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Evaluation of Kinetic Pathway for the Biodegradation of Congo red using *Asperigillus* species and Optimization of Physiochemical Parameters by Response Surface Methodology

Sathish Sundararaman*, D. Joshua Amarnath, S. Sivasubramanian

Department of Chemical Engineering, Sathyabama University,
Rajiv Gandhi Road, Chennai 600 119, India.

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Decolorization of Congo red dye was investigated by using dual microbial organism isolated from textile effluent. The effective fungal strain with dye degrading ability was isolated from the effluent treatment plants and was identified as *Asperigillus Terreus* and *Asperigillus Fumigates* based on morphological and 16SrDNA sequencing. Analysis for identification of intermediate compounds confirmed the presence of quinone compounds, salicylic acid and catechol, based on this possible pathway for the degradation of dye was proposed. Optimization of temperature, pH, initial concentration of dye and time was investigated by using Response Surface Methodology (RSM). Central Composite Design (CCD) was carried out with 30 experiments, nearly 98.70% of decolorization was observed at 35ÚC, pH 7.0 and at the initial concentration of 500 mg/l in 72 h. The values of R^2 = 0.9924, adjusted R^2 = 0.9811 and predicted R^2 = 0.9961 which reveals that experimental values are in good agreement with predicted values.

Key words: Dye degradation, Optimization, Response Surface Methodology, Central Composite Design.

Large numbers of chemically different dyes in significant proportion appears in textile wastewater. Among these, azo dyes are prominent used in textile dying because of their chemical stability and versatility. Azo dyes are high rating of persistent, due to its aromatic structure[1]. The removal of colour from the textile industrial wastewaters is a major environmental concern[2]. Several physiochemical methods have been suggested for the treatment of dye contaminated wastewater but not widely used because of the high cost and secondary pollution generation[3]. Compared with physical and chemical processes, bio-friendly approaches have been the main focus for remediation of dye contaminated wastewater since they require lower costs, eco-friendly and produce less toxic metabolites[4]. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms[5]. Many microorganisms are capable of degrading azo dyes, including bacteria, fungi[6]. Generally, temperature, pH, time and initial concentration of dye are the most important parameters that significantly influence the biodegradation process[7].

RSM is a tool, used to investigate a combined effect of several variables and to determine optimum conditions for a multivariable system. RSM has been extensively used in biotechnology for optimization of physical condition, medium composition in fermentation process. RSM consists of mathematical and statistical techniques that can be used to define the relationship between the response and the independent variables[8]. A fungal treatment of dyes is an economical and feasible alternative to the

* To whom all correspondence should be addressed.
E-mail: sathish.chemical@sathyabamauniversity.ac.in

present treatment technologies[9]. In the present study degradation of Congo red is carried out by using micro-organism isolated from textile effluent[10]. RSM using CCD was applied for the decolorization process to identify the possible interactions and to determine the optimum operational conditions. The physical parameter such as temperature, pH, time and initial concentration of the dye has been carried for optimization.

MATERIALS AND METHODS

Dye stuff and Chemicals

The dye Congo red was obtained from Sigma Aldrich, India. The chemicals used for the preparation of reagent, solutions, media were purchased from Jiangsu Huaxi International trade Co. Ltd., China. Solvents used in the studies were purchased of AR grade MERCK, Mumbai.

Effluent sample

Textile effluent sample were collected from a CETP located at Ayyampet, Kanchipuram, Tamilnadu. The effluent was collected in airtight container. The collected effluent was filtered through Whatman grade no. 1 filter paper (particle retention of 11µm) to remove suspended particles[11]. The pH of the filtered effluent was maintained at 7.0 and stored at 4ÚC to prevent contamination.

Isolation of micro organism

1ml of effluent was transferred into 9 ml of distilled water in sterile test tubes. The stock solution was by serially diluted to get concentration ranging from 0.1 ml of sample from each dilution was spread on potato dextrose agar (PDA) plates with the help of L-rod[12]. The petriplates were incubated at room temperature for 5 days. After the incubation period colonies of fungal strains was observed. Pure fungal isolates were further subculture on the PDA plates. After growth of the colonies, the plates were stored in refrigerator and served as stock cultures. A mycelium disc of 1.2 cm diameter obtained from a 4 to 5 days old culture plates of fungus were transferred to 25 ml PDA in a 250 ml conical flask and incubated at room temperature for 4 to 5 days. Then the content of each conical flasks were filtered through glass wool. The effective fungal strains were selected for further studies on optimization of physiochemical parameters.

Decolorization experiment

Microbial decolorization of dye was investigated at different physicochemical conditions by using dual microbial organisms[13]. Different dye concentration were prepared in 250 ml Erlenmeyer flasks, each containing 100 ml with following MSM composition in g/l, K_2HPO_4 (1.6), Na_2HPO_4 (0.6), NH_4NO_3 (1.0), NaCl (0.5), $MgSO_4.7H_2O$ (0.1) ,$CaCl_2$ $2H_2O$ (0.1)[14]. CCD was employed to analyses the optimal conditions for degradation of dye by dual culture. The optimization experiment was carried out in 500 ml Erlenmeyer flask with 100 ml MSM by four chosen independent process variables at three levels as shown in **Table 1**. Temperature in $^{R"}$C (A), pH (B), initial concentration in mg/l (C) and degradation time in h (D).

The cultures were transferred to fresh nutrient medium containing dye was incubated at 37°C, under static conditions for three days[15]. After incubation, 10 ml of the culture media were withdrawn, centrifuged at 10000 rpm for 15 minutes in a centrifuge at room temperature to separate the cell mass. The supernatant was used for analysis of decolourisation[16]. Absorbance of the supernatant withdrawn at different time intervals was measured at the maximum wavelength for the dye[17] ($\lambda_{max} = 530$ nm) in the visible region on a Schimadzu UV-Visible spectrophotometer (UV 1800) and Fourier transform infrared spectroscopy (FTIR). The percentage of decolourisation was calculated from the difference between initial and final values of absorbance[18].

$$\text{Percentage decolourisation} = \frac{A_i - A_f}{A_f} \times 100$$

Where A_i and A_f are the initial and final level of absorbance.

Identification of Metabolites

GC-MS technique was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount

of each component was calculated by comparing its average peak area to the total areas. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique patterns.

RESULTS AND DISCUSSION

Morphological Analysis

In this study the fungal screening on Congo red degradation was performed. About 10 strains was isolated among them the two fungal strain shows the remarkable ability in decolorizing the dye. These dual fungal strains used for extensive studies and was identified on the basis of morphological characteristics and 16S rDNA sequences were initially analyzed at NCBI server were *Asperigillus Terreus* (KJ522845) and *Asperigillus Fumigates* (KJ522846) **(Fig. 1).** *Fumigates* colonies show suede-like surface consisting of a dense felt of conidiophores. Conidial heads are typically columnar and uniseriate. *Terrus* colonies are typically suede-like and cinnamon-buff to sand brown in color conidial heads is compact, columnar and biseriate.

Effects of physiochemical parameters

Decrease in percentage decolorization was found at lower initial concentration 250 mg/l as well as higher concentration 750 mg/l for the dual culture **(Fig. 2a).** It may due to the metabolites formed during the process of decolorization by cultures may significantly increase the initial concentration of dye increases up to 500 mg/l and further it start decreases. The optimal concentration for dye decolorization was found to be 500 mg/l.

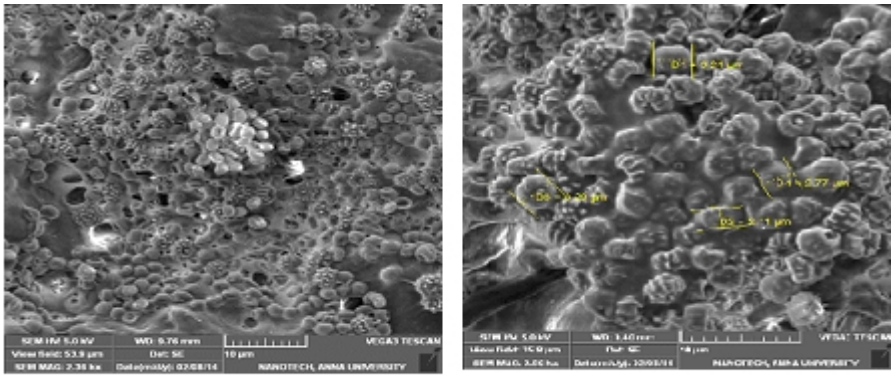
Fig. 2b shows that the percentage degradation of dye increased from 55% to 98.5% as the temperature increased from 25p C to 35p C. Further increase in the temperature decreased the extent of degradation by the fungal strains. This might be due to inhibition of enzyme secretion at higher temperature[19, 20]. An incubation temperature of 35ÚC was found to be optimum for enhanced degradation of dye by using dual strains.

Decrease in percentage decolorization was observed at lower time 36 h as well as higher time 108 h for the organisms **(Fig. 2c).** It is thought that metabolites formed during the process of decolorization by cultures may significantly increase the time for degradation increases up to 72 h and further it start decreases. The optimum time for dye decolorization was found to be 72 h.

pH is the important factor affecting the metabolism of organisms. Microbial strains are able to decolorize the dye at broad range of pH **(Fig. 2d).** Decrease in percentage decolorization was observed at higher pH for the dual culture. It is thought that metabolites formed during the process of decolorization by cultures may significantly increase the pH of culture medium towards alkaline[21]. The optimum pH for dye decolorization was found to be 7.

Analysis of intermediate products

The bio-transformed metabolites were characterized by FTIR. The results of FTIR of Congo red and the sample obtained after decolourisation experiments show various peaks The dye before decolourisation experiments display peaks at 3406, 2151, 1675, 1156, 1077, 776 cm^{-1} for - NH (primary or secondary amine) stretching, alkenes stretch, -N=N- stretching, -S=O, respectively. The IR spectra of degradation product displays peak at 3389, 2141, 1645, 1049, 756 cm^{-1} (**Fig 3**) show that the -N=N- group peak is shifted to 1645 cm^{-1} indicating that the azo bond was undergoing certain changes. The appearance of new peak at 1049 cm^{-1} and disappearance of peak at 1156 cm^{-1} due to the vibration of aromatic amines or may be due to vibration of carboxylic acids and -S=O stretch. The result shows that the dual fungal organisms also degrade the dye. The GCMS of the dye before and after degradation was shown in **Fig. 4 & 5**. On analyzing the major peaks obtained in GC at the specific retention time the metabolites are found and the proposed pathway was shown in **Fig. 6.** The ethyl acetate extract containing the degradation products of Congo red was subjected to GCMS analysis. The result shows that the presence of quinone might have been formed from the diol which undergoes series of reaction such as azo reduction, desulfonation and oxidative deamination. The first step in the degradation pathway may be the reduction of azo group followed by the process of desulfonation[22] resulting in the formation of aromatic amines.



(a) (b)

Fig. 1. SEM image of the fungal organism (a) *Asperigillus Terreus* (KJ522845) and (b) *Asperigillus Fumigates* (KJ522846)

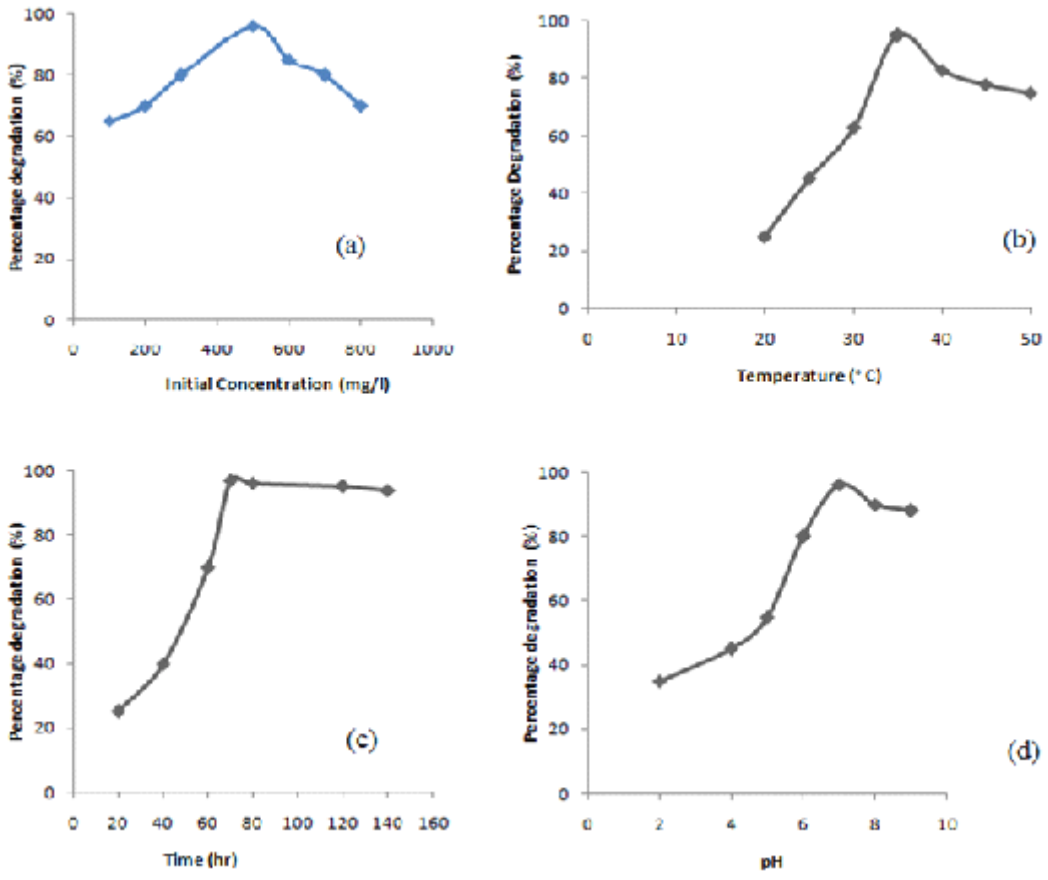


Fig. 2. Effect of parameters affecting degradation (a) initial concentration (b) temperature (c) time and (d) pH

These aromatic amines need oxygen for further breakdown to initiate metabolic degradation result in the formation of diols as an intermediate on further oxidation leads to the formation of quinone which was confirmed by the peaks obtained at the R_T values of 10.63 and 11.9. This quinone might have been undergoing decarboxylation yields salicylic acid whose presence was confirmed from the spectra. Salicylic acid could easily convert into the products that enter into the pathway of tricarboxylic (TCA) cycle.

Table 1. Coded Value of physical parameters

A	Temperature (°C)	30	35	40
B	pH	6	7	8
C	Initial concentration of dye (mg/l)	250	500	750
D	Time (h)	36	72	108

Regression Analysis

The regression equation contain four linear term (A, B, C, D), four square term (A^2, B^2, C^2, D^2), and six cross-interactions (AB, AC, AD, BC, BD, CD) terms plus 1 block term. The empirical mathematical model was tested with the ANOVA with 5% level of significance. The ANOVA was used for checking the significance of the second-order models. The statistical significance of the second-order model equation was determined by F-value **(Table 2)**. In general, the calculated F-value should be greater than the tabulated F-value to reject the null hypothesis, where all the regression coefficients are zero[23].

Table 2. Design table for Congo red degradation by dual culture

Run	Temperature (°C)	pH	Initial concentration (mg/l)	Time (h)	% Decolourisation (Actual)	% Decolourisation (Predicted)
1	30	8	750	96	80.5	80.2
2	35	5.59	500	72	92.4	91.5
3	30	8	250	96	81	81.2
4	40	6	750	96	79	79.5
5	30	6	750	96	80	80.6
6	30	6	250	48	80.5	81.0
7	35	7	500	72	99.6	99.5
8	40	8	750	96	82.3	82.6
9	35	7	500	105.94	90	90.5
10	35	7	500	38.06	91.5	91.8
11	30	6	750	48	81	81.6
12	35	7	500	72	98.6	99.2
13	35	7	146.45	72	90	91.0
14	35	8.41	500	72	93	93.5
15	30	8	250	48	80	80.3
16	40	8	250	48	82	82.5
17	40	6	250	48	82	82.3
18	40	6	250	96	82	82.3
19	35	7	500	72	99.5	99.7
20	35	7	500	72	98.7	99.0
21	40	6	750	48	81.5	81.8
22	40	8	750	48	82.5	83.0
23	40	8	250	96	82.4	82.9
24	35	7	853.55	72	90	90.5
25	42.07	7	500	72	86	87.0
26	27.93	7	500	72	85	85.5
27	30	8	750	48	81.5	81.9
28	35	7	500	72	99.7	99.8
29	35	7	500	72	99.5	99.7
30	30	6	250	96	83	83.2

Percentage degradation
$= 99.01 + 0.30A + 0.20B - 0.23C - 0.15D + 0.39AB - 0.16AC - 0.24AD + 0.46BC + 0.075BD - 0.54CD - 6.56A^2 - 2.96B^2 - 4.31C^2 - 3.94D^2$

Multiple regression analysis was carried out considering full quadratic model equation on the responses to evaluate the adequacy of fit and results are reported in **Table 3**. The coefficient of determination (R^2- values) for the model equation are $R^2 = 0.9961$, adjusted $R^2=0.9924$ and Predicted $R^2= 0.9811$ for the responses were reported. These values suggested that the predicted values are linear relation with experiment values were shown in **Fig. 7**

Interaction effects on operating parameters at optimum conditions

The interactive effect of two independent variables on percentage degradation can be shown on 3D surface plot and 2D contour plot. The effect of two independent variables with another variable at fixed level on the dye degradation by *Asperigillus Terreus* (KJ522845) and *Asperigillus Fumigates* (KJ522846) was shown. **Fig. 8a** shows that as initial concentration (C) and time (D) increases, percentage degradation increased up to optimum level. As initial concentration increased from 250 to 500 mg/l and time increased from 36 to 72 h an increase in percentage degradation was observed. After that a decrease in the percentage degradation was observed with increase in initial concentration and time. The maximum percentage degradation of 98.5% was found to be at 72 h, initial concentration 500 mg/l. The further increase in initial concentration or pH decreases the percentage degradation.

Effect of two variables pH (B) and temperature (A) on degradation is shown in **Fig. 8b** as temperature and pH increases, percentage degradation increased up to optimum level. As temperature increased from 30ÚC to 35ÚC and pH increased from 4 to 7 an increase in percentage degradation was observed. After that a decrease in the percentage degradation was

Table 3. ANOVA for Congo red degradation

Response	1	Percentage degradation			
	ANOVA for Response Surface Quadratic Model				
	Analysis of variance table [Partial sum of squares - Type III]				
Source	Sum ofSquares	D_f	MeanSquare	FValue	p-valueProb > F	
Model	1548.08	14	110.58	272.12	< 0.0001	Significant
A-Temperature	2.9	1	2.9	7.13	0.0174	
B-pH	0.82	1	0.82	2.02	0.176	
C-Intial Concentration of Dye	1.06	1	1.06	2.6	0.1275	
D-Time	0.43	1	0.43	1.05	0.3217	
AB	2.4	1	2.4	5.91	0.028	
AC	0.16	1	0.16	0.39	0.5398	
AD	0.9	1	0.9	2.22	0.1569	
BC	3.42	1	3.42	8.42	0.0109	
BD	0.09	1	0.09	0.22	0.6447	
CD	4.62	1	4.62	11.38	0.0042	
A^2	402.17	1	402.17	989.7	< 0.0001	
B^2	82.01	1	82.01	201.82	< 0.0001	
C^2	173.72	1	173.72	427.51	< 0.0001	
D^2	144.83	1	144.83	356.42	< 0.0001	
Residual	6.1	15	0.41			
Lack of Fit	4.92	10	0.49	2.1	0.214	not significant
Pure Error	1.17	5	0.23			
Cor Total	1554.17	29				
R^2	0.9961					
Adjusted R^2	0.9924					
Predicted R^2	0.9811					
Adeq Precision	43.882					

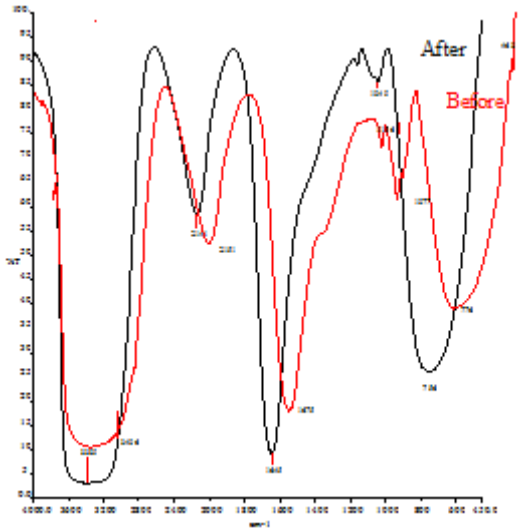


Fig. 3. FT-IR before and after degradation

observed with increase in temperature and pH .The maximum percentage degradation of 97.57% was found to be pH 7, temperature 35ÚC. The further increase in temperature or pH decreases the percentage degradation by fungal strains.

Fig. 8c shows the interactive effect on the initial concentration ratio (C) and temperature (A). As initial concentration increased from 250 to 500mg/l and temperature increased from 30ÚC to 35ÚC an increase in percentage degradation was observed. After that a decrease in the percentage degradation was observed with increase in initial concentration and temperature. The maximum percentage degradation of 97.57%was found to be temperature 35ÚC, initial concentration 500 mg/l. The further increase in initial concentration or pH decreases the percentage degradation by dual culture.

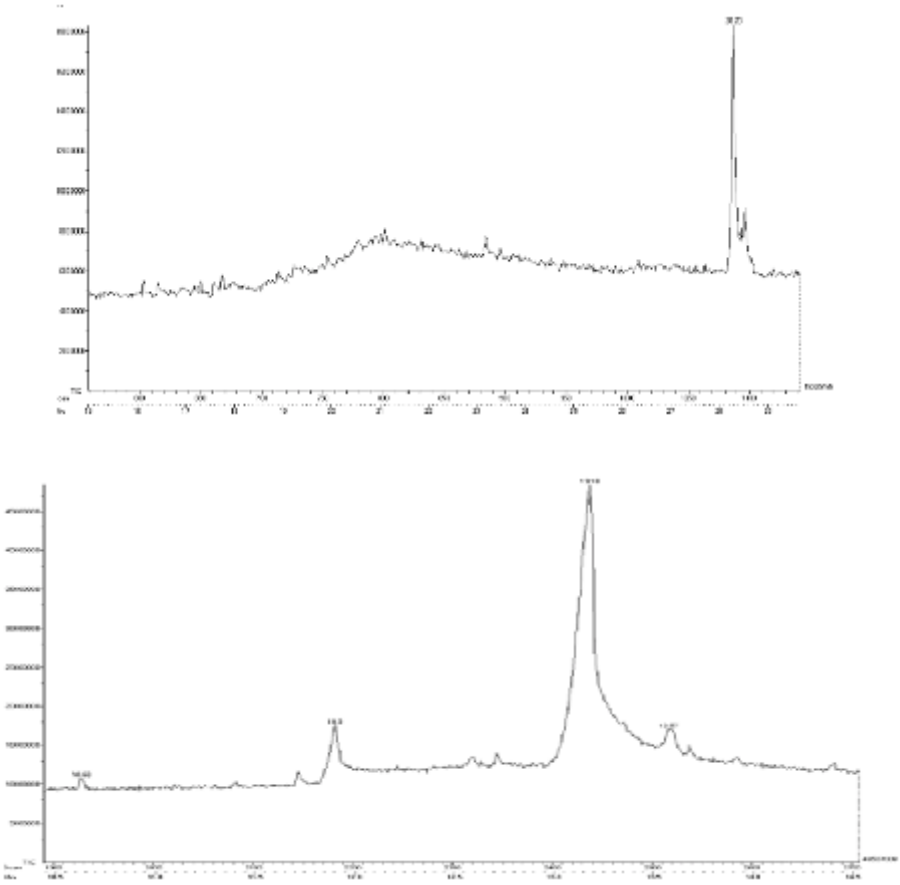


Fig. 4. GC Curve for the dye solution (a) before degradation (b) after degradation

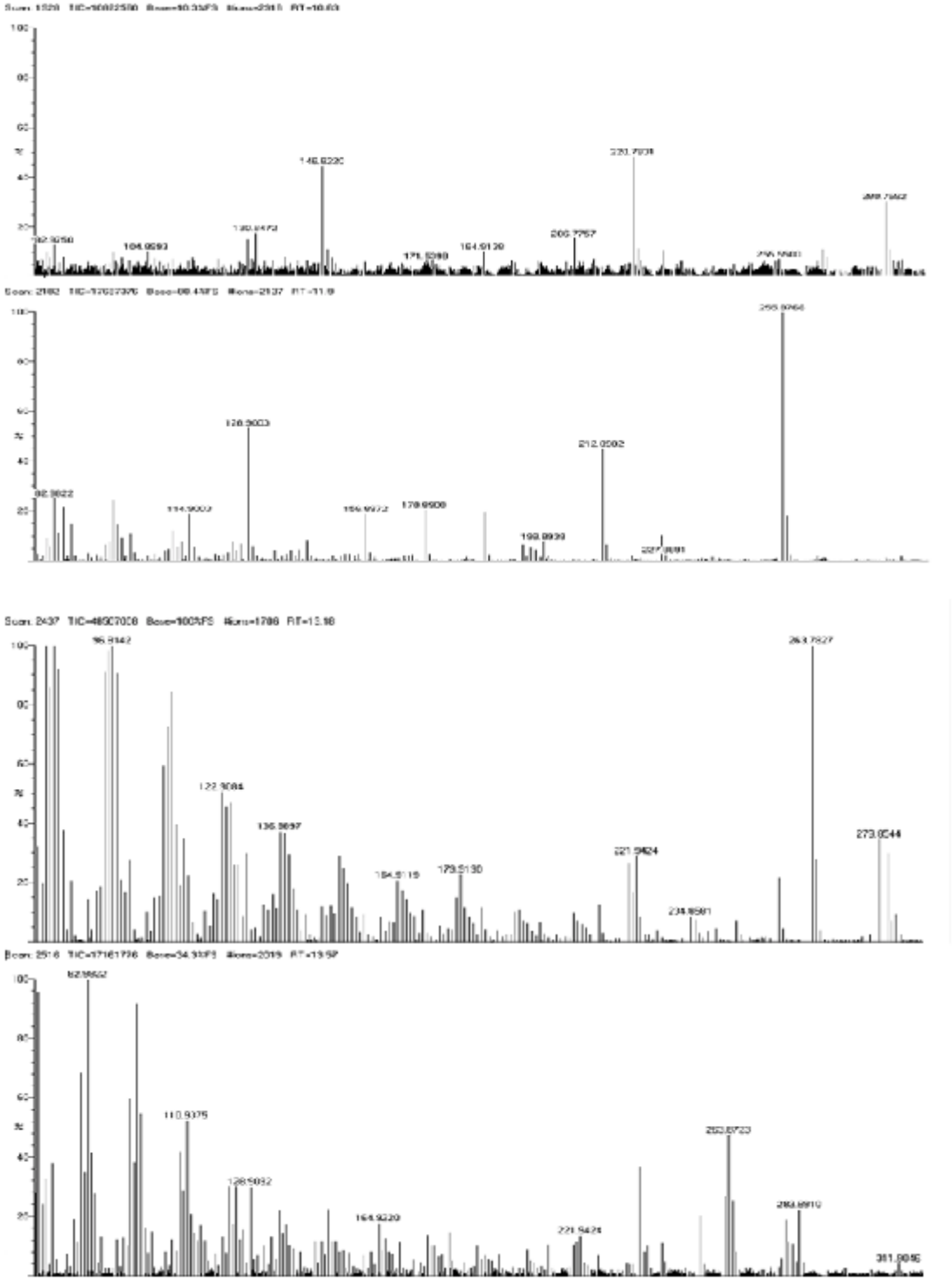


Fig. 5. Mass Spectra of the metabolites obtained from the degradaed sample

Effect of time (D) and temperature (A) was illustrated in **Fig. 8d** that as time and temperature increases, percentage degradation increased up to optimum level. As time increased from 36 to 72 h and temperature increased from 30ÚC to 35ÚC an increase in percentage degradation was observed. After that a decrease in the percentage degradation was observed with increase in time and temperature. The maximum percentage degradation of 98% was found to be temperature 35ÚC, time 72 h. The further increase in time or temperature decreases the percentage degradation by microbial consortium.

Fig. 8e shows that as initial concentration (C) and pH (B). With increase in initial concentration from 250 to 500 mg/l and pH increased from 5 to 7 an increase in percentage degradation was observed. After that a decrease in the percentage degradation was observed with increase in initial concentration and pH. The maximum percentage degradation of 96.70% was found to be pH 7, initial concentration 500 mg/l. The further increase in initial concentration or pH decreases the percentage decolourisation by microbial strains.

Fig. 8f show interactive effect of pH (B) and time (D). As time increased from 36 to 72 h and pH increased from 6 to 8 an increase in percentage degradation was observed. After that a decrease in the percentage degradation was observed with increase in time and pH. The maximum percentage degradation of 98.57%was found to be pH 7, time 72 h. The further increase in time or pH decreases the percentage degradation by fungal culture**.**

Validation of model

Validation of the statistical model was carried out by running the experiment with the initial concentration of 500mg/l, pH 7 at 35ÚC for 72 h gives 98.7% decolourisation experimentally. The linear correlation plot between the adjusted R^2 and predicted R^2 indicate that the experimental values are in good agreement with predicted values as shown. Therefore, the models were found to be adequate in representing the response data of percentage degradation the dye can be further used for analysis and prediction purposes.

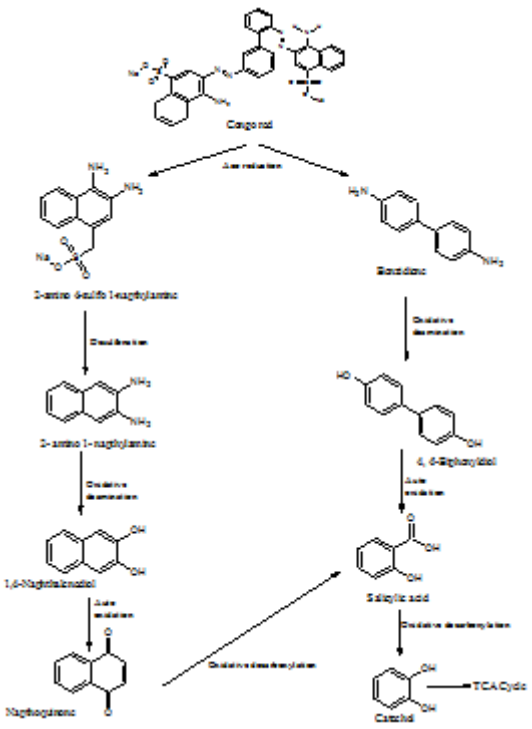


Fig. 6. Proposed pathway for degradation

CONCLUSIONS

The optimization of the decolorization of the Congo red by dual culture was studied. The maximum colour removal was achieved at initial dye concentration of 500 mg/l. The dual organism was found to decolorize the dye at broad range of

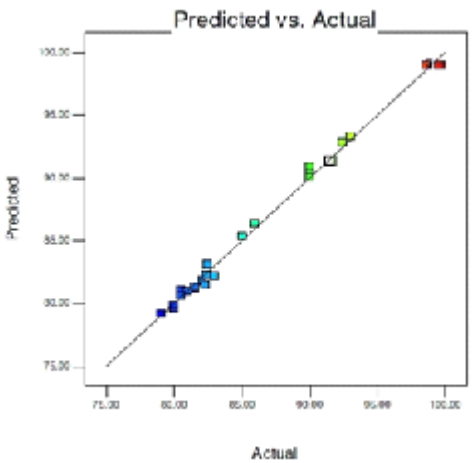


Fig. 7. Graphical comparison between experimental and predicted percentage of degradation

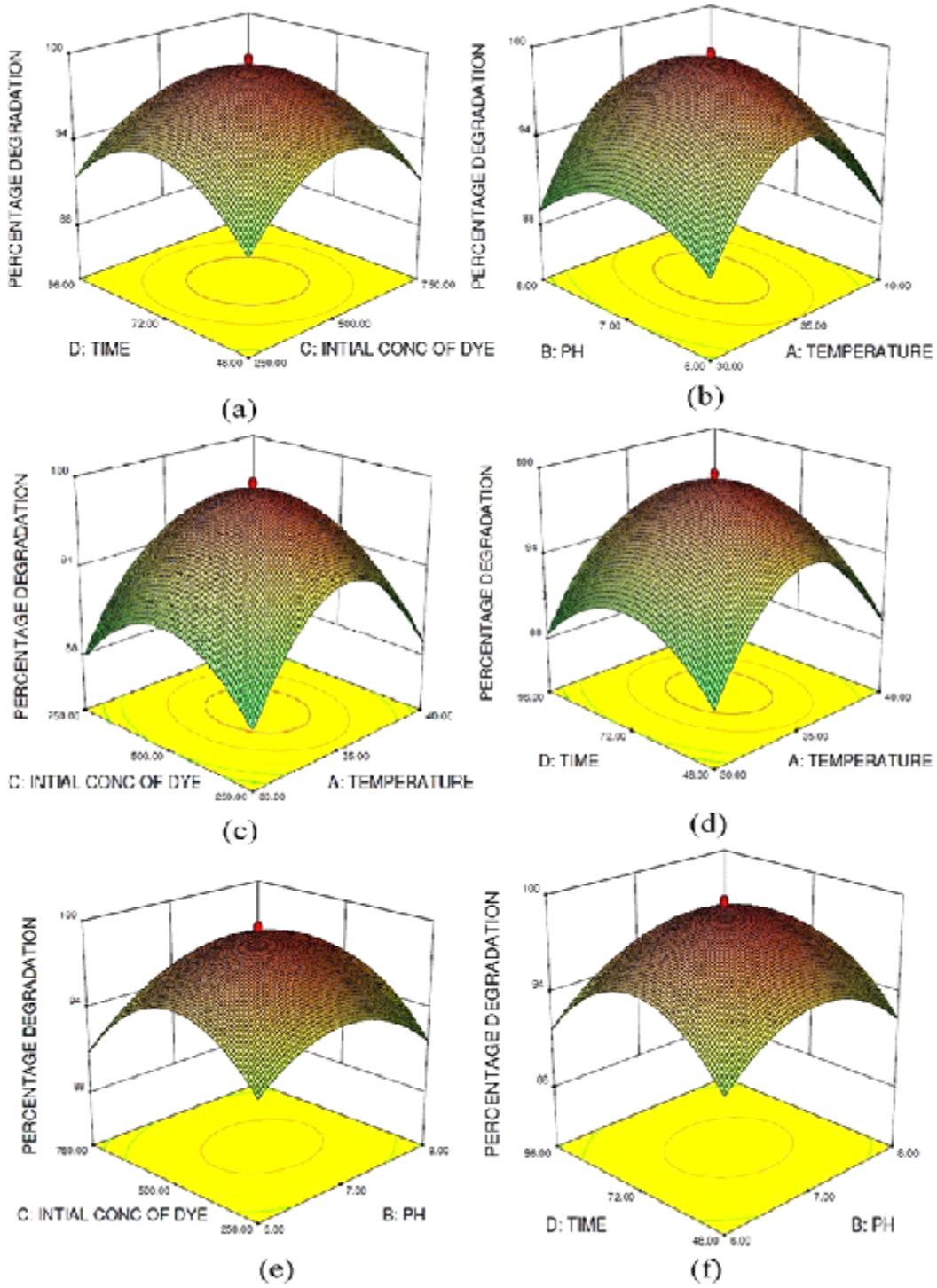


Fig. 8. (a) Interactive effect of Time & Concentration (b) effect of pH & Temperature (c) effect of Concentration & Temperature (d) effect of Time & Temperature (e) Interactive effect of Concentration & pH (f) effect of Time & pH on percentage degradation

pH, however optimum pH for dye decolorization was found to be 7. The optimum time for dye decolorization was found to be 72 h at 35ÚC. The identification of metabolites from the degradation of dye allowed the proposition of the degradation pathway. The obtained results demonstrate that the use of these microorganisms has an enormous potential to degrade the dye containing wastewater. So, these microorganisms can be used for treating textile wastewaters, particularly for water recycling.

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REFERENCES

1. Xu, M., Guo, J., Zeng, G., Zhong, X., Sun, G., Decolorization of anthraquinone dye by Shewanella decolorationis S12. *Applied Microbiology and Biotechnology,* 2006; **71:** 246.
2. Shertate, R.S, Thorat P.R., 2013. "Biodecolorization and degradation of textile diazo dye reactive blue 171 by marinobacter sp. nb-6 – a bioremedial aspect" *International Journal of Pharmacy and Biological Sciences,* 2013; **3 (1)**: 330.
3. Fu Y. and Viraraghavan T., Fungal decolorization of dye wastewaters: a review. *Bioresource Technol,* 2001; **79**: 251.
4. Sri Kumaran, N. and Dharani, G., Decolorization of Textile Dyes by White Rot Fungi Phanerocheate chrysosporium and Pleurotus sajor-caju. *Journal of Applied Technology in Environmental Sanitation,* 2011; **1(4):** 361.
5. Kaushik P, Malik A, Fungal dye decolorization: recent advances and future potential, *Environment International,* 2009; **35**: 127.
6. Aileen C. Jalandoni Buan, Anna Lynn A. Decena-Soliven, Ernelea P. Cao, Virginia L. Barraquio , and Wilfredo L. Barraquio, Characterization and Identification of Congo Red decolorizing Bacteria from Monocultures and Consortia, *Philippine Journal of Science,* 2010; **139 (1)**: 71.
7. Sathish.S, Joshua Amarnath D, Experimental Studies on Decolourization of Azo Dye in Textile Effluent by using Bio Remediation Technique. *International Journal of ChemTech Research,* 2014; **6 (12):** 5114.
8. Sivasubramanian.S, Karthick Raja Namashivayam.S, Optimization of parameters for phenol degradation using immobilized Candida tropicalis SSK01 in batch reactor, *Journal of Environmental Biology,* 2014; **35**: 531.
9. Aleksander Pavko, Fungal Decolourization and Degradation of Synthetic Dyes, Some Chemical Engineering Aspects, University of Ljubljana, Slovenia, **65**; 2011
10. Rajendra Ramasany, Hasab Abdelbagi, Muhammad Ahmed and Sundaram S. Karthik, 2012. Development of microbial consortium for the biodegradation and biodecolorization of textile effluents. *Journal of Urban and Environmental Engineering,* 2012; **6 (1):** 36.
11. Khelifi E., Bouallagui H., Touhami Y., Godon J. J. & Hamdi, M., Enhancement of textile wastewater decolourization and biodegradation by isolated bacterial and fungal strains. Desal. *Water Treatm,* 2009; **2:** 310.
12. Harshad S. Lade, Tatoba R. Waghmode, Avinash A. Kadam, Sanjay P. Govindwar, Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium, *International Biodeterioration & Biodegradation,* 2012; **72:** 94.
13. Banat, I., Nigam, P., Singh, D., Marchant, R., Microbial decolorization of textile dye containing effluents: a review. *Bioresource Technology,* 2009; **58:** 217.
14. Karunya A, Rose C, Valli Nachiyar C, Biodegradation of the textile dye Mordant Black 17 (Calcon) by Moraxella osloensis isolated from textile effluent-contaminated site, *World J Microbiol Biotechnol,* 2014; **30:** 915.
15. Prigione, V., V. Tigini, C. Pezella, A. Anastasi, G. Sannia, G.C. Varese, Decolourisation and detoxification of textile effluents by fungal biosorption**.** *Water Research,* 2008; **42** (12): 2911.
16. Lokendra Singh and Ved Pal Singh, Biodegradation of Textile Dyes, Bromophenol blue and Congored by Fungus Aspergillus Flavus. Environment & We an *International Journal of Science & Technology,* 2010; **5:** 235.
17. Shah MP, Patel KA, Nair SS, Darji AM, Exploring the strength of Pseudomonas aeruginosa ETL-1942 in decolourisation and degradation of acid orange dye to combat textile effluent: applied aspects. *OA Biotechnology,* 2013; 2**(2):** 12.
18. Amar, A., Telke, S.M., Joshi, S., Jadhav, U., Dhawal, P.T., Sanjay, P. & Goivindwar, S., 2010. Decolorization and detoxification of Congo red and textile industry effluent by an isolated

bacterium Pseudomonas sp. SU-EBT, *Biodegradation,* 2010; **21**(2) : 283.

19. Gou M., Qua Y., Zhoua J., Mab, F., Tana L. Azo dye decolorization by a new fungal isolate, Penicillium sp. QQ and fungal-bacterial cocultures. *Journal of Hazardous Materials* **170**, 314.
20. Senan R.C. & Abraham, T.E., 2004. Bioremediation of Textile Azo Dyes by Aerobic Bacterial Consortium Aerobic Degradation of Selected Azo Dyes by Bacterial Consortium, *Biodegradation,* 2004; **15(4):** 275.
21. Ramya, M., Anusha, B., Kalavathy, S., Devilaksmi, S., Biodecolourization and biodegradation of reactive blue by Aspergillus sp. *African Journal of Biotechnology*, 2007; **6:** 1441.
22. Adosinda M., Martins M., Nelson Lima, Armando J.D. Silvestre, Jo~ao Queiroz M., Comparative studies of fungal degradation of single or mixed bioaccessible reactive azo dyes, *Chemosphere*, 2003; **52:** 967.
23. Kumar Praveen G.N. and Sumangala K. Bhat, Fungal Degradation of Azo dye- Red 3BN and optimization of Physico-Chemical Parameters, *ISCA Journal of Biological Sciences*, 2012; **1**(2): 17.