PRAJÑĀ - Journal of Pure and Applied Sciences Vol. 20: 1 - 9 (2012)
ISSN 0975 - 2595

APPLICATION OF REDOX MEDIATORS ON DECOLORIZATION OF ACID RED 2 BY A BACTERIAL CONSORTIUM-PES

Shilpa Gupte*
Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences
New Vallabh Vidyanagar – 388 121, Gujarat, India

ABSTRACT

A novel bacterial consortium-PES was used on the basis of its rapid decolorization ability of Acid Red dye in contrast to individual strains isolated from decaying wood of *Adansonia digitata*. The cultures were identified by Bio Log 1420 system as well as by 16S rRNA nucleotide sequence. The consortium-PES consisting of three cultures designated as *Pseudomonas aeruginosa*, *Escherichia hermanii* and *Stenotrophomonas maltophilia*. The consortium-PES exhibited 98% decolorization within 22±2 h and maximum decolorization activity was observed in the pH range (6-8), temperature range (25-37 ºC) under static condition. The maximum decolorizing efficiency was observed till 700 mg l-1 Acid Red 2 dye concentration with a supplementation of 1g l-1 glucose. The consortium-PES exhibited efficiency to decolorize 15 structurally different azo dyes. Degradation process was studied through UV-Visible spectroscopy, HPTLC and FTIR. In the presence of redox mediator decolorization performance of Acid Red 2 was significantly enhanced and amongst the various redox mediators used, maximum decolorization was observed with AQS. 1mM concentration of AQS was found to be significant to accelerate decolorization of Acid Red 2 (98%) only in 12 h. and similar extent of decolorization could be achieved within 5 h up to 4 cycles of repeated dye spiking.

Key words: *Bacterial consortium-PES, Azo dye, Decolorization, Biodegradation, Redox mediators, AQS*

INTRODUCTION

Azo dyes are extensively used in textile, dyeing, printing, paper, leather, plastics, paints and cosmetics industries. Approximately one million metric ton of dyes are produced annually out of which azo dyes represent about 70% on weight basis [1]. They are characterized by one or more azo groups (-N=N-). It has been estimated that 10-15 percent of dyes are lost in effluent during dying process which can create a strong impact on the aquatic environment because it is discharged in high quantity and may contain many recalcitrant contaminants [2]. In aquatic systems, the dyes undergo various reactions and alterations to their chemical structures which can result in the formation of new xenobiotic compounds which may be more or less toxic than the parental compounds. Disposal of these dyes into environment cause serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration, solubility of gases and also they may be toxic and carcinogenic due to their breakdown products [1]. Dyes can be removed from wastewater by various physico-chemical methods like adsorption, coagulation-flocculation, oxidation and electrochemical methods [3, 4]. However, these chemical and physical methods have many disadvantages in application, such as high-energy costs, high-sludge production and formation of by-products. Alternately, biological methods have received increasing interest because of their cost effectiveness, less sludge formation, diverse metabolic pathways, versatility of microorganisms and eco-friendly [5, 6]. Microbial decolorization of azo dyes has been reported by many researchers using different microorganisms including aerobic, anaerobic bacteria, fungi and algal sp. [7, 8, 9, 10]. Amongst them the ubiquitous nature of bacteria makes them invaluable tools in effluent treatment. Different pure cultures including Enterobacter sp. EC-13, Citrobacter sp., Rhodopseudomonas palustris and Aeromonas sp. have been successfully used to decolorize azo dyes [11, 12, 13]. However, as compared to pure bacterial cultures, mixed bacterial cultures have been reported to be more efficient for dye decolorization in terms of broad range of compounds attacked as well as higher rates of decolorization can be achieved [14]. An anaerobic mixed culture was utilized for biodegradation of monoazo dye Reactive orange 96 [15]. Similarly, developed consortium HM-4 based on combinations of four selected isolates, efficiently decolorized AR-88 in 20 h whereas the individual isolate took 60 h to achieve complete decolorization [16]. Thus, utilization of microbial consortium offers a considerable advantages over the use of pure cultures in the degradation of dyes since individual pure cultures lack the ability to degrade the azo dyes completely.

In the present study the acclimatized novel bacterial consortium-PES was utilized to assess its ability to decolorize different azo dyes. The effect of various physico-chemical parameters (Temperature, pH, carbon source, dye concentration, static and shaking conditions) on dye decolorization by the bacterial consortium were investigated. Azo dyes are specifically reduced under anaerobic or microaerophilic conditions but its slow rate of decolorization represents a serious problem, which may be addressed by the use of redox mediators. Therefore, the effect of various redox mediators on dye decolorization was also investigated using Acid Red 2 dye as model compound with absorbance maxima at 437 nm.

MATERIALS AND METHODS

Chemicals and Dyes

All chemicals used were of analytical grade. Dyes were procured from Smart Chemicals, Baroda and CAB Chemicals G.I.D.C, Ankleshwar, Gujarat, India. Different azo dyes used in this study are listed in Table 1 with their respective absorbance maxima.

Redox Mediators: AQS (Anthraquinone 2-

*Corresponding author: shilpa_gupte2007@hotmail.com

Sulphonic acid), AQDS (Anthraquinone 2,6-Disulphonic acid), Lawsone (2-Hydroxy-1,4 naphthoquinone), Ethyl viologen and Catechol, Alizarin were purchased from Hi-Media Labs. Mumbai, India.

Isolation of Dye Decolorizing Bacteria

A mixed bacterial culture capable of decolorizing textile dyes was established by repeated sub culturing on Bushnell and Haas medium(BHM) amended with glucose (0.1%, w/v) and Acid Red 2 (100 mg l-1). The medium inoculated with a piece of decaying bark of Adensonia digitata and incubated on orbital shaker (120 rpm) at 30 ºC for 10 days. Repeated transfers were carried out in fresh dye containing media till stable dye decolorizing cultures were obtained showing consistent growth and decolorization during successive transfers. The cultures were isolated from the aforementioned mixed cultures and used for development of a dye decolorizing bacterial consortium. All the bacterial cultures obtained were maintained on Luria agar slants and stored at 4ºC.

Effect of Different Physico-Chemical Parameters on Acid Red 2 Decolorization

The effect of pH on Acid Red 2 decolorization was investigated by varying the pH (4-10) in 250 ml flasks containing 150 ml of BHM, supplemented with 0.1% (w/v) glucose and Acid Red 2 (100 mg l-1). Similarly, the effect of temperature on consortium-PES mediated dye decolorization was determined by accessing the dye decolorization between 25-50 ºC. In order to study the effect of various co-substrates on Acid Red 2 decolorization, the flasks were supplemented with co-substrates like glucose, starch, lactose, sucrose and sodium acetate at a concentration of 1% (w/v). To select an effective concentration of glucose, varying concentration of glucose (0.0.2-2% (w/v)) was added in the medium and percentage decolorization was determined. The effect of initial dye concentration on dye decolorization by consortium-PES was studied by addition of varying dye concentrations of Acid Red 2 (100-700 mg l-1) in BHM media. At regular time intervals 2 ml sample was removed and centrifuged at 10,000 x g for 10 min. All the assays were performed in triplicate with the uninoculated media as a control.

Analytical Methods

The extent of dye decolorization by the microbial culture in BHM broth was monitored spectrophotometrically by measuring absorbance of the dye in the cell free supernatant at absorbance maxima of respective dye. The bacterial cultures uninoculated medium supplemented with only dye was kept as abiotic control. The aliquots of culture supernatants were scanned through a wavelength range of 200-800 using UV-Visible spectrophotometer (Shimadzu UV1800) to monitor the changes in absorbance spectra during decolorization.

Degradation of dye was also monitored by HPTLC using silica gel 60 F254 plates supplied by Merck, Germany. A 10 µl of the samples were spotted on the TLC plates by the micro syringe (linomat V, Camag, Germany). The solvent system used was n-propanol/ethyl acetate/water (5:1:4 v/v). The dye chromatogram was observed by exposure to ultraviolet light (254 nm). FTIR (Fourier Transform Infra Red) analysis of native dye & decolorized dye samples were carried out using Perkin Elmer Spectrophotometer. The samples were mixed with spectroscopically pure KBr (1:99) and pelleted by pressed pellet technique.

Effect of Redox Mediators on Dye Decolorization

Bacterial decolorization profile was studied in the presence of various quinone based compounds like AQS (Anthraquinone 2-Sulphonic acid), AQDS (Anthraquinone 2,6-Disulphonic acid), Lawsone (2-Hydroxy-1, 4 naphthoquinone), Ethyl viologen, Alizarin and Catechol were used. To investigate an effective concentration of AQS, varying concentration of AQS (0.1-1.5 mM) was used and percent decolorization was determined at regular time interval. The effect of AQS was also studied on initial concentrations of Acid Red 2 dye (100-700 mg l-1) at 37ºC under static conditions. Repeated batch decolorization study was carried out to understand the stability and performance of AQS mediated decolorization process.

RESULTS AND DISCUSSION

Isolation, Identification and Development of Bacterial Consortium-PES

Potential dye decolorizing bacteria were isolated from decaying wood material of Adansonia digitata tree. The isolated pure cultures were gram negative, motile rods. The cultures were identified by Bio Log 1420 system as well as 16S rRNA sequence analysis and they were identified as: 1. Escherichia hermannii (Gene Bank Accession No GQ884173), 2. Stenotrophomonas maltophilia (Gene Bank Accession No GQ884174) and 3. Pseudomonas aeruginosa (Gene Bank Accession NoGQ884172) (Figure-1a,b,c).

The consortium constituted with equal proportion of 0.5 O.D of all the three isolates exhibited 96±2 % decolorization of Acid Red 2, while individually each of the isolates in pure culture showed less than 68 % Acid Red 2 dye decolorization. The consortia consisting of two of three isolates in different combinations decolorized 75 to 83 % of Acid Red 2 (100 mg l-1) maximally. Thus, these three isolates exhibited synergism in decolorization of Acid Red 2 dye (Table 2). The advantages of mixed cultures are apparent as some strains can collectively carry out biodegradation of complex organic compounds including dyes that no individual strain can do independently [17, 18]. The consortium consisting of all the three cultures was designated as PES representing 1st letter of individual isolates genus name.

Decolorization of Different Dyes by Bacterial Consortium-PES

The consortium-PES was found to decolorize 11 out of 15 dyes tested with more than 75 % decolorization efficiency, suggesting its suitability as dye remediation strains. Maximum decolorization extent of 98% was recorded for Acid Red 2 dye and for other dyes it varied from 70-91% (Table-1). This variation in the decolorization of different dyes might be due to structural diversity of the dyes [19]. Based on maximum percent decolorization, Acid Red 2 dye was considered as model dye to understand effect of various physico-chemical parameters and nutrients on dye decolorizing ability of bacterial consortium-PES.

Effect of Static and Shaking Conditions on Dye Decolorization

The decolorization efficiency of Acid Red 2 (100 mg l-1) dye was found to be 50% higher in static condition in comparison to shaking conditions. The percent decolorization in static and agitated cultures of PES

consortium was found to be 98% and 50% while growth efficiency was higher under shaking condition than static condition respectively (Figure-2a). These observations suggests that the decolorization performance of consortium-PES was better under oxygen limiting environment and this may be because of reductive cleavage of azo linkage but growth efficiency increased with uniform environment [20, 21]. Dissolved oxygen often inhibited the anaerobic decolorization of azo dyes, because oxygen is a preferable terminal electron receptor over azo groups [22].

Effect of pH on the Dye Decolorization

The bacterial consortium-PES exhibited 85 to 97% decolorization of Acid Red 2 over a wide range of pH from 4 to 10 with maximum decolorization efficiency and growth at pH 7. However, efficiency of decolorization was marginally decreased with further increase in the pH from 7 to 10 (Figure 2b). Similar kind of result was reported for methyl red dye by Klebsiella pneumoniae in the pH range of 6-8 [23]. E.coli and P.luteola, both exhibited maximum decolorization rate over a wide range of pH, 7 to 9.5 [24]. The pH tolerance towards alkaline side is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions [25, 6] and thus the ability of dye bioremediation cultures to decolorize dyes in alkaline pH is significant trait.

Effect of Temperature on Dye Decolorization

The dye decolorization efficiency of bacterial consortium-PES was found to increase with increase in incubation temperature from 25°C to 40°C with maximum decolorization at 37°C (96±2%). Same type of results were also found for growth, consortium exhibited gradual increase in growth from 25°C to 40°C with maximum growth at 37°C. However, percentage decolorization was significantly decreased to 40% at 50°C temperature respectively (Figure-2c). This can be attributed to the detrimental effect of high temperature on cell viability due to denaturation of enzymes involved in decolorization. Such high temperature effect was also reported for decolorization activity of Direct Red 81 by the bacterial consortium NBNJ6 [26] and similar kind of results have also been reported for Pseudomonas sp. which decolorize various dyes; Malachite green, Fast green, Brilliant green, Congo red and Methylene blue at 37 ºC but further increase in temperature declines the percentage of decolorization [27].

Effect of Various Co-Substrates on Decolorization of Acid Red 2 dye

Bacterial consortium exhibited maximum decolorization of Methyl Red dye when glucose was supplemented in the medium. In absence of co-substrate the bacterial culture was unable to decolorize the dye, which indicates the availability of supplementary carbon source seems to be necessary for growth and decolorization of dyes [28]. Hence, various carbon sources like glucose, sucrose, starch, sodium acetate and lactose were supplemented. Glucose and maltose served as efficient co-substrates with the percentage decolorization of 96 and 85% respectively. Lactose, sodium acetate, starch, and sucrose were found to be the poor co-substrate allowing only (35%, 18.2%, 18.5%, and 12%) of decolorization respectively. (Figure 2d). The results confirmed that glucose as co-substrate was found to be the best which is in agreement with earlier studies by other researchers [29, 30]. This finding suggests that consortium-PES had ability to use different carbon sources efficiently but as glucose is an easily metabolizable energy source, creates an environment to enhance decolorization rate of dye. This proves the requirement of co-substrate. Similar results were also reported in case of Bacillus cereus which decolorized Cibacron Red P4B in the presence of sucrose [28, 32, 31].

Significant decolorization (98 %) was observed with 0.1% (w/v) glucose, but further increase in glucose concentration (0.5-2% (w/v)) did not increase decolorization efficiency as well as growth of the bacterial strains (Figure-2e). This may be attributed to metabolism of carbon sources produce organic acids which in turn lowers down the pH of the medium. As each microbial strain and its enzyme are highly specific to pH which decrease the extent of dye decolorization [33, 34].

Effect of Initial Dye Concentration on Dye Decolorization

I Decolorization rate of Acid Red 2 was found to increased proportionally to the concentration up to 700 mg l-1. Further increase in dye concentration did not result in any increased in rate of decolorization (Figure-2(f)) which may be attributed to toxic effect of the dye beyond 700 mg l-1 concentration. Similar observations have been recorded for the decolorization of triphenylmethane dyes at high concentration by Kurthia sp. and for decolorization of reactive textile dye red BLI by Pseudomonas sp. SUK 1 [35, 18]. Kartas et. al reported decrease in decolorization rate with increase in dye concentration with reactive dyes under batch anaerobic condition with mixed microbial culture [36].

Analysis of Decolorization Products

The UV-Visible spectrophotometric analysis of uninoculated medium containing Acid Red 2 showed a peak in a visible region at 437 nm. In the medium inoculated with bacterial consortium-PES, the intensity of absorbance peak for Acid Red 2 dye at 437nm started decreasing after 6 h of incubation, with formation of new peaks at lower wavelength indicating alteration or transformation of the dye structure. This observation suggest further metabolism of dye intermediates and peak representing chromophore group of the dye completely disappears, which corelates well with apparent decolorization of dye. The spectrophotometric observations were further supported by HPTLC yielding four bands with R_f values 0.97, 0.92, 0.81 and 0.74 which fluoresced in U.V. light and differed in migration from Acid Red 2 dye with a Rf value 0.98. No bands were observed corresponding to spots of abiotic control. The FTIR spectra of native dye Acid Red 2 sample displayed the peak at 3056 cm^{-1}, 2808 cm^{-1} and 2855 cm^{-1} indicating the presence of aromatic C-H stretching and aliphatic C-H stretching respectively. The group frequency region shows the peak at 1601 cm^{-1}, 1564 cm^{-1}, 1383 cm^{-1} and 1365 cm^{-1} may indicate the C=C ring vibration and -C-N- stretching respectively. The peak at 1148 cm^{-1} may correspond to $–SO_2$ – group. In contrast, IR of extracted decolorized sample shows removal of different bands and presence of different peaks at 3442 cm^{-1}, 2922 cm^{-1}, 1635 cm^{-1} and 1384 cm^{-1} indicating dye may be converted into aliphatic structure and elimination of SO_2, H_2O and N_2 groups occurred (Fig. 3).

Role of Mediators in Decolorization of Acid Red 2 Dye

The transfer of reducing equivalents from a primary electron donor (co-substrate) to a terminal electron acceptor (azo dye) generally acts as the rate-

limiting step in the anaerobic azo dye reduction process [38]. Redox mediators are flavin based and quinone based organic molecules that can reversibly oxidize and reduce, there by conferring the capacity to serve as electron carrier in multiple redox reactions. The supplementation of redox mediators accelerate the transfer of reducing equivalents to the terminal electron acceptor (azo dye) and also minimize the steric interference of the dye molecule [39]. Bacterial consortium-PES decolorized 98% of Acid Red 2 (100 mg l-1) in presence of AQS as a redox mediator within 12 h, while same extent of decolorization was obtained within 22±2 h in the absence of AQS. The decolorization efficiency was not significantly influenced in presence of other redox mediators, AQDS (90%), ethyl viologen (62%), catechol (60%), lawsone (60%) and alizarin (50%), respectively which determines AQS as an effective electron donor to promote the Acid Red 2 decolorization and the rate of color removal increased by 50% with respect to time (Figure 4a). Similar results of enhancement of decolorization were also obtained with other mediators and the supplementation of mediators accelerates the reaction by lowering the activation energy of the total reaction has been reported by many researchers [40, 41].

Effect of Initial AQS Concentration and Initial Dye Concentration on Acid Red 2 Decolorization

A significant effect on dye reduction was observed with increase in AQS concentration from 0.1 to 1.0 mM. Maximum decolorization (98 %) was obtained with 1mM AQS with in 12 h. Further increase in AQS concentration over 1 mM caused decrease in efficiency of decolorization (Figure 4b), which may be attributed to the toxicity of high concentration of AQS at higher concentration or the limiting availability of electron donors. Such type observation was also reported for AQS mediated decolorization by salt-tolerant *Exiguobacterium* sp. [41].Therefore, 1mM AQS concentration was optimum for achieving maximum efficiency of decolorization. Such a finding is important because redox mediator's addition has a cost and its concentration has been kept to minimum. In the presence of AQS (1 mM), extent of dye decolorization increased as dye concentration increased as compared to original flasks which were not amended with AQS. 89% decolorization of 700 mg l-1 Acid Red 2 was observed after 24 h while only 55% decolorization was obtained after 48 h without AQS (Figure-4c). The obtained results revealed that in the presence of AQS same decolorization efficiency can be achieved in the short time interval.

Repeated Batch Decolorization

In the presence of 1mM AQS concentration, 98% of decolorization was observed within 12 h and then same extent of decolorization could be achieved within 5 h up to 3 cycles of repeated dye spiking. However, the time for decolorization increased gradually to 12 h in last two runs (Figure-4d). The decrease in dye decolorization rate in 4th cycle was found to be due to limiting concentration of glucose or the accumulation of toxic products. Thus, bacterial consortium-PES holds good persistence and stability in repetitive operations. The (0.2 mM) lawsone mediated repeated batch decolorization of Acid Red using E. coli YB strain [42].

CONCLUSION

The study proposes the consortium-PES was more efficient than individual strains for decolorization of azo dyes. The applicability of the consortium-PES was found to decolorize 11 dyes out of 15 dyes tested with more that 75 % efficiency. The HPTLC and FTIR analysis revealed biodegradation of Acid Red 2 dye. The dye decolorizing ability of PES consortium was accelerated with augmentation of dye containing medium with 1mM AQS as redox mediator. Thus, the consortium-PES deserves an attention as a new biomass which can be utilized in the bioremediation of dyes containing wastewater effluents.

Table 1: Maximum Extent of Decolorization of Different Azo Dyes by Bacterial Consortium-PES

Dyes	**λ_{max} (nm)**	**% Decolorization**	**C.I No.**
Methyl Red	437	98	Acid Red 2
Red BS	517	91	Reactive red 111
Reactive Red ME4BL	616	89	Reactive red 195
Reactive Blue ME4BL	601	81	Reactive blue 35
Reactive Black Vs B	639	85	Reactive black 5
Green ME4BL	617	87	Green mix
Acid olive MBGL	567	36	Acid Green 104
R-10	531	85	R-10
Acid Red 131	413	89	Acid red 3BN
Reactive Yellow ME4GL	414	34	Reactive yellow 160
RG Yellow MR	519	28	Reactive yellow 44
Yellow 3RW	521	76	Reactive yellow 49
Reactive Red 12B	534	83	Reactive red 156
Acid Red F2R	510	79	Acid red 144
Reactive Blue RR	549	70	Reactive blue 122

Table 2: Decolorization of Acid Red 2 by Pure Culture of PES Consortium As Well As Combinatorial mixed cultures

Culture Code	% Decolorization
1	50 ± 0.5
2	68 ± 1.5
3	61±0.34
1+2	75 ± 1
2+3	83.5 ±0.51
1+3	78.5 ± 1
1+2+3	96 ± 2

1. Escherichia hermanii 2. Stenotrophomonas maltophilia 3. Pseudomonas aeruginosa

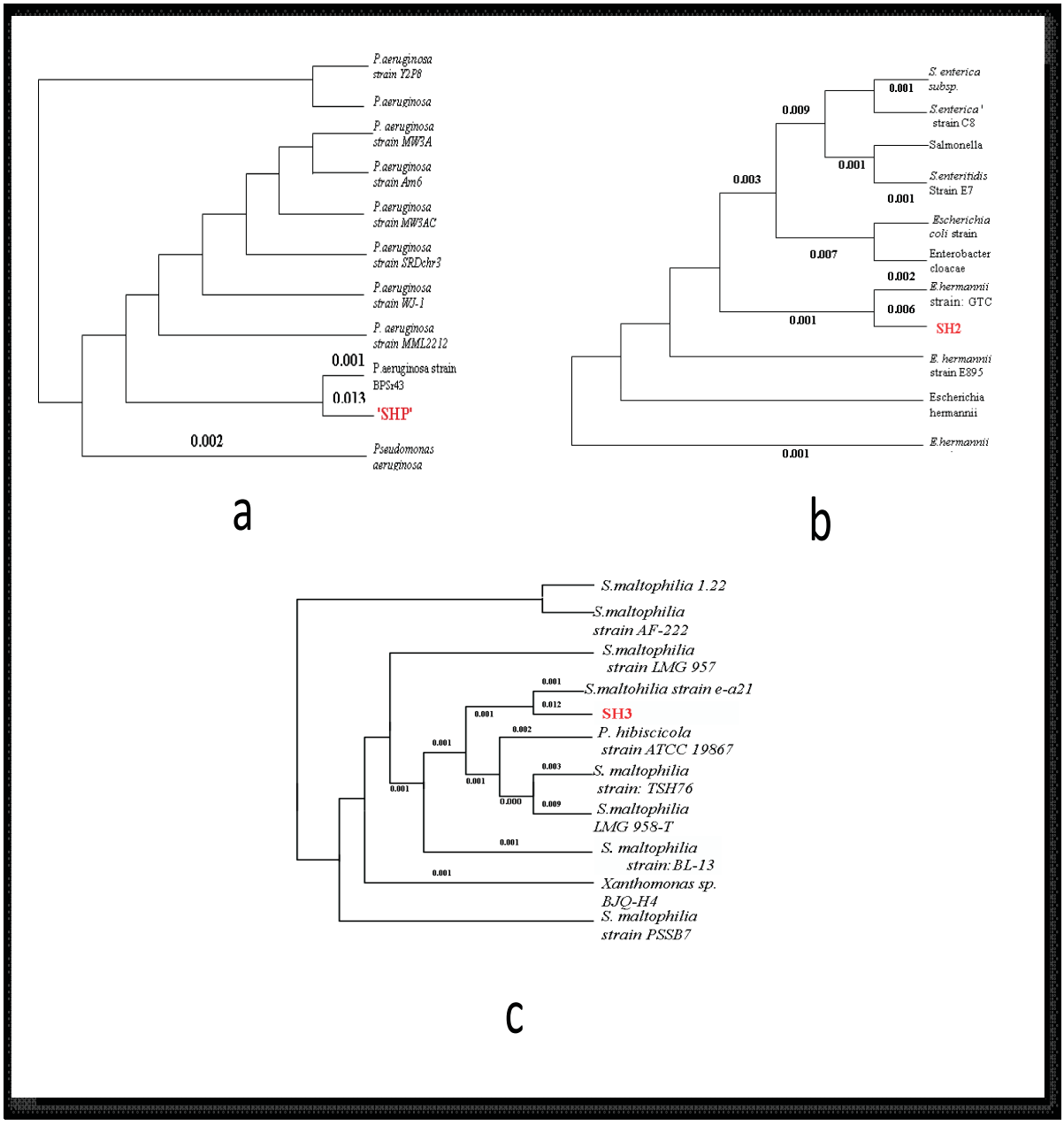

Figure 1 : Phylogenetic Tree made in MEGA 3.1 software using Neighbor Joining Method

(a) P. aeruginosa; **(b)** E. niramanii; **(c)** S. maltophilia

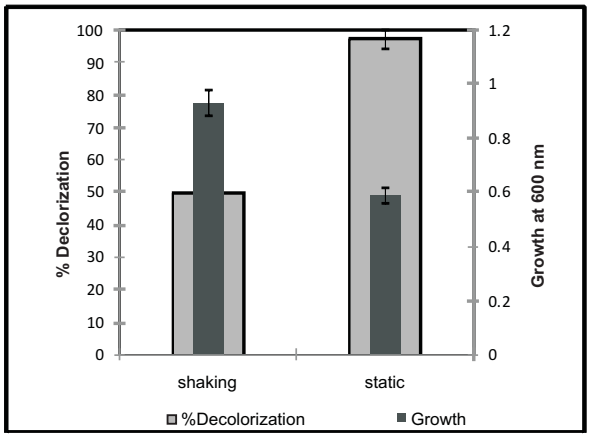

Figure 2(a): Effect of static & shaking condition on Acid Red 2 decolorization

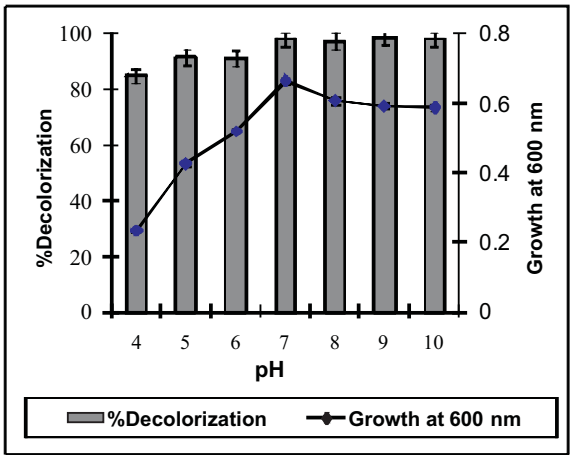

Figure 2(b): Effect of pH on dye decolorization of Acid Red 2 dye

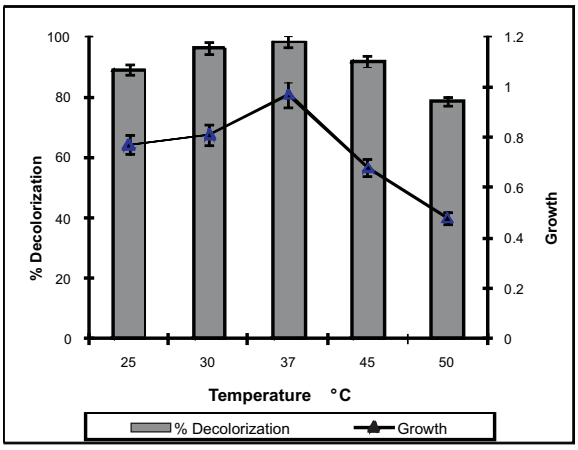

Figure 2(c): Effect of Temperature decolorization of Acid Red 2

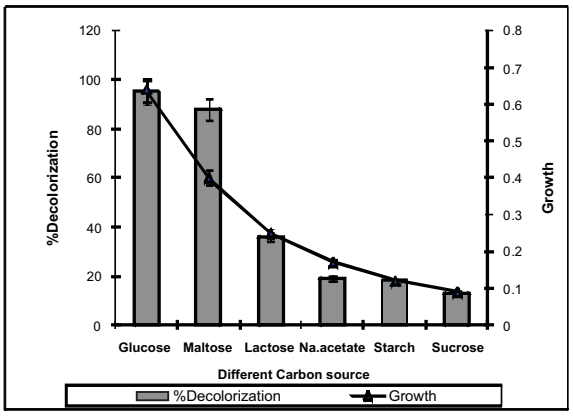

Figure 2(d): Effect of different co-substrates on Acid Red 2 decolorization

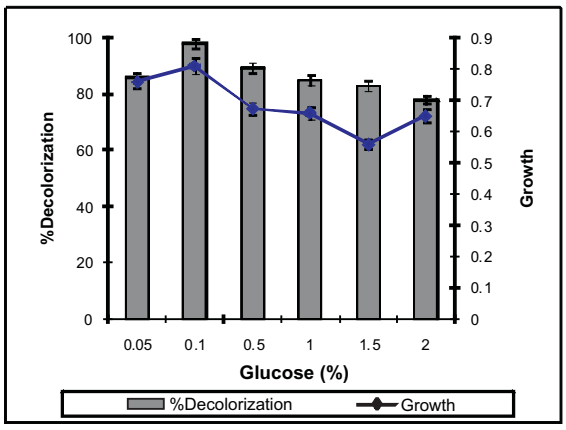

Figure 2(e): Effect of initial glucose concentration on Acid Red 2 decolorization

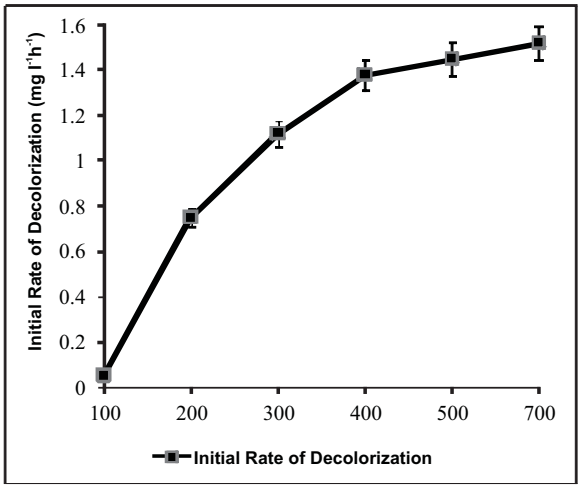

Figure 2(f): Effect of initial dye concentration on dye decolorization of Acid Red 2

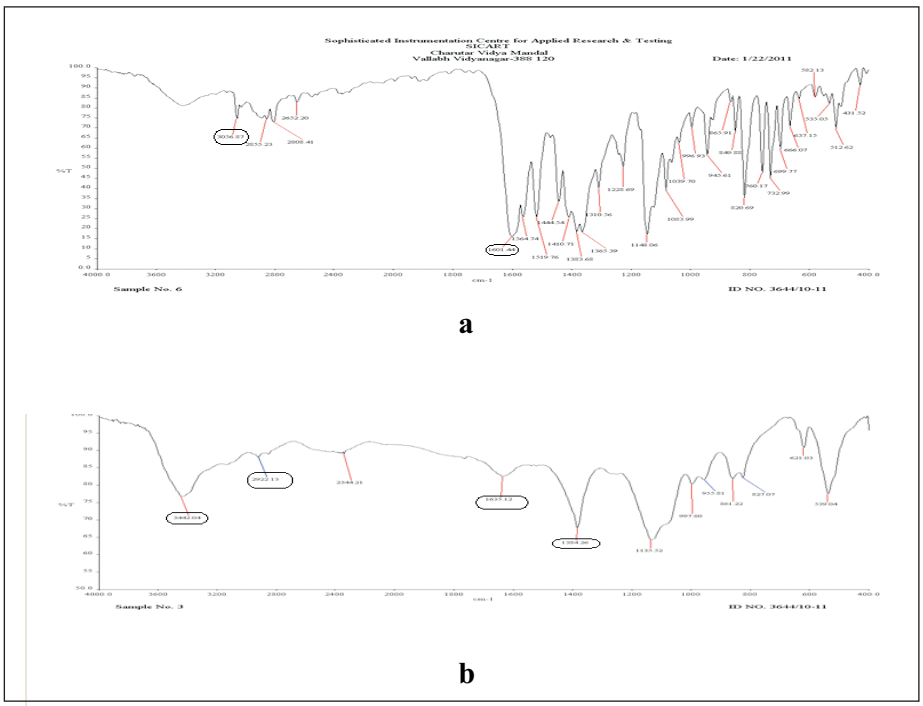

Figure 3 : (a) FTIR spectrum of the Acid Red 2 dye **(b)** FTIR spectrum of the decolorized Acid Red 2 dye

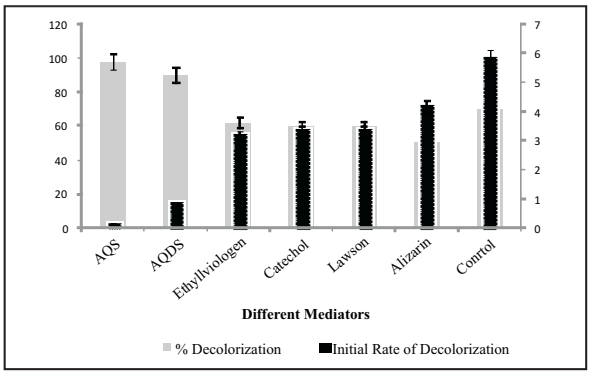

Figure 4(a): Effect of different redox mediators on Acid Red 2 decolorization

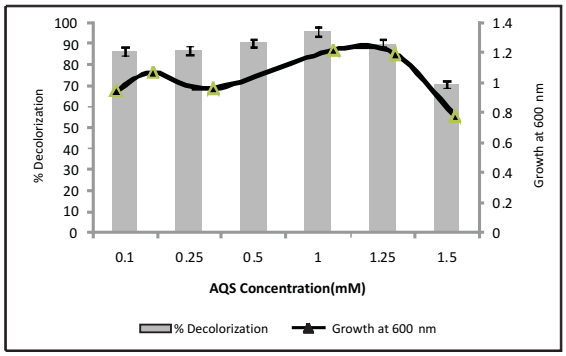

Figure 4(b): Effect of different AQS concentration decolorization

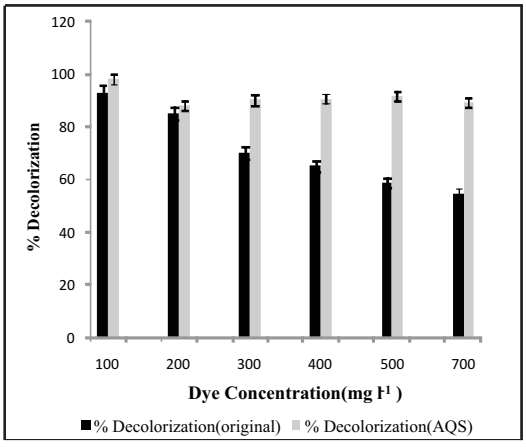

Figure 4(c): Effect of AQS on initial dye concentration of Acid Red 2

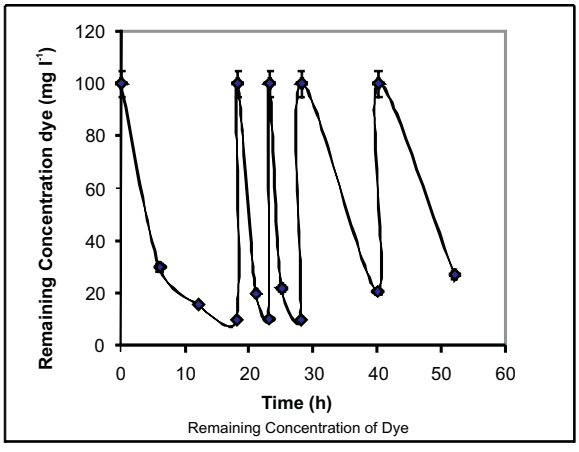

Figure 4(d): Repeated AQS mediated batch decolorization of dye

REFERENCES

[1] Hao, O.J., Kim, H., Chiang, P.C. (2000). Decolorization of wastewater. *Critical Reviews in Environmental Science and Technology*. 30, 449–505.

[2] Sandhya, S. and Vijaya, P.P. (2003). Decolorization and complete Degradation of Methyl Red by a Mixed Culture. *The Enviornmentalist*, 23, 145-149.

[3] Mohanty, S., Dafale, N. and Rao, N.N. (2006). Microbial Decolorization of reactive Black-5 in a two stage anaerobic-aerobic reactor using acclimatized activated textile sludge. *Biodegradation*. 17, 403-413.

[4] Sarioglu, M., Bali, U., Bisgin, T. (2007). The removal of C.I. Basic Red 46 in a mixed methanogenic anaerobic culture. *Dyes and Pigments* 74, 223–229.

[5] Sivaraj, R., Dorthy, A.M. and Venckatesh. R. (2011). Isolation, Characterization and growth kinetics of bacteria metabolizing textile effluent. *Journal of Bioscience and Technology*. 2(4), 324-330.

[6] Fang H., Hu Wenrong, Li Yuezhong, (2004). Investigation of isolation and immobilization of microbial consortium for decolorization of azo dye 4BS. *Water Research*. 38, 3596-3604.

[7] Daeshwar, N., Ayazloo, Khataee and Pourhassan, M. (2007) Biological decolorization of dye solution containing Malachite Green by Microalgae *Cosmarium* sp. *Bioresource Technology*. 98, 1176-1182.

[8] Jadhav, J.P., Parshetti, G.K., Kalme, S.D., Govindwar, S.P. (2007). Decolourization of azo dye methyl MTCC red by Saccharomyces cerevisiae MTCC-463. *Chemosphere* 68,394–400.

[9] Asgher, M., Kausar, S., Bhatti, H.N., Shah, S.A.H., Ali, M. (2008). Optimization of medium for decolorization of Solar golden yellow R direct textile dye by *Schizophyllum commune* IBL- 06. Int. *Biodeterioration. & Biodegradation*. 61, 189–193.

[10] Chang, J.S., Lin, Y.C., (2001). Decolorization kinetics of recombinant E.coli strain harboring azo dye decolorization determinants for Rhodococcus sp. *Biotechnology Letters*. 23, 631-636.

[11] Lui, G.F., J.T. Zhou, J. Wang, Song, Z.Y. and Qv., Y.Y. (2006). Bacterial decolorization of azo dyes by Rhodopsuedomonas palustris. *World Journal of Microbiology and Biotechnology*. 22, 1069-1074.

[12] Wang, H. Su, J.Q., Zheng, X.W., Tian, Y., Xiong, J. and Zheng, T.L., 2009. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by Citrobacter sp. CK3. *Int. Biodeterioration and Biodegradation*. 63, 395-399.

[13] Yoo, E.S., Libra, J. and Adrian, L. (2001) Mechanism of decolorization of azo dyes in anaerobic mixed culture. *Journal of Enviornmental Engineering*. 127, (9) 844-849.

[14] Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S. and Chimni, S.S. (2005). Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize Azo dyes. *Water Research*. 39, 5135-5141.

[15] Banat, I.M., Nigam, P., McMullan, G., Merchant, R. (1997). The isolation of thermophilic bacterial cultures capable of textile dyes decolorization. *Environmental Int*. 23, 547-551.

[16] Fang H., Hu Wenrong, Li Yuezhong (2004). Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. *Chemosphere*. 57, 293-301.

[17] Kalyani, D.C., Patil, P.S., Jadhav, J.P., Govindwar, S.P. (2008). Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1. *Bioresource Technology*. 99, 4635-4641.

[18] Moutaouakkil, A., Zeroual, Y., Dzayri. F.Z., Talbi, M., Lee, K., Blaghen, M. (2003). Bacterial decolorization of the azo dye methyl red by Enterobactero agglomerans *Annals of Microbiology*., 53, 161-169.

[19] Kalme, S.D., Parshetti, G.K., Jadhav, S.U., Govindwar, S.P. (2006). Biodegradation of benzidine based dye Direct Blue-6 by Pseudomonas desmolyticum NCIM 2112. *Bioresource Technology*. 98, 1405-1410.

[20] Pearce, C.I., Lloyd, J.R., Guthrie, J.T. (2003). The removal of colour from textile wastewater using whole bacterial cells: a review. *Dyes and Pigments*, 58, 179–196.

[21] Wong, P.K., Yuen, P.Y. (1998). Decolorization and biodegradation of N, N'-dimethyl p-Phenylenediamine by Klebsiella pneumoniae RS–13 and Acetobacter liquefaciences S-1. *Journal of Applied Microbiology*. 85, 79-87.

[22] Chang, J., and Lin, Y.C. (2001). Decolorization kinetics of recombinant E.coli strain harboring azo dye decolorization determinants for Rhodococcus sp. *Biotechnology Letters*. 23, 631-636.

[23] Aksu, Z. (2003). Reactive dye bioaccumulation by Saccharomyces cerevisiae. Process *Biochemistry* 38, 1437–1444.

[24] Junnarkar, N., Murthy, D.S., Bhatt, N. and Madamwar, D. (2006). Decolorization of diazo dye Direct Red 81 by a novel bacterial consortium. *World Journal of Microbiology and Biotechnology* 22, 163-168.

[25] Mali, P.L., Mahajan, M.M., Patil, D.P., Kulkarni, M.V. (2000). Biodecolorization of members of triphenylmethanes and azo groups of dyes. *Journal of Scientific and Industrial Research* 59, 221- 224.

[26] Ola, I.O., Akintokun, A.K., Akpan, I., Omomowo, I.O. and Areo, V.O. (2010). Aerobic decolorization of two reactive azo dyes under varying carbon and nitrogen source by Bacillus cereus. *African Journal of Biotechnology*. 9(5), 672-677.

[27] Verma, P. and Madamwar, D. (2003). Decolorization of synthetic dyes by a newly isolated

strain of Serratia marsascenes *World Journal of Microbiology and Biotechnology*. 19, 615-618.

[28] Dawkar, V. V., Jadhav, U.U., Jadhav, S.U. and Govindwar, S.P. (2008). Biodegradation of disperse textile dye Brown 3REL by newly isolated Bacillus sp. VUS. *Journal of Applied Microbiology*, 105, 14-24.

[29] Pandey, A., Singh, P., Iyengar, L. (2007). Bacterial decolorization and degradation of azo dyes. *International Journal of Biodeterioration and Biodegradation*. 59, 73-84.

[30] Padmavathy, S., Sandhya S., Swaminathan, K., Subrahmanyam, Chakrabarti, T., Kaul, S.N. (2003). Aerobic Decolorization of Reactive Azo Dyes in presence of Various Co substrates. *Biochemical Engineering*. Q.17 (2), 147-151.

[31] Chen, K.C., Wu, J.Y., Liou, D.J. and Hwang, S.C.J. (2003). Decolorization of the textile dyes by newly isolated bacterial strains. *Journal of Biotechnology*. 101, 57-68.

[32] Nosheen, S., Nawaz, R. Arshad M., and Jamil, A. (2010). Accelerated biodecolorization of reactive dyes with added nitrogen and carbon sources. *Int. J. Agric. Biol.*, 12, 426-430.

[33] Sani, R., Banerjee, U. (1999). Decolorization of Triphenylmethane dyes and textile and dye stuff effluent by *Kurthia* sp. *Enzymology, Microbiology and Technology*. 24, 433-437.

[34] Bras, R., Isabel, M., Ferra, A., Pinherio, H.M., Goncalves, I.C. (2001). Batch tests for assessing decolorization of azo dyes by methanogenic and mixed cultures. *Journal of Biotechnology*. 89, 155-162.

[35] Keharia, H., Patel, H. and Madamwar, D. (2004). Decolorization screening of synthetic dyes by anaerobic methanogenic sludge using a batch decolorization assay. *World Journal of Microbiology and Biotechnology*, 20, 365-370.

[36] Karatas, M., Dursun, S. and Argun, M.E. (2009). Decolorization of reactive dyes under batch anaerobic condition by mixed microbial culture. *African Journal of Biotechnology*. Vol. (8), 6856-6862.

[37] Van der Zee FP, Lettinga. G, Field JA. (2000). The role of (auto) catalysis in the mechanism of anaerobic azo reduction. *Water Science and Technology*. 42:301-308.

[38] Govindwar, S.P. Saratale, R.G., Saratale, G.D. and Chang, J.S. (2011). Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers*. 42, 138-157.

[39] Stolz, A. (2001). Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbilogy and Biotechnology*, 56:69-80.

[40] Cenvantes, F.J. and Van der Zee FP. (2009). Impact and application of electron shuttles on the redox (bio)transformation of contaminants: A review. *Biotechnology Advances*, 27, 256-277.

[41] Qu, Y.Y., Tan, L., Zhou, J.t., Li, A., Gou, M. (2009) Identification and characteristics of a Novel salt-tolerant Exiguobacterium sp. for Azo dyes Decolorization. *Applied Biochemistry and Biotechnology*, 159:728-738.

[42] Liu, G., Zhou, J., Wang, J., Zhou, M., Lu, H., Jin, R.(2009) Acceleration of azo dye decolorization by using quinone reductase activity of azoreductase and quinone redox mediator. *Bioreseource Technology*, 100: 2791-2795.