dye conc. v/s optical density was noted and used to compute the decrease in dye concentration. The following formula was used to get the removal percentage applying absorbance to examine decolorization activity [27].The percent of decolorization was determined by the decrease in absorbance and visual appearance.

Decolourization %

$$= \frac{Initial\ Absorbance\ (A°) - Final\ Absorbance(A)}{Initial\ Absorbance\ (A°)} \times 100$$

Where Aº is the initial absorbance and A is the final absorbance after treatment.

Identification and characterization of microorganisms

A number of pure bacterial isolates were obtained out of which 11 bacterial colonies were selected and were designated as J1, J2. J3, J4 J5, J6, J7, J8, J9, J10, and J11. Out of 11 isolates, 1 isolate was selected on the basis of maximum decolorization of dye in minimum times of hours and, Morphological, Biochemical and Molecular Characterizations (16SrRNA) were confirmed via MTCC, IMTECH Chandigarh, India. The 16S rRNA sequencing of *Bacillus cereus J4* were submitted to NCBI GenBank and assigned the accession number OQ392442, identifying them as *Bacillus cereus J4*.

Factors affecting bacterial decolorization

Optimization of an operating system is necessary to obtain maximum rate of decolorization of azo dyes Methyl Orange (MO). The effectiveness of biological treatment is greatly influenced by several physicochemical parameters e.g. Initial Dye Concentration (10-100mg/ml), Inoculum percentage (0.5- 2.5% v/v), Incubation time (8-72hrs), Temperature (25-50^0C), pH (4-9), Agitation speed (200rpm), Decolourization performance can be enhanced by using different media compositions and supplementing them with various carbon and nitrogen sources. Different Physical parameters i.e. Initial dye concentration, Inoculum%, Incubation time, temperature, pH, and shaking rate [28] were optimized as these factors (parameters) greatly influence the microbial decolourization efficiency. The composition of wastewater from textiles can vary daily and seasonally and typically includes organic compounds, nutrients, sulphur compounds, salts, and various toxic substances [29]. It is possible that the existence of these compounds could impede the process of dye decolorization. Therefore, the impact of each factor on the dye removal must be determined before the treatment of the industrial effluent by biological means.

Optimization of physicochemical parameters using the Plackett–Burman method (PB) and Response Surface Methodology (RSM)

Plackett-Burman design

The Plackett Burman (PB) design was used to identify significant factors (one variable at a time) responsible for removal of Methyl Orange (MO). RSM was employed to ascertain the best response for the decolourization of MO [30, 31].

The Expert software (Version 7.5.7.1, Stat-Ease) was utilized to enhance the decolorization of Methyl Orange (MO) with *Bacillus cereus J4*. Initially, The Plackett-burman (PB) design, is also known as fractional factorial design, [32] was employed to represent the relative significance of various environmental aspects on the dye (MO) removal with bacterial strain. For every variable, eight chosen individual factors were evaluated at both the highest (+) and lowest (-) levels in 11 combinations arranged in accordance with the (PB) design (Table 1). All experiments were performed in triplicate and averages of the dye decolorization taken as responses. Each variable's primary effect was ascertained using the following formula [33].

$E_{xi} = (\{M_{i+} - M_{i-}\}) / N$

E_{xi} stands for the variable major effect in this case.

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M_{i+} and M_{i-} represent the percentage of methyl orange decolorization in trial where independent variables (xi) was present in low and high concentrations, respectively, and N represents the number of trials divided by 2.

Screening using the Plackett-Burman (PB) design

A Plackett-Burman (PB) design along with three central point was used to assess the effects of the culture conditions. [34]. The eight physical factors examined were Initial Dye Concentration (10-100mg/l), Inoculum % (0.5 to 3%v/v), Incubation time (8hrs-72hrs.), pH (4-9), Temperature (25–50ºC), and Agitation speed (0 to 200 rpm). The chemical parameters that were analyzed included extra carbon sources, such as Glucose, Lactose, Sucrose, and Fructose, as well as extra nitrogen sources, such as Ammonium Chloride, Ammonium Sulfate, Peptone, Potassium Nitrate, and Urea, from 0.0% to 3.0% (w/v) concentration. The best results were obtained from lactose and urea (data not included), so they were used throughout the entire study. Based on the findings of experiments where one variable was changed at a time (COVT), all independent factors were set at their fixed values. [30]. Based on PB analysis, four key factors were selected for the Central Composite Design (CCD).

Statistical analysis using Response Surface Methodology (RSM)

Design Of Experiments (DOE): A Central Composite Design was used in this study, which is a standard Response surface Methodology design. RSM facilitates the measurement of the correlations between critical input factors and one or more measurable responses. The three main steps in this optimization process include conducting statistically planned trials, assessing the model's suitability, and figuring out the coefficients in a response-predictive mathematical model. [35]

$Y = (X1, X2 \ldots Xn)$

Table 1 Real and coded values of parameters utilized for Plackett Burman (A) and Response Surface Methodology analysis (B)

Parameters	Unit	Levels	
		-1	+1
A Initial Dye Concentration	mg/l (v/v)	10	100
Inoculum %	g% (v/v)	0.5	3
Incubation Time	Hours	8	72
Temperature	ºC	25	50
pH	Units	4	9
Agitation speed	RPM	0	200
Extra carbon sources	g% (w/v)	0	3
Extra nitrogen sources	g% (w/v)	0	3
B Initial Concentration	mg/l (v/v)	10	100
pH	Units	4	9
Temperature	0C	25	50
Nitrogen source (Urea)	g% (w/v)	0	3

The initial dye concentration (A), initial pH (E), temperature (D), and extra nitrogen source (urea) (H) are the four important factors were employed to raise the percentage decolourization. Table 1 illustrates how the coded numbers +1, 0 and -1 correspond to the upper and lower levels of the variables. Based on practical results, a range of input parameters is selected. This suggests that the total number of tests needed for each of the four parameters is $N = 2n + 2n + nC = 24 + 2 \times 4+6 = 30$

Usually, the CCD comprises nC central run and two (2n) axial runs with two (2n) factorial runs.

Results and Discussion

Characterization of isolated strains

In the present study, a total number of eleven morphologically different isolates J1 to J11 were isolated and screened for Methyl Orange removal efficiency. Only two isolates J4 and J10 were able to decolorize the methyl orange within 48hrs. The selection

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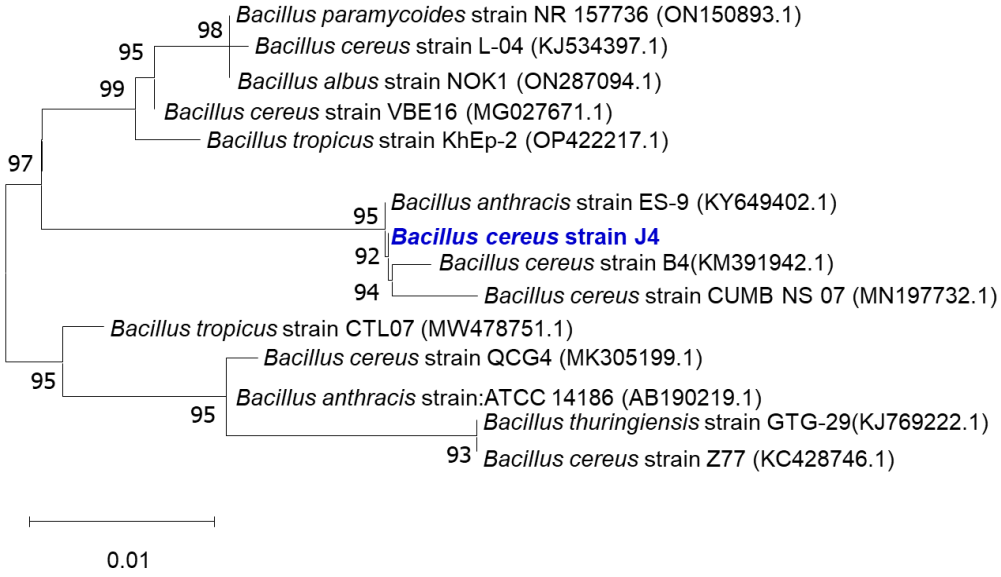
of the best isolate J4 out of J4 and J10 was done by further screening and on the basis of some biochemical testing, and only J4 gave the maximum removal (98%) of Methyl Orange and was chosen for further studies.

Morphological and Biochemical identification

The isolated bacterial strain J4 showed dull creamy colonies on NA plates. It is a small, rod-shaped, non-motile, gram-positive bacterium with a positive MR test and a negative VP test. The J4 strain is unable to utilize citrate, urea, and indole. Additionally, it showed an oxidase +ve and catalase -ve reaction during the study, also J4 showed 100% similarity with *Bacillus cereus* as a result, the J4 was identified as *Bacillus cereus J4* having accession number OQ392442.



Phylogenetic tree of isolated bacterium (J4)

Methyl Orange was shown to be fully decolorized by a unique strain of *Bacillus cereus J4* after 48 hours of incubation. UV-visible spectroscopy was used to confirm the decolorization of Methyl Orange dye (Fig. 1). Degradation was found to be the cause of decolorization of methyl orange. Two subsequent processes following decolorization are degradation and bio adsorption. The outer surface of bacterial cells may confirm dye bio adsorption. When a bacterial cell is being adsorbed, its mass will be colourful; when it is being broken down, the cell will be colourless. After a 12-hour incubation period with dye, the methanol extract of the cell pellets confirmed that the dyes had not been absorbed by the cell biomass (data not shown). Based on this result, it may be concluded that dye decolorization was primarily caused by degradation [36].

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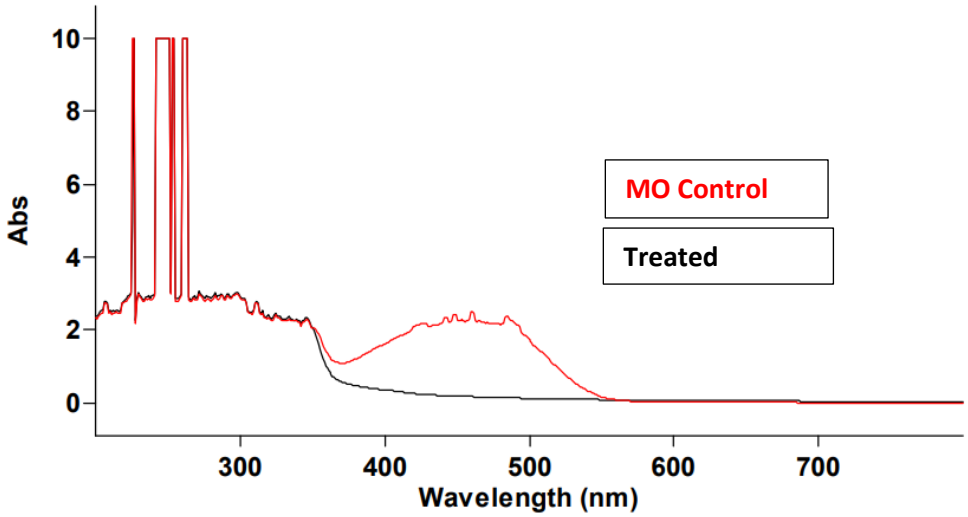

Fig: 1. UV spectra of MO

Statistical optimization of physicochemical parameters affects the dye removal process

Factors affecting the removal of Methyl Orange were screened using the (PB) method. The major impacts in PB design are often significantly confused by two-factor interactions.

The Pareto chart screening results of PB analysis with physical and chemical factors indicated that the initial concentration (A), initial pH (E), temperature (D), and nitrogen source (H) contributed the most to the maximum decolorization of dye Methyl Orange (MO). A significant p-value (<0.05) was also observed in the removal of the dye.

The overall model for the PB design was significant, A number higher than four indicates that the signal is satisfactory for the system.

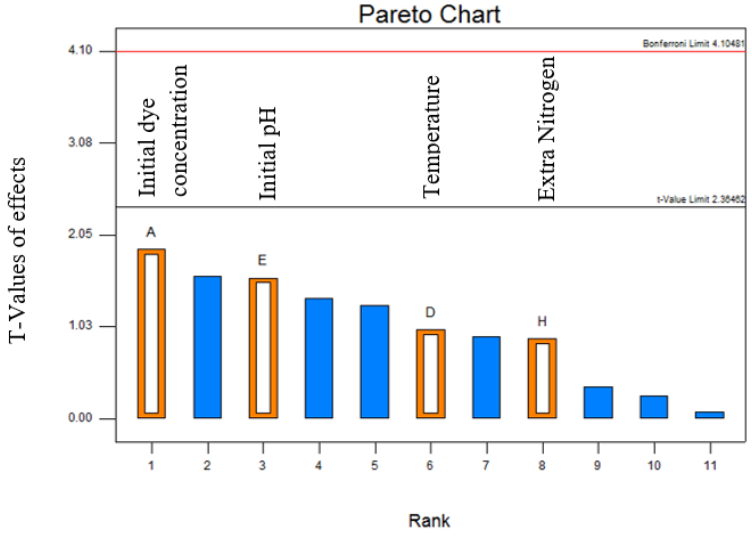

Fig: 2 Pareto Chart

Statistical optimization using RSM

The impact of the four significant variables on the removal of methyl orange was predicted using a Central Composite Design (CCD). Using the Methyl Orange decolourization as a response, RSM was carried out using DOE software. However, the initial dye concentration initial pH, temperature, and additional nitrogen source values had significant positive p values in the PB design, but were disregarded during the CCD design process due to the increased cost and power consumption of controlling these factors on an industrial scale for wastewater treatment. Table 2 shows the list of experiments generated by the software, along with the corresponding figures for dye removal percentage for

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each experiment involving the decolorization of Methyl Orange. To fit the responses, a regression evaluation has been completed. The data was fitted with a quadratic model ($p < 0.001$), as recommended by the software. Additionally, a square root conversion was suggested, resulting in $R^2 = 0.9686$, R^2 Adj = 0.9393, R^2 Pre = 0.8194, and an adequate precision of 16.96. To evaluate the model's suitability, coefficients (R2) and adjusted R2 (R2 Adj) are usually computed. Larger R2 values indicate the proportion of response variations in the model [37]. Therefore, our R2 (0.9894) and R2 Adj (0.9393) values in this instance are satisfactory.

Based on ANOVA (Table 3), the combined impact of pH and initial dye concentration was determined to be the most significant ($p < 0.0012$) in the decolorization of Methyl Orange. The combined relationship between temperature and pH was also important ($p < 0.0094$). The significance value of nitrogen source and the initial dye concentration together is comparatively low when compared to other conjugates, but overall model is significant ($p < 0.0001$). Every single factor has been determined to be significant ($p<0.0001$), with the initial pH being the most influential ($p<0.0007$) in decolorization process (f value =18.29). The three-dimensional responses and the contour diagrams of the previously discussed conjugates are displayed in Fig. 3. Fig. 4 illustrates the predicted versus actual values plot.

Table:2 Experimental runs for decolorization of dye MO using CCD

Run	Initial Dye Concentration (A)	pH (B)	Temperature (C)	Extra Nitrogen source (Urea) (D)	Dye Removal (%)
1	55.00	6.50	37.50	-1.50	99.23
2	55.00	6.50	12.50	1.50	2.11
3	10.00	400	50.00	0.00	12.12
4	55.00	6.50	37.50	4.50	98.04
5	55.00	6.50	37.50	1.50	99.23
6	100.00	4.00	50.00	3.00	10.24
7	100.00	4.00	50.00	0.00	20.11
8	55.00	6.50	37.50	1.50	99.32
9	100.00	9.00	25.00	0.00	97.21
10	55.00	6.50	37.50	1.50	98.27
11	55.00	6.50	37.50	1.50	99.76
12	100.00	9.00	25.00	3.00	50.23
13	145.00	6.50	37.50	1.50	1.11
14	55.00	1.50	37.50	1.50	1.22
15	10.00	9.00	25.00	3.00	22.21
16	55.00	6.50	37.50	1.50	99.87
17	55.00	11.50	37.50	1.50	20.23
18	10.00	9.00	50.00	3.00	1.14
19	10.00	4.00	25.00	3.00	12.15
20	100.00	4.00	25.00	3.00	1.14
21	10.00	4.00	25.00	0.00	22.32
22	10.00	4.00	50.00	3.00	15.11
23	-35.00	6.50	37.50	1.50	1.02
24	100.00	9.00	50.00	0.00	43.24
25	55.00	6.50	37.50	1.50	99.78
26	10.00	9.00	50.00	0.00	11.18
27	100.00	9.00	50.00	3.00	28.17
28	100.00	4.00	25.00	0.00	22.31
29	55.00	6.50	62.50	1.50	1.02

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30	10.00	9.00	25.00	0.00	33.43

The pH and initial dye concentration effects are depicted, along with their correlation, in Fig. 3A. At the lowest values (10 ppm and 4) for initial dye concentration and pH, the dye removal percentage was nearly 39%. However, there was no noticeable difference in the dye removal percentage of 40.3% when the initial dye concentration was raised while maintaining constant pH. At a certain pH, the initial dye concentration rises to a specific point. However, because pH is essential to bacterial physiology, methyl orange elimination reached approximately 85.5% when pH increased towards an alkaline value. This could be due to the fact that methyl orange decolorization increased when the pH was neutral, which is the ideal environment for bacterial survival and function.

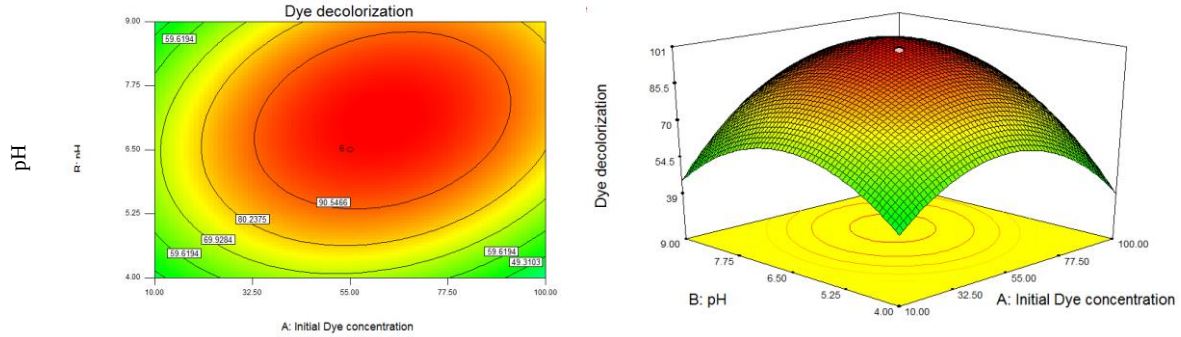
Under constant initial dye concentration, urea was observed to partially improve the removal of Methyl Orange in Fig. 3B. The removal of methyl orange increased by almost 10% from the previous value (71%) when the nitrogen concentration increased from 0.5% to 3%. This suggests that an additional nitrogen source may be enhancing the removal process by enhancing the activities of the bacteria, promoting optimal physiology and survival at a neutral pH, and ultimately speeding up the decolorization of Methyl Orange.

Temperature was found to be a significant factor in relation to the initial dye concentration in Fig. 3C. Initially, there was very low (less than 39%) removal of methyl orange at a temperature of 25–30°C with an initial dye concentration of 10 ppm. When the initial dye concentration was 55.0 ppm and the temperature was 37.5°C, the rate of dye removal increased to 89.5%. Since bacteria could not grow at either the lowest or highest temperatures, this could be the result of their inability to develop at lower temperatures.

Fig. 3D shows the relation between pH and temperature. Meanwhile, the effect of the interaction of pH and temperature had no significant difference on the dye removal efficiency of *Bacillus cereus J4* on Methyl Orange (MO) dye. However, the effect of temperature in the interaction with pH at 37°C enhanced the dye removal of the Methyl Orange dye.

Fig.3E shows the relation between urea and temperature. A significant effect of extra nitrogen source (Urea) at 37 ^{0} C Temperature on dye decolorization of Methyl Orange. This shows that extra nitrogen sources (urea) are acting as removal enhancers by bacterial activities.

The whole study revealed that, initial dye concentration, pH, Temperature and extra nitrogen source (urea) plays a key role in Methyl Orange removal process. The initial aim of this study was to remove as much methyl orange dye as possible from water using *Bacillus cereus J4* in an affordable system. Considering these factors, the following criteria have been chosen to optimize dye removal conditions: initial dye concentration within a specific range.



(a)

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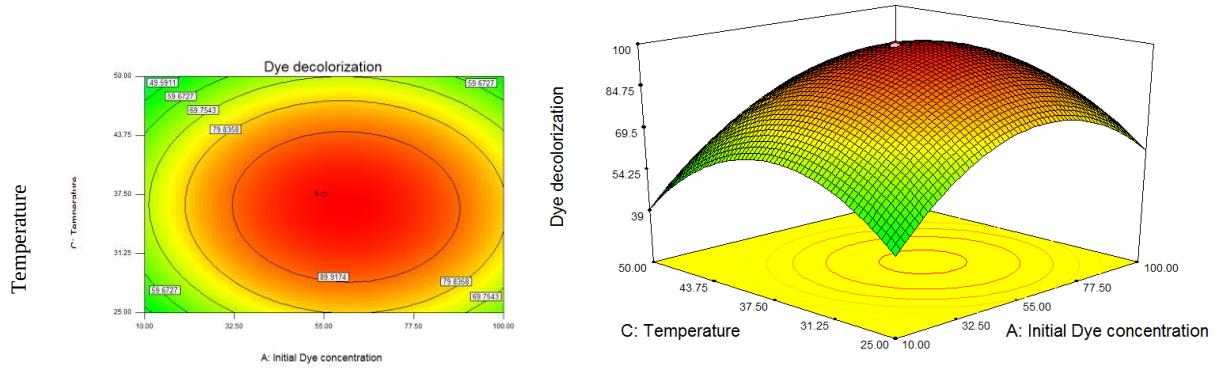

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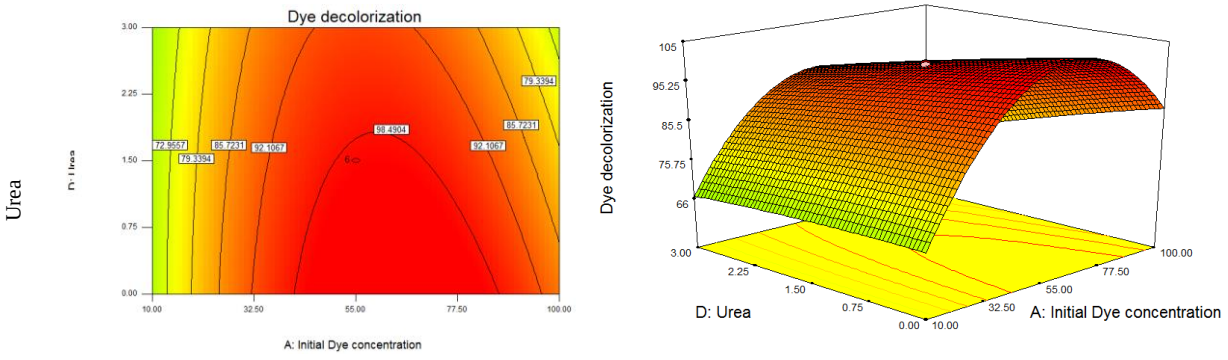

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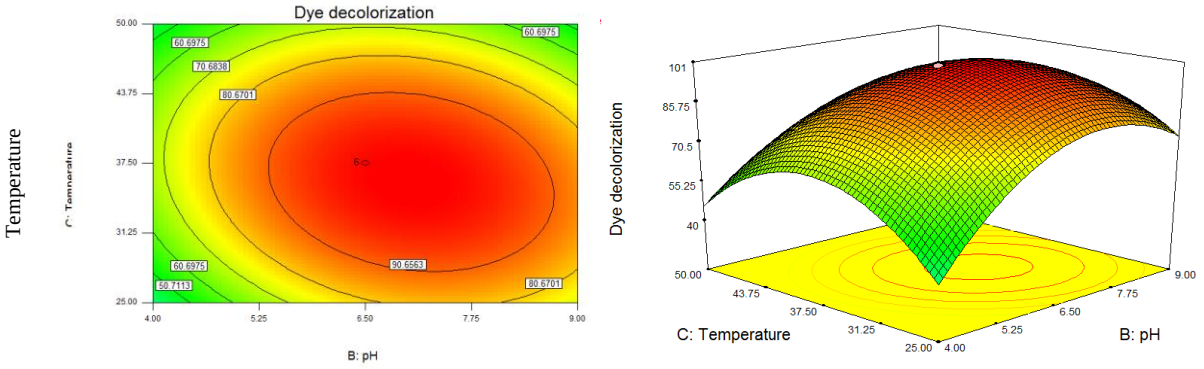
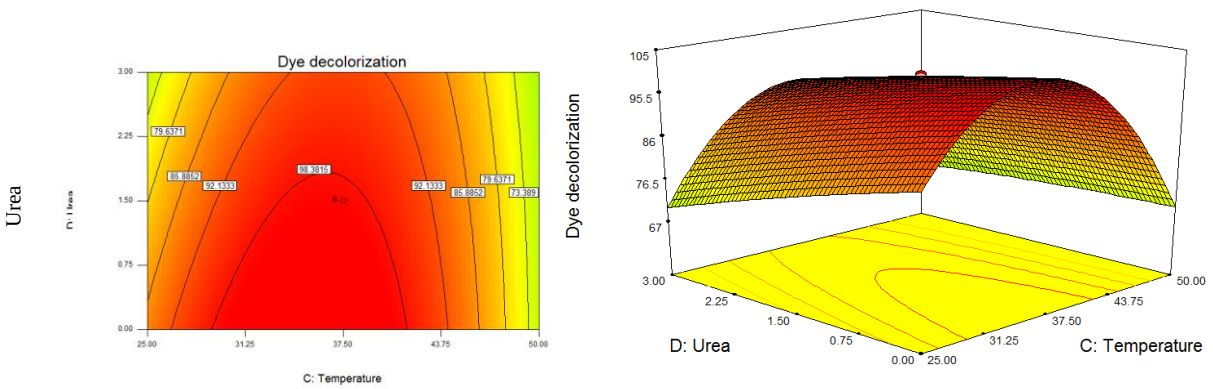


(d)



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(e)

Figure 3 Contour plots correlated to response surface (3D) plots for the interaction of (a) pH vs Initial dye (b) Initial dye vs Temperature (c) Initial dye vs Urea (Extra Nitrogen Source) (d) pH vs Temperature (e) Urea vs Temperature for the decolorization of Methyl Orange using *Bacillus cereus J4*

Table 3

ANNOVA (Analysis of variables)						
Source	Sum Of Square	Degree of freedom	Mean Squares	F-Value	p-Value Prob >F	
Model	46213.62	14	3300.97	33.06	<0.0001	Significant
A-Initial Dye Concentration	854.07	1	854.07	8.55	0.0105	
B-pH	1825.79	1	1825.79	18.29	0.0007	
C-Temperature	618.85	1	618.85	6.20	0.0250	
D-Urea	639.74	1	639.74	6.41	0.0230	
AB	1575.89	1	1575.89	15.78	0.0012	
AC	21.55	1	21.55	0.22	0.6489	
AD	261.23	1	261.23	2.62	0.1266	
BC	885.21	1	885.21	8.87	0.0094	
BD	127.07	1	127.07	0.27	0.2770	
CD	207.00	1	207.00	2.07	0.1704	
A^2	17214.50	1	17214.50	172.43	<0.0001	
B^2	14055.56	1	14055.56	140.79	<0.0001	
C^2	14055.56	1	17043.15	170.71	<0.0001	
D^2	17043.15	1	11.94	0.12	0.7343	
Residual	11.94	15	99.83	-	-	
Lack of Fit	1497.52	10	149.57	414.95	<0.0001	Significant
Pure Error	1495.72	5	0.36	-	-	
Cor Total	1.80 47711.15	29	-	-	-	

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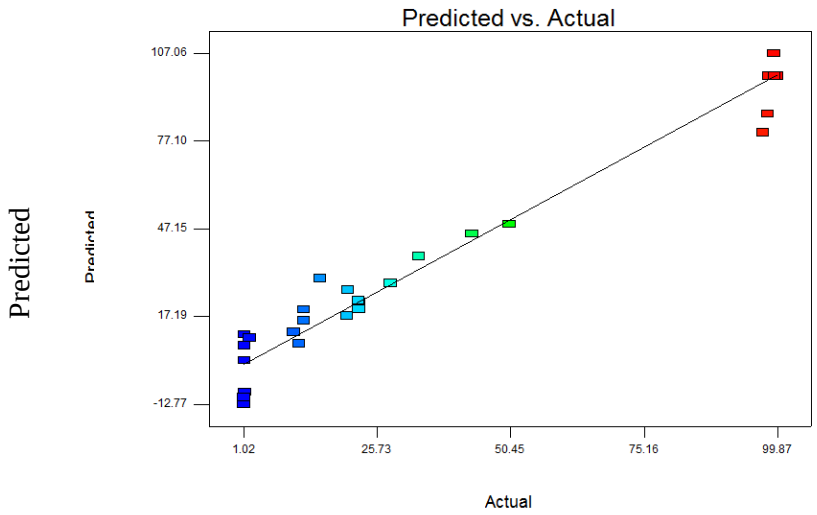

Fig: 3

Initial pH, temperature, and extra nitrogen: all within range; ANNOVA table showed that the optimal model was generated at 50 ppm initial dye concentration, pH 7, 37°C, and 2% extra nitrogen (urea). This model demonstrates an 88.93% removal of methyl orange from an aqueous solution with a desirability of 1. The practical efficacy of the model was confirmed by the 93.05% dye removal obtained in the validation experiment.

In this study eleven bacterial stains isolated from the Textile Industrial area Panipat, Haryana, India. It is evident that *Bacillus cereus J4* can easily remove methyl orange from aqueous solution up to 89.8%. We observed that several physical and chemical parameters significantly influence the rate of methyl orange decolourization. Initial dye concentration, Temperature, extra nitrogen sources (urea), and pH are the key variables for Methyl Orange decolourization, according to statistical analysis for physicochemical factors. Therefore, Bacillus cereus J4 may be a useful agent for the future safe release of methyl orange from industrial effluents into the environment.

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References

1. Saratale, R. G., Saratale, G. D., Chang, J. S., & Govindwar, S. P. (2011) Bacterial decolorization and degradation of azo dyes: a review. Journal of the Taiwan institute of Chemical Engineers 42(1): 138-157. https://doi.org/10.1016/j.jtice.2010.06.006
2. Haseena, M., Malik, M. F., Javed, A., Arshad, S., Asif, N., Zulfiqar, S., & Hanif, J. (2017) Water pollution and human health. Environmental Risk Assessment and Remediation 1(3). http://www.alliedacademies.org/environmental-risk-assessment-and-remediation/ ISSN: 2529-8046
3. Rajan, M. (2014) Impact of dyeing industry effluent on groundwater quality by water quality index and correlation analysis. Journal of Pollution Effects & Control :1-4. http://researchinbiotechnology.com/ar...
4. Ali, H (2010) Biodegradation of synthetic dyes—a review. Water, Air, & Soil Pollution 213: 251-273. DOI 10.1007/s11270-010-0382-4
5. Akarslan, F. and H. Demiralay (2015) Effects of textile materials harmful to human health. Acta Physica Polonica A 128(2B). DOI: 10.12693/APhysPolA.128.B-407
6. Li, Q., Tang, J., Wang, T., Wu, D., Busso, C. A., Jiao, R., & Ren, X. (2017) Impacts of sewage irrigation on soil properties of farmland in China: A review. Solid Earth Discussions : 1-24. https://doi.org/10.5194/se-2017-116

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JCHR (2024) 14(2), 1111-1123 | ISSN:2251-6727

7. Elumalai, S. and G. Saravanan (2016) The role of microalgae in textile dye industrial waste water recycle (phycoremediation).International Journal of Pharma and Bio Sciences 7(4): 662-673.
8. Routoula, E. and S.V. Patwardhan (2020) Degradation of anthraquinone dyes from effluents: a review focusing on enzymatic dye degradation with industrial potential. Environmental science & technology 54(2): 647-664. https://doi.org/10.1021/acs.est.9b03737
9. Kant, R. (2012) Textile dyeing industry an environmental hazard. J Nat Sci 4 (1): 22–26. DOI:10.4236/ns.2012.41004
10. Yang, H.-Y., Liu, J., Wang, Y.-X., He, C.-S., Zhang, L.-S., Mu, Y., & Li, W.-H. (2019) Bioelectrochemical decolorization of a reactive diazo dye: Kinetics, optimization with a response surface methodology, and proposed degradation pathway. Bioelectrochemistry 128: 9-16. https://doi.org/10.1016/j.bioelechem.2019.02.008
11. Singh, R.P., P.K. Singh, and R.L. Singh (2014) Bacterial decolorization of textile azo dye acid orange by Staphylococcus hominis RMLRT03. Toxicology international 21(2): 160. doi: 10.4103/0971-6580.139797
12. Rajee, O. and J. Patterson (2011) Decolorization of azo dye (orange MR) by an autochthonous bacterium, Micrococcus sp. DBS 2. Indian journal of microbiology 51: 159-163. DOI 10.1007/s12088-01-0127-0
13. Chang, J.-S., B.-Y. Chen, and Y.S. Lin (2004) Stimulation of bacterial decolorization of an azo dye by extracellular metabolites from Escherichia coli strain NO3. Bioresource technology 91(3): 243-248. https://doi.org/10.1016/S0960-8524(03)00196-2
14. Sarkar, S., Banerjee, A., Halder, U., Biswas, R., & Bandopadhyay, R. (2017) Degradation of synthetic azo dyes of textile industry: a sustainable approach using microbial enzymes. Water Conservation Science and Engineering 2: 121-131. DOI 10.1007/s41101-017-0031-5
15. Bell, J., Plumb, J. J., Buckley, C. A., & Stuckey, D. C. (2000) Treatment and decolorization of dyes in an anaerobic baffled reactor. Journal of Environmental Engineering 126 (11): 1026-1032. https://doi.org/10.1061/(ASCE)0733-9372(2000)126:11(1026)
16. Sudha, M., Saranya, A., Selvakumar, G., & Sivakumar, N. (2014) Microbial degradation of azo dyes: a review. International Journal of Current Microbiology and Applied Sciences 3(2): 670-690. http://www.ijcmas.com/
17. Du, L.-N., Li, G., Zhao, Y.-H., Xu, H.-K., Wang, Y., Zhou, Y., & Wang, L. (2015) Efficient metabolism of the azo dye methyl orange by Aeromonas sp. strain DH-6*:* characteristics and partial mechanism. International Biodeterioration & Biodegradation 105: 66-72. https://doi.org/10.1016/j.ibiod.2015.08.019
18. Alam, M. Z., Carpenter-Boggs, L., Rahman, A., Haque, M. M., Miah, M. R. U., Moniruzzaman, M., . . . Abdullah, H. M. (2017) Water quality and resident perceptions of declining ecosystem services at Shitalakka wetland in Narayanganj city. Sustainability of Water Quality and Ecology 9: 53-66. https://doi.org/10.1016/j.swaqe.2017.03.002
19. Afrad, M. S. I., Monir, M. B., Haque, M. E., Barau, A. A., & Haque, M. M (2020) Impact of industrial effluent on water, soil and Rice production in Bangladesh: a case of Turag River Bank. Journal of Environmental Health Science and Engineering 18: 825-834. doi: 10.1007/s40201-020-00506-8
20. Prabhakar, Y., A. Gupta, and A. Kaushik (2019) Enhanced decolorization of reactive violet dye 1 by halo-alkaliphilic Nesterenkonia strain: Process optimization, short acclimatization and reusability analysis in batch cycles. Process Safety and Environmental Protection 131: 116-126. https://doi.org/10.1016/j.psep.2019.09.004
21. Huo, Y., Xie, Z., Wang, X., Li, H., Hoang, M., & Caruso, R. A. (2013) Methyl orange removal by combined visible-light photocatalysis and membrane distillation. Dyes and Pigments 98 (1): 106-112. https://doi.org/10.1016/j.dyepig.2013.02.009
22. Youssef, N. A., Shaban, S. A., Ibrahim, F. A., & Mahmoud, A. S. (2016) Degradation of methyl orange using Fenton catalytic reaction. Egyptian Journal of Petroleum 25(3): 317-321. https://doi.org/10.1016/j.ejpe.2015.07.017
23. Haji, S., B. Benstaali, and N. Al-Bastaki (2011) Degradation of methyl orange by UV/H2O2 advanced oxidation process. Chemical Engineering Journal 168(1): 134-139. https://doi.org/10.1016/j.cej.2010.12.050

24. Zhou, W. and W. Zimmermann (1993) Decolorization of industrial effluents containing reactive dyes by actinomycetes. FEMS Microbiology letters 107(2-3): 157-161. https://doi.org/10.1111/j.1574-6968.1993.tb06023.x
25. Garg, S.K. and M. Tripathi (2017) Microbial strategies for discoloration and detoxification of azo dyes from textile effluents. Research Journal of Microbiology 12(1): 1-19. DOI: 10.3923/jm.2017.1.19
26. Bera, S., Sharma, V. P., Dutta, S., & Dutta, D. (2016). Biological decolorization and detoxification of malachite green from aqueous solution by Dietzia maris NIT-D. Journal of the Taiwan Institute of Chemical Engineers 67: 271-284. https://doi.org/10.1016/j.jtice.2016.07.028
27. Akansha, K., D. Chakraborty, and S.G. Sachan (2019) Decolorization and degradation of methyl orange by Bacillus stratosphericus SCA1007. Biocatalysis and Agricultural Biotechnology 18: 101044. https://doi.org/10.1016/j.bcab.2019.101044
28. Kishor, R., Purchase, D., Saratale, G. D., Ferreira, L. F. R., Hussain, C. M., Mulla, S. I., & Bharagava, R. N. (2021) Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium Pseudomonas aeruginosa MZ520730. Journal of Water Process Engineering 43: 102300. https://doi.org/10.1016/j.jwpe.2021.102300
29. Pearce, C., J. Lloyd, and J. Guthrie (2003) The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and pigments 58(3): 179-196. https://doi.org/10.1016/S0143-7208(03)00064-0
30. Saha, N., Samanta, A. K., Chaudhuri, S., & Dutta, D. (2015). Characterization and antioxidant potential of a carotenoid from a newly isolated yeast. Food Science and Biotechnology 24: 117-124.
31. Shah, M. P., Patel, K. A., Nair, S. S., & Darji, A. M. (2013) Microbial decolourization of methyl orange dye by Pseudomonas spp. OA Biotechnology 2(1): 10. DOI 10.1007/s10068-015-0017-z
32. Plackett, R.L. and J.P. Burman (1946) *T*he design of optimum multifactorial experiments. Biometrika 33(4): 305-325. https://doi.org/10.2307/2332195
33. Ghanem, K.M., F.A. Al-Fassi, and A.K. Biag (2012) Optimization of methyl orange decolorization by mono and mixed bacterial culture techniques using statistical designs. Afr J Microbiol Res 6(12): 2918-2928. DOI: 10.5897/AJMR11.1390
34. Haaland, P. D. (2020). Experimental design in biotechnology. CRC press.
35. Dutta, S., Bhattacharyya, A., Ganguly, A., Gupta, S., & Basu, S. (2011) Application of response surface methodology for preparation of low-cost adsorbent from citrus fruit peel and for removal of methylene blue. Desalination 275(1-3): 26-36. https://doi.org/10.1016/j.desal.2011.02.057
36. Ogugbue, C.J. and T. Sawidis (2011) Bioremediation and detoxification of synthetic wastewater containing triarylmethane dyes by Aeromonas hydrophila isolated from industrial effluent. Biotechnology research international 2011. doi:10.4061/2011/967925
37. Chatterjee, S., Chatterjee, T., Lim, S. R., & Woo, S. H. (2011) Effect of the addition mode of carbon nanotubes for the production of chitosan hydrogel core–shell beads on adsorption of Congo red from aqueous solution. Bioresource technology 102(6): 4402-4409. https://doi.org/10.1016/j.biortech.2010.12.117