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Screening of Potential Environmental Isolate with Remazol Brilliant Blue R Dye Metabolizing Activity

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ABSTRACT

The effluents discarded from the rampantly growing textile industries have become a foremost environmental concern. The drawbacks of physical and chemical effluent treatment methods, especially secondary pollution due to formation of sludge, have led to an encouraged implementation of sustainable protocols for disposal of such industrial wastes. In the present study, isolates capable of degrading Remazol Brilliant Blue R (RBBR) dye were isolated from environmental samples and their efficiency was determined by analytical methods including Colorimetry and Gas Chromatography Mass Spectrophotometry (GCMS). Screening of dye metabolizing isolates was done in Minimal Salts Medium with RBBR as a sole source of carbon. Among 9 isolates obtained in this study, the most efficient dye degrader was identified as *Bacillus cereus*. It showed 82.60% decolorization of 0.1% dye in 96h at 30°C. The gas chromatogram of degraded dye metabolites showed 40 peaks compared to 9 observed for standard dye sample confirming degradation of dye molecule. The mass spectroscopic analysis showed presence of fatty acids, amines and alkanes as main components. In conclusion, *Bacillus cereus* isolated in this study efficiently metabolizedrecalcitrant RBBR dye into eco-friendly metabolites and hence has potential in bioremediation of dye polluted soil and water.

Keywords:Bioremediation, Dye degradation, Dye pollution, Effluent, Remazol Brilliant Blue R, Sustainable

INTRODUCTION

The textile and other dye utilizing industries occupy major portion of the market sectors following the industrial revolution in different parts of the world. Consequently, the dye containing wastes form a large proportion of the industrial effluents that require strict regulations regarding treatment and disposal. Since, these steps are tedious and mainly reduce overall industrial profit due to high economic inputs, most industries ignore the norms and standard environmental protocols of proper effluent disposal [1]. These effluents contain mutagenic dye and metal components, salts, phenols, aromatic amines and other toxic chemicals [2]. For this reason, the soil and aquatic ecosystems surrounding industrial areas are drastically compromised. To sustain the ever growing textile and other dye consuming industries, depending on natural pigments is impractical. Synthetic dyes offer advantages like cost effectiveness, higher stability to pH, light and temperature, and color diversity compared to natural pigments and hence they are difficult to replace [3]. Verily, dye pollution will remain a consistently dire environmental threat unless competent natural pigments are synthesized and sustainable biodegradation methods are practiced.

The microbiological method of effluent treatment is one of the sustainable approaches to overcome dye pollution related environmental threat. It basically relies on the use of metabolically active free viable or immobilized cells of microorganisms. These microorganisms can be isolated from dye polluted regions where they naturally adapt to extreme environmental stress conditions by producing necessary enzymes and practicing defense mechanisms. Multiple factors decide the efficiency of microbiological sustainability approaches. These include the stability of cells, presence of multiple enzymes for serial degradation of toxic dye metabolites into nontoxic compounds and preference for dye molecules by the isolate. Hence, live microbial cells are best candidates for carrying out bioremediation processes since they can metabolize xenobiotics into environmental friendly compounds using series of enzymes produced during their biochemical cycles [4, 5]. For the same reason, biotechnological methods like use of purified enzymes or microbial products are discouraged by some researchers. Along with above advantages, the use of free cells is associated with the disadvantage of the fragility of microbial cells due to which they are often not recoverable after effluent treatment [6]. Various natural processes exist to overcome the above challenge without modification of microbial genes. The most widely accepted method involves screening and isolation of microorganisms from dye effluents itself or surrounding soil/

water, for application in various bioremediation strategies. This approach allows skipping the primary enrichment step of microbiological processes since the above environmental conditions allow enrichment of potential strains naturally, which can be best explained with Darwin's theory of '*survival of the best*' species in a given ecosystem. On the other hand, owing to the widespread pollution, dye degrading bacteria persist in diverse environments which are away from industrial areas.

In the present study, potential dye degrading bacteria were screened and isolated from several random sites and their efficiency to metabolize Remazol brilliant blue R (RBBR) was determined using analytical techniques like colorimetry and Gas Chromatography Mass Spectrophotometry (GCMS).

MATERIALS AND METHODS

A. Sample Collection

The soil samples were collected from areas surrounding Sewri petrol pump and Sophia College garden, Mumbai, India. The water samples were collected from Girgaon Chowpatty and Mithi River, Mumbai, India and Ganges River, place, India. These samples were transported from above sites to the laboratory in screw-capped bottles, where they were processed immediately.

B. Chemicals and Culture Medium

The textile anthraquinone dyeRemazol Brilliant Blue R (RBBR) was purchased from Sisco Research Laboratory. Salts used for preparing the Minimal Salt Medium (MSM) were purchased from Hi-Media Laboratories, Mumbai, India. All the chemicals used in the study were of analytical grade.

C. Enrichment of RBBR Dye Degrading Microorganisms

The dye degrading microorganisms from above samples were enriched in MSM[composition in g/l: K_2HPO_4 (7.00), KH_2PO_4 (2.00), $MgSO_4.7H_2O$ (0.1), $(NH4)_2SO_4$ (1.00), Sodium citrate (0.5), traces of yeast extract and glucose (0.1% w/v, pH adjusted to 7+/-0.2] containing 0.1% RBBR with an intention to isolate strains that can utilize the dye as a sole source of carbon. MSM was amended with soil (5% w/v)/ water (5% v/v) samples and 0.1% dye, and incubated at Room Temperature (RT; 30°C) for 7 days under static conditions for enrichment of RBBR degrading microorganisms [7].

D. Screening and Isolation of RBBR Dye Degrading Microorganisms

After enrichment of MSM for a week, the dye degrading microorganisms were isolated on Minimal Salt Agar Medium (MSAM) plates containing 0.1% RBBR. It was prepared by solidifying MSM with 1.5% agar. The plates were incubated at RT for 24 h and then observed for zones of clearance around the isolated colonies. The zone of clearance indicates the ability of isolates to decolorize dyes, which is the preliminary step in biodegradation of dye molecules [8]. Well isolated colonies showing clearance zones were selected for further studies. The cultures were purified by streaking on MSAM plates and maintained on MSAM slants containing 0.1% RBBR.

E. Determination of RBBR Biodecolorization Potential

Potential of dye decolorizing strains isolated from enriched medium was determined using a colorimeter. The isolated strains were inoculated (0.1 OD) in 100ml MSM containing 0.1% RBBR and the medium was incubated at RT for 96 h. The extent of decolorization of media was measured every 24 h up to 96 h by collecting 10 ml aliquots. The cell-free supernatant was obtained by centrifuging aliquots of the inoculated media at 12,000 rpm for 15 min. The decolorization activity was expressed in terms of percentage (%) decolorization and determined by monitoring the decrease in absorbance at the maximum wavelength of dye (575nm). The uninoculated culture medium containing 0.1% RBBR was used as an experimental control and sterile MSM without test culture and dye was set up as sterility control. MSM broth inoculated with potential isolate (and without dye) was maintained as blank.The percentage decolorization was calculated as [9].

$$\% \text{ Decolorization } = \frac{A0 - At}{A0} \; x \; 100 \quad (1)$$

Where, 'A0' is the absorbance of blank and 'At' is the absorbance of test samples.

F. Identification of Potential Strain

The isolate showing maximum decolorization was identified based on cultural, morphological and biochemical tests by comparison of observations with Bergey's manual [10]. The strain identification was done by 16s rRNA analysis, which was outsourced to Codon Life sciences, Goa, India.

G. Determination of RBBR Biodegradation Potential

To confirm biodegradation of dye, the decolorized samples were subjected to GCMS analysis. The cell free supernatant obtained after 96 h incubation was used as sample for the analysis. The biodegradation products were extracted from cells by hydrolysis acidification treatment. It was done by adding an equal volume of ethyl acetate, dried over anhydrous sodium sulfate and dissolved in HPLC-grade methanol [11]. The analysis was carried out at SAIF, IIT, Mumbai, India.

The biodegradation products were identified according to reaction time and mass spectra in the 'National Institute of Standards and Technology' library stored in the GCMS software.

RESULTS AND DISCUSSION

H. Screening of Potential Dye Degrading Microorganisms

On isolation on MSAM, after selective enrichment of dye degraders in MSM, 9 distinct bacterial isolates were selected in our study. The characteristics and details of all isolates are represented in Table 1.Since the dye is toxic to most microbes other than dye degraders, instead of expected clearance zones around colonies, we observed completely decolorized plates after formation of colonies in 24h. A pictorial representation of screening process is shown in Fig. 1.

Table 1: Characteristics of isolates obtained fromdifferent sites

Isolate	Source	Colony characteristics	Gram nature and arrangement
C1	GirgaumChowpatty	White pinpoint circular, flat and opaque colony with entire margin and smooth consistency	Gram positive rods in chains
C2		Grey pinhead sized circular, raised and opaque colony with entire margin and butyrous consistency	Gram negative cocci
M1	Mithi river	White pinhead sized circular flat and opaque colony with irregular margin and smooth consistency	Gram-positive short rods in chains
M2		Bluishwhite pinpoint circular, flat and opaque colony with entire margin and smooth consistency	Gram positive rods
G	Ganges river	Greyish white pinhead sized circular, raised and opaque colony with entire margin and mucoid consistency	Gram positive cocci in chains
P1	Sewri petrol pump	Greyish white pinhead sized circular, raised and opaque colony with entire margin and smooth consistency	Grampositive short rods in chains
P2		White pinpoint circular, flat and opaque colony with entire margin and mucoid consistency	Gram negative cocci
P3		Grey pinpoint circular, raised and opaque colony with entire margin and smooth consistency	Grampositive cocci in pair
S1	Sophia College Garden	White pinhead sized irregular shaped, flat and opaque colony with irregular margin and butyrous consistency	Grampositive short rods in chains

Owing to the widespread textile dye pollution, screening of dye degrading microorganisms have become easier due to natural enrichment of these strains in nature. Similar to our study, Modi et al. [12] used Minimal Basal medium containing 0.1% methyl orange to enrich dye degrading bacteria from soil sample collected from Kamothe, Navi Mumbai, India. However, they used higher concentration of glucose (10%) for screening to support initial biomass build-up that can metabolize the dye more efficiently. Though this procedure is effective, it also results in enrichment of several non- dye degraders due to availability of readily metabolizable carbon source. In another study, 45 isolates were obtained from soil and water samples collected from textile industries in Punjab, India, amongst which 24 isolates showed good potential to degrade wide spectrum of dyes including Black WNN, Blue FNR, Red FN2BL, BlueRC, TURQ Blue and Diresul RDT Black dye.

Unlike our study, microtiter plates were used for screening of isolates for rapid analysis of observations [13]. Compared to the agar plate method used in our study, use of microtiter plates is more practical approach for screening of large amount of samples to identify potential isolates capable of degrading wide range of dyes, as done in above study. Thirty bacterial strains capable of degrading Novacron Red SB were screened from 6 soil/water samples obtained from textile industries in Bangladesh. Among these isolates, 8 strains degraded the dye more efficiently. This study used semi-solid medium for screening process to obtain both aerobic and microaerophilic dye degrading isolates [14]. Considering the oxygen stress in industrial effluents, the above technique can significantly reduce the enrichment and screening time to obtain the most potential strain that can be adapted to bioremediation processes.

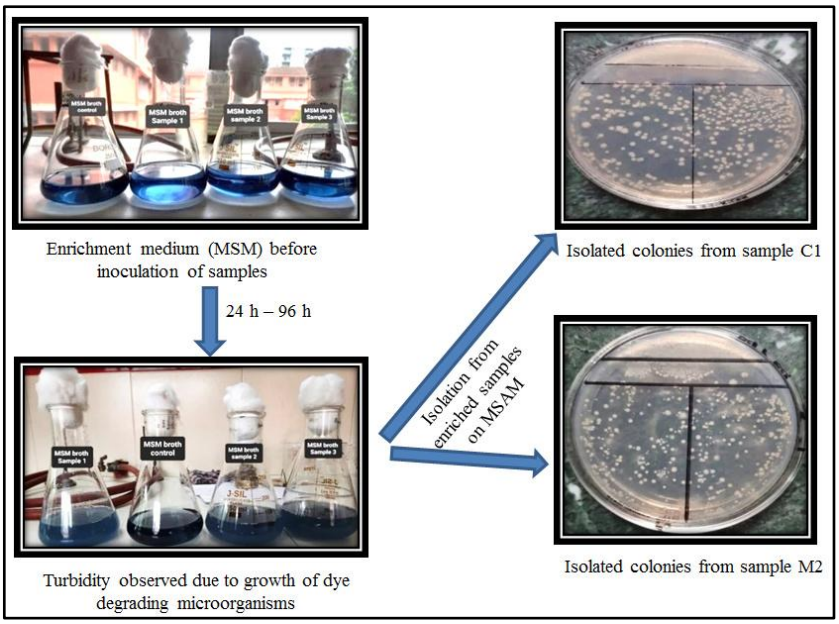

Fig. 1: Steps in screening of RBBR dye degrading microorganisms

Selection and Identification of Potential Isolate with RBBR Biodecolorization Ability

The Fig. 2 represents the extent of dye decolorized by isolates at different time intervals. The observations indicated that isolate M2 decolorized RBBR dye with highest efficiency (82.60% in 96h) compared to other isolates (Table 2).Spore staining of isolate M2 showed presence of spores at both ends of the rods and preliminary tests indicated that the isolate belongs to the Genus *Bacillus*. The 16s rRNA analysis showed 100% identity of this strain with *Bacillus cereus* (NCBI accession no. LRRP01000356).

In literature, several bacterial species capable of decolorizing dye is reported. In an application based study, the cell free extracts of *Aeromonas taiwanensis* completely decolourized a dyed fabric in 21 h on incubation at 37°C and static conditions [12]. An unidentified isolate NF-23 decolorized wide spectrum of dyes to different extent in 72 h on incubation at 35°C, pH 9 and anoxic conditions. Most efficiently, it decolorized 5% dye concentrations of Black WNN and Diresul RDT black followed by Red FN2BL and Blue FNR. Also, enhanced decolorization activity was observed in presence of peptone as co-substrate [13]. Among the 8 potential isolates screened in another study, 3 were identified as *Bacillus* sp. (*Bacillusalvei*, *Bacilluspolymyxa*, *Bacillusmegaterium*) [14]. Also, *Bacillus* strains D1023, D1032 and D1033 isolated from decomposing tree roots decolorized 89.58%, 69.79% and 84.26% of RBBR dye respectively [15]. *Bacillus* sp. is a common environmental isolate which exhibit diverse enzymatic activities. Hence, it is not surprising to obtain these strains commonly from environmental samples.In contrast to these studies, dye decolorization ability of *Neisseria* sp., *Vibrio* sp., *Bacillus* sp., and *Aeromonas* sp. enriched from dyeing effluent samples were negligible in absence of co-substrates (glucose and yeast extract) [16].

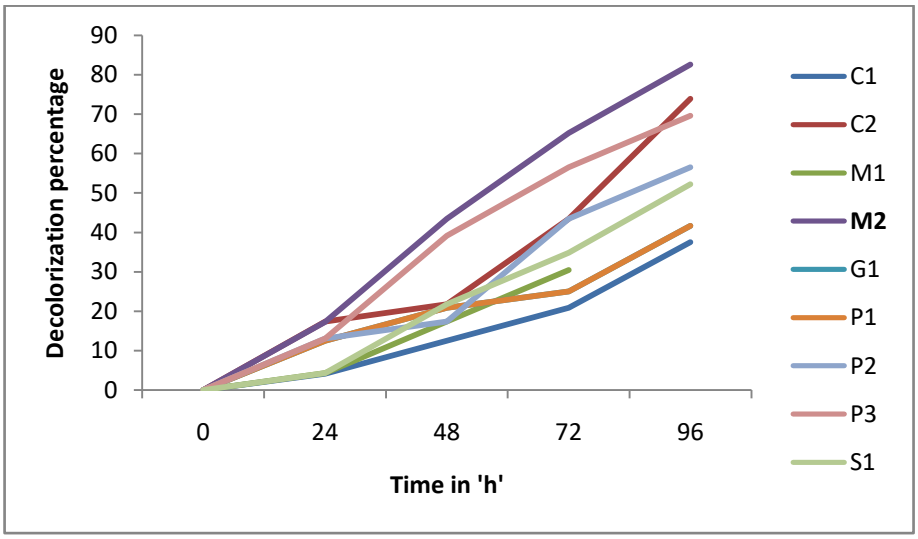

Fig. 2:Dye decolorization by isolates at different time intervals

Table 2: Maximum decolorization of RBBR by isolates

Isolate	Percent (%) decolorization in 96 h
C1	37.51
C2	73.91
M1	60.86
M2	80.60
G	47.82
P1	41.66
P2	56.52
P3	69.56
S	52.17

Determination of RBBR Biodegradation Potential

The Gas chromatogram (Fig. 3) represents the peaks obtained on analysis of standard dye and degraded dye metabolites. The peaks obtained in the Gas chromatogram of dye sample decolorized by *B. cereus* were different from the chromatogram of standard RBBR dye. Overall, the standard dye chromatogram showed 9 peaks and degraded dye metabolites chromatogram showed 40 peaks. Also, none of the peaks observed in standard dye sample were present in degraded dye metabolites chromatogram. This indicates that the dye molecule was effectively degraded in 96 h by *Bacillus cereus*. The identified components corresponding to each peak observed in chromatogram of standard dye and degraded metabolites are represented in Tables 3 and 4 respectively.Majority of the byproducts formed on degradation of dye were fatty acids followed by amines and alkanes (Table 4).

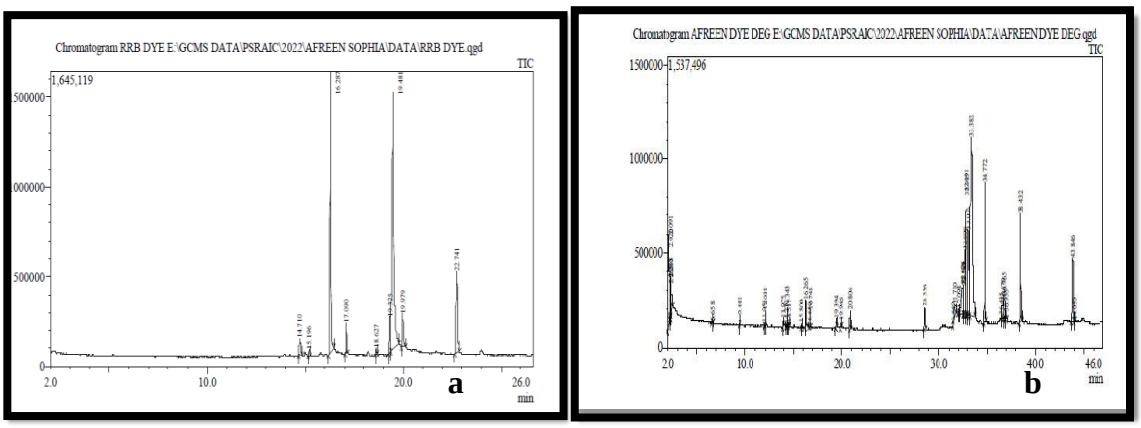

Fig. 3: Gas chromatogram of (a) standard dye and (b) degraded dye metabolites

Table 3: Components corresponding to peaks observed in standard dye

Peak	RTime	Area	Area%	Height	Name
1	14.710	477243	2.46	94585	Phytol, acetate
2	15.196	147833	0.76	35434	3, 7, 11, 15-Tetramethyl- 2- hexadecene- 1-ol
3	16.287	4957978	25.54	1569586	n- hexadecanoic acid
4	17.090	41249	2.53	170264	Octadecenal
5	18.627	117914	0.61	34997	1- nonadecenamine, N, N- dimethyl-
6	19.325	956011	4.93	197381	9, 12- octadecadienoic acid (Z, Z)-
7	19.481	9068712	46.72	1433239	Oleic acid
8	19.979	814802	4.20	185360	Octadecanoic acid
9	22.741	2377733	12.25	455849	3- Diethylamino- 2, 2- dimethyl propionaldehyde

Table 4: Components corresponding to peaks observed in degraded dye

Peak	RTime	Area	Area%	Height	Name
1	2.020	356140	0.72	448091	1-Cyclohexylethylamine
2	2.091	4843596	9.74	497969	(2-Aziridinylethyl)amine
3	2.280	585662	1.18	219426	4-Benzyloxyamphetamine
4	2.318	649920	1.31	214359	Carbamic acid,monoammonium
5	2.365	785895	1.58	166456	(S)-(+)-1-Cyclohexylethylamine

6	6.658	12540	0.03	8272	Dodecane
7	9.481	83266	0.17	51037	Pentadecane
8	11.951	21413	0.04	11650	1-Heptadecene
9	12.038	152221	0.31	80990	Hexadecane
10	13.975	133635	0.27	44475	Tetradecanoic acid
11	14.270	21447	0.04	12904	1-Heptadecene
12	14.343	156944	0.32	94935	Octadecane
13	14.511	60929	0.12	16098	Tridecanal
14	15.800	52002	0.10	24607	7,9-Di-tert-butyl-1- oxaspiro(4,5)deca-6,9-diene-2,8-dione
15	16.265	436298	0.88	139157	n-Hexadecanoic acid
16	16.650	9223	0.02	4750	Trichloroacetic acid,pentadecyl ester
17	16.748	207886	0.42	87394	Pentacosane
18	19.394	168258	0.34	43530	cis-Vaccenic acid
19	19.946	132515	0.27	34286	Octadecanoic acid
20	20.804	398533	0.80	97385	Pentacosane
21	28.556	890659	1.79	110229	Pentacosane
22	31.440	9753	0.02	3396	(S)-(+)-1-Cyclohexylethylamine
23	31.710	797098	1.60	65507	Tetratriacontane
24	32.094	122921	0.25	26958	Tetracosane
25	32.476	1976704	3.97	162225	Tritriacontane
26	32.519	462280	0.93	159118	Tritetracontane
27	32.565	228784	0.46	154987	Oxalic acid, isohexylneopentyl ester
28	32.696	3466184	6.97	343425	Tetracontane
29	32.891	3796869	7.63	653880	Tetracontane
30	32.925	3041787	6.12	631903	Dotriacontane
31	33.075	4276067	8.60	454014	Tetracontane
32	33.382	1220388	24.53	948696	Tetracontane
33	34.772	2274744	4.57	742644	Pentacosane
34	36.435	138131	0.28	16842	Pentatriacontane
35	36.670	771346	1.55	92112	Eicosane, 2-cyclohexyl
36	36.765	1002237	2.01	126177	1-Hentetracontanol
37	36.955	205577	0.41	36488	1-Hentetracontanol
38	38.432	2509507	5.05	572178	Pentacosane
39	43.846	2278110	4.58	333804	Pentacosane
40	44.050	20874	0.04	5265	2-Aminononadecane1-Methyloctadecylamine

The degradation of synthetic dyes occurs due to activity of oxidoreductase enzymes like laccase and lignin peroxidase. These enzymes are present in many microbial species. The presence of fatty acids as one of the common metabolite in our study signifies efficient degradation of dyes because these components can be easily metabolized further by microorganisms through the β oxidation pathway. Though other metabolites obtained in this study including amines and alkanes exhibit toxicity, their effect is relatively less intense compared to dye molecule [17]. Also, the overall concentration of these metabolites was very low compared to fatty acids. Similar to our study, a fatty acid (2-amino benzoic acid) was found to be the major metabolite of methyl red dye on degradation by *Bacillus brevis*, *Bacillus coagulans*, *Bacillus subtilis* and *Lysinibacillusfusiformis*[18]. Biodegradation of methyl red by *Pseudomonas aeruginosa* formed benzoic acid and o-xylene as major metabolites on Day 3 of incubation [19].

CONCLUSION

The process of biodegradation of synthetic dyes is complex and therefore requires combination of strategies for obtaining effective results. Since the microbial ecosystems remain unexplored even after the present scientific advances, one of the best strategies for bioremediation is screening of indigenous strains acclimatized to xenobiotic stress and adapted to degrade these compounds for their own survival. Literature reports many bacterial strains that decolorizes the dye molecule (by partial degradation) and results in formation of equally toxic end products. Unlike these strains, *B. cereus* isolated in our study was capable of metabolizing RBBR into more simple compounds. Thus, it has significant potential in bioremediation of dye polluted sites.

REFERENCES

[1]. T. Schellenberg, V. Subramanian, G. Ganeshan, D. Tompkins, and R. Pradeep, “Wastewater Discharge standards in the evolving context of urban sustainability–The case of India,” Front. Environ. Sc., vol. 8, Article 483657, April 2020.

[2]. T. Shindhal, P. Rakholiya, S. Varjani, A. Pandey, H. H. Ngo, W. Guo, H. Y. Ng, and M. J. Taherzadeh, M. J, "A critical review on advances in the practices and perspectives for the treatment of dye industry wastewater," Bioengineered, vol. 12, pp. 70–87, December 2020.
[3]. H. B. Slama, A. Chenari Bouket, Z. Pourhassan, F. N. Alenezi, A. Silini, T. Oszako, L. Luptakova, P. Golińska, and L. Belbahri, "Diversity of synthetic dyes from textile industries, discharge impacts and treatment methods. Appl. Sc., vol. 11, pp. 6255, July 2021.
[4]. R. Jamee, and R. Siddique, "Biodegradation of synthetic dyes of textile effluent by microorganisms: an environmentally and economically sustainable approach," Eur. J. Microbiol. Immunol., vol. 9, pp. 114–118, October 2019.
[5]. L. R. Pinheiro, D. G. Gradíssimo, L. P. Xavier, and A.V Santos, "Degradation of azo dyes: bacterial potential for bioremediation," Sustainability, vol. 14, pp. 1510, January 2022.
[6]. S. Moyo, B. P. Makhanya and P. E. Zwane, "Use of bacterial isolates in the treatment of textile dye wastewater: A review," Heliyon, vol. 8, pp. e09632, June 2022.
[7]. S. Lalnunhlimi, and V. Krishnaswamy, "Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium," Braz. J. Microbiol., vol. 47, pp. 39–46, February 2016.
[8]. A. U. Jadhav, and C. V. Joshi, "Isolation of Azo dyes degrading microorganisms," J. Emerg. Technol. Innov. Res., vol. 9, pp. c759–c769, August 2022.
[9]. M. M. Sahasrabudhe, R. G. Saratale, G. D. Saratale and G. R. Pathade, "Decolorization and detoxification of sulfonated toxic diazo dye C.I. Direct Red 81 by *Enterococcus faecalis* YZ 66," J. Environ. Health Sci. Eng., vol. 12, pp. 151, December 2014.
[10]. R. E. Buchanan, and N. E. Gibbons, "Bergey's Manual of Determinative Bacteriology," 8th Edition, Williams and Wilkins, Baltimore, 1974, pp. 1268.
[11]. K. Rasool, K. A. Mahmoud and D. S. Lee, "Influence of co-substrate on textile wastewater treatment and microbial community changes in the anaerobic biological sulfate reduction process," J. Hazard. Mat., vol. 299, pp. 453-461, December 2015.
[12]. A. Modi, S. Singh, J. Patki, and N. Padmadas,"Screening and identification of azo dye decolorizers from mangrove rhizospheric soil," Environ. Sci. Pollut. Res. Int., vol. 29, pp.83496-83511, November 2022.
[13]. A. Pokharia, and S. S. Ahluwalia, "Isolation and screening of dye decolorizing bacterial isolates from contaminated sites," Textiles Light Ind. Sc. Technol., vol. 2, pp. 54, April 2013.
[14]. F. Hussain, R. Karim, F. Hossain, and F. Hasan,"Isolation, identification and characterization of dyedegrading bacteria from dyeing industry effluent anddegradation process optimization against NovacronRed SB," Asian J. Agri.& Biol., vol. 6, pp. 461-471, December 2018.
[15]. V. K. Mishraj,H. Sharma, and J. Dubey, "A Comparative decolourisation of RBBRdye and guaiacol degradation byfree and immobilized laccase producing Bacillus spp.," Nat. Env. & Poll. Tech., vol. 14, pp. 319-324, April-June 2015.
[16]. E. Karim, K.Dhar, and T. Hossain, "Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent," J. Gen. Eng. Biotechnol., vol. 16, pp. 375-380, December 2018.
[17]. S. Rajeshkumar, J. Santhoshkumar, R. P. Parameswari, S. Saravanan, S. R. Balusamy, and K. Arunachalam, K, "Degradation of toxic dye and antimicrobial and free radical potential of environmental benign zinc oxide nanoparticles," Bioinorganic Chem. Applicat., vol. 2022, pp. 4513208, July 2022.
[18]. W. M. Abd El-Rahim, H. Moawad, A. Z. Abdel Azeiz, M. J. Sadowsky, "Biodegradation of azo dyes by bacterial or fungal consortium and identification of the biodegradation products," Egypt J. Aquatic Res., vol. 47, pp 269-276, September 2021.
[19]. M. Ikram, M. Naeem, M. Zahoor, A. Rahim, M. M. Hanafiah, A. A. Oyekanmi, A. B. Shah, M. H. Mahnashi, A.A. Ali, N. A. Jalal,F. Bantun, and A. Sadiq, "Biodegradation of azo dye methyl red by *Pseudomonas aeruginosa*: Optimization of process conditions," Int. J. Environ. Res. Public Health., vol. 19, pp 9962, August 2022.