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DEVELOPMENT AND USE OF SOIL BACTERIAL CONSORTIA FOR BIOREMEDIATION OF DYE POLLUTED SOIL AND MUNICIPAL WASTE WATER

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ABSTRACT
Studies were conducted to decolourize C.I Reactive Red 11 (mono azo) and C.I Reactive Yellow 84 (di azo) dyes by developing bacterial consortia isolated from dye contaminated soil. The effect of pH, dye concentration and inoculum size of each consortium (NEERI-R and NEERI-Y) on the decolourization rate was optimized. Maximum decolourization of 85% was achieved at 100 mg/L of C.I Reactive Red 11 and 83% at 100 mg/L of C.I Reactive Yellow 84 after 72 h under shaking condition using 40% inoculum size of the respective consortia. Optimum pH and temperature for decolourization of each dye was 7.0 and 28°C ± 2°C, respectively. The rate of decolourization was enhanced to 89% for C.I Reactive Red 11 and 86% for C.I Reactive Yellow 84 in presence of additional carbon source and 86% for C.I Reactive Red 11 and 85% for C.I Reactive Yellow 84 in presence of additional nitrogen source. However, on addition of carbon and nitrogen sources together, the decolourization rate increased greatly to 94% for C.I Reactive Red 11 and 91% for C.I Reactive Yellow 84. Dye polluted soil and municipal wastewater were decontaminated by directly treating them with the bacterial consortia.

KEYWORDS: C.I Reactive Red 11, C.I Reactive Yellow 84, Bacterial consortium, Decolourization, Municipal wastewater

INTRODUCTION
Azo dye represents the largest class of synthetic dye with a variety of colour and structure (Gharbani *et al*., 2008). They are considered as electron deficient xenobiotic compounds because they possesses azo (N=N) and sulphonic (-So_3-) electron withdrawing groups making the compound less susceptible to oxidative catabolism by bacteria and fungi (Saraswathi and Balakumar, 2009). Their complex aromatic substituted structures make them resistant to degradation under natural conditions (Rajaguru P *et al*., 2000). Due to the poor fixing properties of reactive dyes, as much as 40% of the initial dye remains unfixed and ultimately ends up in the dye bath (Shah 1998) and about 1000 mg/L of dye is present in a typical dye bath (Ince and Tezcanli, 1999). In a textile industry, about 40-60 L of wastewater is generated per kg of cloth dyed (Uygur, 1997). As reported by Khan and Jain (1995), intensive irrigation of agricultural land polluted with various industrial effluents adversely affects soil fertility and plant growth (Khan and Jain, 1995) and are toxic (lethal effect, genotoxic, mutagenic and carcinogenic) to aquatic and terrestrial organisms (Correia *et al*., 1994). The current state of the art technique for treatment of wastewater containing dyes is physio-chemical technique (Churchly, 1994) which has its own limitations resulting in considerable interest in the use of cost-effective, eco-friendly biological system for the treatment of wastewater (Yeh *et al*., 1995). A number of studies have shown that some bacteria and fungi are able to biodegrade and bio adsorbs dyes in textile industry effluents (Pearce *et al*., 2003). In most of the studies, the organisms were *Staphylococcus* sp, E. *coli*, *Bacillus* sp, *Clostridium* sp, and *Pseudomonas* sp (McMullan *et al*., 2001). It has been demonstrated that indigenous microflora (biomass) is significantly better for decontamination than commercially obtained ones (Newman *et al*., 2002). In view of the above, decontamination of soil and water polluted with C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) textile dyes extensively used in the textile industry (Coughlin *et al*., 1999) was attempted by using indigenous bacterial consortium isolated from dye spiked /contaminated soil as it has been estimated that there are 60,000 different bacterial species, most of which have yet to be even named (Reid and Wong 2005). Factors affecting the decolourisation process, such as pH, inoculum size and dye concentration, were also studied for evolving an affordable treatment technology for decontaminating dye polluting environments.

MATERIALS & METHODS
Soil (sample) and dyes
Soil was collected from a plant nursery. The sample was dried, crushed and passed through a 2 mm sieve and stored in air tight glass jars at 25°C until use. The reactive azo dyes, C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) were supplied by Khatau Valabhdas and Co., Mumbai, India. Misstating which were used to inhibit fungal growth for development of the bacterial consortium bought from Cipla Ltd. Mumbai. Soil analysis was done for moisture content (Buurman *et al*., 1996), texture (Bouyoucos, 1962), pH (Singh *et al*., 1999) and electrical conductivity, organic carbon content (Walkely

and Black, 1934). The BOD, COD and TSS of municipal wastewater were also analyzed (Standard Methods for the Examination of Water and Wastewater-21st Ed**,** APHA).

Dye stock preparation and determination of the standard curve

C.I Reactive Red 11 and C.I Reactive Yellow 84 dyes were added into the mineral salt medium with concentration of 1000 mg/L (Seyis and Subasioglu, 2008). Standard curve was prepared using known standard concentrations varying from 10 - 100 mg/L and the absorbance was measured at 545 nm for C. I Reactive Red 11 and 414 nm for C. I Reactive Yellow 84 (Khan and Husain 2007) using a spectrophotometer model Chemito UV2100 (Ansari and Mosayebzadeh, 2010).

Spiking of soil with the dyes

Double spiking of the soil was done with C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) reactive dyes. 200 g of soil was spiked with 1000 mg/kg of each of the dyes separately. Other sets were maintained which were supplemented with 1% glucose as an additional source of carbon along with dye to check for co-metabolizing bacterial species. 1% urea was used as an additional source of nitrogen along with the dye to check for higher nitrogen requiring bacterial species and in one set; both 1% glucose and 1% urea were used along with the dye. The four sets in triplicates were incubated at 28 ± 2 °C for a period of 30 days. The moisture content of all the sets was monitored daily and moisture loss was made up. After 15 days, another dose of 1000 mg/kg of the dyes was added and the soil was further incubated at 28 ± 2 °C. Bacteria present in the spiked soil samples were enriched in a modified growth medium (Li et al. 2008). The modified enrichment medium consisted of yeast extract (0.05%), peptone (0.5%), NaCl (0.5%), $(NH_4)_2SO_3$ (1%), K_2HPO_4 (0.02%), KH_2PO_4 (0.5%) and $MgSO_4.7H_2O$ (0.5%) amended with 1000 mg/L of each of the test dyes separately. K_2HPO_4 and KH_2PO_4 were dissolved in distilled water and autoclaved separately. The final pH of the medium was adjusted to 7.0-7.2. The medium was autoclaved at 121 °C for 15 min. After cooling, 10 g of the spiked soil samples were aseptically added into 250 ml conical flask containing 100 ml of autoclaved enrichment media. Nystatin (0.1 g/mL) was used in one flask to inhibit fungal growth for development of the bacterial consortium (Ndasi and Augustin, 2011). All transfers were performed in aseptic conditions. The flasks were incubated under shaking conditions at 180 rpm (Ndasi and Augustin, 2011) and at a temperature of 28 ± 2 °C for a period of 5 days. After 5 days of incubation, the most effective decolourising bacterial species were screened by spread plating on Mineral Salt Agar amended with 1000 mg/L of the test dyes and incubated for 48-72 h. Bacterial colonies that showed a clear decolourisation zone around them were picked and purified by streaking and reintroduced into 100 mL of freshly prepared enrichment media. The flasks containing the isolated and screened bacterial isolates were incubated at 28 ± 2 °C under agitation at 180 rpm for 3 days (Ndasi and Augustin, 2011). Growth curves of individual bacterial isolates were studied in mineral salt medium with 10 mg/L of Reactive Red 11 and 10 mg/L Yellow 84. Growth of the isolates/monocultures was studied to determine the logarithmic phase. The isolates were grown aerobically (using a rotary shaker) at room temperature. Growth curves of individual bacterial isolates were studied in Mineral Salt Medium (MSM) with 10 mg/L of Reactive Red 11 and Yellow 84 each. For each bacterial isolate, 100 ml medium was taken, autoclaved and then inoculated with 10% of the freshly prepared inoculum. The inoculum was prepared by inoculating a loopful of a 24 h old slant culture into a 100 ml nutrient broth in 250 mL flask and then incubated in a rotary shaker for 24 h at 28 ± 2 ºC. From this mother culture, 10 ml was taken and inoculated to a 100 ml of fresh sterile MSM broth in a 250 ml flask incubated in a rotary shaker at 28 ± 2 ºC. The broth was incubated at 28 ± 2 ºC in a shaker at 180 rpm. An aliquot of culture (5 ml) was taken out in a sterilized tube at regular intervals of 30 min and the absorbance was measured at 545 nm for C. I Reactive Red 11 and 414 nm for C. I Reactive Yellow 84. The growth curve for each isolate was plotted.

Decolourization studies and consortia development

The effect of dye concentration was investigated by using various dye concentrations (mg/L), such as 100, 200, 400 and 800 using different inoculum size, such as 10%, 20% 30% and 40%. Decolourization was also studied using additional carbon and nitrogen sources. The carbon and nitrogen sources used for this study were 1% glucose and 1% urea, respectively. For consortia development, inoculum consisting of 2 mL of each of the five bacterial isolates in the log phase was introduced into separate 100 ml of autoclaved growth medium amended with varying concentrations of dye to obtain 10% inoculum size. Similarly, 20%, 30% and 40% of inoculum sizes were developed. The flasks were incubated under agitation at 180 rpm and temperature of 28 ± 2 °C for 3 days. The control consisted of flask without any microorganisms. All the experiments were carried out in triplicates. Aliquots (5 mL) of the culture media were withdrawn at time intervals of 24 h and centrifuged at 15,000 rpm to get a cell free extract /supernatant. Decolourisation was quantitatively analyzed by measuring the absorbance of the supernatant using a UV-visible spectrophotometer at maximum wavelength (λmax) of 545 nm for C. I Reactive Red 11 and 414 nm for C. I Reactive Yellow 84 (Khan and Husain 2007). The decolourization rate was calculated by the following *i.e*. Dye decolourization (%) = Initial absorbance - Final absorbance/Initial absorbance X 100 (Saratale *et al*., 2006).Parameters, such as temperature and pH were monitored to study their effect on decolourization. Mineral Salt Medium (100 ml each) with 24 h old cultures was inoculated with dye (100 mg/L) and incubated at 28, 37 and 55 °C whereas the effect of pH was studied using MSM with pH values of 4, 7 and 10. pH was adjusted using either HCl (1N) or NaOH (1N). The percentage decolourization was determined over 72 h.

Application of newly developed bacterial consortium (NEERI-R and NEERI-Y) in decolorization of dye contaminated soil and municipal wastewater

The decolorizing ability of the developed bacterial consortium (NEERI-R and NEERI-Y named for patenting purpose) was tested in soil and municipal wastewater. 10 g of sterile and non-sterile soil sample was spiked with 100 mg/kg of C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) reactive dyes separately. NEERI-R and

NEERI-Y was added to separate soil samples so that inoculum size is 40% and incubated at 28 ± 2 °C for 3 days. After incubation, 20 ml of sterile distilled water was added to the flasks which were then kept under shaking conditions for 3 h for the extraction of dyes from soil in water as the reactive dyes were water soluble in nature (Won *et al.*, 2006). Similar experiment was performed using 10 ml of sterile and non-sterile municipal wastewater. The sterile and non-sterile soil and municipal wastewater samples were centrifuged at 3,000 rpm for 15 min. The control for soil as well as municipal wastewater was maintained without the consortia. The clear supernatant was used to measure the absorbance at λmax for the respective dyes as mentioned earlier. Azo dyes being water soluble in nature, 99% of C.I Reactive Red 11 and 98% of C.I Reactive Yellow 84 was recovered from soil and municipal wastewater eliminating the possibilities of the dye getting adsorbed on soil particles.

Statistical analysis

The data was statistically defined by two-way ANOVA using Microsoft Excel. Results of each of the experiments were interpreted depending upon probabilities. Probability (p-value) was less than 0.05 which was found to be significant.

RESULTS & DISCUSSION

A total of 13 morphologically distinct C. I Reactive Red 11 (monoazo) dye decolourizing bacterial isolates and 10 C. I Reactive Yellow 84 (diazo) dye decolourizing bacterial isolates were isolated from the soil. The soil had moisture content of 28%, sandy loam texture, pH of 7 at 25 °C and electrical conductivity of 0.0005 Ms/cm at 24 °C.

TABLE 1: Decolourization (%) of C.I Reactive Red 11 at different dye concentrations (100, 200, 400 and 800 mg/L) using varying inoculums levels (10%, 20%, 30% and 40% inoculum size) in triplicates (values in parentheses represent SE)

C.I Reactive Red 11

Inoculum size	100 mg l^{-1}	200 mg l^{-1}	400 mg l^{-1}	800 mg l^{-1}
10%	59 (±1.17)	50(±0.85)	40 (±0.76)	8 (±0.62)
20%	72 (±0.71)	63 (±0.43)	54 (±0.95)	31 (±0.65)
30%	82 (±0.36)	74 (±0.40)	64 (±0.36)	42 (±0.29)
40%	85 (±0.30)	79 (±0.39)	68 (±0.52)	53 (±0.42)

Two-way ANOVA for C.I Reactive Red 11

Source of Variation	SS	df	MS	F	P-value	F crit
Inoculum size	5742.23	3	1914.1	2296.9	2.00243E-37	2.911
Dye concentration	17666.9	3	5888.9	7066.8	3.27065E-45	2.911
Interaction	26.68	9	2.96	3.56	0.003686789	2.19
Within	26.67	32	0.83			
Total	23462.48	47				

TABLE 2: Decolourization (%) of C.I C.I Reactive Yellow 84 at different dye concentrations (100, 200, 400 and 800 mg/L) using varying inoculums levels (10%, 20%, 30% and 40% inoculum size) in triplicates (values in parentheses represent SE)

C.I Reactive Yellow 84

Inoculum size	100 mg l^{-1}	200 mg l^{-1}	400 mg l^{-1}	800 mg l^{-1}
10%	60 (±0.52)	49 (±1.51)	40 (±1.06)	10 (±0.33)
20%	70 (±1.07)	59 (±0.89)	47 (±0.06)	20 (±0.65)
30%	78 (±1.72)	72 (±1.27)	52 (±0.53)	30 (±0.46)
40%	83 (±1.84)	76 (±0.71)	61 (±0.89)	38 (±0.33)

Two-way ANOVA for C.I Reactive Yellow 84

Source of Variation	SS	df	MS	F	P-value	F crit
Inoculum size	4513.22	3	1504.41	366.56	7.61223E-25	2.91
Dye concentration	15990.22	3	5330.08	1298.70	1.73101E-33	2.91
Interaction	161.0209	9	17.9	4.36	0.000900275	2.19
Within	131.33	32	4.1042			
Total	20795.81	47				

The organic carbon content of the soil was 4.1%. Amongst the various isolates, 5 bacterial isolates, each for monoazo and diazo dye, showed higher decolourization and were used for further study. The dye decolourizing bacterial isolates named as R5, R6, R7, R8 and R13 (NEERI-R consortium) for monoazo dye and Y2, Y3, Y8, Y9 and Y10 (NEERI-Y consortium) for diazo dye showed a distinct zone of decolorization around them. Detailed characteristics of the bacterial isolates are presented in Tables 4 and 5. For C. I Reactive Red 11 (monoazo) dye decolourization, the bacterial isolate R5 initiated its log phase after 3 h of incubation having absorbance 0.455, R6 initiated its log phase after 5 h with an absorbance of 0.259, R7 and R8 initiated its log phases after 6 h having absorbances of 0.226 and 0.584, respectively. R13 initiated its log phase after 3 h with an absorbance of 0.032. All the monoazo dye decolourizing bacterial isolates showed enhanced growth in presence of dye. Of all the 5 bacterial isolates, R5 and R8 were fast growing as compared to R6 and R7 while R13 was the slowest growing bacterial isolate for C. I Reactive Yellow 84 (diazo) dye decolourization, the bacterial isolates Y2, Y3, Y8, Y9 and Y10 initiated its log phase after 1 h 30 min with absorbances of 0.198, 0.209, 0.178, 0.432, 0.155, respectively. The diazo dye decolourizing bacterial isolates showed faster growth in presence of dye as a sole source of carbon and energy.

TABLE 3: Comparision of decolourization (%) of C.I Reactive Red 11 and C.I Reactive Yellow 84 (100 mg l^{-1}) between individual bacterial isolates and the combination of all as a consortia (40% inoculum size)

Characteristics	R5	R6	R7	R8	R13
Size	Medium	Punctiform(tiny)	Medium	Large	Punctiform(tiny)
Colony Shape	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire	Entire
Elevation	Raised	Flat	Convex	Flat	Flat
Consistency	Smooth	Smooth	Smooth	Smooth	Mucoid
Opacity	Opaque	Opaque	Opaque	Opaque	Translucent
Colour	Red center with grey periphery	Pink center with creamish periphery	Light pink	Pinkish-red	Cream
Gram staining	Gram negative bacilli	Gram negative cocci	Gram negative cocco-bicillary	Gram negative rods	Gram positive cocci in clusters

TABLE 4: Characteristics of C.I Reactive Red 11 (monoazo) dye decolourizing bacterial isolates

C. I Reactive Red 11						Consortium
Bacterial isolate	R5	R6	R7	R8	R13	NEERI-R
Decolourization (%)	20.82	21.54	20.82	25.5	20.82	85
C. I Reactive Yellow 84						Consortium
Bacterial isolate	Y2	Y3	Y8	Y9	Y10	NEERI-Y
Decolourization (%)	9.54	10.25	12.65	14.31	15.27	83

TABLE 5: Characteristics of C.I Reactive Yellow 84 (diazo) dye decolourizing bacterial isolates

Characteristics	Y2	Y3	Y8	Y9	Y10
Size	Punctiform (tiny)	Medium	Medium	Medium	Medium
Colony Shape	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire	Entire
Elevation	Flat	Raised	Raised	Raised	Raised
Consistency	Mucoid	Smooth	Smooth	Smooth	Smooth
Opacity	Translucent	Opaque	Opaque	Opaque	Opaque
Colour	Cream	Yellow center with grey periphery	Grey	Yellow	Pink center and grey periphery
Gram staining	Gram positive cocci in clusters	Gram positive cocci	Gram negative cocco-bacillary	Gram negative bacilli in chains	Gram negative cocco-bacillary

From the results shown, NEERI-R and NEERI-Y bacterial consortia could decolourize high concentrations of C.I Reactive Red 11 and C.I Reactive Yellow 84, respectively. The developed bacterial consortium efficiently decolourized different concentrations of dyes i.e. 100, 200, 400 and 800 mg/L using 10, 20, 30 and 40% inoculum sizes with a decolourizing efficiency varying from 60–85% (Tables 1 and 2). The decolourization activity of the consortium was strongly inhibited at higher concentration of dye while the percentage decolourization increased with increasing inoculum sizes. The NEERI-R consortium was capable of decolourizing 85% of C.I Reactive Red 11 while the bacterial consortium NEERI-Y consortium decolourized 83% of the C.I Reactive Yellow 84 at dye concentrations of 100 mg/L using 40% inoculum size in 72 h utilizing the dye as the sole source of carbon and energy. Two-way ANOVA was done showing significant difference in decolourization by using different dye concentration and inoculum size. The decolourization rate was also dependant on the combined effect of the dye concentration and inoculum size which was significant at p value < 0.05 (Tables 1 and 2). However, the decolourization activity of the consortium was strongly inhibited at higher concentrations of dye mainly due to the toxicity imposed by heavy metals (metal complex dyes) and/or the presence of non-hydrolyzed reactive groups dye at higher concentration (Kalme et al. 2007). Synergism of the bacterial interaction among these isolates is obvious as the decolourization rate of C.I Reactive Red 11 and C.I Reactive Yellow 84 using NEERI-R and NEERI-Y bacterial consortium respectively was higher than that of the individual isolates (Table 3). According to Rajguru et al (2000), the compounds with xenobiotic characteristic require unusual catabolic activities, which may not be found in a single microorganism. Pearce et al. (2003) pointed out that higher degree of biodegradation could be expected when the co-metabolic activities within the microbial community complements each other. The ability of microorganisms to degrade azo dyes is generally correlated with their ability to synthesize enzymes, such as lignin-degrading exoenzymes, which are affected by environmental factors, such as pH, temperature and substrate concentration (Schliephake *et al*., 2000). The most suitable temperature for C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) dye decolourizing bacterial consortium was found to be 28 ± 2 °C. Further increase in the incubation temperature from 37 ± 2 °C to 55 ± 2 °C resulted in reduction in decolourization activity of the culture (Figure 1).

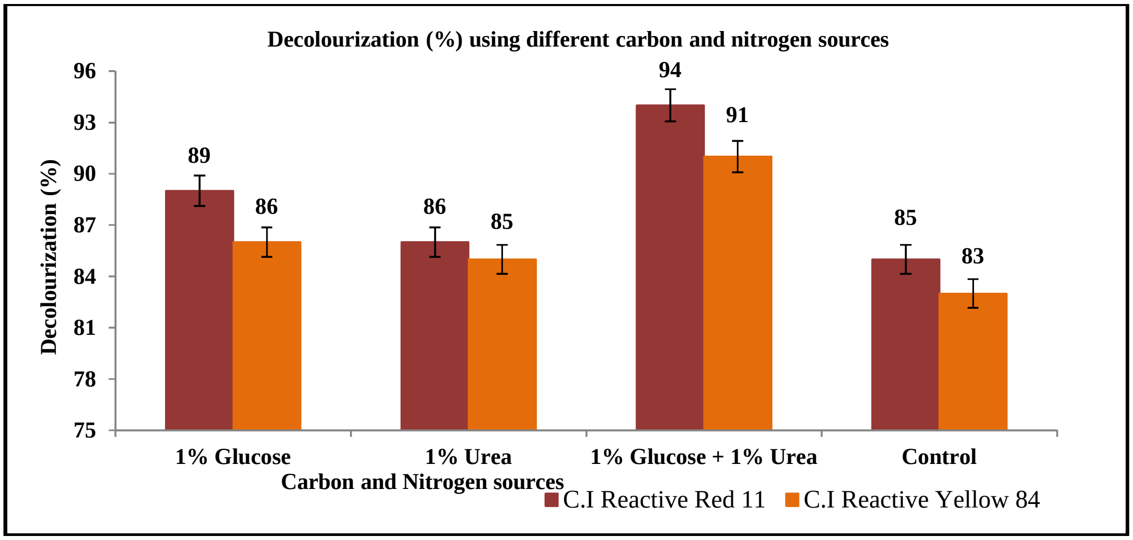

FIGURE 1: Decolourization (%) of C.I Reactive Red 11 and C.I Reactive Yellow 84 (100 mg/L) using consortia (40% inoculum size) using carbon and nitrogen sources and control sample having the consortia but with no additional carbon and nitrogen source.

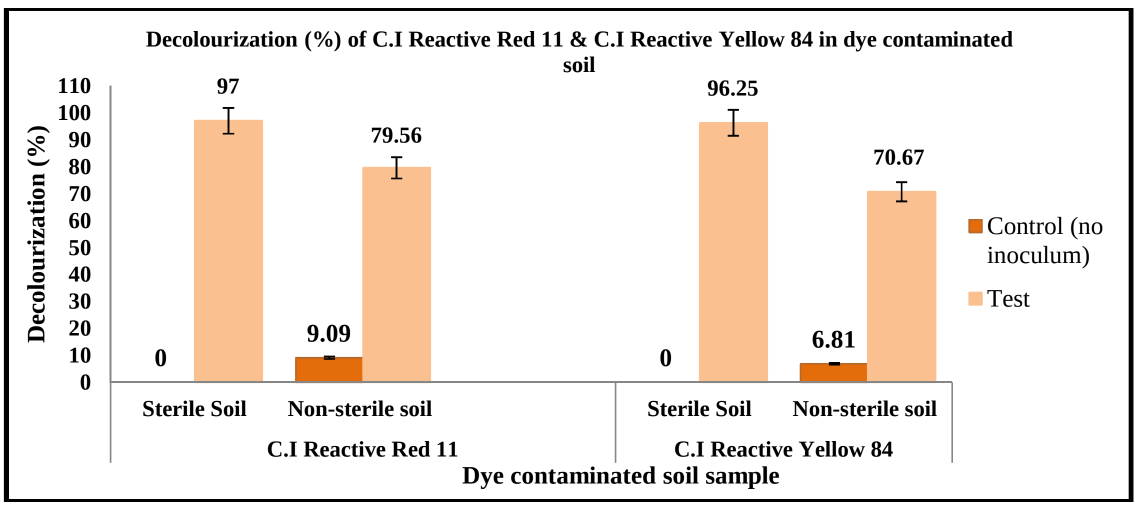

FIGURE 2: Decolorization (%) of C.I Reactive Red 11 and C.I Reactive Yellow 84 dyes in sterile and non-sterile dye (100 mg/kg) contaminated soil samples (test) with the consortia (40% inoculum size) and control soil sample having no consortia.

This might be due to the loss of cell viability or denaturation of the enzymes responsible for decolourization at higher temperature (Cetin and Donmez, 2006).The maximum pH for degradation of many bacterial species lies between neutral or slightly alkaline pH (Asgher *et al*., 2008). The optimal pH for colour removal is often between 6.0 and 10.0 for most of the dyes (Chen *et al*., 1999). In the present study, decolourization of monoazo and diazo dye was favoured at neutral pH 7 while lesser decolourization was observed at alkaline pH 10 (Figure 2). The decolourization of C.I Reactive Red 11 and C.I Reactive Yellow 84 could be accomplished as the organisms were capable of utilizing the dye as a source of carbon for survival. However, the reduction of the azo dyes also depends on the availability and type of a co-substrate which acts as an electron donor for the azo dye reduction. The rate of decolourization of C.I Reactive Red 11 and C.I Reactive Yellow 84 dye was enhanced to 89% and 86% respectively in the presence of 1% glucose. The effect of 1% urea as a nitrogen source was also tested for decolourization. Nitrogen is a major constituent of cells and is essential for bacterial growth and enzyme production. However, some studies showed that nitrogen rich culture may inhibit the colour removal ability by bacteria (Banat *et al*., 1996). Zissi and Lyyberators (2001) pointed out that ammonium ions may compete with the azo bond for electrons and hence inhibit the reduction of the azo chromophore. Therefore, lower nutrient concentration is enough to act as co-metabolite and provides necessary nutrient for bacterial growth and enzyme production. In the presence of 1% glucose and 1% urea together, the rate of decolourization drastically increased to 94% and 91% for C.I Reactive Red 11 and C.I Reactive Yellow 84 dye, respectively (Figure 1) (Plates 1 and 2). Thus, it is evident that of the various types of interactions between bacterial populations, competition for carbon and nitrogen is often the major determinant for exploring the azo dye decolorizing ability of the indigenous bacterial species in the soil environment. The bacterial consortium NEERI-R and NEERI-Y efficiently decolourized the dye contaminated sterile and the non-sterile soil (Figure 2) and municipal wastewater (Figure 3) sample spiked with 100 mg/L of C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) dye, separately using 40% of the consortium as inoculum**.**

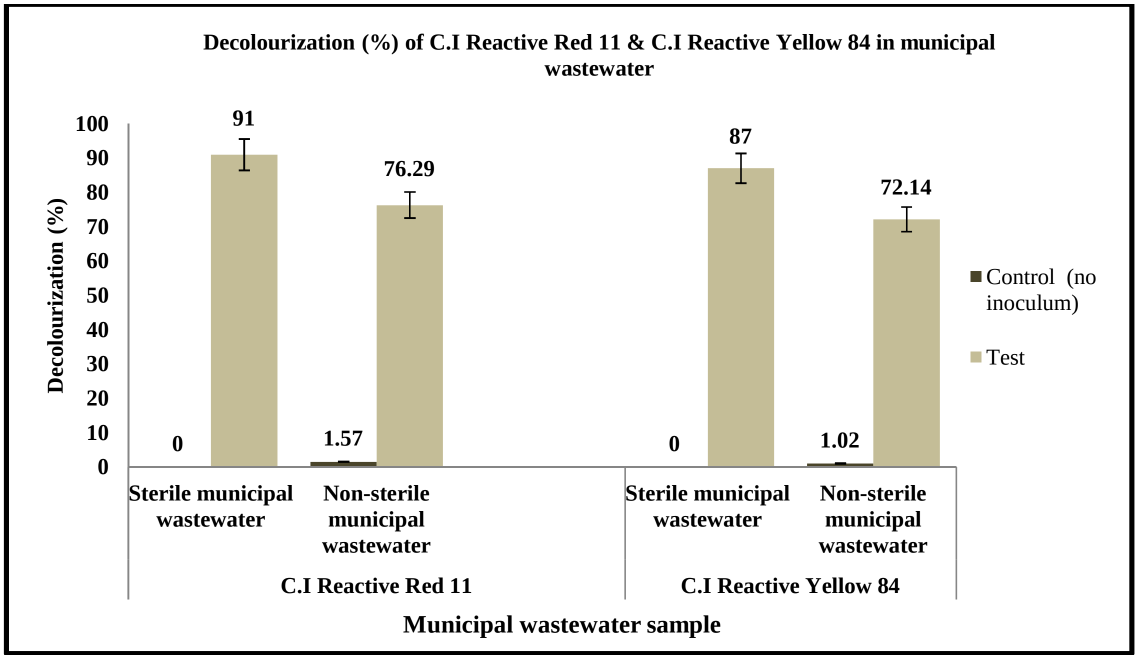

FIGURE 3: Decolorization (%) of C.I Reactive Red 11 and C.I Reactive Yellow 84 dyes in sterile and non-sterile dye (100 mg/l) contaminated municipal waste water samples (test) with the consortia (40% inoculum size) and control municipal waste water sample having no consortia.

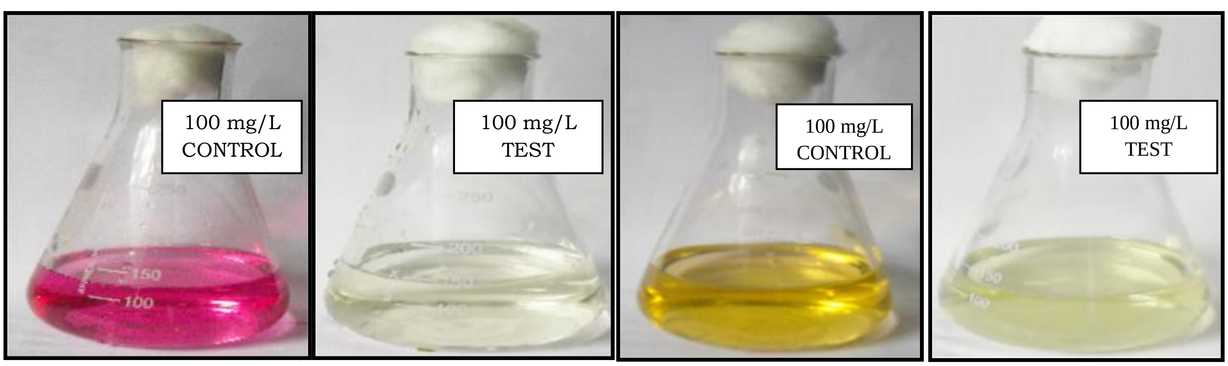

PLATE 1: Decolourization of C.I Reactive Red 11 (100 mg/L) in the test flask using 40% inoculum size of the NEERI-R consortium and with 1% glucose and 1% urea.

The BOD, COD and TSS of the municipal wastewater were 162 mg/L and 332.8 mg/L and 105 mg/L, respectively while the soil was same as used for isolation purpose. The control sample (without the inoculum) having dye contaminated sterilized soil and municipal wastewater showed no decolourization indicating the absence of any chemical or photodegradation of the test dyes. In non-sterile soil and municipal wastewater without the inoculum showed low decolourization indicating the presence of dye degraders but probably in smaller numbers whereas, in non-sterile soil and municipal wastewater with the bacterial consortia decolourization was observed but it was less than that in the sterile soil and municipal wastewater with the bacterial consortium indicating competition for carbon and nitrogen source between the indigenous micro flora and the added bacterial consortium. A much higher degree of decolourization in non-sterile condition could be further achieved by increasing the amount of carbon and nitrogen source. This is supported by the positive results which were observed in the study when additional carbon and nitrogen source was provided to the bacterial consortium (Figure 1). This is a significant observation and offers scope for further research.

CONCLUSION

The bacterial consortia (NEERI-R and NEERI-Y, named for patenting purpose) showed high potential for decolourizing C.I Reactive Red 11 (monoazo) and C.I Reactive Yellow 84 (diazo) dyes. Decolorization increased with an increase in inoculum size. Optimum decolourization of 85% for C.I Reactive Red 11 (mono azo) dye and 83% for C.I Reactive Yellow 84 (diazo) dye was achieved when the pH was 7, temperature of 28 ± 2 ºC and the dye concentrations was 100 mg/L while the inoculum size was 40%. But, since the bacterial consortium was capable of decolourizing 53% of C.I Reactive Red 11 and 30% of C.I Reactive Yellow 84 even at 800 mg/L, it can be suggested that higher percentage of decolourization can be attained even at higher dye concentrations by increasing the supply of carbon (1% glucose) and nitrogen (1% urea) source together. The bacterial consortium successfully decolourized the dyes in non-sterile dye contaminated soil and municipal wastewater spiked with 100 mg/L of the respective dyes upto 70-79% using 40% inoculum size. It can thus be concluded that the bacterial consortium can be effectively used in the aerobic treatment of these reactive dyes in soil and municipal wastewater. Further research needs to be carried out on how to use the consortia on a larger scale for decolorisation purpose for example either through lodging on a suitable support media for waste water treatment,etc.

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