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Decolorization of Textile Dye by *Brevibacillus laterosporus* (TS5) and Influencing Factors Optimization through Response Surface Methodology

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Abstract

The dye removal bacteria *Brevibacillus laterosporus* (TS5) was isolated from dye contaminated soil, and it's identified by 16S rDNA sequencing method. The prospective bacterial strain exhibited a highest decolorization (97.8%) in Luria-Bertani broth medium. Among the operational factors, Plackett-Burman design, experimental results indicated that pH, incubation period, and yeast extract significantly contributed for the dye decolorization. Also, dye concentration, starch, temperature, and inoculum size noted as insignificant factors on dye decolorization. Central composite design applied for optimization of important factors to enhance the dye decolorization by Brevibacillus laterosporus (TS5). The optimal values of significant factors were determined by the Response surface methodology (RSM) as follows: 0.60% (w/v) yeast extract, 7.23 pH and 61.45 hrs incubation period, which assisted for Brevibacillus laterosporus (TS5) to attain 90.66% dye removal. *Brevibacillus laterosporus* (TS5) showed 90.08% decolorization in validation experiments by the support of optimal factors, and implies that explored strain could be a suitable candidate for bioremediation of dye containing effluents.

1. Introduction

The disposal of waste from textile industries is considered as major environmental problem in the worldwide. Particularly, the discharging of textile industry effluent into the ecosystem is hazardous one, as it contains bio-recalcitrant dye stuffs **(Sarayu and Sandhya 2012)**. Textile effluent includes a mixture of dyes, organic and inorganic chemicals **(Doble and Kumar 2005)**. Dyes are recognized as a first pollutant in textile waste water, since it possesses higher visibility in colored effluent **(Kilic *et al.* 2007)**.

Azo dyes are normally recognized as the most imperative group of synthetic dyes. They are carcinogenic aromatic compound that disturbs the transparency, gas solubility nature of water bodies, and inhibits the growth of aquatic plants **(Rodriguez Couto *et al.* 2009)**. Some of the azo dyes induce bladder cancer, chromosomal aberrations in humans **(Medvedev *et al.* 1988)**. The effluents containing complex structure of azo dyes makes it difficult to treat in conventional methods **(Jadhav *et al.* 2016).**

Various physical and chemical technologies are employed in textile industries for the removal of dye molecules from colored effluents **(Yaseen *et al.* 2018)**. They are utilizing the large amount of chemicals, and electricity for their mode of action, which produce the secondary pollutants **(Karthik *et al.* 2015; Sweety *et al.* 2018)**. These secondary pollutants described as sludge, inorganic salts, metals, and carcinogenic substance possess risk to human health and aquatic fauna **(Vikas and Sandip 2013)**. From these points, the investigation aimed on environmental friendly methods for removal of dyes using microorganisms. Several textile dye decolorization studies have been reported with bacteria, fungi, and yeast.

Among microbial decolorization, the use of bacteria is comparatively faster, economical, and can be applied to wide range of dye degradation and mineralization **(Saratale *et al.* 2011)**. Bacterial dye degradation mainly occurs in aerobic, anaerobic, anoxic, microaerophilic, and sequential process **(Ajaz *et al.* 2019)**. Generally, azo dye reduction take place in bacteria through synthesizing of azoreductase enzyme and cleavage of azo bonds under oxygen limited conditions **(Coughlin *et al.* 2003)**.

The most important cultural factors are carbon source, nitrogen source, dye concentration, pH, inoculum size, temperature, and the incubation period is dynamically affected the efficiency of microbial activity and decolorization processes **(Garg *et al.* 2017)**. Consequently, their relevant factor optimization is prerequisite one, prior to real effluent studies, to avoid any inhibitory effect on dye degradation process. Therefore, the necessity found in the screening of robust bacteria which are able to adopt, and decolorize the textile effluent in an adverse condition. Also, key factors optimization by response surface methodology (RSM) approach can enhance the performances of bacterial dye decolorization and its effectiveness **(Mohana *et al.* 2008)**.

Mostly statistical tools employed to simplify the operations, chemical consumption, and the time involved in a process. Moreover, RSM is an efficient chemometric empirical approach that can be used to eliminate insignificant variables, and to study the relationship between a set of significant factors in optimized

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process **(Chen, 1994)**. In this view, the present study focused on the exploration of textile dye decolorizing bacteria *Brevibacillus laterosporus* (TS5) isolated from the dye contaminated soil, and its dye decolorization efficacy was enhanced via optimization by screening of influencing factors in dye removal process with Plackett-Burman design, followed by application of response surface methodology with central composite design.

2. Material and methods

2.1 Screening of dye decolorizing bacteria

The dye contaminated soil sample was collected from a textile dyeing industry located in Tirupur district of Tamil Nadu, India. The bacterial colonies were isolated and enumerated by nutrient agar medium (pH 7.0±0.2) containing, 5 (g/l) Peptone, 5 (g/l) Sodium chloride, 3 (g/l) Yeast extract, 20 (g/l) Agar. The pour plate method employed plates were incubated at 35±2 °C, and 45±2 °C for 24 - 48 hrs incubation. The decolorizing ability of bacterial colonies was determined in Luria Bertani agar plates containing, 10 (g/l) Casein enzymic hydrolysate, 5 (g/l) Yeast extract, 10 (g/l) NaCl, 15 (g/l) Agar, amended with various concentrations (50, 100, 150, 200 and 250 mg/l) of remazol golden yellow (RNL) dye, and incubated at 37 °C for 4 days. The bacterial colonies which showing clear zones on the agar plates were selected for decolorization studies.

2.2 Identification of dye decolorizing bacteria

The dye decolorizing bacterial strain TS5 was identified by 16S rDNA sequencing method. The genomic DNA isolation, extraction, PCR amplification, and 16S rDNA sequencing was carried out in Xcelris Labs Ltd, Ahmedabad, India. The generated 16S rDNA sequences were compared to sequences of the NCBI server using BLASTN tool. The closely related sequences were aligned with multiple alignment program Clustal W. Phylogenetic tree was constructed by the neighbour-joining (NJ) method in MEGA version 5. The sequences have been deposited in NCBI GenBank (http://www.ncbi.nlm.nih.gov) under accession number JQ885974.

2.3 Effect of nutritional substrates in dye decolorization

The effect of co-substrates on dye decolorization using *Brevibacillus laterosporus* (TS5) was carried out in the various broth compositions (100 ml each) Luria Bertani broth (g l^{-1}): Casein enzymic hydrolysate 10.0, yeast extract 5.0, NaCl 10.0; Yeast extract broth (g l^{-1}): yeast extract 5.0, NaCl 5.0; Bushnell and Hass broth (g l^{-1}): $MgSO_4$ 0.2, K_2HPO_4 1.0, $CaCl_2$ 0.02, $FeCl_3$ 0.05, NH_4NO_3 1.0 (remazol golden yellow dye concentration, 100 mg/l). About 1 ml of inoculum with an optical density of 1.0 was inoculated. Experimental flasks were incubated statically at 37 °C for 3 days with abiotic control. The samples were withdrawn at 24 hrs intervals, and centrifuged at 5000 rpm for 20 min. The absorbance value of the cell free supernatant was analyzed in UV spectrophotometer (Cyberlab UV-100 USA) at 412 nm. Triplicate experiments were performed in simultaneously, and decolorization percentage was calculated using the Equation (1).

$$\text{Decoloriza tion } (\%) = \frac{\text{Initial absorbance value - Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Equation (1)

2.4 Plackett- Burman design - Screening of significant factors for dye decolorization

The Plackett–Burman design, a fractional factorial design, was used to select the most important factors that influencing in remazol golden yellow dye decolorization by strain *Brevibacillus laterosporus* (TS5). **Table 1** provides the information about testing factors range and their actual values. The levels of the factors were selected based on previous literatures, selective carbon and nitrogen sources were obtained from preliminary experiments. The effect and relative importance of each factor on dye decolorization was determined in 12 combinations of Plackett- Burman design matrix shown in **Table 2**, which are based on the following first-order model Equation (2)

Table 1. Actual values of the factors Plackett-Burman design screening

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

Table 2. Plackett-Burman design for screening of major factors on dye decolorization

Run order	Starch % (w/v)	Yeast extract % (w/v)	pH	Temperatures (°C)	Inoculum size % (v/v)	Dye concentrations (mg/l)	Incubation periods (hrs)	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

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

Equation (2)

Where Y is the response (Percentage of dye decolorization), X_i is factor levels, i is factor number, β_0 is the model intercepts term, βi is the linear effect, βii *is* the squared effect, βij is the interaction effect between X_i and X_j on dye decolorization process. As per the design, experiments were carried out in flask culture under static incubation. All the runs were performed in triplicate, and the averages of decolorization percentage were considered as the response. Statistical analysis of the Plackett-Burman design results were performed by using statistical software Minitab Version 15.0.

2.5 Response surface methodology - Optimization of dye decolorization

The concentrations of yeast extract, incubation period, and pH were the significant factors for effective dye decolorization was identified from results of Plackett–Burman design. Response surface methodology was used to study the optimal region of significant factors, and their interactions on remazol golden yellow dye decolorization using strain *Brevibacillus laterosporus* (TS5), a full factorial central composite design was employed. Each significant factor was studied at five levels (-α, -1, 0, +1 and +α) and their actual values are shown in **Table 3**. Where $\alpha = 2^{n/3}$; here "n" corresponds to the number of factors and "0" corresponds to the central point. The Equation (3) was adopted to calculate the actual values of significant factors.

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

Equation (3)

Table 3. Actual values of significant factors in central composite design

Variables	Unit	Five levels of variables				
		-α (-1.68179)	-1	0	1	+α (+1.68179)
Yeast extract	% (w/v)	-0.20681	0.1	0.55	1	1.306807
pH	-	3.636414	5	7	9	10.36359
Incubation Period	Hrs	7.636972	24	48	72	88.36303

The experimental model of central composite design was given in **Table 4**. The other cultural factors were maintained in constant range, such as, sodium chloride 0.5% (w/v), starch 0.55% (w/v), dye concentration 200 mg/l, inoculum size 7.5% (v/v), and temperature 37 °C. The experimental results obtained from the central composite design were established in second order polynomial model Equation (4) for the prediction of decolorization efficiency, and the significant factor interactions within testing range.

$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$

Equation (4)

Where Y is the response, β_0 is the intercept term, β_1, β_2 and β_3 are linear coefficient of tested factors, 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 factors. All the experiments were carried out in triplicate, and the statistical software package Minitab Version 15.0 was used to mathematical interpretation of experimental results. The significant factors optimal value was established from Central composite design results with response surface analyzer. Under optimal values of these key factors, the validation study was performed, and enhancement of dye decolorization efficiency was evaluated by comparing the experimental results with predicted values

Table 4. Central composite design for optimization of dye decolorization

Run Order	Pt type	Blocks	Yeast extract % (w/v)	pH	Incubation Period (hrs)
1	1	1	0.1	5	24
2	1	1	1	5	24
3	1	1	0.1	9	24
4	1	1	1	9	24
5	1	1	0.1	5	72
6	1	1	1	5	72
7	1	1	0.1	9	72
8	1	1	1	9	72
9	-1	1	-0.20	7	48
10	-1	1	1.31	7	48
11	-1	1	0.55	3.63	48
12	-1	1	0.55	10.36	48
13	-1	1	0.55	7	7.63
14	-1	1	0.55	7	88.36
15	0	1	0.55	7	48
16	0	1	0.55	7	48
17	0	1	0.55	7	48
18	0	1	0.55	7	48
19	0	1	0.55	7	48
20	0	1	0.55	7	48

3. Results and Discussion

3.1 Isolation and Screening of dye decolorizing bacteria

A total of 42 mesophilic and thermotolerant bacterial strains were isolated and enumerated from the dye contaminated soil. The development of a significant number of bacteria in the dye contaminated soil denotes its ability to degrade the toxic dye substances **(Rajee *et al.* 2011)**. Among these, eighteen isolates made the clear zone around the colony in Luria-Bertani agar plates amended with 100mg/l concentration of remazol golden yellow dye. The colony which showed >1.0 cm of clear zone was found to be effective dye decolorizer **(Tiwari *et al.* 2012)**. Whereas, the isolated bacteria TS5 proficiently showed the clear zone with diverse concentrations of dye amended plates. **Wang *et al.* (2012)** stated that indigenous microorganisms have the capability to decolorize various classes of dyes with high efficiency.

3.2 Identification of dye decolorizing bacteria

The BLAST analysis of the 16S rDNA amplicon, indicated that the strain TS5 was belonging to the member of *Brevibacillus* genus. The phylogenetic relationship of strain TS5 with other *Brevibacillus* species was shown in **Figure 1** exhibited 100% similarity with *Brevibacillus laterosporus* IAM 12465 (D16271) from the GenBank database. The bacterial strain was identified as *Brevibacillus laterosporus* (TS5). Earlier on this, few studies reported the enzymatic role of *B. laterosporus* in the textile dye decolorization **(Gomare *et al.* 2009a and Kurade *et al.* 2011)**.

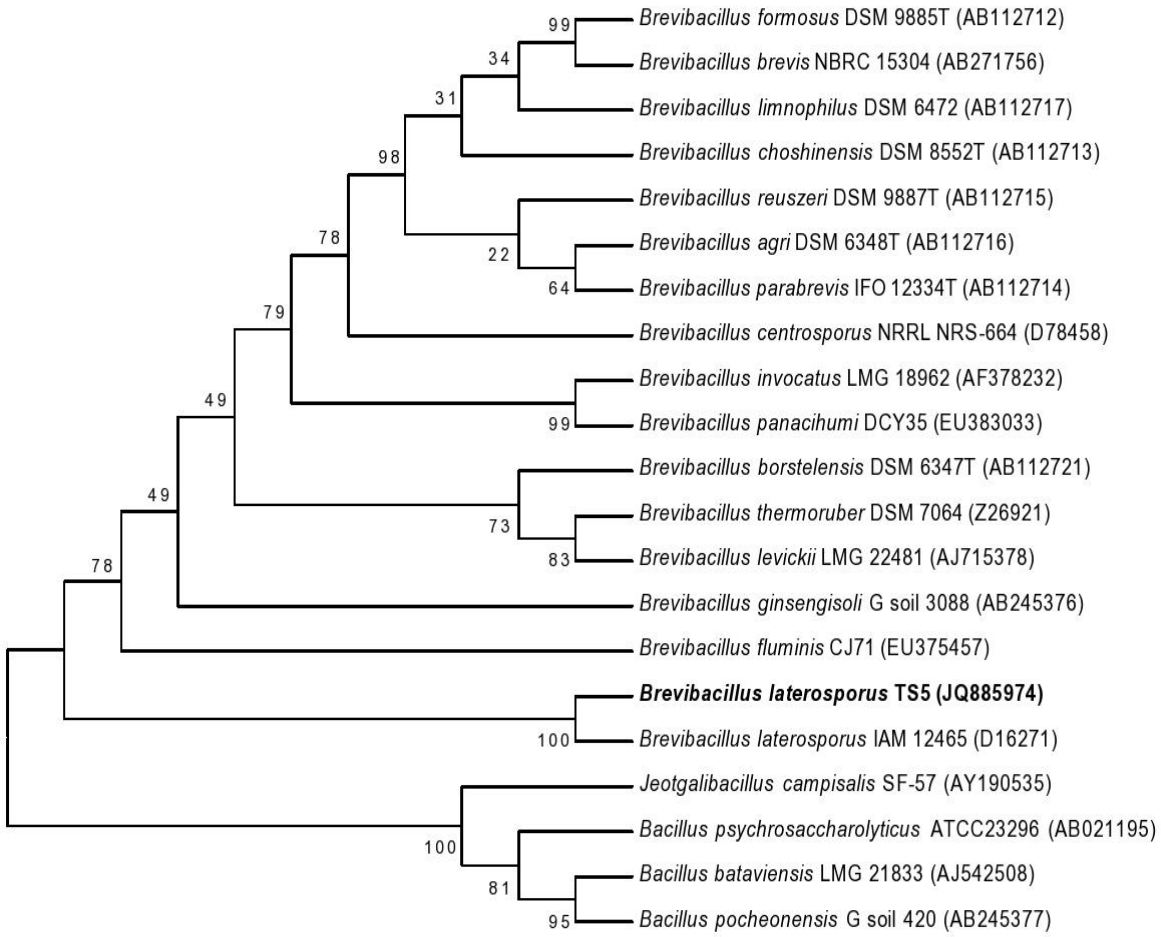


Figure 1. Phylogenetic tree of *Brevibacillus laterosporus* (TS5) showing relationship between selected bacterial strains. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 replicates. Brackets represents sequence accession numbers.

3.3 Effect of nutritional substrates

Suitable substrates of broth composition on decolorization by *Brevibacillus laterosporus* (TS5) were investigated in batch studies. The dyes are recalcitrant to biodegradation in nature, and it's not acting as a nutritional source for microorganisms **(Lellis *et al.* 2019; Jamee *et al.* 2019)**. Thus require additional co-substrates for its degradation, and mineralization **(Dos Santos *et al.* 2007)**. In the present study, *Brevibacillus laterosporus* (TS5) showed 2.55% and 29.31% of dye reduction in yeast extract broth composition at 24 hrs intervals. On this, incubation time extended up to 72 hrs, and the 73.6% decolorization was found. **Moosvi *et al.* (2007)** observed that 25% removal of Reactive Violet 5R (100ppm) by bacterial consortium JW-2 with yeast extract broth composition. The absence of carbon substances in the yeast extract medium confirms the reason for lower decolorization of dyes. Whereas, 25.49%, and 97.8% dye removal observed in Luria-Bertani broth, and its components found as effective nutritional substrates. Casein enzymic hydrolyzate in LB broth enhanced the bacterial growth characteristics and decolorization ability. The Luria-Bertani broth combined nutritional composition indicates the induction for an efficient dye decolorization. Similarly, *Marinobacter* sp. strain HBRA effectively decolorized the 100 mg/l concentration of Direct Blue-1 dye in LB broth under static conditions **(Arun Prasad *et al.* 2013)**. Diversified results revealed that broth composition playing a vital role in dye decolorization experiments. There was no sign of decolorization was observed in bushnell hass broth medium without nutritional substrates. It indicates the inability of bacteria for utilizing the dye as a sole of energy, and agreed with many confirmed reports that the obligate requirement of co-substrate for proficient dye decolorization **(Karim *et al.*2018)**.

3.4 Screening of significant factors on dye decolorization

The influence of operational factors in remazol golden yellow decolorization by *Brevibacillus laterosporus* (TS5) was scrutinized in Plackett-Burman design and the results are ranging from 0.68 to 53.69% **(Table 5)**, it reflected the significant effect of factors in dye removal process. The diverse culture conditions were responsible for the decolorization performance of *Brevibacillus laterosporus* was denoted by **Gomare *et al.* (2009c)**. Standardized effects in a pareto chart **(Figure 2)** illustrates the significant factors of dye decolorization, which was yeast extract, pH and incubation period respectively. Analysis of estimated effects and regression coefficient represents the factors significance level **(Table 6)**. The coefficient value of yeast extract, pH, and incubation period indicates the positive effect accumulated via increased their concentration. The availability of carbon/nitrogen sources determines the rate of dye decolorization, since it acts as an

electron donor for the reduction of the dye **(Kurade *et al.* 2011)**. **Waghmode *et al.* (2012)** found that addition of yeast extract regenerated the NADH, which acts as an electron donor for decolorization of Rubin GFL. Similarly in presence of yeast extract, *A. hydrophila* effectively decolorized, and reduced RED RBN dye into azo bonds **(Chen *et al.* 2003)**. Microbial decolorization of dyes is generally reported in neutral to alkaline pH range. **Kurade *et al.* (2012)** also reported that neutral pH and 40 °C temperature was an effective factor for significant decolorization of Rubine GFL by *Brevibacillus laterosporus*. Thus the influence of finest pH on dye decolorization could be a supportive factor in industrial scale application. On the other hand, concentration of dye, starch, temperature, and inoculum size indicates the insignificant condition for dye decolorization. Beyond the optimum temperature, loss of its cell viability, and inhibition of oxido-reductive enzyme activities lead the reduced decolorization **(Kurade *et al.* 2012)**. In general, lower decolorization was observed with higher dye concentration demonstrates the toxicity of dye molecules effect on microorganisms. **Kurade *et al.* (2013a)** reports the decreased Disperse Brown 118 decolorization by *B. laterosporus* with an increasing of initial dye concentration. In the present study, minimum decolorization with decreasing inoculum volume was observed, because to lesser bacterial cells rapidly entered the death phase from the exponential growth phase, and cause the declined decolorization. **(Kolekar *et al.* 2008)**. **Gomare *et al.* (2009b)** reported the better decolorization of Navy Blue-3G by exponential phase of *B. laterosporus*, as at this stage produced the highest amount of biotransformation enzymes for their dye degradation activity. Starch is a carbon source often utilized in the various processes of textile industries **(Ramesh babu *et al.* 2007)**. Thus the use of starch as a co-substrate by bacteria would be favorable for the removal of dye from textile effluents. However, previous studies stated that carbon sources like glucose and starch have an inhibitory effect on the enzyme system responsible for dye decolorization **(Kurade *et al.* 2011)**. Hence, the interactive effect of factors could be a reason for distinguished observation noted in this study, compared to previous reports.

Table 5. Plackett-Burman design decolorization (%) results

Run order	Starch % (w/v)	Yeast extract % (w/v)	pH	Temperatures (°C)	Inoculum size % (v/v)	Dye concentrations (mg/l)	Incubation periods (hrs)	Percentage decolorization	
								Experimental	Predicted
1	1.0	0.1	9	30	5	100	72	19.43	17.59
2	1.0	1.0	5	45	5	100	24	18.90	23.03
3	0.1	1.0	9	30	10	100	24	38.48	34.83
4	1.0	0.1	9	45	5	300	24	6.19	6.01
5	1.0	1.0	5	45	10	100	72	35.30	31.03
6	1.0	1.0	9	30	10	300	24	18.46	22.54
7	0.1	1.0	9	45	5	300	72	53.69	48.05
8	0.1	0.1	9	45	10	100	72	18.67	26.31
9	0.1	0.1	5	45	10	300	24	1.63	0.20
10	1.0	0.1	5	30	10	300	72	0.68	0.91
11	0.1	1.0	5	30	5	300	72	29.48	33.12
12	0.1	0.1	5	30	5	100	24	5.56	3.37

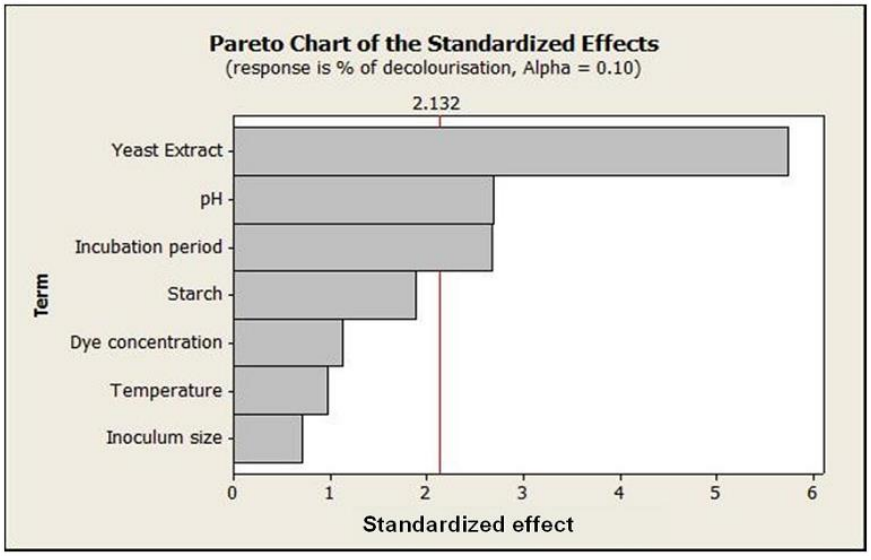


Figure 2. Effects of operational factors on dye decolorization in Pareto chart

Table 6. Statistical analysis of Plackett-Burman design

S. No	Variables	Effects	Coef	SE Coef	T	P
1	Constant	-	20.400	2.036	10.02	0.001*
2	Starch	-7.697	-3.848	2.036	-1.89	0.132
3	Yeast extract	23.407	11.703	2.036	5.75	0.005*
4	Ph	10.983	5.492	2.036	2.70	0.054*
5	Temperature	3.947	1.973	2.036	0.97	0.387
6	Inoculum size	-2.930	-1.465	2.036	-0.72	0.512
7	Dye concentration	-4.597	-2.298	2.036	-1.13	0.322
8	Incubation period	10.933	5.467	2.036	2.68	0.055*
	R-Sq = 93.08% R-Sq (adj) = 80.98%					

*Significant

Usually large t-value associated with low P-value specifies the high significance of the model term. The significant factors effect on the decolorization process is explicated in regression equation number 5. Determination of correlation coefficient (R^2 = 0.9308) is nearer to 1, demonstrates the model was fittest one. It showed a good statistical simulation between the experimental and predicted response, which implies that the model can be explain up to 93.08% variation in the experiment.

Y= 20.400 - 3.848 × starch + 11.703 × yeast extract + 5.492 × pH + 1.973 × temperature -1.465 × inoculum size - 2.298 × dye concentration + 5.467 × incubation period
Equation (5)

ANOVA of linear model explains the factors affecting on remazol golden yellow decolorization **(Table 7)**. The fisher's F – test value of the response is 7.69 and its delivered that the model was significant one, the value of Probability (P) > F is less than 0.034. The operational factors P-value were less than 0.10 indicates, that the model and those factors are highly significant.

3.5 Optimization of dye decolorization

From the results of Plackett-Burman design, yeast extract, pH and incubation period found as the most important factors and their levels were optimized in response surface methodology (RSM) for maximum decolorization of remazol golden yellow by *Brevibacillus laterosporus* (TS5). The experimental results of % dye decolorization involving central composite design were varied from 5.62 to 87.15% **(Table 8)**.

Table 7. Analysis of variance for Plackett-Burman design

S.No	Source	DF	Seq SS	Adj SS	Adj MS	F	P
1	Main effects	7	2677.72	2677.72	2677.72	7.69	0.034*
2	Starch	1	177.72	177.72	177.72	3.57	0.132
3	Yeast extract	1	1643.62	1643.62	1643.62	33.04	0.005*
4	pH	1	361.90	361.90	361.90	7.27	0.054*
5	Temperature	1	46.73	46.73	46.73	0.94	0.387
6	Inoculum size	1	25.75	25.75	25.75	0.52	0.512
7	Dye concentration	1	63.39	63.39	63.39	1.27	0.322
8	Incubation period	1	358.61	358.61	358.61	7.21	0.055*
9	Residual error	4	198.99	198.99	49.75	-	-
	Total	11	2876.71				

*Significant

Table 8. Central composite design decolorization (%) results

Trails	Yeast extract (X_1)	pH (X_2)	Incubation period (X_3)	Percentage decolorization		Residual
				Experimental	Predicted	
1	0.1	5	24	10	12.52	-2.52
2	1	5	24	8.5	6.08	2.41
3	0.1	9	24	6.45	1.67	4.77
4	1	9	24	5.62	5.60	0.01
5	0.1	5	72	17.5	19.55	-2.05
6	1	5	72	24	30.81	-6.81
7	0.1	9	72	25.5	29.95	-4.45
8	1	9	72	52.07	51.58	0.48
9	-0.20	7	48	8.45	6.89	1.55
10	1.31	7	48	21	19.66	1.33
11	0.55	3.64	48	7.5	3.14	4.35
12	0.55	10.36	48	10	11.47	-1.47
13	0.55	7	7.63	24	27.76	-3.76
14	0.55	7	88.36	79	72.34	6.65
15	0.55	7	48	85.65	86.63	-0.98
16	0.55	7	48	87	86.63	0.36
17	0.55	7	48	86	86.63	-0.63
18	0.55	7	48	86.5	86.63	-0.13
19	0.55	7	48	87.15	86.63	0.51
20	0.55	7	48	87	86.63	0.36

The decolorization result (%) was fitted with second order polynomial model equation (6) to explain the confidence of dye removal percentage. The estimated regression coefficient for the model was given in **Table 9**. The small p-value corresponding to larger t-value explains that the factors and model term are significant. Also, correlation coefficient (R^2) was found to be 99.18% that reveals the presence of a good correlation between studied and predicted decolorization (%).

$$Y= 86.632 + 3.796 \times X_1 + 2.478 \times X_2 + 13.253 \times X_3 - 25.933 \times X_1^2 - 28.045 \times X_2^2 - 12.931 \times X_3^2 + 2.592 \times X_1 \times X_2 + 4.425 \times X_1 \times X_3 + 5.313 \times X_2 \times X_3 \quad \textbf{Equation (6)}$$

Where Y is response (percentage of dye decolorization), X_1, X_2 and X_3 were the coded values of yeast extract, pH and incubation period respectively. In analysis of variance determined data **(Table 10)**, the calculated F-value = 134.59 and probability value P=0 shows the model as a significant one. The enhanced effects, linear (p=0.000), quadratic (p=0.000) and interaction (p=0.006) are found between the factors in dye removal. The significant factors interaction and their effects on dye decolorization were graphically shown in response contour plots **(Figure 3a, b, c)**.

Table 9. Estimated regression coefficients of CCD results

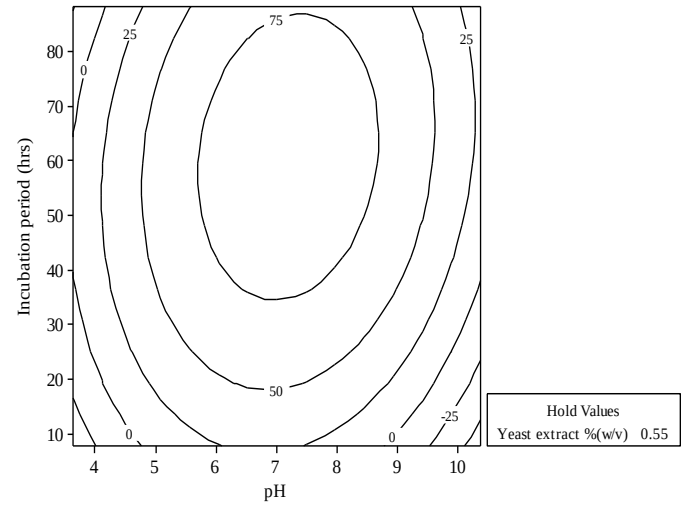
S. No	Variables	Coef	SE Coef	T	P
1	Constant	86.632	1.785	48.534	0.000*
2	Yeast extract	3.796	1.184	3.206	0.009*
3	pH	2.478	1.184	2.093	0.063*
4	Incubation period	13.253	1.184	11.191	0.000*
5	Yeast extract*Yeast extract	-25.933	1.153	-22.494	0.000*
6	pH*pH	-28.045	1.153	-24.326	0.000*
7	Incubation period*Incubation period	-12.931	1.153	-11.216	0.000*
8	Yeast extract*pH	2.592	1.547	1.675	0.125
9	Yeast extract*Incubation period	4.425	1.547	2.860	0.017*
10	pH*Incubation period	5.313	1.547	3.433	0.006*
R-Sq = 99.18% R-Sq (adj) = 98.44%					

*Significant

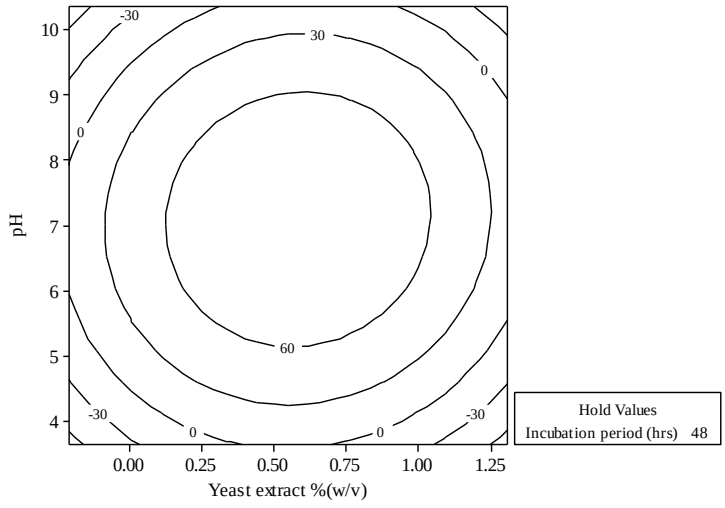
Table 10. Analysis of variance for central composite design

S. No	Source	DF	Seq SS	Adj SS	Adj MS	F	P
1	Regression	9	23201.6	23201.6	2577.95	134.59	0.000*
2	Linear	3	2679.5	2679.5	893.18	46.63	0.000*
3	Square	3	20085.9	20085.9	6695.29	349.55	0.000*
4	Interaction	3	436.2	436.2	145.40	7.59	0.006*
5	Residual error	10	191.5	191.5	19.15	-	-
6	Lack-of-fit	5	189.7	189.7	37.93	100.88	0.000*
7	Pure error	5	1.9	1.9	0.38	-	-
	Total	19	23393.1	-	-	-	-

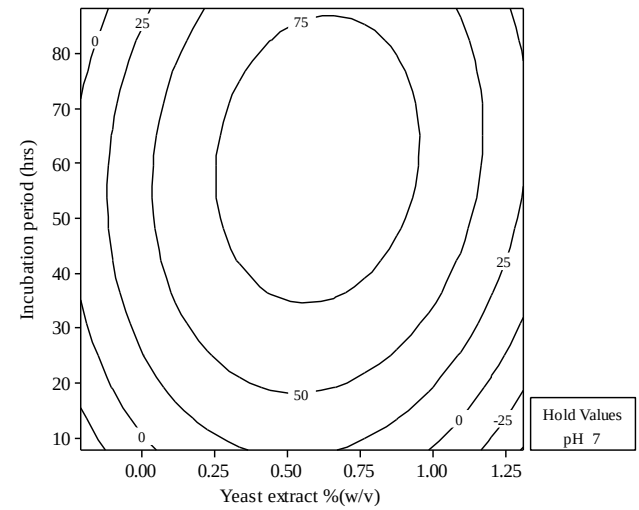
*Significant



(a)



(c)



(b)

Figure 3. Response contour plots on decolorization efficiency (%); (a) Effect of incubation period and pH on dye decolorization (%), (b) Effect of incubation period and yeast extract on dye decolorization (%), (c) Effect of pH and yeast extract on dye decolorization (%).

The response surface methodology predicated optimal values of significant factors were 0.60% (w/v) yeast extract, 7.23 pH and 61.45 hrs incubation period, which capable to yield 90.66% removal of remazol golden yellow dye by *Brevibacillus laterosporus* (TS5). In the optimal values validation, the *Brevibacillus laterosporus* (TS5) using predicted concentrations of significant factors attained the color removal by 90.08%, and it's agreed with expected result (90.66%). Whereas, *Brevibacillus laterosporus* MTCC 2288, was reached 74% color removal for Golden Yellow HER dye by pH 7 and 30 °C temperature **(Gomare *et al.* 2009c)**. Under optimized condition, Acid Red GR color removal by *Dyella ginsengisoli* LA-4 achieved at 98.36%, its resemblance to predicted value 98.47% from RSM **(Zhao *et al.* 2010)**. These studies support that the RSM was effective and reliable for optimization of dye decolorization. It was found that a range of pH mainly affects the bacterial decolorization rate. Similar to present results, the 93.06% of Direct Orange 39 (Orange TGLL) color removal occurred at pH 7 by *Pseudomonas aeruginosa* **(Jadhav *et al.* 2010)**. Microorganisms show differences in the uptake of nutritional sources, as it depends upon their dye degradation mechanisms. The optimization study confirmed the yeast extract contribution, and their significant role in the dye decolorization by *Brevibacillus laterosporus* (TS5), and that carbon source was found as insignificant one. It indicates the carbon sources have an inhibitory effect on the dye decolorizing enzymes. **Senthilkumar *et al.* (2012)** reported that *Pseudomonas* sp. effectively decolorizes the reactive dyes with RSM accounted concentrations of dye, carbon source, and nitrogen source. Likewise, decolorization was found between 3 to 4% in the presence of glucose and starch, as cells preferred the utilization of additional carbon substrates, and ensured the dye derivatives was not functioning as carbon source **(Kurade *et al.* 2011)**. While in extend incubation hours, cultured cells could augment the various biotransformation enzymes responsible for dye decolorization. Moreover, the maximum decolorization of Navitan Fast Blue S5R was found in the late exponential growth phase of *Pseudomonas aeruginosa* **(Valli Nachiyar *et al.* 2003)**. Inspite of, **Abd el-rahim *et al.* (2003)** reported that low decolorization efficiency of bacteria at the end of 21 days incubation, which was 20% for direct yellow and 25% for red dyes (10 mg/l). Since, the toxic nature of dye intermediates, and their concentration varied among dyes inhibits the growth of bacteria, the subsequently reduced decolorization occurred. These are the findings denotes the operational factors influence, and significant factor optimized levels enhances the decolorization rate of *Brevibacillus laterosporus* (TS5).

Conclusion

The present study demonstrated that the decolorization potentiality, and statistical optimization of bacterium *Brevibacillus laterosporus* (TS5) for an effective dye decolorization process. The precise ranges of various operational factors affect the dye decolorization rate of bacteria. Plackett-Burman design approach eliminated the insignificant factors from multiple factors of the optimization process. In these, the contribution of starch seems to be less effective in promoting decolorization. However, decolorization augmented with the addition of yeast extract as a nitrogen source. It indicates the bacterial cells, either utilized dye as carbon source instead of added starch. The bacteria showed a maximum dye removal capacity with neutral pH highlights their positive interaction of dye molecules. The robust dye decolorization obtained with assistance of significant factors, optimal level, and confirms the important factors interactions on decolorization. Hence, this result suggested that the isolated bacteria and their optimized condition could be an effective role in the removal of dyes from textile waste water treatment system.

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Declaration of interest

The authors reports no conflicts of interest.

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