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Isolation and characterization of dye effluent discoloration bacteria from industrial wastewater

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ABSTRACT

The worldwide rapidly developing in industrialization and urbanization have been leads to discharge of effluents and wastewater that causing of environmental pollution and hazardous to the aquatic animals. In moreover, major industrial effluents of different dye concentration hugely affected to agriculture soil and ground water. Bioremediation is the major key to treatment of wastewater and ecofriendly achievement. In this method effectively removal of dye effluents that are involved heterologous biological compounds. In the present study focused on the industrial effluents decolorization by used potential bacterial consortium of two bacterial strains such as *Bacillus* sp and *Pseudomonas* sp. The bacterial strains were isolated from industrial wastewater. Suitable decolorization was observed at 37°C for 15 days incubation to be conformed UV–Vis analysis. In furthermore, the decolorized end product was performed phytotoxicity assessment using of *Vigna radiata* seeds which evaluated toxicity of decolorized end product of wastewater.

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1. Introduction

Raising globalization, urbanization and rapid development of industrialization have been causes different kinds of environmental pollutions. Among various industries, the textile dying industries discharge large volume of waste water after dyeing process (Zollinger, 1987). It is estimated that around 10 - 15% of the dyes are lost in the effluent during the dyeing processes (Kadir *et al.,* 2009). Dyes are classified to the basis of chemical structure of chromophore there are 20 -30 different groups of dyes like azo (monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are the most important groups (Chen et al., 2011). The majority of industrial important azo dyes belong to the following classes: acid dyes, basic dyes, direct dyes, Disperse dyes, mordant dyes, reactive dyes and solvent dyes (Wanyonyi et al., 2017). Dyes make up an abundant class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as –C=C-, ─N═N─ and ─CH═, which are responsible for colors (Molinari et al., 2004).

Around 10,000 different dyes with an annual production of more than 7 $\times 10^5$ metric tons worldwide are commercially available and are extensively used in textile processing, paper, printing, pharmaceutical, food and other industries (McMullan et al., 2001; Siddeeg et al., 2020). The textile effluents containing of various toxic substances namely suspended detergents, surfactants, carcinogenic amines, formaldehyde and organochloride pesticides associated with dyes (Jadhav et al., 2010). During the fabrication process approximately 10% of the dyes have not fixed with fibers, it released into the environment (Asad et al., 2007). The release of untreated effluents from the dyeing industries causes a major threat to the environment and reduces sun light penetration of the stream. These effluents are toxic to fish and mammalian life and to inhibit the activity and growth of microorganisms. It alters the pH, increases the biological oxygen demand (BOD) and chemical oxygen demand (COD) and gives the rivers intense colorations. It also affects the soil fertility and plant growth (Afrad et al., 2020). In humans the azo dyes are capable of producing intestinal cancers, cerebral and skeletal abnormalities in foetuses (Saha and Rao, 2020). Moreover, disposal of textile waste water on an open land contaminates the subsoil water, so that drinking water gives color as well as bad taste. Dissolved substances in industrial effluents alter the chemical and biological status of the soil and water, which may affect growth and productivity of plants and alters the color and quality of the water bodies has been proved to be hazardous to aquatic organisms (Lade et al., 2015). Toxic compounds from dye effluent get into aquatic organisms and ultimately reach man through food chain to cause various physiological disorders like hypertension, skin cancer, sporadic fever, renal damage and cramps etc. They possess toxicity like lethal effect, genotoxicity,

mutagenicity, and carcinogenicity to plants and (Puvaneswari et al., 2006).

There are several physical and chemical treatment methods are used for dyes like adsorption, sedimentation, floatation, coagulation, polymerization, flocculation, reverse osmosis, ultra-filtration, ionization, radiation, reduction, oxidation, electrolysis and ion exchange are extensively used. These methods are not environmentally friendly and produces enormous amount of secondary pollutants and are cost-effective (Roy et al., 2018). Biological treatment is often the most economical alternative compared to physical and chemical processes. Biological methods like stabilization, aerated lagoons, trickling filter, activated sludge, anaerobic digestion and bio- augmentation etc. The biological methods have lower cost but it having large land space and less effective (Sheam et al., 2020). The removal of textile carcinogenic pollutants is the major task of the bioremediation. The ability of microorganisms to decolorize and metabolize dyes has long been known and the use of bioremediation based technologies for treating textile waste water has attracted interest (McMullan *et al.*, 2001). Several microorganisms have been found to decolorize and mineralize azo dyes. Many microorganisms belonging to different taxonomic group of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourize azo dyes (parmar, 2014; Hassan et al., 2013; Zabin et al., 2011,). Biodegradation systems of color removal through the use of bacteria have been shown to be highly effective.

The bacterial metabolism of azo dyes is initiated by a reductive cleavage of the azo bond in most cases, which results in the formation of amines. Azoreductase enzymes responsible for decolourization of azo dyes are purified from several bacterial strains. These reductive processes have been studied in some aerobic bacteria, which grow on azo compounds. Bioreactor is a engineered device, usually a vessel, used to direct the activity of a biological catalyst to achieve a desired chemical transformation. Basic principles of process control, fermentation monitoring, dissolved oxygen, P^H, temperature, monitoring, subtract (Glucose). In current research aimed to focus on isolation and characterization of effluent decoloring bacteria from the textile effluent wastewater. Two bacterial strains were screened for potential degradation of effluents industrial dye, after that 15 days incubation. The decolorized by-product was performed phytotoxic study that revealed toxicity assessment of the end by-product.

2. Materials and methods

2.1. Sample collection

Textile dye effluent waste water was collected from in Edapadi, Salem district, Tamil Nadu, India. Effluent was collected

using sterile container and aseptically transported to the laboratory and stored at 4ºC for further use.

2.2. Isolation of dye degrading bacteria

Collected effluent was mixed with sterile 100mL Bushnell Hass broth and incubated at 37ºC for 7 days. 1 mL of the above broth sample was serially diluted and spread on nutrient agar plates for the isolation of dye degrading bacterial strains and incubated at 37ºC for 24 h. After that incubation period, fifteen bacterial strains were isolated and stored at 4 ºC. The selected bacterial strains were noted as KADB01 to KADB15.

2.3. Identification of bacteria strain

The isolated bacterial strains were identified by following staining and biochemical methods of Gram staining, spore staining, motility, oxidase, indole, methyl red voges-prosjauer, citrate utilization, urease and carbohydrate fermentation test. The results further were compared with manual of systematic bacteriology.

2.4. Screening of dye degrading bacterial strain

The isolated bacterial strains were screening for the selection of efficient dye degradation activity. In briefly described, LB agar medium was prepared and directly added into the different concentration of azo dye solution under the ambient temperature and properly mixed. After that process immediately poured into the petri plates and allowed to solidification. Then respective isolated bacterial strains were streaked on the plates and incubated at 37ºC for 24 h. The efficiency of bacterial dye degradations were observed based on the zone of inhibition (Kabeer et al., 2019).

2.5. Bioreactor study

Bioreactor is an engineered device, usually a vessel, used to direct the activity of a biological catalyst to achieve a desired chemical transformation. Bioreactor of 4L capacity was designed for lab scale study for the treatment of dye contaminated water. The bioreactor was filled with 4L of the dye contaminate water to which 1% of bacterial consortium was added. Temperature was maintained at room temperature and continuously aerated with constant agitation using stirrer at 250 rpm. The samples were collected from various parameters such as pH, CFU and Optical density was measured by UV spectrophotometer. The percentage of dye degradation was calculated by following formula.

% degradation = Initial absorbance value-final absorbance value/ Initial absorbance value x100

Fig. 1 Laboratory scale bioreactor

2.6. Phytotoxicity assay

The soil was taken in a 250ml of sterile plastic cups and 4 *Vigna radiata* seeds were placed into each cup at 1.5 cm depth. All the cups were irrigated regularly with 5 ml of untreated as negative control and bioreactor treated dye effluent. The seeds were allowed to germinate and seed germination was assessed on every day of the experiment. At the end of 10^{th} day shoot length and size of the leave was measured. Control set was carried out using distilled water to replace the textile dye effluent at same time.

3. Results and discussion

Industrial dye contaminated water sample was collected and physicochemical parameters analyzed such as color, oder, $P^{H,}$

Table 1 Physicochemical parameters of industrial dye

S. No	Parameters	Dye contaminated water sample
1	Color	Red
2	Oder	Dyeing
3	P^{H}	8
4	Temperature	36
5	OD Value	0.052

Table 2 Biochemical characterization of isolated bacterial strains

S. No	Tests	Gram's Staining	Spore	Motility Test	Indole Test	Methyl red Test	VP Test	Citrate Test	Urease Test	Catalase Test	Oxidase Test	Sugar fermentation	Tentative Genus
1	KDDB01	+	+	+	-	-	-	-	-	-	+	-	*Bacillus* sp
2	KDDB02	+	+	+	-	-	-	-	-	-	+	+	*Micrococcus* sp
3	KDDB03	+	+	+	-	-	+	-	-	+	-	+	*Bacillus* sp
4	KDDB04	+	-	+	-	-	-	-	-	+	+	-	*Pseudomonas* sp
5	KDDB05	+	+	-	-	-	-	-	-	+	+	+	*Bacillus* sp
6	KDDB06	+	+	+	-	+	+	+	-	-	+	+	*Bacillus* sp
7	KDDB07	+	+	+	-	-	+	-	+	+	-	-	*Staphylococcus* sp
8	KDDB08	+	-	-	+	-	+	-	+	+	-	-	*Staphylococcus* sp
9	KDDB09	+	+	+	+	+	-	-	-	-	-	+	*Bacillus* sp
10	KDDB010	-	-	+	+	+	-	-	-	+	+	+	*Pseudomonas* sp
11	KDDB011	+	-	+	-	-	-	-	-	-	+	-	*Micrococcus* sp
12	KDDB012	+	-	-	-	+	-	-	-	-	-	-	*Enterococcus* sp
13	KDDB013	-	-	-	+	+	-	-	-	+	-	+	*Klebsiella* sp
14	KDDB014	+	-	-	-	+	-	-	+	-	+	-	*Micrococcus* sp
15	KDDB015	+	+	+	-	-	-	-	+	+	+	-	*Bacillus* sp

temperature, OD value were observed and shown in Table 1. The dye degrading bacterial strains were isolated from dye effluent sample by using spread plate technique. The totally fifteen bacterial strains were isolated and biochemical characterized, the results were revealed in the Table 2. Similar report revealed, textile wastewater showed abnormal coloration of black turquoise blue colored. The pH determined the collected test sample was slightly acidic to neutral (Bhatia et al., 2018).

3.1. Screening of dye degradation bacteria

Luria Bertani Agar medium incorporated with azo dye and the bacterial isolates were inoculated. The maximum zone of clearance of the isolated strains were taken as an indicator of the dye degradation bacteria its shows in Table 3, the colony size and zone of clearing size were measured in millimeters. Several researchers have been reported, similar results as related to zone of clearing on the agar plate, its indication of the dye degradation ability of the selected bacteria (Shazia et al., 2017).

Table 3 Zone of clearance dye degradation bacteria

S. No	Strain No	Zone of clearance (mm)
1	KDDB01	0
2	KDDB02	16
3	KDDB03	12
4	**KDDB04**	25
5	**KDDB05**	27
6	KDDB06	0
7	KDDB07	12
8	**KDDB08**	23
9	KDDB09	13
10	KDDB010	0
11	**KDDB011**	21
12	KDDB012	11
13	KDDB013	0
14	KDDB014	12
15	KDDB015	0

3.2. Construction of consortium

The *Bacillus* sp and *Pseudomonas* sp were used for the construction of consortium due to the synergistic effects with each other.

3.3. Dye decolourization

The dye decolourization by the bacterial consortium was detected by the following optical density value of the bioreactor

Table 4 Dye effluent decolourization measurement by UV spectrophotometer

UV Spectroscopic OD values of the treated sample

SL.No	Days	Treated sample	Untreated sample
1.	1	0.052	0.052
2.	2	0.050	0.052
3.	3	0.048	0.052
4.	4	0.047	0.052
5.	5	0.046	0.052
6.	6	0.045	0.052
7.	7	0.044	0.052
8.	8	0.040	0.052
9.	9	0.039	0.052
10.	10	0.038	0.052
11.	11	0.037	0.052
12.	12	0.035	0.052
13.	13	0.034	0.052
14.	14	0.033	0.052
15.	15	0.032	0.052

treated dye effluent shows in Table 4. Our bacterial consortium degraded 38% percentages of dye within fifteen days of treatment. Similar research was conducted by Lalnunhlimi et al.,(2016) for the decolorization of mixture dyes using of *Bacillus cereus* has showed 93.37% of highest decolorization within 5 days. Another related study was reported by Karim et al. (2018), the decolorization experiment using of five frequently employed textile azo dyes by different bacterial isolates strains of EK13 (41%), EK7 (41%) and EK6 (42%) for incubation after 6 days in Bushnell-Haas (BH) broth culture medium (Lalnunhlimi et al., 2016).

3.4. Phytotoxicity

The microbial treated dye effluents was applied to study the phytotoxicity of green gram (Vigna radiate) plant such as germination capability, shoot length and root length. The result was compared with control tape water it's shown in Table 5. In comparison of similar research reported by Nouren et al., 2017, using of yellow 4 dye by-product for phytotoxicity experiment. *Zea mays* seeds treated with degraded by-product of test samples and untreated DY4 dye samples. The germination percentage was calculated based on the root and shoot lengths of germinated seeds were compared with control samples. The result suggested 50% of negatively affected then compared to treated samples, which is confirmed exhibit degradation revealed in the test samples (Karim et al., 2018).

Table 5 Phytotoxicity assessment of treated dye effluent

Shoot length measurement (cm)				
Sl. No.	**Days**	**Treated water**	**Tape water**	**Untreated water**
1	1	0	0	0
2	2	0	0	0
3	3	0.5cm	0.5cm	0
4	4	0.7cm	0.9cm	0
5	5	1.0cm	1.2cm	0
6	6	1.5cm	1.8cm	0
7	7	2.0cm	2.5cm	0
8	8	2.3cm	3.0cm	0
9	9	2.6cm	3.4cm	0.5cm
10	10	3.0cm	3.7cm	0.7cm
11	11	3.4cm	4.1cm	0.8cm
12	12	3.8cm	4.6cm	0.9cm
13	13	4.2cm	5.0cm	1.0cm
14	14	4.6cm	5.5cm	1.5cm
15	15	5.0cm	5.9cm	1.7cm

4. Conclusions

The textile industries are produced large amount of wastewater effluents which hazardous to ecosystem, because of their higher concentration of dyes mixtures. Although bacterial decolorization or degradation of textile effluent is a challenging process, but nowadays many researchers have been reported microbial mediated degradation of textile effluent. In the current research study, consortium of two bacterial strains such as *Bacillus*

sp and *Pseudomonas* sp were potential effluent decolorization was screened through the plate assay method. The consortium of two bacterial strains was observed maximum growth at 37°C and pH 7.0. The construction of consortium with two bacterial isolates is able to decolor of industrial effluent dye up to 38% for 15 days incubation. The seeds germination was revealed less toxic effect in treated samples and also enhancing of plant growth. These results suggested the potential applicable of these two bacterial strains for the treatment of wastewater in the future.

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Conflicts of Interest: None

References

1. Zollinger, H. Synthesis, Properties and Application of Organic Dye and Pigments. Color Chemistry, VCH New York, 1987 pp.92-102.
2. Kadir, T., Zuhal, T. Decolorization of direct dye in textile wastewater by ozonization in a semi-batch bubble column reactor. Desalination, 242(2009) 256-263.
3. Chen, Z., Jin, X., Chen, Z., Megharaj, M., Naidu, R. Removal of methyl orange from aqueous solution using bentonite-supported nanoscale zero-valent iron. J. Colloid Interface Sci. 361 (2011) 601–663.
4. Wanyonyi, W. C., Onyari, J. M., Shiundu, P. M., Mulaa, F. J. Biodegradation and detoxification of malachite green dye using novel enzymes from bacillus cereus strain km201428: kinetic and metabolite analysis. Energy Procedia 119 (2017) 38–51.
5. Molinari, R., Pirillo, F., Falco, M., Loddo, V., Palmisano, L. Photocatalytic degradation of dyes by using a membrane reactor. Chemical Engineering Process 43 (2004)1103-1114.
6. McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robinson, T., Nigam, P., Banat, I.M., Marchant, R., and Smyth, W.F. Microbial decolorization and degradation of textile dyes. Appl Microbial Biotechnol. 561 (2001) 81-87.
7. Siddeeg, S. M., Tahoon, M. A., Mnif, W., Ben Rebah, F. Iron oxide/chitosan magnetic nanocomposite immobilized manganese peroxidase for decolorization of textile wastewater. Processes 8 (2020) 5.
8. Jadhav, J. P., S. S. Phugare, R. S. Dhanve and S. B. Jadhav. Rapid biodegradation and decolorization of direct orange 39 (orange TGLL) by an isolated bacterium Pseudomonas aeruginosastrain BCH. Biodegradation, 21 (2010) 453–463.
9. Asad, S., Amoosegear M. A., Pourbabaee, A. A., Sarboloukki, M. N., Dastgheib, M. Decolonization of textile azo dyes by newly isolation halophilic and halotolerant bacteria. Bioresour Technol 98 (2007) 2082-2088.
10. Afrad, M. S. I., Monir, M. B., Haque, M. E., Barau, A., Haque, M. M. Impact of industrial effluent on water, soil and rice production in Bangladesh: a case of Turag river

bank. J. Environ. Health Sci. Eng. 18(2020) https://doi.org/10.1007/s40201-020-00506-8.

11. Saha, P., Rao, K. Biotransformation of Reactive Orange 16 by alkaliphilic bacterium Bacillus flexus VITSP6 and toxicity assessment of biotransformed metabolites. Int. J. Environ. Sci. Technol. 17 (2020) 99–114.
12. Lade, H., Kadam, A., Paul, D., Govindwar, S. Biodegradation and detoxification of textile azo dyes by bacterial consortium under sequential microaerophilic/aerobic processes. EXCLI J. 14 (2015) 158. doi:10.17179/excli2014-642.
13. Puvaneswari, N., Muthukrishnan, J., Gunasekaren, P. Toxicity assessment and microbial degradation of azo dyes. Indian Journal of Experimental Biology.,vol.44 (2006)618-626.
14. Roy, D. C., Sahan, A. K., Sikdar, B., Rahman, M., Roy, A. K., Prodhan, Z. H., Tang, S. S. Biodegradation of crystal violet dye by bacteria isolated from textile industry effluents. PeerJ 6 (2018) e5015
15. Sheam, M., Haque, Z., Nain, Z. Towards the antimicrobial therapeutic and invasive properties of Mikania micrantha Knuth : a brief overview. J. Adv. Biotechnol. Exp. Ther. 3 (2020) 92–101.
16. Parmar, P. R. Decolorization of acridine red dye by the fungi *Aspergillus species*. Journal of Scientific and Innovative Research, 3(2014) 454-459.
17. Hassan, M. M., Alam, M. Z. and Anwar, M. N. Biodegradation of textile Azo dyes In Effluent. International Research Journal of Biological Sciences, 2(2013):27-31.
18. Zabin, K., Bagewadi, Amithkumar, G., Vernekar, Aishwarya, Y., Patil, Abhijit, A., Limaye, Vandana,M., Jain. Biodegradation of industrially important textile dyes by actinomycetes isolated from activated sludge. Research Article, Biotechnol. Bioinf . Bioeng, 1(2011):351-360.
19. Kabeer, F. A., John, N., Abdulla, M. H. Biodegradation of malachite green by a newly isolated *Bacillus vietnamensis* sp . MSB17 from continental slope of the Eastern Arabian Sea: enzyme analysis, degradation pathway and toxicity studies. Bioremediat. J. 0 (2019) 1–9. https://doi.org/10.1080/10889868.2019.1671790.
20. Bhatia, D., Sharma, N.R., Kanwar, R., Singh, J. Physicochemical assessment of industrial textile effluents of Punjab (India), Appl. Water Sci 8, (2018) 83. https://doi.org/10.1007/s13201-018-0728-4.
21. Shazia, N., Haq, N. B., Munawar, I., Ismat , B., Shagufta, K., and Yusra, S. By-product identification and phytotoxicity of biodegraded Direct Yellow 4 dye, Chemosphere, 167 (2017) 474-484.
22. Lalnunhlimi, S., Krishnaswamy, V. Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium, Brazilian Journal of Microbiology, 47 (2016) 39-46.
23. Karim, M.E., Dhar, K., Hossain, M.T.,. Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent. J. Genet. Eng. Biotechnol, 16 (2018) 375-380. doi:10.1016/j.jgeb.2018.02.005.