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Degradation of Direct Red 28 by *Alcaligenes* sp. TEX S6 Isolated from Aeration Tank of Common Effluent Treatment Plant (CETP), Pali, Rajasthan

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ABSTRACT

Pali city in Rajasthan has been a major environmental concern in the terms of extensive water pollution caused by the textile industries. The textile effluents are characterized by remarkably strong colour, high pH, BOD and COD. The major culprits are the direct diazo dyes and one such dye is Direct Red 28. To develop an effective bioprocess for dye degradation, bacteria were screened from different stages of the common effluent treatment plant situated in Mandia Road, Pali. The most potential bacteria was an isolate from aeration tank and characterized as *Alcaligenes* sp. TEX S6 by 16S rDNA sequencing. The dye removal efficacy of the strain was expressed as a reduction in absorbance maxima of the dye. The strain removed the dye (0.15 g/L) up to 86% within 48 hours of static incubation utilizing fructose and peptone at 37ºC and pH 7. The inoculum concentration had no effect on the decolourisation process. A significant increase in bioefficacy of the strain was observed with respect to abiotic control. TLC chromatogram and FTIR spectra of the pure dye compound and the decolourised dye was suggestive of enzymatic degradation in accordance with biodecolourisation.

INTRODUCTION

Rapid industrialization has undoubtedly led to tremendous growth of economy, but at the same time has proved to be catastrophic for environment. Textile industry is the major consumer of synthetic dyes and utilizes enormous volumes of water for its different processes (Shah 2014a) and likewise generates huge volumes of wastewater (Saranraj et al. 2010), which in most cases is discharged directly into the adjoining surface waters (Rajendran et al. 2011, Ponraj et al. 2011). Pali, a district headquarters located in the south western Rajasthan, is known for its textile dyeing and printing work. Approximately, 800 textile dyeing and printing units situated at Pali discharge about 49 million litres per day (MLD) effluents in the Bandi River which owes its origin in Luni basin (Rathore 2012). The untreated effluents flow regularly in the dry bed of river about 45 km downstream Pali city (Khandelwal & Chauhan 2005). Dyes are widely used in the textile, rubber, paper, printing, colour photography, pharmaceuticals, cosmetics and many other industries. Amongst these, azo dyes represent the largest and most versatile class of synthetic dyes (Keharia et al. 2004). Dyes usually have a synthetic origin and complex aromatic molecular structures contribute to their stability and impart recalcitrance to them. Azo dyes have found widespread use in textile industries because of the ease in synthesizing them as compared to their natural counterparts and are designated as major environmental contaminants (Shah et al. 2013).

Azo dyes are difficult to treat by conventional wastewater treatment. Existing treatment strategy for textile effluent utilizes neutralization of alkaline pH, coagulation, flocculation followed by aerobic biological treatment, but complete removal of recalcitrant dyes from effluents is not attainable, which may be attributed to the colour fastness and stability of dyes to degradation (Anjaneyulu et al. 2005). Biotreatment, most popularly known as bioremediation is the microbial clean up approach which leads to biomineralization and biotransformation of toxic chemicals to less harmful forms by using microorganisms. In addition to being cost-effective, the most satisfactory aspect of biotreatment is its being environmental friendly with minimal sludge generation (Chen et al. 2003). The process of bioremediation gains momentum if the microbes used in the bioprocess are adapted to the system rather than using the non adapted forms. Screening of potential microorganisms based on their specificity and adaptability which governs the microbial activity is a critical step in devising an effective bioprocess.

A number of bacterial isolates *Pseudomonas* sp. (Isik & Sponza 2003, Perumal et al. 2012, Soundararajan et al. 2012, Raja et al. 2013, Shah 2014b), *Proteus* sp., *Salmonella* sp., *Klebsiella* sp. (Saranraj et al. 2010, Perumal et al. 2012), *Aeromonas hydrophila* (Chen et al. 2003, Bumpus 2004), have been reported for their role in dye decolourisation process. The present study is aimed at optimizing decolourisa-

tion of azo dye, which forms the main constituent of textile wastewater generated by textile industries located at Pali, by indigenous bacterial species to attain an accelerated bioprocess.

MATERIALS AND METHODS

Chemicals and Dye Used

All chemicals and reagents used in the study were of analytical grade. The dye used in the study was of commercial grade and procured from the local market with the specifications mentioned in Table 1.

Screening and Characterisation of Indigenous Bacteria

Indigenous bacterial strains were isolated from textile effluent and qualitatively screened by plate assay (Shah 2014b). For plate assay a thin layer of cotton was wrapped onto a match stick and sterilised at 121°C for 15 minutes. A single colony was picked from the pure culture and aseptically transferred onto the plates as single spots containing BHA and incubated at 37°C for 24 to 48 hours. Following incubation, clear zones around spots were observed and diameter around spots was measured in mm and compared with uninoculated plates which served as negative or abiotic controls. Identification of strain was carried out by 16S rDNA sequencing. Upon isolation of DNA, fragments of 16S rDNA gene were amplified by PCR. Forward (27F) and reverse (1542R) DNA sequencing reaction of PCR amplicon was carried out with forward and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyser. The 16S rDNA gene sequence was used to carry out BLAST.

Acclimatization of Screened Isolate

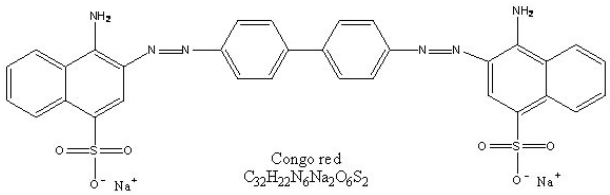
Inoculum preparation: The screened isolate was initially grown in nutrient broth (NB) with composition (g/L): Beef extract-3g, NaCl-5g, Peptone-5g and incubated at 37°C for 24 to 48 hours under agitated conditions (120 rpm) till a desired ($O.D_{660}$= 0.6) has attained (Suizhou et al. 2006).

Growth curve of isolate: The actively growing strain ($O.D._{660}$ = 0.6) in nutrient broth was inoculated as monoculture 1% v/v in a 250 mL Erlenmeyer flasks containing 100 mL of Bushnell & Haas Broth (BHB) with the following composition (g/L): Magnesium sulphate = 0.2; Calcium chloride = 0.02; Monopotassium phosphate = 1.0; Dipotassium phosphate = 1.0; Ammonium nitrate = 1.0; Ferric chloride = 0.05; pH = 7.0, supplemented with diazo dye in a minimum concentration (0.1g/L) and were incubated at 37°C for 24 to 48 hours under agitated conditions (120 rpm). Negative biotic controls were also maintained which were devoid of dye under study. $O.D._{660}$ for both the set of experiments was monitored at regular intervals.

Dye Decolourisation by Optimization of Process Parameters by OFAT Approach

Decolourisation of diazo dye by bacteria was studied in a cell free extract (CFE). Aliquots of 1.5 mL were withdrawn in 2 mL vials from the monocultures of acclimatized bacterial strains. The aliquots were then centrifuged at 10,000 rpm for 15 minutes. The decolourisation activity was monitored spectrophotometrically in cell free extract at the absorption maxima of the dye (λ_{max} = 495.5 nm) and measured as % decolourisation of dye:

Table 1: Physico-chemical properties of Direct Red 28 (Shinde & Thorat 2013)

S.No	Specification	Properties
1	CAS number	573-58-0
2	Molecular mass	696.66g/mol
3	Molecular formula	$C_{32}H_{22}N_6Na_2O_6S_2$
4	IUPAC name	Disodium 4-amino-3-[4[4-(1-amino-4-sulfonato-naphthalen-2-yl) diazenylphenyl]diazenyl-naphthalene-1-sulphonate
4	Molar extinction-coefficient	45,000[L]/[mol].[cm]
5	Absorption maxima	490-495 nm
6	Chromopheric group	(Azo)-N=N-
7	Chemical structure	 Congo red $C_{32}H_{22}N_6Na_2O_6S_2$

$$\%\ decolourisation = \frac{InitialO.D. - finalO.D}{initialO.D} * 100$$

For optimizing the process, one factor at a time (OFAT) approach was used in which one parameter under consideration was varied keeping other factors constant (Fig. 1). The parameter under consideration was fixed in a decolourisation assay and expressed as percent decolourisation as explained earlier. The process was repeated until all the parameters were optimized (Moharrery et al. 2012).

Incubation hour: Actively growing strain (O.D. $_{660}$ = 0.6) was inoculated in 100 mL BHB in 250 mL Erlenmeyer flasks amended with dye in the concentration (0.1 g/L) and incubated for 24, 48, 72 and 96 hours.

Temperature: Different temperatures, psychrophilic (4°C), mesophilic (27°C, 37°C) and thermophilic (60°C) were selected to study their effect on decolourisation whilst keeping other factors constant.

pH: The effect of pH on dye decolourisation was considered by varying the pH in the range from 2-10.

Carbon sources: Different carbon sources were used like starch, dextrose, sucrose, fructose, mannitol, glucose+ fructose. These carbon sources were amended into the medium in the concentration of 0.1% w/v prior to sterilisation.

Nitrogen sources: Different nitrogen sources were used like yeast extract, tryptone, peptone, beef extract and urea. These nitrogen sources were amended into the medium in the concentration of 0.1% w/v prior to sterilisation.

Inoculum percent: Different inoculum concentrations of 0.1%, 0.5 %, 1%,1.5%, 2% and 2.5% v/v were used in the medium.

Dye concentration: To study the effect of dye concentration on decolourisation, different concentrations of the dye were used in the range (100-400) mg/L.

Incubation condition: To study the effect of incubation condition on the process of decolourisation, two variations in incubation conditions were studied, static and shaking.

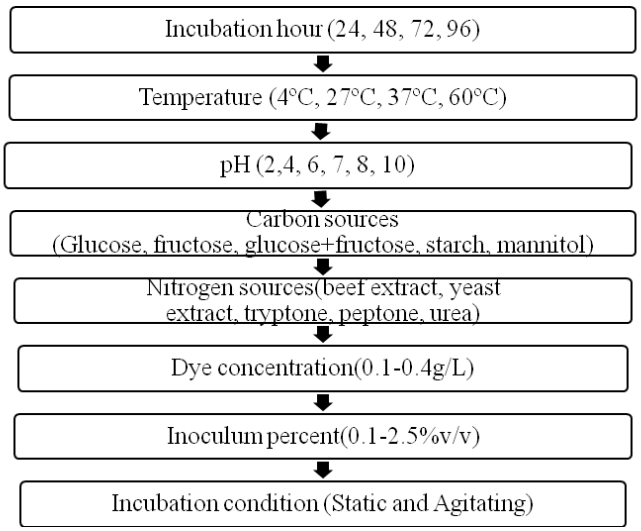


Fig. 1: Different process parametres for OFAT approach for optimisation of decolourisation process.

DYE DEGRADATION STUDIES

Thin Layer Chromatography

For initial conformational change in the di azo moiety after the treatment, thin layer chromatography (TLC) was performed with decolourised broth. The bacterial isolate was allowed to grow till the maximum decolourisation has attained. 5 mL samples were withdrawn from the decolourised broth culture in a sterile 10 mL vial. The sample was centrifuged at 10,000 g or 10,258 rpm for 15 minutes at 4°C and Cell Free Decolourised Supernatant (CFDS) was extracted with equal volume of ethyl acetate. A pinch of sodium sulphate (Na_2SO_4) was added to the extract. The extracts were evaporated to dryness and further dissolved in 1 mL of methanol. The control sample, which was only devoid of inoculum, was processed in a similar manner. 100 µL of samples were loaded as spots using micropipette on commercially available TLC plates coated with silica gel 60F 254 on aluminium foil having dimensions 1-0 cm × 5 cm × 0.25 mm (Merck, Germany). The solvent system used was water: ethanol: acetone (4:4:1) for Direct Red 28. Upon drying, the plates were observed under UV transilluminator (365 nm) (Mali et al. 2000).

FTIR Spectroscopy

Biodegradation of Direct Red 28 was finally monitored by FTIR spectroscopy. For this 100 mL sample was procured after decolourisation. Centrifugation was carried out at 10,000 rpm for 15 minutes and the metabolites were extracted from CFDS using equal volume of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum flash evaporator. The treated Congo Red dye was characterized by Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum 65) and compared with abiotic control and reference dye. The samples were mixed with spectroscopically pure KBr in the ratio of 1:100 and pressed to obtain IR-transparent pellet. The pellet was placed in sample holder and the analysis was carried out in the mid IR region of 500-4000 cm^{-1} with 16 scan speed (Shinde & Thorat 2013).

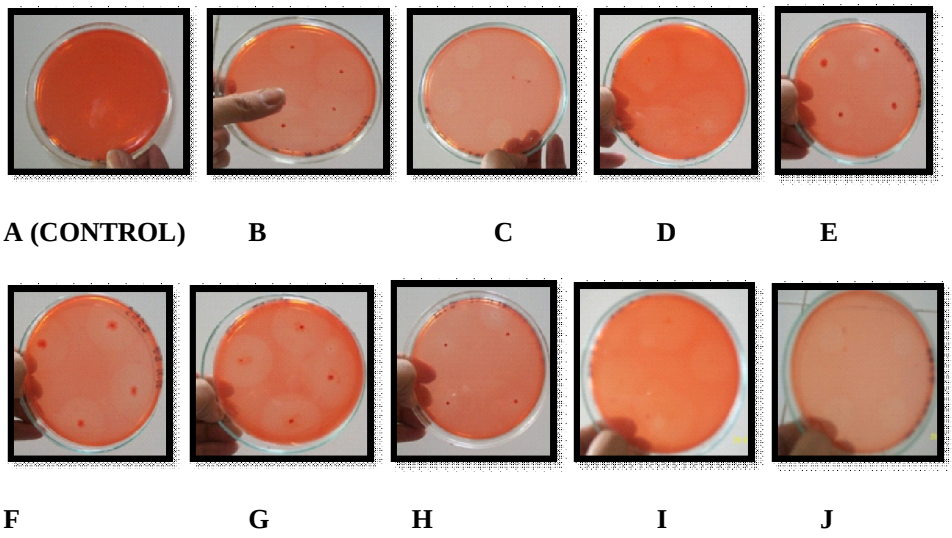


Fig. 2A: Abiotic control B-J:Plate assay of different strains representing clear zone around colonies (mm).

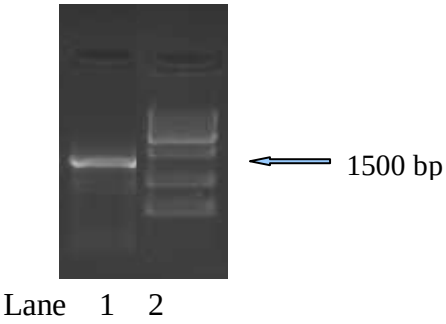


Fig. 3: Gel Image of 16S rDNA amplicon (Lane 1: 16S rDNA amplicon and; Lane 2: DNA marker).

Statistical Analysis

Data were analysed by one-way analysis of variance (ANOVA) with the Tukey-Kramer multiple comparisons test.

RESULTS

Screening of Indigenous Bacteria

A total of 9 bacterial isolates were screened at different stages of CETP for their dye decolourising potential which was expressed in terms of appearance of clear zones around colonies (Fig. 2A-J).

Strain G, encoded as Tex S6, exhibited maximum zone of clearance (0.6 mm) around colonies as compared with abiotic control, and was selected for further dye degradation studies.

Identification of Strain Tex S6

Molecular characterization of strain G revealed a single discrete PCR amplicon band of 1500 bp on agarose gel (Fig. 3). Based on the nucleotide homology and phylogenetic analysis, the strain was identified as *Alcaligenes* sp. TEXS6 (GenBank Accession Number: KF534470.1) (Fig. 4).

Acclimatization and Growth Curve Analysis of the Isolate

The effect of dye on bacterial growth profile indicated that in the presence of dye bacteria, growth was accelerated significantly ($p<0.01$), presumably utilizing dye as the sole source of energy when compared with the negative biotic control (Fig. 5). The blue line indicates growth in presence of dye and red line depicts bacterial growth in absence of dye. A significant increase in bacterial growth was observed with increase in duration of incubation.

Dye Decolourisation Studies by Process Optimization

Effect of incubation hour on decolourisation process: The duration of incubation plays a significant role in a microbially mediated decolourisation process, which may be attributed to optimal growth of the species in concern. A significant increase ($p<0.01$) in decolourisation was observed with increase in duration of incubation from 24 hours to 96 hours (Fig. 6) with 1% v/v inoculum size under static conditions with an initial dye concentration of 0.1 g/L, with no additional carbon and nitrogen sources and pH of the medium was fixed to 7 and temperature 37°C. The acclimatized strain had attained growth exponentially after 48 hours

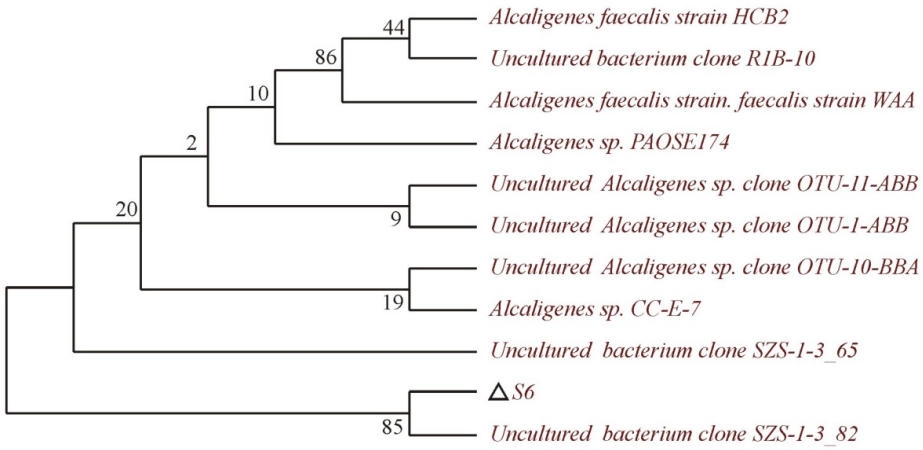


Fig. 4: Phylogenetic tree of Strain Tex S6.

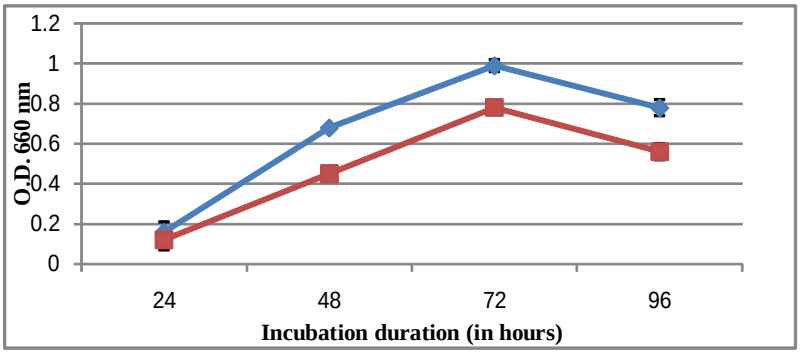


Fig. 5: Effect of Direct Red 28 on growth of *Alcaligenes* sp. TEX S6.

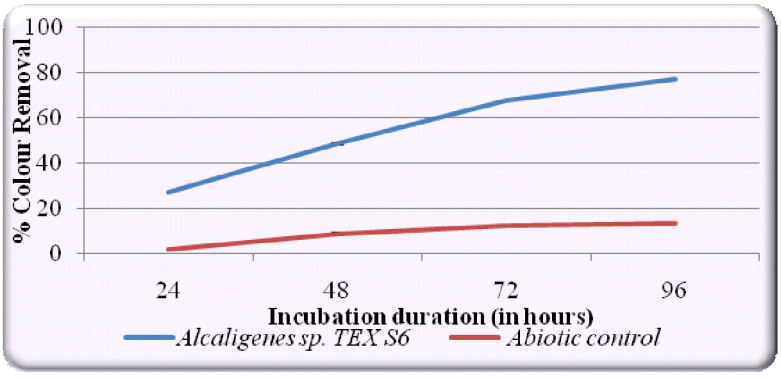


Fig. 6: Effect of incubation duration on decolourisation by *Alcaligenes* sp. TEX S6.

of incubation as elucidated earlier and dye removal efficiency was related to the growth profile of *Alcaligenes* sp. TEX S6. After 24 hours, the dye removal efficiency of the strain was found to be 26.78% which was continuously increased up to 67.41% after 72 hours. Although, a decline in growth was observed after 96 hours of incubation, but a significant increase in dye removal efficiency of the strain (77.03%) was attainable, which may be attributed to biosorption of the dye by biomass during the early stationary phase of growth. The abiotic control had witnessed minimal decolourisation in the range of 1.78%-13.48% after 96 hours of incubation.

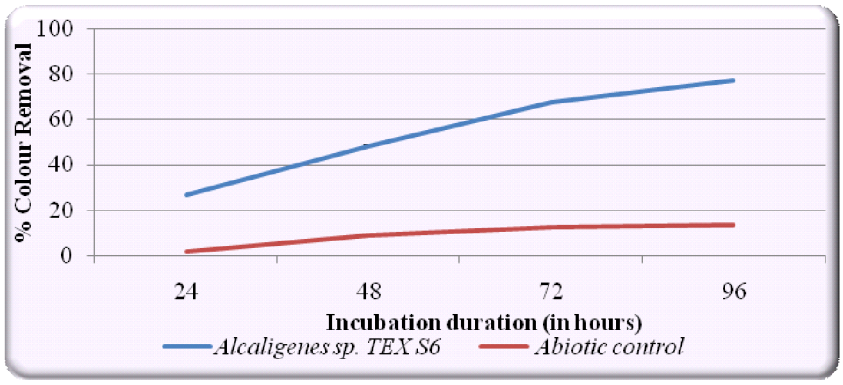


Fig. 7: Effect of temperature on decolourisation by *Alcaligenes* sp. TEX S6.

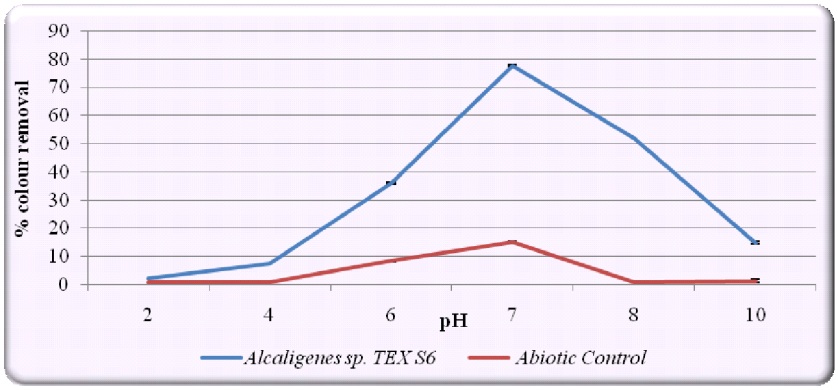


Fig. 8: Effect of pH on decolourisationby *Alcaligenes* sp. TEX S6.

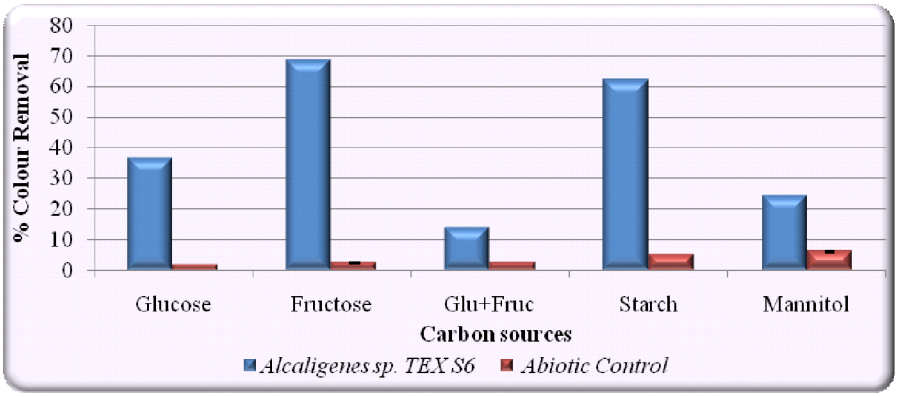


Fig. 9: Effect of different carbon sources on decolourisation by *Alcaligenes* sp. TEX S6

Effect of temperature on decolourisation process: Temperature is the quintessential factor which governs the microbial growth and enzymatic activity. The most pronounced bacterial activity is observed in mesophilic range (25°C-40°C). In our study, the best temperature for optimum decolourisation (72%) was found to be 37°C attained after 72 hours for *Alcaligenes* sp. TEX S6 with 1% v/v inoculum size under static conditions with an initial dye concentration of 0.1 g/L, with no additional carbon and nitrogen sources and pH of the medium at 7, followed by 63.9%

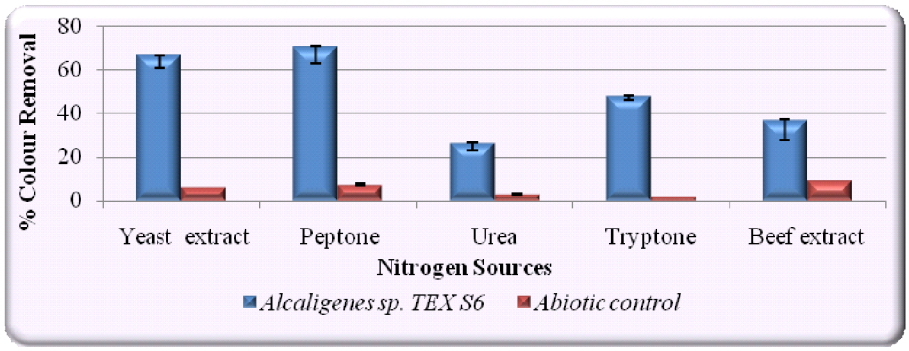


Fig. 10: Effect of different nitrogen sources on decolourisation by *Alcaligenes* sp. TEX S6.

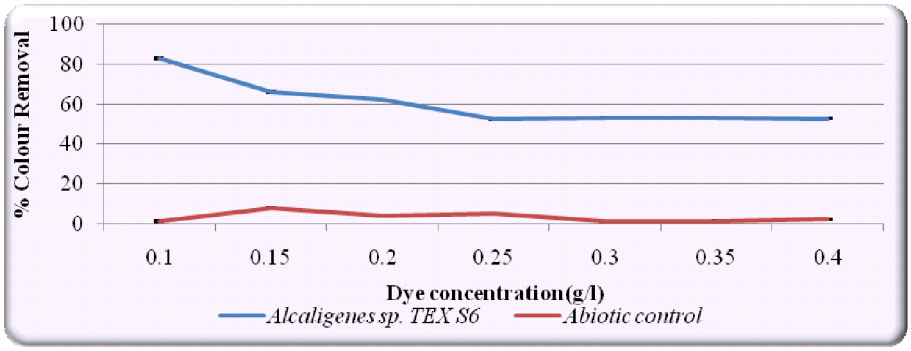


Fig. 11: Effect of dye concentration on decolourisation by *Alcaligenes* sp. TEX S6.

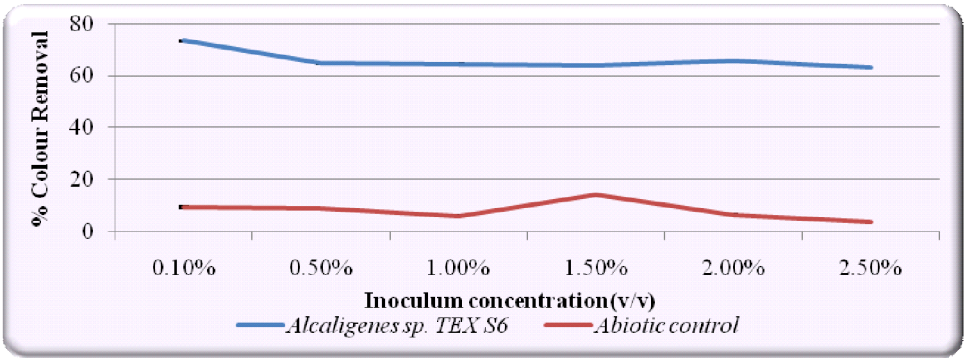


Fig. 12: Effect of inoculum size(v/v)on decolourisation by *Alcaligenes* sp. TEX S6.

(27°C) and negligible decolourisation was attained at both psychrophilic (4.63%) and thermophilic (14.06%) range under same physiological conditions which was found to be statistically different from abiotic control group (Fig. 7).

Effect of pH on decolourisation process: The variation in pH of the growth medium results in change in activity of bacteria, and hence the bacterial growth rate as well as decolourisation also gets affected. Bacteria are active over certain range of pH. The optimum pH for the growth is the same for the dye decolourising activity as it is mainly the metabolic process. A significant difference in colour removal efficacy of *Alcaligenes* sp. TEX S6 was observed at different pH values (Fig. 8). The maximum colour removing efficiency of 65.5% was observed at pH 7 at 37°C and after 72 hours of static incubation with no additional carbon and nitrogen wherein the concentration of dye was 0.1 g/L. It

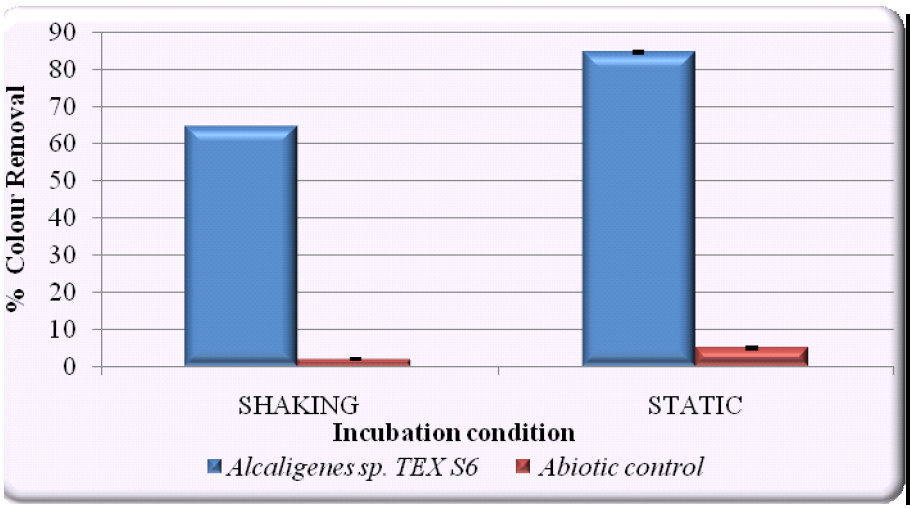


Fig. 13: Effect of incubation condition on decolourisation by *Alcaligenes* sp. TEX S6.

Fig. 14: Colour removal by *Alcaligenes* sp. TEX S6 with respect to abiotic control.

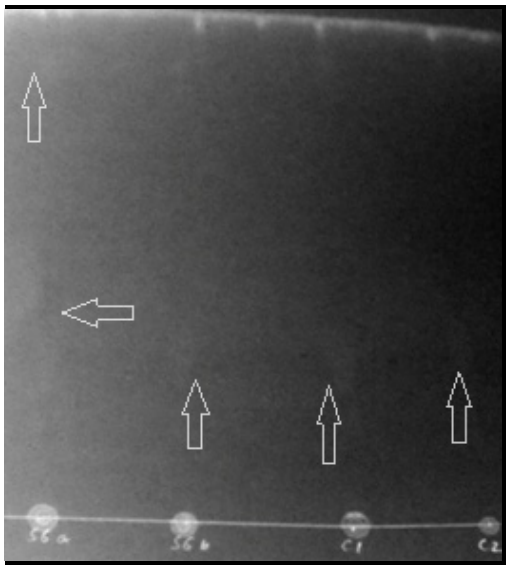


Fig. 15: TLC chromatogram representing degradation of Direct Red 28 by *Alcaligenes* sp. TEX S6 (Lane 1 and Lane 2: Decolourised medium; Lane 3 and Lane 4: Abiotic control).

was followed by 51.95% at pH 8; 35.97% at pH 6; 14.8% at pH 10; 7.4% at pH 4 and 2.34% at pH 2.

Effect of different carbon sources on the process of decolourisation: Textile effluent is usually considered as being deficient in carbon (Bhatt et al. 2012). The decolourisation process was optimized for effective biodecolourisation in presence of wide range of carbon sources like glucose, fructose, glucose and fructose in combination, starch, mannitol and lactose. Significant changes in decolourisation on utilization of different carbon sources by *Alcaligenes* sp. TEX S6 was observed. Maximum decolourisation of 68.6% at concentration of 0.1 g/L of the dye was attained within 72 hours of static incubation wherein the pH of the medium was 7, incubation temperature 37°C, and inoculum size 1% v/v in presence of fructose (1% w/v) as a co-substrate. This was followed by starch (61.9%), glucose (36.6%), mannitol (24.34%), glucose and fructose in combination (14.15%) under similar culture conditions. Very less decolourisation (1.8-6%) was observed for abiotic controls under similar culture conditions (Fig. 9).

Effect of nitrogen sources on the process of decolourisation: Nitrogen is an important factor which governs the microbial growth and henceforth the process of decolourisation which is directly or indirectly linked to microbial growth. Amongst various nitrogen sources like yeast extract, beef extract, tryptone, peptone and urea; maximum decolourisation (70.41%) by utilization of peptone and dye (0.1g/L) was attained within 72 hours of static incubation, wherein the pH of the medium was 7, incubation temperature 37°C, inoculum size 1% v/v and fructose as a co-substrate. It was followed by yeast extract (64.98%), tryptone (48%), beef extract (37%), urea (26.33%) under similar culture conditions. Statistically significant differences in decolourisation were found between abiotic control (2-9%) and different nitrogen substrates (Fig. 10).

Effect of dye concentration on the process of decolourisation: The effect of dye concentration on the decolourisation of dye by *Alcaligenes* sp. TEX S6 was studied over a range of 0.1 g/L to 0.4 g/L. A significant decrease in decolourisation was observed with increase in dye concentra-

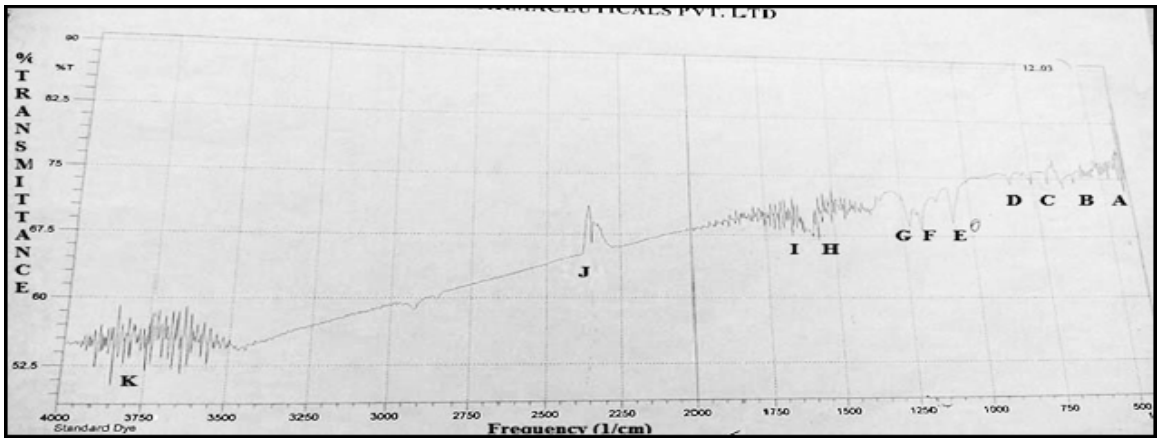


Fig. 16a: Interferogram of standard dye compound (DR 28).

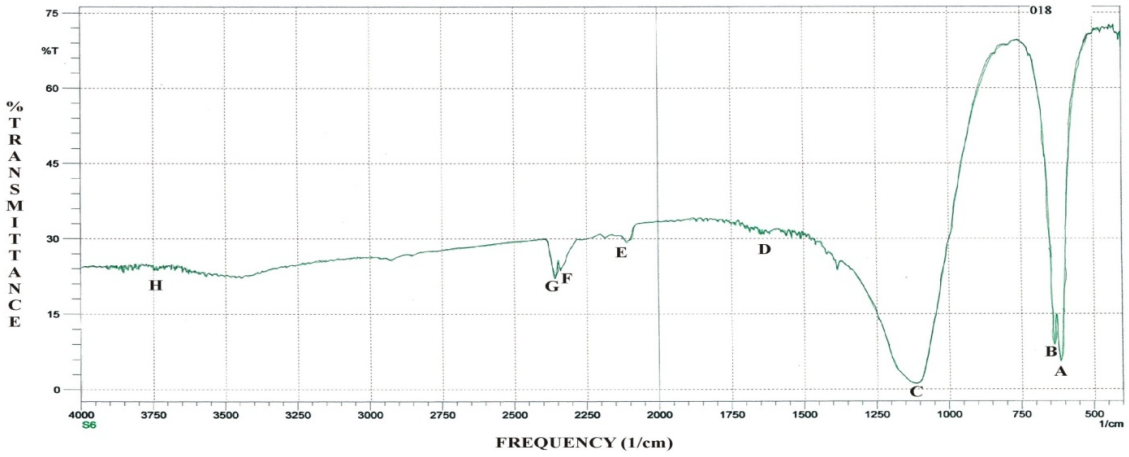


Fig. 16b: Interferogram of DR 28 treated by *Alcaligenes* sp. TEX S6.

tion from 0.1 g/L to 0.25 g/L. Further, the decolourisation attained for the dye concentration in the range from 0.25 g/L to 0.4 g/L was found to be nonsignificant and almost steady. Maximum decolourisation of 83.1% of dye at 0.1 g/L was attained after 72 hours of static incubation, wherein the pH of the medium was 7, incubation temperature 37°C, inoculum size 2.5% v/v with fructose and peptone as co-substrates. Under similar conditions of incubation, decolourisation attained with 0.15 g/L (66.09%); with 0.2 g/L (62.4%); with 0.25 g/L (52.37%); with 0.3 mg/L (53.1%); with 0.35 g/L (52.76%) and with 0.4 g/L (52.62%). Significant differences in decolourisation with respect to abiotic control (1.2-7.9%) were appreciable (Fig. 11).

Effect of inoculum size on the process of decolourisation: The inoculum size is also an important factor upon which the decolourisation is dependent. With increase in inoculum size from 0.1% v/v to 2.5% v/v, the decolourisation efficiency of *Alcaligenes* sp. TEX S6 marginally increased. After 72 hours of incubation under static conditions at 37°C, pH 7, fructose and peptone (both 1% w/v) with 0.1% v/v, the decolourisation of dye (0.1 gm/L) was found to be 63.31% which was non significantly increased to 67.98% at 2.5% inoculum size; although significant difference in decolourisation by abiotic controls (4-14.4%) was observed (Fig. 12).

Effect of incubation condition on the process of decolourisation: A significant difference in the decolourisation potential of *Alcaligenes* sp. TEX S6 was observed when incubation conditions were varied from static to agitating. After 72 hrs of incubation under static conditions at 37°C, pH 7, fructose and peptone (both 1% w/v) with 2.5 % v/v inoculum concentration, the decolourisation of dye (0.1 g/L) was

found to be 84.7%, whereas keeping all the culture conditions same with a variation in incubation condition from static to agitating, decolourisation was found to be 64.66% (Table 13 & 14).

Dye Degradation Studies

Thin layer chromatography: The dye decolourisation study of *Alcaligenes* sp. TEX S6 was further supported by thin layer chromatography (TLC) analysis. Upon observation under UV light (365 nm), TLC chromatogram comprised of four lanes (Fig. 15). Lane 1 and Lane 2 comprised of treatment group which represented biotic decolourised medium, whereas Lane 3 and Lane 4 comprised the abiotic control which was devoid of the strain. The spot corresponding to R_f value of Direct Red 28 = 0.35 (Table 2) was observed in abiotic control and no spot corresponding to this R_f value was observed in the decolourised medium indicating that decolourisation was due to its degradation into unidentified intermediates. Additionally, in Lane 1 and Lane 2, two spots corresponding to R_f values 0.87 and 0.51 (Table 2) were observed, possibly indicating the degradation of dye by induced enzymes like plausibly azoreductases.

Fourier Transform Infra Red (FTIR) spectroscopy: FTIR spectrum of the parent dye compound essentially revealed peaks at 515-690 cm^{-1} (A- alkyl halides); 610-700 cm^{-1} (B- alkynes); 720-725 cm^{-1} (C- alkanes); 1050 cm^{-1} (D- aliphatic amines); 1150 cm^{-1} (E- alkyl halides); 1200 cm^{-1} (F- aliphatic amines); 1250 cm^{-1} (G- aromatic amines); 1550 cm^{-1} (H- nitro compounds) which is characteristic feature of an azo group (-N=N-); 1600 cm^{-1} (I- aromatics); 2250-2500 cm^{-1} (J- nitriles and alkynes) (Fig. 16 a). The FTIR spectrum of metabolites obtained after decolourisation of Direct Red 28 by *Alcaligenes* sp. TEX S6 showed complete disappearance of peaks at 1550 cm^{-1} (C) characteristic of -N=N- group indicates that the dye has been degraded by bacteria and possibly the azo bond has been cleaved by its enzymatic activity. Additional peaks at 2050 cm^{-1} (D), indicative of C=C for acetylinic compounds stretching vibration, indicates formation of an additional product or metabolite of bacterial activity (Fig. 16b).

DISCUSSION

The bacterial strain *Alcaligenes* sp. TEX S6 isolated from activated sludge of CETP exhibited a tremendous potential in degrading the dye (84.7%) under optimized process parameters like incubation conditions and effect of co substrates. Previously, *Aeromonas hydrophila* strain DN322 was isolated from activated sludge of textile printing wastewater treatment plant, Guangzhou, China, and found to decolourise a wide variety of synthetic dyes including azo, anthroquinone and triphenylmethane (Suizhou et al. 2006). A multitude of reports pertaining to decolourisation of textile dyes by *Alcaligenes* sp. isolated from textile effluent and soil contaminated with dyes are widely available hence suggesting *Alcaligenes* sp. to be a promising isolate for dye decolourisation studies (Pandey & Dubey 2012, Sethi et al. 2012, Palani Velan et al. 2012, Vivekanandan et al. 2013). Our study revealed that *Alcaligenes* sp. TEX S6 decolourised dye within 72 hours of incubation. The duration of incubation is an important factor which governs the process of decolourisation, which is a catabolic process carried out by enzymes or biosorption as the process is found to be growth linked (Sridevi & Rao 2013). Complete degradation of Congo Red had been attained by *Bacillus megaterium* DMZ 32 (X60629) in 16 hours of incubation, isolated from sugarcane industrial wastewater (Pradhan et al. 2011). In our study, the optimum temperature for effective decolourisation by *Alcaligenes* sp. TEX S6 was found to be 37°C which supports the findings of previous studies conducted. Maximum decolourisation of Remazol Black B by *Bacillus* ETL-2012 was observed at 37°C (Shah 2013). The hydrogen ion concentration has a profound effect on the efficacy of the decolourisation process and the optimal pH for obliteration of colour from the system usually ranges from 6-10 (Kilic et al. 2007). The optimum pH in our study was in accordance with previous studies where optimum pH for removal of Congo Red by *Pseudomonas stutzeri* ETL-4 has been found to be 7 (Shah 2014b). The azo dye reduction and the rate is dependent upon the presence and availability of a co-substrate because it acts as an electron donor (Derle et al. 2012). The rate of azo reduction process also depends on the type of co-substrate used and chemical structure of azo dye. In our study, the best co-substrates were found to be fructose (70.41%) and peptone (68.6%). 100% decolourisation of Reactive Red R195 was attained by *Micrococcus glutamicus* NCIM 2168 in the presence of sucrose followed by 90.05% in presence of glucose while 88-89% of decolourisation was shown in presence of yeast extract and peptone (Leelangkriangsak & Borisut 2012). The dye concentration in which 83.1% decolourisation was attained by *Alcaligenes* sp. TEX S6 was 0.1 g/L (Verma & Madamwar 2003) which suggests that decolourisation decelerates with increase in dye concentration, which may be attributed to stronger inhibition at high concentration. In-

Table 2: Summary of TLC chromatogram indicating presence of additional spots in biotic group.

Groups	Solvent(cm)	Spots(cm)	R_f value
Biotic (Lane 1 and 2)	13.4	11.7, 6.9	0.87, 0.51
Abiotic (Lane 3 and 4)	13.4	4.8	0.35

oculum size also plays an important role in decolourisation activity as the process is growth linked. In our study, the inoculum size, which yielded 67.98% decolourisation by *Alcaligenes* sp. TEX S6, was found to be 2.5% v/v. A 4% v/v inoculum has been used to attain decolourisation of textile effluent by *Bacillus subtilis* and *Streptococcus faecalis* isolated from activated sludge (Rajeshwari et al. 2012). The literature suggests that the process of decolourisation is more favoured in static conditions as opposed to agitating conditions, because under aerobic conditions azo dyes are generally resistant to attack by bacteria (Hu 1994), and our study was also in accordance with decolourisation being on a higher side under static incubation conditions. *Bacillus boro-niphilus* showed about 100% decolourisation of Reactive Yellow 145 within 9 hours under static conditions (Derle et al. 2012). Whereas as opposed to our findings, Shah (2014b) reported 61.8% of Congo Red under agitating conditions as opposed to 57% under static conditions.

Thin layer chromatography preliminarily investigates the degradation of dyes which may be associated with expression of enzymes. Our TLC chromatogram was suggestive of dye degradation as the R_f value of abiotic control sample was 0.35, whereas that of decolourised dye was found to be 0.57 and 0.81. Previous study on degradation of Reactive Red 195 by *Micrococcus glutamicus* NCIM 2168 suggested R_f value of pure dye as 0.9, whereas the degraded product showed R_f value 0.6 (Sahasrabudhe et al. 2012).

FTIR studies conducted to confirm the degradative properties of indigenous microbes have been phenomenal in suggesting the role of inducible enzymes, which carry out the metabolic process of dye degradation. Our study revealed that the dye was degraded as the peak at 1587.66 cm^{-1} for N=N stretching vibrations, is characteristic of the diazo bond and its complete disappearance suggests the degradation into acetylinic components exhibiting stretching vibration at 2050 cm^{-1} which was in accordance with the study conducted previously aimed at establishing the bioefficacy of *Fusarium* sp. to degrade Congo Red dye (Shinde & Thorat 2013).

CONCLUSION

This work was carried out to screen, characterize the isolated bacteria from activated sludge of a common effluent treatment plant. The most potential strain was found to be *Alcaligenes* sp. TEX S6 identified by 16S rDNA sequencing. The strain removed 86.7% of Direct Red 28 dye which finds its common usage in textile industry. FTIR analysis revealed that the dye had been removed from the culture medium which was expressed as reduction in absorption maxima of dye. The process of decolourisation was attributed to degradation of dye.

Hence, there is an urgent need for simple and cost-effective treatment methods for this problem of water pollution caused by untreated or partially treated textile effluents released in surface waters. Microbial degradation with decolourisation of dyes gives us a hope to circumvent this problem as it is environment friendly, cost-effective and produce no harmful intermediates.

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