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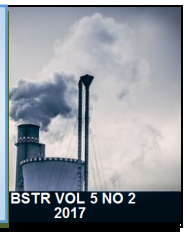

Isolation and Culture Medium Optimisation Using One-Factor-At-Time and Response Surface Methodology on the Biodegradation of the Azo-Dye Amaranth

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ABSTRACT

Isolate JR1 was isolated from the polluted textile industry activities site in the Juru Penang area. This bacterium was characterized as a gram-positive Bacillus bacterium and also gave a positive biochemical test for catalase test and oxidase test. The isolate JR1 gave a maximum decolourization of Amaranth dye under static conditions with the rate of decolorization of 98.82%. Seven variables which are pH, temperature (°C), ammonium acetate (g/L), glucose (g/L), sodium chloride (g/L), yeast (g/L) and dye concentration (ppm) was run by using Plackett-Burman design for the effective parameter of the decolourization of Amaranth. From the seven variables, three effective variables which were ammonium acetate, glucose, and dye concentration were further optimized by using a central composite design. The optimum value of ammonium acetate concentration at 0.74 g/L, glucose concentration at 3.0 g/L and a dye concentration at 58.1 ppm gave the highest percentage of decolourization. Thus, this isolate could provide an alternate solution in removing toxic dyes from environments.

INTRODUCTION

The use of dyes is an important aspect where dyes are use in clothes, food, and also the production of medicines [1]. These dyes are produced from natural origins such as plant, animal and insect. Dyes can be basic, acidic, azo and metal complex dyes depend on the different structural in dyes [2]. Azo dyes contain various structures such as diazotized amine attached to the other amine or phenol group by the important azo bond linkage (-N=N-) [3]. This aromatic group can produce various intensities of colour with different shades for the same dyes. Some azo dyes may contain more than one azo bond depends on the type of azo dyes [4]. Examples, mono-azo dye presence with one azo bond, two in diazo and three in triazo bond linkage [5]. Mostly important azo dyes used in industry can be classed as acid dyes, basic dyes, direct dyes, disperse dyes, mordant dyes, reactive dyes and solvent dyes [6].

The main problem is the treatment utilised to remove azo dye in wastewater from the textile industry. This is because azo dyes has high solubility in water [7]. This will increase the water pollution due to the textile industries discharging azo dye as effluent into the water sources such as river after the dyeing process. This may lead to eutrophication and may cause toxicity and carcinogenicity to the aquatic life. This contaminant has to be removed before discharging into water bodies or on land [8]. Researchers have used physical, chemical and biological treatment method to decrease amounts of azo dyes that discharge from wastewater [9]. The examples of very popular physical method include adsorption and commonly uses activated carbon. Chemical method is more widely used compared to the physical method such as Fenton's reagent [10].

Water-pollution control is presently one of the major areas of scientific activity. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable. Effluent discharge from textile and dyestuff industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health concerns to environmental regulatory agencies. Colour removal, in particular, has recently become of major scientific interest, as

indicated by the multitude of related research reports. During the past two decades, several physicochemical decolourization techniques have been reported, few, however, have been accepted by the textile industries. Their lack of implementation has been largely due to high cost, low efficiency and inapplicability to a wide variety of dyes [11].

The ability of microorganisms to carry out dye decolourization has received much attention. Microbial decolourization and degradation of dyes are seen as a cost-effective method for removing these pollutants from the environment. Recent fundamental work has revealed the existence of a wide variety of microorganisms capable of decolorizing an equally wide range of dyes [12–15]. In this review, we have examined biological decolourization of dyes used in textile industries and report on progress and limitations [5,6].

The most important aspect of biological treatment of dye effluent is eco-friendliness. This is because the dye changes to a simpler inorganic compound which must not be lethal to life and the environment. Basic steps to decolourize and degrade dye is by breakdown the azo bond. Treatment system should be able to complete the mineralization of azo dyes under anaerobic and aerobic conditions [6]. Response surface methodology (RSM) is a statistical method for searching the optimal conditions for run experiment [16]. RSM can give advantages such as time-consuming and reduce the cost because many variables can be done simultaneously [17]. There are many types of design in the RSM but the most popular is the central composite design (CCD) [18].

MATERIALS AND METHODS

Chemicals and Culture Media

The isolated bacteria were purified by sub-culturing them in mineral salt medium (MSM) containing in g/L of distilled water, $(NH_4).2SO_4$ (0.4), KH_2PO_4 (0.2), K_2HPO_4 (0.4), NaCl (0.1), Na_2MoO_4 (0.01), $MgSO_4.7\ H_2O$ (0.1), $MnSO_4.H_2O$ (0.01), $Fe_2(SO_4)_3.H_2O$ (0.01), yeast extract (1) . The pH of the medium was adjusted to pH 7 using 2 M sodium hydroxide (NaOH) and hydrochloric acid (HCl). The medium was sterilised using HARISHIMA autoclave for 20 min at 120˚C and glucose solution 10 g was autoclaved separately [19]. After autoclaved, the MSM and glucose were mixed together at room temperature. The Amaranth solution was added into the MSM after autoclaved to a final concentration of 50 ppm. Chemicals used in the media were obtained from Fisher (Malaysia) and Sigma, Aldrich USA.

Source of Bacteria

Soil and water samples were collected from various known dyes contaminated sites from Juru, Penang. Soil samples were collected from a depth of 5 cm from the top, while water samples were taken using collecting tube at a depth of about 7-10 cm from the top. Samples were kept in sterilised polycarbonated screw cap tubes and stored at 10℃ until further use.

Spectrophotometer analysis of dye degradation

The absorbance of azo-dye Amaranth dye at 520nm was determined photometrically. Sample medium was centrifuged first using micro-centrifuge (Sigma-Aldrich, USA) at 10, 000 g at room temperature for 10 min. The supernatant was taken for the determination of the decolourised percentage of Amaranth dye. The difference in the absorbance reading was used to calculate the percentage of decolourisation using the following formula;

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance - final absorbance}}{\text{Initial absorbance}} \text{ x } 100\%$$

Screening for dye-degrading bacteria.

A soil sample of 1g and 1mL water samples were transferred into sterilised MSM containing 50 ppm of Amaranth dye and incubated in the room temperature on a rotary shaker at 150 rpm for 10 days. The absorbance reading for decolourisation of Amaranth dye was taken every 24 h at an absorbance of 520 nm. The samples from decolourized dye solutions were streaked on a nutrient agar plate and incubated for 24 h at room temperature. The bacterial isolates were chosen and differentiated into different morphology. Each different isolate was incubated in MSM containing 50 ppm of Amaranth dye. The isolate giving maximum decolourisation value was selected and used for further decolourisation experiment [17].

Effect of agitation

The selected isolate bacteria were screened in a different condition which is in a static condition and shaking condition at 150 rpm incubated at room temperature for 24 h. The decolourisation was observed and the absorbance reading was taken using spectrophotometer at maximum wavelength 520 nm. The best condition for maximum decolourisation was be used for the further experiment. The decolourisation study was photometrically at 520 nm.

Effect of different carbon and nitrogen source

Different sources of carbon and nitrogen were screened which promotes better decolourisation of Amaranth dye. MSM was incorporated with different carbon and nitrogen sources in a split set of experiment. The carbon sources were used for this study was glucose, fructose, galactose, arabinose, lactose, sucrose maltose and dextrin were used at an initial concentration of 10 g/L. Meanwhile, the nitrogen sources were urea, ammonium chloride, potassium nitrate, ammonium sulphate, ammonium acetate, alanine, glutamic acid, aspartate and phenylalanine at 0.4 g/L concentration. The experiment was run in triplicate. The decolourisation study was photometrically at 520 nm [20].

Statistical approach on dye degradation

Plackett- Burman design

The Design Expert software version 6.0.7 was used for the statically design experiment and data analysis [19,21]. Each variable was assigned two levels namely a high actual value and a low actual value. the pH had a lower limit 6.0 and an upper limit of 8.0. The temperature was varied between 27 and 35 ºC. The dye concentration was varied between 50 and 250 ppm. The low and high actual value of ammonium acetate and sodium chloride were 0.0 and 1.0 g/L respectively. Glucose was varied between 0.0 to 0.5 g/L and the yeast concentration varied between 0.1 and 0.5 g/L. These seven variables were conducted in duplicate by 12 runs of an experiment on the static conditions for 24 h. The greater effect of variables on decolourisation Amaranth dye was further optimized using CCD.

Central Composite design

Significant screened factors (p-value < 0.05) that affect the percentage of decolourisation were chosen for further optimisation using a central composite design. Each set of experiment was preceded in triplicate with individual control. The cells were removed by centrifugation employing only the supernatant for decolourisation study. The design of the

experiment and the statistical analysis of data were conducted using Design Expert software version 6.0.8.

Data analysis

Statistical analysis was done using Design-Expert® software (Design-Expert® 6.0.8 Portable version) which covers multiple regressions and ANOVA. The analyzing process was carried out by means of Fisher's F test and Student's t-test whereby the determination of the significances of regression coefficients of variables was carried out by Student's t-test.

RESULTS AND DISCUSSION

Bacterial isolation and cultivation

From **Fig.1**, isolate JR1 showed the highest decolourisation with 92.4% dye decolourisation at day 4 compared with rest of the isolates. Isolate 19, BJ3, and 34 exhibited a respective decolourisation percentage of 81.3, 58.3, and 60.7 percent. Isolate JR1 shows better ability in term of decolourisation of 50 ppm amaranth dye compared to rest of the isolates. Full decolourisation occurs at day 10 for isolate JR1, followed by isolate 34. Isolate 19 shows no significant in decolourisation rate at day 7 to 10, which maintained at 78%. Meanwhile, isolate BJ3 achieved the highest decolourisation at day 9 with the rate of 80%. Therefore, isolate JR1 was chosen for further investigation.

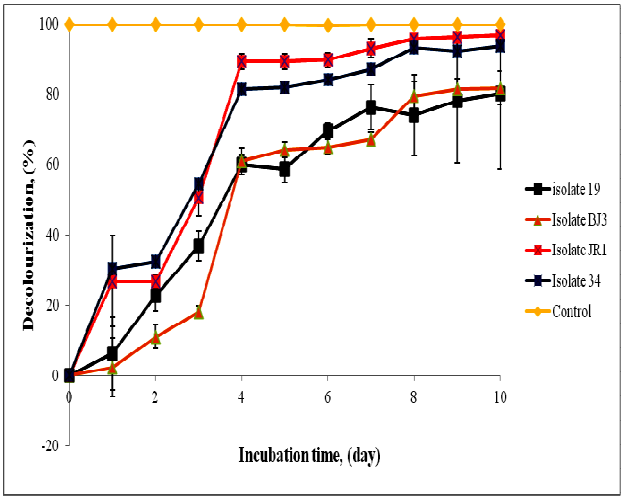

Fig. 1. The decolourisation by four isolates named as isolate 19, BJ3, JR1 and 34 at 50 ppm initial dye concentration within 10 days of incubation period. Error bars indicate the standard error of the mean n= 3.

Characterization of isolate JR1

Isolate JR1 has a bacillus structure with a non-motile feature. Gram staining shows that isolate JR1 is Gram-negative since the bacteria is pink coloured [22]. This is due to the peptidoglycan layer of isolate JR1 cell absorbing the safranin and retaining the pink colour. Biochemical test from **Table 1** shows that the isolate JR1 give positive results for the catalase test. This means that during the aerobic respiration, isolate JR1 can produce hydrogen peroxide which is toxic to the bacteria itself. The isolate JR1 can also produce the enzyme catalase to degrade hydrogen peroxide and produce water and give bubbles. The oxidase test shows positive results by this isolate JR1. This means that it has the ability to produce cytochrome oxidase that catalyzes the oxidation of a reduced cytochrome by molecular oxygen which can be shown by the appearance of dark purple colour. **Table 1** also shows a negative blood agar test means that the isolate JR1 is Gamma hemolysis which means that no lysis of red blood cells results in the appearance of the medium surrounding the colonies.

Table 1. Characterization of azo dye Amaranth degrading isolate JR1.

Characteristics	Isolate JR1
morphology	cocci
motility	-
Gram-reaction	-NE
Catalase test	+
Oxidase test	+
Blood agar test	-

Effect of Agitation

The dye decolourisation of azo dye Amaranth was studied under different conditions which are in shaking and static conditions at the initial concentration of dye at 50 ppm (**Fig. 2**). Based on **Fig. 2**, isolate JR1 shows the highest decolourisation of 50 ppm Amaranth dye in static conditions compared to shaking conditions. Static conditions resulted in an almost decolourisation of the dye with 98.92 % while shaking condition resulted in a decolourisation rate by half with the percentage of 47.95%. This is because shaking conditions will provide oxygen and will block the azoreductase to obtain electrons for azo bond cleavage. While under the static conditions the electron is available from NADH to azoreductase to cleave the azo bond and decolourize the azo dye.

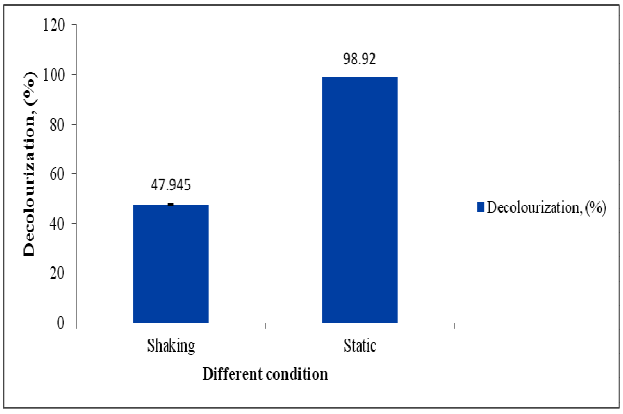

Fig. 2. The effect of agitation on the dye decolourisation efficiency of isolate JR1 on different incubation condition illustrating decolourisation azo dye Amaranth at 24 h. Error bars indicate the standard error of the mean n= 3.

Effect of carbon sources

Different carbon sources based on the three major groups which are monosaccharides: glucose, fructose, galactose, and arabinose, disaccharides: lactose, sucrose and maltose, and the polysaccharide dextrin were used at an initial concentration of 50 ppm in the Amaranth media to further study the effect of Amaranth degradation efficiency of isolate JR1 (**Fig. 3**). Glucose was used as a carbon source in the initial experiment. The optimum glucose concentration was 10 g/L. Other carbon source tested were fructose, lactose, arabinose, maltose, galactose, sucrose, and dextrin at 10 g/L for each carbon sources. Based on the graph above there was no significant difference ($p>0.05$) between the sources based on ANOVA because all carbon source supported isolate JR1 growth and gave more than 90 % of decolourisation of Amaranth. Carbon source that gives the highest percentage of decolourisation was arabinose with 99.1 % of Amaranth decolourization. For the further Amaranth decolourisation experiment, we can choose either one of the carbon sources since all the carbon sources can

support bacteria growth. Based on the studies of Stolz et al. in 2001, they show several isolates that decolourize azo compound that need glucose as carbon and energy source such as *Bacillus subtilis* and *Pseudomonas stutzeri.*[23].

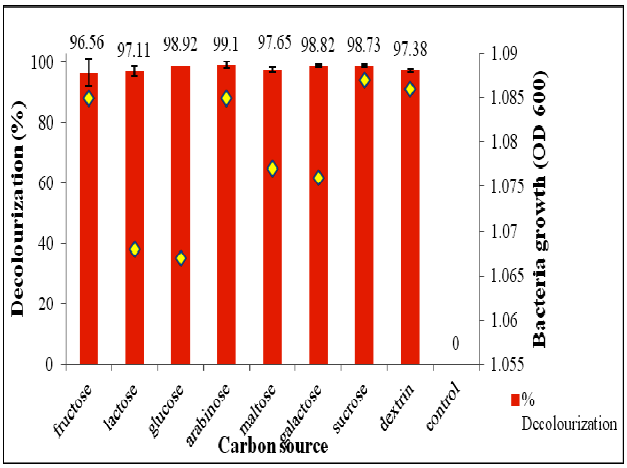

Fig. 3. The effect various carbon source in aiding the decolourisation of 50 ppm Amaranth dye at 24 h. Error bars indicate the standard error of the mean n= 3.

Effect of nitrogen source

Nine different type of nitrogen sources with the final concentration 0.4 g/L was studied on its effect aiding decolourisation of 50 ppm Amaranth dye (**Fig. 4**).

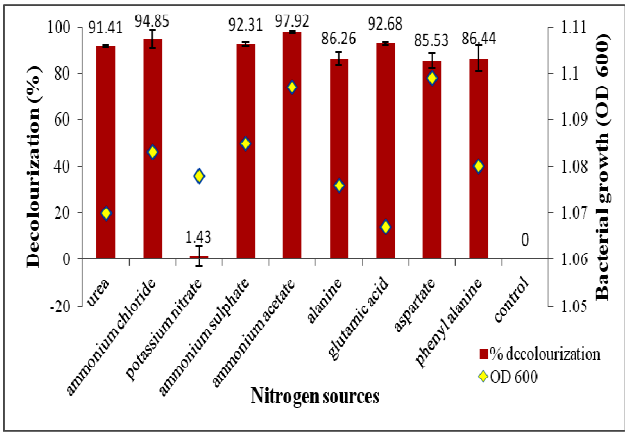

Fig. 4. The effect various nitrogen source in aiding decolourisation of 50 ppm Amaranth dye at 24 h. Error bars indicate the standard error of the mean n= 3.

The nitrogen sources studied were urea, ammonium chloride, potassium nitrate, ammonium sulphate, ammonium acetate, alanine, glutamic acid, aspartate and phenylalanine. Ammonium acetate of 97.92 g/L gives the highest percentage of Amaranth dye decolourization, followed by ammonium chloride with 94.85 g/L decolourisation rate. However, based on ANOVA analysis, there were no significant differences between ammonium acetate and ammonium chloride in aiding decolourisation of Amaranth dye. Meanwhile, potassium nitrate of 1.43 g/L gives the lowest percentage of Amaranth dye decolourization.

Plackett-Burman Analysis

Placket-Burman design was utilised to identify which parameter gave significant effect on decolourisation of Amaranth by isolate JR1. **Table 2** shows the F-value of 11.72 meaning that the model is significant with the Prob > F value of 0.0157 which is less than 0.0500. There is only a 1.57 % chance that a "Model F-Value" this large could be due to noise. The R^2 value is 0.9535 which is desirable as it is close to 1 and show that the 95.35 % the variability in response could be explained by the model. The standard deviation, (SD) is 11.41 show that the model is fit because the smaller the SD the more fit the model. The coefficient of variation, (CV) indicates the degree of precision with which the experiment are compared [24].

The higher the value of CV is, the lower the reliability of the experiment. Based on the table 1 show the low value of CV which is 15.97 that indicates the reliability of the experiment. The "Pred R-Squared" of 0.5815 is not as close to the "Adj R-Squared" of 0.8721 as one might normally expect. This may indicate a large block effect of the experiment. The "Adeq Precision" measure the signal to noise ratio. A ratio greater than 4 is desirable as it indicates adequate model discrimination (Ani Idris, 2008). In this **Table 2.** show, the value of "Adeq Precision" is 8.649 is above than 4. Since the "Prob > F" less than 0.05 and the model is significant so in this experiment parameter C, D and G are a significant model term and can be further optimized by using Central Composite Design, (CCD) [19]

Table 2. The Anova For Plackett - Burman. R^2 is 0.9535, C.V is 15.97.

Source	Sum of Squares	Df	Mean Square	F Value	Prob > F	Remarks
Model	1067.87	7	1523.98	11.72	0.0157	Sig
A	404.96	1	404.96	3.11	0.1524	
B	901.51	1	901.51	6.93	0.058	
C	5443.85	1	5443.85	41.85	0.0029	
D	1673	1	1673	12.86	0.023	
E	614.19	1	614.19	4.72	0.0955	
F	1.93	1	1.93	0.015	0.909	
G	1628.44	1	1628.44	12.52	0.0241	
Residual	520.30	4	130.08			
Cor total	11188.17	11				
S.D	11.41		R-Squared			0.9535
Mean	71.41		Adj R-Squared			0.8721
C.V	15.97		Pred R-Squared			0.5815
PRESS	4682.71		Adeq Precision			8.649

Table 3. Regression analysis of Central Composite design data for Amaranth dye decolourisation by isolate JR1. Observed R^2 was 0.9676, C.V was 1.47.

Source	Sum of Squares	Df	Mean Square	F value	Prob>F	
Model	576.59	9	64.07	33.19	<0.0001	Sig
A	28.74	1	28.74	14.89	0.0032	
B	4.44	1	4.44	2.30	0.1603	
C	267.35	1	267.35	138.52	<0.0001	
A^2	23.59	1	23.59	12.22	0.0058	
B^2	41.37	1	41.37	21.44	0.0009	
C^2	67.62	1	67.62	35.03	0.0001	
AB	0.062	1	0.062	0.032	0.8612	
AC	0.010	1	0.010	5.335 e-003	0.9432	
BC	0.072	1	0.072	0.037	0.8511	
Residual	19.30	10	1.93			
Lack of fit	19.30	5	3.89			
Pure Error	0.000	5	0.000			
Cor Total	595.89	19				
S.D	1.39	R^2		0.9676		
Mean	94.32	Adj R^2		0.9385		
C.V.	1.47	Pred. R^2		0.6064		
PRESS	234.55	Adeq Precision		18.914		

Central composite design

The ANOVA gives the values of the model that can explain whether this model fits the variation observed in Amaranth decolourisation with designed parameter level [25]. Based on **Table 3**, the model F-value of 33.19 indicates the model significance. There was statistically 0.01% chance that this F-value of the model could be occurring due to noise. The model A, B and C are linear, model AB, AC and BC show the interaction between the parameter and model A^2, B^2, C^2 shows the quadratic term. The F-value and Prob>F for the lack of the fit test of the model is 0.0 which both indicates non-significant lack of fit and confirmed the model predictability [26]. The predicted R^2 values are 0.6064 is not as close the adjusted R^2 value of 0.9385 and this might be because of a large block of parameter effect.

The interactions between the parameter measured by R^2 show value of 0.9676, which is desirable as it is close to 1. This means that 96.76 % the variability in the response could be explained by the model. The R^2 value also indicates that the actual and predicted value of percentage decolourisation of Amaranth was very close [27]. The preciseness of experiments confirmed by value C.V of 1.47 indicates the greater reliability of experiments performed since the lower the C.V. the more fit the model of the experiment [28,29]. The measured signal noise indicated adequate precision which a desirable ratio should be greater than 4 [30]. The adequate precision value of 18.914 is shown greater than 4 confirmed that the adequate model discrimination. The SD value of 1.39 indicated the model shows the actual and predicted value of percentage decolourisation is very close. The residual value of 19.30 indicates the model strong compliance with a predicted response.

Response surface plot of central composite design (CCD)

The 3D response surface and 2D contour plot show results as glucose and ammonium acetate concentration increased, the percentage of decolourisation azo dyes Amaranth also increase to a certain extent (**Fig. 8**). Both plot shows that glucose concentration ranging from 2.5 to 3.0 g/L and ammonium acetate concentration ranging from 0.6 to 0.74 g/L was a most effective interaction between this parameter withhold value of dye concentration at 150 ppm. Both plots show the strong interaction between these two parameters for decolourisation of azo dye Amaranth. The value on the line of the 2D contour is the value of percentage decolourisation of Amaranth and the contour plot show the elliptical shape give more confirmed the effective interaction of this parameter.

The interaction between dye concentration and glucose concentration with the hold value of ammonium acetate is 0.5 g/L (**Fig. 9**). Based on the response surface show that there was high percentage decolourisation at low dye concentration with high glucose concentration. However, the percentage decolourisation is low even the glucose concentration is high in the high dye concentration. Based on the contour plot the better decolourisation of Amaranth at dye concentration range 55.0 to 60 ppm within glucose concentration range between 2.5 to 3.0 g/L. The graph contour also gives the elliptical shape that indicates this both parameters have effective interaction.

Fig. 10 shows a response surface plot of ammonium acetate concentration against dye concentration at the fixed level of glucose concentration value of 2.5 g/L. While considering the dye and ammonium acetate concentration had an antagonistic effect. This means by greater decolourisation occur when ammonium acetate concentration higher within the low dye concentration. Based on the contour show the optimal value of ammonium acetate range between 0.6 to 0.75 g/L and dye concentration range between 50 to 150 ppm.

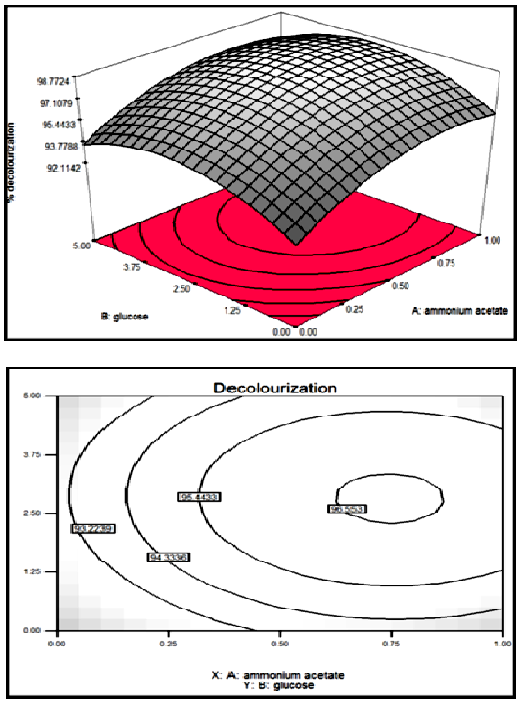

Fig. 8. The 3D contour shows the effect of glucose and ammonium acetate concentration on the decolourisation of Amaranth dye by isolate JR1 while dye concentration maintained at 150 ppm.

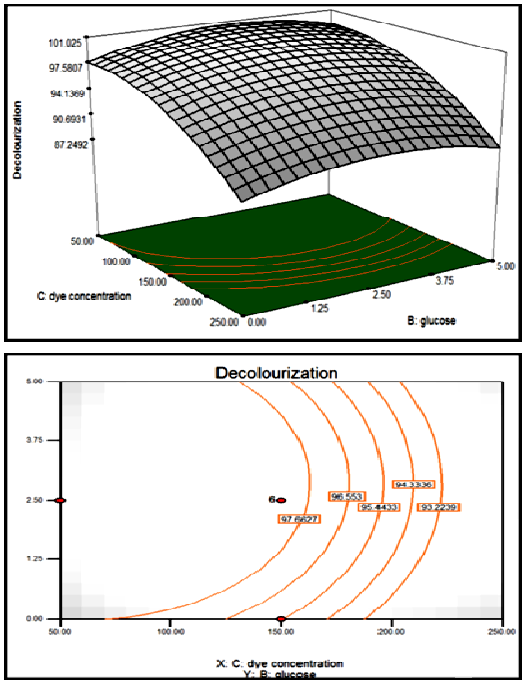

Fig. 9. The 3D contour shows the effect of dye and glucose concentration on the decolourisation of Amaranth dye by isolate JR1 while ammonium acetate maintained at 0.5 g/L.

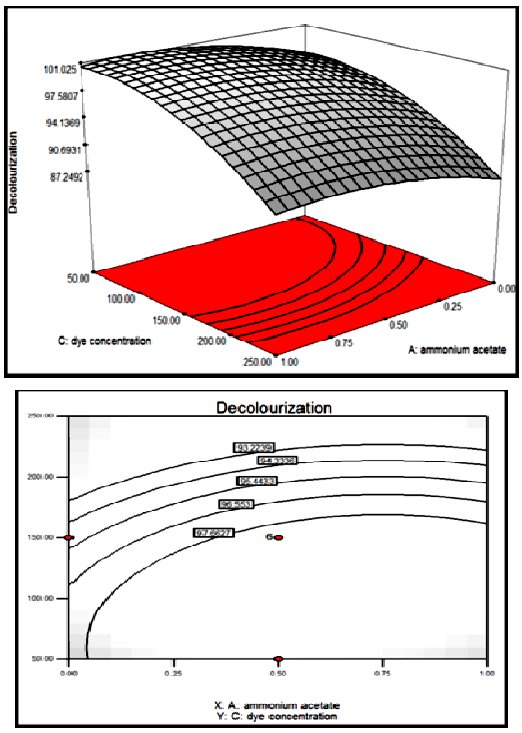

Fig. 10. The 3D contour shows the effect of dye and ammonium acetate concentration on the decolourisation of Amaranth dye by isolate JR1 while glucose concentration maintained at 2.5 g/L.

Percentage decolourisation vs. deviation from reference point

The perturbation graph indicates the optimal values of the tested variables [31]. Based on **Fig. 11** show that the optimal value of these three parameters is ammonium acetate concentration at 0.74 g/L, glucose concentration at 3.0 g/L and dye concentration at 58.1 ppm to give the highest percentage of decolourization. Based on the graph, the more the point of interaction of these three parameters close to 1.0 the more effective the optimal value for this interaction.

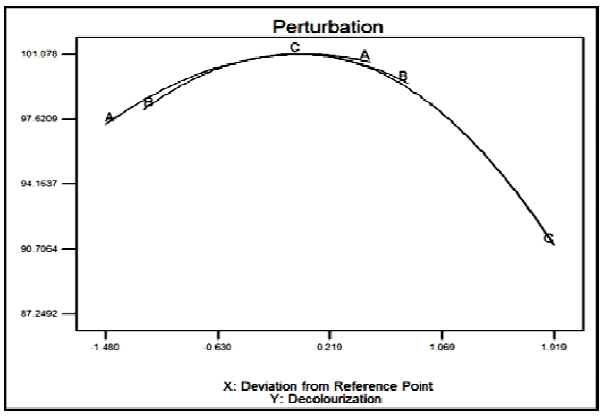

Fig. 11. Perturbation graph showing the optimum values of the tested variables.

Conclusion

From this study, an azo dye Amaranth degrading-bacterial isolate JR1 was isolated from the textile industrial site in Juru, Penang area. The characterization of JR1 shows that the JR1 is bacillus bacteria. Optimization studies on this isolate JR1 were successful by using the response surface methodology (RSM). The optimal condition that gives maximum decolourisation ammonium acetate concentration at 0.74 g/L, glucose concentration at 3.0 g/L and dye concentration at 58.1 ppm. For the future studies, identification of isolate JR1 by using 16S rRNA gene and sequencing method will be carried out. Besides that, it is suggested that combining aerobic and anaerobic bacteria for a better decolourisation and degradation of azo dye Amaranth should be carried out. Finally, it is recommended that in the future, the isolation of bacteria that can use azo dye as carbon and nitrogen source to reduce colour and degrade azo dye should be carried out as decolorization does not mean a complete mineralization.

REFERENCE

1. Pereira L, Alves M. Dyes—Environmental Impact And Remediation. InEnvironmental protection strategies for sustainable development 2012 (pp. 111-162). Springer Netherlands
2. Rauf MA, Meetani MA, Hisaindee S. An overview on the photocatalytic degradation of azo dyes in the presence of Tio_2 doped with selective transition metals. Desalination. 2011 Aug 2;276(1):13–27.
3. Feng W, Nansheng D, Helin H. Degradation mechanism of azo dye C. I. reactive red 2 by iron powder reduction and photooxidation in aqueous Solutions. Chemosphere. 2000 Oct 31; 41(8):1233-8.
4. Khadijah O, Lee KK, Mohd Faiz Fa. Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. Malays J Microbiol. 2009 Jan 1;5(1):25–32.
5. Hu TL. Kinetics of azoreductase and assessment of toxicity of metabolic products from azo dyes by *Pseudomonas luteola.* Water Sci Technol. 2001 Jan 1;43(2):261–9.
6. Banat IM, Nigam P, Singh D, Marchant R. Microbial decolorization of textile-dyecontaining effluents: A review. Bioresour Technol. 1996 Dec 1;58(3):217–27.
7. Shaul GM, Holdsworth TJ, Dempsey CR, Dostal KA. Fate of water soluble azo dyes in the activated sludge process. Chemosphere. 1991 Jan 1;22(1):107–19.
8. Karkmaz M, Puzenat E, Guillard C, Herrmann JM. Photocatalytic degradation of the alimentary azo dye Amaranth: mineralization of the azo group to nitrogen. Appl Catal B Environ. 2004 Aug 10;51(3):183–94.
9. Chen KC, Wu JY, Liou DJ, Hwang SCJ. Decolorization of the textile dyes by newly isolated bacterial strains. J Biotechnol. 2003 Feb 27;101(1):57–68.
10. Solozhenko EG, Soboleva NM, Goncharuk VV. decolourisation of azo dye solutions by Fenton's oxidation. Water Res. 1995 Sep 1;29(9):2206–10.
11. Santos VP, Pereira MFR, Faria PCC, Órfão JJM. Decolourisation of dye solutions by oxidation with H_2O_2 in the presence of modified activated carbons. J Hazard Mater. 2009 Mar 15;162(2):736–42.
12. Chung KT, Stevens SE. Degradation azo dyes by environmental microorganisms and helminths. Environ Toxicol Chem. 1993 Nov 1;12(11):2121–32.
13. Barragán BE, Costa C, Márquez MC. Biodegradation of azo dyes by bacteria inoculated on solid media. Dyes Pigments. 2007;1(75):73–81.
14. Cripps C, Bumpus JA, Aust SD. Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium.* Appl Environ Microbiol. 1990 Apr 1;56(4):1114–8.
15. Zhao X, Hardin IR. HPLC and spectrophotometric analysis of biodegradation of azo dyes by *Pleurotus ostreatus*. Dyes Pigments. 2007 Jan 1;73(3):322–5.
16. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008 Sep 15;76(5):965–77.
17. Tripathi A, Srivastava SK. Biodegradation of orange G by a novel isolated bacterial strain *Bacillus megaterium* ITBHU01 using response surface methodology. Afr J Biotechnol. 2012 Jan 1;11(7):1768–81.

18. Ayed L, Khelifi E, Jannet Hb, Miladi H, Cheref A, Achour S, Et Al. Response surface methodology for decolorization of azo dye methyl orange by bacterial consortium: Produced enzymes and metabolites characterization. Chem Eng J. 2010;165(1):200–8.
19. Karamba KI, Ahmad SA, Zulkharnain A, Syed MA, Khalil KA, Shamaan NA, et al. Optimisation of biodegradation conditions for cyanide removal by *Serratia marcescens* strain AQ07 using one-factor-at-a-time technique and response surface methodology. Rendiconti Lincei. 2016 Sep 1;27(3):533–45.
20. Salem ANM, Ahmed MA, El-Shahat MF. Selective adsorption of amaranth dye on Fe_3O_4/MgO nanoparticles. J Mol Liq. 2016 Jul 1;219:780–8.
21. Ibrahim HM, Elkhidir EE. Response surface method as an efficient tool for medium optimisation. Trends Appl Sci Res. 2011 Feb 1;6(2):121–9.
22. Beveridge TJ. Use of the gram stain in microbiology. Biotech Histochem. 2001 Jan 1;76(3):111–8.
23. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol. 2001 Jul 1;56(1–2):69–80.
24. Vimalashanmugam K, & Viruthagiri T. Response surface methodology optimization of process parameters for xylanase production by *Aspergillus fumigatus* in SSF using central composite design. Parameters. 2012 Nov;2(6):227-287.
25. Daneshvar N, Khataee AR, Rasoulifard MH, Pourhassan M. Biodegradation of dye solution containing malachite green: Optimization of effective parameters using Taguchi method. J Hazard Mater. 2007 May 8;143(1):214–9.
26. Kansal HK, Singh S, Kumar P. Parametric optimization of powder mixed electrical discharge machining by response surface methodology. J Mater Process Technol. 2005 Dec 1;169(3):427–36.
27. Körbahti BK, Rauf MA. Application of response surface analysis to the photolytic degradation of basic red 2 dye. Chem Eng J. 2008 May 1;138(1):166–71.
28. Halim SFA, Kamaruddin AH, Fernando WJN. Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer studies. Bioresour Technol. 2009 Jan 1;100(2):710–6.
29. Mohammed N, Ahmad WA. Application of response surface methodology (RSM) for optimizing removal of Cr (VI) wastewater using Cr (VI)-reducing biofilm systems. Malays J Fundam Appl Sci. 2014;6(1):15–21.
30. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley Interdiscip Rev Comput Stat. 2010 Mar 1;2(2):128–49.
31. Seeman M, Ganesan G, Karthikeyan R, Velayudham A. Study on tool wear and surface roughness in machining of particulate aluminum metal matrix composite-response surface methodology approach. Int J Adv Manuf Technol. 2010 May 1;48(5–8):613–24.