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Optimization of prime parameters for textile dye decolorization by design of experiments (DOEs) using *Lysinibacillus fusiformis* M1

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ABSTRACT

Bacterial strain M1 isolated from soil contaminated with dye was found to be a potential dye decolorizer in the screening studies. The strain was identified as *Lysinibacillus fusiformis* by 16S rDNA sequencing method. While, the bacteria required 72 h to achieve maximum decolorization of dye in yeast extract broth, it needed 48 h of incubation for achieving 97.1% decolorization in Luria Bertani broth. Plackett–Burman design (PBD) was used to study the effect of most important variables that influence the dye decolorization process under static conditions. In PBD model, 12 experiments were made with seven process variables and the remazol golden yellow decolorization was within the range from 1.01 to 69.29%. From statistically analyzed data, lactose, yeast extract, and pH were found as most important variables that influence the dye decolorization process. These three variables and their combined effect of levels was studied by response surface methodology for an optimization of dye decolorization through central composite design and the outcome varied from 2.05 to 83.80%. The optimized level of lactose, yeast extract, and pH was found to be 0.40% (w/v), 1.30% (w/v), and 7.5, respectively, to achieve maximum dye decolorization of remazol golden yellow (89.66%) in validation of model. This study specifically explored factors influencing the dye decolorization through statistical tools and suggests their appropriate level with interactions playing a key role for an effective dye decolorization process.

Keywords: Remazol golden yellow; Statistical optimization; Textile dye decolorization; Carbon and nitrogen sources; Plackett–Burman design; Central composite design

1. Introduction

Rapid industrialization and urbanization are leading to large-scale environmental pollution through the release of wide range of noxious waste from diverse industries. Among them, textile industries generate huge amount of wastewater with synthetic dyes and chemical additives [1]. Worldwide annually, it is estimated that 1,00,000 commercially accessible dyes are available in the market and more than 2,80,000

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tones of textile dyes are discharged into natural ecosystem [2]. Revenue from dye market in the last five years was US$23 billion and still it is expected to increase up to 2% per annum in the next decade. China, Korea, and Taiwan predominantly use dispersible dyes, whereas manufactures in India generate enormous amount of reactive dyes due to easy and cheaper availability of intermediates. These reactive dyes are most hazardous group of chemicals present in textile effluent adding 10–50% of unfixed dyes released into the environment [3].

The discharge of textile effluent containing dyes into the environment leads to eutrophication and the presence of high biochemical oxygen demand can cause rapid depletion of dissolved oxygen level in the surface water along with ground water pollution [4]. Physical and chemical technologies such as photolysis, photocatalytic degradation, membrane filtration, ultrafiltration, advanced oxidation, suspended or supported photocatalysis, electrophotocatalysis, flocculation, coagulation with alum, ferric chloride, magnesium, carbon, polymer, mineral sorbents or biosorbents, and chemical oxidation are available for the removal of color and COD from dye wastewater [5].

The conventional process of physical and chemical methods for textile effluent treatment is inadequate and generating large amount of secondary contaminants in the natural ecosystem [6]. The microbial treatment method is novel, less expensive, and complete mineralization of dyes was achieved by potential micro-organisms [7]. Many researchers reported that bacteria belonging to a different taxonomic group decolorized the dyes effectively under laboratory condition. In converse, the higher amount of saline concentration with diverse groups of dyes and chemicals in textile effluent induces plasmolysis and reduces the cellular activity of bacterial cells, and biodegradation of synthetic dyes is mainly affected by numerous environmental parameters [8].

Due to these limitations, there is a need to explore more potential dye decolorizing bacterial strains for an effective textile wastewater treatments. The main drawback in using distinct bacteria for bioremediation is it does not survive in high toxic and unfavorable environment condition. It is essential to find a balance of various physicochemical parameters that influence the maximal growth of bacteria and thereby facilitate maximal rate of dye decolorization. Optimization of these parameters will pave the way for bioremediation of environment contaminated with dyes. Researchers until now have assessed variation in one-factor-at-time method. This method involves changing one independent factor at a time while keeping the other factors at a constant level [9]. Conventional optimization method is highly laborious, time consuming often failed to find the true optimized condition and specific interaction of independent factors or in combination process [10].

A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach [11]. Hence, statistical designs of experiments known by design of experiments (DOE) are helpful tools to learn more information about the optimum conditions in a small number of experiments [12]. Mostly, it is used to reduce the chemical usage by optimizing the most important parameters and the time involved in a process [13]. DOEs methods frequently used for process analysis and modeling such as full factorial requires multilevel experiments, a partial factorial requires fewer experiments than a full factorial, and response surface methodology (RSM) [14].

Initially, 2 k-factorial Plackett–Burman design (PBD) is used to evaluate the output and find out the effect of significant factors influence in a system [15], furthermore, allowing them to determine the optimum values through RSM. RSM uses mathematical and statistical formulas to estimate linear and quadratic effects of significant factors or independent variables and their interaction on the final response [16].

Considering the advantages and application of DOE, the present study was designed to isolate and identify the potential dye decolorizing strains and to optimize the environmental factors like carbon source, nitrogen source, dye concentration, pH, inoculums size, temperature, and incubation period to enhance the decolorization process in aqueous media using PBD followed by RSM (Central Composite Design [CCD]).

2. Materials and methods

2.1. Isolation and screening of dye decolorizing bacteria

The dye-contaminated soil samples were collected in pre-sterilized polyethylene bags from the textile industries located in Erode and Tiruppur districts of Tamil Nadu, India. Soil samples were transferred to the laboratory within 2 h and stored at 4˚C for bacterial isolation. Pour plate technique was employed to enumerate the bacteria using nutrient agar. The inoculated plates were subsequently incubated at 35 ± 2˚C and 45 ± 2˚C for isolation of mesophilic and thermotolerant bacteria under 24–48 h incubation. Further, distinct bacterial colonies were subjected to decolorization efficiency on the solid medium and liquid culture assay.

All the bacterial strains were individually tested for their ability to decolorize remazol golden yellow (RNL) dye amended with various concentrations (50, 100, 150, 200, and 250 mg/l) in Luria Bertani agar

plates containing (g/l), 10 g of casein enzymic hydrolysate, 5 g of yeast extract, 10 g of sodium chloride, and 15 g of agar. To the prepared plates, test strains were placed by spot inoculation and incubated at 37˚C for 4 d. After incubation, the plates were observed and clear zones around the bacterial colonies were further subjected to quantitative studies [17].

2.2. Identification of dye decolorizing bacteria

The strain M1 was selected as potential dye decolorizing bacterium and identified at species level by 16S rDNA sequencing method. The genomic DNA isolation, PCR amplification, and 16S rDNA sequencing were carried out in Xcelris Labs Ltd, Ahmedabad, India.

2.3. PCR amplification and 16S rDNA sequencing

The fragments of 16S rDNA gene were amplified by PCR from the isolated DNA. The total PCR mixture volume was 20 µl containing genomic DNA, PCR buffer, primer, deoxynucleotide triphosphate, DNA polymerase, and molecular grade water. Before starting the amplification process, a brief spin was given to the PCR tube to settle down the materials to the bottom of the tube. PCR conditions consisted of 25–35 cycles of 95˚C (2 min), 42˚C (30 s), and 72˚C (4 min) plus one additional cycle with a final 20 min chain elongation. PCR-amplified product of 1500 bp was observed in agarose gel electrophoresis. The amplification products were purified on Centricon 100 columns (Amicon), by following the specifications of the manufacturer, followed by ethanol precipitation. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT V3.1 cycle sequencing kit on ABI 3730Xl Genetic Analyzer.

2.4. Phylogenetic analysis and Genbank accession

Aligner software was used to generate the consensus sequence 1326 bp of 16S rDNA from forward and reverse sequence data. Further the sequence was edited by bioEdit program. The obtained sequence was compared with the existing sequences deposited in the NCBI sever (www.ncbi.nlm.nih.gov/BLAST) using BLASTN tool. On the basis of maximum identity score, closely related sequences were selected and aligned with multiple alignment software program Clustal W. Phylogenetic tree was constructed using the software package MEGA Version 5 by neighbor-joining (NJ) method based on bootstrap analysis (1,000 replicates). The nucleotide sequences were deposited in NCBI GenBank, (http://www.ncbi.nlm.nih.gov) under the accession number JX316003.

2.5. Quantitative estimation of dye decolorization in diverse broth composition

The efficacy of screened dye decolorizing bacteria was quantitatively evaluated by liquid culture assay. Prospective dye degrading bacterial inoculums were prepared in 250-ml Erlenmeyer flask containing 100-ml nutrient broth medium having the same composition as mentioned above except agar and incubated for 24 h at 37˚C. One ml of 24 h grown dye decolorizing bacterial strain was inoculated in the different composition of broths. Luria Bertani broth containing (g/l), 10 g of casein enzymic hydrolysate, 5 g of yeast extract, 10 g of sodium chloride; yeast extract broth containing (g/l), 5 g of yeast extract, 5 g of sodium chloride; Bushnell Haas broth containing (g/l), 0.2 g of magnesium sulfate, 1 g of dipotassium hydrogen phosphate, 0.02 g of calcium chloride, 0.05 g of ferric chloride, 1 g of ammonium nitrate at 100 mg/l concentration of remazol golden yellow dye. These flasks were incubated at 37˚C under static condition for 3 d. Control flasks were also maintained with the same medium without bacterial inoculum. After 24 h of incubation, the culture decolorizing broths were withdrawn aseptically and centrifuged at 3,000 rpm for 20 min and filtered through 0.45-µm membrane filter in order to separate the bacterial biomass. The absorbance value of the filtered cell free supernatant was used to determine the percentage of decolorization using UV spectrophotometer (Cyberlab UV-100 USA) at 412 nm for remazol golden yellow dye. All the decolorization studies were carried out in triplicate and mean values were subjected to decolorization analysis. The decolorization rate was calculated using the following equation:

$$\%\text{ of decolorization} = \frac{\text{Initial absorbance value} - \text{Final absorbance value}}{\text{Initial absorbance value}} \times 100 \quad (1)$$

2.6. Optimization of environmental parameters on dye removal through statistical tools

DOEs was a statistical application that enables researchers to reveal the insight knowledge of individual and interactive effects of multiple factors that could have an effect on the net results. Consequently, researchers can able to fix them and produce higher yield robust designs prior to implementation of any process. DOEs which involves screening up to "$n-1$"

variables in just "n" number of trails, besides estimation of the regression coefficients and prediction of the fittest model of the response were done using statistical software Minitab Version 15 [18]. All the experiments were carried out as per the design culture conditions in a basal broth medium along with their duplicates.

2.7. Plackett–Burman design

Based on DOEs module, initially, optimization process was carried out by PBD experiments used to identify the significant and insignificant parameters of dye decolorization employing *Lysinibacillus fusiformis* M1. It was assumed that there was no interaction between the different operational parameters in the range of consideration. 2 k-factorial PBDs dealing with a total number of 12 experiments, includes seven assigned independent variables and two unassigned independent variables. The test levels of the variables were selected based on the preliminary experiments and their actual values were given in Table 1.

Table 2 depicts the Plackett–Burman experimental design and their levels of each variable used in the dye decolorization, which are based on the following first-order model:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ij} X_i^2 + \Sigma\beta_{ij} X_i X_i \tag{2}$$

where Y is the response or dependent variable, in another term to denote is estimated target/percentage of dye decolorization, X_i is independent variable levels, i is independent variable number, β_0 is the model intercepts term/the constant process effect on the total/constant coefficient, βi is the linear effect/variable estimates/linear coefficient, βii is the squared effect/quadratic effect of X_i/quadratic coefficient, βij is the interaction effect/corresponding coefficients/the interaction effect between X_i and X_j on dye decolorization process.

2.8. Statistical analysis of PBD

Triplicate experiments were carried out as per the PB design in static Erlenmeyer flasks incubation and the averages of percentage decolorization were taken as responses. The positive or negative magnitude of the coefficient value indicates that the corresponding impact on dye decolorization process and the coefficient value close to zero implies a small or no effect [19]. The p-values ($p < 0.10$) were used as a tool to justify the significance of each of the coefficients and to understand the interactions between the significant variables affecting the dye decolorization process [5]. Regression coefficient (R^2) should be at least 80% for a good fittest model [20]. The statistical significance of the variable and model was also determined by F-test for analysis of variance (ANOVA) and the variables with higher than 90% confidence level were considered as significant one.

2.9. Central composite design

Optimization of the experimental conditions for decolorization of dye was achieved using RSM (CCD). RSM can be used to illustrate the individual and cumulative effect of the test variables on the response and to find out the mutual interaction effect between the cluster of controlled test variables and their subsequent effect on the response [21]. The significant independent variables lactose, yeast extract, and pH were identified based on the results of PBD. These three factors covered five levels ($-\alpha$, −1, 0, +1, and $+\alpha$) of CCD and their actual values with 20 experimental runs are mentioned in Tables 3 and 4. Where $\alpha = 2^{n/3}$; here "n" was the number of variables and "0" corresponded to the central point. The actual value of each variable/factor was calculated using the following equation [22]:

$$\text{Coded value} = \frac{\text{Actual value} - (\text{high level} + \text{low level})/2}{(\text{High level} - \text{low level})/2} \tag{3}$$

Table 1
Actual values of test variables for PBD

Test variables	Lactose % (w/v)	Yeast extract % (w/v)	pH	Temperature (°C)	Inoculums size % (v/v)	Dye concentration (mg/l)	Incubation period (h)
Low level (−)	0.1	0.1	5	30	5	100	24
High level (+)	1.0	1.0	9	45	10	300	72

Table 2
PBD experiments for optimization of RNL dye decolorization

Run order	Lactose % (w/v)	Yeast extract % (w/v)	pH	Temperature (℃)	Inoculums size % (v/v)	Dye concentration (mg/l)	Incubation period (h)	DV-1	DV-2
1	1.0	0.1	9	30	5	100	72	1	1
2	1.0	1.0	5	45	5	100	24	1	1
3	0.1	1.0	9	30	10	100	24	−1	1
4	1.0	0.1	9	45	5	300	24	−1	−1
5	1.0	1.0	5	45	10	100	72	−1	−1
6	1.0	1.0	9	30	10	300	24	1	−1
7	0.1	1.0	9	45	5	300	72	−1	1
8	0.1	0.1	9	45	10	100	72	1	−1
9	0.1	0.1	5	45	10	300	24	1	1
10	1.0	0.1	5	30	10	300	72	−1	1
11	0.1	1.0	5	30	5	300	72	1	−1
12	0.1	0.1	5	30	5	100	24	−1	−1

Notes: DV 1 and 2—Dummy variable; +1 denoted for high concentration; −1 denoted for low concentration.

Table 3
Actual values of the variables in CCD

Variables	Unit	Five levels of variables				
		$-\alpha$ (−1.68179)	−1	0	1	$+\alpha$ (+1.68179)
Lactose	% (w/v)	−0.20681	0.1	0.55	1.0	1.306807
Yeast extract	% (w/v)	−0.20681	0.1	0.55	1.0	1.306807
pH	–	3.636414	5	7	9	10.36359

Table 4
CCD experiments for optimization of RNL dye decolorization

Run order	Pt type	Blocks	Lactose % (w/v)	Yeast extract % (w/v)	pH
1	1	1	0.1	0.1	5
2	1	1	1.0	0.1	5
3	1	1	0.1	1.0	5
4	1	1	1.0	1.0	5
5	1	1	0.1	0.1	9
6	1	1	1.0	0.1	9
7	1	1	0.1	1.0	9
8	1	1	1.0	1.0	9
9	−1	1	−0.20	0.55	7
10	−1	1	1.31	0.55	7
11	−1	1	0.55	−0.20	7
12	−1	1	0.55	1.31	7
13	−1	1	0.55	0.55	3.64
14	−1	1	0.55	0.55	10.36
15	0	1	0.55	0.55	7
16	0	1	0.55	0.55	7
17	0	1	0.55	0.55	7
18	0	1	0.55	0.55	7
19	0	1	0.55	0.55	7
20	0	1	0.55	0.55	7

The other individual variables play an vital role in the study was sodium chloride 0.5% (w/v), dye concentration 200 mg/l, inoculums size 7.5% (v/v), temperature 37˚C, and incubation period 48 h act as a constant factor in this phase of 20 trials. The culture at 18 h (OD 610 nm = 0.5) produced on a nutrient broth was used to inoculate the experimental flasks as per the CCD.

2.10. Statistical analysis of CCD

Consequently, the correlation and interrelationship of dependent variable (response) between three independent variables were determined by fitting the following linear quadratic model (Eq. (4)) to the data conquered from CCD experiments [23].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \cdots \quad (4)$$

where Y is the measured response (percentage of decolorization)/dependent variable, β_0 is the intercept term, β_1, β_2, and β_3 are linear coefficient of three independent variables, β_{11}, β_{22}, β_{33} are quadratic coefficient, β_{12}, β_{13}, β_{23} are interaction coefficient, and X_1, X_2, X_3, X_4 are coded independent variables.

The quality of fit of the experimental model was expressed by the determination of coefficient R^2 and its statistical significance was determined by F-test. The predicted optimal values of the selected variables were obtained by solving the regression equation and by analyzing the response contour plots [24].

2.11. Validation of the optimized values

In the validation study, the maximum decolorization level (response) was verified by testing the predicted culture condition/optimal point of potential dye degrading bacteria under static incubation condition. The decolorized level of broth was observed at the end of incubation period as described above and compared with the predicted results of the CCD.

3. Results and discussion

3.1. Isolation and screening of textile dye decolorizing bacteria

A total of 96 bacterial strains were isolated from the dye-contaminated soil samples using nutrient agar medium. High bacterial load exist with this sample is probably due to the continuous enrichment of wastewater [25]. All the bacterial strains were examined for their dye degradation ability at different concentrations (50–250 mg/l) of textile dye which are detailed in materials and methods. The reductions in the dye color proportionate to increase in the growth of the organism indicate positive strains capable of degrading the dye [26]. Among them, 20 strains showed clear zones around the bacterial culture grown on the agar plates amended with 100 mg/l concentration of remazol golden yellow dye. Ultimately, the bacterial strain M1 was found to be the most-efficient dye degrader in all the concentrations of dye and it was subjected to further studies. Chen et al. [27] clearly stated that micro-organisms isolated from textile industry sites contain capability to survive in the presence of toxic dyes.

3.2. Identification of dye decolorizing bacteria

Partial sequence analysis of 16S rDNA revealed that the strain M1 was closely related to the genus *Lysinibacillus* and the nucleotide sequence was deposited in the GenBank database under the accession number JX316003. The phylogenetic tree was constructed from evolutionary distances by the NJ method (Fig. 1). The strain M1 has 100% sequence similarity with *L. fusiformis* DSM2898^T and it was identified as *L. fusiformis*. The dye decolorization of *Lysinibacillus* strain M1 found in our studies is similar to those found in few other studies. To our knowledge, only limited reports are available on decolorization of textile dyes using *Lysinibacillus* sp. In a study, potential azo dye degrading *Lysinibacillus* sp. V3DMK were isolated from textile industry dye-contaminated soil and sequences were deposited in Genbank with the accession number JF975599 [28]. Moreover, the work of Anjaneya et al. [29] revealed that the *Lysinibacillus* sp. AK2 from textile effluent-contaminated soil was capable of decolorizing sulfonated azo dye.

3.3. Quantitative effect of various compositions on dye decolorization

The bacterial strain M1 that showed maximum clear zones was assessed for dye removal competence in different compositions of aqueous medium. During quantitative analysis, broth compositions used in decolorizing study play a significant role in the dye removal process [30]. At the end of incubation, the strain M1 showed maximum dye decolorization in Luria Bertani broth composition (72.73% in 24 h and 97.1% in 48 h) followed by yeast extract broth (83.91% decolorization in 72 h incubation), whereas minimum decolorization 72.85 and 78.19% was found between 24 and 48 h incubation period. In contrast, bacterial strain

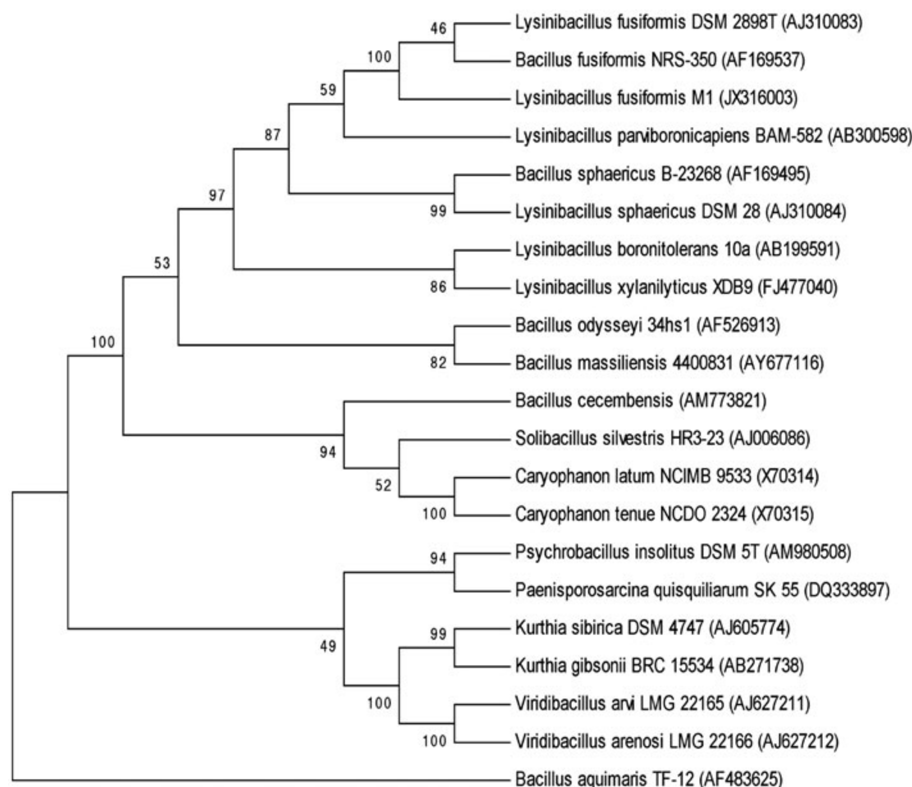

Fig. 1. Phylogenetic tree of *Lysinibacillus fusiformis* M1 showing relationship between other bacterial strains. The percent numbers at the nodes indicate the levels of bootstrap support based on NJ analyses of 1,000 replicates. Brackets represents sequence accession numbers.

does not have ability to grow and decolorize the remazol golden yellow dye even beyond the stipulated incubation period in the Bushnell Haas broth composition. Maximum dye decolorization was found within short period of incubation in Luria Bertani broth was attributed to the amendment of casein enzymatic hydrolysate as vitamin source for their growth and metabolic activities. Amendment of yeast extract leads to regeneration of NADH to act as electron donor for an enhanced decolorization [31]. Yeast extract was utilized a carbon and nitrogen sources by bacteria in dye removal metabolism [32]. These results clearly proves that the nutrients incorporated composition enhanced the growth of bacterial strains and decolorization performance was rapid than minerals only integrated Bushnell Haas Broth composition. This explains the necessity of co-substrates for an effective dye removal rate was already reported by Moosvi et al. [33].

3.4. Effect of environmental parameters on dye removal through PBD

The optimized region of parameters involved in textile dye remazol golden yellow decolorization was determined by DOE using *L. fusiformis* M1. Initially, PBD screening was made and the results are depicted in Table 5. There was a wide variation in the dye decolorization, ranging from 1.01 to 69.29%. These output deviations revealed that the importance of significant variables optimization to achieve maximum color removal.

3.4.1. PBD analysis of estimated effects and regression coefficients

The statistical analysis of regression coefficients, student's *t*-test and *p*-value of seven independent variables is given in Table 6. On dye decolorization, the variable coefficient value indicates that the increase in concentration of yeast extract, pH, temperature, and incubation period showed a positive influence. Chen et al. [27] reported *Lysinibacillus* sp. exhibited a higher level of dye removal with an increase in the yeast extract concentration. The maximum decolorization of dye was observed at 40°C and beyond this level a reduced decolorization rate was noticed. Anjaneya et al. [29] illustrated that thermophilic point of temperature leads to the loss of microbial activity and

Table 5
PBD screening of significant parameters for dye decolorization using *L. fusiformis* M1

Run order	Lactose % (w/v)	Yeast extract % (w/v)	pH	Temperature (˚C)	Inoculum size % (v/v)	Dye concentration (mg/l)	Incubation period (h)	Percentage decolorization	
								Experimental	Predicted
1	1.0	0.1	9	30	5	100	72	18.81	19.92
2	1.0	1.0	5	45	5	100	24	18.01	17.15
3	0.1	1.0	9	30	10	100	24	40.86	33.55
4	1.0	0.1	9	45	5	300	24	8.54	9.80
5	1.0	1.0	5	45	10	100	72	16.03	16.88
6	1.0	1.0	9	30	10	300	24	4.45	11.75
7	0.1	1.0	9	45	5	300	72	69.29	59.18
8	0.1	0.1	9	45	10	100	72	23.67	31.33
9	0.1	0.1	5	45	10	300	24	1.87	2.99
10	1.0	0.1	5	30	10	300	72	1.01	−8.67
11	0.1	1.0	5	30	5	300	72	30.78	40.97
12	0.1	0.1	5	30	5	100	24	14.91	13.38

Table 6
Statistical analysis of PBD on remazol golden yellow decolorization

S. no	Variables	Effect	Coef	SE Coef	*T*	*p*
1	Constant		20.691	3.137	6.60	0.003*
2	Lactose	−19.095	−9.548	3.137	−3.04	0.038*
3	Yeast extract	18.455	9.227	3.137	2.94	0.042*
4	pH	13.808	6.904	3.137	2.20	0.093*
5	Temperature	4.408	2.204	3.137	0.70	0.521
6	Inoculum size	−12.095	−6.047	3.137	−1.93	0.126
7	Dye concentration	−2.698	−1.349	3.137	−0.43	0.689
8	Incubation period	11.832	5.916	3.137	1.89	0.132

Notes: $R^2 = 88.48\%$; R^2 (adj) = 68.31%.
*Significance ($p<0.10$).

inactivation of enzyme responsible for decolorization. Higher concentration of dye made the inhibitory effect on bacterial growth and reduced the decolorization efficiency [34]. On the other side, decreasing the other variables such as lactose, inoculums size, and dye concentration had a negative influence. Waghmode et al. [35] found that lactose and dextrose acting additional carbon source hinder the decolorization of Rubin GFL and textile effluent. Reduced decolorization with decreasing inoculums size was observed due to lesser bacterial cells rapidly entered in stationary phase and subsequently to death phase [36].

Usually, larger *t*-value associated with low *p*-value specifies a high significance of the model term. From this statistically analyzed data, lactose, yeast extract, and pH were found as most important variables that influence the dye decolorization by *L. fusiformis* M1. The effect of significant variables on decolorization process is explained by statistical model in regression (Eq. (5)) and it can be used to predict the decolorization rate by any desired combination of seven variables in the experimental range. This was explained by the model and revealed a good agreement between the experimental results and predicted values calculated from the model.

$$Y = 20.691 - 9.548 \times \text{lactose} + 9.227 \times \text{yeast extract} + 6.904 \times \text{pH} + 2.204 \times \text{temperature} - 6.047 \times \text{inoculums size} - 1.349 \times \text{dye concentration} + 5.916 \times \text{incubation period} \quad (5)$$

Determination of correlation coefficient ($R^2 = 0.8848$) being nearer to 1 denote a good statistical simulation between the experimental and predicted responses, which implies that the model can explain up to 88.48% variation in the experiment and fittest one.

Pareto chart of the standardized effects was demonstrated for the magnitude of variables that were important for dye decolorization and the main effect of variables estimates present on the horizontal axis (Fig. 2). The Pareto chart also shows a vertical line representing statistical significance ($p = 0.10$). According to their significance level, the screened independent variables main effects are rank structured and in the order of lactose, yeast extract, and pH, respectively.

3.4.2. Analysis of variance

ANOVA of linear model was used to interpret the effect of independent variables on remazol golden yellow decolorization (Table 7) and it does explain the presence of variations from the results caused by each variable relative to the total variation. The variables, whose p-value was less than 0.10, indicates that the model and variables are highly significant one.

3.5. Central composite design

PBD identified important variables and their combined effect of levels was studied by RSM for optimization of remazol golden yellow decolorization through CCD, the strain *L. fusiformis* M1 showed that dye removal rate was varied from 2.05 to 83.80% (Table 8).

The second-order polynomial model (Eq. (6)) was adapted with experimental results by multiple regression analysis and the response of independent variables was present in an empirical model and

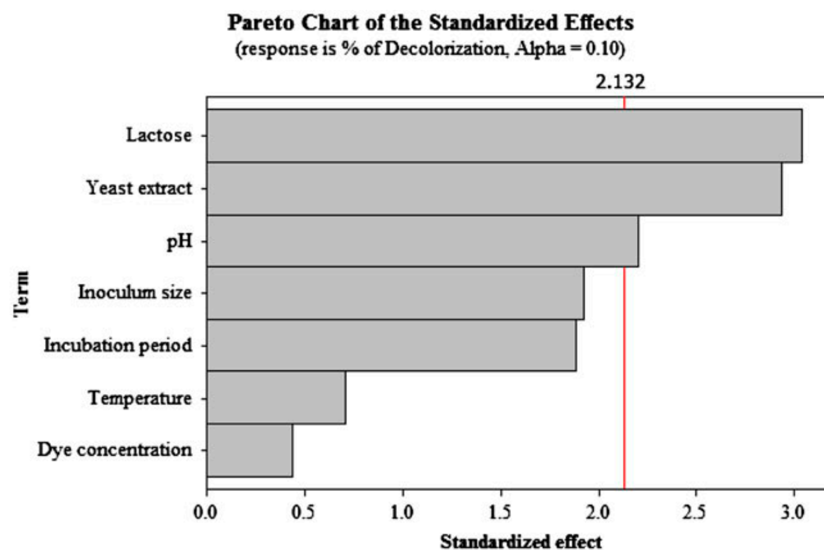

Fig. 2. Effects of variables on percentage of dye decolorization in Pareto chart.

Table 7
ANOVA for effect of variables on dye decolorization using *L. fusiformis* M1

S. no	Source	DF	Seq SS	Adj SS	Adj MS	*F*	*p*
1	Main effects	7	3,626.60	3,626.60	3,626.60	4.39	0.086*
2	Lactose	1	1,093.76	1,093.76	1,093.76	9.26	0.038*
3	Yeast extract	1	1,021.76	1,021.76	1,021.76	8.65	0.042*
4	pH	1	572.01	572.01	572.01	4.84	0.093*
5	Temperature	1	58.30	58.30	58.30	0.49	0.521
6	Inoculum size	1	438.87	438.87	438.87	3.72	0.126
7	Dye concentration	1	21.84	21.84	21.84	0.18	0.689
8	Incubation period	1	419.97	419.97	419.97	3.56	0.132
9	Residual error	4	472.33	472.33	118.08	–	–
	Total	11	4,098.93				

*Significance ($p<0.10$).

Table 8
CCD with predicted values for effective dye removal

Trails	Lactose (X_1)	Yeast extract (X_2)	pH (X_3)	Percentage decolorization		Residual
				Experimental	Predicted	
1	0.1	0.1	5	5.084	9.93	−4.84
2	1.0	0.1	5	59.74	48.32	11.41
3	0.1	1.0	5	57.39	41.97	15.41
4	1.0	1.0	5	54.6	48.92	5.67
5	0.1	0.1	9	5.51	0.31	5.19
6	1.0	0.1	9	6.93	11.46	−4.53
7	0.1	1.0	9	67.92	68.45	−0.53
8	1.0	1.0	9	63.89	48.16	15.72
9	−0.20	0.55	7	23.18	26.98	−3.80
10	1.31	0.55	7	30.64	42.21	−11.57
11	0.55	−0.20	7	39.17	38.22	0.94
12	0.55	1.31	7	79.7	96.02	−16.32
13	0.55	0.55	3.64	3.74	14.94	−11.20
14	0.55	0.55	10.36	2.05	6.22	−4.17
15	0.55	0.55	7	82.13	82.64	−0.51
16	0.55	0.55	7	82.67	82.64	0.02
17	0.55	0.55	7	83.34	82.64	0.69
18	0.55	0.55	7	83.8	82.64	1.15
19	0.55	0.55	7	83.34	82.64	0.69
20	0.55	0.55	7	83.25	82.64	0.60

Table 9
Estimated regression coefficients for percentage of decolorization

S. no	Variables	Coef	SE Coef	T	p
1	Constant	82.648	4.615	17.907	0.000*
2	Lactose	4.525	3.062	1.478	0.170
3	Yeast extract	17.185	3.062	5.612	0.000*
4	pH	−2.593	3.062	−0.847	0.417
5	Lactose × lactose	−16.987	2.981	−5.699	0.000*
6	Yeast extract × yeast extract	−5.488	2.981	−1.841	0.095*
7	pH × pH	−25.478	2.981	−8.547	0.000*
8	Lactose × yeast extract	−7.862	4.001	−1.965	0.078*
9	Lactose × pH	−6.809	4.001	−1.702	0.120
10	Yeast extract × pH	9.026	4.001	2.256	0.048*

Notes: R^2 = 93.48%; R^2 (adj) = 87.61%.
*Significance (p<0.10).

Table 10
ANOVA for dye decolorization

S. no	Source	DF	Seq SS	Adj SS	Adj MS	F	p
1	Regression	9	18,359.2	18,359.2	2,039.91	15.93	0.000*
2	Linear	3	4,404.9	4,404.9	1,468.29	11.47	0.001*
3	Square	3	12,437.2	12,437.2	4,145.72	32.37	0.000*
4	Interaction	3	1,517.1	1,517.1	505.71	3.95	0.043*
5	Residual error	10	1,280.6	1,280.6	128.06	–	–
6	Lack-of-fit	5	1,278.9	1,278.9	255.78	729.68	0.000*
7	Pure error	5	1.8	1.8	0.35	–	–
	Total	19	19,639.8	–	–	–	–

*Significance (p<0.10).

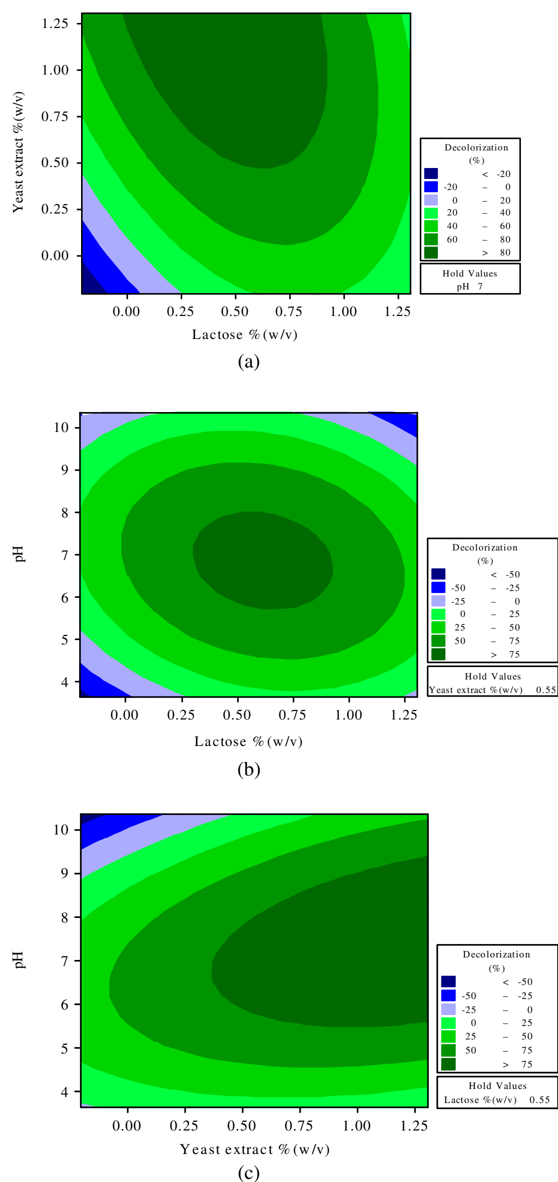

Fig. 3. Contour plots between the variables for dye removal (%), (a) interactive effect of yeast extract and lactose concentration on dye removal (%), (b) interactive effect of pH and lactose concentration on dye removal (%), and (c) interactive effect of pH and yeast extract concentration on dye removal (%).

estimated regression coefficient for the model is given in Table 9.

$$Y = 82.648 + 4.525 \times X_1 + 17.185 \times X_2 - 2.593 \times X_3 - 16.987 \times X_1^2 - 5.488 \times X_2^2 - 25.478 \times X_3^2 - 7.862 \times X_1 \times X_2 - 6.809 \times X_1 \times X_3 + 9.026 \times X_2 \times X_3 \quad (6)$$

where Y is dependent variable or predicted response for dye decolorization, X_1, X_2, and X_3 were the coded values of independent variables as lactose, yeast extract, and pH. The magnitude of coefficient for overall effect of the variables noticed the presence of high significance ($p = 0$) on decolorization process. Correlation coefficient (R^2) was found to be 93.48%, thus higher R^2 value demonstrated that presence of good correlation between the variables and dye decolorization process.

In analysis of variance, the calculated F-value = 15.93 and p-value = 0 indicates that the model is highly significant to predict the experimental results for remazol golden yellow decolorization (Table 10). The individual, quadratic, and interaction effects were also present in variables and it was found to have high significance on remazol golden yellow decolorization.

Graphical representations of responses obtained by regression equation are illustrated in Fig. 3. The contour plots lines signify the relationship and interaction effect of two variables with varying concentrations on dye decolorization, whereas the third variable was apprehended in the middle level. These plots were made to know about the changes in response surface region, particularly, plots used to predict the decolorization competence with different rate of the tested variables [37]. A contour plot indicates the type of interactions present between the tested variables and response.

Optimal concentrations of important variables: lactose, yeast extract, and pH were found to be 0.40% (w/v), 1.30% (w/v), and 7.5 for maximum decolorization of remazol golden yellow using *L. fusiformis* M1. As per this response region, strain *L. fusiformis* M1 experimentally attained 89.66% decolorization, which is concurrent with expected decolorization 99.21% found in response surface plots. *L. fusiformis* M1 requires minimum amount of carbon source due to the preference of the cells in assimilating the dye compound as a carbon source [38]. *Lysinibacillus* sp. RGS showed decolorization up to 90% in the broad pH range (7.0–9.0) but maximum decolorization was achieved at pH 7.0. The broad pH stability shown by this strain in decolorization gained much attention in textile wastewater treatment since it carry higher pH [39]. Likewise, *Lysinibacillus* sp. strain AK2 decolorized metanil yellow over a broad pH range 5.5–9.0 and the optimum value was found as 7.2 [29]. Correspondingly, the dye removal rate was excellent in using *Lysinibacillus* sp. KMK-A at neutral pH condition stated by Chaudhari et al. [40]. With increase in yeast extract concentration (0.1–5 g/l) in reactive violet 5R dye removal, *Lysinibacillus* sp. V3DMK achieved complete decolorization at the optimal point of 1 g/l yeast

extract was correlated with our studies [28]. These are the efficient reports with sufficient results synchronizing with our findings and support the prospective application of *L. fusiformis* M1 on textile wastewater treatment process.

4. Conclusions

The role of *L. fusiformis* in textile dye decolorization with high efficacy is very less. This experiment clearly demonstrates that the bacterial strains from dye-contaminated soil are naturally resistant to the toxic chemicals in the contaminated site. Several environmental factors such as pH, temperature, dye concentration, and co-metabolic nutritional sources influences textile dye decolorization process. The individual carbon and nitrogen sources have different impact on the utilization of dye as a co-substrate by *L. fusiformis* M1. The use of optimized culture along with nutrients to increase the decolorization rate of remazol golden yellow seems to be a dynamic approach. The optimization process has to be found favorable point of each individual factor for a satisfied operational condition of the progression. Studying effect of various factors on bacterial textile dye decolorization through DOE was a suitable practice to begin the favored culture condition to achieve maximum dye removal in real effluent treatment. From the above point, it could be concluded that isolated dye decolorizing bacteria *L. fusiformis* M1 would be a suitable candidate for the treatment of textile wastewater in a large-scale level.

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