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Decolorization of Remazol Brilliant Blue Dye by *Escherichia coli* NG188 under Optimized Conditions

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THE OBJECTIVE of this study was to degrade Remazol Brilliant Blue (RB) textile dye by using *Escherichia coli* NG188 isolated from the industrial wastewater effluent. The optimum RB degradation (%) was obtained at; 40°C, inoculum size 5%, and pH 8 under static condition. The influence of nutritional factors on dye degradation via Plackett-Burman statistical design was investigated. On the basis of the calculated *t*-values, P values and confidence level: glucose and dye concentration had confidence levels above 90% and hence were considered the significant parameters that influence dye degradation by *Escherichia coli* NG188. A verification experiment was carried out to evaluate the accuracy of the applied Plackett-Burman statistical design. The decolorization percentage showed a 1.24 fold increase in the degradation rate of the dye compared to the average of the basal condition results. The complete decolorization of the RB dye was reported using the Response Surface Methodology (RSM). The biodegradation of RB was confirmed by UV–visible spectroscopic and FTIR analysis.

Keywords: *Escherichia coli* NG188, Plackett-Burman, Remazol Blue, Response Surface Methodology (RSM), Statistical design.

Introduction

Water is a vital requirement of life and used for various household as well as industrial activities. It is one of the most essential natural resources, unfortunately exploited the most. Because of the unrestricted and excessive exploitation of water, the whole world is facing water disasters. Rapid industrialization caused the release of pollutants to the water bodies, which including several xenobiotics (Meng et al., 2015; Li et al., 2019; El-Dougdoug et al., 2020). This deteriorates the quality as well as the quantity of water and makes it unsafe for further use. Dyes are an important class of synthetic organic compounds used in many industries, such as textile, printing, cosmetic, food, and drug processing industries. These dyes are mutagenic, carcinogenic and very toxic in nature and have negative impact on the living organisms (Dos Santos et al., 2007; Wang et al., 2018; Simões et al., 2019). Due to the globalization of the world market; textile industries are facing a challenge in the field of quality and productivity.

During dyeing process, majority of dyes remain unbound to fabrics and are released with wastewater into the environment (Mishra & Maiti, 2018). A dye house effluent contains 0.6-0.8g/L dye, but the pollution it causes is mainly due to durability of the dyes in the wastewater system (Jadhav et al., 2007). Therefore, it is necessary to develop technologies and an effective treatment for degradation and decolorization of dyes in such effluents.

The microbial degradation of textile dyes became a key research area in environmental sciences (Li et al., 2019). The dye degrading microorganisms are ubiquitous but are generally

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isolated from wastewater effluents (Nawahwi et al., 2013; Pelosi et al., 2014; Forss et al., 2017) and soils (Shah et al., 2013; Lovato et al., 2017). The microbes can adapt to the toxic wastes and develop new resistant mechanisms, they have the ability to biodegrade many toxic chemicals into less harmful compounds (Das & Mishra, 2017), in addition to that they are easy to grow and the use of their isolated enzymes for textile dyes degradation is not expensive in relative terms because there is no need for high purity levels in treating effluents. Many microorganisms have been isolated and explored for their ability and capacity to degrade dyes (Li et al., 2019). Others have been modified by the genetic engineering tools to obtain "super and faster degraders". Many bacterial, algal and fungal species have developed the ability to absorb and/or biodegrade dyes (Ghanem et al., 2012; Noman et al., 2019). So, most of the studies on dye biodegradation have focused recently on the bacteria and fungi, in which bacteria are found to be more effective and efficient (Khehra et al., 2005; Lade et al., 2012; Velayutham et al., 2018).

On the other side, the statistical designs, such as the Plackett-Burman design and response surface methodology (RSM) can enhance biodegradation efficiency by optimizing all the relevant parameters at once (Das & Mishra, 2017), which is better than optimizing one parameter at a time. Plackett-Burman design provides an effective and fast way to identify the most important factors among a large number of variables, thereby giving significant information on each parameter. Response surface methodology (RSM) helps evaluate the important factors and building models to study the interactions between the variables or desirable responses (Ghanem et al., 2015, 2016).

Therefore, the objective of this research was to isolate dye degrading bacteria from local environment and screen their potentiality to degrade Remazol Brilliant Blue dye under optimized conditions using Plackett-Burman and Box-Behnken statistical experimental designs.

Materials and Methods

Tested organisms and culture conditions

The bacterium was isolated from the wastewater effluent at the bleaching stage from Tetco Company for textile dyeing in Kafr El Dawar in Egypt. It has been identified as *Escherichia* coli NG188, on the basis of partial 16S rRNA sequence submitted to the Genbank under accession number KU043041. The bacterium was maintained on LB-agar slant (g/L), yeast extract 5, tryptone 10, NaCl 10, agar, 15. If not otherwise mentioned, the decolorization experiments were performed in 250ml Erlenmeyer flasks containing (50ml) minimal salt medium (MSM) **(**Patel et al., 2012) supplemented with Remazol Blue (RB) (50mg/L) dye, 5% inoculum (v/v) and incubated at 40°C temperature under static condition for 3 days. The pH was adjusted by 1N NaOH or 1N HCl. All decolorization experiments were performed in triplicates.

Decolorization potential

Growth, as well as, RB degradative capabilities of *E. coli* NG188 in liquid medium was carried out using MSM **(**Patel et al., 2012) supplemented with 50mg/L Remazol Blue. The mediumwas inoculated with 5% of respective bacterial inoculum (OD_{600}= 1). All the flasks were incubated under static condition at 37°C. Aliquots were withdrawn periodically and analyzed for growth and dye degradation.

For the degradation analysis, 2.0ml aliquot of decolorized medium was centrifuged at 10,000g for 15min and the supernatant from each sample was read at A_{600} using UV-Visible spectrophotometer (OPTIMA). Uninoculated flasks were kept as a control. The percentage of dye removal was calculated as mentioned by Zabłocka et al. (2015):

$$\text{Decolorization \%} = (A0-A)/A0\times100$$

where, A0 represents the initial concentration, while A represents final concentration.

For growth determination in relation to decolorization percentage the cell pellet collected after centrifugation of culture filtrate at 10,000g for 15min, were re-suspended in distilled water has the same volume of the supernatant and was mixed in vortex and the OD_{600} was measure by OPTIMA spectrophotometer.

Optimization of physicochemical parameters

The effect of physicochemical parameters, such as aeration (static and shacked (200rpm) conditions), initial dye concentration (50-1000mg/L), temperature (30-45°C), pH (6-12), and inoculum size (1-9%), on RB degradation were studied.

Optimization of nutritional factors using statistical experimental design

Plackett-Burman statistical experimental design

Application of the statistical design was carried out in a "two phases "of optimization approach. The first step was to evaluate the relative importance of the various constituents in the culture media and selecting levels of variables that have the significant influences on the degradation process, the second was the verification of the experiments to validate results under specific optimized experimental conditions.

Seven independent variables were screened in twelve combinations, organized according to the Plackett–Burman design matrix in the results section (Table 1). Evaluate each variable at two levels, low (-) and high (+). The main effect of each variable can be calculated by using the following standard equation :

$$\text{Main effect} = [\sum R(H) - \sum R(L)]/ N$$

where R(L) and R(H) are the observations of trials where independent variables were present in low (L) and high (H) concentrations, respectively and N is number of trials divided by 2.

Data analysis of the results of Plackett-Burman experimental

Excel (Microsoft Office, 2010) was used for the experimental design and all statistical analysis. The variables with confidence levels about 90% were considered to influence RB degradation.

Box-Behnken design

In the second phase of medium formulation for optimum RB decolorization, the Box-Behnken experimental design (Box & Behnken, 1960) was applied. In this model, the most significant independent variables, namely; K_2HPO_4 (X_1), KH_2PO_4 (X_2) and glucose (X_3) are included and each factor can be examined at three different levels, low (-), high (+) and central or basal (0).

The independent variables for *E. coli* NG188 were K_2HPO_4, KH_2PO_4 and glucose. Fifteen combinations and their observations were fitted to the following second order polynomial model:-

$$Y= b_0 + b_1X_1+ b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23} X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where, Y is the dependent variable RB decolorization percentage (%) X_1, X_2 and X_3 are the independent variables; b_0 is the regression coefficient at centre point; b_1, b_2 and b_3 are linear coefficients; b_{12}, b_{13} and b_{23} are second-order interaction coefficients; and b_{11}, b_{22}, and b_{33} are quadratic coefficients.

The values of the coefficients were calculated using Microcal Origin 8.0724 software and the optimum concentrations were predicted using Microsoft Excel 2010. The coefficient of determination R^2, determine the quality of the fit of the polynomial model equation. Three-dimensional graphical representations were also constructed using Statistica 7 software to reflect the effects as well as the interactions of independent variables on the objective.

Analytical methods

The degradation of dye was monitored using a T70/Vis Spectrometer PG Instruments Ltd. from 100nm to 1000nm, and the λ_{max} for RB was 600nm. The changes in the functional groups of RB after treatment were analyzed using Fourier transformed infra-red spectrometer, in which the culture medium was centrifuged at 10,000g for 15min to remove the suspended particles. The supernatant was once again centrifuged to ensure that the supernatant was free of bacterial cells and was used for extraction of metabolites using an equal volume of ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness. The FT-IR analysis of extracted metabolites was done using spectrophotometer and compared with control dye in the IR range of 500-4000cm^{-1}. The samples were mixed with spectroscopically pure KBr pellets, fixed in sample holder and the analysis was carried out.

Results and Discussion

Quantitative estimation for RB degradation

The kinetics of *E. coli* NG188 cells to decolorize RB dye was investigated during growth on MSM medium. The results presented in Fig. 1 indicate that RB had a little inhibitory effect on *E.coli* NG188 growth.

Growth on MSM liquid medium for Escherichia coli MG188 in the presence and absence of RB and RB decolorization kinetics at 37C and pH 7

The data also show that the exponential increase in cell density was accompanied by a

gradual decrease in color intensity suggesting that the degradation of the cells to RB dye is paralleled to biomass formation. *Escherichiacoli* NG188 was able to decolorize 33.5% of RB dye after 72hrs of incubation.

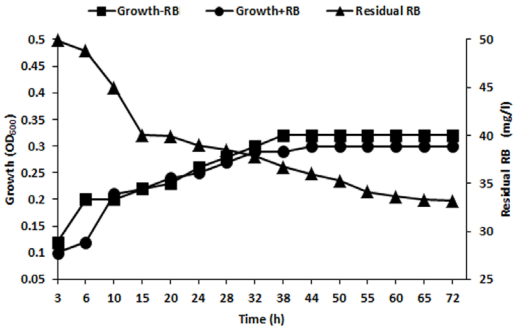


Fig. 1. Growth on MSM liquid medium for *Escherichia coli* NG188 in the presence and absence of RB and RB decolorization kinetics at 37°C and pH 7.

Factors affecting RB decolorization

Effect of static and shaking conditions

The aeration factor is a very important factor in the degradation of the dye, it is greatly affect the dye decolorization as it was reported by manystudies that the static conditionsmore efficient than the shaking conditions (Parshetti et al., 2007; Telke et al., 2008; Velayutham et al., 2018).

This investigation was undertaken to select the best culture conditions that allow the highest decolorization percentage. The decolorization percentage for *E. coli* NG188 under static conditions was 33% and showed almost 3.1 folds increase compared to that incubated under shaken conditions.

It was reported that Modi et al. (2010) had also proved that the degradation of Reactive Red 195 dye by *Bacillus cereus* M1 was under static conditions (97%) higher than in shaken conditions (8%). Similarly, Olukanni et al. (2013) showed that *Bacillus thuringiensis* RUN1 could degrade 72.84±3.25% of the Congo Red under static conditions. Also, Lim et al. (2013) proved that *Enterococcus faecalis* strain can degrade 98% of Acid Orange 7 under static conditions. On the contrary, Olukanni et al. (2009) found that the time for Methyl Red decolorization by *Micrococcus* strain was reduced from 24hrs under static conditions to 6hrs under shaking conditions.

Effect of temperature on dye decolorization

Temperature is an important factor affecting growth and metabolic activity of microorganisms. The decolorization of dye efficiency was found to increase up to the optimum temperature and then decreased. A decline performance after the optimum temperature is usually due to denaturation of enzyme responsible for decolorization or the loss of cell viability (Kolekar et al., 2008; Saratale et al., 2011; Solis et al., 2012).

It was also reported that the dye degradation depended largely on the microbial optimum growth temperature (Stolz, 2001; Khan et al., 2009).

The data obtained revealed that, the temperature effect on the decolorization of RB was significant for *E. coli* NG188. It was observed that there is an increase in decolorization of RB with increase in temperature and it was optimum at 40°C, in which *E. coli* NG188 decolorized 59% of RB after 3 days of incubation and above or below this temperature decolorization was decreased (data not shown). These results showed that there is no thermal deactivation of decolorization activity under operational temperatures. Therefore, *E. coli* NG188 could acclimatize to broad range of temperature.

Similarly, Oturkar et al. (2013) reported that the best degradation (98%) of Reactive Red 141 by *Bacillus lentus* BI377 was at 40ºC. Also, Kurade et al. (2013) found that the best degradation (100%) of Remazol Red was at 40ºC. The data obtained are also agreed with Cao et al. (2013) who found that the best degradation of Napthol Green B by *Shewanella oneidensis* MR-1 was at 40ºC and it decreased by increasing the temperature to 45ºC. Jadhav et al. (2011) also reported that the best degradation (97%) of Remazol Red by *Pseudomonas aeruginosa* was at 40ºC while it was (72 %) at 10°C and 82% at 30°C. Dawkar et al. (2009) also reported that the best degradation (94%) of Navy Blue 2GL by *Bacillus* sp. VUS was at 40ºC. On the contrary, Gopinath et al. (2009) found that the best degradation (100%) of Congo Red by *Bacillus* sp. was at 37ºC. Lu et al. (2010) reported that the best degradation (50%) of Amaranth by *E. coli* k12 was at 37ºC while Chang et al. (2004) found that the optimum temperature of Reactive Red 22 by *E. coli* NO_3 was at 28ºC under the static conditions.

Effect of the dye concentration

The effect of the initial concentration of RB on the percentage (%) of decolorization was studied at 40°C and the results obtained are shown in Fig. 2. In the present study, RB was degraded without any co-substrate and *Escherichia coli*. NG188 was found to withstand even at higher concentrations of RB (1000mg/L), this confirms that, *E. coli* NG188 could tolerate high toxicity. Jang et al. (2005) stated that, the rate of decolorization reaction is inversely proportional to the initial dye concentration, as the dye concentration increases, decolorization rate decreases (Ali, 2010; Saratale et al., 2011).

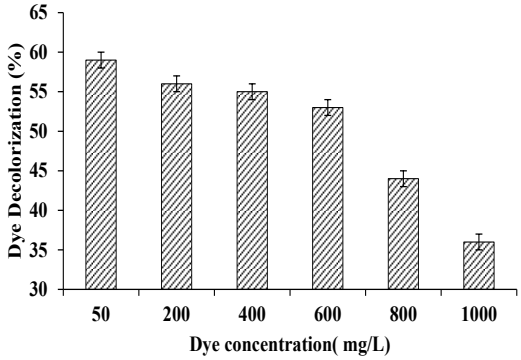


Fig. 2. Effect of different concentrations of RB on the decolorization % by *E. coli* NG188 at 40°C after 3 days of incubation under static conditions using MSM with pH 7.

The maximum decolorization was 59% and this was observed with an initial dye concentration of 50mg/L. The % of decolorization was efficient at lower dye concentrations. Similarly, Anjaneya et al. (2011) found that *Lysinibacillus* sp. effectively decolorizes 100% of Metanil Yellow at 200mg/L, but was only able to decolorizes 62% of Metanil Yellow at 1000mg/L after 24hrs of incubation. Also, Ayed et al. (2011) observed that *Sphingomonas paucimobilis* decolorized completely Methyl Red at 750mg/L and only 38% of a 1000mg/L dye solution was decolorized by this microbe.

The decreased decolorization efficiency at high dye concentrations may be due to the toxicity of the dye to bacteria and/or inadequate biomass concentration for the uptake of higher concentrations of dye or inhibition of the enzyme system (Chen et al., 2003). Dyes which have reactive groups as sulfonic acid (SO_3H) on their aromatic rings are highly inhibited the growth of microorganisms at higher concentrations and the increase in number of carbon atoms of the dye decreases the decolorization % (Solis et al., 2012).

Effect of inoculum size

The size of bacterial inoculum is one of the factors affecting dye decolorization (Rajeswari et al., 2014). The data obtained showed that RB decolorization by *E.coli* NG188 increased steadily as the inoculum size increased reaching the highest decolorization upon using 5% inoculum.It was noticed that as the inoculum size increases above 5%, the decolorization % decreases. This is due to the fact that increasing in the inoculum size increases the growth and growth related activities of bacterial culture, but there could be reduction in dye removal rate due to the nutrient limitation (Nikhil et al., 2012).This data is matching with that reported by Olaganathan & Patterson (2012) who observed that the optimum inoculum size for the decolorization of the Vat Blue 4 was 5%. Also, Tripathi & Srivastava (2011) found that 5% was the optimum inoculum size for the decolorization of Acid Orange 10 by *Pseudomonas putida* MTCC 102. On the contrary, Nikhil et al. (2012) reported that 3% inoculum size was the optimum for the Reactive Red M8B dye. Rajeswari et al. (2014) reported that 20% inoculum size was the optimum for the biodegradation of the reactive dyes by *Lysinbacillus sphaericus* RSV-1.

Effect of initial pH

The pH of the culturing media is a very important factor in the degradation of the dye due to the dependence of the enzymatic activity on the pH level. The major effect of pH may be attributed to the transport of dye molecules across the cell membrane, which may be considered as the rate limiting step for the decolorization (Lourenço et al., 2000).

Escherichia coli NG188 was able to decolorize the dye in broad range of pH (6-12) (data not shown). It showed maximum dye decolorization at pH 8. The decolorization % decreased at nearly neutral pH (6-7) and at alkaline pH (9-12). The results suggest that pH variation had a significant effect on the decolorization of RB dye by *E. coli* NG188. The highest decolorization, 72%, was observed at pH 8. With further increase in pH, the decolorization % was decreased gradually. Several authors reported that the optimum pH for dye decolorization at the alkaline range. Shah et al. (2013) found that the best biodegradation of Crystal Violet by *Bacillus subtilis* ETL-2211 was at pH 8.

Similar observations was also reported by Mane et al. (2008) who found that the maximun degradation of the Reactive Blue-59 by *Streptomyces krainskii* SUK-5 was at pH 8**,** however, Jadhav et al. (2008) reported that the pH has no significant effect on degradation of the Brilliant Blue G by *Bacillus* sp. Jadhav et al. (2012) also has found that the optimum pH for the decolorization of Remazol Orange was 8 by *Pseudomonas aeruginosa* BCH, while Morrison et al. (2012) found that the optimum degradation of Direct Blue 15 by *Clostridium perfringens* was at the alkaline range pH 9 and Kurade et al. (2013) also reported that the optimum pH for dye decolorization of Remazol Red by *Brevibacillus laterosporus* was at pH 9.

Optimization of nutritional factors using statistical experimental design

Plackett–Burman design

The Plackett–Burman design with two levels of concentrations for seven different variables were carried out according to the experimental matrix as shown in Table 1 and the percentage of decolorization was determined as a response. The maximum dye decolorization (81%) was achieved in trial number 8, while the minimum dye decolorization (27%) were observed in trial number 6. Main effects of the examined variables on dye decolorization were calculated and presented in Table 2. A large contrast mean, either positive or negative, indicates that a factor has a large impact on RB decolorization %; while a mean close to zero means that a factor has little or no effect. When the sign of the effect of the tested variable is positive, the influence of the variable on dye decolorization is greater at a high level. When the sign is negative, the effect of the variable is greater at a low level. It was found that all of the tested variables except $(NH_4)_2SO_4$ had a positive effect on dye decolorization. The *t*- test for any individual effect allows an evaluation of the probability of finding the observed effect purely by chance. Some investigators find that confidence levels greater than 85 % are acceptable (Lu et al., 2011). In this study, variables with confidence levels above 90% were considered significant. Based on the statistical analysis of confidence level of 7 variables (Table 2), K_2HPO_4 and glucose had confidence levels above 90% and hence were considered the significant parameters influence dye decolorization by *E. coli* NG188.

The coefficient (R^2) determine the goodness of fit of the model. In this case, the R^2 value was calculated to be 0.9856 for *E. coli* NG188, indicated that 98.56% of the total variability in the response could be explained by this model and only 1.43% of the total variation were not explained. A regression model with R^2 closed to 1.0 is considered as having a very high correlation (Yong et al., 2011). Therefore, the present R^2 value reflected a very good fit between the observed and predicted responses and implied that the model is reliable for predicting dye decolorization. After applying the ANOVA statistical test, it was found that the first order models for dye decolorization was satisfactory, the polynomial model equation was proposed to calculate the optimum levels of these variables for dye decolorization can be written as:

$Y= 49.8 - 0.50\ (NH_4)_2SO_4 + 5.25\ K_2HPO_4 + 3.25\ KH_2PO_4 + 1.75\ MgSO_4.7H_2O + 2.25$ NaCl + 18.0 Glucose + 2.00 dye concentration.

where; Y represents dye decolorization percent

TABLE 1. Plackett–Burman design matrix for seven variables with coded values along with observed results for screening of significant factors affecting dye decolorization by *E. coli* NG188.

Run order	Experimental values							Decolorization %
	$(NH4)_2SO_4$	K_2HPO_4	KH_2PO_4	$MgSO_47H_2O$	NaCl	Glucose	Dye conc.	
1	-1 (0.5)	-1 (1)	-1 (0.5)	1 (1)	1 (10)	1 (10)	-1 (25)	61
2	1 (3)	-1 (1)	-1 (0.5)	-1 (0.05)	-1 (1)	1 (10)	1 (100)	56
3	-1 (0.5)	1 (10)	-1 (0.5)	-1 (0.05)	1 (10)	-1 (1)	1 (100)	36
4	1 (3)	1 (10)	-1 (0.5)	1 (1)	-1 (1)	-1 (1)	-1 (25)	30
5	-1 (0.5)	-1 (1)	1 (3)	1 (1)	-1 (1)	-1 (1)	1 (100)	31
6	1 (3)	-1 (1)	1 (3)	-1 (0.05)	1 (10)	-1 (1)	-1 (25)	27
7	-1 (0.5)	1 (10)	1 (3)	-1 (0.05)	-1 (1)	1 (10)	-1 (25)	70
8	1 (3)	1 (10)	1 (3)	1 (1)	1 (10)	1 (10)	1 (100)	81
9	0 (1)	0 (6)	0 (1)	0 (0.1)	0 (5)	0 (5)	0 (50)	56

TABLE 2. Statistical analysis of Plackett–Burman design by *Escherichia coli* NG188.

Variables	Main effect	*t*-value	Regression coefficient	Confidence level (%)
Intercept	-	-	49.8	-
$(NH_4)_2 SO_4$	-1.0	-0.2	-0.5	43
K_2HPO_4	10.5	2.3	5.3	97
KH_2PO_4	6.5	1.4	3.3	90
$MgSO_4.7H_2O$	3.5	0.7	1.8	74
NaCl	4.5	1.0	2.3	80
Glucose	36.0	7.7	18.0	100
Dye concentration	4.0	0.9	2.0	79

Validation of the model

Based on the data obtained from Plackett-Burman experimental results, the following composition (g/L) is predicted to be near optimum for decolorization of RB by *Escherichia coli*. NG188, $(NH_4)_2SO_4$, 0.5; K_2HPO_4, 10; KH_2PO_4, 3; $MgSO_4.7H_2O$, 1; NaCl, 10; glucose, 10; and dye concentration, 100mg/L.

In order to determine the accuracy of the applied Plackett-Burman screening test, a verification experiment was carried out in triplicate. The predicted near optimum levels of dependent variables were examined and compared to the basal condition settings (72% decolorization). The applied near optimum condition resulted in dye degradation of approximately 92% by *Escherichia coli*. NG188, within an incubation period of 54hrs. This resulted in a 1.27 folds increase in the dye degradation compared to the average results obtained under the basal condition.

Optimization of dye decolorization by Box-Behnken design

The variables identified by Plackett-Burman design were further optimized by Response Surface Methodology (RSM) using Box-Behnken design experimental plan. The three key variables were examined at three different levels (-, 0, +). However, data shown in Table 3 exhibits various combinations used and corresponding RB decolorization % for *E.coli* NG188. The amounts of remaining components in all assemblies were the same as those in the pre-optimized medium of Plackett-Burman design.

TABLE 3. Box-Behnken design for the most significant three variable that affected dye decolorization by *E. coli* NG188.

Trial	Variable (g/L)			Decolorization%	
	K_2HPO_4	KH_2PO_4	Glucose	Observed	Predicted
1	1 (15)	0 (3)	1 (15)	94	95.25
2	0 (10)	-1 (4.5)	1 (15)	90	90.25
3	-1 (5)	0 (3)	1 (15)	78	76.75
4	0 (10)	1 (1.5)	-1 (5)	90	89.75
5	1(15)	0 (3)	-1 (5)	82	83.25
6	0 (10)	0 (3)	0 (10)	81	80.83
7	0 (10)	0 (3)	0 (10)	80.5	80.83
8	0 (10)	0 (3)	0 (10)	81	80.83
9	-1 (5)	1 (1.5)	0 (10)	81	82.5
10	0 (10)	-1 (4.5)	-1 (5)	77	77.25
11	-1 (5)	0 (3)	-1 (5)	81	79.75
12	-1 (5)	-1 (4.5)	0 (10)	83	84
13	1(15)	-1 (4.5)	0 (10)	91	89.5
14	1(15)	1 (1.5)	0 (10)	100	99
15	0 (10)	1 (1.5)	1 (15)	86	85.75

The maximum experimental value for dye decolorization achieved by *E. coli* NG188 was 100%, while the predicted response based on RSM was estimated to be 99% (Table 3). The close correlation between the experimental and predicted data indicates the appropriateness of the model. The analysis of variance (ANOVA) for the response quadratic model is presented in Table 4.

The model was significant at the 90% confidence level and the quality of the model can also be checked using various criteria. The calculated regression equation for the optimization of media constituents assessed dye decolorization (Y) as a function of these variables. By applying quadratic regression analysis on the experimental data, the following equations were found to explain dye decolorization:

$$Y= 102 - 3.87\ X_1 - 9.89\ X_2 + 0.683\ X_3 + 0.367\ X_1X_2 + 0.150\ X_1X_3 - 0.567\ X_2X_3 + 0.118\ X_1^2 + 2.20\ X_2^2 - 0.0017\ X_3^2$$

where, Y is the dependent variable RB decolorization % obtained after 3 days of incubation; X_1, X_2 and X_3 are the concentrations of the independed variables as shown in Table 3.

The coefficient of determination, i.e., R^2, was 0.978. Normally, a regression model having an R^2 value higher than 0.9 is considered to have a very high correlation and a model with an R^2 value between 0.7 and 0.9 is considered to have a high correlation (Bonett & Price, 2005). That, the R^2 value of 0.978 reflected a good fit between the observed and predicted responses (Table 3) and it was reasonable to use the regression model to analyze the trends in the responses.

Using a confidence interval of 90%, the analysis suggested that, the factors that affected the response significantly using *E. coli* NG188 were all the factors and their interactions (since they gave P value < 0.05) except for the concentration of glucose and its square (P value > 0.05). According to the polynomial equation, all these factors except the concentration of K_2HPO_4, KH_2PO_4 and the interaction between KH_2PO_4 and glucose, had a positive effect on RB decolorization. This could also be seen from the response surfaces where higher responses were observed for high concentration of K_2HPO_4 and KH_2PO_4 (Fig. 3A), high concentration of K_2HPO_4 and glucose (Fig. 3B) and high concentration of KH_2PO_4 and relatively low concentration of glucose (Fig. 3C). It is important to understand why three components that were identified as critical for *Bacillus* sp. *NG13* and *E. coli* NG188, i.e., K_2HPO_4, KH_2PO_4, glucose and RB dye concentration might be important for the decolorization percentage. The effect of KH_2PO_4 and K_2HPO_4 is likely due to HPO_4^{-2} and PO_4^{-2} that phosphate is a component of nucleic acids, nucleotides, including ATP and phospholipids (Khan et al., 2009). The effect of glucose is likely because carbon compounds are the sources of carbon skeleton and energy for bacterial cell growth. While the inhibitory effect of high concentration of RB dye is likely because, dyes which have reactive groups as sulfonic acid (SO_3H) on their aromatic rings are highly inhibited the growth of microorganisms at higher concentrations and the increase in carbon concentration of the dye decrease the decolorization % (Solis et al., 2012).

TABLE 4. Analysis of variance for the fitted quadratic polynomial model.

	Coefficients	Standard Error	*t* Stat	P value
Intercept	**101.83**	**8.09**	**12.59**	**0.00**
X_1	-3.87	0.82	-4.69	0.01
X_2	-9.89	2.75	-3.60	0.02
X_3	0.68	0.82	0.83	0.45
X_1X_2	0.37	0.11	3.39	0.02
X_1X_3	0.15	0.03	4.62	0.01
X_2X_3	-0.57	0.11	-5.24	0.00
X_1^2	0.12	0.03	3.50	0.02
X_2^2	2.20	0.38	5.87	0.00
X_3^2	0.00	0.03	-0.05	0.96

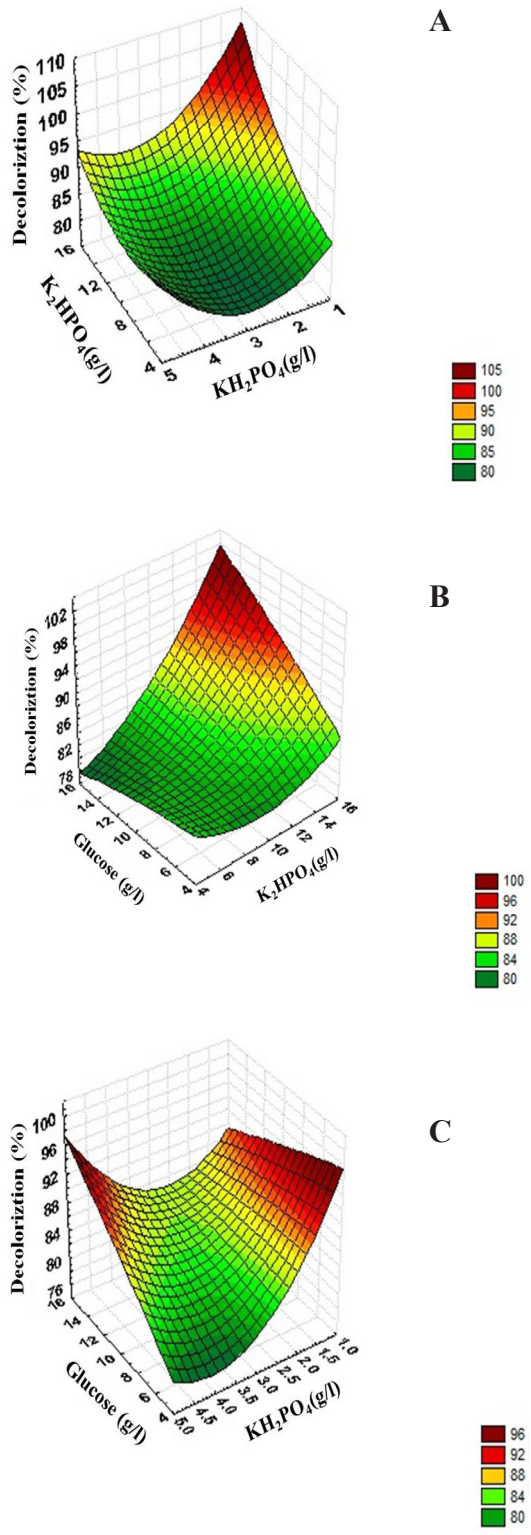


Fig. 3. Response surface of the interaction of (A) KH_2PO_4 and K_2HPO_4, (B) K_2HPO_4 and glucose and (C) KH_2PO_4 and glucose on RB decolorization % (Y) by *E. coli* NG188.

The optimum response for RB decolorization by *Escherichia coli* NG188 was observed at trial number 14, since it gave a 100% decolorization, hence, the suggested optimum medium is (g/L): $(NH_4)_2SO_4$, 0.5; K_2HPO_4, 15; KH_2PO_4, 4.5; $MgSO_4.7H_2O$, 1; NaCl, 10; glucose, 15; dye concentration, 100 mg/L.

Spectrophotometric analysis

UV vis analysis

The UVv is analysis of RB dye showed that the λ max for RB dye was 600nm. However, after complete decolorization by *E. coli* NG188, the maximum wavelength for RB shifted from the visible light range towards the UV range (Fig. 4). The complete disappearance of the absorbance peaks in the visible region indicates the complete decolourization and effective degradation of RB by *E. coli* NG188. After decolorization, the appearance of a new peak in the UV spectra indicated the formation of other metabolites (Shah et al., 2013).

FTIR analysis

It was shown from the FT-IR analysis that, some peaks disappeared from the region 400-1600cm^{-1}, which indicates that there are breakingdown in C=C and C=O (Shobana & Thangam, 2012) and the broad band in 3600cm^{-1} (Fig. 5B) indicates the formation of –OH group (Ismail et al., 2013) as a result to the biological treatment by the bacterial isolate *E. coli* NG188. The results have further confirmed the biodegradation of the Remazol Blue dye by *E. coli* NG188. FTIR spectral comparison between the dye and its degradation products produced by the bacterial strain after decolorization indicates that the RB degraded into different metabolites (Fig. 5 A, B).

Conclusion

Remazol Blue can be efficiently decolorized by *E. coli* NG188. The decolorization process is markedly influenced by the composition of the cultivation medium and the concentration of Remazol Blue. The culture conditions were considerably optimized using the statistical experimental designs of Plackett-Burman and Box-Behnken. Moreover, Spectrophotometric analysis confirmed the biodegradation of the RB into different metabolites.

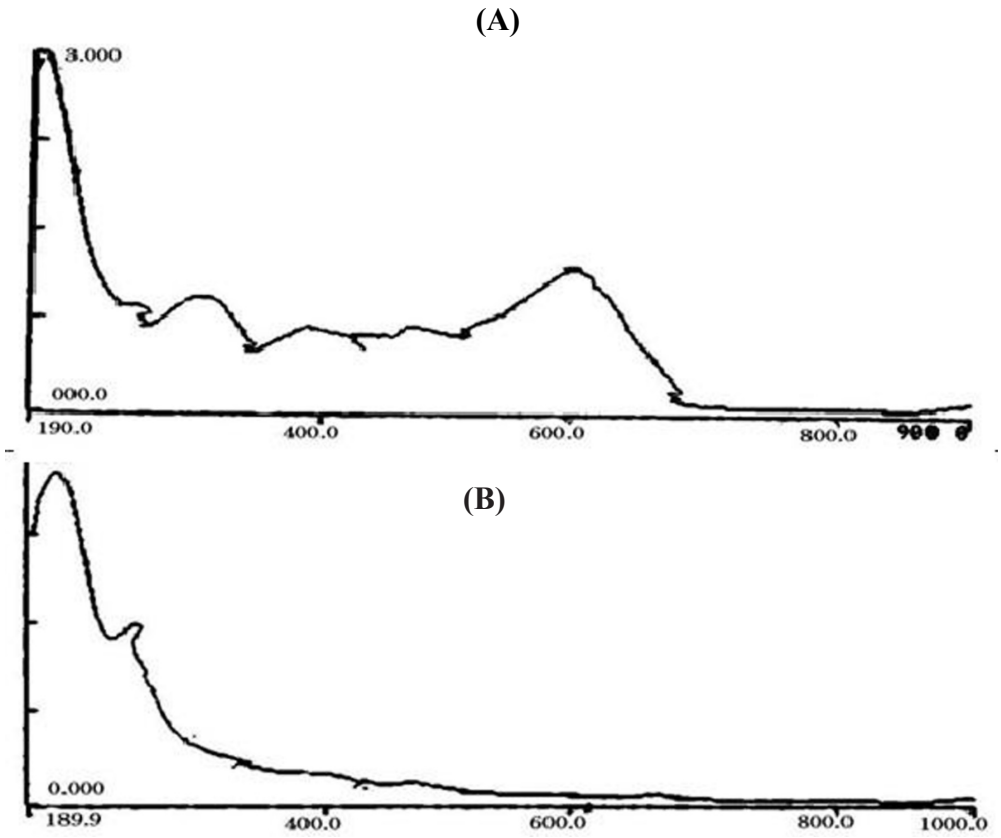
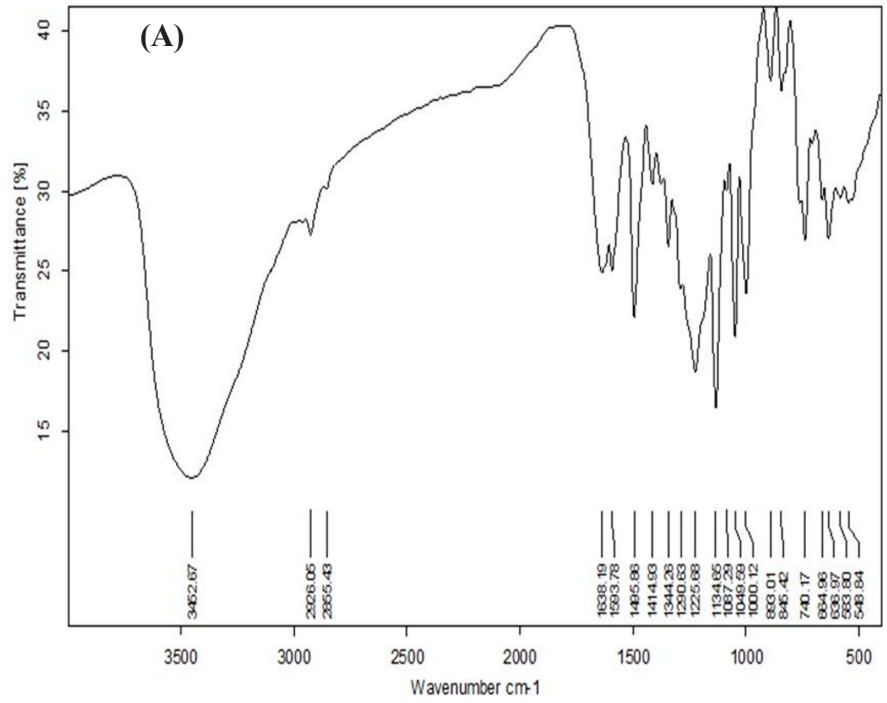


Fig. 4. UV-Visible spectra of the RB dyes before the biological treatment (A), and after treatment by *E. coli* NG188 (B).



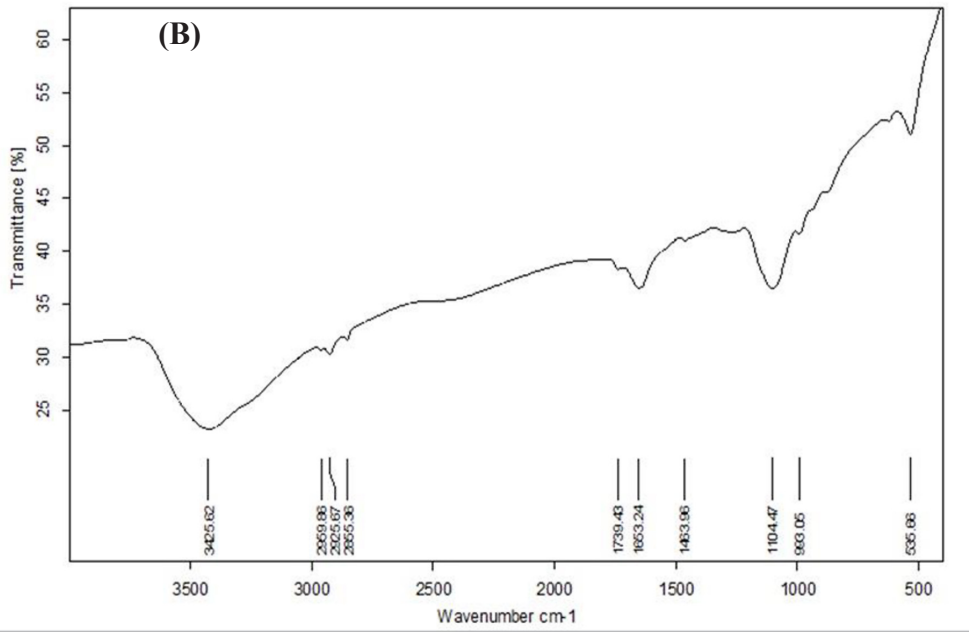


Fig. 5. FTIR spectra of the RB dye before the biological treatment (A), and after treatment by *E. coli* NG188 (B).

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ازالة صبغة الريمازول الازرق بواسطة *Escherichia coli* NG188 تحت الظروف المثلى

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الهدف من هذه الدراسة هو تكسير صبغة الريمازول الأزرق، المستخدمة فى االنسيج، بواسطة البكتيريا *Escherichia coli* NG188 المعزوله من مياه الصرف الصناعي. النسبة الأمثل لإزالة اللون كان عند درجة 40 درجة مئوية ، حجم اللقاح 5% ، ودرجة الحموضة 8 في حالة ساكنة. تم دراسة تأثير العوامل الغذائية على إزالة لون الصبغة عن طريق التصميم الإحصائي بلاكيت برمن. على أساس قيم *t* المحسوبة ، قيم P ومستوى الثقة: كان تركيز الجلوكوز والصبغ مستويات ثقة أعلى من 90% ، وبالتالي تم اعتبار المعلمات الهامة التي تؤثر على تدهور الصبغة بواسطة *Escherichia coli* NG188. تم إجراء تجربة تحقق لتقييم دقة التصميم الإحصائي المطبق "بلاكت برمن". أظهرت نسبة إزالة اللون زيادة قدرها 1.24 مرة في معدل تدهور الصبغة مقارنة بمتوسط نتائج حالة القاعدية. تم الإبلاغ عن إزالة الصبغة الكاملة لصبغة الريمازول الأزرق باستخدام منهجية سطح الاستجابة (RSM). تم تأكيد التحلل الحيوي للريمازول الأزرق من خلال التحليل الطيفي للأشعة فوق البنفسجية والمرئية.