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DECOLORIZATION AND DEGRADATION OF TEXTILE DYES BY *STENOTROPHOMONAS MALTOPHILIA* RSV-2

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Short Profile

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ABSTRACT:
Dye waste effluent samples were collected from textile dyeing unit as well as CETP located in Tirupur. Strains having decolorizing ability of various textile reactive dyes were obtained from primary, secondary screening and acclimatization study. *Stenotrophomonas maltophilia* RSV-2 strain could able to degrade the mixed dyes were isolated and identified based on biochemical and 16S ribosomal RNA gene sequence study. Optimization of yeast extract composition, pH, temperature, salinity and biomass composition was determined. Decolorization performance of individual dyes using two different substrates was studied.

KEYWORDS
decolorization, dyewaste effluent, Stenotrophomonas maltophilia, textile dyes.

INTRODUCTION

Environmental pollution is one of the major problems of the modern world. On one hand, industrialization is necessary to satisfy the needs of the world's overgrowing population but on the other hand, it threatens life on earth by polluting the environment. The problem of environmental pollution is increasing day by day due to the release of xenobiotic substances into water, soil and air. These substances include organic compounds (pesticides, dyes, polymers etc.) and heavy metal ions. The damage caused by these pollutants to plants, animals and humans cannot be neglected and hence strategies must be developed to solve the problem of environmental pollution on priority basis (Ali and Muhammad 2008). In India, an average mill discharges about 1.5 million litres of contaminated effluent per day, which leads to chronic and acute toxicity (Sheth and Dave, 2009).Considering both the volume generated and the effluent composition, textile industry wastewater is rated as the most polluting among all industrial sectors (Mezohegyi et al., 2009).Approximately 10,000 different dyes and pigments are used industrially, and over 0.7 million tons of synthetic dyes are produced annually, worldwide. In 1991, the world production of dyes was estimated 668, 0002 of which azo dyes contributed 70 %. During dying process, a substantial amount of azo dye is lost in a wastewater. The routine use of dyes in day-to-day life is increasing because of rapid industrialization, most widely in textile, rubber, and enamel, plastic, cosmetic and many other industries (Raffi et al. 1997). Dye wastewaters are usually treated using physicochemical methods such as flocculation, coagulation, adsorption, membrane filtration, precipitation, irradiation ozonization and Fenton's oxidation (Lodha and Choudhari, 2007). Recently, coupled chemical–biological treatment technologies used for the mineralization and/or decolorization of dyes as these technologies are most acceptable for the recalcitrant compounds like dyes (Fongsatitkul *et al.*, 2004).These methods are effective but may generate significant amounts of chemical sludge, whose disposal in secure landfill increases process cost (Kumar *et al.*, 2005).

METHODS

Sample collection

Effluent samples were collected from textile dying unit and CETP located in Tirupur. In our previous study, the effective decolorizing strains were isolated based on the primary, secondary screening and acclimatization study (Rajeswari et al., 2011). Ten different mixed reactive dyes were utilized for adaptive study namelyYellow ME4GL, Blue RR, Red RR, Yellow RR, Red M5B, Blue MR, Deep Black RR, Yellow MERL, Red ME4BL and Golden Yellow MR. All the experiments were performed in Mineral Salts Medium (MSM) of pH 7.0 contained (g L-1) the following composition NaCl (1.0), $CaCl_2 \cdot 2H_2O$ (0.1), $MgSO_4 \cdot 7H_2O$ (0.5), KH_2PO_4 (1.0) and Na_2HPO_4 (1.0).

Identification of the strain

Strains were identified based on biochemical and 16S ribosomal RNA gene sequence study. A pure colony of the bacterium was grown in LB medium until log phase growth was obtained. The resulting growing bacteria were obtained with centrifugation. Genomic DNA from this isolate was

extracted with the Bacterial Genomic DNA Isolation Kit (RKT09). The rDNA fragment of ~1.4kb was amplified using high –fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward, reverse primer.The Forward and reverse Prokaryotes 16s rRNA specific primers were16s Forward Primer:5'-AGAGTRTGATCMTYGCTWAC-3 and 16s Reverse Primer:5'-CGYTAMCTTWTTAC GRCT-3'. The reaction mixture contained 1µl of template DNA, 400 ng of each 16s forward and reverse primers, 4 µl of dNTPs (2.5 mM), 10 µl of 10x DNA polymerase assay buffer,1.5mM of MgCl2 as final concentration, 1 µl of Taq DNA polymerase enzyme (3U/ µl), water to make the final volume as 100 µl. The PCR conditions were Initial denaturation of 94 for 5 min, Denaturation of 94 for 30 sec, Annealing of 55 for 30 sec, Extension of 72 for 2 min and Final extension of 72 for 15 min. Number of Cycles were 35.The reaction was carried out in Thermal Cylcer ABI2720. The sequencing mixture contained 1 µl of Template (100ng/ µl), 2 µl of Primer (10pmol/ µl), 3 µl of Milli Q water and the conditions were Initial Denaturation of 96°C for 1min followed by Denaturation of 96°C for 10 sec followed by Hybridization 50 °C for 5 sec finally Elongation of 60 °C for 4 min for 25 cycles. The nucleotide sequence was determined automatically using Big Dye Terminator v3.1 Cycle Sequencing Kit in the ABI PRISM 3130- Genetic Analyzer with universal primers. DNA sequence analyses were performed using the Blast search and was aligned with sequences from other bacteria in the GenBank database of NCBI using CLUSTAL W .The phylogenetic tree was constracted using Mega 4.0 software

Optimization of physico chemical parameters

Effect of yeast extract (0.05, 0.1, 0.2, 0.4, 0.8 % (w/v), pH (3, 5, 7, 9 and 11), Temperature (28, 30, 37, 45, and 50ºC), Salinity (1, 3, 5, 7, 9 %) and biomass concentration (4, 8, 12, 16, and 20 %) on decolorization was studied. The culture was inoculated in Nutrient broth medium incubated at 30 ºC (temperature which found at the time of sample collection) for 12 hours .After incubation, cells was pelleted (6000×g for 20 min) and the growth was monitored spectrophotometrically at 600nm. About 1.0 OD at 600 nm was utilized for optimization parameter study. The medium to inoculums was maintained at 50:1 ratio. All the Experiments were performed in Mineral salt medium and triplicates were maintained for all the experiments.

Effect of individual dyes on decolorization ability by the strain

The decolorization ability of the strain was tested against individual dyes with the concentration of 1000 mg/l. two different substrates were utilized namely yeast extract and soya chunk powder . The absorbance was measured at its maximum wavelength against a blank. The percentage of decolorization was calculated as follows:

% Decolorization = Initial absorbance - Observed absorbance/Initial absorbance x 100

RESULTS AND DISCUSSION

Dyes of different structures are often used in the textile processing industry, and, therefore, the effluents from the industry are markedly variable in composition. A non specific biological process may be vital for treatment of the textile effluents containing a mixture of dyes (Chen et al., 2003).According

to the reports (Knapp and Newby, 1995; Sani and Banerjee, 1999) decolorization of dyes by bacteria can be due to adsorption to microbial cells or to biodegradation. The Adaptive study was conducted by adding dyes in increasing concentration in the decolorized medium and the nature of the strain in high concentration of mixed dyes reavealed, that the strain could decolorized the reactive mixed dyes up to 2100 ppm concentration of in 67 h with 58 percentage.The decolorization of 100 ppm concentration was achieved in 5 h and 20 min with 94 percent decolorization. As the dye concentration increased the decolorization performance decreased. The results were elaborated in (table 1). The decolorization rate was found to be 18.07 mg h-1. Decline in decolorization rate may be due to the poisonous effect of the dye on bacteria, obstruction of active sites of azoreductase enzyme by complex dye structure and/or insufficient production of biomass for decolorization of higher concentrations of dye (Shah and Rao 2011). Adaptation of a microbial community towards toxic or recalcitrant compounds is found to be very useful in improving the rate of decolorization process (Dafale et al., 2008). The adaptation of microorganisms to higher concentrations of pollutants is called acclimatization and leads to forced or directed evolution (Ali, 2010). An adaptation procedure was used to acclimatize the microbes (Ogawa et al., 1981) to an increasing concentration of methyl red as the carbon source (Adedayo et al., 2004). The single flask procedure of adaptation (Watson, 1993) without transfer of microorganisms was employed. Moreover, the time required for decolorization increased with increasing dye concentrations. This might be attributed to the toxicity of dye to bacterial cells through the inhibition of metabolic activity, saturation of the cells with dye products, inactivation of transport system by the dye or the blockage of active site of azoreductase enzymes by the dye molecules (Mabrouk and Yusef, 2008).

Charcteristics of *Stenotrophomonas maltophilia*

The strain is Gram Negative in nature The biochemical test result showed that the strain gave negative for Indole, V-P test, Citrate Utilization Test, Sucrose, Xylose, Mannitol, Arabinose, Hydrolysis of starch, Catalase, Gelatin liquefaction and Phenylalanine deaminase and positive for TSI Test (A/A), Glucose, Lactose, Oxidase and Nitrate reduction test(table:2). Presence of growth at temperature (10–50°C) and NaCl (2.0–7.0%) was observed. 16S rRNA phylogenetic analysis also BLAST n result reavealed that strain belongs to *Stenotrophomonas maltophilia*. The sequence was deposited in the GenBank database under accession number JF502570 (fig:1) . A bacterial strain AAP56, isolated from a polluted soil (from Kelibia city) and identified as *Stentrophomonas maltophilia*, was particularly interesting for its ability to decolorize recalcitrant dyes of an industrial effluent: SITEX Black (Galai et al., 2010). This strain can also decolorize some synthetic dyes: Methylene Blue, Toluidine Blue, Methyl Green, IndigoBlue, Neutral Red, Congo red, Methyl Orange and ReactivePink (Galai et al. 2009).A rod-shaped, gram-negative bacterium *Stenotrophomonas* sp. SMSP-1 was isolated from the sludge of a wastewater treating system of a pesticide manufacturer. Strain SMSP-1 could hydrolyze methylparathion to p-nitrophenol (PNP) and dimethyl phosphorothioate but could not degrade PNP further. Strain SMSP-1 was able to hydrolyze other organophosphate pesticides, including fenitrothion, ethyl parathion, fenthion, and phoxim, but not chlorpyrifos (Shen et al., 2010).

Effects of yeast extract concentration on decolorization

Effects of yeast extract concentration on maximum decolorization was studied by adding the medium with 0.05, 0.1, 0.2, 0.4, 0.8 % of yeast extract along with 100 ppm of mixed dye in 100 ml containing MSM. The maximum decolorization was obtained in medim containing 0.8% of yeast extract. Percentage decolorization ranged from 35.35-98.04 in increasing concentration of yeast extract . The highest biomass was found to be 1.935 OD in 0.8% medium. The increases in concentration of yeast extract result in increase growth rate. Eventhough our isolate performed maximum decolorization at 0.8% yeast extract(fig:2a). Considering the expensive nature of yeast extract, we performed our further experiments having only 0.4% of yeast extract to minimize the effect of cost. The metabolism of yeast extract is considered essential to the regeneration of NADH that acts as the electron donor for the reduction ofazo bonds .It had also been found that increasing yeast extract concentrations (from 0 to 10 g l_{-}^{1}) resulted in higher decolorization rates, and the decolorization rates reached a plateau as yeast extract was higher than 8 g l_{-}^{1} (Chen et al., 2003). *Bacillus* sp. VUS decolorized azo dye Navy blue 2GL in 48 h at static anoxic condition in yeast extract medium. The time required for 94% decolorization of 50 mg/l dye was 48 h. Yeast extract was best medium for faster decolorization than other media (Dawkar et al., 2009).The use of yeast extract for increased decolorization of dye has also been reported (Chang and Lin 2000; Khehra et al., 2005).

Effect of pH on decolorization

The effect of pH on decolorization was investigated in the pH range of 3, 5, 7, 9, and 11. It was found that change in pH significantly affect the decolorization rate. The isolates shown good growth at pH 7 and 9, while at 3,5 was found to be inhibitory for the growth. Bacterial cultures generally exhibit maximum decolorization at pH values near 7.0. the percentage of decolorization and biomass OD was found to be 94.83, 96.44 and 1.219, 0.725 at pH 7 and 9 respectively(fig 2b). Our isolates could able to decolorize the dye even in the pH of 11, but the incubation time required for the process took 3-4 days to achieve maximum decolorizaion of 70-80% in both the isolates (data not shown). So these indigenous isolate can be effectively utilized for real textile dyes having the pH of neutral to alkaline condition. A significant increase in decolorization was observed as the pH increased from 7 to 9. However, relatively a rapid decrease in decolorization was found when pH was increased from 9 to 11. It is thought that the pH effect may be more likely related to the transport of dye molecules across the cell membrane, which was considered as the ratelimiting step for the decolorization (Lourenco et al., 2000).

Effect of temperature on decolorization

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 influence of temperature on decolorization was performed in the temperature range of 26ºC, 30ºC, 37ºC, 45ºC, and 50ºC. The maximum decolorization was found to be 99.42, 99.9 and 85.21 in the temperature range of 30ºC, 37ºC and 45ºC respectively. The biomass OD was found to be in the range of 0.9-1.000 in that temperature (fig 2c). The temperature conditions studied in the present investigation were selected based on the average high and low temperatures of the concerned zone.

Optimal temperature to decolorize mixed azo dye for RSV-1 and RSV-2 strains was 30°C and 37°C. However, decolorization rate in the strains dropped sharply as the temperature increased from 45 to 50°C. After that, there was a significant decrease in the decolorization of dye as the temperature increased from 45 to 50°C. Most textile and other dye effluents are produced at relatively high temperatures and hence temperature will be an important factor (Srinivasan and Viraraghavan 2010).

Effect of salinity on decolorization

Wastewaters from textile processing and dyestuff manufacture industries contain substantial amounts of salts in addition to azo dye residues (Khalid et al., 2008). Salt concentrations up to 15–20% have been measured in wastewaters from dyestuff industries (EPA 1997). Hyper-salinity chemical industry wastewaters often contain a range of chemicals, which are recalcitrant to biodegradation. For example, Reactive dyes are very soluble by design and, as a result, not all are used up by textile fibers during the dyeing process and therefore end up with the discharge from dye houses (Pearcea et al., 2003). Strain achieved maximum decolorization and the biomass OD of 93.03, 82.72 and 0.927 and 0.835 at 1 and 3 NaCl concentrations respectively (fig 2d). The maximum decolorization was achieved only after 6th day of incubation. Wastewaters from textile processing and dye stuff manufacture industries contain substantial amounts of salts in addition to azo dye residues (Khalid et al., 2008). Salt concentrations up to 15–20% have been measured in wastewaters from dyestuff industries (EPA 1997). Thus, microbial species capable of tolerating salt stress will be beneficial for treating such wastewaters.

Effect of biomass concentration on decolorization

Effect of biomass concentration on decolorization was performed by adding the medium with 1.0 OD culture of 4, 8, 12, 16, and 20 % concentration inoculum. When the concentration of yeast extract increased, the growth and percentage decolorization also increased. RSV-2 achieved 51.11, 58.37, 92.16, 94.15 and 98.14 % decolorization at inoculum concentrations of 4, 8, 12, 16 and 20% respectively and the OD was 1.13 and 1.77 at 16 and 20% respectively. There was no significant difference in percentage removal at 12, 16 and 20% inoculum concentrations and hence 20 % inoculum concentration was selected for further experiments (fig 2e).A trend of increase in decolorization was noticed with increase in inoculums concentration. However, beyond 10% (v/v) inoculum size, rate of increaseing decolorization was not very significant (Mathew and Madamwar 2004).

Effect of yeast extract and soya chunk powder on decolorization of individual dye by *Stentrophomonas maltophilia*

Strain was inoculated in optimized MSM contains yeast extract, pH, temperature, dye concentration and inoculam concentration of 0.4%, 7.0, 37°C, 1000 mg l^{-1}, and 1.0 OD respectively. Static condition was maintained throughout the experiment. The ten structurally different reactive dyes with the same concentration (1000 mg l^{-1}) used in this study were most of the dyes efficiently decolorized by RSV-2 in 24 h. A maximum decolorization extent of 90% was recorded in Reactive Yellow MERL, Red ME4BL and Golden Yellow MR, and for the other Blue RR, Red M5B and Deep Black RR dyes, the value varied from 80-90%. Yellow RR and Yellow ME4GL achieved 52% and 14% respectively (table

3). No significant different was observed when soya powder used. But the decolorization percentage of blue MR was slightly increased to 37%.The time required for decolorization and total percentage decolorization was varied for different dyes. Variation in decolorization efficiency and time required for decolorization may be due to structural differences of dyes (Paszczynski et al., 1992). M. glutamicus strain was applied to decolorize a mixture of ten reactive dyes (Reactive Green 19A, Reactive Yellow 17, ReactiveRed 2, Reactive Orange 4, Reactive Blue 171, Reactive Orange 94, Reactive Blue 172, Reactive Red 141, Reactive Red 120, and ReactiveBlue 59) at a concentration of 50 mg $l_{\_}^{1}$ and 37°C under static condition. Moreover showing a 63% decolorization within 72 h (Saratale et al., 2009).

CONCLUSION

The effective strain having a potential decolorizing ability was isolated from dye waste effluent. The strain can be effectively utilized for the treatment of real textile dye effluent containing high concentration of dyes with optimized physic chemical parameters.

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Table: 1 Adaptive study in mixed dye

Step	Total dye added in medium (ppm)	Time (h)	Decolorization (%)
1	100	5.20	94
2	200	2.45	92
3	300	4.30	86
4	400	8.10	71
5	500	15.00	63
6	600	32.00	58
7	600	-	-
Total	2100	67	77.33

Table: 2 Morphology, Cultural and Biochemical Characterization of isolated Strains

Biochemical and culture conditions	RSV-2
Gram staining	Gram-negative
Indole	-
Methyl red	+
V-P test	-
Citrate Utilization Test	-
TSI Test	A/A
Glucose	+
Lactose	+
Sucrose	-
Xylose	-
Mannitol	-
Arabinose	-
Hydrolysis of starch	-
Catalase	-
Oxidase	+
Gelatin liquefaction	-
Nitrate reduction	+
Phenylalanine deaminase	-
Growth at temperature (10–50°C)	+
Growth on NaCl (2.0–7.0%)	+
Morphology under Microscope	
Cell type (shape)	Short Rods
Color	Yellowish
Surface	Smooth/mucoid
Arrangement	cluster
Density	Opaque
Elevation	Convex
Motility	Positive

+ Positive reaction, - negative reaction

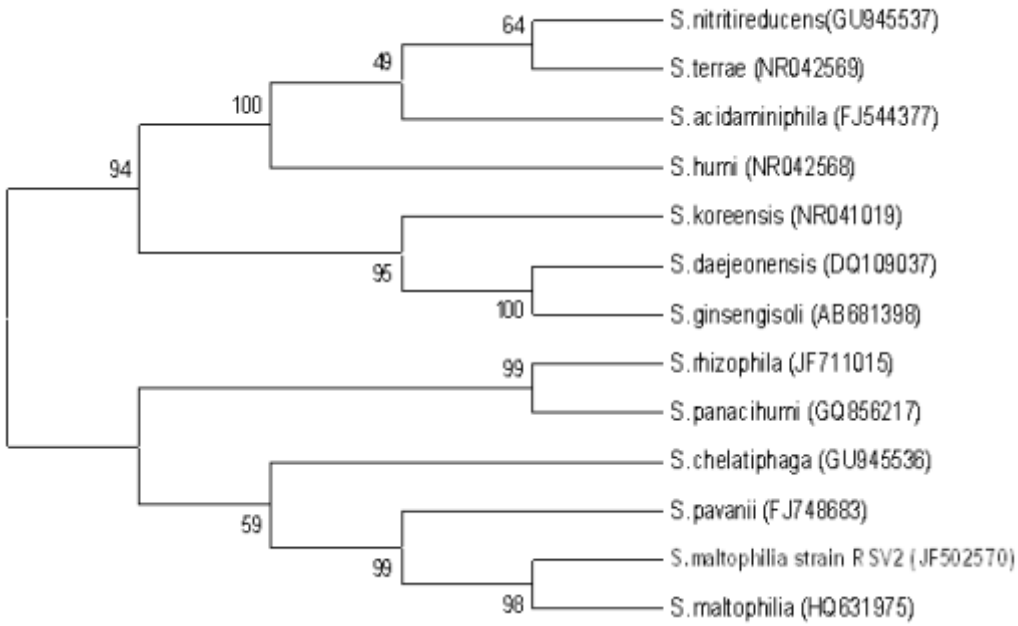

Fig: 1 Phylogenetic analysis of 16 s rRNA sequence of Stenotrophomonas maltophilia strain RSV-2 using MEGA-4.0 software. The percent numbers at the nodes indicate the levels of ootstrap support based on neighbor-joining analyses of 500 replicates. Brackets represent sequence accession

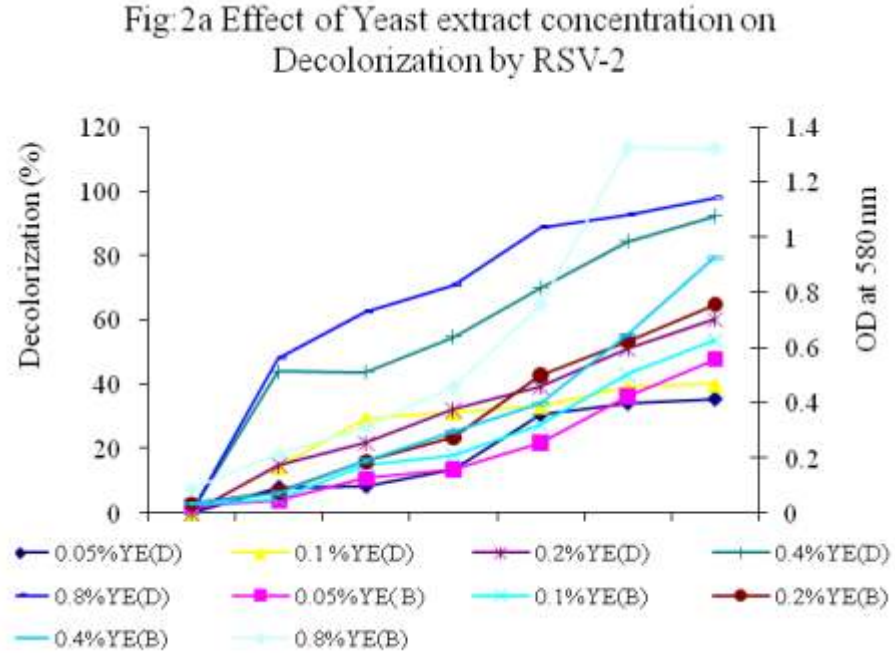

Fig:2 b Effect of Various pH Ranges on Decolorization by RSV-2

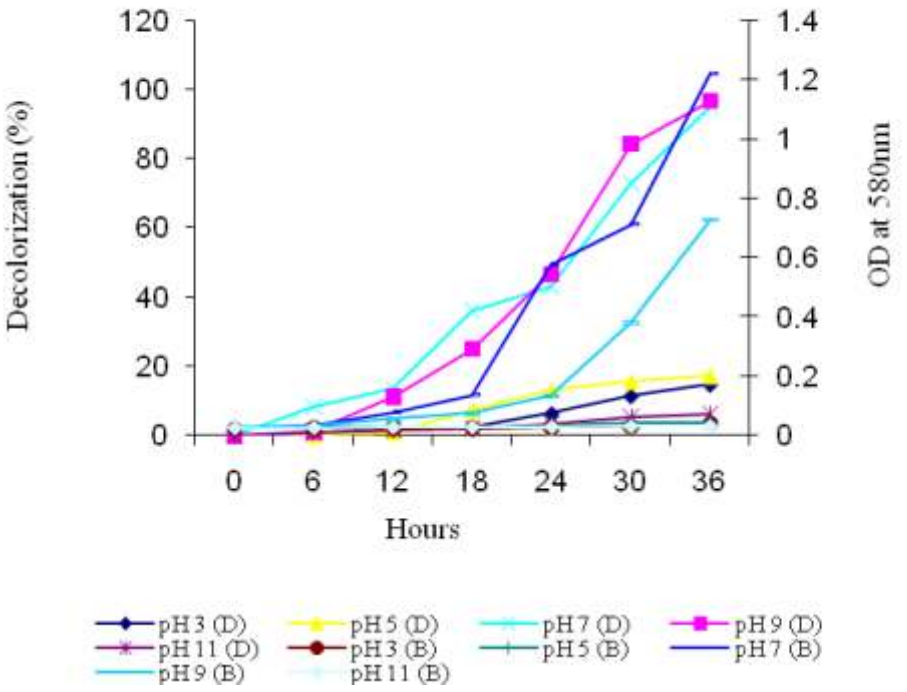

Fig :2 c Effect of Various Temperature on Decolorization by RSV-2

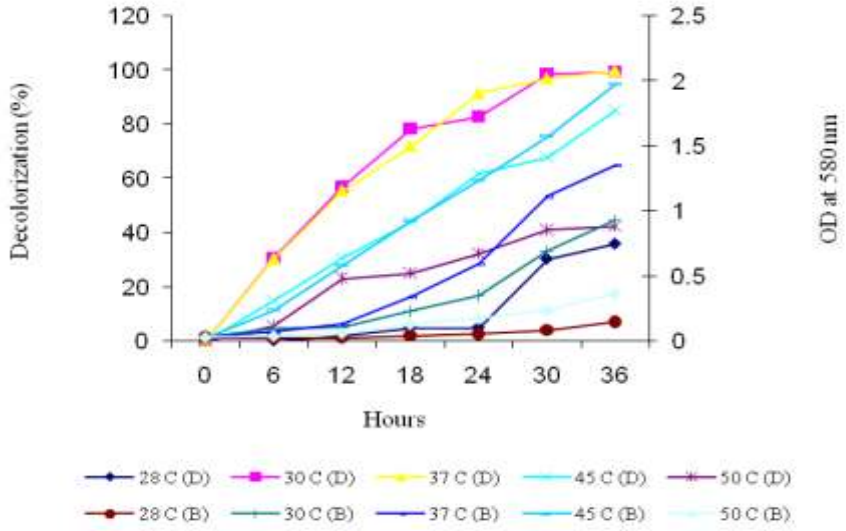

Fig :2 d Effect of NaCl Concentration on Decolorization by RSV-2

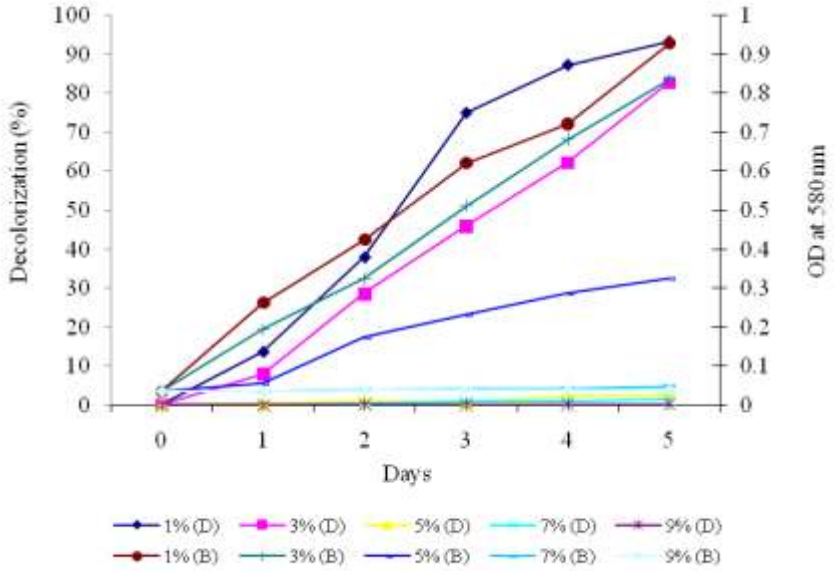

Fig :2 e Effect of Biomass on Decolorization by RSV-2

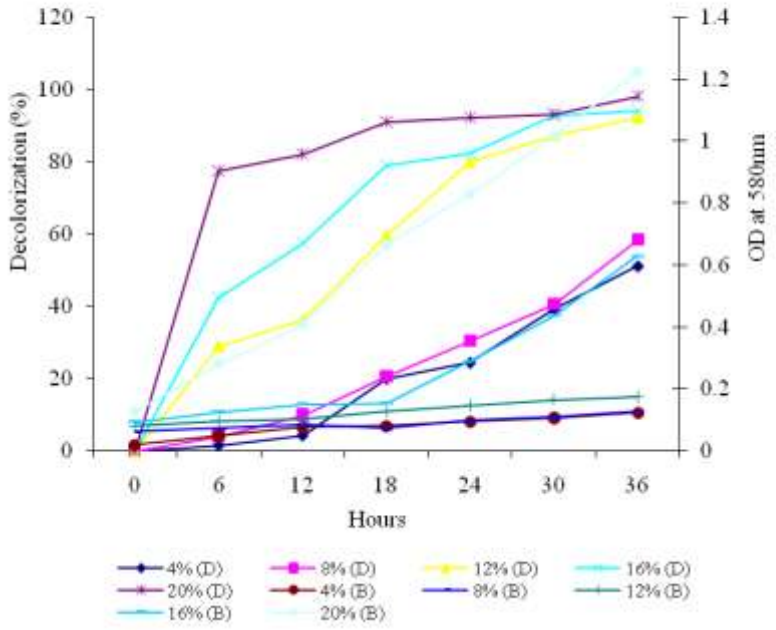

Table: 3 Decolorization of individual dyes by Stenotrophomonas maltophilia RSV-2

Name of reactive dyes	Percent Decolorization	
	Yeast	Soya
Yellow ME4GL	14±0.47	02±1.69
Blue RR	88±1.14	86±0.47
Red RR	62±0.47	58±3.55
Yellow RR	52±0.47	68±0.47
Red M5B	82±1.24	79±0.94
Blue MR	23±1.69	37±0.94
Deep Black RR	88±2.62	85±1.69
Yellow MERL	96±0.94	77±1.63
Red ME4BL	93±1.69	94±0.94
Golden Yellow MR	96±0.94	92±0.47

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