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ORIGINAL PAPER

Bacteria-mediated bio-degradation of reactive azo dyes coupled with bio-energy generation from model wastewater

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Abstract

The wastewaters produced from dyeing process in textile industries contain xenobiotic mixed reactive dyes that have negative impact on ecosystem when discharged as untreated/partially treated. The present study aims to degrade mixed dyes (reactive red 21 (RR21) and reactive orange 16 (RO16)) present in wastewater by *Pseudomonas aeruginosa* 23N1. The process optimization and the effect of experimental parameters (like amount of co-substrate, pH and temperature) on process performance are investigated through response surface methodology. To understand the extent of degradation of dye compounds, metabolites extracted from treated water samples were investigated using UV–visible, FTIR and GC-MS analyses. The results reveal that the bacteria could significantly reduce ADMI value of wastewater by ~ 87% against the initial mixed dyes containing aqueous solution (50 mg/L each of RR21 and RO16 dyes). The analysis of extracted metabolites from treated water sample indicates the utilization of dye compounds as nutrient by bacteria. The bacteria might exhibit satisfactory ADMI reduction in the presence of initial ≤ 100 mg/L Cr(VI) concentration. The bio-degradation is performed under microbial fuel cell in the presence of co-substrate yeast extract and found very much promising in terms of faster ADMI reduction and energy production. The maximum output voltage generation of 790 ± 5 mV and power density 940.61 ± 5 mW/m^2 are recorded during decolourization of mixed dye-laden real wastewater in a microbial fuel cell. The bacteria studied here confirm the effective bio-decolourization of real textile wastewater.

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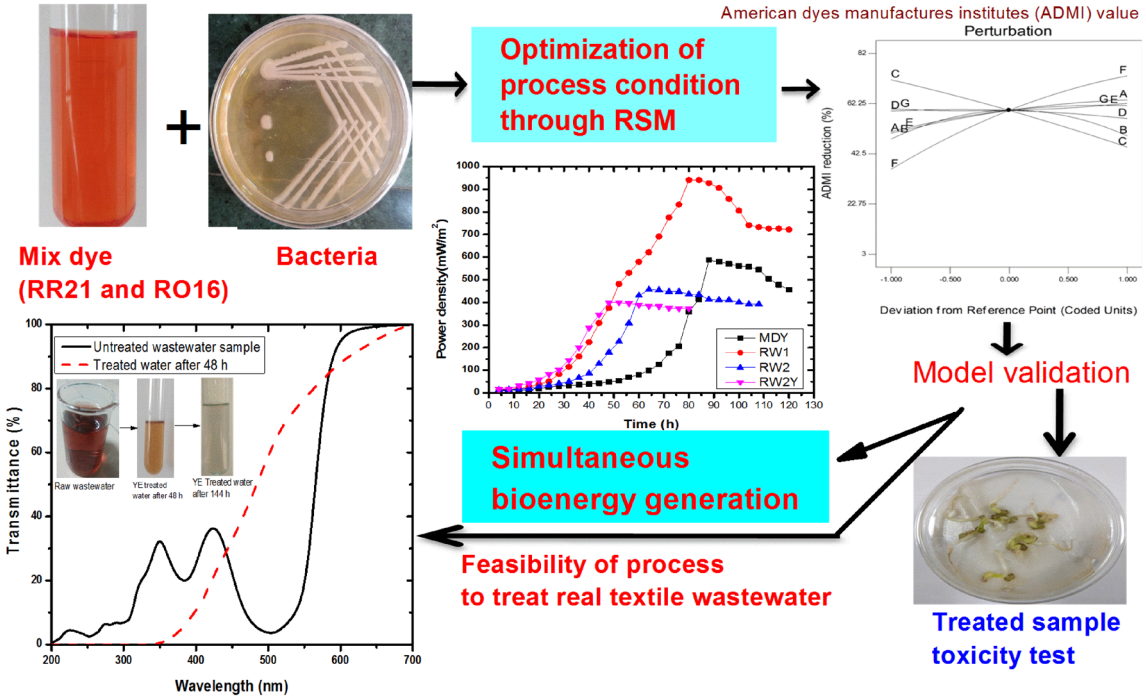
Graphic abstract



Keywords Bacteria · Wastewater treatment · Dye · Salt tolerant · Bacteria microbial fuel cell

Introduction

The colourants are being used since prehistoric time for artistic, fibre additive, communicative and esthetical purposes (Paz et al. 2017). Earlier, colourants were extracted from flowering plants, alga and insects, which are considered as renewable, sustainable eco-friendly sources (Shahid et al. 2013). However, the ever increased demand of colourants has led to excessive exploitation of natural resources. Moreover, the biological colourants have certain drawbacks like less stable, low brightness and insufficient fixation capacity with fibres, which could be accomplished by the use of synthetic dyes (Chaudhari et al. 2017). The synthetic dyestuffs belong to major complex organic groups like azo, triphenylmethane and anthraquinone, which differ in physical and chemical properties related to their applications (Mishra and Maiti 2018a). Among the colourants, reactive azo dyes are widely used in textile dyeing process due to their better stability and fixation property with fibres (Maqbool and Abid 2016). Considerably, more than 15% of these reactive azo dye molecules could not fix with fibres and lost through effluents generated from industrial dying processes (Shafqat et al. 2017; Hameed and Ismail 2018). These dyes are carcinogenic and mutagenic, which have several detrimental effects on the surrounding ecosystem and human health on its exposure (Roy et al. 2018). Along with dyestuffs, the textile effluent contains high concentration of salt (sodium chloride ~4 g/L) and chromate (Cr(VI)) ions that are used to improvise the binding of dye molecules with fibres (Liu et al. 2017; Yaseen and Scholz 2019). Therefore, effective wastewater treatment technologies need to be investigated for detoxification and decolourization of such effluents loaded with dyes, salt and heavy metal ions. The conventional treatment processes are found to be insignificant for decolourization of synthetic reactive dyes, because these complex compounds might easily pass through this system and remain unaffected or partially altered (Keskin et al. 2015). Currently, the biological process involving with biotic organisms like bacteria, fungi and plants is used to degrade or detoxify the different dye compounds from wastewater (Huang et al. 2015; Hussain et al. 2017). Although this process is eco-friendly, inexpensive and efficient to treat textile wastewater, but fungi and plants show certain drawbacks as those require long period for satisfactory growth and bio-degradation of dyes (Chakraborty et al. 2018). Comparatively, the bacterial species could colonize and degrade wide classes of dyestuffs within short period of time and have better adaptability in adverse environments (Ma et al. 2014). The bacterial cells might utilize dye compounds as only nutritional source (Mishra and Maiti 2019a). The

metabolic degradation of reactive azo dyes by bacterial species is followed through mineralization of dye molecules that reduces the chance of production of toxic secondary amines (Naseer et al. 2016). The bacterial enzymes like azoreductase, laccase and peroxidase are commonly involved in azo dye degradation (Telke et al. 2015; Mishra and Maiti 2019b, c). In most of the reported studies, azoreductase has been found as chief enzyme involved in cleavage of azo bonds (N = N) (Chacko and Subramaniam 2011). Moreover, the satisfactory activity of bacteria could be achieved under the optimal process condition. Therefore, the process parameters like temperature, concentration of nutrients and dissolve oxygen content should be optimized to enhance the growth and performance of bacterial cells for dye degradation (Garg et al. 2015). In this regard, response surface methodology (RSM) is commonly used to explore the integrative effects of process parameters on the interested process response.

Among all bacterial species investigated for azo dye degradation, *Pseudomonas sp.* has been found as more efficient bio-remediation agent (Bedekar et al. 2014; Mishra and Maiti 2018b). In recent trend, the industries demand of hybrid wastewater treatment and energy generation from the same treatment process might reduce the cost of treatment and provide energy for various industrial operations (Ilamathi and Jayapriya 2018). In this regard, the microbial fuel cells (MFCs) are being investigated for hybrid wastewater treatment with energy recovery (Lovley 2006; Miran et al. 2016). The microorganism promotes the oxidative conversion of chemical energy into electrical energy. The bio-conversion of organic compounds to bio-energy could vary due to physicochemical composition, concentration of the organic/inorganic compounds and source of wastewater (Mohan et al. 2010).

In this study, *P. aeruginosa* 23N1 is investigated to decolourize the wastewater containing mixed azo reactive orange 16 (RO16) and reactive red 21 (RR21) dyes. Meanwhile, these toxic reactive azo dyes are generally used for colouring the fabrics in textile industries. Previously, optimization of process parameters for effective decolourization of individual RR21 (Mishra and Maiti 2018c), and RO16 (Mishra and Maiti 2018d) dye has been reported from our laboratory. In this study, the optimization of process condition to decolourize the wastewater containing mixed RR21 and RO16 dyes is carried out using RSM-based central composite design (CCD) method. Progressing further, it is the first kind of the study that reports the degradation mechanism of individual and mixed reactive RR21 and RO16 dyes by strain 23N1, which is elaborately discussed on the basis of analysis of end metabolites through FTIR and GC-MS techniques. Furthermore, concentration of total dissolved carbon (TOC) of untreated and treated wastewaters containing dye molecules is estimated to verify the extent of utilization of dye as carbon source by bacteria. The effect of Cr(VI) on performance of remediation system is investigated to ascertain a good response of bacteria even in the presence of toxic heavy metal ions. The two-chambered microbial fuel cell (MFC) is examined to generate the energy during mixed dye-laden wastewater treatment process, which could be a useful application for reduction in overall operating cost of water treatment. Thus, bio-treatment of textile wastewater as well as production of electrical energy could be a promising approach for wastewater treatment. Various co-substrates have been used to promote decolourization of dye solutions, but role of co-substrates in the process bio-decolourization coupled with microbial fuel cell is not explored in the literature. In this study, the role of co-substrate in power generation from MFCs has been explored. Further, few studies might report the bio-decolourization using wastewater containing mixed dyes and heavy metal ions. The toxicity analysis of the end products of bio-treatment is performed by estimating the germination rate of *Vigna radiata* seeds, to assess the toxicity of treated water. The capability of bacteria to decolourize real textile wastewater is assessed to ensure its feasibility to treat industrial effluents.

Materials and methods

Dyes and chemicals

The reactive azo dye chemicals used in this study, namely reactive orange 16 (RO16, CAS number 12225-83-1), reactive red 21 (RR21, CAS number 11099-79-9) and 99% $K_2Cr_2O_7$, were provided by Sisco Research Laboratories Pvt. Ltd., Delhi, India. The other chemicals (such as NaCl and yeast extract) were supplied from Himedia Laboratories, Mumbai, India. The individual dye stock solution of 1000 mg/L was prepared by dissolving 1 g of dye powder in 1000 mL double distilled water (DDW), while the stock solution of Cr(VI) was prepared by dissolving 2.835 g of $K_2Cr_2O_7$ in 1 L DDW. The mixed dyes/Cr(VI) solution for respective experimental steps was prepared by mixing and diluting the required volume of each dye stock solution in 1000 mL DDW, for example: 50 mL of RR21 dye stock solution is mixed with the same volume of RO16 dye stock solution and diluted to 1000 mL DDW, assuming individual dye concentration would be obtained as 50 mg/L each. The initial pH of experimental dye solutions was maintained by using 1 N solutions of NaOH and HCl.

Bacterial culture conditions

The pure culture of bacteria *P. aeruginosa* 23N1 is supplied by National Centre for Cell Sciences (NCCS), Pune, India. The bacterial cells were cultured in HK34b nutrient agar medium. The bacterial inoculums for experimental

study were routinely cultured in Erlenmeyer flask (volume 100 mL) supplemented with 50 mL nutrient broth (yeast extract 0.35% (w/v) and peptone 2% (w/v)) and incubated at 30 °C for 24 h under orbital shaking at 150 rpm (Mishra and Maiti 2018c). This bacterial inoculation volume is used in % (v/v) for subsequent experiments. The experiments for optimization of dye decolourization process condition were carried out in 100-mL Erlenmeyer flask and incubated for 48 h under static-isothermal condition.

Instrumental analysis and data evaluation

It should be noted that there is no standard analytical technique available to estimate the individual dye concentration from mixed dyes solution. In this regard, American dyes manufactures institutes (ADMI) index value of mixed dyes solution is evaluated, which is based on UV–visible transmittance data of coloured solution. The samples were collected from cultured experimental flask and then centrifuged at ~ 10080 RCF for 20 min to separate the bacterial biomass from supernatant. The supernatant solution was analysed through UV–visible spectroscopy to obtain the transmittance of residual dye solution. The change in ADMI value (in %) to treated dye aqueous solution compared to untreated dye solution signifies the extent of decolourization, which can be calculated by using the following formula:

$$\text{ADMI reduction } (\%) = \frac{\text{ADMI}_{\text{in}} - \text{ADMI}_{\text{f}}}{\text{ADMI}_{\text{in}}} \times 100\%$$

where ADMI_{in} represents the initial ADMI value of untreated dyes solution and ADMI_{f} denotes the final ADMI value of treated dyes solution. The diphenylcarbazide method (given as per APHA 2011) is followed to analyse the concentration of Cr(VI) at 540 nm (λ_{max}) in the experimental samples using UV–visible spectroscopy. TOC concentration of the experimental dye solutions was analysed using TOC analyser (model TOC-L, Shimadzu). To confirm the degradation of dye molecules, the experimental solutions were analysed using FTIR and GC-MS techniques. In this regard, supernatant solution was mixed in equivalent volume of ethyl acetate to extract the metabolites. This extract was separated and dried over anhydrous Na_2SO_4. The ethyl acetate was removed to obtain powder metabolites using rotary evaporator at 72 °C. Thereafter, the extracted metabolites powder was dissolved in methanol (HPLC grade) for further analysis. These prepared solutions were analysed using FTIR and GC-MS.

Toxicity analysis of metabolites

The dye-contaminated untreated and treated water samples were used to germinate the seeds of *V. radiata*, under laboratory condition. The healthy seeds were collected from local market, which were primarily washed with DDW before use. These seeds were laid on a filter paper dipped in 4 mL of the corresponding water samples, in petri plates (size 100 × 15 mm). The seeds containing petri plates were kept in the dark at temperature 24 ± 1 °C for 120 h, to promote the germination of seeds. In addition, an equal number of seeds were grown in the control experiment (only DDW) to compare the analysis. The phytotoxicity of treated water sample was estimated in relation to normal seed germination rate using the methodology as reported by Kurade et al. (2016) and Priac et al. (2017).

Statistical analysis

The optimization of process condition for mixed dyes decolourization was executed based on experimental matrix obtained through RSM-based CCD method using Design-Expert software (version 7.1.6, Stat-Ease Inc., Minneapolis, USA). Small type CCD with 5 centre points, one axial point, one factorial point, along with block value of 1, and alpha value of 2 were selected in this study. For optimization of process condition, the analysis of variance (ANOVA) was performed for experimental decolourization response data of experimental trials. The numeric parameters were varied over five levels as: alpha − 2 and + 2 (axial point), one centre point, and + 1 and − 1 (factorial points), as shown in Table 1.

Bio-energy generation

The generation of bio-energy during decolourization of mixed reactive dye-laden wastewater by strain 23N1 is carried out in "H"-shaped MFC chamber. This MFC was designed using 0.5-cm-thick Plexiglas material. Two cylindrical chambers of equal working volume of 900 mL with dimension (15 cm height × 10 cm diameter) were used in MFC, as shown in Fig. S6. Two graphite rods of dimension (17 cm length × 1.2 cm diameter) were used electrodes in MFC. The proton exchange membrane "Nafion-117 from DuPont" was used in this study. The membrane was

Table 1 Process parameters with their minimum and maximum limits used in CCD to construct experimental steps in this study

Parameter	Units	− 1 level	+ 1 level	− Alpha	+ Alpha
pH		5	9	3	11
Temperature		20	40	10	50
RR21	mg/L	50	100	25	125
RO16	mg/L	50	100	25	125
Salt	g/L	2	6	0	8
Yeast extract	g/L	3	9	0	12
Inoculation volume	%	3	8	0.5	10.5

pre-treated in solution of H_2O_2 (3%) for 1 h at 80 °C and then in solution of 0.5 M H_2SO_4 for 1 h at 80 °C. Open-circuit voltage was monitored using digital multimeter (Mashtech India Pvt. Ltd.), which was connected to 100 Ω external resistances (R) in series. Power density was calculated using the following equation:

$$\text{Power density (mW/A)} = \frac{V^2/R}{A}$$

where V signifies the voltage (mV); A signifies the total surface area of the electrode (m^2); and R signifies the resistance. The real textile wastewater used in this study was collected from textile industry located in the district Saharanpur of Uttar Pradesh, India. The schematic diagram of overall experimentation method adopted to carry out this study is shown in Fig. 1.

Results and discussion

In the reported literature, the decolourization of individual RR21 (Mishra and Maiti 2018c) and RO16 (Mishra and Maiti 2018d) dyes by strain 23N1 has been found to be effective under static-isothermal condition. However, these dyes are found in mixed state in the wastewater generated from dyeing industries. Considering these reported findings, the decolourization of mixed (RR21 and RO16) dyes and optimization of process condition are carried out under static-isothermal condition.

Optimization of dye decolourization process condition

To investigate the ADMI reduction of mixed dyes containing aqueous solutions and to study the effect of process parameters on the performance of bio-decolourization, the experimental scheme is produced through CCD, which exhibits total 41 experimental trials, as shown in Table 2. The lower and higher limits of process parameters are defined as per the result obtained during the preliminary study (reported in Mishra and Maiti 2018c, d). The ADMI reduction percentages for all experimental trials are obtained in the range 3.5% (lowest) to 81.1% (highest). These response data are used in analysis of variance (ANOVA) to assess the reliability of derived quadratic model equation to predict the ADMI reduction percentage of wastewater containing mixed dyes under defined process condition. The experimental data are found to be strongly correlated with model-predicted values of respective experimental trial, with correlation coefficient (R^2) value of 0.998 that ensures the well fitness of data for practical use. The ANOVA analysis of experimental data is shown in Table 3, which signifies that the response model is statistically significant with F value and p value of 59.08 and 0.0001, respectively. The adjusted R^2 value, predicted R^2 and adequacy precision value of model are obtained as 0.981, 0.953 and 25.04, respectively, which indicate better

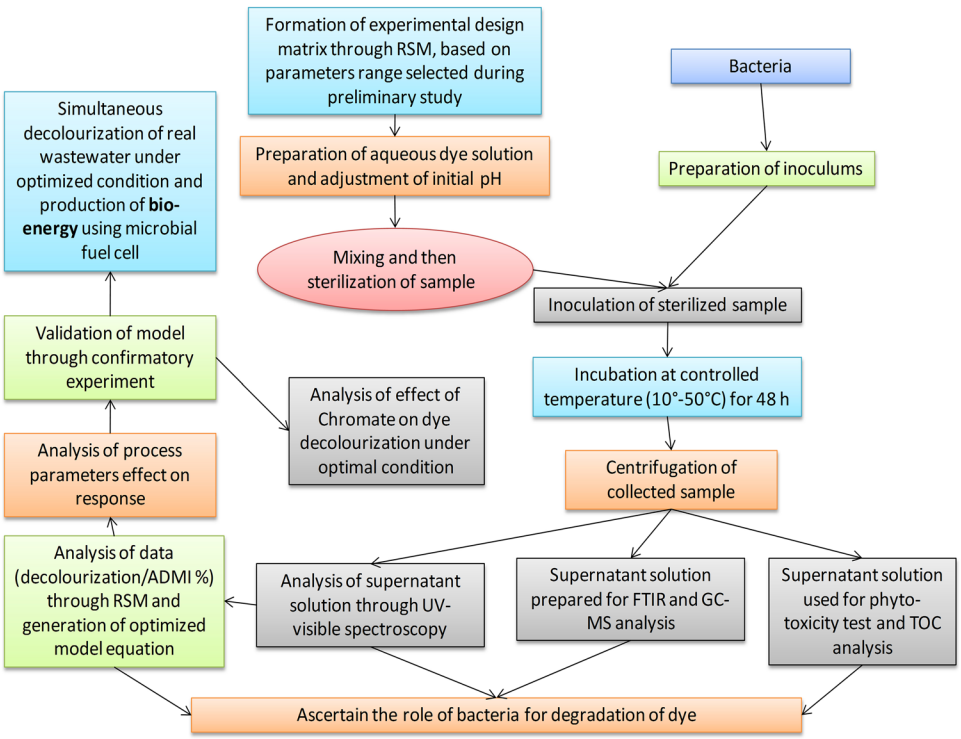


Fig. 1 Schematic flow diagram of the experimental procedure

Table 2 Experimental steps produced through CCD for optimization of mixed dyes decolourization process with respective ADMI reduction value

S. No.	pH	Temperature (°C)	RR21 (mg/L)	RO16 (mg/L)	Salt (g/L)	Yeast extract (g/L)	Inoculation volume (%)	ADMI reduction value (%)	
								Experimental	Predicted
1	9	40	100	50	6	9	8	56.81	56.78
2	9	40	50	100	2	9	3	67.22	67.19
3	9	40	50	100	6	3	8	69.40	69.36
4	9	20	100	100	2	9	8	20.11	20.08
5	5	40	100	50	2	9	3	52.91	52.88
6	9	20	50	50	6	3	3	11.80	11.77
7	5	40	50	100	6	3	8	72.38	72.35
8	9	40	100	100	2	9	3	53.89	53.86
9	9	20	100	50	2	3	8	5.00	4.97
10	5	20	50	50	6	9	3	33.06	33.03
11	5	40	50	100	2	3	3	57.45	57.41
12	9	40	100	50	6	3	3	43.90	43.84
13	9	20	50	100	2	3	3	6.84	6.81
14	5	20	100	50	2	3	8	4.75	4.72
15	5	40	50	50	2	9	8	71.15	71.12
16	9	20	50	50	2	9	8	25.19	25.16
17	5	40	50	50	6	9	8	69.36	69.33
18	9	20	50	100	6	9	8	41.87	41.84
19	5	20	100	100	6	3	8	9.38	9.35
20	5	20	100	100	6	9	3	20.88	20.82
21	5	40	100	100	2	3	8	24.14	24.11
22	5	20	50	50	2	3	3	9.09	9.06
23	3	30	75	75	4	6	5.5	36.55	36.64
24	11	30	75	75	4	6	5.5	62.81	62.91
25	7	10	75	75	4	6	5.5	15.60	15.70
26	7	50	75	75	4	6	5.5	18.06	18.15
27	7	30	25	75	4	6	5.5	81.07	81.16
28	7	30	125	75	4	6	5.5	28.13	28.22
29	7	30	75	25	4	6	5.5	55.48	55.57
30	7	30	75	125	4	6	5.5	49.56	49.65
31	7	30	75	75	0	6	5.5	36.13	36.22
32	7	30	75	75	8	6	5.5	56.57	56.66
33	7	30	75	75	4	0	5.5	3.49	3.58
34	7	30	75	75	4	12	5.5	76.98	77.07
35	7	30	75	75	4	6	0.5	64.07	64.16
36	7	30	75	75	4	6	10.5	68.42	68.51
37	7	30	75	75	4	6	5.5	62.81	59.56
38	7	30	75	75	4	6	5.5	64.07	59.56
39	7	30	75	75	4	6	5.5	56.97	59.56
40	7	30	75	75	4	6	5.5	59.05	59.56
41	7	30	75	75	4	6	5.5	55.48	59.56

Table 3 Analysis of variance of mixed dyes decolourization experimental response data

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*p* value Prob > *F*	
Model	22602.49	35	645.79	59.08	0.0001	Significant
A:pH	344.99	1	344.99	31.56	0.0025	
B:temperature	3.02	1	3.02	0.28	0.6214	
C:RR21	1401.35	1	1401.35	128.21	< 0.0001	
D:RO16	17.50	1	17.50	1.60	0.2615	
E:salt	208.79	1	208.79	19.10	0.0072	
F:yeast extract	2700.07	1	2700.07	247.04	< 0.0001	
G:inoculation volume	9.49	1	9.49	0.87	0.3942	
AB	269.12	1	269.12	24.62	0.0042	
AC	591.27	1	591.27	54.10	0.0007	
AD	16.58	1	16.58	1.52	0.2729	
AE	586.68	1	586.68	53.68	0.0007	
AF	9.68	1	9.68	0.89	0.3898	
AG	230.48	1	230.48	21.09	0.0059	
BC	47.93	1	47.93	4.39	0.0904	
BD	2.71	1	2.71	0.25	0.6399	
BE	233.35	1	233.35	21.35	0.0057	
BF	9.91	1	9.91	0.91	0.3847	
BG	167.32	1	167.32	15.31	0.0113	
CD	49.67	1	49.67	4.54	0.0862	
CE	154.68	1	154.68	14.15	0.0131	
CF	35.59	1	35.59	3.26	0.1310	
CG	2.35	1	2.35	0.22	0.6624	
DE	44.02	1	44.02	4.03	0.1010	
DF	2.19	1	2.19	0.20	0.6732	
DG	0.00	1	0.00	0.00	0.9938	
EF	0.91	1	0.91	0.08	0.7843	
EG	33.62	1	33.62	3.08	0.1398	
FG	689.07	1	689.07	63.04	0.0005	
A^2	191.44	1	191.44	17.52	0.0086	
B^2	3629.49	1	3629.49	332.07	< 0.0001	
C^2	47.34	1	47.34	4.33	0.0919	
D^2	96.50	1	96.50	8.83	0.0311	
E^2	343.73	1	343.73	31.45	0.0025	
F^2	738.87	1	738.87	67.60	0.0004	
G^2	91.54	1	91.54	8.38	0.0340	
Residual	54.65	5	10.93			
Lack of fit	0.21	1	0.21	0.02	0.9080	Not significant
Pure error	54.44	4	13.61			
Correlation total	22657.13	40				

accuracy of model for prediction of ADMI reduction percentage of mixed reactive dyes solution.

The major influential model terms are found as *A*, *C*, *E*, *F*, *AE*, *AC*, *AB*, *AG*, *BE*, *BG*, *CE*, *FG*, A^2, B^2, F^2, D^2, E^2 and G^2. The quadratic model equation produced through multiple regression analysis of experimental response data is as follows:

$$\begin{aligned}\text{ADMI reduction (\%)} =& 59.56 + 6.57A + 0.61B - 13.24C - 1.48D + 5.11E + 18.37F \\ &+ 1.09G - 11.02AB + 10.79AC - 1.78AD + 22.78AE - 2.76AF \\ &- 10.01AG - 6.23BC - 1.48BD + 9.71BE - 6.15BF - 7.49BG \\ &+ 6.53CD + 11.47CE + 3.93CF - 3.02CG - 7.31DE - 1.25DF \\ &- 0.049DG + 1.26EF + 9.97EG - 28.89FG - 2.45A^2 - 10.66B^2 \\ &- 1.22C^2 - 1.74D^2 - 3.28E^2 - 4.81F^2 + 1.69G^{2.}\end{aligned}$$

The diagnostic plots give better understanding of the feasibility of model and are shown in Fig. S1 of supplementary information (SI). Moreover, the insignificant model terms from the above equation are ignored through backward elimination regression method and alpha out value 0.1. The resultant model equation is obtained as follows:

$$\begin{aligned}\text{ADMI reduction (\%)} =& 59.59 + 5.86A + 0.039B - 12.12C - 1.61D + 6.35E + 17.23F \\ &+ 1.05G - 13.26AB + 10.36AC + 22.41AE - 7.39AG - 6.61BC \\ &+ 9.42BE - 4.15BF - 5.08BG + 5.14CD + 13.52CE + 2.73CF \\ &- 5.66CG - 9.97DE + 8.82EG - 29.72FG - 2.46A^2 - 10.67B^2 \\ &- 1.23C^2 - 1.75D^2 - 3.29E^2 - 4.82F^2 + 1.68G^2\end{aligned}$$

During the regression analysis, the coefficient of factor F is obtained as 17.23, which is highest among all individual process parameters, shown in Table S1 of SI. The insignificant factors are removed through backward elimination regression method using alpha value of 0.1 to exit, and the modified model equation is obtained as:

$$\begin{aligned}\text{ADMI reduction (\%)} =& 59.59 + 5.86A + 0.039B - .12C - 1.61D + 6.35E + 17.23F \\ &+ 1.05G - 13.26AB + 10.36AC + 22.41AE - 7.39AG - 6.61BC \\ &+ 9.42BE - 4.15BF - 5.08BG + 5.14CD + 13.52CE + 2.73CF \\ &- 5.66CG - 9.97DE8.82EG - 29.72FG - 2.46A^2 - 0.67B^2 \\ &- 1.23C^2 - 1.75D^2 - 3.29E^2 - 4.82F^2 1.68G^2\end{aligned}$$

Perturbation plot shows the steeper increment in the slope of factor *F*, as shown in Fig. S1f of SI, which reveals that the amount of yeast extract has major influence on ADMI reduction of mixed reactive azo dyes solution. However, the decrement in perturbation slope and negative coefficient values of factor *C* (− 12.12) and *D* (− 1.61) reveal that the increase in dye concentration significantly reduces the response. This might be due to the suppressed growth and activity of bacteria at higher toxicity level of medium. The pH (factor *A*) and salinity (factor *E*) of the medium have shown positive effect on the response (coefficient values of 5.86 and 6.35, respectively), while among the interactive terms, factor *AE* has shown the highest positive coefficient of 22.41.

The interactive effect of model terms could be better analysed through contour plots constructed through ANOVA analysis as shown in Fig. 2. From Fig. 2a, it is revealed that the strain 23N1 is salt tolerant and exhibits higher dye decolourization even in alkaline medium, which has proved here as a beneficial characteristic (capability to treat alkaline textile wastewater) of biotic agent. The significant elliptical plot of interaction between salt and temperature is shown in Fig. 2b, which signifies that these bacteria express its capability to reduce ADMI value under salty environment, when incubation temperature of medium is maintained around 30 °C (suitable temperature for microbial growth and activity).

Similar interactive trend is obtained between pH and temperature, as shown in Fig. 2c. These illustrations are reinforced by interactive effect of inoculation volume with pH and temperature on ADMI reduction percentage as shown in Fig. 2d, e, respectively. It could be observed from Fig. 2d, e that the ADMI reduction percentage increases with decrease in inoculation volume at alkaline pH and at the temperature range of 30–35 °C. This signifies that the small inoculation volume (~ 3%) could be sufficient to achieve higher ADMI reduction in alkaline culture medium, when incubated at the temperature range of 30–35 °C. The co-substrate in the culture medium enhances the bacterial growth and its activity (Imran et al. 2016). The requirement of lower amount of co-substrate reduces the operational cost of treatment. The interaction behaviour of inoculation volume and yeast

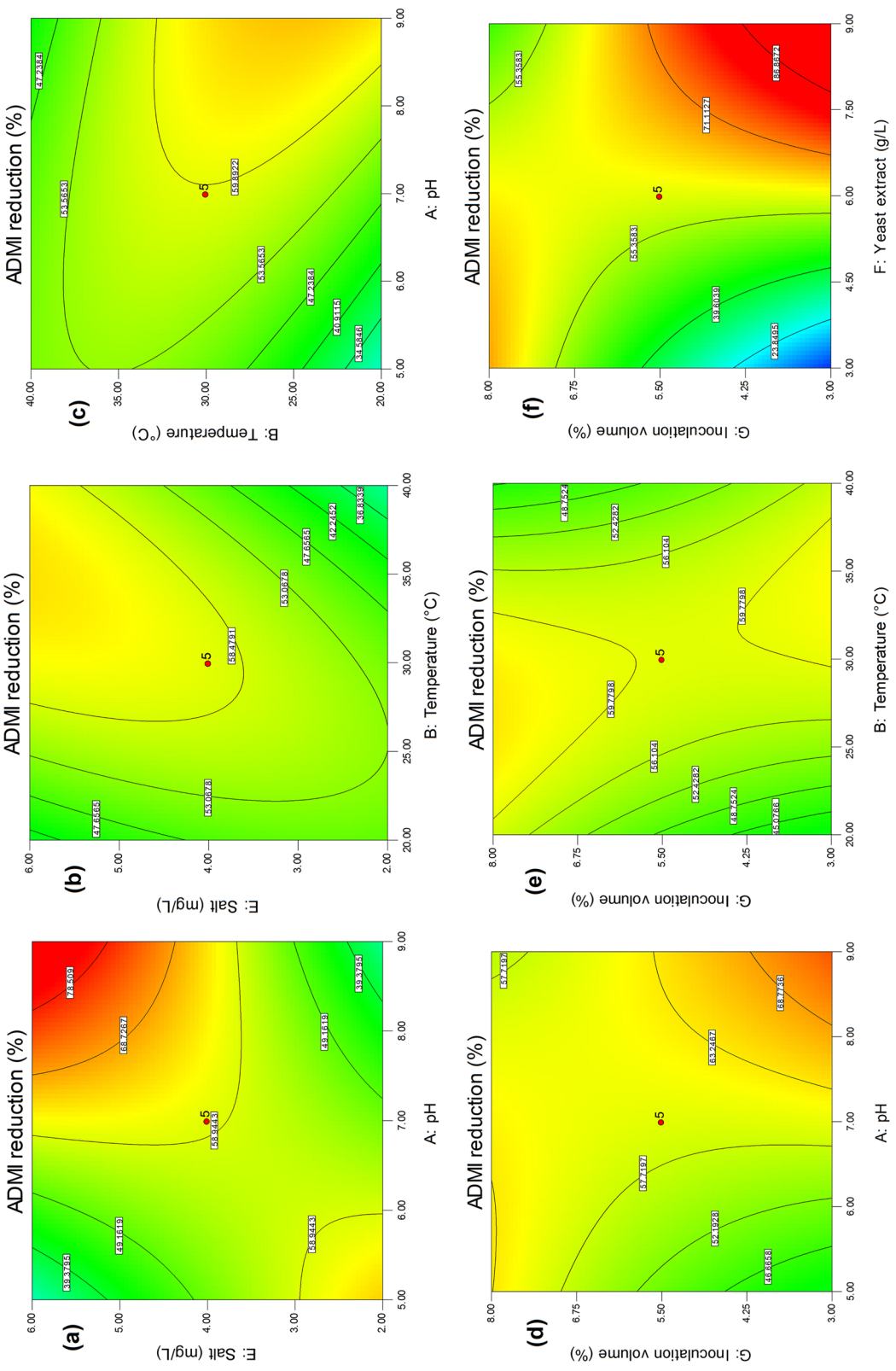


Fig. 2 Contour plot of interactive process parameters: **a** pH and salt; **b** temperature and salt; **c** pH and temperature; **d** pH and inoculation volume; **e** temperature and inoculation volume; **f** yeast extract and inoculation volume

Table 4 Process condition of model confirmatory experiments and response

S. no.	pH	Temperature (°C)	RR21 (mg/L)	RO16 (mg/L)	Salt (g/L)	Yeast extract (g/L)	Inoculation volume (%)	ADMI reduction value (%)	
								Predicted	Experimental
1	5.13	40	50	75	4.18	8.41	7.41	79.5	79.9 ± 0.5
2	8.59	40	50	75	5.89	6.95	3.24	74.37	74.6 ± 0.4
3	8.78	40	75	50	3.26	8.98	3.56	77.72	77.1 ± 0.4
4	8.96	40	75	50	5.79	6.39	5.26	74.18	75.3 ± 0.5
5	8.31	40	50	50	5.62	7.49	5.63	81.55	80.8 ± 0.5

extract is represented in Fig. 2f, which reveals that ADMI reduction percentage increases with increment in amount of yeast extract, which is supplied as co-substrate in dye decolourization medium. In a culture condition, the low ADMI reduction response of system with large inoculation volume might be due to the achievement of early metabolic and growth saturation stage by bacteria without consuming dye molecules as food supplement.

The suitability of quadratic model equation for prediction of ADMI reduction percentage of mixed RR21 and RO16 dyes solution in a given condition is confirmed through the confirmatory experiments. A total of five confirmatory experiments are investigated to compare with the corresponding model-predicted response values, which are enlisted in Table 4. The actual experimental ADMI response data are found in strong correlation with the corresponding model-predicted values.

Degradation of reactive azo dyes

The mechanism of dye decolourization is investigated through the analysis of metabolites extracted from treated dye water samples and compared with the corresponding raw solutions. Initially, the decolourization of dye is confirmed through analysis of change in UV–visible absorbance spectra between treated and untreated dye solutions used to validate derived quadratic model for RR21 decolourization (confirmatory experiments 1, reported by Mishra and Maiti 2018c) and RO16 dye decolourization (confirmatory experiments 2, reported by Mishra and Maiti 2018d) as shown in Fig. 3a, b, respectively. It could be clearly observed that the absorbance peak at maximum wavelength of RR21 (λ_{max} at 537 nm) and RO16 dye (λ_{max} at 493 nm) obtained during analysis of untreated dye solution is completely diminished

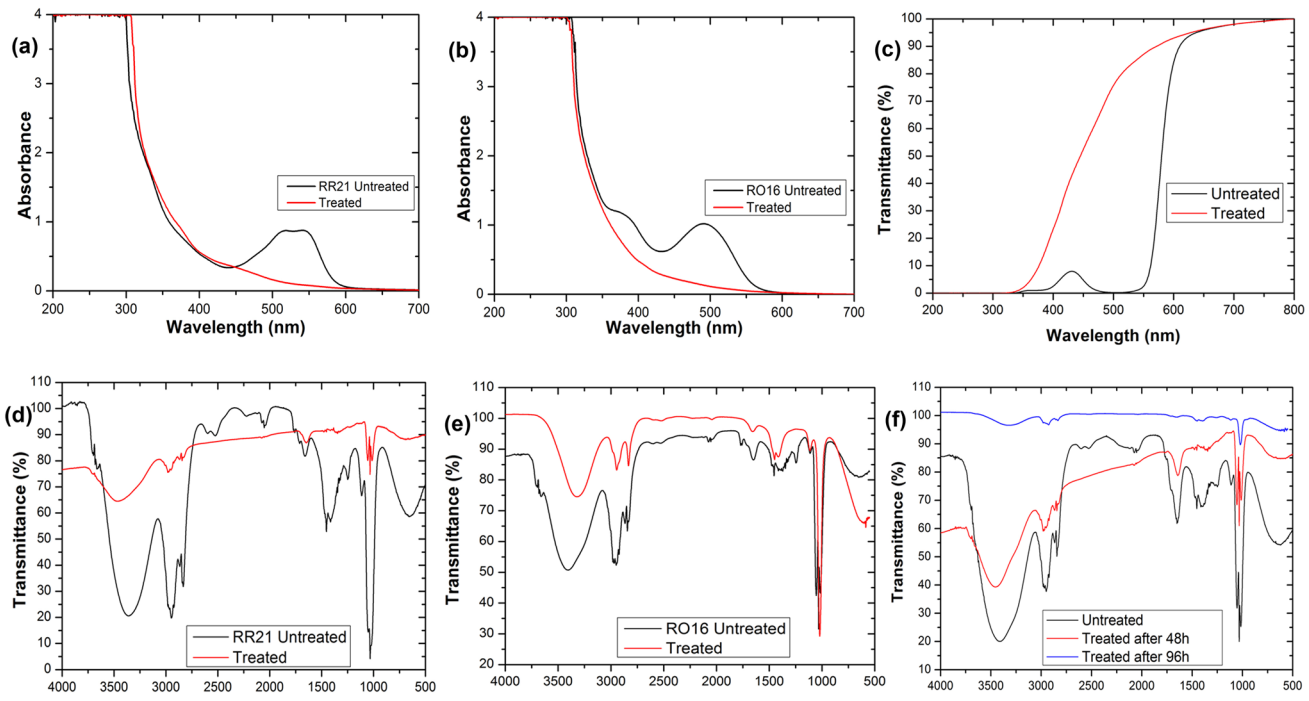


Fig. 3 Change in absorption and transmittance spectra of untreated and treated dye solutions: **a** UV–visible absorption spectra of RR21 dye; **b** UV–visible absorption spectra of RO16 dye; **c** UV–visible transmittance spectra of mixed dyes; **d** FTIR spectra of RR21 dye; **e** FTIR spectra of RO16 dye; **f** FTIR spectra of mixed dyes

for treated solution and no other absorbance peak appeared. Evidence of mixed dyes degradation could be confirmed with the change in UV–visible transmittance spectra between treated and untreated dye solutions as represented in Fig. 3c. These results indicate that the dye decolourization could be either due to bio-transformation/degradation or bio-accumulation of dye by bacteria. Although it is well known that during the bio-accumulation of dye molecules by bacteria, coloured biomass cells appear at the end (Holkar et al. 2018). However, it is observed in this study that the bacterial cells do not exhibit any change in morphological colour as compared to biomass cells in control experiments (without dye). Hence, the metabolite present in supernatant of treated water is investigated through FTIR and GC-MS techniques, to assess the extent of degradation of initial dye molecules. The FTIR transmittance spectra of RR21, RO16 and mixed dyes solution are shown in Fig. 3d, e, f, respectively, which shows similar peaks in all six samples. The major transmittance peaks in FTIR spectra of untreated sample are found as: 2950–2750 cm^{-1} (C–H stretch), 3500–3200 cm^{-1} (N–H stretch), 1600–1700 cm^{-1} (aromatic C = C stretch and C = N stretch), 1450–1200 cm^{-1} (phenol C–O stretch), 2160 cm^{-1} (C–S or S–O stretch), 750–850 cm^{-1} (C–Cl stretch) and 1050–1150 cm^{-1} (alkoxy C–O stretch and S = O stretch). After treatment, FTIR transmittance peaks of aromatic C = C, C–H stretch, N–H stretch, C = N stretch, C–Cl stretch and S = O stretch are drastically reduced, while the peaks of phenol C–O stretch, S–H stretch and S–O stretch almost disappear. The FTIR analysis of treated mixed dyes aqueous solution after 96 h of incubation period exhibits small transmittance peak of C–O stretch, while other peaks have almost disappeared, shown in Fig. 3f. The disappearance of functional group peaks in treated solution confirms the bio-degradation of dye molecules by strain 23N1. Furthermore, to confirm the bio-degradation, the treated dye solution collected after incubation period of 48 h (used for FTIR analysis) is used for GC-MS analysis. The GC-MS analysis of untreated and treated RR21 dye water samples have shown several transmittance peaks at different retention time as shown in Fig. S2 of SI. To verify the degradation of dye chromophore, GC-MS analyses of yeast extract and salt containing water samples are used for comparative assessment of m/z spectra as shown in Fig. S3 of SI.

The GC-MS analysis of raw RR21 dye solution exhibits m/z spectra peak at retention time 4.2 min, which corresponds to m/z spectra of un-metabolized RR21 dye molecule. These peaks completely disappear after treatment of RR21 dye solution, and several new peaks of unidentified metabolites appear near by the retention time of raw dye molecule. In addition, unidentified peaks at retention time 5 and 5.8 min are detected for untreated samples, which do not appear after bio-degradation of dye. During the analysis of treated RR21 dye solution, several other peaks at retention time 19.9, 20.8, 23.4, 24.2, 30.7 and 32.6 min are detected, which indicate the formation of new lower molecular weight metabolites. GC-MS analysis of untreated and treated RO16 dye solution exhibits several m/z spectra peaks at different retention time as shown in Fig. S4 of SI. The GC-MS analysis of raw RO16 dye solution has showed peaks at retention time 40 min, which could be the m/z spectra of RO16 dye. These peaks disappear and new peaks appear near by the retention time after treatment. The other peaks of unidentified compositions are also observed at retention time 6.3, 8.0, and 42.3 min, which are not obtained after treatment of dye solution. Interestingly, several new peaks appeared after treatment at retention time 4, 24.7 and 27.2, which could be due to formation of new lower molecular weight metabolite through bio-degradation of RO16 dye. Bouraie and Din (2016) have reported that the microbial decolourization of reactive azo dyes begins with reduction of azo chemical bond or mineralization of dye molecule and then complete degradation of intermediate aromatic amines, when incubated under static-isothermal condition. In this study, the GC-MS analyses of treated azo dye samples reveal that RR21 and RO16 dye compounds have been most likely broken down into unidentified lower molecular weight compounds. The dye chromophores are degraded due to breaking of azo bond and other bonds, which leads to the decolourization of water samples by bacteria. The intermediate metabolite formed after degradation of RR21 dye might be 3-acetoxy-5-acetoxymethyl-4-cyano-2-meth, while the intermediates formed during degradation of RO16 dye might be pentan-2-one, 4-(2-naphthylsulphonyl)-4-M. Bedekar et al. (2014) have investigated the RO16 dye biodegradation pathway mediated by *Lysinibacillus sp.* RGS. They have proposed that the RO16 is catabolized into end metabolites of 4-hydroxy-5,8-dihydronaphthalene-2-sulphonate and 2-[(3-aminophenyl) sulphonyl] ethanol by bacteria, which confirmed the detoxification of RO16 dye. Furthermore, the GC-MS analysis of metabolites extracted from the treated mixed dyes solution (confirmatory experiment 5) exhibits m/z spectra at retention time 5.2, 11.6, 13.4, 15.3, 17.2, 20.3, 24.1, 31.3, 33.6, 34.7 and 35.2 min (shown in Fig. S6 of SI), which indicate the conversion of mixed dyes compound into smaller metabolites. Comparative analysis of m/z spectra of untreated and treated mixed dyes samples indicates that disappearance of peaks and reduction in intensity of the existing peaks after degradation of dyes by strain 23N1 as shown in Fig. S4 of SI.

The involvement of azoreductase enzyme in degradation of RR21 and RO16 dyes can be ensured through the analysis of cell-free supernatant solution of treated samples, as per the experimental protocol reported by Telke et al. (2010). The 2 mL reaction mixture containing methyl red (4.45 µM), NADH (100 µM) and potassium phosphate buffer (1.7 mL, 20 mM, pH 7.5) is inoculated by 0.1 mL

supernatant solution, followed by incubation at 30 °C for 12 h. The UV–visible absorbance spectral analysis of colour reaction mixture at λ_{max} 430 nm indicates the decrease in absorbance of inoculated sample after 12-h incubation, which confirms the involvement of azoreductase enzyme in degradation of dyes. Furthermore, the TOC concentration of initial untreated mixed dyes water supplemented with yeast extract (confirmatory experiment 5) is analysed as 3492 mg/L, which is reduced by 45% after treatment. This indicates the utilization of dye as a carbon source by bacteria during treatment process. The remaining TOC concentration could be removed through ultra/nanofiltration membrane technique, whose study is under progress and the details will be reported in future from our laboratory.

Biomass growth in dye containing culture media

The biomass growth of strain 23N1 in three culture media for different dyes combinations is investigated through measurement of optical density at 600 nm (OD600) using UV–visible spectroscope. The OD600 measurement methodology is a well-known technique to estimate the density of bacterial cell in culture media. The biomass growth is assessed in 50 mg/L solutions of individual RR21 and RO16 dyes. The process condition is adopted as: temperature 40 °C, pH 9, yeast extract 8 g/L, salt 4 g/L and 0.5% inoculation volume (v/v). The change in OD600 value of dye containing culture media with respect to time (h) is illustrated in Fig. 4. In RR21 dye solution, the maximum absorbance is obtained as 2.09, while it is 2.25 for RO16 dye solution. Comparatively, the bacterial density is higher for RO16 containing sample than RR21 dye solution, which indicates that RR21 dye is more toxic as compared to RR21 dye.

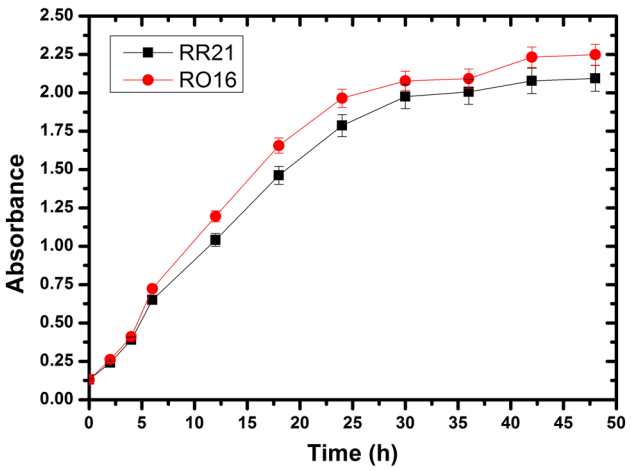


Fig. 4 Measurement of optical density at 600 nm (OD600) using UV–visible spectroscope

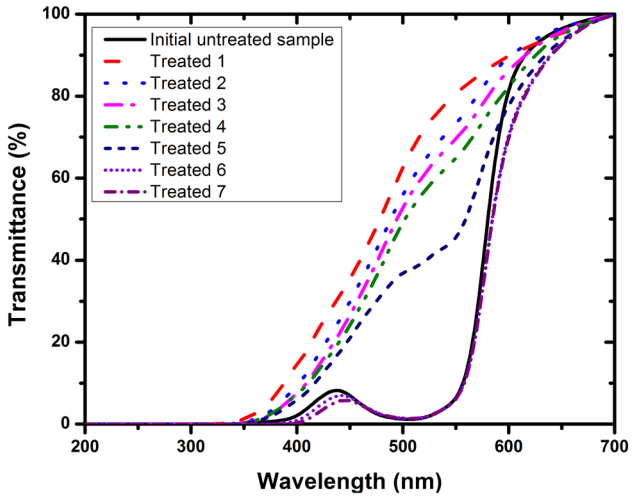


Fig. 5 Change in UV–visible transmittance spectra due to effect of Cr(VI) on ADMI reduction of mixed dyes water sample. *Note*: Initial untreated sample denotes the spectra of mixed dyes water sample; treated 1, 2, 3, 4, 5, 6 and 7 represent the spectra of treated water sample containing initial Cr(VI) concentration 0 mg/L, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L and 150 mg/L, respectively

Effect of chromate

The chromium salt is generally used, as mordant in the industrial dyeing process. The co-existence of chromate (Cr(VI)) with dye in textile wastewater increases the toxicity and makes it resistant to microbial growth. The effect of Cr(VI) (initial concentration range: 25–150 mg/L) on ADMI reduction performance of strain 23N1 is assessed using process condition of confirmatory experiment 5 (mentioned in Table 4). The change in UV–visible transmittance spectra of mixed dyes containing water sample due to the presence of Cr(VI) ions is shown in Fig. 5, which clearly indicates the decrease in transmittance of treated water containing Cr(VI). The initial and final ADMI values with reductions of ADMI values in percentages of experiments (confirmatory experiment 5) containing different Cr(VI) concentrations are illustrated in Table 5.

The ADMI reduction percentage decreases from 81.07 to 63.16% with respect to an increase in initial Cr(VI) concentration from 25 to 100 mg/L. The presence of Cr(VI) concentration ≥ 125 mg/L could be lethal and exhibits only 10.76% ADMI reduction, which might be due to unfavourable higher toxicity of medium for bacterial growth and its activity. This result indicates that the bacterial strain 23N1 could sustain even if initial Cr(VI) concentration lies between 0 and 100 mg/L and exhibits satisfactory ADMI reduction. The literature shows that the Cr(VI) concentration of < 50 mg/L is commonly found in textile wastewater (Chaudhari et al. 2017); thus, strain 23N1 could be used satisfactorily for textile wastewater treatment.

Table 5 Initial and final ADMI values with ADMI reduction percentage of experimental water sample (5 confirmatory experiments) and respective Cr(VI) concentration

S. no.	Initial Cr(VI) concentration (mg/L)	Initial ADMI of untreated water sample	Final ADMI after treatment	ADMI reduction (%)
1	0	498.30 ± 5	94.31 ± 8	81.07 ± 0.5
2	25	463.49 ± 8	156.95 ± 8	66.14 ± 0.3
3	50	470.73 ± 6	170.66 ± 5	63.75 ± 0.5
4	75	483.87 ± 8	176.53 ± 8	63.52 ± 0.5
5	100	492.46 ± 7	181.41 ± 6	63.16 ± 0.3
6	125	507.40 ± 8	452.81 ± 8	10.76 ± 0.5
7	150	519.23 ± 7	463.88 ± 87	10.66 ± 0.4

Table 6 Characteristics of real textile wastewater

S. No.	Parameters	Analysed value	
		RW1	RW2
1	pH	4.9 ± 0.1	7.9 ± 0.1
2	Total dissolved solid (mg/L)	4950 ± 0.04	3850 ± 0.04
3	Total suspended solid (mg/L)	355 ± 0.04	335 ± 0.03
4	Electrical conductivity (μS/cm)	5865 ± 0.02	4665 ± 0.04
5	Initial ADMI	467.95 ± 0.4	492.21 ± 0.5
6	Cr(VI) (mg/L)	Not assessed	25.6 ± 0.05

Simultaneous decolourization of wastewater and production of bio-energy using microbial fuel cell (MFC)

In MFCs, the decolourization of synthetic mixed dyes aqueous solution and real industrial wastewater is carried out in chamber 1 at room temperature (~ 30 °C) for 120 h under static condition, while chamber 2 is filled with 100% DDW as shown in Fig. S6 of SI. The bio-decolourization process produces protons (H^+) and electrons (e^-) through redox reactions that facilitates the power generation (Ilamathi and Jayapriya 2018). The defined process conditions selected for the four different experiments are as: sample "MDY" represents mixed dyes aqueous solution containing yeast extract and salt (same as confirmatory experiment number 5); sample "RW1" represents real wastewater (physiochemical characteristics are enlisted in Table 6); sample "RW2" represents real textile wastewater containing Cr(VI); sample "RW2Y" represents real wastewater supplemented with 8 g/L yeast extract as co-substrate.

Each experimental sample (above stated) is inoculated with 4% (V/V) bacterial inoculums. The output voltage is monitored using digital voltmeter at the time interval of 4 h as shown in Fig. 6a. The power density is evaluated using voltage data, shown in Fig. 6b. The maximum voltage and power density in the experiment using sample MDY is recorded as 624 mV and 586.85 mW/m^2 after 88 h of incubation period, which is probably obtained through degradation of dyes in saline medium by bacteria. The bio-electricity generation potential of bacteria is estimated

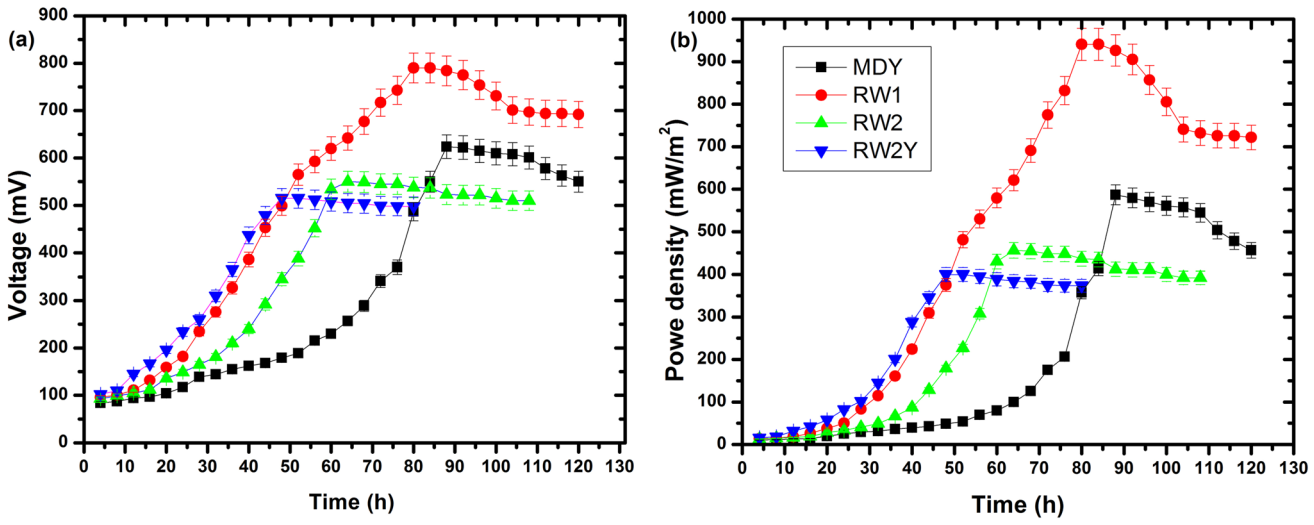


Fig. 6 Open-circuit voltage and power density generation during mixed dyes degradation in microbial fuel cell: **a** voltage generation; **b** power density generation

through MFC using two different samples of RW1 and RW2, from which the maximum voltages for two water samples are recorded as 790 mV (power density 940.61 mW/m^2) after 88 h and 550 mV (power density 455.91 mW/m^2) after 64 h treatment, respectively. This variation in voltage generation between samples RW1 and RW2 might be due to different composition of dyes and other ionic constituents. It has been observed that the reduction % of ADMI values for experiment using samples RW1 and RW2 is obtained as 76.0% after 120 h and 71.4% after 108 h of incubation period, respectively. In order to achieve satisfactory ADMI reduction percentage and estimate the bio-electricity generation potential of bacteria, the voltage generation is examined using sample RW2 supplemented with yeast extract (coded as: RW2Y). The maximum voltage in RW2Y is recorded as 515 mV (power density 399.73 mW/m^2) after 48 h with ADMI reduction of 77.32 ± 0.4%, which reveals that bacteria enhance the bio-decolourization activity in the presence of co-substrate and promotes the generation of energy within short period of time. Previously, Sun et al. 2013 have reported the increase in power generation and decolourization of Congo red dye on the addition of redox mediator in bacteria-driven MFC. However, the amount of power generation and decolourization of dyes vary with different co-substrates and the effluent composition (Cao et al. 2010; Nayak et al. 2018). The decline in power generation after maximum gain could be due to reduction in generation of electrons in chamber 1. It is well known that the glucose, acetate and ethanol are most commonly used co-substrate in such processes (Sun et al. 2013). However, in our study, the glucose has shown negative influence on decolourization response of reactive dyes by bacterial strain 23N1 (Mishra and Maiti 2018c, d). This study reveals that the yeast extract as co-substrate promotes the decolourization of mixed dyes as well and enhances the rate of power generation though MFCs.

Although the selection of co-substrate for effective decolourization of dyes depends on the dye composition in the water, strain 23N1 requires peptone with yeast extract for decolourization of MO dye (reported in earlier study of Mishra and Maiti 2018b). Thus, this study suggests that the degradation of mixed dyes (RR21 and RO16) compounds by strain 23N1 could be coupled with power generation in the presence of yeast extract as only co-substrate. However, a detailed study is required to optimize the process condition for high-power generation with faster decolourization of textile dye wastewater, which might be explored in the separate study.

Furthermore, the decolourization of RR21, RO16 and other unknown dyes containing wastewater RW1 is investigated under optimal process condition as: yeast extract 9 g/L, pH 9, inoculations volume 4% and incubation time 48 h at temperature 40 °C under static condition. The considerable

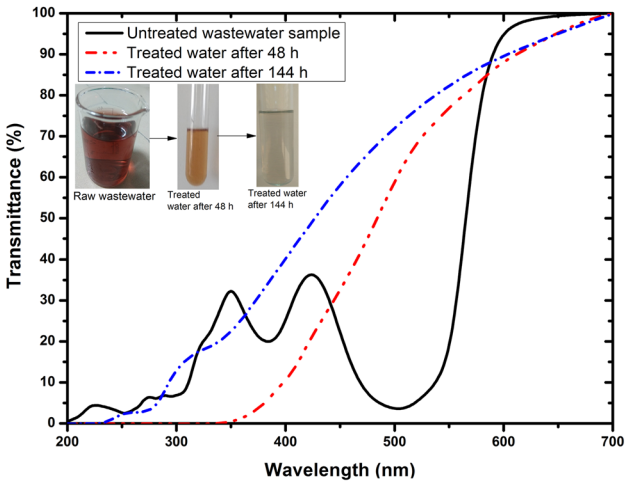


Fig. 7 Change in UV–visible transmittance spectra of untreated and treated real textile wastewater. (*Note*: Treated water produced after bacterial treatment of wastewater using yeast extract-supplemented medium)

changes in UV–visible transmittance spectra of treated water sample are observed compared raw feed, as shown in Fig. 7. The wider peaks observed at wavelength range 450–550 nm (includes both λ_{max} of RR21 at 537 nm and λ_{max} of RO16 at 493 nm) for untreated sample completely diminish in treated sample, which indicates the decolourization of dyes. The ADMI value for treated water sample obtained from this experiment and ADMI reduction percentage is estimated are 142.12 ± 0.8 and 69.63% ± 0.5, respectively. This result signifies the feasibility of strain 23N1 for treatment of industrial wastewater along with electrical power generation.

Toxicity analysis of metabolites

The treated mixed synthetic aqueous dye solution (confirmatory experiment 5) and real textile wastewater RW2 are used to investigate the germination rate of *V. radiata* seeds under controlled laboratory conditions. During the analysis of germination rate, it is observed that treated aqueous dye solution and treated RW2 water promote the germination of seeds, which exhibit 100% germination rate compared to control test as shown in Fig. S7a and S7b, respectively, of SI. The growing seeds do not express any morphological disorder, which indicates non-toxicity of end metabolites in the treated water samples. The average length of radicals germinated in the presence of treated waters from synthetic water and raw wastewater samples is estimated as 5.8 and 5.5 cm, respectively, which is comparatively better than radical grown in control (average length 4.4 cm). Thus, it confirms that the end metabolites produced after treatment of mixed dyes sample are non-toxic and might support in the growth of vegetation.

Table 7 Comparative study of bacteria-mediated dye decolourization

Mixed dyes	Optimal process condition (IDC, pH, Temp., OT)	Bacterial species	ADMI removal (%)	Reference
Remazol red, brown 3-REL, rubine GFL, Scarlet RR, methyl red, golden yellow HER and brilliant blue GL	Static medium (70 mg/L, 6–8, 40 °C, 24 h)	*Brevibacillus laterosporus* MTCC2298	87	Kurade et al. (2011)
Reactive black-5, reactive yellow 3G-P and reactive red 3B-A	Anaerobic medium (100 mg/L, 10, 35 °C, 36 h)	*Clostridium bifermentans* SL186	90	Joe et al. (2008)
Reactive purple, reactive pink MB, malachite green and reactive red-M5B,	Aerobic medium (200 mg/L, 7, 37 °C, 8 days)	*Bacillus cereus*	70.8	Maheswari and Sivagami (2016)
Acid blue and acid red	Aerobic medium (100 mg/L, 7, 37 °C, 72 h)	*Pseudomonas aeruginosa*	90	Lucious et al. (2014)
Reactive red 21 and reactive orange 16	Static medium (50 mg/L, 8.2, 40 °C, 96 h) using jack fruit seed powder as co-substrate	*Pseudomonas aeruginosa* SVM16	90.2	Mishra et al. 2019
Reactive red 21 and reactive orange 16	Static medium (50 mg/L, 8.3, 40 °C, 48 h) using yeast extract as co-substrate	*Pseudomonas aeruginosa* 23N1	80.8	In this study

IDC initial individual dye concentration, *Temp*. incubation temperature, *OT* operational time)

The few studies related to decolourization of mixed dyes (of different chromophore) by bacterial species have been reported in recent past, shown in Table 7. Joe et al. (2008) have investigated the capability of *Clostridium bifermentans* SL186 to bio-decolourize mixed dyes (reactive black-5, reactive yellow 3G-P and reactive red 3B-A) in anaerobic condition. They have found that the bacteria could effectively reduce ADMI value by 90% of mixed dyes solutions in wide range of pH (6–12). However, they suggested that the bacteria require additional glucose as carbon source along with yeast extract in dye decolourization medium that increases chemical cost of the process. Kurade et al. (2011) used *Brevibacillus laterosporus* MTCC 2298 to decolourize the mixture of structurally dissimilar dyes (rubine GFL, Remazol red, brown 3-REL, golden yellow HER, scarlet RR, brilliant blue GL and methyl red) in static micro-aerobic medium containing yeast extract, peptone and additional carbon sources. The authors reported that the bacteria could reduce ADMI value by 87% of dye chromophore through cellular oxidative–reductive metabolism involving enzymes like veratryl alcohol oxidase, tyrosinase, NADH-DCIP reductase and azo reductase. Lucious et al. (2014) carried out the decolourization of mixed dyes (acid blue and acid red) using *P. aeruginosa* in glucose-supplemented medium under aerobic condition. The authors reported that the bacteria exhibited maximum 90% ADMI reduction efficiency. Maheswari and Sivagami (2016) investigated the decolourization of mixed dyes (reactive red M5B, malachite green, reactive purple and reactive pink MB) from textile industrial effluent by *Bacillus subtilis* and *B. cereus* in aerobic condition. They reported that the *B. cereus* exhibited comparably higher dye degradation performance (70.8%) than *B. subtilis.* Mishra et al. (2019) investigated the decolourization of mixed dyes (RR21and RO16) solution by *P. aeruginosa* in jack fruit seed powder-supplemented medium. The authors reported that bacterial could significantly reduce ADMI by 90.2% after 96 h. However, the same bacterial growth and decolourization activity were significantly reduced in the presence of metal ions in the culture medium. Based on the literature available, it has been observed that the bacterial species require additional carbon sources to sustain the dye toxicity stress of the culture medium. The reported bacterial species exhibit certain limitations like requirement of additional carbon source, negative effect of metal ions and long incubation period for effective decolourization of dyes. In this study, the bacteria strain 23N1 exhibits satisfactory dye decolourization efficiency in the absence of additional carbon source other than yeast extract and the presence of metals ions than other bacterial species. This study would give good idea to researchers for investigating potential bacterial species and explore the optimization of process condition for wastewater treatment along energy generation.

Conclusion

This study is carried out to optimize the decolourization process condition of mixed (reactive red 21 (RR21) and reactive orange 16 (RO16)) dyes containing aqueous solutions by *P. aeruginosa* 23N1, through response surface

methodology. Further, the metabolites extracted from the treated water sample are used to investigate the extent of degradation of RR21 and RO16 dyes in water samples containing either individual dye or mixed dyes. The non-toxicity of end metabolites is assessed through germination test of *V. radiata* seeds in medium supplemented with treated mixed dyes water sample. The quadratic model equation generated through analysis of variance for mixed dyes ADMI reduction percentage data is found to be statistically significant ($p < 0.05$) and suitable to predict the bio-decolourization efficiency of the process. The strain 23N1 exhibits maximum ADMI reduction percentage of $81.08 \pm 0.4\%$ for mixed dye-laden water sample. The yeast extract is required to be used as co-substrate by bacteria to decolourize dyes, which is highly salt tolerant (~6 g/L NaCl) and exhibits better performance in alkaline medium. The strain 23N1 could sustain Cr(VI) concentration up to 50 mg/L and exhibit satisfactory ADMI reduction of ~67%. The yeast extract as co-substrate is found to be very effective to increase the rate of bio-degradation of dyes and to achieve maximum voltage generation in microbial fuel cell within very short period of time. The maximum voltage generation of 790 mV and power density 940.61 mW/m^2 are noticed during degradation of mixed dye-laden real wastewater in microbial fuel cell. The results reveal that the both complex dyes are utilized as nutrient by bacteria and converted into simpler organic compounds, which could promote the 100% healthy germination rate of *V. radiata* seeds that signify the non-toxicity of end metabolites. Thus, the microbial fuel cell could be a promising technology for bio-decolourization of textile wastewater as well as bio-energy generation.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interest.

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