International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 8; August 2017; Page No. 5212-5217
DOI: http://dx.doi.org/10.24327/ijcar.2017.5217.0674

Research Article

DECOLORIZATION OF REACTIVE BLACK B BY *PAENIBACILLUS DENDRITIFORMIS* STRAIN CS_2a_4

Nishant Junnarkar[1,2]* and Neepa Pandhi[1]

[1]Department of Microbiology, Shree M. & N. Virani Science College, Rajkot, GUJARAT, India 360005
[2]School of Life Sciences, Central University of Gujarat, Gandhinagar, Gujarat, India 382030

ARTICLE INFO

Article History:

Received 19th May, 2017
Received in revised form 14th June, 2017 Accepted 26th July, 2017
Published online 28th August, 2017

Key words:

Paenibacillus dendritiformis, azo dye, Reactive Black B, decolorization.

ABSTRACT

Azo dyes are one of the group of synthetic dyes extensively used in textile, paper, pharmaceutical, cosmetic and other industries. These pose environmental hazard if released untreated in environment. Reactive Black B (RBB) dye decolorizing bacterial strains were isolated from the samples collected from the vicinities of dye manufacturing industries. Amongst these, *Paenibacillusdendritiformis*strain CS_2a_4 was found to be the potent decolorizer and hence, medium composition and cultural conditions optimization was attempted to improve RBB decolorization by CS_2a_4 strain. Optimization of the cultural conditions and co-substrates concentration in decolorization medium, resulted in the reduction of decolorization period for RBB from 40h to 16h by the culture. CS_2a_4 could decolorize RBB upto 350 ppm efficiently (>80%) within 16-30h. Spectral analysis and TLC analysis of decolorized medium revealed the transformation of RBB dye into unknown intermediates.

INTRODUCTION

Synthetic dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutics, cosmetics and other industries (Almeida and Corso, 2014; Dellamtrice *et al*., 2017). The chemical classes of dyes employed more frequently on industrial scale are the azo, anthraquinone, sulfur, indigoid, triphenylmethyl (trityl), and phthalocyanine derivatives. Amongst these, azo dyes represent the largest and most versatile class of synthetic dyes (Keharia *et al*., 2004).

And for sulfonated azo dyes, both aromatic sulfonic and azo groups confer to their xenobiotic nature as these are rare among natural products (Junnarkar *et al*., 2006). Approximately 10-15% of the dyes are released into the environment during manufacturing and usage. Since some of the dyes are harmful, dye-containing wastes pose an important environmental problem (Verma & Madamwar, 2003). These dyes are poorly biodegradable because of their structures and treatment of wastewater containing dyes usually involves physical and/or chemical methods such as adsorption, coagulation-flocculation, oxidation, filtration and electrochemical methods. However, these methods are expensive and have operational problems. Furthermore, dyestuffs cannot be converted to CO_2 by physical and chemical methods. Complete degradation of dyestuff can only be accomplished by chemical or biological oxidation (Kapdan *et al*., 2000).

Corresponding author:* **Nishant Junnarkar
Department of Microbiology, Shree M. & N. Virani Science College, Rajkot, GUJARAT, India 360005

Several species of bacteria and fungi have been reported to decolorize and degrade textile dyes and have been employed in the treatment of dye bearing wastewaters.

Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970s with reports of *Bacillus subtilis* (Horitsu *et al*.,1977), then *Aeromonashydrophila* (Idaka & Ogawa 1978) followed by *Bacillus cereus* (Wuhrmann*et al*., 1980). Numerous bacteria capable of dye decolorization, either in pure cultures or in consortia, have been reported (Banat *et al*., 1996; Rajaguru*et al*., 2000; Coughlin *et al*., 2002; Pearce *et al*., 2003; Verma & Madamwar 2003; Junnarkar *et al*., 2006).

In this article, studies on decolorization of Reactive Black B (RBB, C.I. Reactive Black 5) by *Paenibacillusdendritiformis*strain CS_2a_4 in pure culture is reported. Optimization of cultural conditions was attempted for rapid decolorization of RBB by CS_2a_4.

MATERIALS AND METHODS

Chemicals and Organisms

All chemicals used were of analytical grade. The common name of all the dyes have been used for convenience. Reactive azo dye Reactive Black B (RBB), a diazo dye, was procured from Meghmani Chemicals Ltd. GIDC, Vatva, Ahmedabad, Gujarat, India. Structure of RBB is mentioned in Fig. 1.

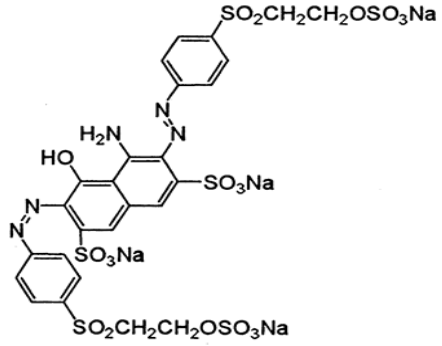

Fig 1 Structure of Reactive Black B (C. I. Reactive Black 5)

Bacterial culture *Paenibacillusdendritiformis* Strain CS_2a_4 was used for decolorization studies in detail. It was maintained on Nutrient Agar at 4ºC.

Medium

Bushnell-Haas Medium (BHM) containing the following in gL^{-1}: $MgSO_4$, 0.2; $CaCl_2$, 0.02; KH_2PO_4, 1.0; K_2HPO_4, 1.0; $(NH_4)_2NO_3$, 1.0; $FeCl_3$, 0.05, pH 7; supplemented with glucose (0.1 g%, w/v) and yeast extract (0.5 g %, w/v) and RBB (100 ppm), was used for enrichment of dye decolorizing bacteria from the samples.For isolation Nutrient Agar Medium was used. BHM + Glucose, 0.1 g% + Yeast Extract, 0.5g%; now is referred as Bacterial Decolorization Medium (BDM) further in the text.

Enrichment, Isolation and Screening of Dye Decolorizing Bacteria

Samples Collection

The soil and wastewater samples were collected from effluent-contaminated sites (22º 97'63.6"N, 72º 63.3"E) in the vicinities of dyeing industries located in GIDC Phase IV, Vatva, Ahmedabad, Gujarat, India and from the vicinities of dyeing industry (21º76'28.5"N,70º 63'08.7"E) located in Jetpur, Dist. Rajkot, Gujarat, India.1% suspension of the samples (soil, w/v and wastewater, v/v) were prepared in sterile distilled water and these were used for enrichment and isolation of dye-decolorizing bacteria using Bacterial Decolorization Medium (BDM).

Enrichment of dye decolorizing bacteria

For enrichment of dye decolorizing bacteria, 100 mL of BDM in 250 mL Erlenmeyer flasks were inoculated with 2 mL of 1% suspension and incubated on an orbital shaker (150 rpm), as well as, under stationary conditions at 37°C. Samples, which showed decolorization in liquid media, were repeatedly tested further for decolorization ability by transferring the enriched culture in fresh BDM till stable dye decolorizing cultures were obtained, showing consistent growth and decolorization in every successive transfer.

Isolation and Screening of dye decolorizing bacteria

The samples that showed consistent growth and decolorization in dye-containing medium were transferred on to Nutrient agar plates. This resulted in isolation of 22 different bacterial cultures, which were further tested for their abilities to decolorize the RBB dye in pure culture. Gram staining of the bacterial isolates was carried out to determine the morphology and Gram reaction. Amongst the several isolates, bacterial culture CS_2a_4 exhibited maximum decolorization in short duration and hence was selected for further studies.

Molecular Identification of bacterial strain CS_2a_4

The bacterial isolate CS_2a_4 was subjected to 16S rRNA gene sequencing by Sanger's method. DNA isolation was performed using XcelGen Bacterial gDNA Mini Kit (Cat No.: XG2411-01). The 16S rRNA gene was amplified using universal primers, 27F: 5'AGAGTTTGATCCTGGCTCAG3' and 1492R: 5'ACGGCTACCTTGTTACGACTT3'. The amplified PCR products were then subjected to sequenceing by BigDye Terminator v 3.0 cycle-sequencing kit on the ABI 3730 XL sequencer (Applied Biosystems, USA) using Foundation Data Collection Sofware v 3.0. The DNA sequence was assembled using Sequencing Analysis Software v 5.3 (Applied Biosystems, USA).

Online BLAST analysis on NCBI site revealed its 99% similarity with *Paenibacillusdendritiformis.* The sequence was submitted in GenBank under the accession number KU601317.

Decolorization Study and Quantification

Decolorization studies were performed in 250 mL flasks containing 100 mL of BDM amended with 100 ppm of RBB and each isolate was inoculated in it at the rate of 5% (v/v) inoculum volume. Flasks were then incubated at 37°C under static as well as shaking condition till decolorization was observed. Upon decolorization, thedecolorized samples were centrifuged at 8000 rpm for 10 min at 4°C. The supernatants were collected and absorbance was recorded at the absorption maxima of RBB (597nm) using double beam UV-Vis Spectrophotometer (UV-100, CyberLab, USA). Dye removal was expressed as percent decolorization using the following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial } A_{597} - \text{Final } A_{597}}{\text{Initial } A_{597}} \times 100$$

A_{597} = Absorbance at 597nm

Optimization of Cultural Conditions

Cultural conditions were optimized by varying C-sources (glucose, sucrose, galactose, fructose and peptone) and N-sources (yeast extract, meat extract and ammonium sulfate) in BHM, co-substrate concentration (Glucose: 0.1-1.0 g% & Yeast Extract: 0.1-0.5%), inoculum size (5%, 10%, 15% & 20%; v/v), initial pH of the decolorization medium (pH 4-10), incubation temperature (20, 25, 30, 35, 37, 40 and 45ºC) and initial dye concentration (50-500 ppm) in the decolorization medium. Once decolorization was achieved, the % decolorization was recorded. The experimental sets were run in triplicates, mean and standard deviation values were calculated.Decolorized samples obtained were further subjected to Spectral Analysis and TLC analysis.

Decolorization of RBB by CS_2a_4 culture under optimized cultural conditions

All the optimum cultural conditions were compiled and decolorization of RBB dye was assessed in optimized Bacterial Decolorization Medium (BDMO).

Spectral analysis of decolorized samples

Supernatants of decolorized samples were subjected to spectral analysis using double beam UV-Visible

spectrophotometer (UV-100, CyberLab, USA) in the UV and Visible range (200-800 nm).

TLC analysis of decolorized samples

The decolorized medium was collected in centrifuge tubes, centrifuged at 8000rpm for 10min. and the supernatant was subjected to extraction of degradation products using ethyl acetate as the solvent (1:1). The ethyl acetate extracts were dried in oven at 50°C and the residue was subjected to TLC analysis using propane-2-ol: liquor ammonia (7:3) as the solvent system.

RESULTS AND DISCUSSION

Isolation and Screening of RBB decolorizing bacteria

Isolation of bacterial cultures from the enriched cultures resulted in 22 different isolates. These were subjected to screening for the potent RBB decolorizing bacterial strain, where the isolate exhibiting maximum % decolorization in shorter incubation period was selected for further studies. The isolated bacterial strains exhibited % decolorization of RBB (100 ppm) in BDM, in the range of 92-45% in 40-70h of incubation at 37ºC in static condition (Table 1). Among these isolates, bacterial strain CS_2a_4 exhibited 92% decolorization (Fig. 2) in 40h and hence it was selected for further studies on RBB decolorization.

Table 1 Decolorization of RBB by different bacterial isolates in Bacterial Decolorization Medium under static conditions at 37°C.

Sr. No.	Culture code	Gram's staining reaction	% Decolorization	Incubation period (h)
1	PPc1	Gram negative	85.3	50
2.	PPc2	Gram negative	83.3	50
3.	PPa1	Gram negative	83.3	50
4.	PPa2	Gram negative	81.8	50
5	PPb1	Gram negative	78.2	72
6	PPb2	Gram negative	75.4	50
7	S3a1	Gram negative	79.3	72
8	S3a2	Gram negative	80.6	50
9	S3b1	Gram negative	72.1	72
10	S3b2	Gram negative	64.8	50
11	CS_2a_1	Gram variable	75.2	72
12	CS_2a_4	Gram variable	92.3	40
13	CS_2a_3	Gram variable	87.6	50
14	W1	Gram positive	78.2	72
15	W2	Gram positive	66.8	50
16	W7	Gram positive	73.2	72
17	W8	Gram positive	75.2	72
18	W9	Gram positive	78.9	50
19	B6	Gram negative	84.2	72
20	B10	Gram negative	81.3	72
21	B11	Gram negative	72.8	72
22	B12	Gram negative	82.6	50

Fig 2 BDM containing RBB (100ppm) and medium decolorized by bacterial culture CS_2a_4 under static condition at 37°C.

Molecular Identification of CS_2a_4

16S rRNA gene sequence of bacterial culture CS_2a_4 was submitted in GenBank under the accession number KU601317. Online BLAST analysis of the sequence on NCBI site exhibited 99% similarity with *Paenibacillusdendritiformis* and is now further referred as *Paenibacillusdendritiformis*strain CS_2a_4 in the text.

Effect of co-substrate on dye decolorization

Among the different C-sources (Fig. 3) and N-sources (Fig. 4) supplemented in BHB, CS_2a_4 exhibited maximum decolorization of RBB dye when glucose (84.5% Decolorization in 26 h) and yeast extract (71% Decolorization in 22 h) were supplemented in BHB. Majority of reports indicate obligate requirement of labile carbon source for the functioning of dye decolorizing bacteria (Banat *et al.*, 1996). The biodegradation of dyes without any supplement of carbon or nitrogen sources is very difficult (Sani and Banerjee, 1999). Azo dye decolorization by mixed as well as pure cultures generally requires complex organic sources, such as yeast extract, peptone, or a combination of complex organic sources and carbohydrates (Chen *et al.*, 2003; Khehra *et al.*, 2005).

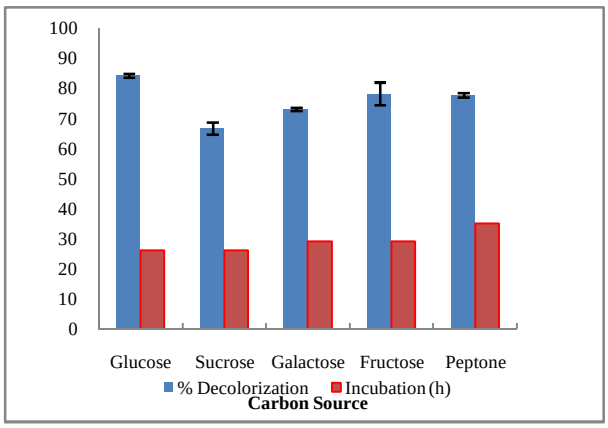

Fig 3 Effect of different C-sources as co substrate on decolorization of 100 ppm RBB dye byCS_2a_4 (inoculum volume 10%, pH 7.0) at 37ºC under static condition.

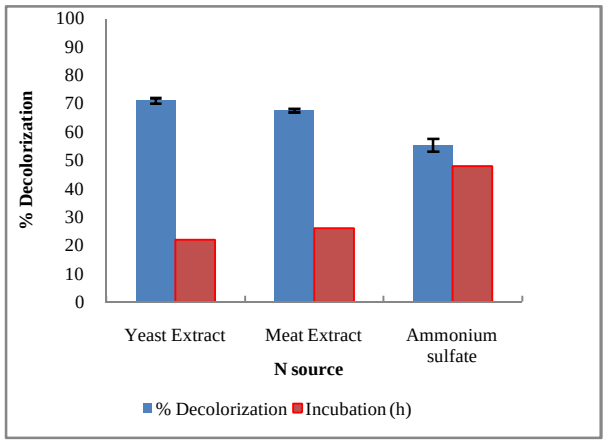

Fig 4 Effect of co substrate on decolorization of 100 ppm RBB dye byCS_2a_4 (inoculum volume 10%, pH 7.0) at 37ºC under static condition.

Effect of glucose and yeast extract concentration on decolorization

To optimize the concentration of glucose for maximum and rapid decolorization, glucose concentration was varied from

0.1 to 1 g% in BDM. Rapid and maximum % decolorization was observed at 1.0 g% (91% Decolorization in 24 h). Decolorizing efficiency of culture increased with increase in glucose from 0.1 to 1.0 g% in decolorization medium (Fig. 5). Similarly, concentration of yeast extract was varied from 0.1 g% to 0.5 g% (Fig. 6), to optimize its concentration in decolorization medium. Maximum % Decolorization was observed at 0.5 g% (93% Decolorization in 24 h). Thus, all the remaining experiments were performed at 1.0 g% glucose and 0.5 g% yeast extract.

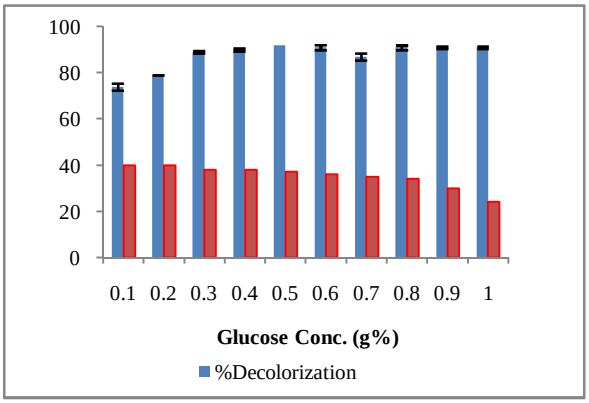

Fig 5 Effect of glucose concentration on decolorization of 100 ppm RBB dye by CS_2a_4 (Inoculum volume 10%, pH 7.0) at 37ºC under static condition.

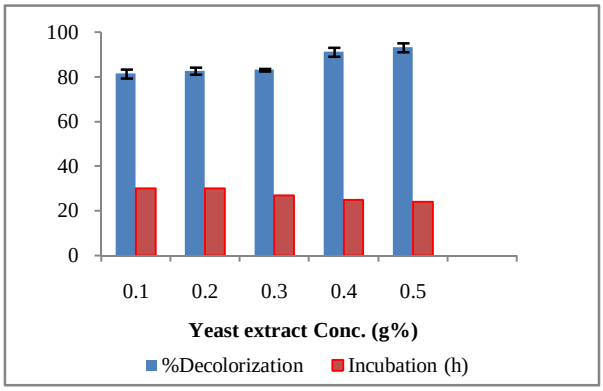

Fig 6 Effect of yeast extract concentration on decolorization of 100 ppm RBB dye by CS_2a_4 (Inoculum volume 10%, pH 7.0) at 37ºC under static condition.

For efficient reduction of azo dyes, a source of electron donors and redox mediators are essential (Khan *et al*., 2014). Here, glucose might have been used as electron donor by the culture for reduction of RBB. Moreover, redox mediators such as NADH might have been involved in reduction of azo bond by azo-reductases. Azo dye decolorization by mixed as well as pure cultures generally requires complex organic sources, such as yeast extract, peptone, or a combination of complex organic sources and carbohydrates (Chen *et al*., 2003; Khehra *et al*., 2005). During decolorization of azo dyes via reduction of azo bonds, it was reported that reducing equivalents from various carbon sources are transferred to the dye (Saratale *et al*., 2011). In addition to the type of the carbon source, it is important to consider the amount of the source because it must be sufficient to meet microbial growth requirements and achieve decolorization. However, high carbon concentrations lead to low decolorization because the microorganisms utilize the carbon source preferentially to the dye (Solis *et al*., 2012).

Effect of pH on dye decolorization

The culture could decolorize the dye in the range of 60-80 % Decolorization over a wide range of pH (Fig. 7). However, it exhibited rapid decolorization at pH 7. Thus; all the remaining experiments were performed at pH 7.

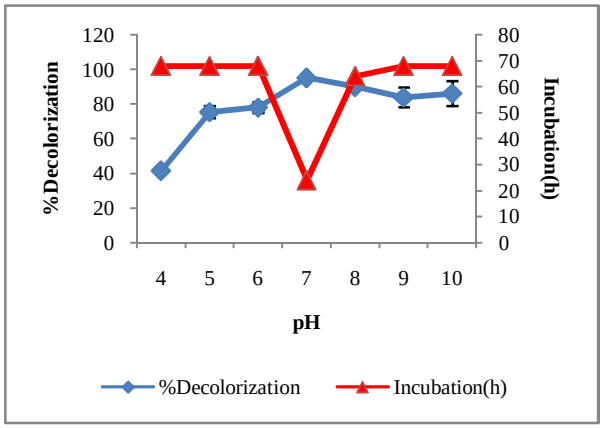

Fig 7 Effect of initial pH of medium on decolorization of 100 ppm RBB dye by CS_2a_4 (inoculum size 10%) at 37ºC under static condition.

The medium pH has a major effect on the dye decolorizing efficiency of bacterial cultures and the optimal pH for color removal had been reported between 6.0 and 10.0 (Chen *et al*., 2003; Gou *et al*., 2009; Kilic *et al*., 2007). Junnarkar *et al*. (2006) reported maximum decolorization of Direct Red 81 by bacterial consortium NBNJ6 at pH 7. Chang & Lin (2001) also reported that, both *E. coli*&*P. luteola*, exhibited best decolorization rate at pH 7 with constant decolorization rates up to pH 9.5. The rate of color removal is higher at the optimum pH and tends to decrease rapidly at strongly acidic or strongly alkaline pH.

Effect of temperature on dye decolorization

Incubation temperature was varied from 20ºC to 45ºC. Maximum decolorization was observed at 37ºC (91.33% in 24 h), whereas; above and below this optimum temperature lower % decolorization was observed (Fig. 8). It was observed that the decolorization rate of azo dyes increased up to the optimal temperature, and afterwards there was a marginal reduction in the decolorization activity. This decline at higher temperatures can be attributed to the loss of cell viability or the denaturation of an azo reductase enzyme (Saratale *et al*., 2011).

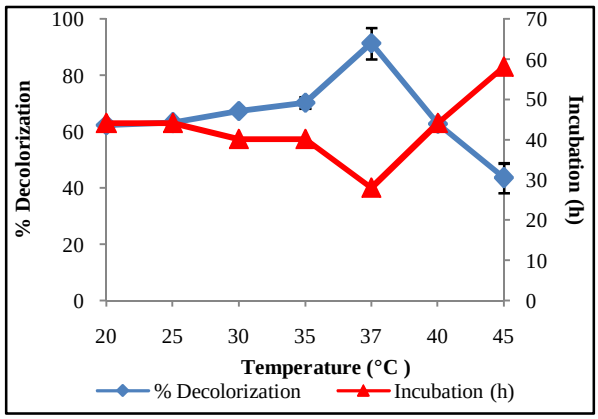

Fig 8 Effect of incubation temperature on decolorization of 100 ppm RBB dye byCS_2a_4 (inoculum size 10%, pH 7) at 37ºC under static condition

Incubation temperature is a key factor which affects the metabolic rate of the microorganisms. At optimum temperature, enzymes involved in dye decolorization are maximally functional. Most of the dye decolorizing bacterial strains had their optimum temperature at or near 37 ºC. Junnarkar *et al.* (2006) and Dave and Dave (2009) have reported that there was no considerable decolorization at temperatures above 40ºC, under agitating condition.

Effect of Inoculum size on dye decolorization

To find out the optimum inoculum needed for rapid and maximum decolorization by our organism, decolorizing ability was tested at different inoculum sizes viz. 5%, 10%, 15% and 20% (v/v). The decolorization period decreased with increase in the inoculum size (Fig. 9). At higher inoculum volume as number of cells are more, the extracellular enzymatic activities also tend to be higher, making easier for the cells to utilize external 'C' and energy sources as well as co-metabolize dye molecules present in the growth medium. Meng *et al.* (2012) reported faster decolorization of Acid Red 27 in presence of higher initial cell concentration. Junnarkar *et al.* (2006) reported 20% inoculum size to be the optimum one for attaining maximum decolorization rate for Direct Red 81 by the consortium NBNJ6. However, there was no proportionate increase in the percentage of decolorization with increase in the inoculum size of *Kurthia* sp. when inoculated in textile effluent (Sani & Banerjee, 1999). Dave and Dave (2009) have reported that beyond 25% up to inoculum size of 50% (v/v), time required for decolorization of Acid Red 119 did not decrease considerably, which indicated that 25% (v/v) was the optimum inoculum size.

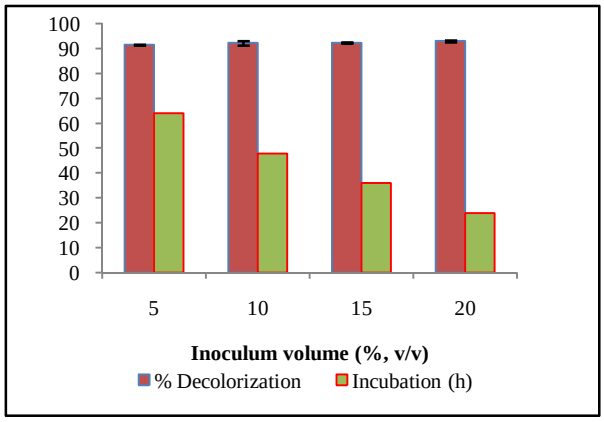

Fig 9 Effect of inoculums size (5%-20%, v/v), on decolorization of 100 ppm RBB dye by CS_2a_4 (pH 7.0, yeast extract 0.5%, glucose 1.0%) at 37ºC under static condition.

Effect of initial dye concentration on dye decolorization

The influence of dye concentration on the decolorization ability of the organism was investigated considering their occurrence in varying amounts in effluents. The decolorizing activity of the CS_2a_4 culture was assessed by providing RBB dye at different initial concentrations in optimized BDM (BDMO), varying from 50-500 ppm. With increase in dye concentration, time required for decolorization decreased with reduced % decolorization (Fig. 10). Similar results were observed in the bacterial decolorization of various reactive azo dyes (Kalyani *et al.*, 2008). Further increase in dye concentration resulted in reduction in decolorization rates. Similar observations were recorded by Kapil *et al.* (2009).

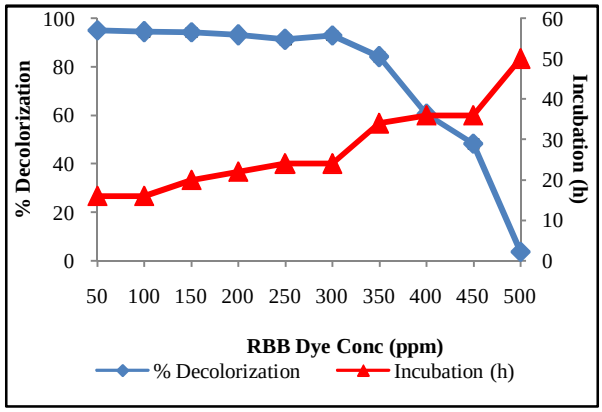

Fig 10 Effect of initial dye concentration (50-500 ppm), on decolorization of RBB by CS_2a_4 in BDMO.

It is observed thorough several literatures that increasing the dye concentration gradually decreases the decolorization rate, probably due to the toxic effect of dyes on individual bacteria and/or inadequate biomass concentration (or improper cell to dye ratio), as well as blockage of active sites of azoreductase by dye molecules with different structures (Jadhav *et al.*, 2008; Sani and Banerjee, 1999; Saratale *et al.*, 2009; Tony *et al.*, 2009).

Moreover, as shown in Fig. 10, decolorization period was reduced to 16h (94%), indicating the improved performance of the culture in BDMO.

Spectral analysis of decolorized medium

Spectral analysis of decolorized samples in the UV-Vis range (200-800 nm) revealed the shift of the peaks from visible range to UV range (Fig.11), which attributes to the formation of aromatic amines during transformation of the dyes by the culture in BDMO.

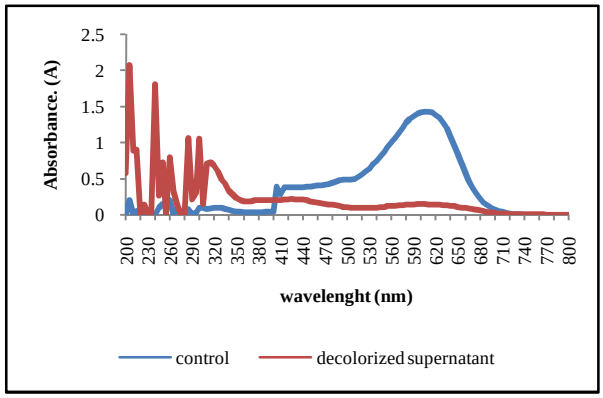

Fig 11 Spectral analysis of control (BDMO + 100 ppm RBB) and decolorized medium supernatant in UV-Visible range.

TLC analysis of decolorized medium supernatant

RBB dye decolorization by $CS_2a_4$was further supported by TLC analysis. When the dye chromatogram was observed in UV light, fluorescent blue colored bands with R_f values: 0.9, 0.95, 0.8 and 0.75 were observed in the lane of decolorized medium supernatant. Whereas the R_f values of bands observed in control (uninoculated medium) lane were 0.7 and 0.97; no such corresponding spots were observed in decolorized sample lane, indicating that decolorization was due to degradation or transformation of intact dye molecule into unknown intermediates.

CONCLUSION

Among the 22 bacterial isolatesobtained, *Paenibacillusdendritiformis* CS_2a_4 was found to be the potent decolorizer of Reactive Black B (RBB), under static conditions. Optimization of its cultural conditions resulted in the reduction of decolorization period for RBB from 40h to 16h. CS_2a_4 could decolorize RBB upto 350 ppm efficiently (>80%) within 16-30h. Spectral analysis and TLC analysis of decolorized medium revealed the transformation of RBB dye into unknown intermediates. Thus, the culture exhibited immense potential for its application in treatment of RBB dye bearing wastewater. Further, toxicity analysis of the products formed upon transformation of the dye by CS_2a_4 culture needs to be revealed.

References

Almeida, E. J. R. and Corso, C. R. 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.

Banat, I. M., Nigam, P., Singh, D. and Marchant, R. 1996. Microbial Decolorization of Textile- Dye-Containting Effluents: A Review, BioresTechnol, 58: 217-227.

Chang, J. and Lin, C. 2001. Decolorization Kinetics of a Recombinant *Escherichicoli* Strain Harboring Azo-Dye-Decolorizing Determinants from *Rhodococcus* sp., *Biotechnol Lett,* 23: 631-636.

Chen, K. C., Wu, J. Y., Liou, D. J. and Hwang, S. C. J. 2003. Decolorization of the Textile Dyes by Newly Isolated Bacterial Strains, *J Biotechnol,* 101: 57-68.

Coughlin M.F., Kinkle B.K., Bishop P.L. 2002. Degradation of acid orange 7 in an aerobic biofilm Chemosphere 46: 11-19

Dave, S and Dave, R. 2009. Isolation and characterization of Bacillus thuringiensis for Acid red 119 dye decolourisation, *BioresourTechnol*, 100: 249-253.

Dellametrice, P. M., Silva-Stenico, M. E., Beraldo de Moraes, L. A., Flore, M. F., Monteiro, R. T. R. 2017. Degradation of textile dyes by cyanobacteria, *Braz JMicrobiol,* 48: 25-31.

Gou, M., Qu, Y., Zhou, J. Ma, F. and Tan, L. 2009. Azo Dye Decolorization by a New Fungal Isolate, Penicillium sp. QQ and Fungal-Bacterial Cocultures, *J Hazard Mater*, 170: 314-319.

Horitsu, H, Takada, M., Idaka, E., Tomoyeda, M. & Ogawa, T. 1977. Degragation of *p*-aminoazobenzene, *Europ J AppMicrobiol*,4: 217-224.

Idaka, E. & Ogawa, Y. 1978. Degradation of azo compounds by *Aeromonashydrophila*var. 2413, *J Society Dyers Color*,94: 91-94.

Jadhav, S. U., M. U. Jadhav, A. N. Kagalkar, and Govindwar, S. P. 2008. Decolorization of Brilliant Blue G Dye Mediated by Degradation of the Microbial Consortium of *Galactomycesgeotrichum* and *Bacillus* sp., *J Chin Inst Chem. Engrs*, 39: 563-570.

Junnarkar, N., Murty, D. S., Bhatt, N. S. & Madamwar, D. 2006. Decolorization of diazo dye Direct Red 81 by a novel bacterial consortium. *World J of MicrobiolBiotechnol*. 22: 163-168.

Kalyani, D. C., Telke, A. A., Dhanve, R. S. and Jadhav, J. P. 2008.Ecofriendly Biodegradation and Detoxification of Reactive Red 2 Textile Dye by Newly Isolated *Pseudomonas* sp. SUK1, J Hazard Mater, 163: 735-742.

Kapdan I.K, Kargi, F., McMullan, G. & Marchant, R., 2000. Decolorization of textile dyestuffs by a mixed bacterial consortium, *Biotechnol Lett*, 22: 1179-1181.

Keharia, H., Patel, H. & Madamwar, D. 2004. Decolorization screening of synthetic dyes by anaerobic methanogenic sludge using a batch decolorization assay, *World J MicrobiolBiotechnol,* 20: 365-370.

Khan, Z., Jain, K., Soni, A. and Madamwar, D. 2014. Microaerophilic degradation of sulphonated azo dye-Reactive Red 195 by bacterial consortium AR1 through co-metabolism, *IntBiodeterior Biodegradation*, 94:167-175.

Khehra, M. S., H. S. Saini, D. K. Sharma, B. S. Chadha, and S. S. Chimni 2005. Comparative Studies on Potential of Consortium and Constituent Pure Bacterial Isolates to Decolorize Azo Dyes, *Water Res*, 39: 5135-5141.

Kilic, N. K., Nielsen, J. L. Yuce, M. and Donmez, G. 2007. Characterization of a Simple Bacterial Consortium for Effective Treatment of Wastewaters with Reactive Dyes and Cr(VI), *Chemosphere,* 67: 826-831.

Meng, X., Liu, G., Zhou, J., Q. Fu, S. and Wang, G. 2012. Azo dye decolorization by *Shewanellaaquimarina* under saline conditions, *BioresourTechnol*, 114: 95-101.

Pearce, C. I., Lloyd, J. R. & Guthrie, J. T. 2003. The removal of colour from textile wastewater using whole bacterial cells: A review, Dyes Pigm, 58: 179-196.

Rajaguru, P., Kalaiselvi, K., Palanival, M. & Subburam, V. 2000. Biodegradation of azo dyes in sequential anaerobic-aerobic system, *App MicrobiolBiotechnol*, 54: 268-273.

Sani, R. K. and Banerjee, U. C. 1999. Decolorization of Triphenylmethane Dyes and Textile and Dyestuff Effluent by *Kurthia*sp, *Enzyme MicrobTechnol*, 24: 433-437.

Saratale, G. D., Saratale, R. G., Chang, J. S. and Govindwar, S. P. 2011. Fixed-bed decolorization of Reactive Blue 172 by *Proteusvulgaris* NCIM-2027 immobilized on *Luffacylindrica* sponge, *IntBiodeterBiodeg*, 65: 494-503.

Saratale, R.G., Saratale, G.D., Chang, J.S., Govindwar, S.P., 2009. Decolorization and biodegradation of textile dye Navy blue HER by *Trichosporonbeigelii* NCIM- 3326, *J of Hazard Mater*, 166: 1421-1428.

Solis, M., Solís, B., Pérez, H. I., Manjarrez, N., Flores, M. 2012. Microbial decolouration of azo dyes: A review, *Process Biochem*, 47: 1723-1748.

Tony, B. D., Goyal, D. and Khanna, S. 2009. Decolorization of Textile Azo Dyes by Aerobic Bacterial Consortium, *IntBiodeterior Biodegradation*, 63: 462-469.

Verma, P. and Madamwar, D. 2003. Decolourization of synthetic dyes by a newly isolated strain of *Serratia marcescens, World J MicrobiolBiotechnol*, 19: 615-618.

Wuhrmann, K., Mechsner, K.L. & Kappeler, T. 1980. Investigation on rate determining factors in the microbial reduction of azo dyes, *European J ApplMicrobiol*, 9: 325-338.

****