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# EFFECT OF MULTIPLE FACTORS ON AZO DYE DECOLORIZATION USING A MODERATE HALOPHILIC BACTERIUM *EXIGUOBACTERIUM AURANTIACUM* (ESL52)

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***Key words :*** *Carbon sources, Nitrogen sources, Central composite design, Plackett-Burman design, Textile dye decolorization.*

**Abstract –** Halo-tolerant dye decolorizing bacteria ESL52 was isolated from a textile sludge contaminated with dye and the strain was identified as *Exiguobacterium aurantiacum* using 16S rRNA sequencing method. This strain showed 96.78 % decolorization efficiency within 48 hours in LB broth. The significant factors that influence dye decolorization process were obtained using Plackett-Burman design: starch, yeast extract and pH. These factors were optimized using central composite design, which was followed by response surface methodology (RSM). The dye removal rate was found to vary from 5.06 to 88.30%. The established optimal concentration of the variables was found to be as follows: starch - 0.49% (w/v); yeast extract - 0.60% (w/v); and pH - 7.10. This optimal condition yielded 88.26% of decolorization under experimental setup, which was in agreement with the predicted value of 89.73%. This study confirmed that the specific interactions of co-substrates with pH would be a key factor for effective decolorization of remazol golden yellow dye under halophilic culture condition.

## INTRODUCTION

The availability of freshwater has become a serious alarm worldwide, due to the decline in water quality. Imbalance in the accumulation of nutrients in water bodies due to the serious input of wastewater from domestic, agricultural and industrial sectors has evoked an alarming condition (Ma *et al.,* 2013; Stavenhagen *et al.,* 2018). Azo-dyes constitute the largest and the most versatile class of dyes used in textile, paper, food, cosmetic and pharmaceutical industries due to its high stability, cost effectiveness and color range. About 60 – 70% of the produced azo dyes are consumed by textile industries (Chacko and Subramaniam, 2011). More than 100,000 marketable dyes are accessible globally and around $7 \times 10^5$ tones of the dye ingredients are manufactured annually for the dyeing processes (Das and Mishra, 2017). Among the various dyes in market sectors, reactive dyes such as anthroquinone and triphenylmethane are generally used in the dyeing processes (Sudha *et al.,* 2014).

Mixed reactive dyes in water are resistant to evaporation when exposed to light and other chemicals. According to an assessment done by the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry, about 90% of the dyes used in textile industries have an LD50 value greater than 2000 mg $kg^{-1}$. The discharge of such dyes into the surrounding can cause significant environmental pollution and serious health risk (Ayed *et al.,* 2017). Azo dye chromophores are resistant to photo-oxidation therefore they bio-accumulate quickly. They affect the photosynthesis of aquatic plant and consequently the ecosystem (Wang *et al.,* 2012). Azo dyes and their breakdown products pose a serious health hazard: leading to devastating health effects such as nausea, hemorrhage, ulceration of the skin and mucous membrane and adverse affects on the kidney, reproductive system, liver, brain and central nervous system (Tyagi *et al.* 2001; Jin *et al.* 2007). Recently various physical, chemical and advanced chemical oxidation treatment methods have been

urbanized to eliminate the incommodious dyes from wastewater efficiently (You *et al.*, 2010). On the other hand, these methods have various pitfalls during practice, such as lack of complete removal of dye and generation of hazardous secondary waste (Olukanni *et al.*, 2010). Instead, biological treatment techniques have been successful and effective in the decolorization and biotransformation of toxic dyes into non-toxic products with cost-effective operation and maintenance (Ji *et al.*, 2012; Maqbool *et al.*, 2016; Hussain *et al.*, 2017).

However, effective microbial degradation of dyes is dependent on many factors such as survival, movement, adaptability, and the chemical composition (Ahmed *et al.*, 2016). Hence, novel microbial strains with better adaptability, survival, and activity are necessary for the degradation of azo dyes. The efficiency of biological treatment system is influenced by operational parameters such as pH, temperature, dye concentration, inoculum size and nutrients (Peternel *et al.*, 2007). Therefore, prior to field application, the effect of aforementioned parameters on color removal process must be investigated and duly optimized. Microbial decolorization of dyes and the effect of its dependent factors are usually studied by varying one-factor-at-time. Design of experiments (DOEs) is an efficient chemo-metric empirical approach; encompassing the Plackett-Burman (PBD) design and response surface methodology (RSM) to determine the optimal conditions for a multivariable process (Rasha *et al.*, 2018). Moreover it is a valuable tool to understand the presence of interactions between the investigated factors (Korbathi and Rauf, 2008).

This paper attempts to study the isolation of salt tolerant bacteria *Exiguobacterium aurantiacum* (ESL52) from textile sludge, for the decolorization of textile dye remazol golden yellow in an aqueous medium. The screening of significant parameters and their effect on dye decolorization was studied using Plackett-Burman design, which was followed by response surface methodology to obtain the optimal condition by implementing central composite design.

## MATERIALS AND METHODS

### Isolation and screening of halophilic bacteria for azo dye decolorization

Salt tolerant bacterium was isolated from the dye contaminated sludge of a textile industry located in Erode, Tamil Nadu, India. The sample was subjected to serial dilution and pour plate technique was performed on salt tolerant medium (pH 7.0±0.2) containing peptone 4 g $L^{-1}$, NaCl 50 g $L^{-1}$, $NH_4Cl$ 1 g $L^{-1}$, $NaHCO_3$ 1 g $L^{-1}$, $K_2HPO_4$ 0.2 g $L^{-1}$, $MgSO_4.7H_2O$ 0.2 g $L^{-1}$, yeast extract 3 g $L^{-1}$, and agar 20 g $L^{-1}$. The plates were incubated at 35 ± 2°C and 45 ± 2 °C for the isolation of mesophilic and thermo-tolerant bacteria (Somasegaran and Hoben, 1994). Distinct bacterial colonies, showing productive growth in salt tolerant medium were further purified on the same media and to screen for dye removal ability. The selective isolates were screened for their ability to decolorize remazol golden yellow (RNL) dye at various concentrations (50, 100, 150, 200 and 250 mg $L^{-1}$) in Luria Bertani agar plates containing casein enzymic hydrolysate: 10 g $L^{-1}$, yeast extract: 5 g $L^{-1}$, NaCl: 10 g $L^{-1}$ and agar: 15 g $L^{-1}$. Test isolates were inoculated as spots and incubated at 37 °C for 4 days. The isolates showing clear zones around the colonies were preferred for quantitative studies.

### Identification of dye decolorizing bacteria

The strain was identified by isolating genomic DNA from the potential dye decolorizing bacteria followed by 16S rRNA sequencing at Xcelris Labs Ltd, Ahmedabad, India.

### Phylogenic tree analysis and Genbank accession

Aligner software was used to generate the consensus sequences of 16S rRNA from forward and reverse sequence data. Closely related sequences were obtained from NCBI sever (www.ncbi.nlm.nih.gov/BLAST) using BLAST tool and aligned using multiple alignment software program: Clustal W (Shahid *et al.*, 2017). A phylogenetic tree was constructed by using the neighbor - joining (NJ) method in MEGA version 5.0 based on bootstrap analysis (1,000 replicates). The nucleotide sequence was then deposited in NCBI GenBank with an accession number: JX014269.

### Effect of diverse broth composition on azo dye decolorization

About 100 mL of Luria Bertani broth (containing 10 g $L^{-1}$ casein enzymic hydrolysate, 5 g $L^{-1}$ yeast extract, 10 g $L^{-1}$ NaCl), yeast extract broth (containing 5 g $L^{-1}$ yeast extract, 5 g $L^{-1}$ NaCl) and Bushnell and Hass broth (containing 0.2 g $L^{-1}$ $MgSO_4$, 1 g $L^{-1}$ $K_2HPO_4$, 0.02 g $L^{-1}$ $CaCl_2$, 0.05 g $L^{-1}$ $FeCl_3$, 1 g $L^{-1}$ $NH_4NO_3$) were prepared. One mL of overnight grown bacterial culture ESL 52 was inoculated with

remazol golden yellow dye at 100 mg $L^{-1}$ concentration. The flasks were incubated at 37 °C for 3 days under static conditions. The control flask was maintained without bacterial inoculation. After 24 hours of incubation, the optical density (OD) values of the decolorizing broth samples was analyzed by using UV spectrophotometer (Cyberlab UV-100 USA) at 412 nm. Experiments were carried out in triplicate and the obtained mean values were subjected to decolorization analysis. The dye decolorization percentage was determined by using the following formula (1):

% decolorization= (Initial OD-Final OD)/Initial OD×100 .. (1)

**Optimization of azo dye decoloriztion through Design of Experiments (DOEs) tools**

Design of experiment (DOEs) is a statistical application which involves the screening of up to 'n-1' variables in just 'n' number of trails to obtain the individual and interactive effects of multiple factors considered in a process. "Goodness of fit" of the constructed model was determined using Minitab Version 15 (Tripathi *et al.* 2012).

**Plackett-Burman Design**

A 2k-factorial Plackett-Burman design was used to identify the significant parameters for dye decolorization using the isolated salt tolerant bacteria. Sample points in the experimental design were carried out in triplicate using basal broth medium by maintaining the abiotic factors at constant under static condition. Positive or negative coefficient value indicated corresponding impact on dye decolorization and zero implied small or no effect (Du *et al.* 2012). The p-values <0.10 was used to understand the significant variables on dye decolorization process and to justify the significance of coefficients (Khelifi *et al.* 2012). The regression coefficient ($R^2$) of values greater than ±0.8 was taken into consideration for the level of significance (Joglekar and May, 1987). The statistical significance of the model was also determined by F-test for performing analysis of variance (ANOVA) in a net result of dye decolorization.

**Response surface methodology (RSM)**

Response surface methodology (RSM) was performed to find the optimal region of significant parameters to obtain maximum azo dye decolorization. In this study, central composite design (CCD) was carried out to illustrate the mutual interaction effect of the significant parameters and their subsequent effect on the bacterial dye decolorization (Galai *et al.* 2012). Significant parameters such as starch, yeast extract and pH were identified by PBD. The parametric levels are shown in Table 3, where $\alpha = 2^{n/3}$; "n" corresponds to the number of parameters and "0" corresponds to the central point in central composite design (Table 4). The actual values of each parameter were calculated by using the following formula (2) (Paul *et al.* 1992).

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

Other significant parameters, namely, 0.5% (w/v) sodium chloride, 200 mg $L^{-1}$ dye concentration, 18 h culture (OD 610nm = 0.5) with an inoculum size of 7.5% (v/v), 37 °C temperature and 48 h incubation period were kept constant. Post-generation of model, the predicted optimum conditions were experimentally verified in triplicate.

## RESULTS AND DISCUSSION

**Isolation and screening of dye decolorizing bacteria**

The total heterotrophic bacterial population of textile industry sludge was enumerated by colony counter and the number of bacterial colonies was estimated to be $9 \times 10^4$ CFU $g^{-1}$. Mariappan *et al.*, (2003) have reported a higher number (13.2 to 32 × $10^7$ CFU $g^{-1}$) of THB population in dye contaminated soil. The dye contaminated sludge was alkaline in nature, possibly due to the presence of surfactants, salts and other chemicals that are employed in the dyeing process, which in turn influenced the flora of the sludge (Araujo and Monteiro, 2006). The isolation medium was designed to be hypertonic; which in conjunction with higher incubation temperatures, allowed the selective growth of salt – tolerant and thermo – tolerant flora (Tiwari *et al.* 2013). The dye decolorization efficiency of distinct bacterial strains was determined at different concentrations (50-250 mg $L^{-1}$) of textile dye in Luria bertani medium. Twenty colonies showed zones of decolourization at 100 mg $L^{-1}$ concentration of remazol golden yellow dye amended plates. Of the 20 colonies, isolate ESL52 was able to produce the clearest zone of decolorization, which prompted for further investigation of the strain.

**Identification of dye decolorizing bacteria**

Phylogenic identification of bacterial strain was performed by sequencing the 16S rRNA. The phylogenetic tree shown in (Fig. 1.) was constructed by applying the neighbor-joining method by using GenBank database. The strain portrayed 93% homology to *Exiguobacterium mexicanum* 8N (AM072764) and 92% homology to *Exiguobacterium aurantiacum* DSM 6208 (DQ019166). A previous study on *Exiguobacterium* sp. TL (EU159578) isolation from the soil surface near pharmaceutical industries was reported by Tan *et al.* 2009. Dhanve *et al.*, (2009) reported *Exiguobacterium* sp. RD3 (EF541141) from textile waste water contaminated soil for decolorization of reactive yellow 84A dye at high salt concentrations. However, there are no reports on the isolation of *Exiguobacterium* sp. from textile sludge are available.

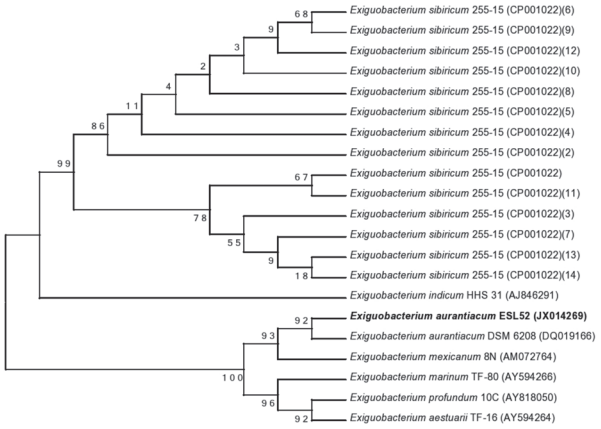



**Fig. 1.** Phylogenetic tree of *Exiguobacterium aurantiacum* ESL52 showing relationship between selected bacterial strains. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 replicates. Brackets represents sequence accession numbers.

**Effect of various compositions on dye decolorization**

The variation in decolorization ratio was observed by experimenting the effect of different composition on dye degradation at different time intervals. *Exiguobacterium aurantiacum* (ESL52) showed 47.28% decolorization ratio at the end of 24 h incubation period in Luria Bertani broth; while 48 hrs, the bacterial cells achieved a maximum decolorization of about 96.78%. Similar results of decolorization were obtained with moderate salt tolerant bacteria *Marinobacter* sp. (HBRA) which completely decolorized Direct Blue 1 in the Luria Bertani broth (Arun Prasad *et al.* 2013).

The occurrence of maximum dye removal ratio in LB broth may be attributed to the amendment of casein enzymic hydrolyzate as a vitamin source, which contributes to increase in the cell growth and metabolism. The percentage of decolorization was observed to be better in co-substrate amended media (Khehra *et al.* 2005). Decolorization of 8.72 and 29.12% were observed using yeast extract broth at 24 and 48 h incubation respectively, while further incubation lead to 57.55% decolorization. Similar results have been reported by using *Halomonas* strain (GTW) under the same experimental conditions (Guo *et al.*, 2008). According to Hu (1994), the addition of an organic nitrogen source to yeast extract may trigger NADH regeneration which in turn produces more effective dye removal.

**Screening of central factors on dye decolorization through Plackett-Burman design**

Screening of significant parameters for dye removal using *Exiguobacterium aurantiacum* (ESL52) was accomplished by using Plackett-Burman design (Table 5). These results suggest that the degree of decolorization is highly influenced and varies significantly based on the incubation conditions. The regression coefficients of tested factors on decolorization are shown in Table 6. These experimental results suggest that the decolorization increases with an increase in the values of yeast extract, pH, inoculums size and incubation period. *Exiguobacteriuam* sp. strain attained highest decolorization (90%) of X-3B azo dye using 6% (v/v) inoculum size in the late exponential growth phase (Tan *et al.* 2009).

Inoculum size and pH are the most influential parameters in dye removal mechanism. It has been

**Table 1.** Actual values of variables for Plackett-Burman design

| Test variables | Starch% (w/v) | Yeast Extract % (w/v) | pH | Temperature (°C) | Inoculums size % (v/v) | Dye concentration (mg $L^{-1}$) | 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.** Plackett-Burman design for screening of significant parameters in dye decolorization

| Run order | Starch % (w/v) | Yeast extract % (w/v) | pH | Temperature (°C) | Inoculums size % (v/v) | Dye concentration (mg $L^{-1}$) | 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 |

DV 1 & 2 - Dummy variable;
+1 denoted for high concentration; -1 denoted for low concentration

**Table 3.** Actual values of the significant parameters in central composite design

| Variables | Unit | Five Levels of variables | | | | |
|---|---|---|---|---|---|---|
| | | -α (-1.68179) | -1 | 0 | 1 | + α (+1.68179) |
| Starch | % (w/v) | -0.20681 | 0.1 | 0.55 | 1 | 1.306807 |
| Yeast Extract | % (w/v) | -0.20681 | 0.1 | 0.55 | 1 | 1.306807 |
| pH | - | 3.636414 | 5 | 7 | 9 | 10.36359 |

**Table 4.** Central composite design for optimization of azo dye decolorization

| Run Order | Pt Type | Blocks | Starch % (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 | 3.63 |
| 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 |

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

| Run order | Starch % (w/v) | Yeast extract % (w/v) | pH | Temperature (°C) | Inoculum size % (v/v) | Dye concentration (mg $L^{-1}$) | Incubation period (h) | Percentage decolorization | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Experimental | Predicted |
| 1 | 1.0 | 0.1 | 9 | 30 | 5 | 100 | 72 | 10.27 | 12.93 |
| 2 | 1.0 | 1.0 | 5 | 45 | 5 | 100 | 24 | 5.69 | 4.86 |
| 3 | 0.1 | 1.0 | 9 | 30 | 10 | 100 | 24 | 60.14 | 47.28 |
| 4 | 1.0 | 0.1 | 9 | 45 | 5 | 300 | 24 | 1.77 | 4.54 |
| 5 | 1.0 | 1.0 | 5 | 45 | 10 | 100 | 72 | 15.34 | 16.16 |
| 6 | 1.0 | 1.0 | 9 | 30 | 10 | 300 | 24 | 4.90 | 17.75 |
| 7 | 0.1 | 1.0 | 9 | 45 | 5 | 300 | 72 | 49.29 | 47.22 |
| 8 | 0.1 | 0.1 | 9 | 45 | 10 | 100 | 72 | 30.58 | 36.29 |
| 9 | 0.1 | 0.1 | 5 | 45 | 10 | 300 | 24 | 1.24 | 3.90 |
| 10 | 1.0 | 0.1 | 5 | 30 | 10 | 300 | 72 | 1.05 | 8.14 |
| 11 | 0.1 | 1.0 | 5 | 30 | 5 | 300 | 72 | 30.25 | 32.31 |
| 12 | 0.1 | 0.1 | 5 | 30 | 5 | 100 | 24 | 5.60 | 10.07 |

**Table 6.** Statistical analysis of Plackett-Burman design

| S. No | Variables | Effect | Coef | SE Coef | T | P |
|---|---|---|---|---|---|---|
| 1 | Constant | - | 18.01 | 3.329 | 5.41 | 0.006* |
| 2 | Starch | -23.01 | -11.51 | 3.329 | -3.46 | 0.026* |
| 3 | Yeast extract | 19.18 | 9.59 | 3.329 | 2.88 | 0.045* |
| 4 | Ph | 16.30 | 8.15 | 3.329 | 2.45 | 0.071* |
| 5 | Temperature | -1.38 | -0.69 | 3.329 | -0.21 | 0.845 |
| 6 | Inoculum size | 1.73 | 0.87 | 3.329 | 0.26 | 0.808 |
| 7 | Dye concentration | -6.52 | -3.2 | 3.329 | -0.98 | 0.383 |
| 8 | Incubation period | 9.58 | 4.79 | 3.329 | 1.44 | 0.224 |
| | R-Sq = 88.02% | R-Sq (adj) = 67.04% | | | | |

*Significant

previously reported by Kamle *et al.,* (2007) and is also reconfirmed in our study that the bacterial strains require an optimal pH for effective dye decolorization. However, higher concentration of starch, temperature and dye concentration had a negative influence on dye decolorization especially by affecting the growth and metabolism of the bacterial cells (Jadhav *et al.,* 2008). At temperature above 40 °C, salt tolerant bacteria showed decreased decolorization ability, probably due to the inactivation of corresponding enzymes (Khalid *et al.* 2008).

When 0.1% of starch was employed as a carbon source, 62.5% of navy blue HE2R dye removal was attained using *Exiguobacterium* sp. RD3 (Dhanve *et al.,* 2008). The Pareto chart indicates that standardized effect, where a vertical line implies the statistical significance (p = 0.10) of factors corresponding to a major effect on the horizontal axis (Fig. 2). The influence of the variable parameters can be mathematically expressed by equation (3):

Y= 18.01 - 11.51 × starch + 9.59 × yeast extract + 8.15 × pH - 0.69 × temperature + 0.87 × inoculum size - 3.26 × dye concentration + 4. 79 × incubation period —-(3)

Analysis of variance (ANOVA) to study the influence of a variable on the degree of decolorization is shown in Table 7.

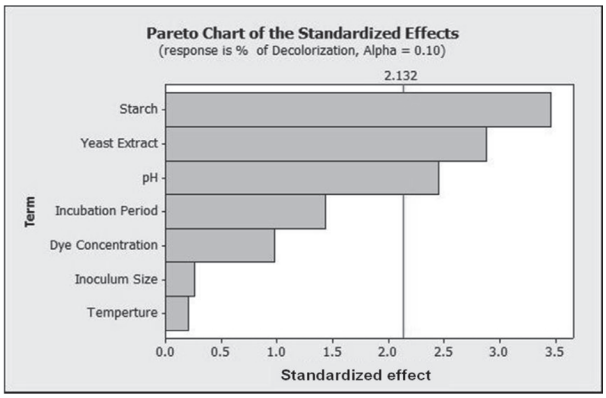



**Fig. 2.** Effects of variables on percentage of dye decolorization in Pareto chart

**Optimization of significant factors through central composite design**

The screened significant factors (starch, yeast extract and pH) and their combined effect on decolorization were studied by response surface methodology (RSM) through central composite design, as shown in Table 8. Regression analysis and regression coefficient of the model is given in Table 9. The correlation coefficient ($R^2$) was found to be 81.31%, which represents a good correlation between the factors and response. The parametric behavior of the variables can be mathematically expressed as a second – order polynomial, as shown in the equation given below:

$$Y = 89.1628 - 3.8707 \times X_1 + 3.9750 \times X_2 + 3.0656 \times X_3 - 15.4406 \times X_1^2 - 14.7459 \times X_2^2 - 34.7835 \times X_3^2 + 0.4112 \times X_1 \times X_2 - 1.4588 \times X_1 \times X_3 - 0.3587 \times X_2 \times X_3 \ldots\ldots\ldots (4)$$

where, Y is the predicted response of dye decolorization (%), $X_1$, $X_2$ and $X_3$ are the coded values of starch, yeast extract and pH respectively. The ANOVA test indicates a good reliability of the constructed model, as shown in Table 10.

The graphical representation of the response is shown in Fig. 3. The response surface and contour plots examine the interaction that exists between the variables in central composite design. The percentage of dye decolorization at various conditions was represented by the number of lines

**Table 7.** ANOVA analysis for Plackett-Burman design

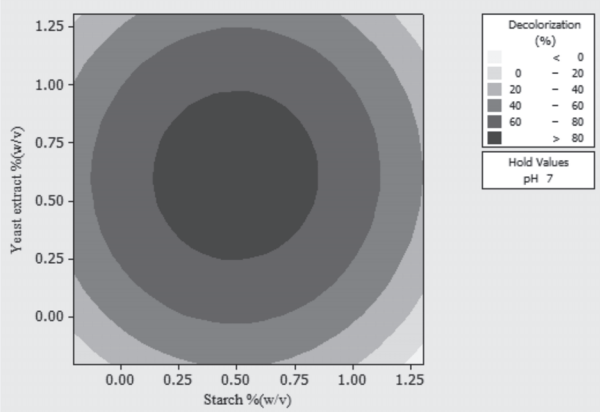
| S. No. | Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|---|
| 1 | Main effects | 7 | 3906.26 | 3906.26 | 558.04 | 4.20 | 0.092* |
| 2 | Starch | 1 | 1588.61 | 1588.61 | 1588.61 | 11.95 | 0.026* |
| 3 | Yeast extract | 1 | 1103.81 | 1103.81 | 1103.81 | 8.30 | 0.045* |
| 4 | pH | 1 | 796.58 | 796.58 | 796.58 | 5.99 | 0.071* |
| 5 | Temperature | 1 | 5.75 | 5.75 | 5.75 | 0.04 | 0.845 |
| 6 | Inoculum size | 1 | 9.00 | 9.00 | 9.00 | 0.07 | 0.808 |
| 7 | Dye concentration | 1 | 127.47 | 127.47 | 127.47 | 0.96 | 0.383 |
| 8 | Incubation period | 1 | 275.04 | 275.04 | 275.04 | 2.07 | 0.224 |
| 9 | Residual error | 4 | 531.91 | 531.91 | 132.98 | - | - |
| | Total | 11 | 4438.17 | | | | |

*Significant

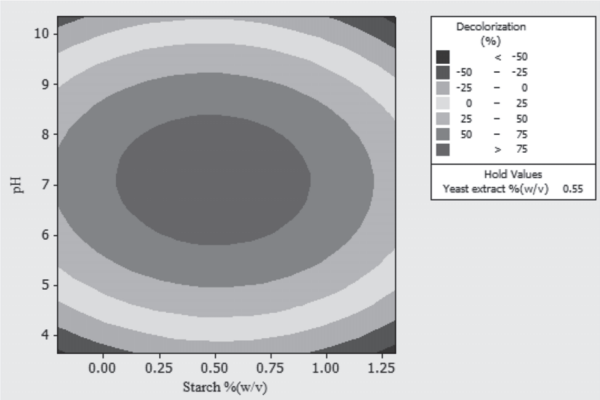
**Table 8.** Central composite design results with predicted values of decolorization

| Trails | Starch ($X_1$) | Yeast extract ($X_2$) | pH ($X_3$) | Percentage decolorization | | Residual |
|---|---|---|---|---|---|---|
| | | | | Experimental | Predicted | |
| 1 | 0.1 | 0.1 | 5 | 7.22 | 19.61 | -12.39 |
| 2 | 1.0 | 0.1 | 5 | 5.98 | 13.97 | -7.99 |
| 3 | 0.1 | 1.0 | 5 | 6 | 27.46 | -21.46 |
| 4 | 1.0 | 1.0 | 5 | 5.06 | 23.46 | -18.40 |
| 5 | 0.1 | 0.1 | 9 | 18 | 29.38 | -11.38 |
| 6 | 1.0 | 0.1 | 9 | 9.58 | 17.90 | -8.32 |
| 7 | 0.1 | 1.0 | 9 | 14 | 35.79 | -21.79 |
| 8 | 1.0 | 1.0 | 9 | 8.57 | 25.95 | -17.38 |
| 9 | -0.20 | 0.55 | 7 | 77.5 | 51.99 | 25.50 |
| 10 | 1.31 | 0.55 | 7 | 55.6 | 38.98 | 16.61 |
| 11 | 0.55 | -0.20 | 7 | 50.25 | 40.77 | 9.47 |
| 12 | 0.55 | 1.31 | 7 | 86.78 | 54.14 | 32.63 |
| 13 | 0.55 | 0.55 | 3.64 | 7.09 | -14.37 | 21.46 |
| 14 | 0.55 | 0.55 | 10.36 | 16.59 | -4.06 | 20.65 |
| 15 | 0.55 | 0.55 | 7 | 88 | 89.162 | -1.162 |
| 16 | 0.55 | 0.55 | 7 | 87.5 | 89.162 | -1.66 |
| 17 | 0.55 | 0.55 | 7 | 88.01 | 89.162 | -1.152 |
| 18 | 0.55 | 0.55 | 7 | 88.05 | 89.162 | -1.11 |
| 19 | 0.55 | 0.55 | 7 | 87.89 | 89.162 | -1.27 |
| 20 | 0.55 | 0.55 | 7 | 88.3 | 89.162 | -0.86 |

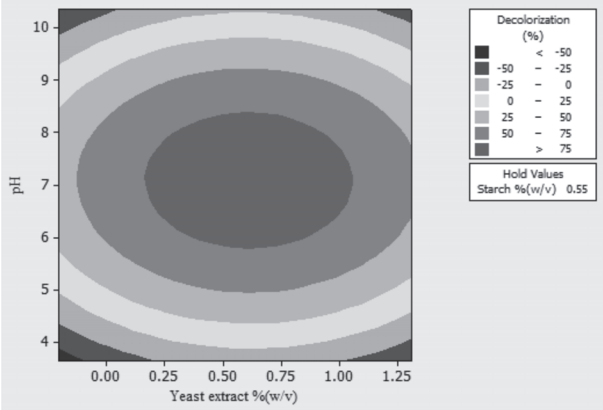
in the inside contour plot (Hasan *et al.* 2009). Response optimizer was applied to predict the optimal concentration of significant factors for maximum decolorization of remazol golden yellow using *Exiguobacterium aurantiacum* (ESL52), which




**(a)** Effect of yeast extract and starch concentration on dye decolorization (%)




**(b)** Effect of pH and starch concentration on dye decolorization (%)




**(c)** Effect of pH and yeast extract concentration on dye decolorization (%)

**Fig. 3.** Response contour plots between variables on decolorization (%)

were 0.49% (w/v) of starch, 0.60% (w/v) of yeast extract and pH 7.10 respectively. The optimization study explores favorable point of each individual factor for a satisfied operational condition of the process (Ravikumar *et al.*, 2006). The predicted optimal point was verified by lab experiments for maximum dye removal. *Exiguobacterium aurantiacum* (ESL52) attained 88.26% of decolorization experimentally, which was in concurrence with the predicted value 89.73%. The addition of carbon alongside nitrogen source sustains a rapid depletion of oxygen from the medium by inducing an earlier growth of cells. In *Exiguobacterium* sp., the latter can be assimilated by using yeast extract with growth promoting carbon sources, this also enhances the decolorization process in anaerobic conditions (Dhanve *et al.*, 2008). In relation to this report, diazo dye reactive yellow 84A (50 mg $L^{-1}$) was decolorized up to 90.05% within 48 h at pH 7 by the *Exiguobacterium* sp. RD3 (Dhanve *et al.*, 2009). Besides, the present study is supported by previous literature where *Exiguobacterium* sp. effectively decolorized several azo dyes at acidulous and neutral pH range (5.4-7.4), other optimized factors employed were 6% (v/v) of inoculation amount and 30 to 40 °C temperature range ( Tan *et al.*, 2009). Similarly, Qu *et al.*, (2010) demonstrated effective decolorization of reactive dark blue K-R by a synergism of fungus with bacterial strain *Exiguobacterium* sp. using response surface methodology. It was shown that about 97% of the 200 mg $L^{-1}$ dye was decolorized in accordance with this optimized condition. In accordance to the previous literature, it has been proved that the application of salt tolerant bacteria *Exiguobacterium aurantiacum* (ESL52) can efficiently decolorize azo dye waste water in alkaline condition with minimal prerequisite of co-substrates.

## CONCLUSION

The dye decolorizing moderate salt tolerant bacteria *Exiguobacterium aurantiacum* (ESL52) was isolated from the textile waste. Various factors such as pH, temperature, inoculum size, dye concentration and co-substrates, which may influence the azo – dye metabolism efficiency were investigated. Design of experiments was applied to understand the behavior of various parameters on the decolorization process. An effective decolorization was observed with subsistence of cells supplemented with carbon and nitrogen sources. It

**Table 9.** Estimated regression coefficients for Central composite design

| S. No | Variables | Coef | SE Coef | T | P |
|---|---|---|---|---|---|
| 1 | Constant | 89.1628 | 9.093 | 9.806 | 0.000* |
| 2 | Starch | -3.8707 | 6.033 | -0.642 | 0.536 |
| 3 | Yeast extract | 3.9750 | 6.033 | 0.659 | 0.525 |
| 4 | pH | 3.0656 | 6.033 | 0.508 | 0.622 |
| 5 | Starch* Starch | -15.4406 | 5.873 | -2.629 | 0.025* |
| 6 | Yeast extract*Yeast extract | -14.7459 | 5.873 | -2.511 | 0.031* |
| 7 | pH*pH | -34.7835 | 5.873 | -5.923 | 0.000* |
| 8 | Starch*Yeast extract | 0.4112 | 7.883 | 0.052 | 0.959 |
| 9 | Starch*pH | -1.4588 | 7.883 | -0.185 | 0.857 |
| 10 | Yeast extract*pH | -0.3587 | 7.883 | -0.046 | 0.965 |

R-Sq = 81.31% R-Sq (adj) = 64.49%

*Significant

**Table 10.** Analysis of variance for central composite design results

| S. No | Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|---|
| 1 | Regression | 9 | 21625.1 | 21625.1 | 2402.79 | 4.83 | 0.011* |
| 2 | Linear | 3 | 548.7 | 548.7 | 182.91 | 0.37 | 0.778 |
| 3 | Square | 3 | 21056.9 | 21056.9 | 7018.97 | 14.12 | 0.001* |
| 4 | Interaction | 3 | 19.4 | 19.4 | 6.47 | 0.01 | 0.998 |
| 5 | Residual error | 10 | 4970.8 | 4970.8 | 497.08 | - | - |
| 6 | Lack-of-fit | 5 | 4970.5 | 4970.5 | 994.10 | 14437.17 | 0.000* |
| 7 | Pure error | 5 | 0.3 | 0.3 | 0.7 | - | - |
| | Total | 19 | 26595.9 | - | - | - | - |

*Significant

was found that higher dye concentration and temperature hinders the decolorization process, probably due to the accumulation of dye intermediates in the decolorizing broth. The optimized factors effectively improved the percentage of remazol golden yellow dye removal in aqueous medium. These results suggest that *Exiguobacterium aurantiacum* (ESL52) can potentially be applied to large scale decolorization processes of textile waste water.

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