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ORIGINAL ARTICLE

Biodegradation optimization and metabolite elucidation of Reactive Red 120 by four different *Aspergillus* species isolated from soil contaminated with industrial effluent

Fuad Ameen[1] · **Fatimah Alshehrei**[2]

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Abstract Azo dyes are recalcitrant owing to their xenobiotic nature and exhibit high resistance to degradation processes. In the present study, different *Aspergillus* species (*A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*) isolated from soil samples contaminated with industrial effluent, collected from Jeddah, Saudi Arabia, were analyzed for azo dye, Reactive Red 120 (RR120) biodegradation. The physicochemical parameters such as carbon (sucrose) and nitrogen (ammonium sulfate) sources, pH, and temperature affecting the biodegradation of RR120 were optimized using central composite design–response surface methodology (CCD-RSM). The maximum RR120 degradation was found to be 84% (predicted) at the optimum level of sucrose (11.73 g/L), ammonium sulfate (1.26 g/L), pH (5.71), and temperature (28.26 °C). Further, the validation results confirmed that the predicted values are in good agreement with the experimental results for RR120 degradation by *A. flavus* (86%), *A. fumigatus* (84%), *A. niger* (85%), and *A. terreus* (86%). The metabolic product of RR120 after biodegradation by different *Aspergillus* species was identified as sodium 2-aminobenzenesulfonate. The present study suggests that *Aspergillus* species are good candidates for azo dye-loaded effluent treatment.

Keywords *Aspergillus* · Biodegradation · Metabolic product · Reactive Red 120 · Sodium 2-aminobenzenesulfonate · Response surface methodology

✉ Fuad Ameen
fhasan@ksu.edu.sa

[1] Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

[2] Department of Biology, Umm Al-Qura University, Makkah 24382, Saudi Arabia

Introduction

Synthetic dyes are extensively used in the textiles, pharmaceuticals, cosmetics, printing, leather, food, and paper industries. The dye-loaded effluent emanating from these industries are considered as one of the most serious water pollutions, with reports that approximately 100 tons of used dyes per annum are discharged into water streams worldwide (Yagub et al. 2012). Reactive azo dyes, in particular, are widely utilized in textile dyestuffs, owing to their simple dying procedures and good stability during washing procedures (Spadaro et al. 1992). The majority of these dyes and their transformed products are highly toxic and mutagenic to biotic communities (Benigni et al. 2000; Poonkuzhali et al. 2011; Sathishkumar et al. 2013). Therefore, treatment of the dye-loaded effluent without causing secondary pollution is essential to protect the ecosystems receiving the effluent (Sathishkumar et al. 2014).

In recent decades, several physicochemical and biological treatment techniques have been reported for the remediation of reactive azo dyes (Sathishkumar et al. 2012; Adnan et al. 2016; Saadon et al. 2016). Among these techniques, biological treatments have received a great deal of interest owing to their minimal impact on the ecosystem and their cost-effectiveness. Although bacterial treatment is economical and simple, there is a problem associated with the formation of toxic aromatic amines during the degradation process (Vyrides et al. 2014). Treatment with ascomycota has proven to be promising for reducing costs and providing an eco-friendly process, owing to the utilization of natural redox mediators in catalyzing the enzymatic mechanism, which are produced by the fungus itself (Rodríguez-Couto 2012; Adnan et al. 2015).

In the present study, Reactive Red 120 (RR120) dye was selected as a model dye for the degradation analysis, because it is removed during processing operations and is significantly present in textile industry wastewater (Suwannaruang et al.

2015). In addition, four different species of the ascomycota fungi genus *Aspergillus* isolated from effluent-contaminated soil samples near weaving factories in Jeddah, Saudi Arabia, were chosen, owing to their remarkable properties of rapid growth and high percentages of RR120 degradation. Further, optimum conditions including carbon and nitrogen sources, pH, and temperature were assessed using central composite design–response surface methodology (CCD-RSM). Finally, the metabolic product obtained from RR120 after biodegradation by *Aspergillus* species was identified as sodium 2-aminobenzenesulfonate. To our knowledge, this is the first study to demonstrate this new and non-hazardous metabolic product of RR120 biodegradation by *Aspergillus* species.

Materials and methods

Chemicals

RR120 was procured from Sigma Aldrich (St. Louis, MO, USA). Ammonium acetate and methanol [high-performance liquid chromatography (HPLC) grade] were purchased from Merck (Kenilworth, NJ, USA). All other chemicals and reagents used in the present study were of analytical grade.

Sample collection

Soil samples were collected in sterilized tubes at different locations from effluent-contaminated soil near weaving factories (sediment had slight dye contamination) located in the industrial city of Jeddah, Saudi Arabia. This city has an area greater than 12 million m^2 and contains 552 industrial activities, including food, mineral, and chemical processing industries.

Screening of fast-growing and rapid RR120 degrading fungal isolates

Different fungal strains isolated from the collected soil samples were inoculated into RR120 dye-amended potato dextrose agar (PDA) medium and incubated at 27 °C. Fungal growth and RR120 decolorization efficiency were then regularly monitored based on the color change of the RR120-amended medium. A non-inoculated dye–PDA plate was maintained as a control. The most efficient RR120 degrading fungal strains were selected for further molecular identification and dye degradation.

Identification of fungi

The selected isolates were examined under macroscopic and microscopic observation. Subsequently, molecular identification of the selected fungal strains was performed by 18S rRNA sequencing. In brief, the genomic DNA isolated from the selected fungal strain was amplified using polymerase chain reaction (PCR) with ITS1 (5′-TCCGTAGGTGAACC TGCGG-3′) and ITS2 (5′-GCATCGATGAAGAACGCAGC -3′) utilized as universal primers. The reaction was assisted by the addition of $MgCl_2$, PCR buffer, heat-stable Taq polymerase, dNTPs mixture, and DNA template. The PCR process underwent a series of thermal cycling: 1 cycle at 94 °C for 3 min, 25 cycles at 94 °C for 30 s and 50 °C for 30 s, and concluded with gene amplification at 72 °C for 10 min (Saroj et al. 2014). The amplified genes were then cloned into pGEM-T Easy (Promega) before being sent to Seeing Bioscience Corporation, Taiwan, for identification. The nucleotide alignment and phylogenetic tree were constructed based on the comparison of the resulting sequence with the known gene sequences from the National Center for Biotechnology Information (NCBI) GenBank database.

RR120 biodegradation and optimization by RSM

In the present study, pure RR120 biodegradation fungi inoculums (2%) were mixed with mineral salt medium broth (MSMB). The MSMB contained the following components (g/L): potassium dihydrogen phosphate 0.5, potassium hydrogen phosphate 0.5, calcium chloride 0.1, ferrous sulfate 0.07, magnesium sulfate 1.0, potassium chloride 0.5. The pH of the medium was adjusted (according to RSM design) with 1N sodium hydroxide or 1N hydrochloric acid before autoclaving at 121 °C for 15 min. In a preliminary study, most significant carbon (glucose, sucrose, fructose, lactose, mannitol, and starch) and nitrogen (casein, peptone, sodium nitrate, ammonium chloride, ammonium sulfate, and ammonium nitrate) sources were screened using Plackett–Burman (PB) design. Based on the PB screening results, sucrose and ammonium sulfate were selected as suitable carbon and nitrogen sources, respectively, for further optimization study. In addition, the most significant parameters such as pH and temperature were considered for optimization with fixed initial concentration of RR120 (100 ppm), agitation (120 rpm), and incubation period (7 days).

Generally, optimization experiments could be done by empirical or statistical methods. The empirical method is time-consuming, incomplete, and does not necessarily enable an effective optimization. In this study, CCD-RSM was applied to optimize the dye degradation process by the isolates. This statistics-based optimization method is a powerful experimental design to recognize the performance of composite systems (Coman and Bahrim 2011; Balan et al. 2012; Fabiszewska et al. 2015; Chang et al. 2017). Interestingly, CCD-RSM, which involves full factorial search by examining simultaneous, systematic, and efficient variation of important variables, was applied to model the optimization process, identify possible interactions, higher order effects, and determine the

optimum operational conditions (Sathishkumar et al. 2015; Krishnan et al. 2016). The independent variables used in this study for optimization were sucrose (g/L) (X_1), ammonium sulfate (g/L) (X_2), pH (X_3), and temperature (°C) (X_4) at five levels (+2, +1, 0, −1, and −2), as shown in Table 1. The percentage of RR120 degradation by selected fungal strains was considered as the dependent variable. According to the CCD matrix, 30 runs were carried out to achieve the response (actual) and the central point was replicated six times to determine the experimental error (Table 2). The data obtained were fitted to a second-order polynomial as follows (Eq. 1):

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=j}^{a} X \sum_{j=1+1}^{4} b_{ii} x_{ij} \quad (1)$$

where Y is the percentage of RR120 degradation, b_0, b_i, b_{ii}, and b_{ij} are constant coefficients, and x_i are uncoded independent variables. The data from the RSM experiments performed were analyzed and interpreted using Design-Expert 10 (Stat Ease, Minneapolis, MN, USA). Three main analytical steps, analysis of variance (ANOVA), a regression analysis, and the plotting of the response surface, were performed to obtain an optimum condition for the RR120 degradation. To verify the dye degradation results predicted by the model, a validation experiment was performed with the predicted values of independent variables.

Measurement of RR120 biodegradation

The RR120 dye degradation experiments were carried out under optimized conditions, and, further, the residual RR120 in the culture medium was analyzed based on the method described by Wang et al. (2009), with minor modifications. Briefly, 1 mL of culture medium was collected every 24 h and centrifuged at 8000 × g for 10 min. The RR120 biodegradation was then determined by measuring the absorbance at 595 nm using a UV-visible spectrophotometer (DR 2700, Hach, Loveland, CO, USA). The percentage of RR120 biodegradation (BD %) was calculated using the following equation (Eq. 2):

$$\mathrm{BD}\,(\%) = \frac{A_0 - A_t}{A_0} \times 100 \quad (2)$$

where, A_0 refers to the initial absorbance, A_t is the absorbance after incubation, and t is the incubation time.

After biodegradation of RR120, the fungal mycelium was removed from the culture by filtration. The supernatant obtained from the culture was utilized to extract the metabolites with an equal volume of ethyl acetate. The mixture was shaken, 5 mL of the mixture was transferred into a 50-mL separatory funnel, and 5 mL of ethyl acetate was added. The separatory funnel was shaken vigorously for approximately 2 min with periodic venting to release vapor. Further, the organic layer was allowed to separate for 10 min and was recovered into a 50-mL beaker. The aqueous layer was re-extracted twice with 2 mL of ethyl acetate and the combined extract was dried by passing through a funnel containing anhydrous sodium sulfate. The dried extract was dissolved in methanol (HPLC grade) and used for the identification of metabolites by HPLC and gas chromatography–mass spectrometry (GC-MS) analysis. HPLC analysis was carried out using a Waters 1525 instrument (Waters Associates Inc.) on a C18 column (Symmetry, 150 mm) by the isocratic method using the gradient of methanol with a flow rate of 1 mL min^{-1} for 10 min and UV photodiode array detector (model 2996) at 254 nm. A total of 10 mL of filtered sample was manually injected into the injector port.

The identification of metabolites formed after RR120 biodegradation was performed using a Thermo Trace DSQ II GC-MS. The ionization voltage was 70 eV. The column temperature program was set as follows: 50 °C hold for 3 min, 10 °C min^{-1} to 180 °C hold for 1 min, and 10 °C min^{-1} to 280 °C hold for 3 min. Helium was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. The RR120 biodegradation products were detected by comparison of the retention time and fragmentation pattern, as well as with mass spectra from the NIST spectral library support stored in the GC-MS solution software (version 1.10 beta, Shimadzu). Analysis of all samples was performed in triplicate. The data were calculated using SPSS Statistics 17.0 software. The significant difference between the treatments and the control was detected based on least significant difference at $P < 0.05$.

Results and discussion

Isolation and identification of RR120 decolorizing fungi

Twenty fungal isolates were obtained from the collected soil samples. Among these, four fungal strains (isolates B, D, H, and K) were selected for further RR120 biodegradation studies, based on superior growth performance and RR120 degradation efficiency. Subsequently, these isolates were identified by molecular and microscopic techniques as *A. flavus* (B), *A. fumigatus* (D), *A. niger* (H), and *A. terreus* (K).

RR120 biodegradation optimization by RSM

To enhance the rate of RR120 biodegradation by the isolates (*A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*), carbon (sucrose) and nitrogen (ammonium sulfate) sources, temperature, and pH were optimized according to the CCD-RSM matrix. Almost all the selected *Aspergillus* species' RR120

Table 1 Coded and uncoded values of independent variables used in the central composite design (CCD) design

Variables	Range of variables and level					Step change value (ΔZi)
	−2	−1	0	+1	+2	
Glucose (g/L)	2	6	10	14	16	4
Ammonium sulfate (g/L)	0.5	1	1.5	2	2.5	0.5
pH	5.0	5.5	6.0	6.5	7.0	0.5
Temperature (°C)	20	25	30	35	40	5

degradation efficiencies were found to be similar (data not shown). The mean value of RR120 degradation by all the selected *Aspergillus* species results are shown in Table 2. The results show that the percentage of RR120 degradation corresponded to the combined effect of the four selected independent variables in their specified ranges. The RR120

Table 2 Range and level of variables in the full factorial CCD matrix and response

S. no.	Glucose (g/L)	Ammonium sulfate (g/L)	pH	Temperature (°C)	Response (dye degradation, %)	
	X_1	X_2	X_3	X_4	Observed (Y_j)	Predicted ($\hat{Y}_j$)
1	−1	−1	−1	−1	64	65
2	+1	−1	−1	−1	77	79
3	−1	+1	−1	−1	67	65
4	+1	+1	−1	−1	70	72
5	−1	−1	+1	−1	54	56
6	+1	−1	+1	−1	73	74
7	−1	+1	+1	−1	54	52
8	+1	+1	+1	−1	65	65
9	−1	−1	−1	+1	67	69
10	+1	−1	−1	+1	77	77
11	−1	+1	−1	+1	72	75
12	+1	+1	−1	+1	78	79
13	−1	−1	+1	+1	61	60
14	+1	−1	+1	+1	78	79
15	−1	+1	+1	+1	64	63
16	+1	+1	+1	+1	73	75
17	−2	0	0	0	41	39
18	+2	0	0	0	66	62
19	0	−2	0	0	76	78
20	0	+2	0	0	73	69
21	0	0	−2	0	80	80
22	0	0	+2	0	73	69
23	0	0	0	−2	70	71
24	0	0	0	+2	77	75
25	0	0	0	0	79	80
26	0	0	0	0	78	80
27	0	0	0	0	79	80
28	0	0	0	0	80	80
29	0	0	0	0	81	80
30	0	0	0	0	80	80

The observed dependent responses are the mean of all isolated *Aspergillus* sp.

degradation varied markedly in the range of 41–81%. These results suggest that the selected variables strongly affect the RR120 degradation process.

The statistical analysis of the CCD-RSM experimental results were performed using Design-Expert 10 software to generate response surface modeling and optimization of the process variables. Table 3 illustrates the statistical significance for RR120 degradation assessed by Fisher's (F) test and ANOVA for the response surface quadratic model. The ANOVA result shows that the fitted second-order response surface model was highly significant with the F-test = 68.38 ($P < 0.0001$). The model reliability was confirmed by the determination of coefficient R^2 (0.9846), adjusted R^2 (0.9702), and predicted R^2 (0.9205), which suggests that about 1% of the total variation cannot be explained by the model. The R^2 values had advocated a high correlation between both the experimental and predicated values, which indicates that the regression model gives a good explanation of the relationship between the independent variables and the dependent variable. The insignificant lack-of-fit test (F-value = 3.06) also confirms that the model was perfectly fitting to the experimental data. In addition, the adequate precision ratio was found to be 34.862, which indicates an adequate signal. Therefore, this model can be used to navigate the design space. Table 3 shows that the individual terms (x_1, x_3, x_4), squared terms (x_1^2, x_2^2, x_3^2, x_4^2), and interactive terms (x_1x_2, x_1x_3, x_2x_4, x_3x_4) of variables were highly significant for the RR120 degradation. The final equation in terms of coded variables for the RR120 degradation is as follows:

$$Y = +79.50 + 5.75x_1 - 0.58x_2 - 2.67x_3 + 2.50x_4 - 6.69x_1^2 - 1.44x_2^2 - 0.94x_3^2 - 1.69x_4^2 - 1.87x_1x_2 + 1.50\ x_1x_3 - 0.25\ x_1x_4 - 0.75\ x_2x_3 + 1.00\ x_2x_4 + 0.88\ x_3x_4 \quad (3)$$

Equation 1 was used to facilitate the plotting of response surfaces in order to ascertain the interactive effects of the independent variables for RR120 degradation. Two-dimensional (2D) plots are the graphical representations of the regression equation that was generated for the pairwise combination of the four variables, while keeping the other two at their center point value. The interactions between the variables can be inferred from the shapes of the contour plots. Circular contour plots indicate that the interactions between the variables are negligible. In contrast, elliptical plots indicate evidence of the interactions. Figure 1a–f shows that the RR120 degradations achieved were highly influenced by all the investigated variables. Further, the results confirm that the percentage of RR120 degradation was increased up to a certain level for all of the investigated factors; however, beyond that, degradation was decreased.

The analysis of the residuals was performed to judge the adequacy of the developed model. Figure 2 demonstrates that none of the studentized residuals had a value higher than 1, and, also, all of the residuals fell within the acceptable range, thus certifying that the model is good. The predicted maximum RR120 degradation of 84% was derived from RSM

Table 3 Analysis of variance (ANOVA) results for the equation using Design-Expert 10

Source	Degrees of freedom	Sum of squares	Mean square	F-value	Prob. > F
Model	14	2499.53	178.54	68.38	<0.0001*
X_1	1	793.50	793.50	303.89	<0.0001*
X_2	1	8.17	8.17	3.13	0.0973
X_3	1	170.67	170.67	65.36	<0.0001*
X_4	1	150.00	150.00	57.45	<0.0001*
X_1^2	1	1226.68	1226.68	469.79	<0.0001*
X_2^2	1	56.68	56.68	21.71	0.0003*
X_3^2	1	24.11	24.11	9.23	0.0083*
X_4^2	1	78.11	78.11	29.91	<0.0001*
X_1X_2	1	56.25	56.25	21.54	0.0003*
X_1X_3	1	36.00	36.00	13.79	0.0021
X_1X_4	1	1.00	1.00	0.38	0.5453
X_2X_3	1	9.00	9.00	3.45	0.0831
X_2X_4	1	16.00	16.00	6.13	0.0257
X_3X_4	1	12.25	12.25	4.69	0.0468
Residual	15	39.17	2.61		
Lack of fit	10	33.67	3.37	3.06	0.1144
Pure error	5	5.50	1.10		
Cor. total	29	2538.70			

*Significant; SD: 1.62; R^2: 0.9846; adj R^2: 0.9702; pred R^2: 0.9205

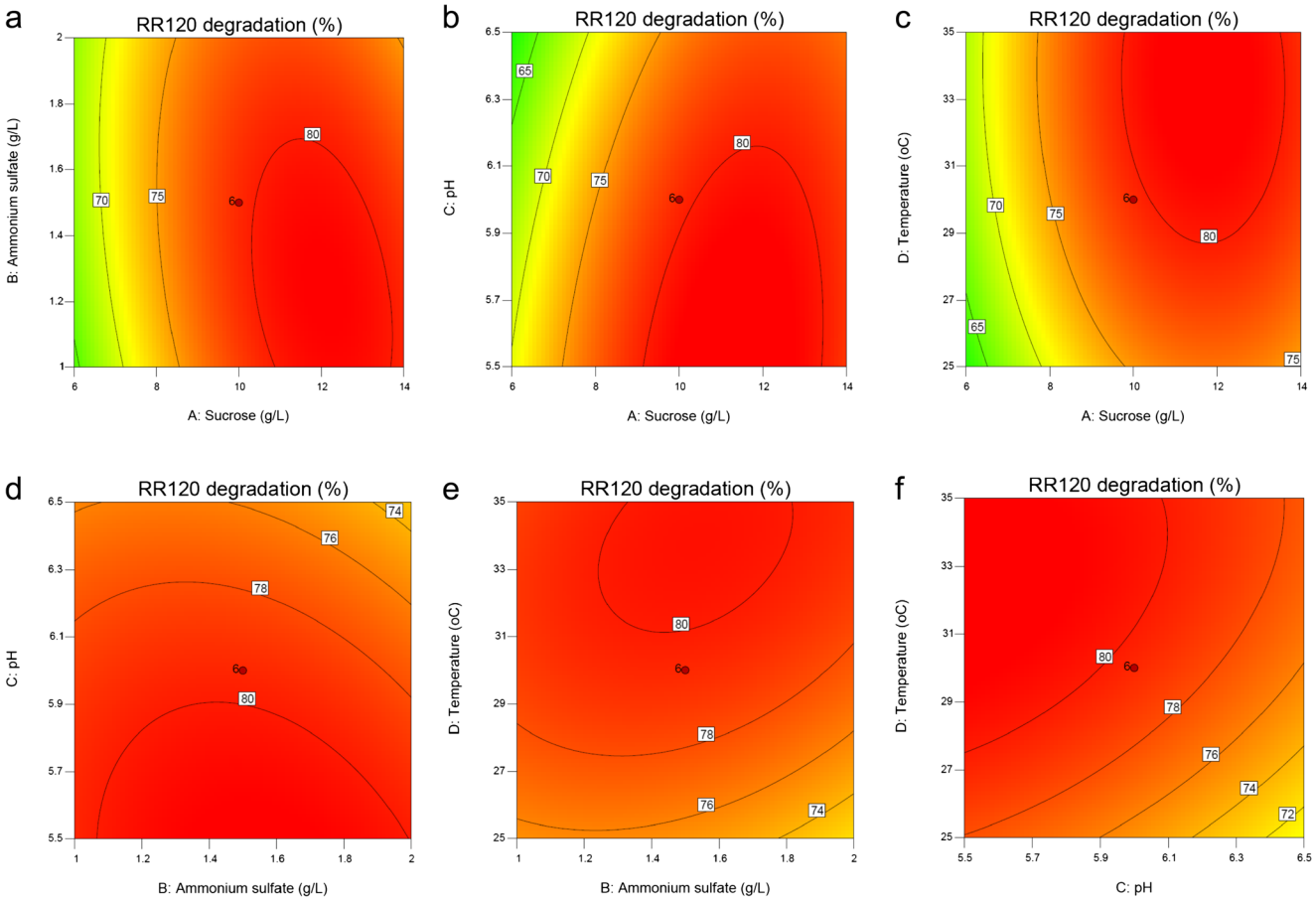

Fig. 1 Contour plot for the RR120 degradation by *Aspergillus species*. Interaction of: **a** sucrose with ammonium sulfate, **b** sucrose with pH, **c** sucrose with temperature, **d** ammonium sulfate with pH, **e** ammonium sulfate with temperature, and **f** pH with temperature

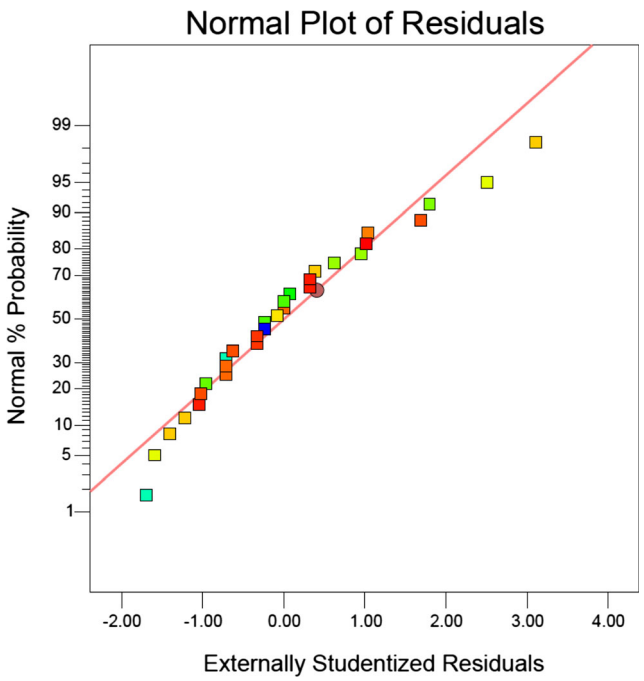

Fig. 2 The studentized residuals and normal % probability plot of RR120 degradation by *Aspergillus* species

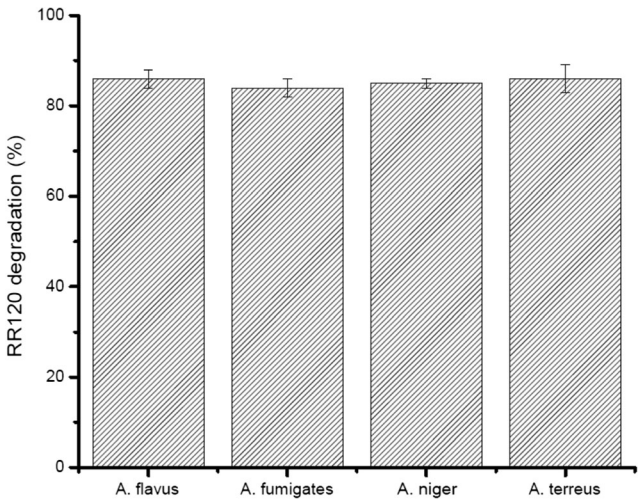

Fig. 3 RR120 degradation by *Aspergillus* species under response surface methodology (RSM) optimized conditions [sucrose (11.73 g/L), ammonium sulfate (1.26 g/L), pH (1.26 g/L), and temperature (28.26 °C) with fixed initial concentration of RR120 (100 ppm), agitation (120 rpm), and incubation period (7 days)]

regression at the optimum levels of sucrose, ammonium sulfate, pH, and temperature of 11.73 g/L, 1.26 g/L, 5.71, and 28.26 °C, respectively. Further, in order to validate the predicted optimum results, the RR120 degradation experiments (replicates) were performed with optimized combinations of the independent variables [sucrose 11.73 g/L, ammonium sulfate 1.26 g/L, pH 5.71, and temperature 28.2 °C with fixed initial concentration of RR120 (100 ppm), agitation (120 rpm), and incubation period (7 days)], as described above. The RR120 degradation experimental results were found to be 86%, 84%, 85%, and 86% for *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*, respectively (Fig. 3). This confirms that the predicted values are in good agreement with the experimental results. Similarly, Sharma et al. (2009) observed 84.8% of diazo dye Acid Red 151 (AR 151) decolorization at the optimum pH of 5.5 and temperature of 30 °C using RSM.

Generally, the addition of carbon and nitrogen sources to the culture medium enhances the efficacy of microorganisms to break down azo dyes. Ambrosio and Campos-Takaki (2004) observed maximum degradation of reactive azo dyes in medium containing sucrose as the carbon source. Zhang et al. (1999) confirmed that the support of media by a carbon source would work as a co-substrate to enhance the biodegradation of azo dye by fungal isolates. Ryu and Weon (1992) reported that the maximum degradation of azo dyes by *A. sojae* occurred in the presence of additional ammonium sulfate (1.8 g/L) as the nitrogen source. Zheng et al. (1999) indicated that the addition of ammonium ions to the culture medium could enhance dye decolorization by the fungal isolate. Ganesh et al. (1994) observed that a decrease in fungal efficacy to degrade azo dyes depends on an increase in acidic or alkaline

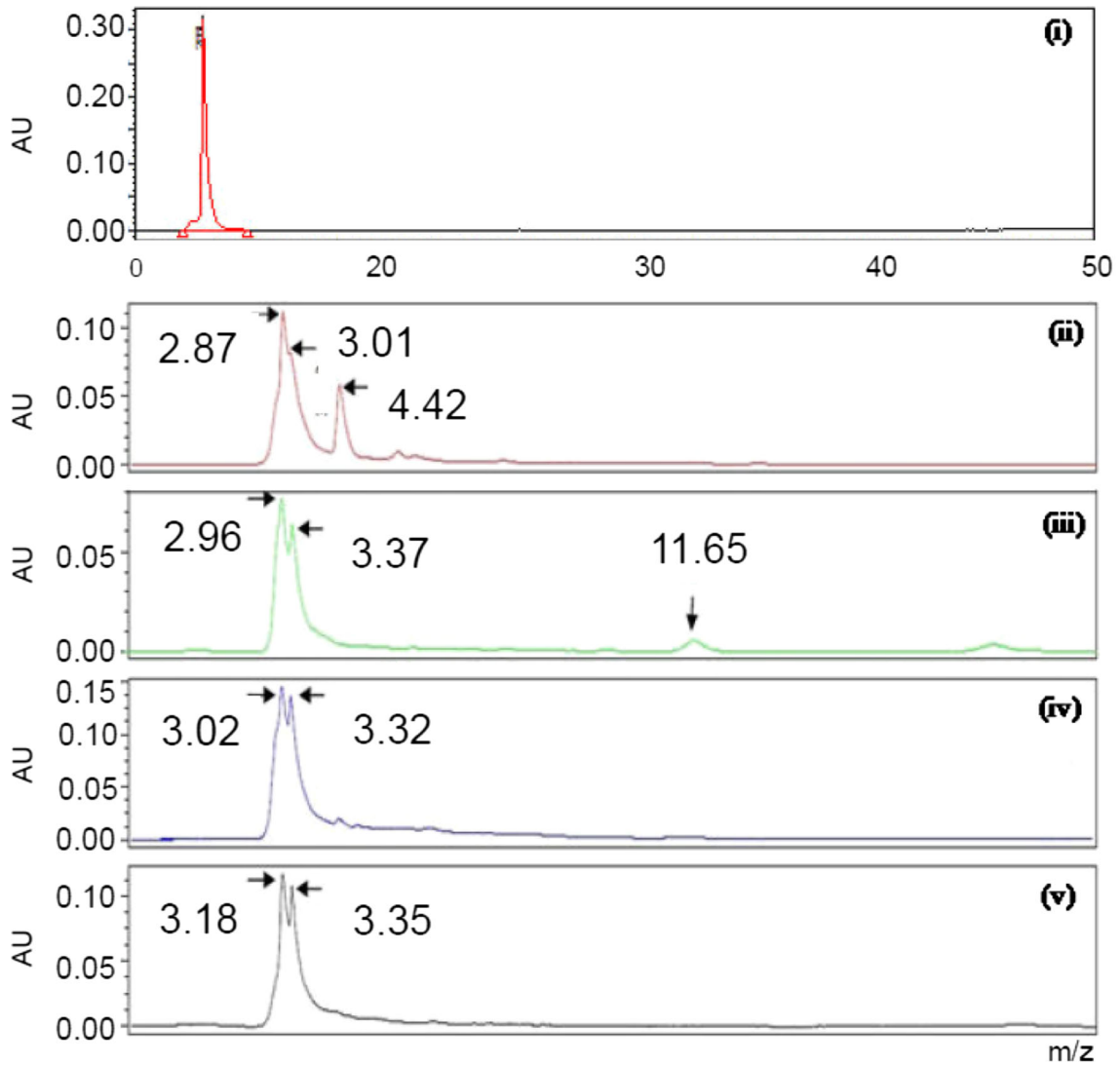

Fig. 4 HPLC chromatogram of (i) RR120 and their metabolites after biodegradation by (ii) *A. flavus*, (iii) *A. fumigatus*, (iv) *A. niger*, and (v) *A. terreus*

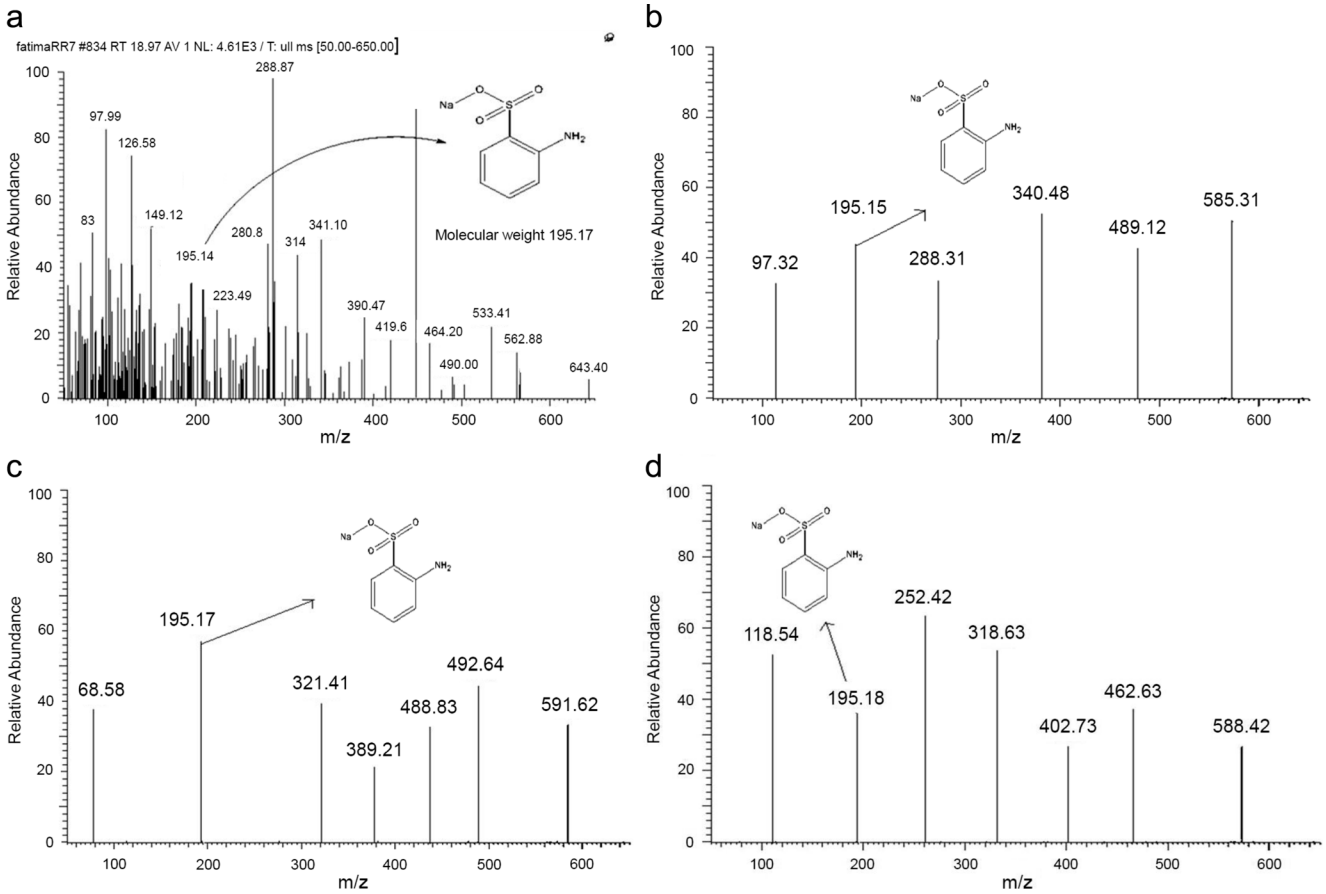

Fig. 5 Mass spectrum (*m*/*z*) of sodium 2-aminobenzenesulfonate produced from the biodegradation of RR120 by isolates of: **a** *A. flavus*, **b** *A. fumigatus*, **c** *A. niger*, and **d** *A. terreus*

conditions for optimum environments suitable for the growth of fungus. The dye degradation was considerably decreased with increasing pH and temperature. This might be due to enzyme denaturation, which reduces the amount

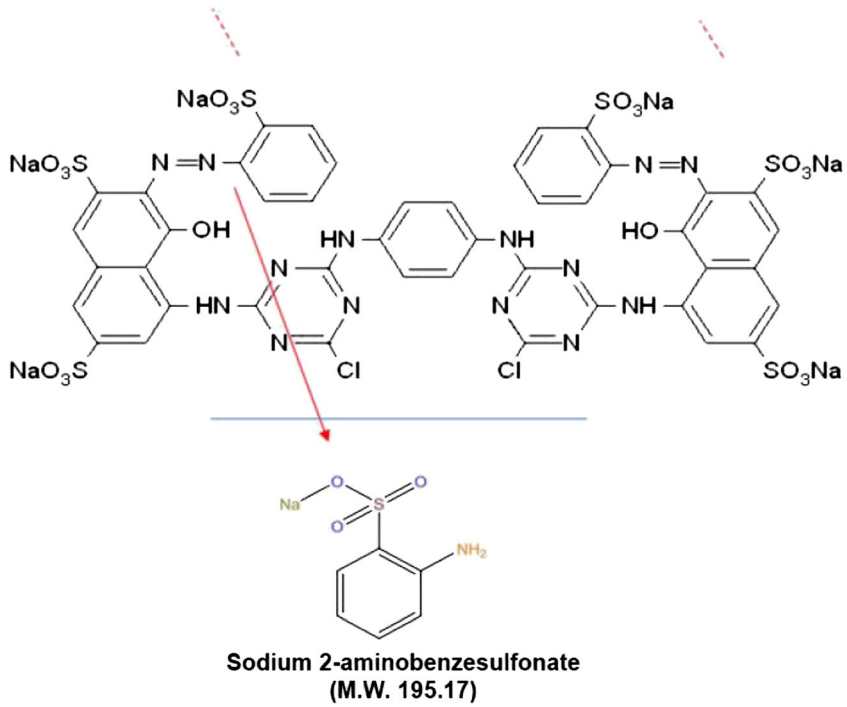

Fig. 6 Proposed mechanism of RR120 biodegradation by four different *Aspergillus* species

of cell viability for extracellular enzymatic activities by biodegrading the structure of targeted dye molecules (Khan et al. 2013).

Identification of RR120 metabolic intermediates

The biodegradation intermediates formed by fungal isolates after the 7-day incubation period were assessed using HPLC analysis. The HPLC chromatogram in Fig. 4 shows that the peak which appeared at 2.91 *m*/*z* for RR120 disappeared and new peaks formed at low mass ions after biodegradation by *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*. These results indicate that RR120 was broken down into many residues due to the enzymatic process of *Aspergillus* species.

The metabolites of RR120 biodegradation by each of the *Aspergillus* species were detected using GC-MS analysis. RR120 has a high molecular weight of 1469.98. After *Aspergillus* species treatment, the azo bond (N = N) could break down and a low mass compound appeared, as shown in Figs. 5a–d and 6. The results demonstrate that a peak after RR120 biodegradation by *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus* was observed at 195.14, 195.15, 195.17, and 195.18 *m*/*z*, respectively, corresponding to sodium 2-aminobenzenesulfonate. Previously, Almeida and Corso (2014) reported that azo reductase enzyme produced by *A. terreus* was degraded by azo dye. Jin and Ning (2013) observed dye degradation by laccase enzyme produced from *A. fumigatus*. Thus, in the present study, laccase and azo reductase might be involved in the transformation of RR120 to sodium 2-aminobenzenesulfonate.

Conclusions

Aspergillus species such as *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus* were predominantly present in the industrial effluent contaminated soil. This study suggests that central composite design–response surface methodology (CCD-RSM) was an appropriate method to optimize the suitable culture conditions for maximum Reactive Red 120 (RR120) degradation by *Aspergillus* species. The predicted and experimental values were very close, which reflected the accuracy and applicability of the model. Sodium 2-aminobenzenesulfonate, the metabolite resulting from RR120 biodegradation by all *Aspergillus* species, was less toxic to parental compounds. The present study concludes that *Aspergillus* species have an enormous potential to degrade the textile dyes and resolve the problem of unnecessary dyes present in the effluents of textile industries. However, further detailed pilot scale studies are required for actual industrial applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Disclosures The manuscript does not contain clinical studies or patient data.

References

Adnan LA, Sathishkumar P, Mohd Yusoff AR, Hadibarata T (2015) Metabolites characterisation of laccase mediated reactive black 5 biodegradation by fast growing ascomycete fungus *Trichoderma atroviride* F03. Int Biodeterior Biodegrad 104:274–282

Adnan LA, Hadibarata T, Sathishkumar P, Mohd Yusoff AR (2016) Biodegradation pathway of Acid Red 27 by white-rot fungus *Armillaria* sp. F022 and phytotoxicity evaluation. CSAWAC 44:239–246

Almeida EJ, Corso CR (2014) Comparative study of toxicity of azo dye Procion Red MX-5B following biosorption and biodegradation treatments with the fungi *Aspergillus niger* and *Aspergillus terreus*. Chemosphere 112:317–322

Ambrosio ST, Campos-Takaki GM (2004) Decolorization of reactive azo dyes by *Cunninghamella elegans* UCP 542 under co-metabolic conditions. Bioresour Technol 91:69–75

Balan K, Sathishkumar P, Palvannan T (2012) Decolorization of malachite green by laccase: optimization by response surface methodology. J Taiwan Inst Chem Eng 43:776–782

Benigni R, Giuliani A, Franke R, Gruska A (2000) Quantitative structure–activity relationships of mutagenic and carcinogenic aromatic amines. Chem Rev 100:3697–3714

Chang SH, Wu CH, Wang SS, Lin CW (2017) Fabrication of novel rhamnolipid-oxygen-releasing beads for bioremediation of groundwater containing high concentrations of BTEX. Int Biodeterior Biodegrad 116:58–63

Coman G, Bahrim G (2011) Optimization of xylanase production by streptomyces sp. P12-137 using response surface methodology and central composite design. Ann Microbiol 61:773–779

Fabiszewska AU, Kotyrba D, Nowak D (2015) Assortment of carbon sources in medium for yarrowia lipolytica lipase production: a statistical approach. Ann Microbiol 65:1495–1503

Ganesh R, Boardman GD, Michelsen D (1994) Fate of azo dyes in sludges. Water Res 28:1367–1376

Jin X, Ning Y (2013) Laccase production optimization by response surface methodology with *Aspergillus fumigatus* AF1 in unique inexpensive medium and decolorization of different dyes with the crude enzyme or fungal pellets. J Hazard Mater 262:870–877

Khan R, Bhawana P, Fulekar MH (2013) Microbial decolorization and degradation of synthetic dyes: a review. Rev Environ Sci Biotechnol 12:75–97

Krishnan J, Kishore AA, Suresh A, Madhumeetha B, Prakash DG (2016) Effect of pH, inoculum dose and initial dye concentration on the removal of azo dye mixture under aerobic conditions. Int Biodeterior Biodegrad. doi:10.1016/j.ibiod.2016.11.024

Poonkuzhali K, Sathishkumar P, Boopathy R, Palvannan T (2011) Aqueous state laccase thermostabilization using carbohydrate polymers: effect on toxicity assessment of azo dye. Carbohydr Polym 85:341–348

Rodríguez-Couto S (2012) A promising inert support for laccase production and decolouration of textile wastewater by the white-rot fungus *Trametes pubescesns*. J Hazard Mater 233–234:158–162

Ryu BH, Weon YD (1992) Decolorization of azo dyes by *Aspergillus sojae* B-10. J Microbiol Biotechnol 2:215–219

Saadon SA, Sathishkumar P, Yusoff ARM, Wirzal MDH, Rahmalan MT, Nur H (2016) Photocatalytic activity and reusability of ZnO layer synthesised by electrolysis, hydrogen peroxide and heat treatment. Environ Technol 37:1875–1882

Saroj S, Kumar K, Pareek N, Prasad R, Singh RP (2014) Biodegradation of azo dyes Acid Red 183, Direct Blue 15 and Direct Red 75 by the isolate *Penicillium oxalicum* SAR-3. Chemosphere 107:240–248

Sathishkumar P, Arulkumar M, Palvannan T (2012) Utilization of agro-industrial waste *Jatropha curcas* pods as an activated carbon for the adsorption of reactive dye remazol brilliant blue R (RBBR). J Clean Prod 22:67–75

Sathishkumar P, Balan K, Palvannan T, Kamala-Kannan S, Oh B-T, Rodríguez-Couto S (2013) Efficiency of *Pleurotus florida* laccase on decolorization and detoxification of the reactive dye Remazol Brilliant Blue R (RBBR) under optimized conditions. CSAWAC 41:665–672

Sathishkumar P, Kamala-Kannan S, Cho M, Kim JS, Hadibarata T, Salim MR, Oh BT (2014) Laccase immobilization on cellulose nanofiber: the catalytic efficiency and recyclic application for simulated dye effluent treatment. J Mol Catal B Enzym 100:111–120

Sathishkumar P, Hemalatha S, Arulkumar M, Ravikumar R, Mohd Yusoff AR, Hadibarata T, Palvannan T (2015) Curcuminoid extraction from turmeric (*Curcuma longa* L.): efficacy of bromine-modified curcuminoids against food spoilage flora. J Food Biochem 39:325–333

Sharma P, Singh L, Dilbaghi N (2009) Response surface methodological approach for the decolorization of simulated dye effluent using *Aspergillus fumigatus fresenius*. J Hazard Mater 161:1081–1086

Spadaro JT, Gold MH, Renganathan V (1992) Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 58:2397–2401

Suwannaruang T, Rivera KKP, Neramittagapong A, Wantala K (2015) Effects of hydrothermal temperature and time on uncalcined TiO_2 synthesis for reactive red 120 photocatalytic degradation. Surf Coat Technol 271:192–200

Vyrides I, Bonakdarpour B, Stuckey DC (2014) Salinity effects on biodegradation of reactive black 5 for one stage and two stages sequential anaerobic aerobic biological processes employing different anaerobic sludge. Int Biodeterior Biodegrad 95:294–300

Wang H, Su JQ, Zheng XW, Tian Y, Xiong XJ, Zheng TL (2009) Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3. Int Biodeterior Biodegrad 63:395–399

Yagub MT, Sen TK, Ang HM (2012) Equilibrium, kinetics, and thermodynamics of methylene blue adsorption by pine tree leaves. Water Air Soil Pollut 223:5267–5282

Zhang FM, Knapp JS, Tapley KN (1999) Decolourisation of cotton bleaching effluent with wood rotting fungus. Water Res 33:919–928

Zheng Z, Levin RE, Pinkham JL, Shetty K (1999) Decolorization of polymeric dyes by a novel *Penicillium* isolate. Process Biochem 34:31–37