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Biodegradation of Acid orange 10 dye by bacterial strain *Bacillus* sp.

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ABSTRACT

A novel bacterial strain was isolated from industrial effluent samples and evaluated as a decolorizing microorganism. The isolated bacterium identified as *Bacillus* sp. on the basis of morphological and biochemical tests. The physical parameters such as temperature, pH, aeration, dye concentration and inoculum size were optimized for 100% decolorization of Acid orange 10 dye by isolated *Bacillus* sp. Optimization of various parameters like temperature 40°C, pH 8.5 and inoculum size 10% were enhances the decolorization process of Acid orange 10 dye at static condition. The isolated *Bacillus* sp. was able to decolorize Acid orange 10 dye at temperature 60°C and pH 8.5, which indicates that isolated *Bacillus* sp. was thermo-alkalophilic in nature and could efficiently decolorize the dye even at higher concentration of up to 700 ppm at static condition. Based on the results of the present study, authors concluded that newly isolated microorganism *Bacillus* sp. was thermo-alkalophilic and found to be potential candidate for decolorization of Acid orange 10 dye.

KEYWORDS: *Bacillus* sp., Acid orange 10, textile effluent, decolorization

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INTRODUCTION

Organic chemicals that own color are known as dyes and are the important industrial coloring chemical compounds. Dyes are classified by (i) chromophore groups in their chemical structures as azo dyes, anthraquinone dyes and phthalocyanine dyes etc. and (ii) their usage or application method as disperse dyes for polyester and reactive dyes for cotton[1]. Azo (monoazo, diazo, triazo and polyazo), anthraquinone, triarylmethane and phthalocynine dyes are main groups of dyes. Azo dyes absorb light in the visible region because of their chemical structure and which is characterized by one or more azo bonds (-N=N-)[2]. Globally, 2.8×10^5 tons of textile dyes are poured into water ecosystem every year[3]. Azo dyes are the most common (more than 3000 different varieties) of all textile dyes produced because of their easier biosynthesis, chemical stability and the diversity of colors available as compared to natural dyes[4]. About 80% of azo dyes are used in the dyeing process of textile industries. They are widely used in the textile, leather, food, paper, cosmetics and pharmaceutical industries. It has been estimated that about 10-15% of the dyes used in dyeing process goes unbound with the textile fibers and are discharged into the environment[5].

Release of dye containing effluent derived from various industrial practices into water bodies and surrounding industrial areas is of major concern which have several adverse effects on life including decreased aquatic photosynthesis, ability to exhaust dissolved oxygen and toxic effect on flora, fauna and humans. Presence of dyes in the textile effluent causes an unpleasant appearance by imparting the color and also their breakdown products (amines) are toxic, carcinogenic and mutagenic[6,7]. There are many reports on the use of physico-chemical methods for color removal from dye containing effluents[8,9]. These methods include adsorption, chemical treatment, coagulation and ion pair extractions, but they are linked to problems such as high cost and produce large amounts of sludge after treatment which requires safe disposal.

Numerous studies available with emphasis on the use of microorganisms to degrade dyes, suggest that biodegradation is an eco-friendly and cost-effective method for dye containing wastewater treatment[10,11]. The enzymatic approach offers alternative strategy for decolorization/degradation of azo dyes from wastewater over conventional physico-chemical treatments as it ends with less sludge production and is also cost effective. There are several enzymes involved in removal of azo dyes which proved an effective molecular weapon for decolorization of azo dyes[12]. With these views the present

study was carried out to study the effect of various physical parameters on dye decolorization by the newly isolated bacterium of *Bacillus* sp.

MATERIALS AND METHODS:

Chemicals and media

Acid orange 10 dye was obtained from Shailaja textile industry, Sholapur Maharashtra, India. Dehydrated culture medium obtained from Hi-media and other chemicals used were of analytical grade. Bushnell and Haas medium (BHM) mineral salts medium of the following composition used for the dye decolorization studies were as follows $MgSO_4$ 0.2, K_2HPO_4 1.0, $CaCl_2$ 0.02, $FeCl_3$ 0.05, NH_4NO_3 1.0 (g/l) supplemented with or without glucose (0.1% w/v) and yeast extract (0.05% w/v). The pH of the medium was adjusted to 7.0, autoclaved at 121°C for 20 m and inoculated with bacterial strain. The decolorization studies were carried out in 250 ml conical flasks containing 100 ml BHM mineral slats medium with respective azo dyes.

Isolation and identification of Acid orange 10 decolorizing bacteria

The Acid orange 10 dye decolorizing bacterium was isolated from the soil sample collected from industrial effluent. 10 g of effluent soil sample was suspended in 100 ml of complete medium broth supplemented with Acid orange 10 (100 mg/l) individually and acclimatized for 5 days at 30°C at 150 rpm. The acclimatized soil sample serially diluted and plated appropriate dilution on BHM agar plat (pH 7.0) containing Acid orange 10 (100 mg/l). All the bacterial isolates were studied by inoculating them in complete medium broth supplemented with dye. The inoculated medium was incubated at 30°C (and or 37°C) under shaking condition at 150 rpm for 1-5 days. The decolorization was visually observed and the isolates showing considerable decolorization of the dyes were selected for further investigation. The selected bacterial isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology[13].

Decolorization assay

2 ml aliquots of cell-free decolorized supernatant were collected at regular intervals of time from the media and centrifuged at 10000 rpm for 10 m to remove the cells by their interfere with the measurement. The supernatant was used for spectrophotometric analysis (ANTHELE-SECOMAM France) at wavelength of 300-700 nm. The dye Acid orange 10 maximum absorbance was at 480 nm. The efficiency of decolorization was expressed as the percentage ratio of the decolorized dye

concentration to that of the initial one. The percent decolorization was determined by using the below formula[5,14]. Uninoculated control was used to compare abiotic colour loss during the experiment.

$$D= [A^0-A^1)/A^0] \times 100$$

Where,

D - Decolorization (%) ; A^0 - Initial dye absorbance before decolorization; A^1 - Final absorbance after decolorization

Effect of temperature, pH, inoculum size, dye concentration and shaking on dye decolorization

Various operational and environmental conditions were standardized by varying the particular parameter and keeping the other parameters constant. Following parameters were standardized and their effects were observed on decolorization of Acid orange 10. The effect of different parameters such as temperature 20-50°C, pH 4-10, inoculum size 1-10%, dye concentration 50-1000 mg/l and shaking 50-200 rpm on decolorization in 100 ml of culture media containing 30 ppm of Acid orange 10 dye.

Identification of metabolites

The complete decolorized medium of Acid orange 10 was centrifuged at 10,000 rpm for 15 m. The pH was adjusted to 7 and 200 ml of the supernatant was extracted twice with 500 ml diethyl ether. The remaining aqueous layer was acidified to pH 2 by 1N HCl and extracted twice with 500 ml of diethyl ether. The acidic and basic extracted fractions were pooled and evaporated over anhydrous Na_2SO_4 and under reduced pressure at 30°C. The residue was dissolved in 500 µl of Methanol.

Thin layer chromatography (TLC)

Preliminary identification was made by TLC of extracted compounds. The glass plate of 200×100×2 mm (length×breadth×thickness) coated with 40% (w/v) aqueous slurry of silica gel- G with binder were used for carrying out TLC. 10 µl of extracted fractions and standard Acid orange 10 dye was loaded on glass plate coated with silica gel and the solvent system chloroform:ethanol (70:30) was used for developing the TLC plate. The dye and products chromatogram was observed by exposing the plate to Iodine vapors. The metabolites were identified by diazotization and carbylamines test.

RESULTS AND DISCUSSION

Biological treatment of textile azo dyes has been proved to be the best method due to its ability to degrade almost all dye stuff and also overcome many dis-advantages posed by the physico-chemical processes.

Isolation and identification of Acid orang-10 decolorizing bacteria

The bacterial species capable of decolorizing of Acid orange 10 were isolated from different sources using Bushnell-Hass medium containing 100 ppm dye at 37°C. Among the several isolated bacterial strains, one strain was selected based on its ability to decolurize Acid orange 10 with in 16 h at 37°C of 100 ppm dye concentration and used for further studies. The isolated bacteria was identified as *Bacillus* sp. based on cultural, morphological, staining and physiological tests.

Table 1 Morphological and biochemical characteristics of *Bacillus* sp.

Test	Result
Gram's Staining	+
Shape	Rod
Motility	Motile
Indole Production	-
Methyl Red	-
Voges-Prausker	-
Citrate utilization	+
Catalase	+
Oxidase	-

The morphological and biochemical characteristics observations are shown in Table 1. and similar kind of observations were reported by Ponraj *et al*[15]. Ewida (2014) isolated dye decolorizing bacterium from textile wastewater samples were collected from Moket Mac textile factory, Sharqia Governorate, Egypt. Enrichment culture technique was used to isolate AR 337 dye decolorizing bacteria[16]. The bacterial isolates were originated from the dye contaminated textile wastewater of local industry, so they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with azo dyes[17]. Decolorization and degradation of azo dyes may take place by two methods either adsorption on the microbial biomass

(biosorption) or biodegradation of the dyes by the living cells. Decolorization process of Acid orange 10 by *Bacillus* sp. was not due to adsorption, because when the bacterial cell mass collected treated with solvents of methanol or chloroform didn't release of any of the color from the cells. Numerous studies available with emphasis on the use of microorganisms to degrade dyes suggest that biodegradation is an eco-friendly and cost-effective method for dye containing waste water treatment[10,11]. Bacteria mediated decolorization and degradation of azo dyes involve azo-reductase assisted breakdown of azo bond (-N=N-) under anaerobic condition which results in the formation of colorless hazardous aromatic amines[16], which are further removed aerobically or anaerobically[17]. Horistu *et al*[20] first reported *Bacillus subtilis* culture capable of degrading azo dyes and after that *Aeromonas hydrophilia*[21] and *Bacillus cereus*[22] were reported to degrade azo dye. Recently, Lalnunhlimi and Krishnaswamy reported alkaliphilic bacterial consortium of *Bacillus flexus* strain NBN2 (SY1), *Bacillus cereus* strain AGP-03 (SY2), *Bacillus cytotoxicus* NVH 39198 (SY3) and *Bacillus sp.* L10 (SY4) which is capable of decolorizing direct blue 151 and direct blue 31 up to 97.57% and 95.25% respectively in 5 days[23].

Effect of incubation time on dye decolorization

The absorption maximum of Acid orange 10 obtained by scanning in UV-Visible spectrophotometer and maximum peak obtained at 480 nm (Figure 1). The samples were removed at different intervals of time at 6, 12, 18 and 24 h and scanned for λ_{max} of Acid orange 10 dye. The results showed that gradually disappearance of the λ_{max} peak (480 nm) to complete (100%) disappearance of peak at 24 h incubation.

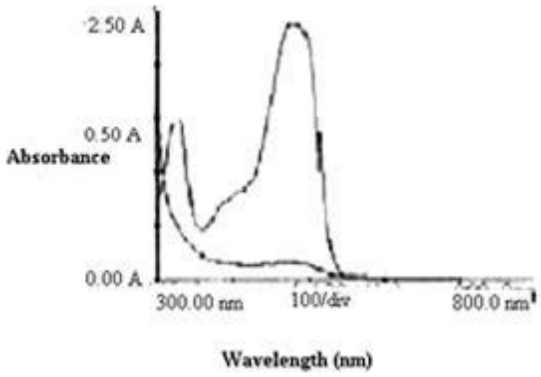

Figure 1. Effect of RPM on dye decolorization by *Bacillus* sp.

These observations clarified that the disappearance of the Acid orange 10 dye in its original form due to modification or breakdown of dye by newly isolated *Bacillus* sp. Time take by newly isolated *Bacillus* sp. in the present study was too less than previously reported studies. Jothimani and Prabhakaran have reported 59% dye removal from dyeing industry effluent using *Pseudomonas* and *Bacillus* species after

4 days of incubation[24]. Shah *et al* reported decolorization of direct blue dye in 168 h by *Bacillus* sp. ETL-1979[25] and 96.4% decolorization of black WNN (100 mg/l) was attained in 48 h under optimized condition with free cells of *paenibacillus alvei* MTCC 10625[26]. Decolorization of direct blue 151 at the concertation of 200 mg/l was reported to decolorize by 95.25% in five days according to Lalnunhlimi *et al* by bacterial consortium (*B. cytotoxicus*, *Bacillus sp.* and *B. flexus*)[21].

Effect of shaking on dye decolorization

Aeration either favors or inhibit microbial decolorization of azo dye. To study the effect of aeration on decolorization of Acid orange 10, *Bacillus* sp. cells were cultured in nutrient broth containing dye under static and shaking cultural conditions. At the shaking speed of 50 rpm, the rate of decolorization was equal to static conditions for 100% decolorization of Acid orange 10 dye (36 h). However further increase in speed of shaking at 100, 150 and 200 rpm time taken for 100% decolorization was increased to 48 h, 72 h and 96 h respectively, indicating that the decolorization process was inhibited upon increase of aeration (Figure 2).

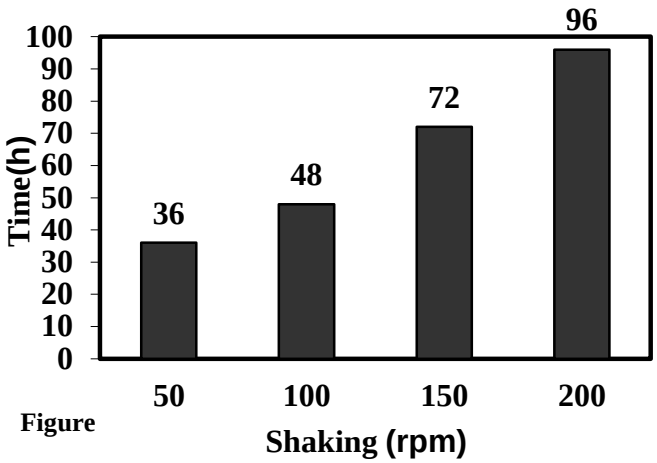

Figure 2. Effect of RPM on dye decolorization by *Bacillus* sp.

Inhibition of dye decolorization at higher rpm in shaking condition indicates decolorization process was inhibited in presence of higher concentration of oxygen whereas, lower rpm (50 rpm) and at static condition enhances the percent of decolorization and indicates lower concentration of oxygen was enhances decolorization. The results of present study are in concurrence with results reported by Verma and Madamwar wherein the authors have reported that under static conditions decolorization was high and in agitation decolorization was neglizable[27]. The mechanism of bacterial degradation of azo dyes to

their corresponding amines is initiated by a reduction of azo linkage with the aid of low specificity cytoplasmic azoreductase. Azoreductase mediated degradation of azo dyes is inhibited by the presence of oxygen because oxygen was a preferable terminal electron acceptor over the azo groups in the oxidation of reduced electron carriers such as NADH[28]. Under shaking conditions, the presence of oxygen deprives the azoreductase from receiving electrons required for azo bond cleavage, whereas under static conditions, these electrons are readily available to the enzyme from NADH to decolorize the azo dyes[29].

Effect of inoculum size on dye decolorization

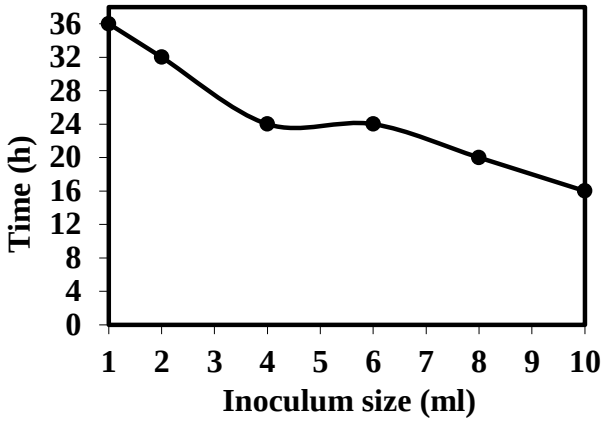

Figure 3. Effect of inoculum size on dye decolorization by *Bacillus* sp.

The effect of different inoculum size of newly *Bacilllus* sp. on the decolorization of Acid Orange 10 (300 ppm) indicated that the increase in the inoculum size from 1-10% progressively decreased the time required for the complete decolorization. The minimum inoculum size of 1% required 36 h for 100% decolorization of Acid orange 10, as the inoculum size increased the time taken for complete decolorization of Acid orange 10 dye was decreased from 36 hrs at 1% to 16 hrs at 10% of inoculum size (Figure 3). The percentage of decolorization of azo dye has linear relationship with inoculum size. The results of the present study are in corroborated with findings of Kumar *et al*[30]. Whereas Gurulakshmi *et al* reported maximum dye decolorization by *Bacillus subtilis* strain was achieved with 20% inoculum size[31] and *B. megaterium* removed 91% of Acid red 337 dye concentration of 500 mg/l within 24 h when the inoculum size was 10% wt./v, solution pH was 7 and the incubation temperature was 30°C[32].

Effect of temperature on dye decolorization

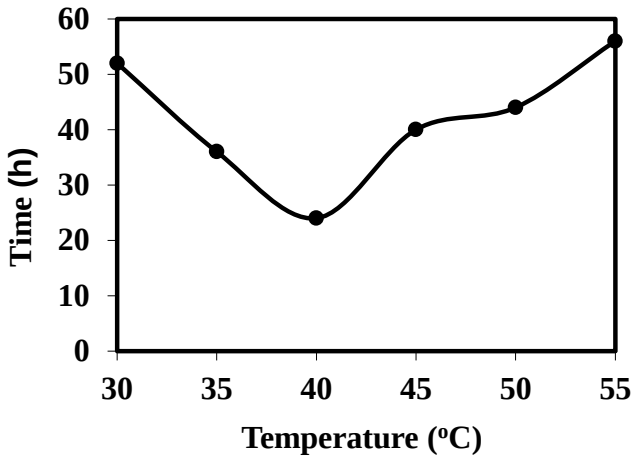

Figure 4. Effect of temperature on dye decolorization by *Bacillus* sp

The optimum temperature required for the complete decolorization of Acid orange 10 was found to be between 35-45°C. At 40°C the 100% decolorization was found within the shortest time of 24 h, hence 40°C could be taken as the optimum. At 20°C the decolorization of Acid Orange 10 was very slow and it took almost 72 h for 100% decolorization. At 60°C the time required increased to more than 72 h while above 60°C complete decolorization did not occurred. It is significant to note here that, *Bacillus* sp. was capable of effecting complete decolorization of 100 ppm dye even at 60°C, indicating its thermophilic nature (Figure 4). Kumar and Sawhney reported that the *Bacilus substilis* (RA 29) showed 95.67% of Congo red decolorization at 37°C[33]. It must be noted that, the optimum temperature for production of an enzyme (in this case azo reductase enzyme) does not always coincide with that for growth[34]. Bayoumi *et al*., reported the optimum incubation temperature for maximum color removal percentage for the two azo dyes Acid orange 7 and Direct blue 75 was 35°C by any of the two strains *Com. acidovorans*-TM1 or *Bur.cepacia* TM5[35].

Effect of pH on dye decolorization

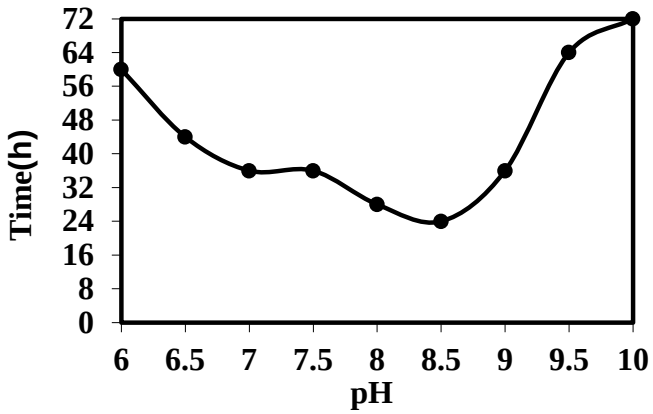

Figure 5. Effect of pH on dye decolorization by *Bacillus* sp.

The results of the present study depict that newly isolated *Bacillus* sp. was capable of 100% decolorization of Acid orange 10 with a wide range of pH from 6.0 to 9.5. The maximum decolorization takes place at pH 8.5 and 300 ppm of the dye was decolorized within 24 hours. The results depict that the isolated *Bacillus* sp. was alkalophilic in nature. At pH 5.50 and below and also at pH 10 100% decolorization could not be achieved (Figure 5). These findings are in agreement with the studies in which maximum decolorization of Methyl Red was achieved by *Micrococcus* strain R3 in pH range of 6-8[36] and the decolorization of Acid Orange dye by *Staphylococcus hominis* RMLRT03 strain was found in the pH range of 6-8[37]. Bayoumi *et al* reported that neutral and slightly basic pH values would be more favorable for decolorization process of azo dyes[35].

Effect of dye concentration on dye decolorization

Newly isolated *Bacillus* sp. decolorize 100 ppm of dye completely within 16 h of incubation. Whereas 100% decolorization of 200 ppm of Acid orange 10 dye was found at 24 h, further increase in dye concentration to 300, 400, 500, 600 and 700 ppm time required increased to 36, 44, 52, 64 and 74 h respectively. The isolated *Bacillus* sp. decolorize the Acid orange 10 dye completely up to a maximum concentration of 700 ppm in 74 h incubation period. A dye concentration above 700 ppm was not completely decolorized even after extended incubation period (Figure 6). These studies have shown the negative effect of increasing dye concentration from optimum level on dye decolorizing efficiency[38-41]. Karunya *et al* reported that the dye concentration at 200 mg/l was completely decolorized in 48 h time by *pseudomonas aerusinosa* but above this concentration there was not much change in the decolorization level[42]. *Alcaligenes aquatilis* was found to decolorize only 82% of 10 mg/l concentration

of Synazol red 6HBN after incubation of 4 days at 37 °C and pH 7[43]. The time taken for the complete decolorization increased with increase in the concentration of Acid orange 10 by *Bacillus* sp.

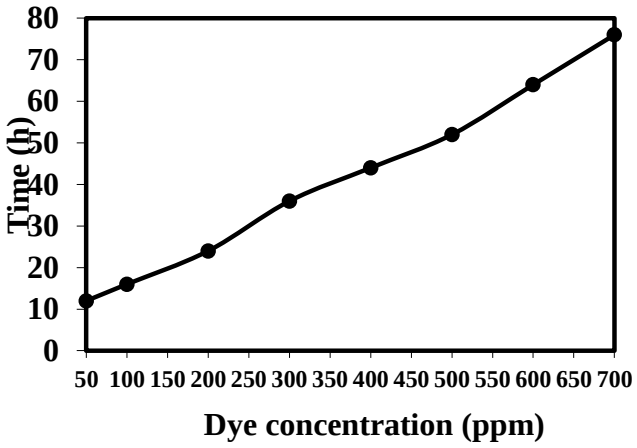

Figure 6. Effect of dye concentration on dye decolorization by *Bacillus* sp.

Identification of metabolic intermediates

The dye degraded products separated on TLC plates using solvents propanol:water:acetic acid (80:19:01). The separated products of TLC plates were exposed to iodine vapors showed two degraded products with 0.75, 0.56 Rf values, whereas controlled the Rf values is 0.50 (Figure 7). Each degraded product was analyzed by diazotization and carbolamine test, the Rf values 0.75, 0.56 give positive results and confirmed as aromatic amines. This clearly indicates that decolorization was due to degradation of dyes into intermediate products. The initial step in bacterial degradation of dye is reductive cleavage of -N=N- (azo) bond leading to formation of colorless aromatic amines.

Figure 7. Identification Acid orange 10 dye degraded product on TLC (Rf values of control dye and decolorized sample).

CONCLUSIONS

The *Bacillus* sp. isolated from effluent soil sample was able to decolorize Acid orange 10 dye at temperature 60°C and pH 8.5, which indicates that isolated *Bacillus* sp. was thermo-alkalophilic in nature and could efficiently decolorize the dye even at higher concentration of up to 700 ppm at static condition. Hence the microorganism *Bacillus* sp. was found to be potential candidate for decolorization of Acid orange 10 dye.

REFERENCES

1. Singh P, Iyengar L and Pandey A. Bacterial decolorization and degradation of azo dyes. In: Singh, S.N., (ed.) microbial degradation of xenobiotics. Springer, Heidelberg Dordrecht London New York, 2012;101-131.
2. Chang JS, Chou C, Lin YC, Lin PJ, Ho JY, Hu TL. Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*. Water Research. 2001; 35(12):2841-50.
3. Jin XC, Liu GQ, Xu ZH, Tao WY. Decolorization of a dye industry effluent by *Aspergillus fumigatus* XC6. Applied microbiology and biotechnology. 2007;74(1):239-43.
4. Chang JS, Chen BY, Lin YS. Stimulation of bacterial decolorization of an azo dye by extracellular metabolites from *Escherichia coli* strain NO3. Bioresource technology. 2004;91(3):243-8.
5. Asad S, Amoozegar MA, Pourbabaee A, Sarbolouki MN, Dastgheib SM. Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresource technology. 2007; 98(11):2082-8.
6. Mugdha A, Usha M. Enzymatic treatment of wastewater containing dyestuffs using different delivery systems. Sci Rev Chem Commun. 2012; 2(1):31-40.
7. Xu M, Guo J, Cen Y, Zhong X, Cao W, Sun G. *Shewanella decolorationis sp*. nov., a dye-decolorizing bacterium isolated from activated sludge of a waste-water treatment plant. International Journal of Systematic and Evolutionary Microbiology. 2005; 55(1):363-8.
8. Golob V, Vinder A, Simonič M. Efficiency of the coagulation/flocculation method for the treatment of dyebath effluents. Dyes and pigments. 2005; 67(2):93-7.
9. López-Grimau V, Gutierrez MC. Decolorization of simulated reactive dyebath effluents by electrochemical oxidation assisted by UV light. Chemosphere. 2006; 62(1):106-12.

10. Vitor V, Corso CR. Decolorization of textile dye by *Candida albicans* isolated from industrial effluents. Journal of industrial microbiology & biotechnology. 20081; 35(11):1353-7.
11. Pajot HF, Delgado OD, de Figueroa LI, Farina JI. Unraveling the decolorizing ability of yeast isolates from dye-polluted and virgin environments: an ecological and taxonomical overview. Antonie van Leeuwenhoek. 2011; 99(3):443-56.
12. Singh S, Chatterji S, Nandini PT, Prasad AS, Rao KV. Biodegradation of azo dye Direct Orange 16 by *Micrococcusluteus strain* SSN2. International journal of environmental science and technology. 2015; 12(7):2161-8.
13. Sneha J, Gomashe AV, Sonal A. Decolorization potential of *Bacillus sp*. for removal of synthetic textile dyes. International journal of current microbiology and applied sciences. 2014;3(12):83-8.
14. Dong X, Zhou J, Liu Y. Peptone-induced bio decolorization of reactive brilliant blue (KN-R) by *Rhodocyclus gelatinosus* XL-1. Process Biochemistry. 2003; 39(1):89-94.
15. Ponraj M, Gokila K, Zambare V. Bacterial decolorization of textile dye-Orange 3R. International journal of advanced biotechnology and research. 2011; 2(1):168-77.
16. Ewida AYI. Biodegradation of Alachlor and Endosulfan using environmental bacterial strains. World Applied Sciences Journal. 2014;32(4):540–547.
17. Shanmugaraju V, Rajan PC. (2018). A Review on decolourization of Azo dye by micro – organisms. International Journal of Comprehensive Research in Biological Sciences. 2018; 5(1):0-29.
18. Van der Zee FP, Villaverde S. Combined anaerobic–aerobic treatment of azo dyes—a short review of bioreactor studies. Water research. 2005; 39(8):1425-40.
19. Joshi T, Iyengar L, Singh K, Garg S. Isolation, identification and application of novel bacterial consortium TJ-1 for the decolorization of structurally different azo dyes. Bioresource technology. 2008; 99(15):7115-21.
20. Horitsu H, Takada M, Idaka E, Tomoyeda M, Ogawa T. Degradation of p-Aminoazobenzene by *Bacillus subtilis*. European journal of applied microbiology and biotechnology. 1977;4(3):217-24.
21. Idaka E, Ogawa T, Horitsu H, Tomoyeda M. Degradation of azo compounds by *Aeromonas hydrophila var*. 24B. Journal of the Society of Dyers and Colorists. 1978;94(3):91-4.

22. Wuhrmann K, Mechsner KL, Kappeler TH. Investigation on rate—Determining factors in the microbial reduction of azo dyes. European journal of applied microbiology and biotechnology. 1980;9(4):325-38.
23. Lalnunhlimi S, Krishnaswamy V. Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. Brazilian journal of microbiology. 2016;47(1):39-46.
24. Jothimani P, Prabakaran J. Influence of bacterial system on the decolorization of dye effluent under enrichment techniques. In State Level Seminar in Recent Developments in Applied Microbiology, Tamil Nadu Agricultural University. Coimbatore, pp-25-26 1998.
25. Sha MP, Patel KA, Nair SS, Darji AM. An application of response surface methodology in microbial degradation of azo dye by *Bacillus subtillis* ETL-1979. Am J Microbiol Res. 2014;2:24-34.
26. Pokharia A, Ahluwalia SS. Decolorization of Xenobiotic Azo Dye-Black WNN by Immobilized *Paenibacillus alvei* MTCC 10625. Int. J. Environ. Bioremed. Biodegrad. 2016;4:35-46.
27. Verma P, Madamwar D. Decolourization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. World Journal of Microbiology and Biotechnology. 2003;19(6):615-8.
28. Chang JS, Lin YC. Fed-batch bioreactor strategies for microbial decolorization of azo dye using a Pseudomonas luteola strain. Biotechnol Progress. 2000;16:979–85.
29. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol. 2001;56:69–80.
30. Kumar K, Dastidar MG, Sreekrishnan TR. Effect of process parameters on aerobic decolorization of reactive azo dye using mixed culture. World Acad. Sci. Eng. Technol. 2009;58:952-5.
31. Gurulakshmi M, Sudarmani DN, Venba R. Biodegradation of leather acid dye by *Bacillus subtilis.* Adv Biotech. 2008;7:12-9.
32. 32Ayman YIE, Marwa EE, Azza AZ. (2019) Complete degradation of azo dye acid red 337 by *Bacillus megaterium* KY848339.1 isolated from textile wastewater. Water Science. 2019;33(1):154-161.
33. Kumar A, Sawhney R. Identification of *Bacillus subtilis subsp*. *subtilis* "RA-29", a congo red decolorizer using 16S rDNA sequencing. Researcher. 2011;3:18-22.

34. Sodhi HK, Sharma K, Gupta JK, Soni SK. Production of a thermostable α-amylase from *Bacillus sp.* PS-7 by solid state fermentation and its synergistic use in the hydrolysis of malt starch for alcohol production. Process Biochemistry. 2005;40(2):525-34.
35. Bayoumi RA, Musa SM, Bahobil AS, Louboudy SS, El-Sakawey TA. Bio decolorization and biodegradation of azo dyes by some bacterial isolates. J. Appl. Environ. Biol. Sci. 2010;1(1):1-25.
36. Olukanni DO, Osuntoki AA, Gbenle GO. Decolourization of azo dyes by a strain of *Micrococcus* isolated from a refuse dump soil. Biotechnology. 2009;8(4):442-8.
37. Singh RP, Singh PK, Singh RL. Bacterial decolorization of textile azo dye acid orange by *Staphylococcus hominis* RMLRT03. Toxicology international. 2014;21(2):160.
38. Sponza DT, Işık M. Reactor performances and fate of aromatic amines through decolorization of Direct Black 38 dye under anaerobic/aerobic sequentials. Process Biochemistry. 2005;40(1):35-44.
39. Saratale RG, Saratale GD, Kalyani DC, Chang JS, Govindwar SP. Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. Bioresource technology. 2009;100(9):2493-500.
40. Hassan MM, Alam MZ, Anwar MN. Biodegradation of textile azo dyes by bacteria isolated from dyeing industry effluent. Int Res J Biol Sci. 2013;2(8):27-31.
41. Olukanni OD, Osuntoki AA, Awotula AO, Kalyani DC, Gbenle GO, Govindwar SP. Decolorization of dyehouse effluent and biodegradation of Congo red by *Bacillus thuringiensis* RUN1. J Microbiol Biotechnol. 2013;23(6):843-9.
42. Karunya A, Rose C. Studies on degradation of textile azo dye, Mordant Black 17 using *Pseudomonas aeruginosa* SUB 7, isolated from textile effluent. International Journal on Applied Bioengineering. 2013;7(1).
43. Ajaz M, Rehman A, Khan Z, Nisar MA, Hussain S. Degradation of azo dyes by *Alcaligenes aquatilis* 3c and its potential use in the wastewater treatment. AMB Express. 2019;9(64):1-12.