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Biodecolorizationof Azo Dye by Microbial Isolates from Textile Effluent and Sludge

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Abstract:

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. Different parameters such as various carbon source, nitrogen source, temperature and pH were optimized for decolourization of Light Red Dye by using bacterial isolates. The present 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. All parameters studied in this paper were found to be effective for all isolates. The selected bacterium shows higher decolourization in shaking condition as compared to static condition. The optimum pH obtained for decolourization of Light Red dye by all bacterial strains was 8.0-8.5. Although, good decolourization efficiency even in alkaline region was observed. The optimum temperature was found to be 55^0C. Enhanced decolourization was observed in presence of glucose as a carbon source and yeast extract & urea as a nitrogen source. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment. High decolourization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

Keywords: Azo dye, Biodecolorization, Light red, *Pseudomonas* sp, static condition

1.0 Introduction:

Different dyes used in textile industry usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to be biodegraded. A great number of dyes and other chemicals are used in textile wet processing. There are more than 10^5 commercially available dyes with over 1×10^6 ton of dyestuff produced annually world-wide (Pandey *et al.,* 2007). Among these available dyes, azo dyes constitute about 70% of all known dyestuffs in the world and represent 70% of total dyes produced per year, thus making them the largest and the most important group of synthetic colorants released into the environment (Ambrosio and Campos-Takaki, 2004). It is estimated that about 10-15% of the total production of colorants is lost during their synthesis and dyeing Processes (J.R. Easton, 1995). Whereas, in the case of reactive dyes almost 50% of the initial dye load is found in the dye bath effluents. The recalcitrance of azo dyes has been attributed to the presence of sulfonate groups and azo bonds, two features generally considered as xenobiotic (Lee and Pavlostathis, 2004). Colored industrial effluent is the most obvious indicator of water pollution and the discharge of highly colored synthetic dye effluents is aesthetically displeasing and cause considerable damage to the aquatic life. Due to increasingly stringent environmental legislation, the textile industry is seeking to develop effective wastewater remediation technologies, especially those that allow colour removal that is largely unaffected by conventional treatment systems (O'Neill *et al.,* 2000). Although several physical-chemical methods have been used to eliminate the colored effluents in wastewater, they are generally expensive, produce large amounts of sludge. More often these conventional modes of treatment lead to the formation of some harmful side products. Interest is therefore now focused on the microbial biodegradation of dyes as a better alternative (An SY *et al*., 2002). Some microorganisms including bacteria, fungi and algae, can degrade or absorb a wide range of dyes (Robinson *et al.*, 2001) .The biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. This method is more

economical and leads to less accumulation of relatively harmless sludge.

Most importantly, biological treatment of dye bath effluents is ecofriendly. It causes mineralization of dyes to simpler inorganic compounds which are not lethal to life forms. 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 (Kapdan*et al*., 2003). So for complete mineralization of azo dyes the microbial population forming part of treatment system should be able to work efficiently under both anaerobic and aerobic conditions. Despite the existence of a variety of chemical and physical treatment processes, bioremediation of textile effluent is still seen as an attractive solution due to its reputation as a low-cost, environmentally friendly, and publicly acceptable treatment technology (Banat *et al*. 1996). A number of biological processes such as bio sorption have been proposed as having potential application in removal of dyes from textile wastewater (Bustard *et al*. 1998).

Screening potential microorganisms is a critical step in the construction of an effective remediation system. At present, a number of studies have focused on microorganisms, which are able to decolourize and biodegrade these dyes. Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of azo dyes (O'Neill *et al*., 2000). Alternatively, dye decolourization using microbial enzymes has received great attention in recent years due to its efficient application (Couto *et al*., 2005). Colour removal processes with active microorganisms have two different simultaneous steps: an adsorption of dyes on the surface of the organisms and a degradation of dyes by the enzymes produced by these organisms (Khalaf, 2008). The Objectives of the present study were: to determine Physico-chemical characterstics of the textile effluent & sludge and to screen for bacteria with the potential to decolorize textile dyes.

2.0 Materials and Methods:

2.1 Sample Collection and Measurement of Physiochemical Parameters

Wastewater samples were collected in screw capped sterilized bottles from Amanishah Nala, Jaipur (Rajasthan). Some physicochemical parameters of wastewater viz., temperature, pH, conductivity, hardness, chloride content, acidity, alkalinity, TDS, free CO_2, and DO were measured (APHA, 1989).

Fig1: Photo showing the site of Sample collection at Amanishah Nala, Jaipur

2.2 Bacterial Isolation and Cultivation

For isolation of bacteria, water and sludge samples were collected as sources for bacteria. Numerous colonies were obtained through serial dilution method. Isolated colonies were then obtained through streaking method on nutrient agar. Each strain was then inoculated into nutrient broth and incubated for 24 h at 37 ^{0}C on a platform shaker at 150 rpm. A 10% (v/v) inoculum was transferred into 250 mL flask containing 100 mL LB media and incubated similarly. After 24 h, 10% (v/v) samples were sub-cultured into fresh LB media containing the respective dyes and further incubated as described above. Strains capable of utilising fresh dyes as a nutrient source were plated on MSM plates and incubated at 37 °C for 24 h. It was from these plates; isolated colonies were taken and repeatedly streaked on nutrient agar to obtain pure cultures. The pure bacterial cultures were subsequently transferred into nutrient broth.

2.3 Screening for Bacteria Decolourising Capability Using Selected Azo Dye

The bacterial isolates were cultivated in nutrient broth 24 hours before screening was done in MSM media. For initial screening, 0.1% (v/v) aliquot of each isolated strain in nutrient broth was inoculated into MSM, each containing 200 µL individual dye solutions. Decolourisation of the dye solution was monitored visually after 24 h incubation. Strains that showed high decolourising potential were chosen to be tested further using dye incorporated in MSM agar plates.

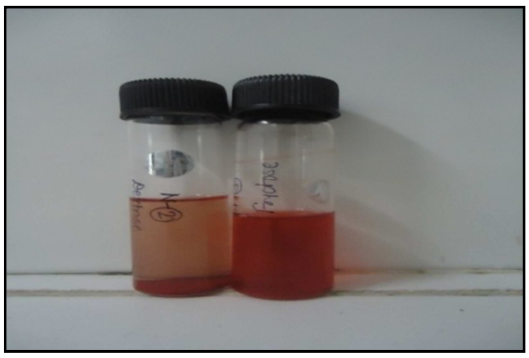

Fig2: Screening of dye degrading bacteria for decolorization

In secondary screening using dye incorporated MSM agar plates, the selected isolates were first inoculated into the nutrient broth for 24 h. The culture was then lawned onto the agar and left for another 24 h before any decolourisation zone was noted. Respective dye incorporated agars without any inoculums were used as controls and the decolourisation were estimated visually by comparing the inoculated plates with those of the control plates after 24 to 72 h. Final screening using selected dyes in MSM liquid media were initially done using smaller volume of samples. Each selected strain was inoculated into flasks containing 10 mL nutrient broth and allowed to grow for 24 h. A sample of 10% (v/v) of the aliquot was then transferred into flasks containing 10 mL of MSM media. Decolourization was studied on various carbon (Glucose, Fructose, Sucrose, Maltose&Mannitol), Nitrogen sources (Yeast extract, Beef extract, peptone, Ammonium sulphate & Urea), different dye concentrations (100–1000 mg/l), pH values (5, 6, 7, 7.5, 8, 8.5 and 9) and temperature (15, 37, 55 and 65 ^{o}C). Growth was monitored spectrophotometrically. The cell pellet obtained upon centrifugation (6000 g for 20 min.) of 5 ml culture, was resuspended in 5 ml distilled water and its absorbance was observed at 660 nm. Decolourization was determined by measuring the absorbance of culture supernatants at the absorbance maxima of the respective dyes. All the experiments were performed in triplicate. The percent decolourization of effluent was determined by using the formula:

$D= [A_0-A_1)/A_0] \times 100$

Where, D- decolourization in %; A_{0-} initial absorbance; A_{1-} final absorbance

3.0 Results and Discussion:

3.1 Physicochemical Characteristics of Wastewater:

Table 1 shows the some physicochemical characteristics of industrial wastewater from where azo dyes degrading bacteria were isolated. The textile effluent and sludge sample was collected from Amanishahnala was black in colour, with pungent smell and pH 6.8 which was in the permissible limits. The temperature of the effluent was very high (31 ^{0}C), Electrical conductivity (EC) of the effluent was quite low (2.56mS). TDS was also high in the sample (500mg/l). There was a high load of COD (0.016mg/ml) and Free CO_2 (74.8mg/l) in the collected sample. A high value of COD will cause depletion of Dissolved oxygen in water.

Table 1: Physicochemical characteristics of Wastewater

Parameter	Textile Effluent	Industrial Effluent Standard
Physical Analysis of Water		
Temperature of water	31^{o}C	30-35^{o}C
Conductivity of water	2.56 mS	2 m S
Chemical Analysis of Water		
pH in the water	6.8	5.5-9
Chloride in water	6.52 mg/ml	1 mg/ml
Acidity of water	205 mg/l	-
Alkalinity of water	420 mg/l	<120
TDS of water	500 mg/l	<500
Organic Constituents in Water		
Free CO_2	74.8 mg/l	22
COD	0.016 mg/ml	-
Bacteriological Analysis of Water		
MPN Test	>= 1600 Coliforms present	<400

3.2 Bacterial Isolation and Cultivation:

A total of 18 cultures of bacteria were isolated, purified and screened for the degradation of azo dyes from textile effluent and sludge sample. Of all the cultures tested, 13 bacterial isolates (four from textile effluent and nine from sludge) were further screened for dyes degradation and finally three bacterial isolates each from textile effluent and sludge were selected on the basis of dyes tolerance i.e., resistance in minimal medium containing 2% of reactive light red dye**. Table 2 and 3** shows different bacterial strains isolated from the textile effluent and sludge which were screened for their ability to decolorize textile dye and the potential strains were morphologically and biochemically characterized for identification. Based on preliminary tests and secondary screening, plating on selective media and biochemical tests, they were identified as *Klebsiella pneumonia* (T), *Pseudomonas florescence* (T) and *Proteus mirabilis* (T) from textile effluent and *Shiegellasp*(S)*, Morganellasp* (S) and *Klebsiella pneumonia* (S) from sludge and were selected for light red dye degradation (**Table 4).**

Table 2: Biochemical characteristics of isolates from textile effluent (T)

Isolates No.	MR	VP	I	Ci	TSI					Cat	Oxi	Identified bacterial species.
					Slant	Mid	Butt	H2S	Gas			
T1	-ve	-ve	-ve	+ve	P	Y	Y	+ve	+ve	+ve	-ve	***Klebsiellapneumoniae***
T5	+ve	-ve	-ve	+ve	P	Y	Y	-ve	-ve	+ve	+ve	***Pseudomonas florescens***
T7	-ve	-ve	-ve	+ve	P	Y	Y	+ve	+ve	+ve	-ve	***E.coli***
T12	+ve	-ve	-ve	+ve	P	Y	Y	-ve	-ve	+ve	-ve	***Proteus mirabilis***

-ve=Negative; +ve= Positive; P=Pink; Y=Yellow

Table 3: Biochemical characteristics of isolates from Sludgesample (S)

Isolates No.	MR	VP	I	Ci	TSI					Cat	Identified bacterial sp.
					Slant	Mid	Butt	H2S	Gas		
S1	+ve	-ve	-ve	+ve	P	Y	Y	+ve	-ve	+ve	***Alcaligenes sp.***
S2	+ve	-ve	-ve	+ve	P	Y	Y	+ve	-ve	+ve	***Salmonella sp.***
S4	+ve	-ve	+ve	+ve	P	Y	Y	+ve	-ve	+ve	***Edwardsiella sp.***
S7	+ve	-ve	-ve	+ve	P	Y	Y	-ve	-ve	+ve	***Shiegella sp.***
S10	+ve	-ve	-ve	-ve	P	Y	Y	+ve	-ve	+ve	***Morganella sp.***
S12	+ve	+ve	+ve	+ve	Y	Y	Y	+ve	-ve	+ve	***Serratiamarscens***
S13	-ve	-ve	-ve	-ve	P	Y	Y	+ve	-ve	+ve	***Pseudomonas florescens***
S16	+ve	-ve	-ve	+ve	Y	Y	Y	+ve	-ve	+ve	***Klebsiella pneumonia***
S20	+ve	-ve	-ve	-ve	P	Y	Y	+ve	-ve	+ve	***Proteus mirabilis***

-ve=Negative; +ve= Positive; P=Pink; Y=Yellow

Table 4: % dye decolorization by bacterial isolates from textile effluent and sludge

Bacterial isolates	Dye Decolorization
Klebsiella sp.(T)	80.00%
Pseudomonas sp(T).	80.00%
Proteus sp. (T)	80.00%
Morganellasp(S).	40.00%
Shiegella sp.(S)	40.00%
Klebseilla sp. (S)	40.00%

3.3 Decolourization at Different Dye Concentration:

The rate of decolourization increased with increase in initial dye concentration from 100 to 1000 mg/L, showed 90.04 % percentage of decolourization for textile effluent and 93.34 % for sludge isolates (**Fig 3**) within 24 hr. Similar results were mentioned by Khalid *et al.* (2008). Dye concentration can influence the efficiency of microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration (Sahasrabudhe and Pathade, 2011). Among textile effluent isolates, *Proteus mirabilis* showed maximum decolorization i.e.

90.04% and all sludge isolates showed equal decolorization (93.34%).

3.3.1 Effect of pH on dye decolourization:

The Hydrogen ion concentration showed profound effect on the biological activities of the organism. *Klebsiella* isolate from textile effluent and sludge exhibited maximum decolourization at pH value 8.5, percentage of decolourization (89.54%) was achieved within 24hr for textile effluent and (93.54 %) for sludge isolates (**Fig 4**). Our culture exhibited decolourization activity in the range of pH 7 to 9. The effect of pH may be related to the transport of dye molecules across the cell membrane, which is considered a rate limiting step for dye decolourization (Ogugbue and Sawidis, 2011).

3.3.2 Effect of temperature on dye decolourization:

Temperature plays an important role in microbial growth and enzyme activity; it is one of the most important parameter taken into consideration for the development of biodecolorization processes. The dye decolourization activity of culture was found to increase with increase in incubation temperature from 15 to 65˚C with maximum activity attained at 55˚C with decrease in decolorization with further increase in temperature. *Pseudomonas* sp gave highest decolourization rate of Light Red dye (97.17 %) at temperature 55˚C after 24hr among textile effluent isolates and *Shiegella* sp (97.17 %) among sludge isolates (**Fig 5**).

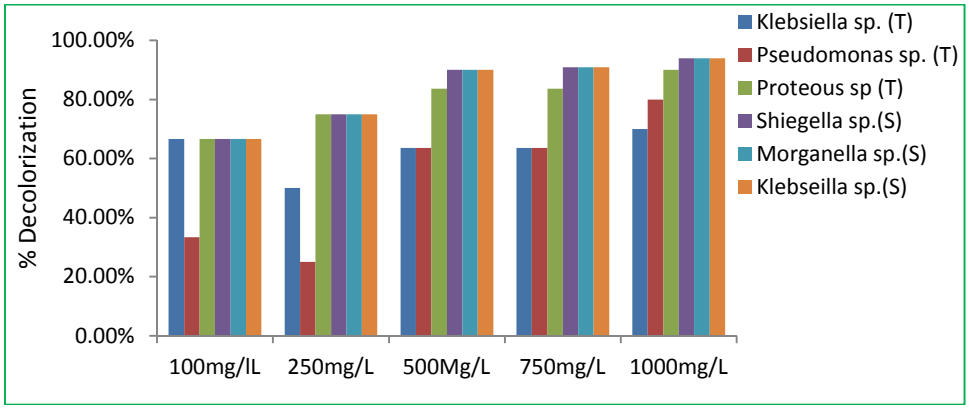

Fig 3: Dye decolourization at different concentration by isolates from textile effluent and Sludge

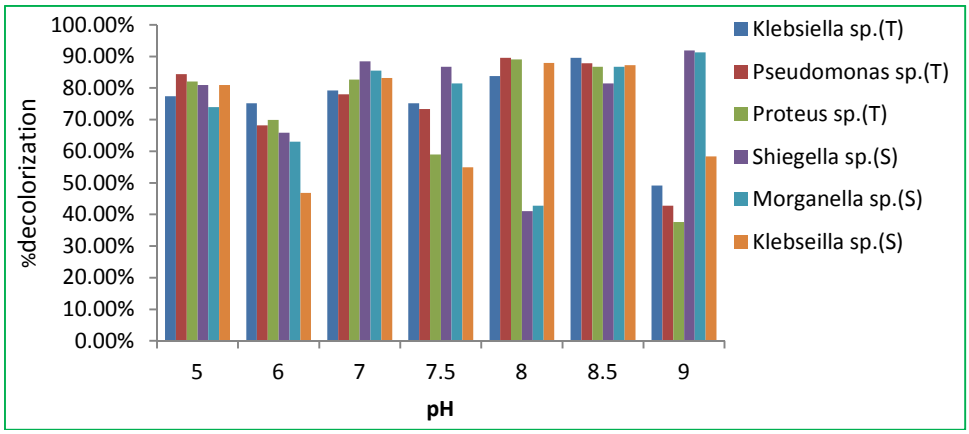

Fig 4: Dye decolourization at different pH by isolates from textile effluent and Sludge

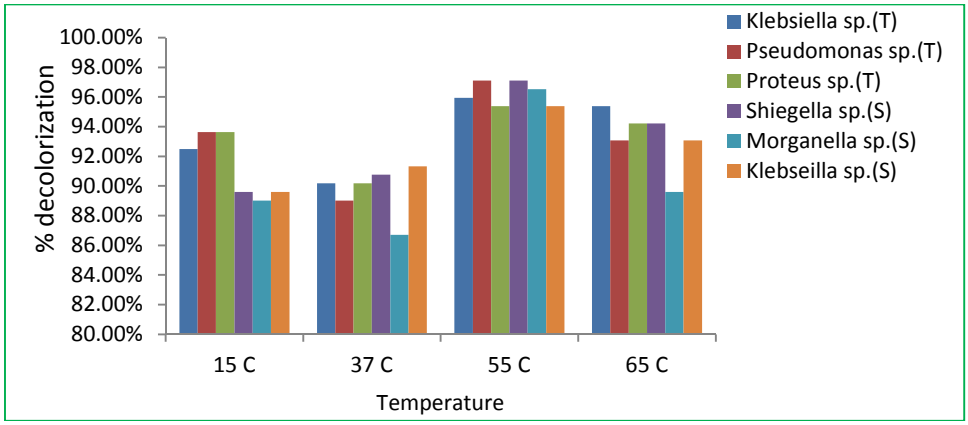

Fig 5: Dye decolorization at different temperature by isolates from textile effluent and Sludge

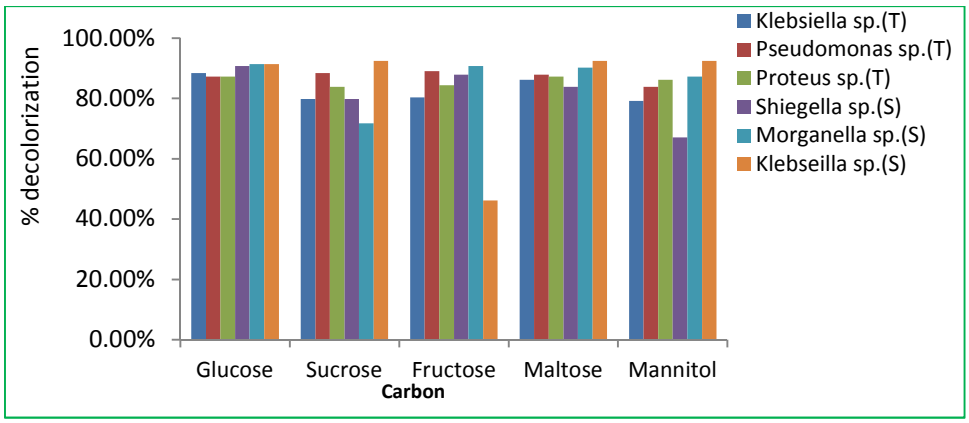

Fig 6: Dye decolourization at different carbon by isolates from textile effluent and Sludge

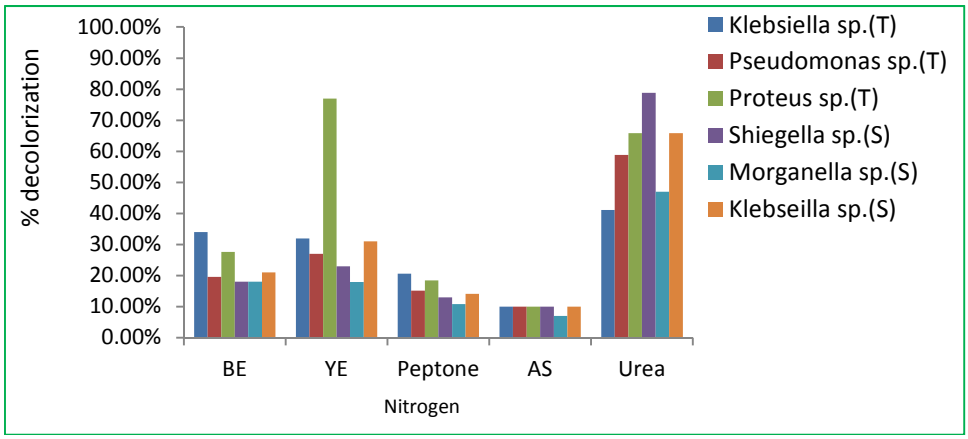

Fig 7: Dye decolourization at different nitrogen by isolates from textile effluent and Sludge

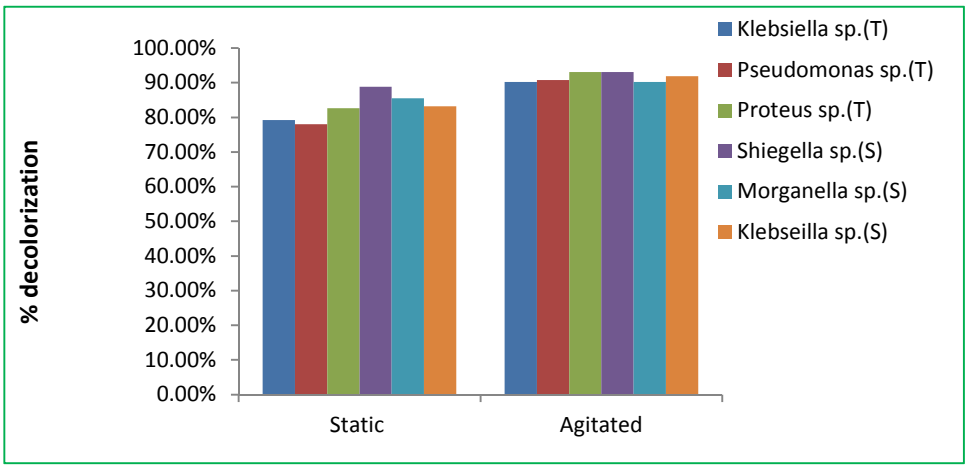

Fig 8: Dye decolourization under aeration and static condition by isolates from textile effluent and Sludge

3.3.3 Effect of carbon source on dye decolourization:

In order to optimize dye decolourization we have studied the addition of glucose, sucrose, maltose, fructose and mannitol. Addition of glucose revealed that all the strains could utilize the dyes, with high percentage of decolourization within 24 hr for *Klebsiella* sp isolate (90 %) from textile effluent and sludge (**Fig 6**). The addition of glucose has helped in the bacterial growth rate and consequently has increased the rate of decolourization (Celik *et al*., 2012). *P. putida* WLY can use glucose as carbon and energy sources and thus, provided sufficient electrons for reductive conditions through the cleavage of the azo bond of X-3B (Yang *et al*., 2011). Glucose showed 95% decolourization in alone and in combination with other nitrogen sources showed 100%, 100% and 98% decolourization respectively (Waghmode *et al*., 2012).

3.3.4 Effect of nitrogen source on dye decolourization:

Light red dye was studied for decolourization activity in liquid medium supplemented separately with different nitrogen sources such as Beef extract, yeast extract, Peptone, Ammonium sulphate and Urea. Amongst studied different nitrogen sources, spectrophotometric analysis revealed that the strains could utilize urea as inorganic nitrogen and yeast extract as organic nitrogen the most effective supplement for promoting higher decolourization efficiency. Dye showed maximum percentage of decolourization within 24 hr by *Proteus mirabilis* isolate (70-75%) from textile effluent and *Klebsiella pneumonia* (30-35%) from sludge in presence of Yeast extract whereas *Proteus mirabilis* (6-65%) from textile effluent and *Shiegella* sp (70-75%) from sludge in presence of Urea (**Fig 7**). Organic nitrogen sources are considered essential media supplements for the regeneration of NADH that act as electron donors for the reduction of azo dyes by microorganisms (Rajeshwari *et al*., 2011). *Bacillus* sp. found most effective decolourization under the presence of yeast extract (Ponraj *et al*., 2011).

3.3.5 Effect of Static and Agitation conditions:

These results indicate that decolourisation is not dependent on biomass concentration but is significantly correlated with dissolved oxygen levels. Maximum decolourisation of 93% was observed under shaking condition by *Proteus mirabilis* isolate from textile effluent and *Klebsiella pneumonia* isolate from sludge compared to 84% in static condition (**Fig 8**). In static incubation, transfer of oxygen is limited to the broth surface, and the cell cultures will most likely sediment to the bottom of the flasks and become rapidly oxygen-depleted (Stolz, 2001; Chen, 2002). In the absence of oxygen, the azo dye acts as sole oxidant or electron acceptor, and its reduction rate is then governed exclusively by the rate of formation of the electron donor, in this case the reduced azo dyes (Wuhrmann *et al*., 1980).

4.0 Conclusion:

Textile effluent and sludge produced by effluent treatment plant is rich source of dye decolorizing bacterial population. The present study confirms

1. The ability of isolated bacterial culture showing 80% decolourization for Light red dye in textile effluents isolates thus suggesting their application for decolourization of dye in industrial waste waters.

2. In case of sludge isolates, all showed 40% decolourization for Light red dye.
3. Effective decolorization of the light red azo dye which is commonly used in the textile industries was observed under shaking condition.
4. Maximum decolorization of light red azo dye was observed at pH 8.5 and 55°C temperature.
5. Enhanced decolourization was observed in presence of glucose as a carbon source and yeast extract and urea as an additional nitrogen source.

These indigenous bacterial strains could be utilized for treatment of dye present in wastewater with high degrading and decolorizing activity against various reactive dyes commonly used in the textile industries. It is proposed that these bacterial species has a practical application potential in the biodegradation of various dye effluents.

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