INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-1, Jan-Mar-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490
Received: 5th Jan-2016 *Revised: 17th Jan 2016* *Accepted: 20th Jan 2016*
Research article

DECOLORIZATION OF ALIZARIN RED S DYE BY BACTERIAL STRAINS ISOLATED FROM INDUSTRIAL EFFLUENTS

Illakkiam D[a], Subha D[b] Ahila V[b], and Geetha N[c]*

Assistant Professor[a], Research Scholars[b], Professor and Head[c]
Department of Biotechnology, Mother Teresa Women's University, Kodaikanal 624 101 TN

ABSTRACT: Bioremediation of textile dyes has been of considerable interest because it is inexpensive, eco-friendly and produces a less amount of sludge. An attempt was made to evaluate the potential of two bacterial strains for decolorization of Alizarin red S were isolated from textile dye effluents. The bacterial strains *Pseudomonas sp*. and *Escherichia coli* were identified by morphologically and biochemically. Physico-chemical parameters like pH, temperature, dye concentration, carbon source, nitrogen source, combination of carbon source and nitrogen source and the effect of immobilized bacterial cells were optimized. The optimal condition for decolorization of Alizarin red S for both *Pseudomonas sp.* and *Escherichia coli* was found to be 1% glucose, 1% peptone, pH 7.0, 37°C, 500 mg/L dye concentration, combination of 1% glucose and 1% peptone and 50 immobilized bacterial cells per 100ml of Mineral Salt Medium. The highest decolorization rate was found to be 69.17% and 78.04 % for *Escherichia coli* and *Pseudomonas sp.* respectively. The results revealed that *Pseudomonas sp.* was found to be more efficient in dye decolorization than *Escherichia coli.*. The FTIR spectrum of control Alizarin red S displayed a peak at 3409.86 cm-1 indicates an OH stretching of phenols. Peaks at 2926.07, 1648.24, 1383.56 and 1066.10 showed CH stretching of alkanes, CC stretching of alkenes, nitro compounds and CO stretching of alcohols, ethers, carboxylic acids and esters respectively. The FTIR spectrum of the products formed after decolorization displayed a peak at 3448.24 showed OH stretching of phenols. Peaks at 1641.04 and 1080.47 indicate a CC stretching of alkenes.
Key words: Textile effluents, Alizarin Red S dye, Decolorization, Dye Degrading Bacteria, *Pseudomonas sp., Escherichia coli*

*Corresponding author: Geetha N, Department of Biotechnology, Mother Teresa Women's University, Kodaikanal 624 101Tamil Nadu, India, E-mail: geethadrbio@gmail.com

INTRODUCTION

Rapid development of industrialization and urbanization leads to several environmental problems such as water scarcity, pollution and population explosion. Various industries such as textile, rubber, paper, leather, plastics, food, pharmaceutical and cosmetics consume wide range of dyes for coloring the products [1]. The effluent from textile industries carries a large number of dyes and other additives which are added during the coloring process [2]. Dyes are aromatic organic compounds with aryl ring structure which makes them more stable, it brings some difficulties to remove in conventional water treatment procedures of these pollutants [3]. Discharge of effluents from various dyeing industry to the water bodies is a major human concern. Among them, Alizarin (1, 2-dihydroxy anthraquinone) a prominent red dye principally used for dyeing textile fabrics and are known to undergo biological or photochemical degradation once they reach the aquatic environment. The parent compounds are less harmful whereas the degradation products may be more harmful and persistent [4]. It is important to safeguard the environment from such contaminants which are a potential hazard to living organisms.

Traditionally broad ranges of methods were applied for the treatment of waste effluents generated from dyeing industries involves light, biological, physical, chemical and activated sludge techniques are very expensive [5,6]. Bioremediation of textile dyes has been of considerable interest because it is inexpensive, eco-friendly and produces a less amount of sludge. Biotechnological tools also have been applied for the degradation of various textile dyes and it was found that up to 70% color removal was noticed with different micro flora [7]. Nowadays many researchers have demonstrated partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, fungi and algae. Preliminary characterization of the bacterial isolate regarding its morphological, biochemical characters and decolorization activity as well as the molecular identity gives useful information with regard to the further application of strain for various purposes [8]. In the present study, an attempt was made to evaluate the potential of bacterial strains for decolorization effluent containing a dye, Alizarin red S with respect to various pH, temperature, carbon (glucose) source, nitrogen (peptone) source and different dye concentration were optimized.

MATERIALS AND METHODS

Chemicals and media

Textile dye, Alizarin Red S and dye effluents were collected from a dying industry located at Erode, Tamil Nadu. All microbiological media and medium ingredients were procured from HiMedia Laboratories, Mumbai (India).

Isolation, screening and identification of dye degrading bacteria

Microbial isolations were carried out by serially diluting textile effluent in sterile distilled water subsequently plated onto nutrient agar medium added with 100mg/l Alizarin red S and incubated at 37° C for 12 hrs. After incubation the presence of dye degrading bacterial colonies were checked by observing the zone of clearance/decolorization on the respective plates [9]. Those colonies that exhibited dye degradation were isolated and maintained in nutrient broth medium. Further the identification of dye degrading bacterial strains were carried out on the basis of morphological and biochemical analysis.

Decolorization Assay

Decolorization activity was expressed in terms of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima (λmax) of respective dyes (i.e. 423nm for Alizarin red S). The un-inoculated nutrient broth supplemented with respective dye was used as reference. The culture suspension was centrifuged at 8,000 rpm for 10 min for removal of the biomass. The degree of decolorization of the tested dye was measured at its respective maximum absorbance wavelength using supernatant by UV-visible spectrophotometer. Based on decolorization potential, strain was selected for further studies. The decolorization assay was calculated according to the following formula [10, 11].

Percentage of Decolorization = [A-B /A] × 100

Where A= Initial absorbance

B= Observed absorbance

Effect of pH and temperature on dye decolorizing activity

The effect of pH on the dye decolorization was determined by measuring the relative activity using different buffers at 37°C. Sodium acetate (pH 4.0 to 5.5), Tris (pH 6.0 to 8.5) sodium carbonate (pH 9.0) buffers were used for the estimation of relative activity at different pH conditions. The maximum activity was considered as 100%, and used as reference in determining relative activities at different pH values. The effect of temperature on the reaction rate was determined by performing the standard reaction at different temperatures in the range of 20-100°C. The relative activity was expressed considering maximum activity as 100%.

Effect of different concentration of dye on dye decolorizing activity

The effect of different concentration of dye was determined by measuring the relative activity using different concentration (100 mg/l, 500 mg/l and 1000 mg/l) at 37° C.

Effect of different concentration of carbon and nitrogen source on dye decolorizing activity

The effect of different concentration of carbon (glucose-0.25g, 0.5g, 0.75g and 1.0g) and nitrogen source (peptone-0.25g, 0.5g, 0.75g and 1.0g) individually and in combination of both (glucose 1.0g and peptone 1.0g) on dye decolorizing activity were monitored at specific wavelength at 37°C.

Fourier Transform Infrared Spectroscopy

The biodegraded dye samples were characterized by FTIR spectroscopy. Infrared spectroscopy experiments were performed using GX-FTIR model, Perkin Elmer, USA spectrophotometer. The samples were mixed with spectroscopically pure KBr (1:99) and pelleted by pressed pellet technique. The changes in % transmission at different wavelength were observed. The data were recorded within a scanning range of 4000-400 cm-1.

RESULTS AND DISCUSSION

Isolation, screening and identification of dye degrading bacteria

The textile dye effluent sample was collected from textile industries located at Erode, Tamil Nadu. Textile industries play a positive role in the Indian economical reformation [12]. The industrial units are now looking forward to cost effective solutions for reduction of pollution. Normally bacterial decolorization is faster and nonspecific. Previously reported an array of bacteria as degraders of dyes such as *Bacillus subtilis, Escherichia coli, Enterococcus* sp., *Pseudomonas* sp., *Rhabdobacter* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Clostridium* sp., *Micrococcus dermacoccus, Acinetobacter* sp., *Geobacillus, Rhizobium, Proteus* sp., *Morganella* sp., *Aeromonas* sp., *Alcaligenes* sp. and *Klebsiellla* [13]. In the present study, the isolated morphologically distinct colonies showed decolorization zone on nutrient agar plate containing Alizarin red S dye. By microscopic and biochemical characters, the bacterial cultures were identified as *Pseudomonas* sp. and *Escherichia coli*.

Effect of pH and temperature on dye decolorization

The effect of pH for both *Escherichia coli* and *Pseudomonas species* on dye decolorization activity was determined in different buffers (pH 3.0 to 9.0). The decolorization rate was increased when pH increased from pH 3.0 (45%) to pH 7.0 (57%) for *Escherichia coli* and from pH 3.0 (47%) to pH 7.0 (61%) for *Pseudomonas species*. Maximal activity of decolorization rate was observed at pH 7.0 i.e 57% and 61% activity were observed for *Escherichia coli* and *Pseudomonas species,* respectively **(Fig.1 a&b)**.

The effect of temperature for both *Escherichia coli* and *Pseudomonas species* on dye decolorization activity was determined in various temperatures (28°C, 37°C and 42°C). Maximal activity of decolorization rate was observed at temperature 37°C. In this temperature *Escherichia coli* and *Pseudomonas species* showed 55% and 64% activity, respectively (**Fig.2 a&b)**. Increase in temperature resulted in marginal reduction in decolorization activity of the bacterial culture. Decline in decolorization activity at higher temperature can be attributed to the denaturation of the azo-reductase enzyme [14]. The decolorization of the dye Acid Orange 10 by *Pseudomonas putida* MTCC 102 showed optimum pH and temperature of 7.0 and 37 °C, respectively [15].

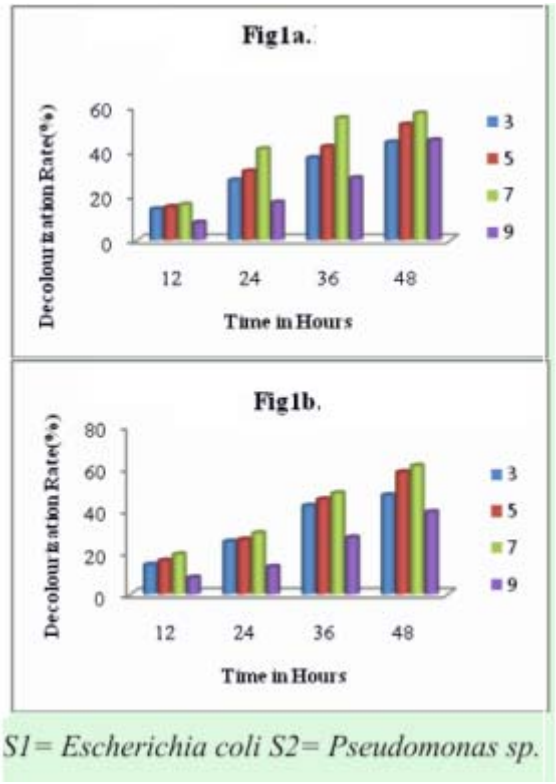

Fig. 1 Effect of dye decolorization at different pH.

(a) S1- Dye decolorization by *Escherichia coli* at different pH. (b) S2- Dye decolorization by *Pseudomonas sp.*at different pH.

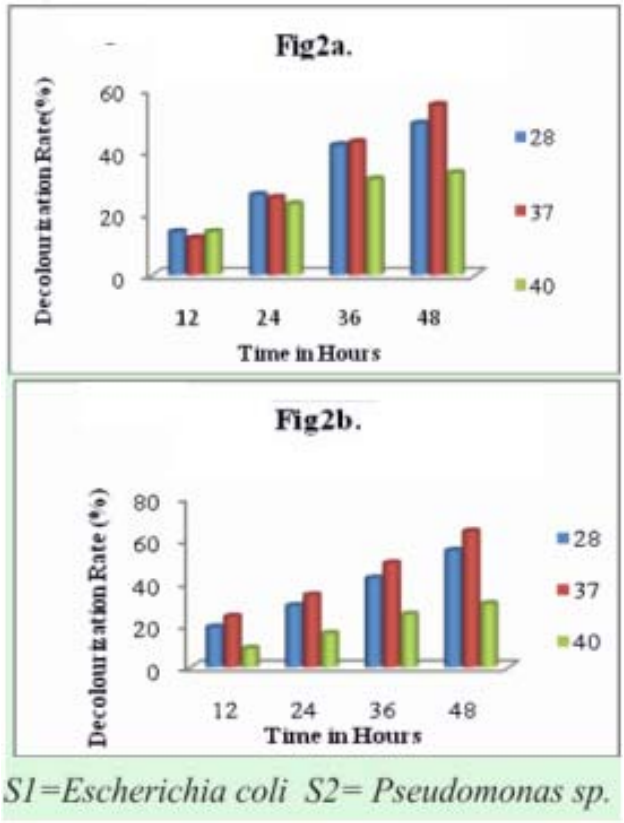

Fig. 2 Effect of dye decolorization at different temperature.

(a) S1- Dye decolorization by *Escherichia coli* at different temperature. (b) S2- Dye decolorization by *Pseudomonas sp*.at different temperature.

Effect of different concentration of dye

Decolorization activity of both *Pseudomonas species* and *Escherichia coli* was studied at different concentrations varying from 100 to 1000 mg/l. The optimum dye concentration for decolorization by *Pseudomonas species* and *Escherichia coli* was found to be 500 mg/l **(Fig.3a&b)**. Further increase in dye concentration resulted in reduction in decolorization rate. Lower decolorization efficiency is due to higher inhibition at high dyestuff concentration [16].

Effect of glucose as carbon source on dye decolorization

Different concentrations of glucose (0.25g, 0.5g, 0.75g and 1.0g) as carbon source were evaluated for dye decolorization. Among various concentrations, the highest concentration i.e 1g/l of glucose showed maximum activity for decolorization of dye for both *Escherichia coli* and *Pseudomonas species* as shown in **Fig.4 a&b**. *Pseudomonas aeruginosa* GSM3 showed complete decolorization in the presence of glucose when compared to lactose and sucrose [17].

Effect of peptone as nitrogen source on dye decolorization

In present study, different concentrations of peptone (0.25g, 0.5g, 0.75g and 1.0g) as nitrogen source were evaluated for dye decolorization. Among them 1g/l of peptone concentration was found to be optimum for both *Escherichia coli* and *Pseudomonas species* to make the dye decolorization process economical **(Fig.5 a&b)**. There is a report that peptone is the ideal nitrogen source for decolorization of Red 3BN dye by *Bacillus sp* [18].

Combined effect of carbon and nitrogen source on dye decolorization

A combination of 1.0g carbon source (glucose) and 1.0g of nitrogen source (peptone) was very effective for dye decolorization of both *Escherichia coli* and *Pseudomonas species* when compared to addition of carbon and nitrogen source separately into the medium **(Fig.6 a&b).**

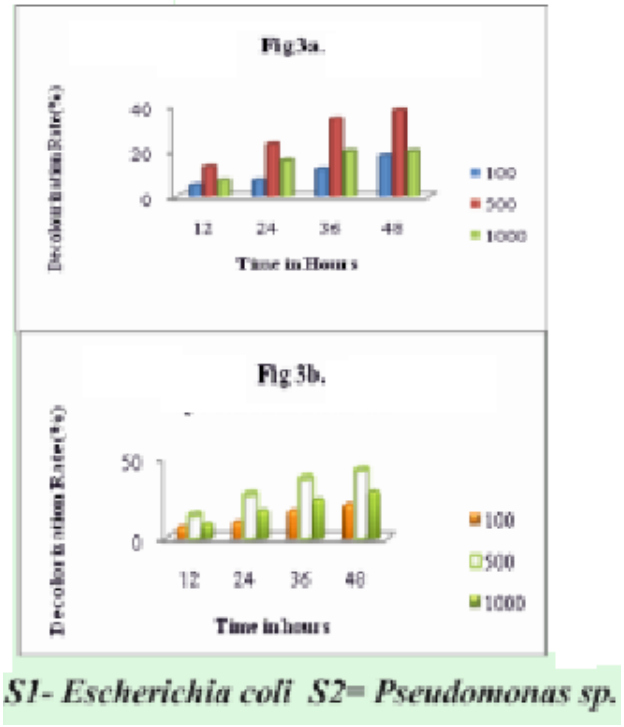

Fig. 3 Effect of different concentration of Dye

(a) S1- Dye decolorization by *Escherichia coli* at different dye concentration. (b) S2- Dye decolorization by *Pseudomonas sp.*at different dye concentration.

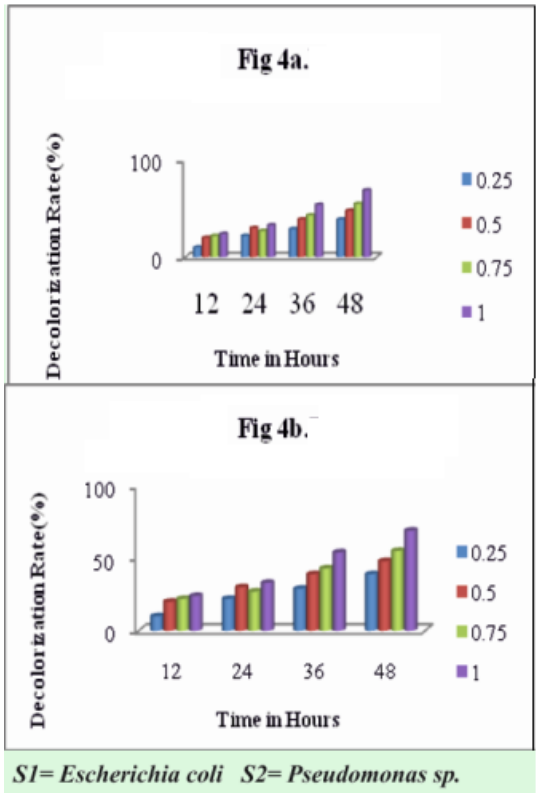

Fig. 4 Effect of glucose as carbon source on dye decolorization

(a) S1- Dye decolorization by *Escherichia coli* at different concentration of glucose as carbon source. (b) S2- Dye decolorization by *Pseudomonas sp.*at different concentration of glucose as carbon source.

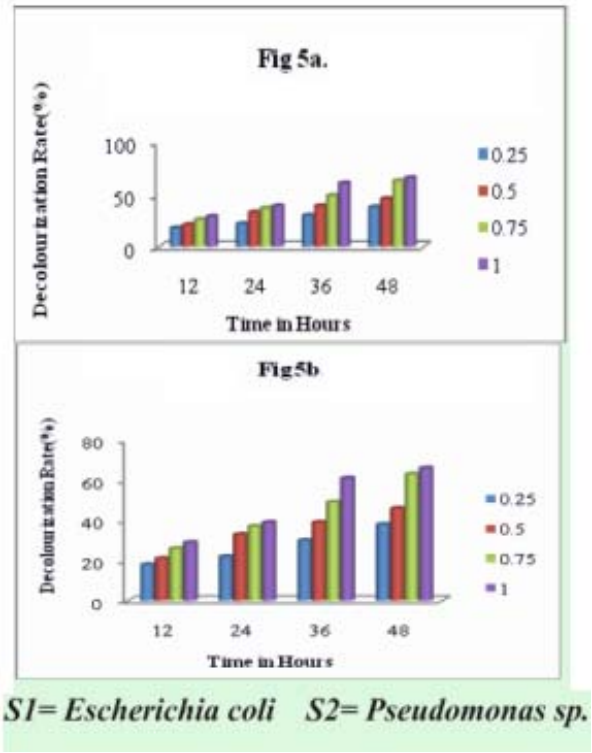

Fig. 5 Effect of peptone as nitrogen source on dye decolorization

(a) S1- Dye decolorization by *Escherichia coli* at different concentration of peptone as nitrogen source. (b) S2- Dye decolorization by *Pseudomonas sp.*at different concentration of peptone as nitrogen source.

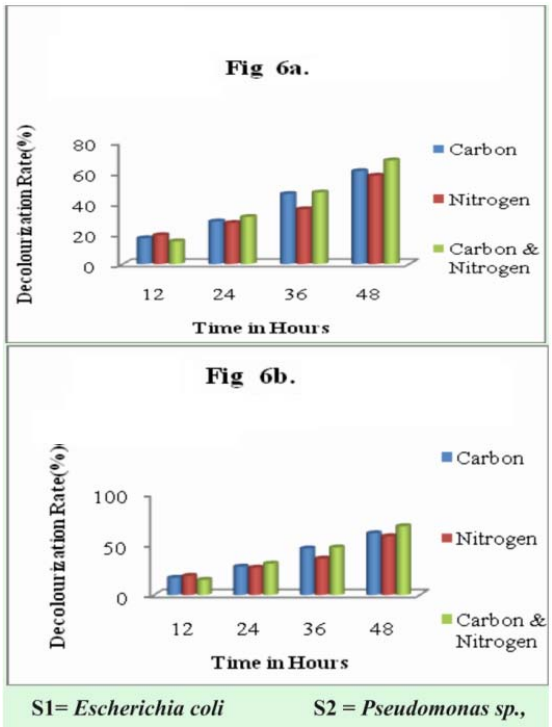

Fig. 6 Combined effect of glucose and peptone as carbon and nitrogen source respectively on dye decolorization

(a) S1- Dye decolorization by *Escherichia coli* at different concentration of peptone as nitrogen source and glucose as carbon source. (b) S2- Dye decolorization by *Pseudomonas sp.* at different concentration of peptone as nitrogen source and glucose as carbon source.

FTIR spectrum of control and sample

The FTIR spectrum of control **(Fig.7a)** displayed a peak at 3409.86 cm-1 indicates an OH stretching of Phenols. Peaks at 2926.07, 1648.24, 1383.56 and 1066.10 showed CH stretching of alkanes, CC stretching of alkenes, Nitro compounds and CO stretching of alcohols, ethers, carboxylic acids and esters respectively. The FTIR spectrum of the products formed after decolorization **(Fig.7b)** displayed a peak at 3448.24 showed OH stretching of phenols. Peaks at 1641.04 and 1080.47 indicate a CC stretching of alkenes. However the FT-IR spectra of degradation product displayed peaks at different positions indicating the complete breakdown of Alizarin Red S dye.

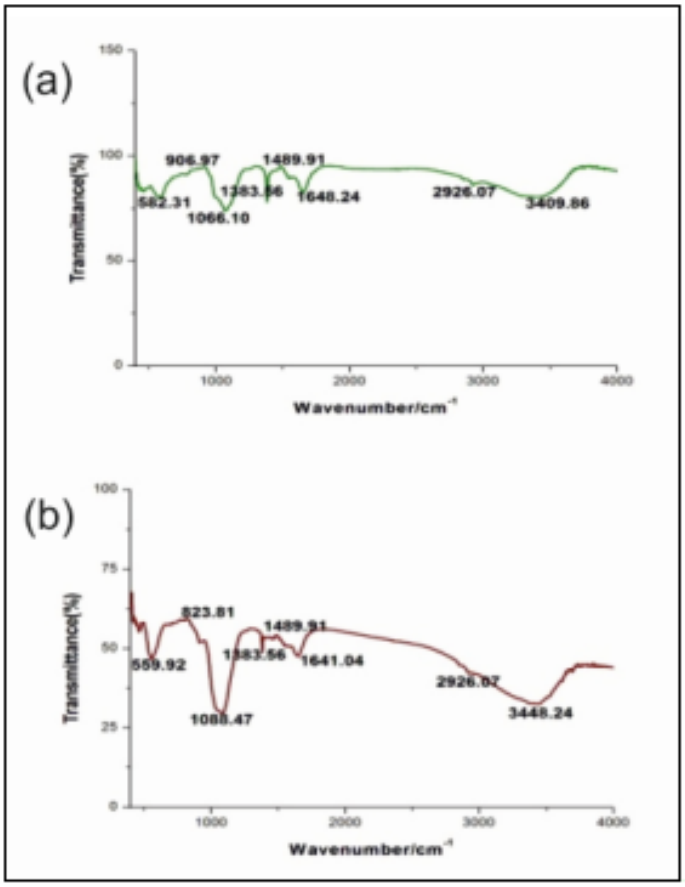

Fig. 7 Fourier Transform Infrared spectroscopy analysis.

(a) FTIR spectrum of control- Alizarin Red S dye

(b) FTIR spectrum of degraded product.

CONCLUSION

The present study confirms the ability of bacteria to degrade the dye, Alizarin red S with degradation efficiency (68%) and (72%) for *Escherichia coli* and *pseudomonas species*, thus suggesting its application for degradation of dye bearing of industrial wastewaters. Presence of a co-substrate (Glucose and peptone), and optimum physical parameters (pH and temperature) are the essential conditions for attaining maximum degradation efficiency. Decolorization of dye was confirmed by UV/VIS spectrophotometer and FTIR. From this study, it has been concluded that *Pseudomonas species* had degraded Alizarin red S effectively, when compared to *Escherichia coli*. Nevertheless, both the species of bacteria can be inferred as good agents for the degradation of Alizarin Red S dye.

ACKNOWLEDGEMENT

Financial assistance received from DST-CURIE, New Delhi is gratefully acknowledged for the purchase of UV-Spectrophotometer and FTIR.

REFERENCES

[1] Ahalya N, Kanamadi R D and Ramachandra T V 2006. Biosorption of Iron (III) from aqueous solutions using the husk of Bengal gram (*Cicer arientinum*). Indian. J. Chem. Technol. 13:122-127.

[2] Wang C, Yediler A, Linert D, Wang Z and Kettrup A. 2002. Toxicity evaluation of reactive dye stuff, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria *vibrio fisheri*. Chemosphere 46:339-344.

[3] Chen K, Wu J, Liou D and Hwang S-C. 2003. Decolorization of the textile dyes by newly isolated bacterial strains. Journal of Biotechnology 101:57–68.

[4] Sanchez-Prado L, Lompart M, Lores M, Garcma-Jares C, Bayona J M and Cela R. 2006. Monitoring the photochemical degradation of triclosan in waste water by UV light and sunlight using solid phase micro-extraction. Chemosphere 65: 1338-1347.

[5] Malik A. 2007. Environmental challenge vis a vis opportunity: the case of water hyacinth. Environ. Int. 33:122–138.

[6] Chung K T and Cerniglia C E. 1992. Mutagenecity of azo dyes: structure activity relationship. Mutation Research 277: 201-220.

[7] Khadijah O, Lee K K, Mohd Faiz F and Abdullah. 2009. Isolation, screening and development of local bacterial consortia with azo dyes decolorizing capability. Malays J Microbiol 5: 25-32.

[8] Grekova-Vasileva M, Popov I, Vassilev D and Topalova Y. 2009. Isolation and characterisation of capable of azo dye decolourisation, Biotechnology and Biotechnol. 23: 318-322.

[9] Khalid A, Arshad M and Crowley D E. 2008. Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. Applied Microbiology and Biotechnology 78: 361-369.

[10] Chen K C, Wu J Y, Liou D J and Hwang S C J. 2003. Decolorization of the Textile Azo Dyes by Newly Isolated Bacterial Strains. Journal of Biotechnology 101: 57-68.

[11] Ali N, Hameed A and Ahmed S. 2009. Physicochemical Characterization and Bioremediation Perspective of Textile Effluent, Dyes and Metals by Indigenous Bacteria. Journal of Hazardous Materials 164: 322-328.

[12] Vasanthy M., Sangeetha M and Geetha A. 2004. Removal of mixed dye (PBBMR and POM2R) using *Bacillus* sp.., and *Alkaligens* sp. Asian Journal of Microbiology, Biotechnology and Environmental Science 6: 579-581.

[13] Sudha M, Saranya A, Selvakumar G and Sivakumar N. 2014. Microbial degradation of Azo Dyes: A review. Int. J. Curr. Microbiol. App. Sci. 2: 670-690.

[14] Pearce C I, Lioyd J R and Guthrie J T. 2003. The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes and pigments. 58: 179-186.

[15] Tripathi A and Srivastava S K. 2011. Eco-friendly treatment of azo dyes: Bio decolorization using bacterial strains. Int J Biosci Biochem Bioinfo. 1: 37-40.

[16] O'Neill C, Hawkes F R, Hawkes D L, Lourenco N D, Pinheiro H M and Delee W. 1999. Colour in textile effluents sources, measurement, discharge consents and simulation. Journal of Chemical and Biotechnology. 74:1009-18.

[17] Mallikarjun C. Bheemaraddi, Channappa T. Shivannavar, Subhashchandra M. Gaddad. 2014. Isolation and characterization of an azo dye reactive red 2 degrading bacteria from dye contaminated soil. Int J Pharm Bio Sci. 4: 711 – 722.

[18] Praveen Kumar G N. and Bhat Sumangala K. 2012. Decolorization of Azo dye Red 3BN by Bacteria. I. Res. J. Biological Sci. 5: 46-52.

INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences