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**RESEARCH ARTICLE**

# COMPARATIVE STUDY ON BIODEGRDATION OF AZO DYES BY *KLEBSIELLA PNEUMONIAE* ISOLATED FROM ENVIRONMENTAL AND CLINICAL SAMPLES

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**ABSTRACT**

The present study investigates comparative study on biodegrdation of azo dyes by *Klebsiella pneumoniae* isolated from environmental and clinical samples. The samples were collected from environmental such as water contaminated with dyes and clinical samples such as throat, pus and urine samples *Klebsiella pneumoniae.* The azo dye used in this study namely Congo red, Methyl red, Methyl orange, Eriochrome black T. *K. pneumoniae* isolated from clinical samples degrades higher percentage of Methyl red (88.76%) and Congo red (66.45%) and least percentage of Methyl orange (7.00%). Whereas the degradation of azo dye by *K. pneumoniae* isolated from water of textile dye effluent, higher percentage of degradation was found against Methyl orange (95.45%) Congo red (76.38%) Eriochrome Black T (73.18%) and least degradation against Methyl red (8.86%). By comparing both samples for the degradation, environmental sample like water was best decolorizer of azo dye compared to clinical samples.



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## INTRODUCTION

Dyes may be defined as substances which, when applied to a substrate, impart colour to the substrate. They adhere on compatible surfaces by physical adsorption, mechanical retention, formation of covalent bond or of complexes with salts or metals, or by solution (Kirk-Othmer 2004). Dyes are substances capable of colouring fabrics which cannot be removed by rubbing or washing (Sharma 2011). The colour of dyes depends on their ability to absorb light in the visible range of electromagnetic radiation (400–700 nm). According to Witt theory, a coloured dye must have a chromophore group and an auxochrome group. Chromophores impart colour to the dye because they are capable of absorbing light in the visible region (e.g., nitro, azo, quinoid groups), while auxochromes deepen the colour when introduced into a coloured molecule. Organic synthetic dyes have been widely used as colorants in different industries such as textile, paper, colour photography, pharmaceutical, food, cosmetics, and photoelectrochemical cell (Forgacs *et al.,* 2004). Based on chemical structure of chromophore there are 20-30 different group of dyes.

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The three most common groups of dyes are azo, anthraquinone and phthalocyanine (Axelsson *et al.,* 2006). The auxochromes can belong to the classes of reactive, acid, direct, basic, mordant, disperse, sulphur, and vat dyes based on method of application (Ibrahim M 1996; Hunger 2009). Dyes contain at least one nitrogen-nitrogen (N=N) double bond, however many different structure exist. For example, in azo dyes, monoazo dyes have only one N=N double bond, while diazo and triazo dyes contains two and three N=N double bonds respectively. Azo dyes are largest group of dyes. They are the largest and most versatile class of dye. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Maximo *et al.,* 2003). Azo dyes are characterized by the presence of one or more azo groups – N = N (Adedayo *et al* .,2004). These are prepared by azo coupling between a diazonium compound and an aniline, phenol or other aromatic compound (Brás R *et al.,* 2005). These dyes constituting 60–70 % of all dyestuff produced (Ali 2010). Azo dyes in purified form are mutagenic or carcinogenic, except for some azo dyes, leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens to human beings (Praveen Sharma *et al* ., 2009). Most of the Methyl red degradation studies revealed in the formation of DMPD (N-N0-dimethyl-p-phenylenediamine) which remains undegraded in the culture and mutagenic in nature (So *et al.,* 1990; Wong and Yuen,

1998). The untreated dyeing effluents that are straightly used in agriculture have a serious impact on environment and human health (Pourbabaee *et al.,* 2006). Generally, azo dyes are not considered to be toxic to humans. Chronic effects are associated with those who work in textile processing units. The cleavage of azo bonds in azo dyes results in formation of aromatic amines, which can act as mutagens. Azo dyes accidentally consumed by humans are biochemically reduced by microbes present in the gastrointestinal tract leading to formation of dye intermediates that act as carcinogens can initiate bladder cancer.

Aromatic amines may further be reduced to nitrenium and carbonium ions which can anchor with DNA and RNA, and consequently end in mutation of the nucleic acids, and formation of tumours (Chequer *et al.,* 2011). In mammals, metabolic reduction of azo dye is mainly due to bacterial activity in the anaerobic parts of the lower gastrointestinal tract. Various organs, especially the liver and the kidney also reduce azo dyes. After azo dye reduction in the intestinal tract, the released aromatic amines are absorbed by the intestine and are excreted in the urine. There could be summarized two different mechanisms for bacterial azo dye decolourization (Pearce CI *et al.,* 2003). Aerobic bacteria usually show a high specificity to dye structures and need to be acclimatized over a long period in the presence of azo compounds to induce azoreductase expression. In contrast, anaerobic decolourization is usually unspecific, and the anaerobic dye removal efficiency is much higher than that of aerobic decolourization. These findings indicate that the anaerobic process is more useful for decolourizing a broad range of azo dyes in wastewater (Seesuriyachan *et al.,* 2007). Biological processes have potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature.

The isolation of potent species and there by degradation is one of the interest in biological aspect of effluents treatmen (Mohan S.V. *et al.,* 2002). The basic step in the decolourization and degradation of azo dyes is breakdown of azo bonds, leading to removal of colour. Azo dyes are known to undergo reductive cleavage whereas the resultant aromatic amines are metabolized under aerobic conditions. Although there are many studies on microbial decolourization of azo dyes by various microbial species, it is necessary that new experiments be conducted for finding new resources and microorganisms with suitable biological properties for decolourization. These new microorganisms which have capability to grow in polluted environment, minimal nutritional requirements and rapid growth can be used to achieve good results in decolourization experiments (Kapdan *et al.,* 2003).

**Sample collection:** The water samples were collected in sterile bottle from Decora Textiles, Mominpura, Nagpur. These water samples were transported to the laboratory within 2 hrs for further process. The clinical samples was collected from pathology labs of Nagpur such as throat, pus and urine samples

**Isolation of *Klebsiella pneumonia*:** Water and clinical samples were streaked on C.L.E.D. Agar plates, EMB plates and MacConkey plates. Plates were incubated at $37^0$C for 24 hours. After 24 hours plates were observed for isolated colonies.

**Identification of *Klebsiella pneumoniae***

**Morphological characteristics**

**Gram character examination** Isolated colony was selected by picking the colony with inoculating loop. Smear was prepared on clean side. A staining technique is performed as per standard procedure. Shape, size, gram character was then observed.

**Motility:** Bacterial motility is an important part in making final identification. Motility of the given isolate was observed by using Hanging Drop Method.

**Cultural characteristics:** CLED, EMB Agar and MacConkey Agar plates were prepared. Water and clinical samples were streaked on the plates and incubated at $37^0$C for 24 hrs. The plates were then observed for colonies.

**Biochemical characteristics:** The staining was followed by use of various reagents and test to get closer to the identification of bacteria. There are many biochemical tests available for bacterial identification. The commonly used biochemical tests are Catalase test, IMViC test, Triple Sugar Iron test, Urease test and Sugar fermentation test (Finegold and Barson, 1986).

**Biodegradation studies:** Biodegradation experiment was carried out in 250 ml flask containing 100ml of nutrient broth. To the flask 0.005gm of dyes was added. Then, the flask is autoclaved at 121° C for 15 minutes. The autoclaved flask is then inoculated with 100µl of bacterial culture. Samples were drawn at every 24 hours interval for observation of degradation of dyes. 5ml of dye solution was taken in eppendroff tube and centrifuged for 20minutes at 8000rpm. Supernatant was used to measure decolorization of dye using colorimeter.

| Azo dyes Used | Wavelength(nm) |
|---|---|
| Congo red | 470 |
| Methyl red | 410 |
| Methyl orange | 463 |
| Eriochrome black T | 612 |

**Formula of percent decolourization:**

% Decolourization= (initial O.D – final O.D/initial O.D)*100

## RESULTS AND DISCUSSION

In this present study, bacterial degradation of azo dye namely Congo red, Methyl red, Methyl orange, Eriochrome black T by *Klebsiella pneumoniae* isolated from clinical sample and from the waste water contaminated with dye collected from Decora textile, Nagpur. The *Klebsiella pneumoniae* was isolated and identified on the basis of morphological, biochemical and cultural characteristics. (Table No. 1and2 and figure 1)

**Biochemical characteristics:** The results of biochemical characteristics of the isolated species of *Klebsiella pneumoniae* were given in the following table:

**Cultural characteristics:** The degradation was expressed in terms of percentage degradation. *K. pneumoniae* isolated from clinical samples degrades higher percentage of Methyl red

**Table 1. Biochemical Characteristics of *Klebsiella pneumonia***

| Strain | Indole | MR | VP | Citrate | Urease | TSI | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Acid | Gas | $H_2S$ |
| *K.pneumoniae* | -ve | -ve | +ve | -ve | +ve | +ve | +ve | -ve |

**Table 2. Sugar fermentation characteristics of *Klebsiella pneumonia***

| Strain | Glucose | | Sucrose | | Mannitol | | Lactose | | Maltose | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Acid | Gas | Acid | Gas | Acid | Gas | Acid | Gas | Acid | Gas |
| *K.pneumoniae* | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve | +ve | -ve |

**Figure 1. Isolated colonies of *Klebsiella pneumoniae* on C. L. E. D Agar & MacConkey Agar**

**Table 3. Biodegradation of azo dyes by *K.pneumoniae* isolated from the clinical sample**

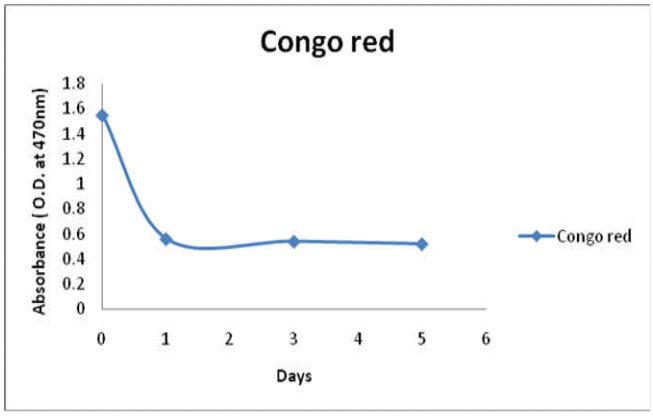
| Azo dyes | Zero time | After 24 hrs | After 72 hrs | After 120 hrs | Total % degradation |
|---|---|---|---|---|---|
| Congo red | 1.55 | 0.56 | 0.54 | 0.52 | 66.45 |
| Methyl red | 1.78 | 0.26 | 0.22 | 0.20 | 88.76 |
| Methyl orange | 1.00 | 1.00 | 0.95 | 0.93 | 7.00 |
| Eriochrome Black T | 0.25 | 0.21 | 0.14 | 0.11 | 56 |

**Table 4. Biodegradation of azo dyes by *K.pneumoniae* isolated from the textile effulent (Environmental Sample - water)**

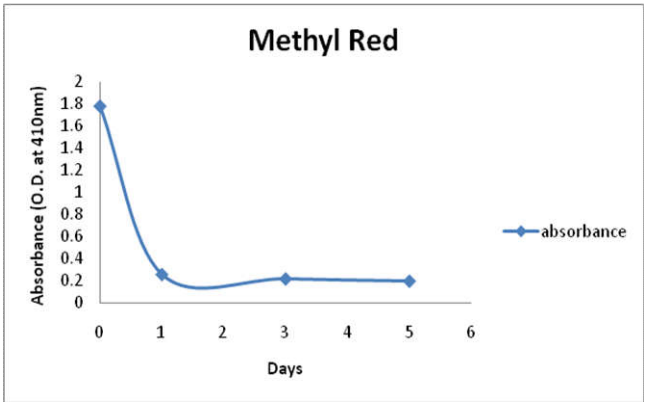
| Azo dyes | Zero time | After 24hrs | After 72hrs | After 120hrs | Total % degradation |
|---|---|---|---|---|---|
| Congo red | 0.72 | 0.47 | 0.20 | 0.17 | 76.38 |
| Methyl red | 0.79 | 0.86 | 0.80 | 0.72 | 8.86 |
| Methyl orange | 1.98 | 1.75 | 0.36 | 0.09 | 95.45 |
| Eriochrome Black T | 1.38 | 1.10 | 0.55 | 0.37 | 73.18 |

(88.76%) and Congo red (66.45%) and least percentage of Methyl orange (7.00%) (Table No. 3). Whereas the degradation of azo dye by *K. pneumoniae* isolated from water of textile dye effluent, higher percentage of degradation was found against Methyl orange (95.45%) Congo red (76.38%) Eriochrome Black T (73.18%) and least degradation against Methyl red (8.86%) (Table No. 3). By comparing both samples for the degradation, environmental sample like water was best decolorizer of azo dye compared to clinical samples (Graph No. 9). The reason behind this was the *K. pneumoniae* present in the environment of water containing harmful azo dyes and due to mutation of this heavy mutagens provoke to *K. pneumoniae* to produce enzyme to degradate these mutagens for the survival of *K. pneumoniae* to become best degradator of azo dyes.

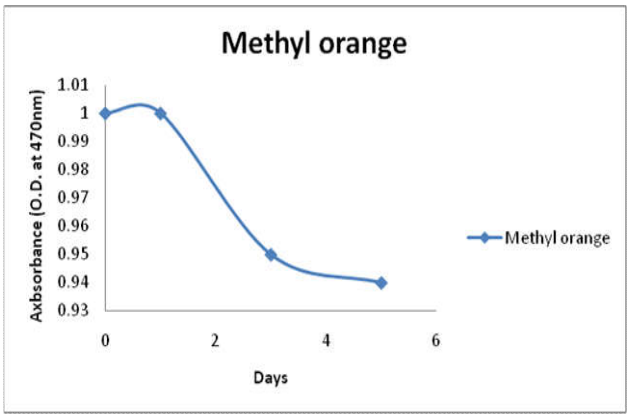
**Biodegradation assay of azo dyes:** Saranraj *et al.,* investigated the decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Five different bacterial species were isolated from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.* In this present isolated *Klebsiella pneumoniae* was from two different sources. The bacterial inoculums were inoculated into flasks containing Direct azo dyes (500 mg/l) with trace amounts of yeast extract, glucose and sucrose and then sterilized and incubated for 4 days. The decolourization was expressed in terms of percentage decolourization. *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. *Klebsiella pneumoniae* (98.44%) was the best decolourizer of Viscose Orange – A. The best decolourizer of Direct Green-PLS was *Bacillus subtilis* (99.05%). *Klebsiella pneumoniae* (87.27%) highly decolourized the Direct Violet-BL. *Escherichia coli* (61.56%) was the best decolourizer of Direct Sky Blue-FF. The best decolourizer of Direct Black-E was *Klebsiella pneumoniae* (92.03%). Bacterial biodegradation was assessed by physicohemical analysis. Sun-Young An *et al.,* studied on Decolorization of triphenylmethane and azo dyes by *Citrobacter* sp. A *Citrobacter* sp., isolated from soil at an effluent treatment plant of a textile and dyeing industry.



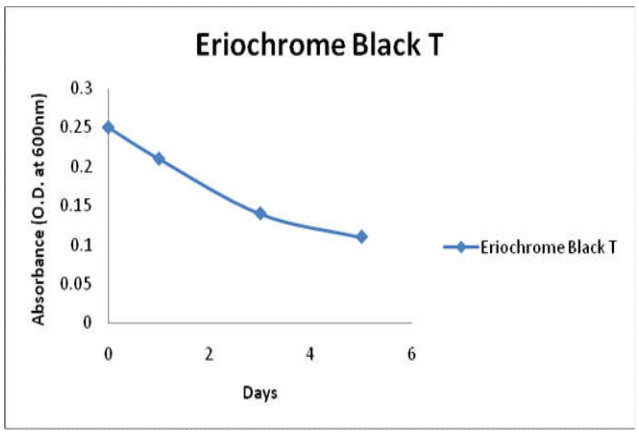

**Graph 1. Biodegradation of Congo red**



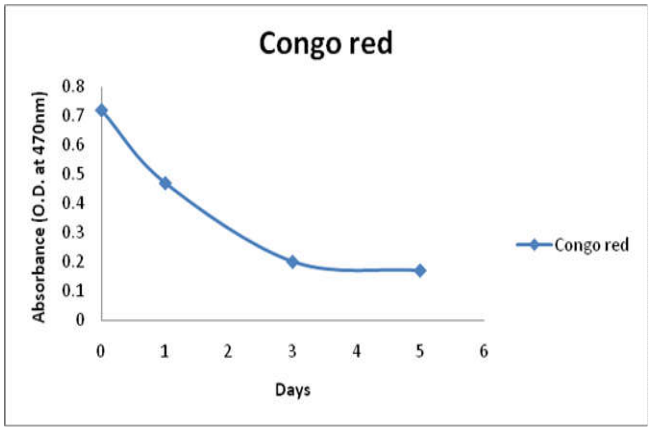

**Graph 2. Biodegradation of Methyl red**



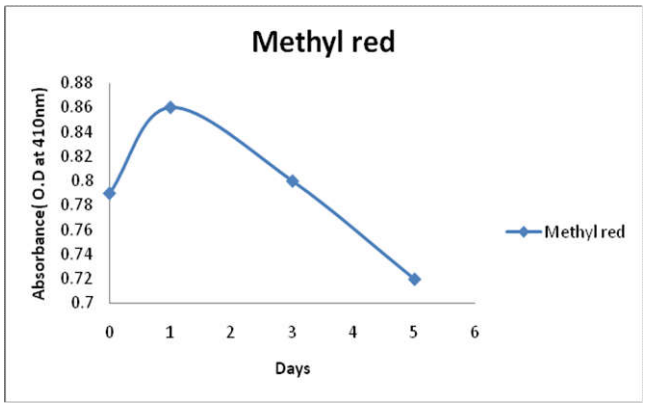

**Graph 3. Biodegradation of Methyl orange**



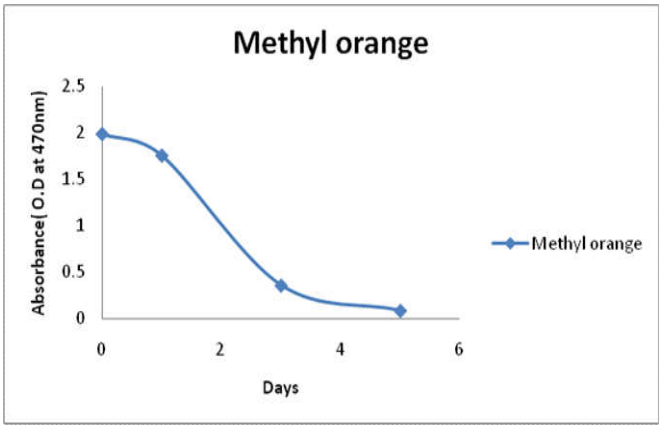

**Graph 4. Biodegradation of Eriochrome black T**



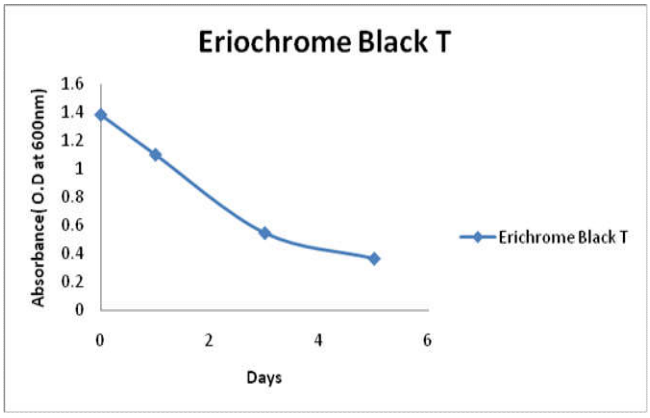

**Graph 5. Biodegradation of Congo red**



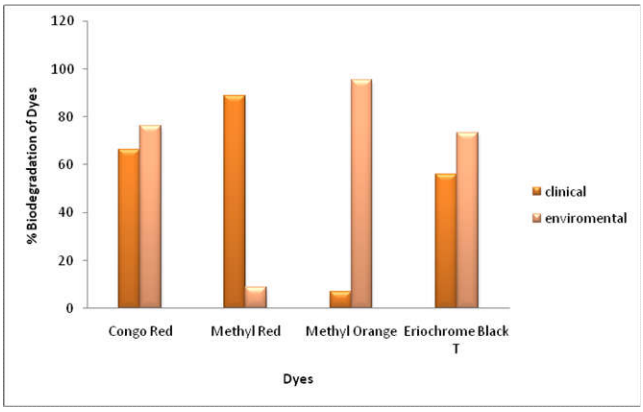

**Graph 6. Biodegradation of Methyl red**




**Graph 7. Biodegradation of Methyl orange**




**Graph 8. Biodegradation of Eriochrome black T**




**Graph 9. Total degradation of azo dyes by Klebsiella pneumoniae (clinical vs environmental)**

**Figure 2. Biodegradation of Congo red**


**Figure 3. Biodegradation of Methyl orange**

More than 90% of Methyl Red at 100 $\mu$M were reduced within 1 hrs and the percentage decolorization of Congo Red were 26%, respectively. In the present study degradation of Methyl red and Congo red by *Klebsiella* sp. shows 8.86% and 76.38%. Daizong Cui *et al.,* studied on Decolourization of azo dyes by a newly isolated *Klebsiella* sp. strain Y3, and effects of various factors on biodegradation. The isolate decolourized Methyl Red, Congo Red, Orange I and Methyl Orange by almost 100% (100 mg/L) in 48 h. The culture exhibited an ability to decolourize repeated additions of dye, showing that the strain could be used for multiple cycles of biodegradation. Azo dyes at high concentrations could be tolerated and degraded by Y3. An almost complete mineralization of Methyl Red and Congo Red at the concentration of 800 mg/L was observed within 48 h.

In present study, the *Klebsiella pneumoniae* isolated from dye contaminated water decolorized Methyl orange by almost 100% (5mg/100ml) and Congo red by 76.38%. Bella Devassy Tony *et al.,* worked on Decolorization of textile azo dyes by aerobic bacterial consortium. The decolorization potential of two bacterial consortia developed from a textile wastewater treatment plant showed that among the two mixed bacterial culture SKB-II was the most efficient in decolorizing individual as well as mixture of dyes. At 1.3 g L_1 starch supplementation in the basal medium by the end of 120 h decolorization of 80–96% of four out of the six individual azo dyes Congo red, Bordeaux, Ranocid Fast Blue and Blue BCC (10 mg L_1) was noted. The culture exhibited good potential ability in decolorizing 50–60% of all the dyes (Congo red, Bordeaux, Ranocid Fast Blue and Blue BCC) when present as a mixture at 10 mg L_1. Sonia Sethi, *et al.,* studied on Biodecolorization of Azo Dye by Microbial Isolates from Textile Effluent and Sludge. The dye decolourizing isolates, *Pseudomonas sp, Klebsiella sp* and *Proteus sp* were isolated from the textile effluent samples and *Shiegella sp, Morganella sp* and *Klebsiella sp* were isolated from sludge collected from AmanishahNala, Sanganer, Jaipur. Study confirms the ability of all textile effluent isolates for decolourization of light red dye showing 80% decolourization whereas sludge isolates showed 40% decolourization under optimum conditions.

Among all the isolates, *Proteus sp* from textile effluent and *Klebsiella sp* from sludge were found to be most efficient in dye decolourization. In the present study the *Klebsiella pneumoniae* from clinical found more efficient against methyl whereas *Klebsiella pneumoniae* from textile effluent found more efficient against methyl orange. Wong and Yuen worked on decolorization and biodegradation of methyl red by *Klebs1ella pneumoniae* RS-13. Bacterial Strains iso1ated from dye-contaminated S1udge decolorized a toxic azo dye, name1y, methy1 red (MR). *K1ebsie11a pneumoniae* RS-13 was se1ected because of its better abilities to comp1ete1y decolorize and degrade MR under aerobic conditions. Effects of physico-chemica1 parameters, such as: temperature and aeration, concentrations of glucose, ethanol, ammonium su1fate and pH of the cu1ture medium on the MR degradation by the bacterium and a previous1y iso1ated aerobic MR-degrading bacterium, *Acetobacter liquefaciens* S-1, were determined. *K. pneumoniae* RS-13 had higher MR degradation ability than *A.liquefaciens* S-1. Under optima1 conditions, *K. pneumoniae* RS-13 comp1ete1y decolorized and degraded 100 mg/l of MR in cultura1 medium. In present study, *Klebsiella pneumoniae* from clinical sample shows 88.76% decolorization and *Klebsiella pneumoniae* from textile effluent shows 8.86% decolorization.

## Conclusion

Application of traditional waste water treatment requires enormous cost and continuous input of chemical which become uneconomical and cause further environmental damage. Hence economical, less costly and eco-friendly techniques using bacteria can be employ for the treatment of waste water treatment contaminated with azo dyes. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. So, by this present study it can be concluded that *Klebsiella pneumoniae* can be used as a good microbial source for the treatment of waste water contaminated with azo dyes. It can be isolate easily from the environment and can be use for biodegradation purpose.

The *Klebsiella pneumoniae* which is isolated from the textile effluent degrade the azo dyes more readily than the clinical isolate.

## REFERENCES


Adedayo, O., Javadpour, S., Taylor, C., Anderson, W.A., and Moo-Young. 2004. Decolourization and detoxification of Methyl Red by aerobic bacteria from a waste water treatment plant. *World Journal of Microbiology*. 20: 545-550.

Ali, H. 2010. Biodegradation of synthetic dyes—a review. Water Air Soil Pollut 213:251–273.

Axelsson, J., Nilsson, U., Terrazas, E., Aliaga, T.A. andWelander, U. 2006. Decolorization of the textile dyes reactive red 2 and reactive blue for using *Bjerekandera*sp. strain Bol 13 in a continuous rotating biologicalcontactor reactor. *Enz. Microbial Technol.* 39: 32-37.

Bella Devassy Tony , Dinesh Goyal , Sunil Khanna, 2009. "Decolorization of textile azo dyes by aerobic bacterial consortium." *International Biodeterioration and Biodegradation* 63 (2009) 462–469.

Brás R, Gomes A, Ferra MIA, Pinheiro HM, Gonc¸ alves IC. 2005. Monoazo and diazo dye decolorisation studies in a methanogenic UASB reactor. *J Biotechnol.,* 115:57–66.

Chequer F, Dortaz D, Oliveira D. 2011. Azo dyes and their metabolites: Does the discharge of the azo dyes into water bodies represent Human and Ecological risks? In: Hauser P (ed) Advances in treating textile effluents, ISBN: 978-953-307-704-8, In Tech.

Daizong Cui, Guofang Li, Min Zhao and Song Han, 2014. "Decolourization of azo dyes by a newly isolated *Klebsiella* sp. strain Y3, and effects of various factors on biodegradation". *Biotechnology and Biotechnological Equipment,* 2014 Vol. 28, No. 3, 478_486.

Forgacs E, Cserhatia T, Oros G (2004) Removal of synthetic dyes from wastewaters: a review. *Environ Int* 30:953–971.

Hunger K. 2009. Industrial dyes—chemistry, properties, applications. Wiley-VCH, Germany

Ibrahim M. Banat, Poonam Nigam, Datel Singh and Roger Marchant 1996. "Microbial decolorization of textile dyecontaining effluents: a review." *Bioresource Technology* 58 (1996) 217-227.

Kapdan, I.K. and Erten, B. 2007. "Anaerobic treatment of saline wastewater by *Halanaerobiumlacusrosei*," *Process Biochemistry* 42.449–453.

Kirk-Othmer. 2004. Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 7, 5th Edition. Wiley-Interscience.

Ponraj, M., Gokila K. and Vasudeo Zambare, 2011. "Bacterial decolorization of textile dye- orange 3r." International *Journal of Advanced Biotechnology and Research* ISSN 0976-2612,Vol 2, Issue 1, 2011, pp 168-177.

Maximo, C., Amorim, M.T.P., and Costa Ferreira, M. 2003. Biotransformation of industrial reactive azo dye by *Geotrichum* sp. *Enzyme and Microbial Technology. 32*: 145-151.

Mohan S.V., Rao C.N., Prasad K.K. and Karthikeyan J. 2002 "Treatment of simulated reactive yellow 22 (azo) dye effluents using *Spirogyra* species." *Waste Manage*ment 22: 575– 582

Pearce CI, Lloyd JR, Guthrie JT 2003." The removal of color from textile wastewater using whole bacterial cells: a review*". Dye Pigment*

Pourbabaee, A.A., Malekzadeh, F., Sarbolouki, M.N., Najafi, F., 2006. Aerobic decolorization and detoxification of a disperse dye in textile effluent by a new isolate of Bacillus sp. Biotechnology and Bioengineering 93, 631–635.

Praveen Sharma, G.R. Chaudry and Thomes Edison, 2009. Mutagenicity testing of some commonly used azo dyes. Applied Environmental Microbiology, 42(4): 641-648.

Saranraj P. Sriram N., Reetha D. 2013." Biological degradation of reactive dyes by using bacteria isolated from dye effluent contaminated soil." *Middle-east journal of scientific research* 17 (12): 1695-1700, 2013.

Seesuriyachan P, Takenaka S, Kuntiya A, Klayraung S, Murakami S, Aoki K. 2007. 'Metabolism of azo dyes by Lactobacillus casei TISTR 1500 and effects of various factors on decolorization." *Water Res*.41:985_992.

So, K., Wong, P., Chang, K., 1990. Decolorization and biodegradation of Methyl red by Acenitobacter liquifaciens. Toxicology Assessment 5, 221–235.

Sonia Sethi, Shubhum, Mohan MukeshMalviya, Neelam Sharma, Saksham Gupta, 2012. BiodecolorizationofAzo Dye by Microbial Isolates from Textile Effluent and Sludge. *Universal Journal of Environmental Research and Technology*, Volume 2, Issue 6: 582-590

Sun-Young An, Sang-Ki Min, In-Ho Cha, Yong-Lark Choi, Young-Su Cho, Cherol-Ho Kim and Young-Choon Lee, 2002. "Decolorization of triphenylmethane and azo dyes by Citrobacter sp." *Biotechnology Letters* 24: 1037–1040, 2002.

Wong, P., Yuen, P., 1998. Decolorization and biodegradation of N,Ndimethyl- p-phenylenediamine by *Klebsiella pneumoniae*RS-13 and Acenitobacter liquifacienss-1. *Journal of Applied Microbiology* 85, 79–87.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