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RESEARCH ARTICLE

REACTIVE DYE DECOLORIZATION BY ALKALOPHILIC BACTERIA ISOLATED FROM BENTONITE MINES

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ABSTRACT

Alkaline soil samples, collected from open mines, having different pH (7±0.3, 9±0.3 and 10±0.3) were used for the isolation of textile reactive dye decolorization study by enrichment. Five potent gram positive reactive Blue RGB dye decolorizing bacilli were isolated and identified during the study, these were designated as A, B, C, D and E for the ease of permutations. Decolorization of Reactive Blue RGB (100, 200, 500 and 1000 mg/L) was studied at pH 9±0.3 at 30±2.0ºC in Nutrient Broth containing glucose. Isolate E was the most efficient Reactive Blue RGB decolorizer, exhibiting 98.38% decolorization of Blue RGB (500 mg/L) in 24 h, with a rate of 20.50 mg/L/h moreover at 1000 mg/L initial dye concentration it could decolorize the dye by 95.10% with a very significant dye removal rate of 39.63 mg/L/h. The consortium containing AE, BE, CE, DE, CDE, EAB and EABC isolates decolorized Blue RGB (500 mg/L) with 98.38, 98.38, 98.09, 84.81, 84.95, 99.28 and 98.00% with an efficient dye removal rate of 20.50, 20.50, 20.44, 17.67, 17.70, 20.68 and 20.42 mg/L/h respectively, during the 1st 24 h of incubation.

INTRODUCTION

One of the main sources of severe pollution worldwide is the textile industries and their dye containing waste waters. The dyeing industry uses 10,000 different textile dyes with an estimated annual production of 7 x 10^5 metric tonnes and 30% of these dyes are used in excess of 1000 tonnes per annum, and 90% of the textile products are used at the level of 100 tonnes per annum or less (Baban *et al*., 2010; Robinson *et al*., 2001; Soloman *et al*., 2009).

The affinity of the dye for the fabric varies depending on the class of the dye and hence, its loss in waste waters could vary from 2% for basic dyes to as high as 50% for reactive dyes. The loss of dyes in wastewaters leads to severe contamination of surface and ground waters in the vicinity of dyeing industries (Ganesh *et al*., 1994; O'Neill *et al*., 1999). About 10-25 % of textile dyes are lost during the dyeing process and 2-20% is directly discharged as aqueous effluents in different environmental components. Water pollution caused by textile dyeing industry is a matter of serious concern due to significant organic matter and the dyeing agents that produce colors. The textile industry is a major consumer of water for its different wet processing operations, is also a major producer of effluent wastewater containing organic surfactants, salts, acids, alkalis, solvents and the residual dyes. The cotton textile industry is a growing industry in India and the wastewater from a typical cotton textile industry is characterized by high values of BOD, COD, color, and pH (ISPCH, 1995). The recycling of treated waste water has been recommended due to the high levels of contamination in dyeing and finishing processes (i.e., dyes, their breakdown products, pigments, dye intermediates, auxiliary chemicals and heavy metals, etc.) (Zaharia and Suteu, 2012). Conventionally wastewater treatments include adsorption, sedimentation, chemical methods, chemi-coagulation, biological methods, and advanced oxidation procedures (Basha *et al.*, 2008; Bayramoglu *et al.*, 2004; Can *et al.*, 2006). However, these approaches are not without their disadvantages. Moreover, absorbents are not reusable in general.

Biological methods, for example, take more time and cannot degrade complicated dye structures (Can *et al.*, 2006). In addition, some commercial dyes are harmful to many microorganisms (Mohan *et al.*, (2007). In Particular, the discharge of dye containing effluents into the water environment is unsuitable, not only because of their color, but also because many of dyes and their breakdown products released in the environment are toxic, carcinogenic or mutagenic to other life forms mainly because of carcinogens, such as benzidine, naphthalene and other aromatic compounds (Suteu *et al*., 2009; Zaharia *et al*., 2009).

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MATERIALS AND METHODS

Dye stuff and chemicals

All the chemicals and media components used in the study were of analytical grade (Hi-Media and S.D. Fine Laboratories). The λ_{max} of Reactive dyes used for the decolorization studies were; Reactive Violet 5 (500 nm), Yellow F3R (475 nm), Yellow RGB (470 nm), Orange W3R (500 nm), Red RGB (500 nm), Blue RGB (530 nm) and Red F3R (505 nm). The dyes were procured from the local dye manufacturing units and Reactive Blue RGB was used as a model dye for the optimization experiments. One gram of dye powder was added in 100 mL sterile distilled water and this stock solution was successively diluted for different experiments respectively.

Sample collection and enrichment of dye decolorizing bacteria

Alkaline soil samples were collected from open mines processing multi mineral soils for bentonite production at Ashapura Mines situated at Miyani village of Abdasa Taluka of Kutch located at latitude 23º 12' N and longitude 60º 44' E. The top layers of unprocessed, processed and soda added soils were collected and transferred to lab in polythene bags. These soils had different alkaline pH (7±0.3, 9±0.3 and 10±0.3) and the isolates were enriched at their respective pH. One gram of soil was added to 10 mL sterile distilled water and 5% (v/v) of soil suspension was inoculated in 20 mL of sterile medium containing g/L: glucose, 5.0; peptone, 10.0; sodium chloride, 5.0; beef extract, 3.0 supplemented with 50 mg/L of respective dye. The pH of the medium was maintained at 7±0.3, 9±0.3 and 10±0.3 respectively. All the tubes were incubated at 30±2.0ºC. After 90-95% decolorization of the added dye, 5% (v/v, containing 2.0×10^7 cells/mL) of each decolorized broth was used as an active inoculum for the further experiment. The experiments for the decolorization were performed in triplicates, using the medium as described above, pH 9±0.3, incubation temperature 30±2.0ºC. Control tubes were maintained with same conditions for each respective experiment and with different dye concentration. The procedure was repeated for 100 mg/L, 200 mg/L, 500 mg/L and 1000 mg/L of Blue RGB dye.

The decolorization ability was assessed for individual isolates and combination of different isolates, at different dye concentration.

Isolation and Preservation of the cultures

Decolorized (90-95%) broth was streaked on Nutrient agar plates for isolation of predominant flora followed by cultural characterization of the isolates. The obtained cultures were preserved at 4-8ºC in refrigerator on Nutrient Agar slants supplemented with 0.5% glucose. The procedure was repeated every month.

Characterization of dye decolorizing isolates

A well isolated colony of the respective isolate was used to study the colony characteristics, cell morphology and gram reaction. Gram reaction and morphological characteristics were investigated using Gram staining Kit (Hi-Media). Microscopic examination was carried out by compound light microscope (LABOVISION). The gram reaction was further confirmed by KOH and vancomycin test; wherein, a single colony of bacterial isolate was placed on concavity slide and a drop of 3% KOH was added and gel formation was checked after few minutes. Vancomycin is a narrow spectrum antibiotic, it specifically inhibits gram positive organisms. 0.1 mL of pure isolate was spread on sterile Nutrient agar medium. Vancomycin antibiotic disc (5 µg/disc) was placed at the center of the plate and incubated at 30±2.0ºC for 24-48 h.

Biochemical characterization

The metabolic characteristics were studied by using various biochemical media (Hi-Media Make), according to Bergey's manual of Determinative Bacteriology (Holt *et al*., 1994). All isolates were examined for the carbohydrate utilization pattern using various carbohydrates viz. L-Arabinose, Cellobiose, Fructose, D- Glucose, Glycogen, Meso-Inositol, Lactose, Mannitol, D- Mannose, Maltose, Melezitose, Melibiose, Raffinose, Rhamnose, Ribose, Salicin, Sorbitol, Sucrose, Starch, Trehalose, D- Xylose. The biochemical characteristics examined included; growth at 45ºC, growth at 65ºC, growth on 7% NaCl media, anaerobic growth, casein hydrolysis test, esculin hydrolysis test, gelatin hydrolysis test, starch hydrolysis test, lipid hydrolysis test, catalase test, urea utilization test, indole production test, citrate utilization test, nitrate reduction test, Voges– Proskauer test, arginine dehydrolase (ADH) test, lysine decarboxylase (LDC) test and ornithine decarboxylase (ODC) test.

Analytical Techniques

For the decolorization study, 3 mL of the uninoculated and inoculated broths were centrifuged at 6000 rpm for 15 min. The decolorization of dye was quantitatively analyzed by measuring the absorbance of the supernatant using spectrophotometer (Systronic 169) at its maximum wavelength, ($\lambda_{max,}$ of 530nm for Blue RGB dye).

The percentage of decolorization of individual isolates and consortia was calculated as follows:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

The dye removal rate was calculated as follows:

$$\text{Dye removal rate, (mg/L/h)} = \frac{\text{\% decolorization x Dye concentration (mg/L)}}{100 \times \text{time for decolorization (h)}}$$

RESULTS

Enrichment of dye decolorizing bacteria

Inoculation of 5% (v/v) of soil suspension to the nutrient medium enriched with 50 mg/L respective dye led to the decolorization of all the seven reactive dyes; wherein, the decolorization obtained after 24 h was; Reactive Violet 5R, 81.09%; Yellow F3R, 79.76%; Yellow RGB, 78.14%; Orange W3R, 71.95%; Red RGB, 66.66% and Red F3R, 65.61%. Reactive Blue RGB was most efficiently decolorized to 98.57% after 24 h with a dye removal rate of 2.05 mg/L/h; hence, Blue RGB was used as a model dye for further study.

Isolation of dye decolorizing bacteria

Five bacterial strains were isolated as the most active Blue RGB dye decolorizing bacteria from enrichment medium i.e., Nutrient broth medium supplemented with 0.5% glucose and Blue RGB (100 mg/L) at pH 9.0±0.3. These five isolates were designated as A, B, C, D, and E.

Characterization of Isolates

Cultural and morphological characterization

All the bacteria were gram positive bacilli, single and in chain form. The cultural characteristics of isolates were as shown in **Table: 1.** None of the isolates exhibited gel formation in the KOH test. This further confirmed the Gram's reaction of all the isolates. The isolates A, B, C, D and E exhibited 23 mm, 22 mm, 18 mm, 17 mm and 18 mm zone of inhibition around vancomycin antibiotic disk after incubation, which further confirmed the Gram's positive nature of all the isolates.

Table 1 Cultural characteristics

Characteristics	Isolates				
	A	**B**	**C**	**D**	**E**
Size	Large	Large	Large	Large	Small
shape	Irregular	Irregular	Round	Irregular	Round
Edge	Uneven	Uneven	Entire	Uneven	Entire
Elevation	Convex	Umbilicate	Flat	Flat	Flat
Opacity	Translucent	Opaque	Opaque	Opaque	Opaque
Pigmentation	No	No	No	No	No
Consistency	Dew drop	Smooth	Dry	Dry	Dry

Carbohydrate Utilization

Table 2 Sugar fermentation test

	A			**B**			**C**			**D**			**E**		
Sugar	Acid	Gas	Growth	Acid	Gas	Growth	Acid	Gas	Growth	Acid	Gas	Growth	Acid	Gas	Growth
L-Arabinose	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
D-glucose	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
Glycogen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Meso- Inositol	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Lactose	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+
Mannitol	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-
D- Mannose	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
Maltose	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
Melezitose	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Melibiose	+	-	+	+	-	+	-	-	+	-	-	+	+	-	+
Raffinose	-	-	+	-	-	+	-	-	+	-	-	+	+	-	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	+	-	+	-	-	+	+	-	+	+	-	+	-	-	+
Salicin	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
Sorbitol	+	-	+	+	-	+	-	-	+	-	-	+	-	-	+
Sucrose	+	-	+	+	-	+	+	-	+	-	-	+	+	-	+
Starch	+	-	+	+	-	+	-	-	+	+	-	+	+	-	+
Trehalose	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+
D- xylose	+	-	+	-	-	+	-	-	+	-	-	+	-	-	+

(+ Positive, - Negative)

On the basis of the sugar fermenting ability (**Table 2)** and the biochemical tests **(Table 3)**, the isolates were identified using the online ABIS Advanced Bacterial Identification Software (http://www.tgw1916.net/bacteria_logare.html) as shown in **Table 4.**

Decolorization of Blue RGB dye by individual isolates and consortia at different dye concentrations.

Decolorization activity of the bacterial isolates A, B, C, D and E were studied using Blue RGB at different concentration viz. 100, 200, 500 and 1000 mg/L, at interval of 24 h, up to 120 h.

At 100 mg/L of Blue RGB, best decolorization efficiency was exhibited by isolate E viz. 96.73%, 99.22%, 99.31%, 99.31%, and 99.31% at time interval of 24 h, 48 h, 72 h, 96 h and 120 h respectively (Table 3.5). Isolate A exhibited 33.56%, 43.35%, 47.61%, 88.45%, 94.69% decolorization. 35.81%, 47.71%, 47.5%, 49.76%, 92.26% decolorization was observed in case of isolate B. The consortium AE gave 68.92%, 77.32%, 94.66%, 94.91%, 94.91%; BE gave 45.38%, 54.39%, 95%, 97.34%, 97.34%; EAB gave 37.5%, 50.73%, 87.5%, 93.3%, 95.84%; and ABCDE gave 11.26%, 32%, 48.03%, 49.43%, 87.02% decolorization after 24 h, 48 h, 72 h 96 h, and 120 h respectively. Amongst the individual isolates, isolate E was found to be most efficient. 100 mg/L of Blue RGB was decolorized in the range of 96.73 to 99.31% when incubated for 24 to 120 h respectively. The dye removal rate during the first 24h of incubation was 4.03 mg/L/h which steadily decreased to 2.07, 1.38, 1.03 and 0.83 mg/L/h during subsequent incubation of 24 h. At 200 mg/L concentration of Blue RGB, isolate E exhibited decolorization in the range of 98.06 – 99.31%, when incubated for 24 – 120 h.

The maximum dye removal rate of 8.17 mg/L/h was observed at 24 h incubation time, with 98.06% decolorization. During the subsequent incubation periods of 24 h, percent decolorization increased to 98.12, 98.87, 98.93 and 99.31%; whereas, the dye removal rate decreased to 4.09, 2.76, 2.07 and 1.66 mg/L/h respectively. Isolates A and B performed fairly well at 200 mg/L concentration of Blue RGB.

Table3 Results of Biochemical test

Sr. No.	Test	A	B	C	D	E
1	Growth at 45ºC	+	+	+	+	+
2	Growth at 65ºC	-	-	-	-	-
3	Growth on 7% NaCl media	-	-	-	-	-
4	Anaerobic growth	+	+	+	+	+
5	Casein hydrolysis test	-	+	-	-	+
6	Esculin hydrolysis test	-	-	-	-	-
7	Gelatin hydrolysis test	-	+	+	Weak	-
8	Starch hydrolysis test	+	+	-	+	+
9	Lipid hydrolysis test	+	+	+	-	+
10	Catalase test	-	-	-	-	-
11	Urea utilization test	-	-	-	-	-
12	Indole production test	-	-	-	-	-
13	Citrate utilization test	+	+	-	-	-
14	Nitrate reduction test	-	-	-	-	-
15	Voges–Proskauer test	-	-	-	-	-
16	Arginine Dehydrolase (ADH) test	-	-	-	-	-
17	Lysine Decarboxylase (LDC) test	-	-	-	+	-
18	Ornithine Decarboxylase (ODC) test	-	-	-	-	-

Table 4 Identification of Isolates using online ABIS Advanced Bacterial Identification software

Isolate	Identification
A	*Bacillus niacini* ~ 83% (acc: 77%)
B	*Bacillus smithii* ~ 80% (acc: 79%)
C	*Bacillus drentensis* ~ 89% (acc: 73%)
D	*Virgibacillus (Bacillus) pantothenicus* ~ 84% (acc: 77%)
E	*Bacillus coagulans* ~ 89% (acc: 79%)

Isolate A expressed decolorization in the range of 98.0 – 99.31%; whereas, isolate B showed decolorization in the range of 98.62 – 99.50% when incubated up to 120 h. The increase in the incubation time was found to be inversely proportional to the dye removal rates expressed by both, isolates A and B. The dye removal rate of isolate A decreased from 8.17 to 4.09 mg/L/h, at an incubation time of 48 h, and further decreased 2.76, 2.07 and 1.66 mg/L/h. Isolate B removed the dye at a rate of 8.22 mg/L/h during the 1st 24 h of incubation which subsequently decreased to 4.12, 2.76, 2.07 and 1.66 mg/L/h respectively.

The decolorization exhibited by combination of isolates is shown in **Table 5**. Consortium AB expressed poor decolorization of 100 mg/L Blue RGB, however, at 200 mg/L concentration, decolorization was obtained in the range of 97.94 – 99.81% at incubation of 24 – 120 h. Similar results were observed with consortium CE, wherein, 200 mg/L Blue RGB was decolorized in the range of 96.62 – 99.37%. Consortium AE, BE and EAB showed lower decolorization of 100 mg/L Blue RGB till an incubation of 48 h, whereas at 200 mg/L concentration good decolorization was expressed at 24 and 48 h incubation time respectively viz. 99.25, 99.25% for AE; 98.12, 98.12 for BE and 98.87, 99.06 for EAB. The maximum decolorization expressed by consortium AE, BE and EAB after 120 h incubation were 99.68, 99.56 and 99.87% respectively. Poor decolorization efficiency was exhibited by consortium AC, AD, BC, BD, CD, ABC, BCD and ABCD.

Isolate E was most efficient in decolorization of Blue RGB dye at 500 and 1000 mg/L concentration (**Table 6).** Isolate E decolorized 98.38% of Blue RGB at a concentration of 500 mg/L in 24 h, at a rate of 20.50 mg/L/h.

Table 5 Decolorization of Blue RGB dye at 100 and 200 mg/L concentration by individual isolate and by the consortium

Isolates/ Consortium	Decolorization (%)									
	Dye concentration (mg/L)									
	100	200	100	200	100	200	100	200	100	200
	24 h		48 h		72 h		96 h		120 h	
A	33.56	**98.00**	43.35	**98.12**	47.61	**99.25**	**88.45**	**99.31**	**94.69**	**99.31**
B	35.81	**98.62**	47.5	**98.87**	47.71	**99.50**	49.76	**99.50**	**92.26**	**99.50**
C	32.09	19.06	43.46	19.75	44.54	22.56	47.92	24.69	56.32	26.44
D	29.27	17.00	32.96	18.12	36.06	18.12	39.95	18.75	42.26	18.75
E	**96.73**	**98.06**	**99.22**	**98.12**	**99.31**	**98.87**	**99.31**	**98.93**	**99.31**	**99.31**
AB	31.08	**97.94**	41.79	**97.94**	49.43	**98.06**	51.50	**98.69**	52.31	**99.81**
AC	24.43	21.87	26.81	23.69	30.68	23.75	33.83	24.81	39.03	31.37
AD	23.54	21.44	25.14	21.50	28.41	21.81	30.71	21.87	49.77	21.93
AE	68.92	**99.25**	77.32	**99.25**	**94.66**	**99.25**	**94.91**	**99.62**	**94.91**	**99.68**
BC	27.03	22.62	43.35	23.50	53.29	25.62	58.32	27.25	58.43	31.43
BD	17.45	23.12	31.62	23.75	43.18	25.18	46.88	25.37	48.96	26.06
BE	45.38	**98.12**	54.30	**98.12**	**95.00**	**98.19**	**97.34**	**99.56**	**97.34**	**99.56**
CD	30.97	15.62	32.96	17.37	34.66	20.12	35.10	21.31	35.33	26.87
CE	36.94	**96.62**	38.55	**96.62**	40.90	**97.62**	44.11	**98.12**	49.30	**99.37**
DE	30.18	79.50	31.51	80.31	33.75	81.06	34.75	81.25	34.75	84.06
ABC	32.05	25.00	42.23	27.75	57.61	28.00	60.74	30.06	60.74	33.87
BCD	21.28	22.87	34.97	23.12	49.43	24.18	51.96	24.37	52.07	28.68
CDE	24.63	82.44	32.96	82.50	33.29	84.18	33.83	84.31	34.18	88.18
DEA	21.73	78.06	23.80	78.12	26.60	78.12	42.96	78.12	53.46	81.87
EAB	37.50	**98.87**	50.73	**99.06**	**87.50**	**99.68**	**93.3**	**99.87**	**95.84**	**99.87**
ABCD	22.30	25.31	23.46	25.43	27.84	26.18	30.71	27.93	35.1	33.12
BCDE	27.36	80.37	33.74	81.06	34.09	81.06	34.75	81.25	37.18	83.87
CDEA	20.83	84.56	25.47	85.12	28.40	85.81	36.14	86.62	36.49	88.75
DEAB	25.56	83.56	28.16	83.62	28.29	83.68	41.45	85.12	54.15	85.81
EABC	21.17	79.00	28.04	79.18	31.81	79.37	33.6	79.50	38.22	82.93
ABCDE	11.26	75.87	32.00	76.31	48.03	77.18	49.43	77.50	**87.02**	80.00

Further incubation increased the decolorization percent i.e. 99.62, 99.62, 99.85 and 99.86%; whereas, the removal rate decreased during the subsequent incubation periods by 1.97, 1.5, 1.3 and 1.25 folds, with a removal rate of 4.16 mg/L/h after 120 h of incubation. The combination AE, BE, CE, DE, CDE, EAB and EABC decolorized 500 mg/L Blue RGB with 98.38,

98.38, 98.09, 84.81, 84.95, 99.28 and 98.00% with a removal rate of 20.50, 20.50, 20.44, 17.67, 17.70, 20.68 and 20.42 mg/L/h respectively, during the 1st 24 h of incubation.

gelling in KOH test and sensitive to Vancomycin. On the basis of cultural, morphological and metabolic characteristics, identification was done

Table 6 Decolorization of Blue RGB dye at 500 and 1000 mg/L concentration by individual isolate and by the consortium

Isolates/ Consortium	Decolorization (%)									
	Dye concentration (mg/L)									
	500	1000	500	1000	500	1000	500	1000	500	1000
	24 h		48 h		72 h		96 h		120 h	
A	15.90	10.61	24.24	19.59	24.81	20.82	25.90	20.82	26.24	20.82
B	11.43	15.71	24.46	20.92	35.38	22.65	35.66	23.27	37.05	23.78
C	13.09	8.37	20.76	14.80	22.43	16.84	22.66	18.47	24.38	19.08
D	44.09	28.06	50.38	30.10	50.47	30.10	51.24	31.33	52.33	32.45
E	**98.38**	**95.10**	**99.62**	**96.73**	**99.62**	**96.73**	**99.85**	**96.73**	**99.86**	**96.73**
AB	11.43	11.02	18.38	15.51	23.86	20.20	25.00	22.96	25.28	23.98
AC	17.23	17.96	24.23	19.49	24.28	22.65	24.28	23.27	26.43	23.88
AD	39.66	21.33	44.42	26.22	45.42	31.84	46.09	32.45	47.05	32.65
AE	**98.38**	37.65	**99.57**	41.84	**99.76**	43.57	**99.81**	45.51	**99.86**	46.12
BC	20.38	8.98	25.81	17.04	25.81	17.35	25.90	17.55	28.48	19.18
BD	39.90	23.47	44.23	26.02	44.28	26.84	45.62	28.78	46.71	29.29
BE	**98.38**	62.55	**99.23**	68.37	**99.57**	68.37	**99.71**	68.37	**99.86**	69.49
CD	47.43	22.86	52.86	26.63	54.52	29.29	57.04	29.8	57.86	30.61
CE	**98.09**	22.04	**99.09**	29.90	**99.28**	31.73	**99.62**	32.24	**99.76**	32.24
DE	**84.81**	61.73	**90.76**	68.78	**91.57**	69.80	**92.38**	70.51	**94.33**	70.92
ABC	11.52	12.96	23.28	14.39	27.09	19.29	29.71	21.53	30.86	23.16
BCD	41.19	19.59	44.71	25.10	45.33	25.61	47.61	26.33	48.00	26.73
CDE	**84.95**	64.80	**90.95**	65.00	**92.76**	65.31	**93.24**	65.31	**93.33**	65.31
DEA	71.05	64.80	70.33	65.51	74.95	65.82	78.14	66.02	79.14	66.33
EAB	**99.28**	30.10	**99.33**	37.55	**99.71**	39.08	**99.80**	39.69	**99.81**	40.31
ABCD	40.33	21.22	42.86	29.08	43.24	30.41	43.57	31.12	45.00	31.33
BCDE	71.95	59.49	76.28	65.00	77.95	65.31	79.90	65.31	80.95	65.51
CDEA	73.76	57.76	79.28	60.51	79.86	60.92	80.95	61.22	81.66	62.04
DEAB	66.66	**65.61**	72.38	**73.16**	74.28	**73.67**	74.66	**75.82**	76.28	**77.24**
EABC	**98.00**	11.84	**99.76**	14.08	**99.76**	17.24	**99.81**	21.53	**99.81**	26.12
ABCDE	67.38	26.94	73.95	20.71	74.76	32.45	75.42	34.69	75.71	37.35

Higher concentration of Blue RGB (1000 mg/L), decreased the decolorization efficiency of all the isolates and their combinations, except isolate E. During the 1st 24 h of incubation, isolate E could decolorize 95.10% with a very significant removal rate of 39.63 mg/L/h. Further increase in the incubation time increased the percent decolorization to 96.73% which remained constant up to 120 h incubation; but the removal rate decreased to 20.15, 13.44, 10.08 and 8.06 mg/L/h during subsequent incubation period. Consortium DEAB exhibited 65.61, 73.16, 73.67, 75.82 and 77.24% decolorization at different incubation periods with a maximum dye removal rate of 27.34 mg/L/h during the 1st 24 h and the subsequent removal rates were 15.24, 10.23, 7.90 and 6.44 mg/L/h. All other isolates and consortia showed lower decolorization and rate.

DISCUSSION

Textile Reactive dyes form strong covalent bonds with the cellulosic fiber in alkaline condition, and as the pH increases ionization with Cellulose increases, thus, increasing the fixation of the dye on the fabric (Chinta and Shrivastava, 2013); thereby the wastewater discharged from the textile dyeing unit has an alkaline pH. The treatment of such wastewater requires the neutralization of pH prior to biological treatment, which is cost intensive. Hence, the isolation of dye decolorizing alkalophilic bacteria was performed from open mines processing multi-mineral soils for bentonite production at pH 9.0±0.3. Five isolates capable of decolorizing Reactive Blue RGB dye were characterized. Morphologically these were Gram positive rods, occurring singly or in chains, without

by ABIS online (http://www. tgw1916.net). Results indicated that the isolates have close similarity with *Bacillus niacini, Bacillus smithii, Bacillus drentensis, Virgibacillus (Bacillus) pantothenicus* and *Bacillus coagulans*. Identification based on similar characterization, using ABIS online software, has been reported by Duggirala *et al*., 2013a. Isolate E (*Bacillus coagulans*) proved to be the most efficient in decolorizing Reactive Blue RGB (100 to 1000 mg/L); however, the decolorization expressed by A, B, AB, CE, DE, CDE, DEA, BCDE, CDEA, DEAB, EABC and ABCDE at 100 and 200 mg/L concentration, was directly proportional to the dye concentration.

Lower concentration (100 mg/L) of dye limited the decolorization percent whereas higher dye concentration (200 mg/L) provided sufficient induction and better decolorization. This pattern was observed in most of the isolates and their combinations albeit at different concentration of dye. Moreover, the percent decolorization was directly proportional to the incubation time. Dye concentration of 500 mg/L and above showed gradual inhibition of decolourization ability of the isolates and their combinations. Sheth and Dave, 2009, have reported a gradual inhibition of decolorization of Reactive Red BS at concentration of 800 ppm and above, by *Pseudomonas aeruginosa* NGKCTS. The dye removal rate exhibited by isolate E and consortium DEAB after 24 h, for Reactive Blue RGB (1000 mg/L) was 39.63 and 27.34 mg/L/h respectively, which was 2.3 and 1.6 folds higher than the removal rate reported by Duggirala *et al.*, 2013b, for Reactive Blue 250 (1000 mg/L) using anaerobic rumen consortium.

CONCLUSION

Five alkalophilic bacterial strains were isolated from open mines processing multi-mineral soils for bentonite production showed most active Reactive Blue RGB dye decolorizing ability at pH 9.0±0.3. These isolates could decolorize 7 different dyes namely Reactive Violet 5, Yellow F3R, Yellow RGB, Orange W3R, Red RGB, Blue RGB and Red F3R. Blue RGB was used as a model dye for decolorization study. Isolate E was found to be the most promising culture for the decolorization of Reactive Blue RGB dye and the best consortium obtained for the same was EAB. Isolate E could decolorize 98.38 % of Reactive Blue RGB (500 mg/L) with a rate of 20.50 mg/L/h, during the first 24 h of incubation; whereas 1000 mg/L dye was decolorized to 95.10% with a significant removal rate of 39.63 mg/L/h. Consortium EAB exhibited 99.28 % decolorization (500 mg/L) and a dye removal rate of 20.68 mg/L/h. The percentage decolorization was found to increase or remain unchanged during the subsequent incubation periods after the 24 h of incubation, whereas the dye removal rate was found to decrease; which could be due to the exhaustion of the nutrients and dye molecules present in the growth medium at the start of the incubation. The isolates are very promising for their application in bioremediation of textile dyeing units' wastewater and scale up of the process.

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