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Research Paper

Biodecolorization and degradation of xenobiotic azo dye - Basic Red 46 by *Staphylococcus epidermidis* MTCC 10623

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Abstract

A critical environmental menace looms on account of excessive discharge of contaminated effluent from the textile industry. In the present study, Staphylococcus epidermidis MTCC 10623 was examined for its potential to decolorize Basic Red 46 dye (BR 46), an extensively used dye in textile industry. S. epidermidis showed 99.6% decolorization of BR 46 at pH 9.0 and 40°C temp. after 6 h incubation. Addition of glucose (0.3%) and ammonium sulfate (0.1%) as carbon and nitrogen source enhanced the decolorization ability. Decolorization of BR 46 with bacterial cells immobilized over polyurethane foam (PUF) and nylon mesh (NM), resulted in 99.7 and 99.6% decolorization after 6 h incubation. UV-Vis and FTIR analysis of metabolites formed after bacterial treatment suggested that decolorization was due to degradation and not owing to adsorption. Phytotoxicity assay of these metabolites had no adverse effects on germination rate of test plants, which revealed that the treated effluent was safe for irrigation.

Keywords: *Azo dye, basic Red 46, biodecolorization, immobilization, phytotoxicity, Staphylococcus epidermidis*.

Introduction

While the textile industry in our country has emerged as one of the largest employer, it has also become the notorious polluter for surface and underground water followed by printing, paper, leather, cosmetic, food industry, pharmaceutical, tannery, chemical, paint and varnish industries [1]. Mushrooming growth of textile industries has phenomenally intensified the use of synthetic organic dyes. Out of the approximate total global production of $7x10^5$ metric tons annually, about two third of these dyes are consumed by the textile industry [2]. Even at 1 mg/L concentration of dyes form major and most hazardous contaminants of textile industrial effluent [3]. In addition, this colored wastewater reduced light penetration and oxygen transfer in water bodies [4-5]. Azo dyes are biorecalcitrant, carcinogenic [6], mutagenic [7] and highly toxic towards humans and animals [4,8]. Variety of physico-chemical techniques such as membrane filtration, coagulation, flocculation, ion exchange, photo-oxidation, electrolysis, ozonation and chemical reduction are in vogue for textile effluent treatment but they are constrained by their limited applicability, high operational and maintenance cost and generation of toxic chemical sludge [9]. Microbial degradation is gaining more attention because of low operating cost, high efficiency and environmental friendly nature [10]. Decolorization of azo dyes by bacterial cultures can be aerobic, anaerobic or microaerophilic [2,11]. Variety of bacteria has been used for biodegradation of azo dyes such as *Morganella* sp. [12], *Lysinibacillus* sp. RGS [13], *Brevibacillus laterosporus* MTCC 2298 [14], *Rhodopseudomonas palustris* 51ATA [15], *Alcaligenes faecalis* PMS-1 [16], *Halomonas variabilis* MTCC 3712 [2], *Staphylococcus epidermidis* MTCC 10623 [17] and *Staphylococcus hominis* RMLRT 03 [18] under microaerophilic and aerobic conditions. We attempted to analyze if the bacterial culture *S. epidermidis* MTCC 10623 isolated from the soil contaminated with textile effluent could be used for bioremediation of Basic Red 46.

Materials and Methods

Dye, media and analytical reagents

Basic Red 46 (BR 46) is a mono azo dye and it was obtained from Ekta Dyeing & Finishing House, Ludhiana, India (λmax-532 nm). Growth medium and chemicals were purchased from Himedia Laboratories and Merck Limited (India) respectively. All other chemicals used in the study were of analytical grade and high purity. The composition of the mineral salt medium (MSM) used in the present study was: $(NH_4)_2SO_4$: 1.0 g/L, K_2HPO_4: 6.0 g/L, KH_2PO_4: 1.0 g/L, NaCl: 5.0 g/L, $MgSO_4.7H_2O$: 0.1 g/L and Glucose: 3.0 g/L [19]. Dye and glucose solutions were filter sterilized and added separately into the MSM at the time of inoculation.

Microorganism and culture conditions

Staphylococcus epidermidis MTCC 10623 was isolated from textile effluent contaminated soil collected from the site of Nahar Oswal Denim, Lalru (India) and screened by micro titer plate method as reported earlier [20]. The pure culture was maintained at 4°C on the nutrient agar slant and cultured in nutrient broth for inoculum preparation for 24 h at 37°C.

Optimization of physico-chemical parameters for the decolorization of BR 46

Optimal conditions for complete decolorization of the BR 46 dye by *S. epidermidis* were investigated by studying the effects of physico-chemical parameters such as static and shaking condition, pH (4-10), temperature (25-50°C), dye concentration (25, 50, 75, 100, 125 and 150 mg/L) and inoculum concentration (1, 3, 5, 7 and 10%). Experiments were performed in 250 mL Erlenmeyer flasks, containing 100 mL of MSM supplemented with graded concentration of BR 46 dye and inoculated with mid-log phase culture of *S. epidermidis*. Uninoculated dye containing medium was incubated simultaneously to account for abiotic decolorization. Samples were withdrawn from flasks after 0, 3, 6, 9 and 12 h for dye decolorization assay.

Effect of different co-substrates on decolorization

Different co-substrates such as carbon (glucose, sucrose, maltose and starch) and nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, urea and yeast extract) were examined for their regulatory effect on decolorization and degradation of BR 46 dye.

Immobilization of *S. epidermidis* on support matrices and decolorization of BR 46 under optimized conditions

Polyurethane foam (PUF) and Nylon mesh (NM) matrices were selected for immobilization because of their easy availability, large surface area and inner filamentous and porous structure. Pieces (1x1x1 cm) of matrices were washed 3-4 times with distilled water and subsequently dried and sterilized. For immobilization, mid-log phase culture (1%) of *S. epidermidis* along with support matrices was inoculated in a 500 mL flask containing 150 mL of nutrient broth (NB) and incubated at 37°C in an orbital shaker for 24 h. Fresh NB medium (25 mL) was added to the flask after every 24 h for 5 days. This provided sufficient nutrient for the growth and enabled the bacterial culture to get entrapped on the support matrices. Thereafter, immobilized *S. epidermidis* was used for decolorization of BR 46 dye in batch system under optimized conditions.

Decolorization assay

Decolorized sample (5 mL) was withdrawn at periodic interval of time, centrifuged at 10000 rpm for 15 min and the decrease in absorbance was measured at λmax (532 nm). The uninoculated dye free medium was used as blank. All assays were performed in triplicate and compared with uninoculated control. The color removal efficiency of bacterial isolate was expressed as per following equation [14].

$$\text{Decolorization (\%)} = [I - F] \times 100 / I$$

Where, I = initial absorbance, F = final absorbance of decolorized sample

FTIR and UV-Vis spectral analysis

After decolorization, bacterial biomass was removed by centrifugating at 10000 rpm for 15 min and supernatant was scanned using UV-vis spectrophotometer (*Spectronix, ST 2800, India*). The degradation of dye was analyzed by the change occurred in spectra of control dye and decolorized samples. For FTIR analysis, supernatant was extracted with equal volume of ethyl acetate. The extracted residue was dried over anhydrous Na_2SO_4 to remove the moisture content [21]. Thereafter, the solvent was evaporated to dryness in a rotary vacuum evaporator (*Khera Instruments Ltd., New*

Delhi, India). The dried metabolite was mixed with spectroscopic pure KBr (1:300) to form pellet and fixed in the sample holder for analysis. FTIR analysis was done in the mid-IR region of 400–4000 cm^{-1} with 16 scan speed using FTIR spectrophotometer (*Shimadzu, 8400 S, UK*) and changes in the frequencies were observed [22].

Scanning Electron Microscopy (SEM) of support matrices immobilized with *S. epidermidis*

For scanning electron micrographs, immobilized support matrices was fixed with 2% glutaraldehyde and 4% formaldehyde solution at room temperature and subsequently washed with 0.15 M phosphate buffer (7.0) for 15 min (3x15). Further, the fixed samples were dehydrated in graded series of ethanol solutions 30, 50, 70 and 90% (3x15 each) and 100% (1x15) for 15 min. The un-inoculated support matrix was also treated as per the described procedure [23]. Micrographs were taken on a scanning electron microscope (*JSM-6150, JEOL, JAPAN*).

Phytotoxicity assay

Phytotoxicity test was performed in order to assess the toxicity of BR 46 dye and its metabolites formed after decolorization by *S. epidermidis*. The test was carried out on common Indian agricultural crops *Triticum aestivum* (monocot plant) and *Vigna radiata* (dicot plant) [24,25]. Ten healthy seeds of *V. radiata* and *T. aestivum* were separately sown into plastic pots containing garden soil. Toxicity study was carried out at room temperature by daily irrigating the pots with 10 mL of BR 46 dye (100 mg/L) and filtrate containing the degraded metabolites after bacterial treatment. Control was maintained by treating seeds with distilled water (daily 10 mL). Germination rate, length of plumule and radicle was recorded after ten days.

Statistical Analysis

All the experiments had three replicates. The results obtained from each set of data have been expressed in terms of mean (average) and standard error by using Microsoft Excel (version Windows 2007).

Results and Discussion

Microorganism

S. epidermidis MTCC 10623 was selected because of its ability to decolorize various dyes being a denizen of soil contaminated with dye effluent. *S. epidermidis* is a gram positive, entire, elevated, opaque, small, off-white, nonmotile and spherical (cocci) bacterium. It is non-spore forming, nitrate reducing, starch hydrolysing, methyl red degrading, catalase +ve, acid producing and salinity tolerant bacterium. From the last two decades a lot of research has been carried out on bacterial degradation of azo dyes from textile effluent. Researchers examined the numerous bacterial strain having high dye decolorizing capability, isolated from the soil contaminated with textile dye effluent [20,18], lake-mud samples [26], activated sludge [27] and roots of plants [6] from wastewater treatment sites indicating the natural adaptation of these isolates to high dye concentration and their survival in the presence of toxic dyes.

Effect of shaking on decolorization of BR 46

S. epidermidis showed 98.8% decolorization of BR 46 (50 mg/L) in static condition after 12 h as compared to only 48.9% under shaking condition (Figure 1). This was much higher than *Pseudomonas putida* SKG 1, which decolorized 69% of Orange II in static condition and only 36.5% under shaking condition after 96 h incubation [28]. Chen et al. and Wang et al. [26,29] obtained slightly better results describing 80 and 60% decolorization by *Aeromonas hydrophila* and *Bacillus* sp. YZU 1 under static condition. The bacterium however showed significant increase in growth under aerobic condition. *Staphylococcus hominis* RMLRT03 strain brought 85.5% decolorization of Acid Orange in static condition which was reduced to 32.47% in shaking condition [18]. These studies supported contentious conclusion that shaking (aerobic) was not congenial for the dye decolorization as oxygen, being a preferable terminal electron acceptor over the azo groups inhibited the anaerobic decolorization. Our results revealed that *S. epidermidis* is a facultative anaerobe while it needs oxygen for growth, microaerophilic condition favor dye degradation.

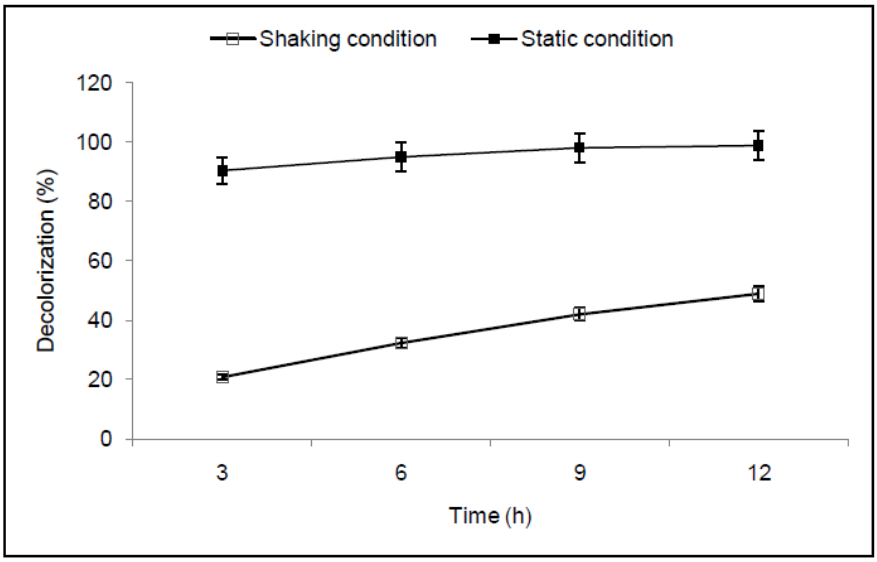

Figure 1: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 under shaking and static condition

Effect of pH and temperature on decolorization of BR 46

S. epidermidis was able to decolorize the dye at a wide pH range of 4-10. Although maximum decolorization (99.8%) was achieved at pH 9.0 after 12 h incubation under static condition, the activity was fairly comparable with pH 5-8 (Figure 2). However, decrease in decolorization was more drastic at pH 4.0 (88.6%). Decolorization of Navy Blue-3G by *Brevibacillus laterosporous* MTCC 2298 in broad pH (7.0-11.0) range corroborated our results [14]. Complete degradation of Reactive Black B, Remazol Blue and Reactive Red RB were observed by *Escherichia coli* and *Pseudomonas luteola* at pH 7.0 with consistent decolorization up to pH 9.5 [30]. *Sphingomonas paucimobilis* also decolorized Methyl Red at pH 9.0 [31]. Mixed bacterial culture (SB4) exhibited more than 85% decolorization of Reactive Violet 5R over a broad range of pH 5-8.5 [32]. Further, *Pseudomonas* sp. RA 20 decolorized 95% of Reactive Black 5 after 24 h incubation at pH 8.0 [33].

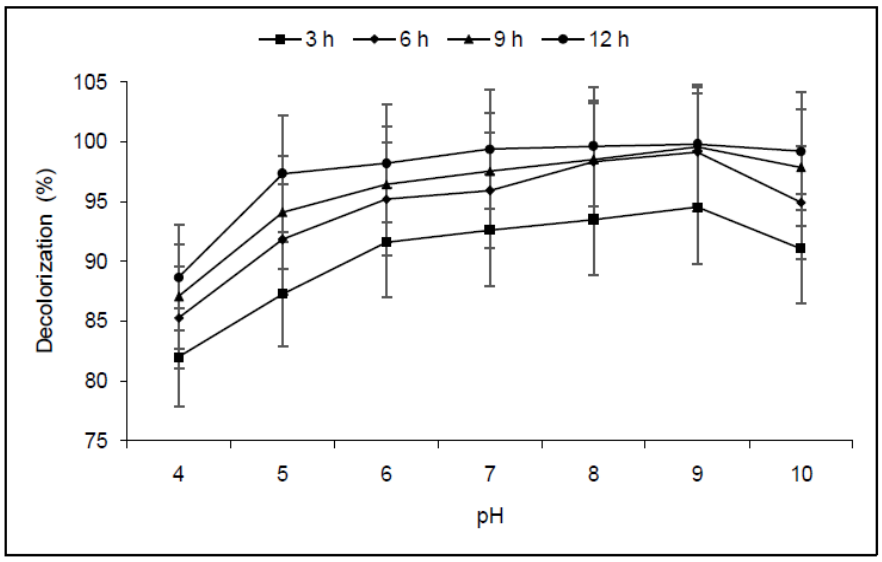

Figure 2: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 at different pH

Prasad et al. [34] also reported complete decolorization of Direct Black 1 at pH 8.0 in 6 h under static condition. Low pH appeared to be an important factor probably by regulating transport of nutrients across the cell membrane, indirectly impacting the decolorization process. Bacterial strains with tolerance or adaptability to a wider range of pH will be more effective in dye decolorization in effluents

with unpredictable pH range [11]. Our results demonstrated that *S. epidermidis* can be used effectively to treat the textile effluent which is generally highly alkaline.

Decolorization steadily increased up to 35°C with incubation period and remained unchanged till 45°C after 12 h of incubation. Further rise to 50°C drastically reduced to dye decolorization activity. Interestingly at 30-45°C temperature range incubation duration have not any significant effect. It varied from 88.4% at 30°C to 97.7% at 45°C (Figure 3). Although, at 50°C, the bacterium showed time dependent signs of recovery it was still lower than at 45°C. Temperature competitively affects growth and physiological activity of bacterium which is reflected in dye decolorization activity [18]. Higher decolorization of BR 46 at 35-45°C and time dependent recovery at 50°C temp. showed thermotolerance nature of the bacterium. Quite a few reports are available on effective decolorization and degradation of azo dyes by bacterial cultures such as *Bacillus* sp. YZU1 [29], *Enterobacter* sp. [35] and mixed culture [32] at 40 and 37°C. Complete decolorization of Direct Blue 1 by *Marinobacter* sp. strain HBRA was observed at 37°C [34]. As much as 92.3 and 97% decolorization of Acid Orange [18] and Black WNN dye [17] were obtained by treating with *Staphylococcus hominis* and *Staphylococcus epidermidis* MTCC 10623 at 35°C. Decolorization of Acid Black 210 [36], Remazol Red [13] and Methyl Red [31] was observed at 30°C by *Providencia* sp. SRS82, *Lysinibacillus* sp. RGS and *Sphingomonas paucimobilis*. Interestingly the bacterial culture used in these reports appear to have been functional well at 30-40°C, as compared to *S. epidermidis* used by us, which performed better at 45°C and showed time dependent adaptability at 50°C.

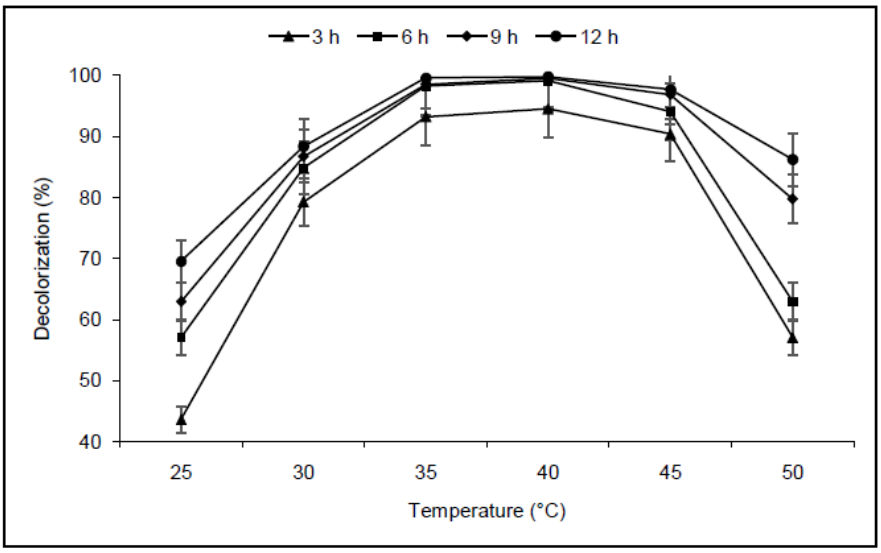

Figure 3: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 at different temperature

Effect of dye concentration and inoculum load

Studies on the dye concentration in the range of 25-150 mg/L on decolorization showed that *S. epidermidis* competitively decolorized 99% of BR 46 up to the concentration of 75 mg/L after 6 h showing marginal reduction to 98.6, 97.8 and 91% at 100, 125 and 150 mg/L dye concentration even after 12 h incubation (Figure 4). The concentration of dye in textile effluent affects the decolorization potential of bacteria through a combination of factors including toxicity of dye and inability of degrading enzymes to identify the substrate [28]. Our strain is effectively removed up to 150 mg/L of BR 46 within 12 h incubation and retained the activity at higher concentrations and extended incubation period. In contrast, although *Alcaligenes faecalis* completely decolorized 50 mg/L of Reactive Orange 13 in 4, it failed to retain the activity at higher concentrations [16]. Decolorization of Direct Blue 1 also decreased from 100 to 30% with increase in dye concentration from 100-500 mg/L [34]. *S. epidermidis* MTCC 10623 decolorized 95% of 100 mg/L Black WNN dye in 24 h, but it did not increase beyond at 250 mg/L even after 72 h incubation [17].

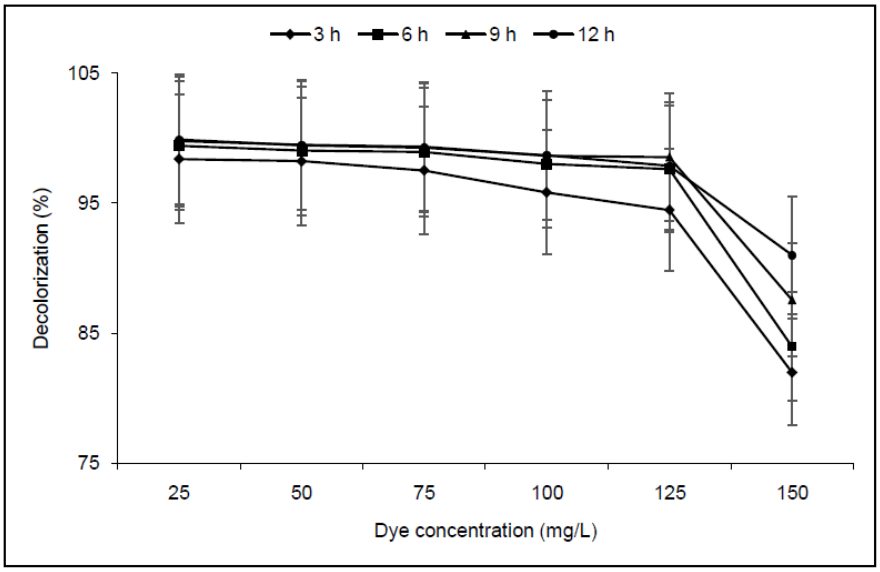

Figure 4: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 at different dye concentration

A direct correlation was observed between inoculum concentration and decolorization by *S. epidermidis*. It was maximum (99.5%) with 5% inoculum after 6 h incubation, whereas same level of decolorization was achieved with 7% inoculum within 3 h. Beyond 7%, decolorization was independent of inoculum concentration (i.e. 10%), whereas only 79.7 and 96% decolorization was observed with 1 and 3% inoculum after 6 h (Figure 5). Hence 5% inoculum was optimum for further experiments and beyond this decolorization was not affected by increase in inoculum concentration significantly. *Pseudomonas putida* SKG1 was able to decolorize (86.9%) textile dye effluent with 4% inoculum in 48 h incubation and beyond this no significant decolorization was observed [28]. The Reactive Black 5 decolorization was 75.1% with 2 mL of inoculum (*Enterobacter* sp. GY1) and increased to 79.0, 81.0, 84.2 and 85.4% with 4, 6, 8 and 16 mL culture respectively within 24 h [37].

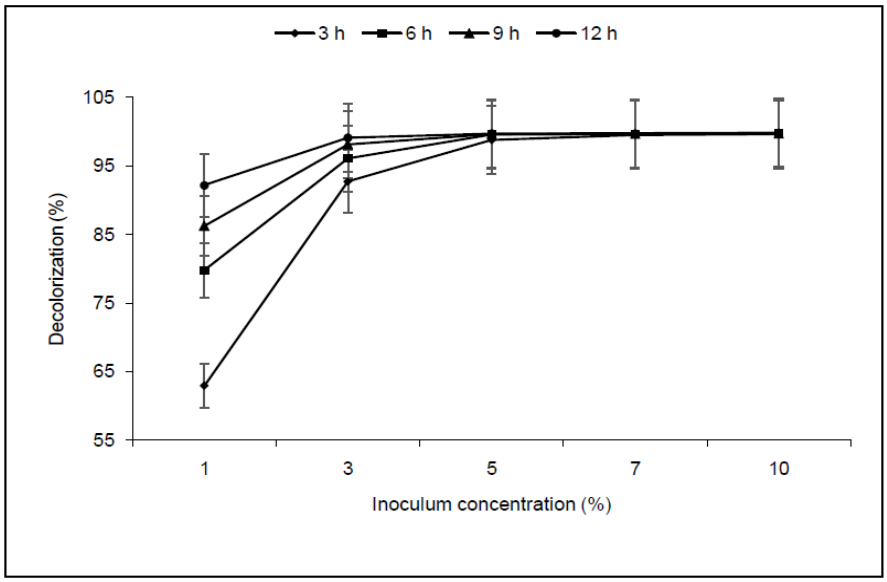

Figure 5: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 at different inoculum concentration

Effect of carbon and nitrogen sources as co-substrates

The presence of co-substrate play a vital role in azo reduction process as it acts as an electron donar. The rate of azo reduction process also depends on the type of co-substrate used and chemical structure of the azo dyes [37]. *S. epidermidis* was able to decolorize only 23% of 100 mg/L of Basic Red 46 after 6 h without any carbon source, whereas on supplementing carbon sources (3 g/L) such

as glucose, sucrose, maltose and starch, decolorization enhanced to 99.5, 92.4, 96 and 98.5% (Figure 6). In the presence of glucose (0.4%), *Pseudomonas putida* SKG1 decolorize 86.9% of orange II [28]. Glucose acts as a better co-substrate and effective reducing agent (electron donar) for degradation process and also promotes the growth of bacteria. Requisite of supplementation of carbon source for enhancement of decolorization was previously reported in several studies [21,38]. Decolorization of Golden Yellow HER enhanced with the supplementation of less concentration glucose [21]. Jain et al. [32] revealed that supplementation of glucose and yeast extract enhanced the decolorization 100%, however only 4% color removed without any co-substrate. *Enterobacter* sp. F NCIM 5545 exhibited strong decolorizing activity with 6 g/L glucose that decreased with increase in glucose concentration to 12 g/L [35], therefore, inhibited the decolorization rate, as at higher concentration, bacteria utilized the glucose for its growth instead for the production of enzyme responsible for the decolorization [1].

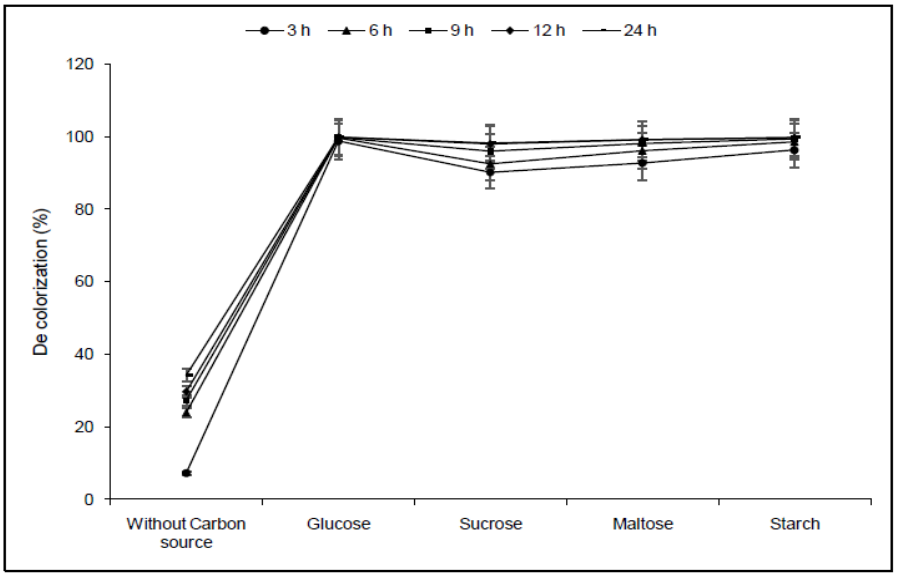

Figure 6: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 with different carbon source as a co-substrate

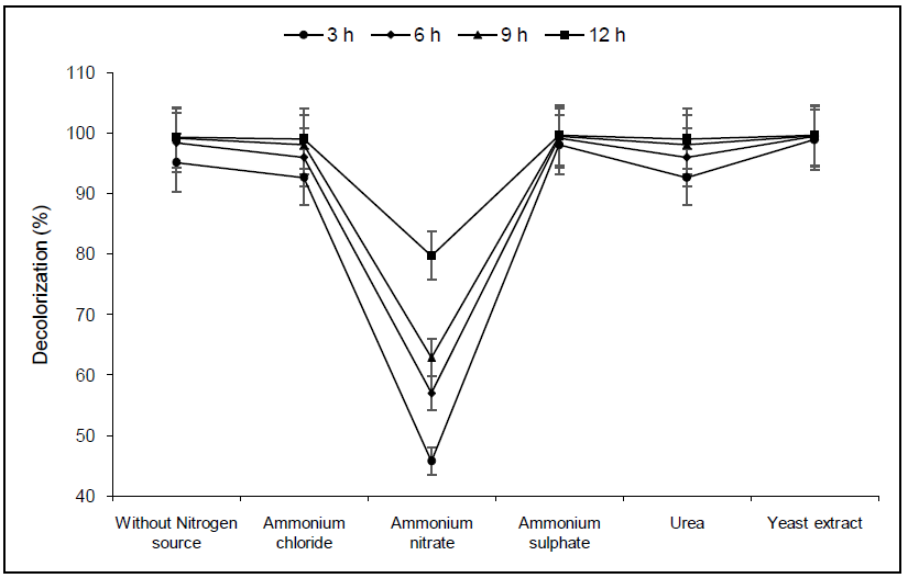

Figure 7: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 with different nitrogen source as a co-substrate

Various nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, urea and yeast extract) were examined to attain maximum decolorization of BR 46 dye and observed more than 99% decolorization with all nitrogen sources, except ammonium nitrate (79.7%) within 12 h (Figure 7)

due to its inhibitory effect on decolorization potential of bacteria, whereas decolorization of Orange II was maximum (92.8%) with ammonium sulphate (0.1%) in comparison to other nitrogen sources [28]. Medium supplemented with molasses and yeast extract also stimulates the microbial growth for the 100% decolorization of Remazol Blue (100 mg/L) [30], however peptone is also reported as the best nitrogen supplement for biodegradation of azo dyes [39].

Decolorization of BR 46 dye by immobilized *S. epidermidis* under optimized conditions in batch system

A number of support matrices such as polyurethane foam [23,40,41], marble chips [38], calcium alginate [42], polyvinyl alcohol [37] and agricultural waste [43] to immobilize bacterial, fungal, yeast and mixed culture for the decolorization of textile dyes reported by many researchers. In the present study, polyurethane foam (PUF) and nylon mesh (NM) were used as support matrices for the immobilization of *S. epidermidis* and investigated 99.8% decolorization of BR 46 (100 mg/L) under optimized conditions (pH 9.0 and temp. 40°C) after 6 h incubation (Figure 8).

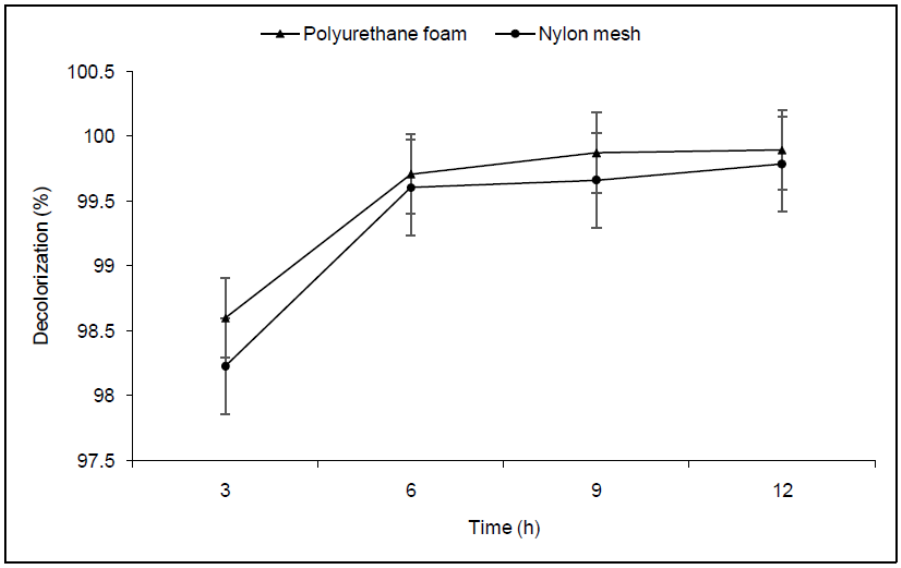

Figure 8: Decolorization of BR 46 by *S. epidermidis* MTCC 10623 immobilized over polyurethane foam and nylon mesh

Similar findings were observed with *Pseudomonas putida* [44], *Bacillus subtilis* and *Pseudomonas oleovorans* [23] immobilized on PUF. PUF specifically considered suitable carrier for whole cell as well as enzyme immobilization in various studies, whereas nylon mesh was not reported for immobilization till now. Lu et al. [41] also investigated effective decolorization of azo dye amaranth by *Escherichia coli* K12 immobilized on anthraquinone -2- sulfonate mediated polyurethane foam. In the present study these matrices were used for immobilization of bacteria as the inner filamentous fibril like structure of nylon mesh and polygonal structure of polyurethane foam provides large surface area for the growth that enhanced the decolorization efficiency.

Scanning electron microscopy (SEM) of immobilized *S. epidermidis*

SEM was performed to check the immobilization of *S. epidermidis* on PUF and NM (Figure 9 A and C), which provides large surface area for the immobilization of bacteria. It is evident from the scanning electron micrographs (Figure 9 B and D) a contiguous layer of cocci shape bacteria attached to the surface of the PUF and NM. Immobilized bacteria efficiently decolorized BR 46 dye within 6 h under optimized condition in batch system and can be used for decolorization of textile dyes in continuous system as well. Khehra et al. [45] evaluated the development of bacterial biofilm on PUF by SEM studies and reported up to 98% decolorization of Acid Red 88. *Pseudomonas oleovorans* and mixed bacterial culture was also immobilized on PUF [23] and marble chips [38] respectively and observed adherence of bacterial cultures to the support surface.

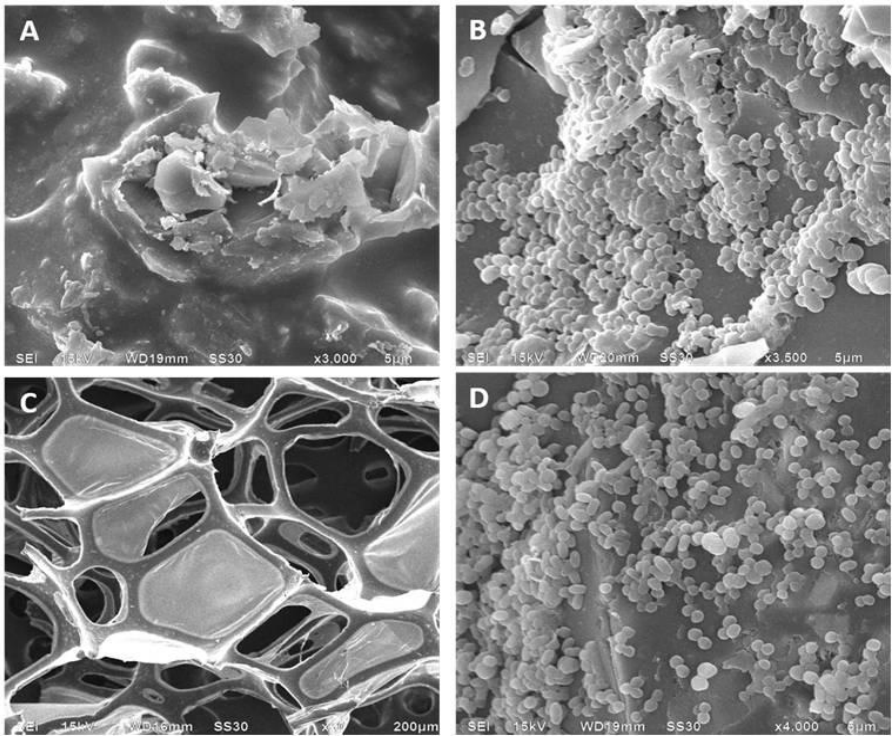

Figure 9: Scanning electron micrograph of support matrices (A) Nylon mesh, (B) *S. epidermidis* MTCC 10623 *immobilized* on NM, (C) Polyurethane foam, (D) *S. epidermidis* immobilized on PUF

FTIR analysis and UV-Vis spectral analysis for biodegradation

The decolorization and biodegradation of BR 46 dye was monitored by FTIR and UV-vis spectroscopy. Spectral analysis of BR 46 dye (λmax 532 nm) (Figure 10) was carried out in the UV-visible range (200-800 nm) to study the degradation of dye. For untreated dye BR 46 dye presented three absorbance peaks at 204, 287 and 532 nm. For treated decolorized sample under microaerophilic condition, the absorbance peak in the visible region completely disappeared after 12 h incubation time, indicating complete breakdown of the native color compound and thus provided strong evidence of the decolorization and degradation process. Absorbance peaks at 204 and 287 nm in the UV region disappeared and were replaced by new peaks at 227 and 255 nm (Figure 10). Decolorization of dye could be either due to adsorption process or by bacterial biodegradation. In the adsorption process, absorption peaks decreased proportionately to each other whereas in biodegradation either the major peak in visible region completely disappears or gives rise to a new peak.

The significant difference in FTIR spectrum of BR 46 dye control (Figure 11) with extracted metabolites clearly indicated the biodegradation of textile dye by *S. epidermidis.* Furthermore, the FTIR spectra of dye control displayed number of absorbance bands at 3445-3057 cm^{-1}, 2926 cm^{-1}, 1672 cm^{-1}, 1603 cm^{-1}, 1450 cm^{-1}, 1400 cm^{-1}, 1253-1288 cm^{-1}, 1235-1092 cm^{-1}, 999-729 cm^{-1}, 637-520 cm^{-1} indicative of -NH, C-H, -C=C-, C-C (aromatic ring), C-H (bend), C-C (aromatic ring), C-N (aromatic ring), C-N (aliphatic), =C-H and C-Br. On comparing the spectra with decolorized sample of BR 46, the absorbance bands at 2922 cm^{-1}, 1099-1026 cm^{-1}, 805 cm^{-1} have enlarged that correspond to C-H stretch, C-N (aliphatic) and C-H bend and two new absorbance bands at 1517 cm^{-1} and 1261 cm^{-1} were observed, indicative of N-O stretch and C-N stretch (aromatic ring). Absorbance bands at 3028 cm^{-1}, 1671 cm^{-1} and 1608 cm^{-1} were shorten correspond to –NH, C=C-, C-C (aromatic ring). Similar findings were reported after bacterial degradation of Acid Black 210 [36], Reactive Blue 19 [35], Orange II [28], Reactive violet 5 [32], Reactive Black B [12] and Reactive azo dyes [5].

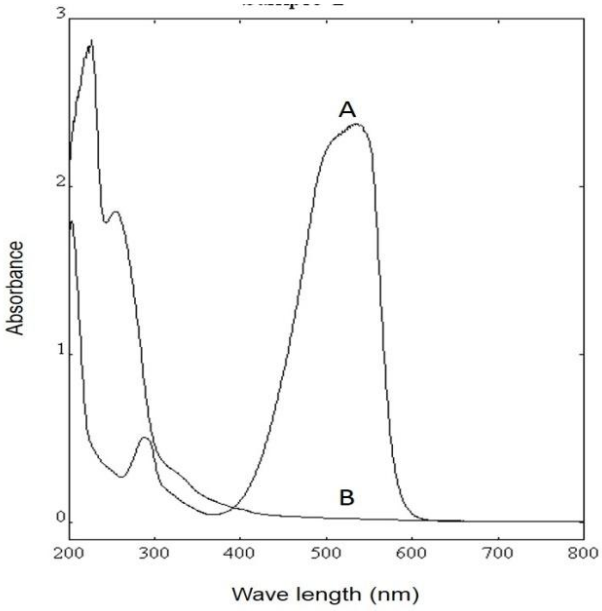

Figure 10: UV-Vis spectra of BR 46 dye after decolorization by *S. epidermidis* MTCC 10623 A: BR 46 dye Control, B: Decolorized sample

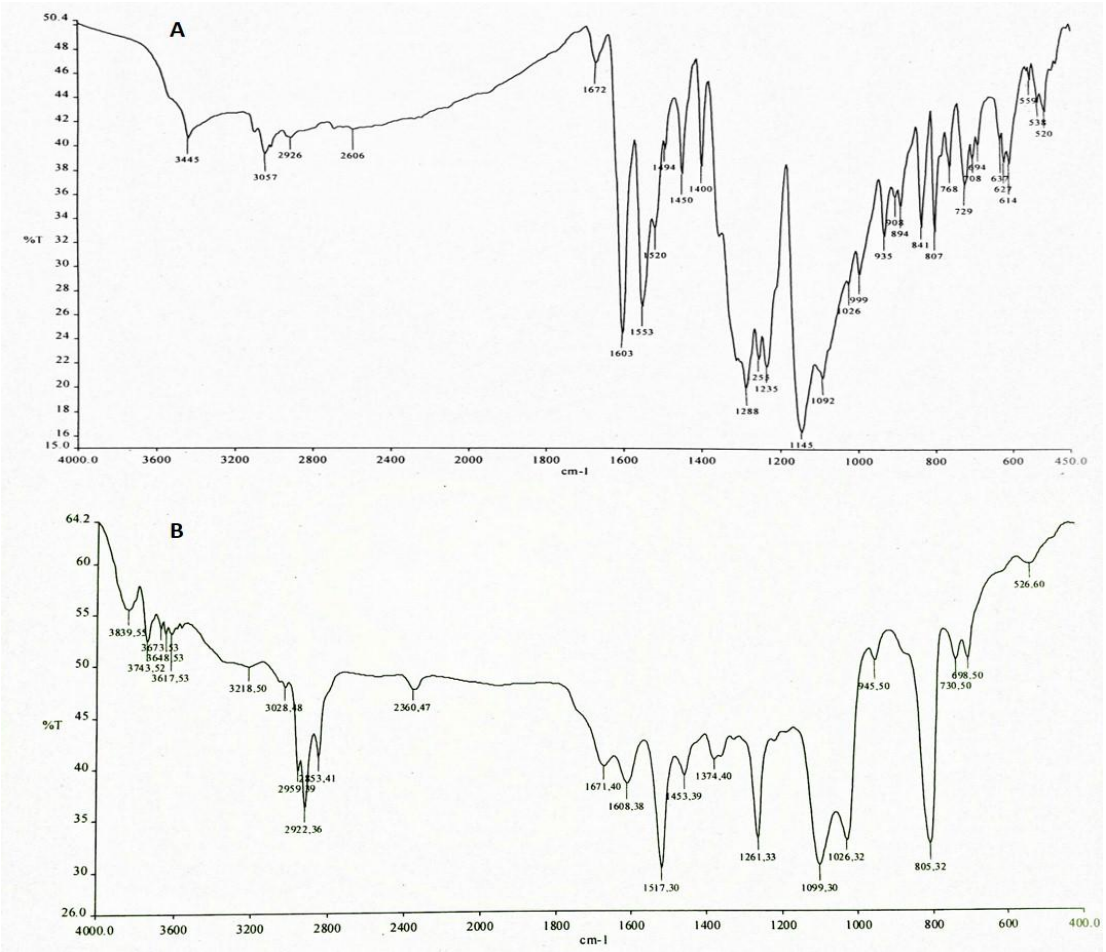

Figure 11: FTIR analysis of (A) BR 46 dye control (B) dye products formed after degradation

Phytotoxicity assay

Release of textile effluent without treatment in natural water bodies pollutes the environment, causes health hazards and harmful to crops when used for irrigation purposes. Use of bioassays for monitoring the toxic effect of dyes as well as its metabolites on plants was revealed by many researchers [24,25]. Phytotoxicity results showed good germination rate as well as significant growth in the plumule and radicle of *Triticum aestivum and Vigna radiata* plants in degraded dye product, as compared to BR 46 dye control (Table 1). Further, germination rate of *Phaseolus mungo* was less with Rubine GFL dye [34] as compared to its degradation product, whereas Scarlet RR (4000 mg/L) [46] inhibited 100% germination of *Triticum aestivum* compared with the metabolite as well as water control. Phytotoxicity assay on *Phaseolus mungo* plants resulting into 100% germination as well as significant growth of plumule and radicle length in metabolite formed after bacterial treatment as compared to Black WNN [17] dye control. Furthermore, 85% inhibition in germination rate of *Phaseolus mungo* plant was observed because of toxicity of reactive azo dyes as compared to 100% germination in water control and treated textile wastewater [5]. Phytotoxicity analysis concluded that dye was toxic to this plant, while the metabolites formed after bacterial degradation was nontoxic, as the plants grown with treated effluent was healthy and continuously growing, signifying the detoxification of dye.

Table 1: Phytotoxicity analysis of BR 46 dye and its degraded products on *Triticum aestivum* and *Vigna radiata* plants after treatment with *S. epidermidis*

Sample	Germination (%)	*Triticum aestivum*		*Vigna radiata*	
		Plumule (cm)	Radicle (cm)	Plumule (cm)	Radicle (cm)
Water control	100	15.74±0.50	10.3±0.36	15.46±1.05	12.03±0.10
BR 46 dye	60	6.50±0.50	3.53±0.15	3.86±0.15	2.33±0.11
Decolorized sample	100	16.25±0.05	10.63±0.50	13.35±1.10	8.98±0.10

Conclusion

S. epidermidis MTCC 10623 isolated from soil sample collected from effluent treatment site of textile industry, showed 99.6% decolorization of BR 46 dye in pH range of 8-10 with 5% inoculum at 40°C up to 50 mg/L dye concentration within 6 h under static condition. Moreover, Immobilized *S. epidermidis* on PUF and NM exhibited more than 99.6% decolorization of 100 mg/L of BR 46 within 6 h under optimized conditions in batch system. UV-vis spectral and FTIR spectroscopic analysis before and after decolorization were confirmatory to degradation of BR 46 dye. Hence this indigenous bacterial culture of *S. epidermidis* could be used as a potential microbe for developing an efficient and effective technology for decolorization and degradation of dyes from aqueous solution.

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References

1. Saratale R.G., Saratale G.D., Chang J.S., Govindwar S.P., Bacterial decolorization and degradation of azo dyes: A review, J. Taiwan Inst. Chem. Eng., 42, 138-157 **(2011)**

2. Balamurugan B., Thirumarimurugan M., Kannadasan T., Anaerobic degradation of textile dye bath effluent using *Halomonas* sp., Bioresour. Technol., 102, 6365-6369 **(2011)**

3. Gupta V.K., Khamparia S., Tyagi I., Jaspal D., Malviya A., Decolorization of mixture of dyes: A critical review, Global J. Environ. Sci. Manage., 1(1), 71-94 **(2015)**

4. Modi H.A., Rajput G., Ambasana C., Decolorization of water soluble azo dyes by bacterial cultures isolated from dye house effluent, Bioresour. Technol., 101, 6580-6583 **(2010)**

5. Balapure K., Bhatt N., Madamwar D., Mineralization of reactive azo dyes present in simulated textile wastewater using down flow microaerophilic fixed film bioreactor, Bioresour. Technol., 175, 1-7 **(2015)**

6. Sharma S., Roy S., Biodegradation of dye Reactive Black -5 by a novel bacterial endophyte, Intl. Res. J. Environ. Sci., 4(4), 44-53 **(2015)**

7. Franciscon E., Mendonca D., Seber S., Morales D.A., Zocolo G.J., Zanoni M.B., Grossman M.J., Durrant L.R., Freema H.S., Umbuzeiro G.A., Potential of bacterial consortium to degrade azo dye Disperse Red 1 in a pilot scale anaerobic-aerobic reactor, Process Biochem., 50, 816-825 **(2015)**

8. Jilani S., Bioremediation application for textile effluent treatment, Middle East J. Sci. Res., 23(1), 26-34 **(2015)**

9. Ayed L., Chaieb K., Cheref A., Bakhrouf A., Biodegradation and decolorization of triphenylmethane dyes by *Staphylococcus epidermidis*, Desalin., 137-146 **(2010)**

10. Solis M., Solis A., Perez H.I., Manjarrez N., Flores M., Microbial decolorization of azo dyes: A review, Process Biochem., 47, 1723-1748 **(2012)**

11. Imran M., Crowley D.E., Khalid A., Hussain S., Mumtaz M.W., Arshad M., Microbial biotechnology for decolorization of textile wastewaters, Rev. Environ. Sci. Biotechnol., 14, 73-92 **(2015)**

12. Pathak H., Soni D., Chauhan K., Evaluation of in vitro efficacy for decolorization and degradation of commercial azo dye RB-B by *Morganella* sp. HK-1 isolated from dye contaminated industrial landfill, Chemosphere, 126-132 **(2014)**

13. Saratale R.G., Gandhi S.S., Purankar M.V., Kurade M.B., Govindwar S.P., Eun Oh S., Saratale G.D., Decolorization and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated *Lysinibacillus* sp. RGS, J. Biosci. Bioeng., 115, 658-667 **(2013)**

14. Gomare S.S., Kalme S.D., Govindwar S.P., Biodegradation of Navy Blue-3G by *Brevibacillus laterosporus* MTCC 2298, Acta Chim. Slov. 56, 789-796 **(2009)**

15. Celik L., Ozturk A., Abdullah M.I., Biodegradation of Reactive Red 195 azo dye by the bacterium *Rhodopseudomonas palustris* 51ATA, Afr. J. Microbiol. Res., 6(1), 120-126 **(2012)**

16. Shah P.D., Dave S.R., Rao M.S., Enzymatic degradation of textile dye Reactive Orange 13 by newly isolated bacterial strain *Alcaligenes faecalis* PMS-1, Intl. Biodeterior. Biodegrad., 69, 41-50 **(2012)**

17. Pokharia A., Ahluwalia S.S., Decolorization of Black WNN dye with *Staphylococcus epidermidis* MTCC 10623, Curr. Trends Biotechnol. Chem. Res., 2(2), 65-73 **(2012)**

18. Singh R. P., Singh P.K., Singh R.L., Bacterial decolorization of textile azo dye Acid Orange by *Staphylococcus hominis* RMLRT 03, Toxicol. Intl., 21(2), 160-166 **(2014)**

19. Mabrouk M.E.M., Yusef H.H., Decolorization of Fast Red by *Bacillus subtilis* HM, J. Appl. Sci. Res., 4, 262-269 **(2014)**

20. Pokharia A., Ahluwalia S.S., Isolation and screening of dye decolorizing contaminated sites, Text. Light Ind. Sci. Technol., 2(2), 54-61 **(2013)**

21. Waghmode T.R., Kurade M.B., Khandare R.V., Govindwar S.P., A sequential aerobic/microaerophilic decolorization of sulfonated mono azo dye Golden Yellow HER by microbial consortium GG-BL, Intl. Biodeterior. Biodegrad., 65, 1024-1034 **(2011)**

22. Vitor V., Corso C.R., Decolorization of textile dye by *Candida albicans* isolated from industrial effluents, J. Ind. Microbiol. Biotechnol., 35, 1353-1357 **(2008)**

23. Silviera E., Marques P.P., Macedo A.C., Mazzola P.G., Porto A.L.F., Tambourgi E.B., Decolorization of industrial azo dye in an anoxic reactor by PUF immobilized *Pseudomonas Oleovorans*, J. Water Reuse Desalin., 18-26 **(2011)**

24. Cui D., Li G., Zhao D., Gu X., Wang C., Zhao M., Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions, J. Hazard. Mater., 221-222, 185-192 **(2011)**

25. Lade H.S., Waghmode T.R., Kadam A.A., Govindwar S.P., Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium, Intl. Biodeterior. Biodegrad., 72, 94-107 **(2012)**

26. Chen K.C., Wua J.Y., Liou D.J., Hwang J., Decolorization of the textile dyes by newly isolated bacterial strains, J. Biotechnol., 101, 57-68 **(2003)**

27. Wang H., Su J.Q., Zheng W.X., Tian Y., Xiong X.J., Xheng T.L., Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by *Citrobacter* sp. CK3, Intl. Biodeterior. Biodegrad., 63, 395-399 **(2009)**

28. Garg S.K., Tripathi M., Singh S.K., Tiwari J.K., Biodecolorization of textile dye effluent by *Pseudomonas putida* SKG-1 (MTCC 10510) under the conditions optimized for mono azo dye Orange II color removal in simulated minimal salt medium, Intl. Biodeterior. Biodegrad., 74, 24-35 **(2012)**

29. Wang Z.W., Liang J.S., Liang Y., Decolorization of reactive black 5 by a newly isolated bacterium *Bacillus* sp. YZU1, Intl. Biodeterior. Biodegrad. 76, 41-48 **(2013)**

30. Cetin D., Donmez G., Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions, Enzyme Microb. Technol., 38, 926-930 **(2006)**

31. Ayed L., Mahdhi A., Cheref A., Bakhrouf A., Decolorization and degradation of azo dye Methyl Red by an isolated *Sphingomonas paucimobilis*: biotoxicity and metabolites characterization, Desalin., 274, 272-277 **(2011)**

32. Jain K., Shah V., Chapla D., Madamwar D., Decolorization and degradation of azo dye- Reactive Violet 5R by an acclimatized indigenous bacterial mixed cultures-SB4 isolated from anthropogenic dye contaminated soil, J. Hazard. Mater., 213-214, 378-386 **(2012)**

33. Hussain S., Maqbool Z., Ali S., Yasmeen T., Imran M., Mahmood F., Abbas F., Biodecolorization of Reactive Black-5 by a metal and salt tolerant bacterial strain *Pseudomonas* sp. RA20 isolated from Paharang drain effluents in Pakistan, Ecotoxicol. Environ. Saf., 98, 331-338 **(2013)**

34. Prasad A.S.A., Satyanarayana V.S.V., Bhaskara Rao K.V., Biotransformation of Direct Blue 1 by a moderately halophilic bacterium *Marinobacter* sp. strain HBRA and toxicity assessment of degraded metabolites, J. Hazard. Mater., 262, 674-684 **(2013)**

35. Holkar C.R., Pandit A.B., Pinjari D.V., Kinetics of biological decolorization of anthraquinone based Reactive Blue 19 using and isolated strain of *Enterobacter* sp. F NCIM 5545, Bioresour. Technol., 173, 342-351**(2014)**

36. Agrawal S., Tipre D., Patel B., Dave S., Optimization of triazo Acid Black 210 dye degradation by *Providencia* sp. SRS82 and elucidation of degradation pathway, Process Biochem., 49, 110-119 **(2014)**

37. Chen G., Huang G.C., Chen L., Chen D., A batch decolorization and kinetic study of Reactive Black 5 by a bacterial strain *Enterobacter* sp. GY-1, Intl. Biodeterior. Biodegrad., 65, 790-796 **(2011)**

38. Tony B.D., Goyal D., Khanna S., Decolorization of Direct Red 28 by mixed bacterial culture in an up flow immobilized bioreactor, J. Ind. Microbiol. Biotechnol. 36(7), 955-960 **(2009)**

39. Bayoumi R.A., Musa S.M., Bahobil A.S., El-Sakaway T.A., Biodecolorization and biodegradation of azo dyes by some bacterial isolates, J. Appl. Environ. Biol. Sci., 1(1), 1-25 **(2010)**

40. Lade H., Govindwar S., Paul D., Mineralization and detoxification of the carcinogenic azo dye congo red and real textile effluent by a polyurethane foam immobilized microbial consortia in an upflow column bioreactor, Intl. J. Environ. Res. Public Health, 12, 6894-6918 **(2015)**

41. Lu H., Zhou J., Wang J., Si W., Teng H., Liu G., Enhanced biodecolorization of azo dyes by anthraquinone-2-sulfonate immobilized covalently in polyurethane foam, Bioresour. Technol., 101(18), 7185-7188 **(2010)**

42. Dastagir, Sindhu S., Naga Padma P., Studies on immobilized yeast for decolorization of azo dyes, Intl. J. Sci. Technol. Res., 3(4), 182-185 **(2014)**

43. Kulandaivel S., Kaleeswari P., Mohanpriya P., Decolorization and adsorption of dyes by consortium of bacteria with agriculture waste, Intl. J. of Curr. Microbiol. Appl. Sci., 3(12), 865-882 **(2014)**

44. Manikandan B., Ramamurthi V., Karthikeyan R., Sundararaman T.R., Biobleaching of textile dye effluent using mixed culture through an immobilized packed bed bio reactor (IPBBR), Modern Appl. Sci., 3(5), 131-135 **(2009)**

45. Khehra M.S., Saini H.S., Sharma D.K., Chadha B.S., Chimni S.S., Biodegradation of azo dye C.I. Acid Red 88 by an anoxic-aerobic sequential bioreactor, Dyes and Pigm., 70, 1-7 **(2006)**

46. Jadhav J.P., Kalyani D.C., Telke A.A., Phugare S.S., Govindwar S.P., Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals and toxicity from textile dye effluent, Bioresour. Technol., 101, 165-173 **(2010)**.