ISSN Online: 2349-7785

Bioremediation of Textile Azo Dye Congo Red Using Bacterial Isolates From Textile Waste Water

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Received 10 October 2014; accepted in revised form 30 October 2014

Abstract: Increasing environmental pollution has compelled the textile industries to investigate into the possible environment-friendly methods of treating industrial waste. Synthetic dyes that are generally used in industries are toxic, mutagenic, carcinogenic and recalcitrant in nature. Microorganisms have the ability to decolorize synthetic dyes used in textile industry for dyeing. Total of nine isolates were selected on the basis of Gram's reaction, colony characteristics and cell morphology. The samples contained only Gram negative species. All isolates were tested for their ability to decolorize the azo dye Congo red (CR). Further, these isolates were optimized for pH, temperature, aeration and varying medium for maximum decolorization of azo dyes. Differences in structures and complexity of the dye led to varied decolourization of different dyes by the same organism. The selected bacterium showed higher decolorization in microaerophilic condition as compared to aerophilic condition. But the percentage decolorization of CR was almost similar in both Glucose-Peptone-Yeast extract (GPY) medium and Minimal Salt Medium (MSM) with varied time period. At 35°C and pH (7 - 8) the isolated bacteria showed higher decolorization. After the dye decolorization, bands on TLC and UV-visible absorbance spectra showed a maximum absorption peak in the visible area which decreased to a minimum level after 48 h of incubation. This proved that the dye was degraded by the bacteria when compared to the control.

Key words: Textile effluent, bacterial isolates, decolorization, azo dyes Congo red, toxicity.

Introduction

Release of hazardous, harmful substances into water bodies is one of the important reasons for environmental pollution [17]. Among various industries, the textile dyeing industry discharges large volumes of waste water into the natural streams [26], which is about 15 % of the dyestuff used during manufacturing and processing operations [15].

Azo dyes used in textile industries are characterized by the presence of one or more azo groups -N = N- [19] which are responsible for their colour. Congo red (sodium salt of benzidinediazo-bis-1-napthylamine-4 sulfonic acid), an azo dye [11] is carcinogenic, toxic and recalcitrant. The microbial metabolic cleavages of azo linkages in the azo dye result in free aromatic amines [16]. Methods for dye removal may be physical, biological, chemical or electrochemical. But, biological methods offer the best alternative for physico-chemical processes as they are cost effective, produce less sludge and is eco-friendly [10,12].

In the present study, an attempt has been made to isolate azo-dye degrading bacteria from textile industry effluent and optimize physico-chemical conditions for higher decolourization.

Materials and methods

Collection of effluent samples

Effluent samples were collected from five different textile industries located at Erode, Tamilnadu. All samples were collected in sterile plastic containers.

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Development of enrichment culture for dye decolorization and isolation of dye decolorizing bacteria

The principle of selective batch culture was used to select isolates that could decolorize the dye. 10 ml of the effluent was added into 100 ml physiological saline in a 500 ml beaker and shaken vigorously. 10 ml was withdrawn from flask and inoculated into 100 ml mineral salt medium (MSM) with 100 mg.l^{-1} dye and incubated at 30±2^{o}C under aerobic conditions. Cultures were observed daily for change in turbidity and decolourization.10 ml of decolorized cultures were inoculated into fresh MSM containing dye and incubated at 30 ±2^{o}C for 72 h. The cultures were serially diluted up to 10^{-6} dilution and then streaked on Luria-Bertani broth supplemented with 1 % of dye incubated at 30 ±2^{o}C for 3 d. The obtained pure cultures were maintained on nutrient agar at 4^{o}C [6].

Identification and characterization of the isolate

The selected dye decolorizing isolates were identified on the basis of their morphological, physiological and biochemical characters according to Bergey's Manual of Systematic Bacteriology [8].

Decolorization assay

MSM was prepared as per Khadijaho [15]. Decolorization was assessed by measuring absorbance of the supernatant at 495 nm [24].

Optimization of environmental factors for efficient decolourization

Factors like temperature, pH, aeration and medium were optimized during the experimentation for maximizing decolorizing efficiency of the isolates. Optimization studies included varying pH (5, 6, 7, 8 and 9) and temperature (15, 25, 35, 45, 50^{o}C). To study the effect of aeration, the flasks were incubated under aerobic and microaerophilic conditions. MSM and GPY broth were used to study the effect of media. GPY broth was prepared as per Joe *et. al.* [13]. Culture conditions remained same. An uninoculated blank was run to check the abiotic decolorization during the experimentation.

Biodegradation analysis

Spectrophotometric [34] and chromatographic [27] analyses (TLC silica gel 60F, Merck, Germany) were carried out for biodegradation studies. The developing solvent system used was ethyl acetate: hexane (2:3 v/v) for biotransformed intermediates/ products and ethyl acetate: methanol (7:3 v/v) for residual dye. The bands of aromatic components were observed under UV Transilluminator (λ_{max} = 365nm) and other bands were observed by exposing the plates to iodine vapour.

Statistical analysis

All the experiments in the study were performed in triplicates and standard error was calculated.

Results and discussion

Isolation and identification of the bacterial isolates

A total of nine bacterial isolates were screened for decolourization and degradation of congo red. Each colony was named as TE1, TE2, TE3, TE4, TE5, TE6, TE7, TE8 and TE9. All the nine bacterial isolates from the enrichment culture were screened for their efficiency to remove congo red from broth. A concentration of 100 mg.l^{-1} of the dye was used in the study. Observations showed 60-81 % decolorization efficiency in 24 h.

Although, some isolates showed about 70 % decolourization by the end of 24 h, some did not even reach 60 %. Hence, it was further incubated to 72 h. A gradual increase in the rate of decolourization from 24 to 72 h was observed. But TE6, TE7 and TE8 showed maximum decolourization by 24 h.

All the isolates were characterized morphologically and biochemically. Microscopic observations revealed that all the isolates were rod shaped and gram negative in nature. All the isolates (TE1-TE9) were positive for KOH, catalase, oxidase, amylase, H_2S, organic acids, citrate and fermented glucose, lactose and sucrose. They were negative for indole and acetoin production but TE1-TE5 were positive for urease. Similar to the present study, several authors have reported isolation and screening of microorganisms capable of decolorizing various azo dyes from

sludge samples collected from wastewater treatment sites contaminated with dyes [6,25,28].

Dubey *et. al.* [6] isolated *Bacillus* species from soil contaminated with untreated textile mill effluent. The isolate utilized Golden Yellow HER an azo dye as the sole source of carbon and nitrogen for which 100 % decolourization was obtained.

Ponraj *et. al.*, [25] studied the ability of species of *Bacillus*, *Klebsiella*, *Pseudomonas* and *Salmonella* in decolorization of textile dye Orange 3R, isolated from a textile industry effluent in Tamil Nadu. They observed that species of *Pseudomonas* and *Bacillus* showed maximum dye decolorization of 89 % by the end of 144 h under optimum condition. Saranraj *et. al.* [28] isolated five different dye degrading bacterial species, *Bacillus subtilis*, *Pseudomonas aeruginosa, Proteus mirabilis,* and *Escherichia coli* from the textile effluent in Tirupur region of Tamil Nadu.

Effect of environmental factors on decolourization

pH

pH ranging from 5-9 was used to find out optimal pH for the decolorization of azo dyes by the selected bacterial isolates. It was observed that an increase in pH from 5 to 7 had positive effect on the decolorization of Congo red. However, decolorization rate in all strains dropped sharply as the pH increased from 8 to 9. Optimal pH to decolorize the dye for all the tested isolates was 7 (Fig.1). pH, temperature, aeration and medium on cell growth and dye decolorization are the most critical factors to be considered. The bacterial isolates showed similar ability for decolorizing Congo red. Many researchers have carried out similar work to investigate effect of pH and temperatures on decolourization of dyes by microorganisms [9,29]. Olukanni *et. al.* [23] and Nosheen *et. al.* [22] documented that dye decolorization behavior of each strain varied with variation in pH. The pH tolerance of the decolorizing bacteria is quite important as the reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and high temperatures [1]. For color removal the optimum pH is often at neutral or slightly alkaline pH [9]. Suwannawong *et. al.* [32] observed that optimal pH for Congo red decolorization was at 6.0 -7.0. The significant suppression of decolorizing activity at different pH and temperatures might be due to loss of cell viability or deactivation of enzymes responsible for decolourization. Decolourization of Congo red increased with pH and has been drastically affected after pH 8.0. Similar observations were reported by Moosvi *et. al.* [21], Tripathi and Srivastava [34] and Bhatt *et al.*[2].

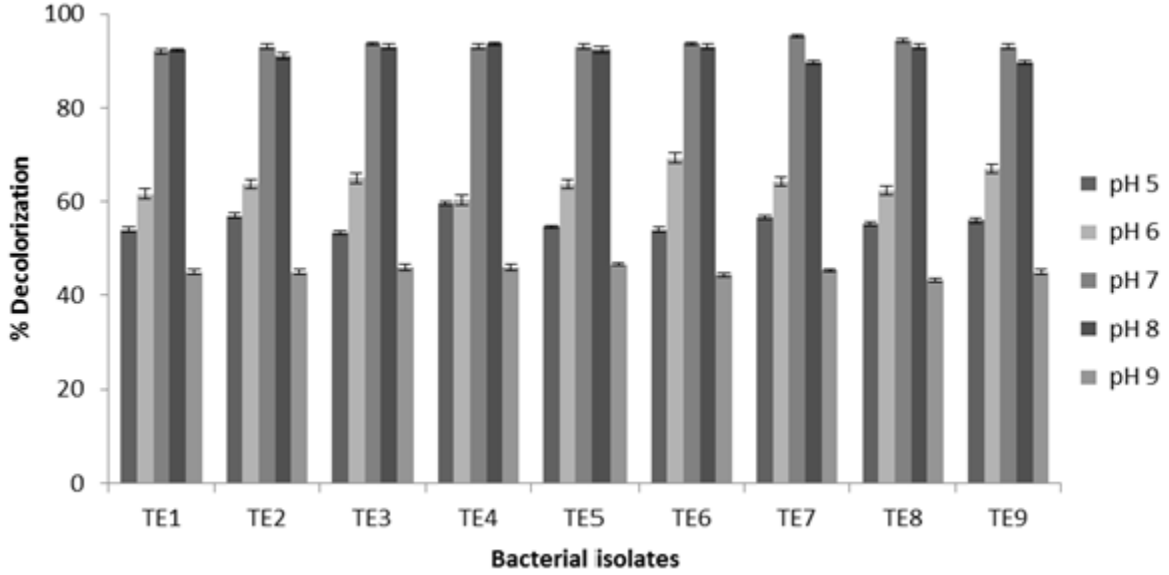

Fig. 1. Effect of pH on decolorization of Congo red by nine bacterial isolates after 72h, dye concentration 100mg. l^{-1} 35 ^{o}C. Results are means of triplicate experiments (Standard Error is indicated with error bars), TE – Textile effluent

Temperature

Incubation temperature ranging from 15-50°C was considered to study the optimal temperature for the decolorization of azo dyes by selected bacterial isolates. It was observed that an increase in the temperature from 15-35°C had positive effect on the decolorization of Congo red. Optimal temperature to decolorize the dye for all tested strains was 35°C. However, decolorization rate in all strains dropped sharply as the temperature increased from 35-50°C. The best decolorization was achieved at temperature 35°C after 72 h (Fig.2). This could be owing to a higher enzyme production and maximal growth conditions of the bacterial culture for its dye decolorization ability. Saratale *et. al*. [30] and Bhatt *et al.*[2]. reported maximum decolorization at 37°C. Decrease in decolorization at higher temperature may be due to the loss of cell viability and thermal deactivation of the enzyme responsible for decolorization [3]. Guo *et. al*. [7] reported optimum range of temperature of 28-35°C for the decolorization of dye using *B. subtilis*. Congo red decolourization at 37°C and increase in temperature beyond 37 °C led to decline in decolourization activity of the strain [18].

Aeration

TE6 showed maximum decolourization (80 %) after 24 h under aeration condition in the MSM. But after 48 h of incubation, the rate of decolourization did not increase much. But it was observed that TE3, TE6 showed 80.7 decolorization and TE1, 2, 4, 5,7, 8 and 9 showed 77.7, 75.7, 71, 78.4, 79, 76 and 78.7 % of decolourization respectively. After 72 h, TE3 showed 81 % of decolorization, TE5, TE6 and TE7 have shown 80 - 80.7 %, whereas TE1, 2, 4, 8 and 9 showed 75.7 - 78.7 % decolourization. Under microaerophilic conditions, TE7 showed maximum decolourization of 93 % (Fig.3).

The results were similar to those of studies on *Escherichia coli* NO_3 [4], *Pseudomonas luteola* [5] and *Rhodopseudomonas palustris* [20].

Under aerobic conditions, the aerobic respiration of the isolate might dominate the utilization of NADH and deprive the azoreductase from obtaining electrons from NADH to decolorize azo dyes [31]. Azo dyes are generally resistant towards bacterial attack under aerobic conditions due to the inhibition of azo bond reduction by oxygen - sensitive azo reductase [29] and may be due to competition of oxygen and the dye compounds for the reduced electron carriers under aerobic conditions [14]. Tamboli *et. al*. [33] also reported that aerobic condition delays the degradation of textile dyes which supports our observation.

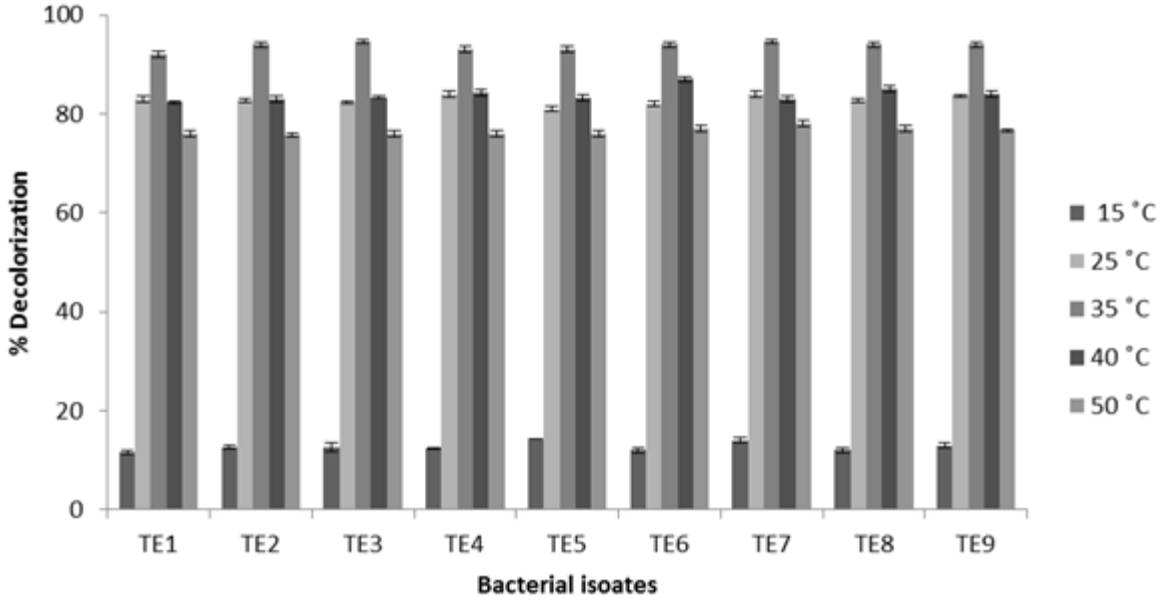

Fig. 2. Effect of temperature on decolorization of Congo red nine bacterial isolates after 72h, dye concentration 100mg. l^{-1} pH: 7. Results are means of triplicate experiments (Standard Error is indicated with error bars), TE – Textile effluent

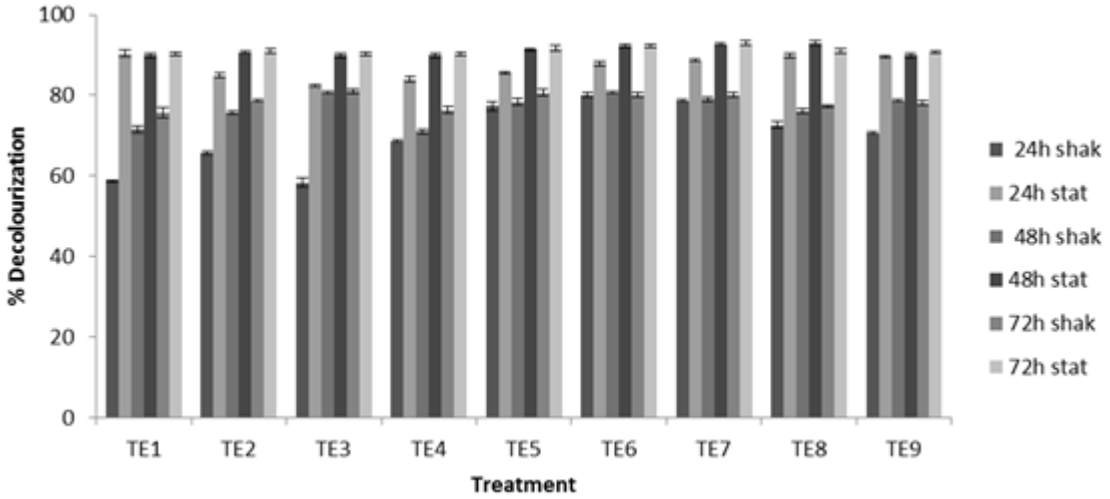

Fig. 3. Effect of aeration on decolorization of Congo red by nine isolates after 24,48 and 72 h of incubation in aerobic condition and microaerophilic condition; dye concentration 100 mg. l^{-1} pH: 7, 35°C. Results are means of triplicate experiments (Standard Error is indicated with error bars), TE – Textile effluent

Media

Effect of medium on cell growth and dye decolourization is one of the most critical factors to be considered. After 72 h of incubation, a maximum of 83 % of decolourization was observed in the GPY medium whereas a maximum decolorization of 81 % was observed in the MSM. On the GPY medium, TE6 and 7 have shown maximum rate of decolourization after 72 h (83 %), whereas in MSM, TE3 has shown maximum rate of 80.7 % of decolourization after 72 h. The rate of decolorization was almost same in both GPY and MSM media (Fig. 4).

Degradation studies of the dyes using the selected isolates

When TLC plates were observed under UV light, brown spots were observed as indicated in Fig. 5(A) were the biotransformed inter-mediate/ products and the spot observed (Fig. 5B) were residual dayes.

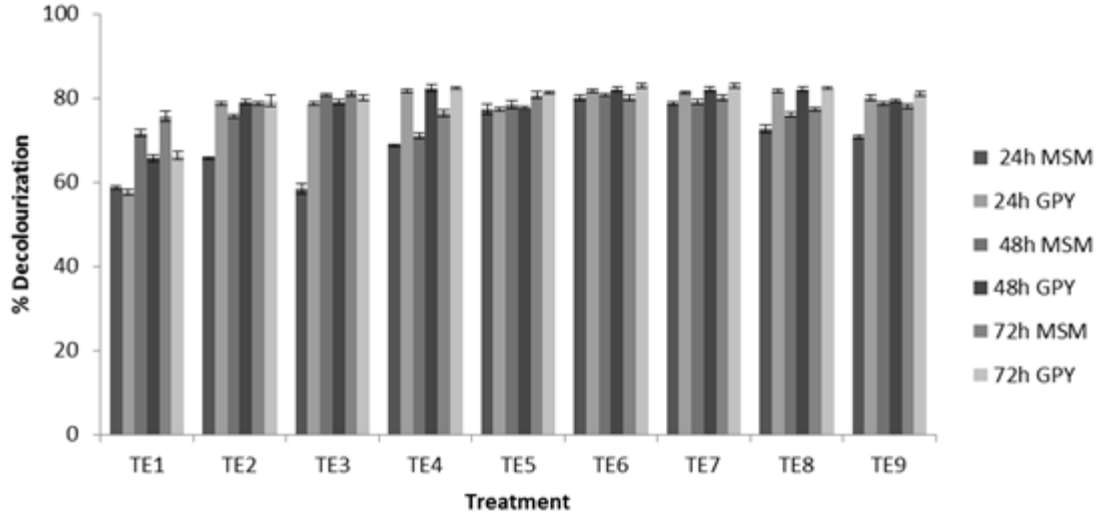

Fig. 4. Effect of media on decolorization of Congo red by nine isolates in mineral salt media and GPY broth (Glucose, peptone, yeast extract) after 24, 48 and 72 h of incubation at 37°C, pH : 7, dye concentration 100mg. l^{-1}. Results are means of triplicate experiments (Standard Error is indicated with error bars), TE – Textile effluent

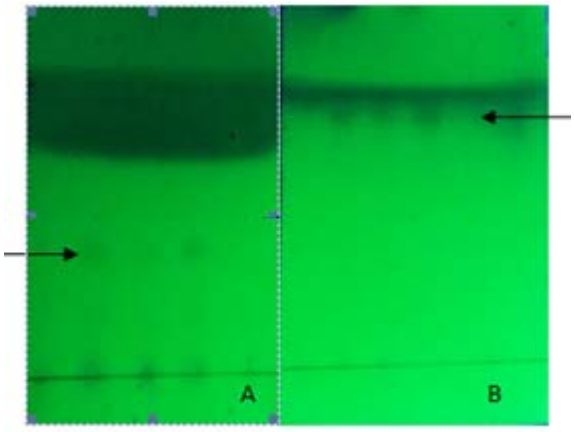

Fig. 5. TLC experiments shows biotransformed intermediates/products (**A**) and residual dye (**B**). (←) Indicate band formation

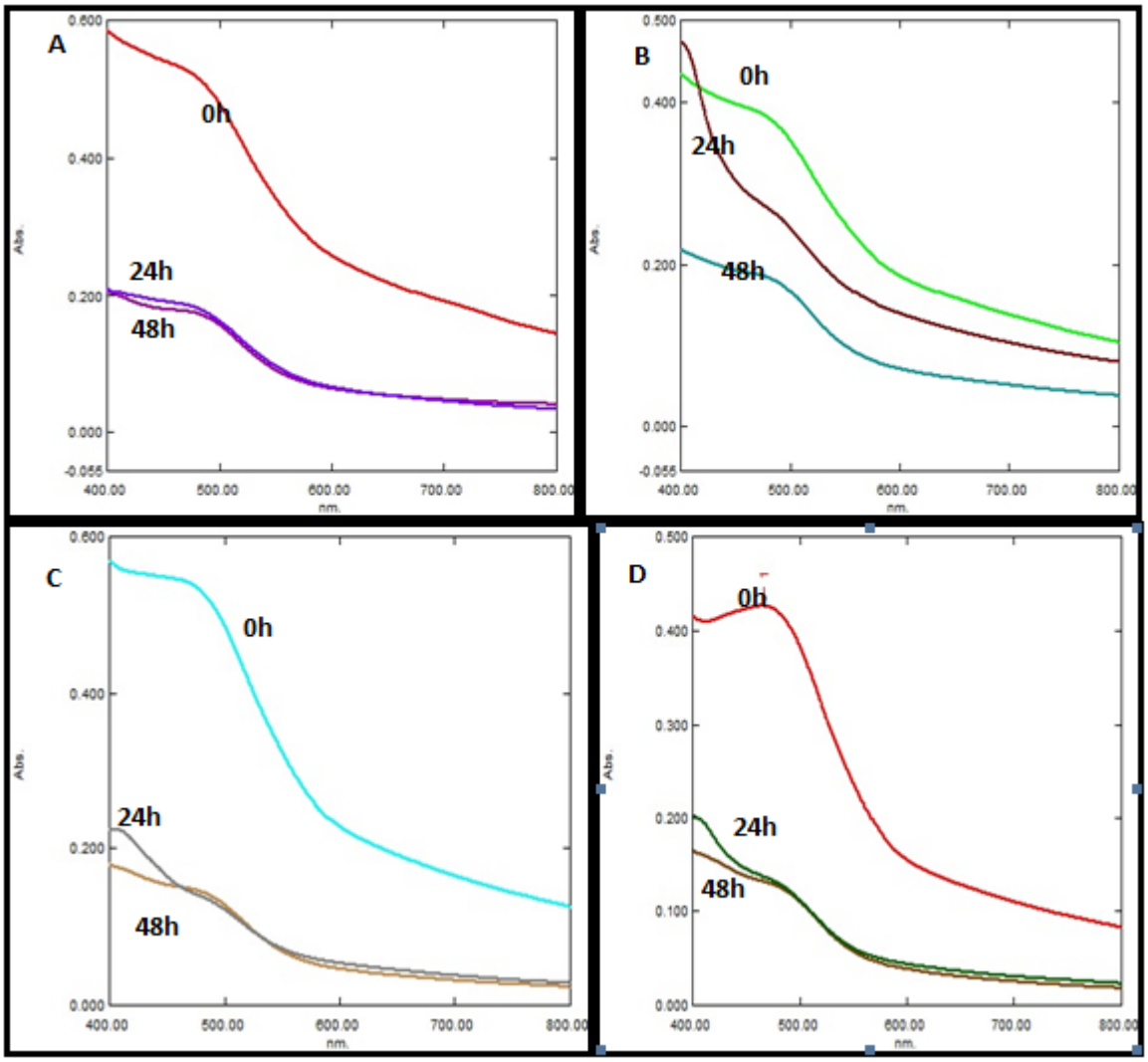

Fig. 6. UV-Vis spectrum of decolorization (%) of Congo red with different bacterial isolates TE1 **(A),** TE2 **(B),** TE6 **(C)** and TE7**(D)** under aerobic conditions

The UV-vis absorption spectrum of CR (at 100 $mg.l^{-1}$) was studied at different time interval, over the range of 200-800 nm which indicated that the maximum absorption was in between 400-500 nm after 0 h but a decline was observed in the peak after 24 h and 48 h (Fig: 6). Similar results were obtained in case of Direct orange 39 [10] and Remazol Black B [13].

Conclusion

In the present study, an attempt was made to examine the potential of different bacterial strains isolated from textile effluent for Congo red degradation. The selected bacterium showed higher decolorization in microaerophilic condition, pH 7 and 35°C temperature. TLC bands and UV-visible absorbance spectrum showed maximum absorption peak in the visible region which decreased to a minimum level after 48 h of incubation. This proved that dye was degraded by the bacterium.

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