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Bioremediation potential of individual and consortium Non-adapted fungal strains on *Azo dye* containing textile effluent

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ABSTRACT

This study investigates the non-adapted individual and consortium fungal strains for the reduction of azo dyes containing textile effluent. About 4 predominant non-adapted fungal strains such as Aspergillus sp., Penicillium sp., Fusarium sp., Rhizopus sp., with potential dye degradation ability were isolated from different niche. It was used to develop consortium for bioremediation efficiency analysis on textile effluent. The Fusarium was not compatible in the consortium though it could give good individual dye reduction. On analyzing with the individual and consortium non-adapted fungal strains of the treatment trials, the consortium fungal strains are found to be the very effective bioremediation ability. The consortium reduced the color up to 74% and reduce other solids upto 50%. This study reveals the standardization of pH, retention time, organic load, incubation time and Inoculum concentration for the effective decolorization of the azo dye containing textile effluent. The GCMS analysis of the non-adapted fungi treated in pH-6, organic loading rate-100%, retention time- 5days, inoculum concentration-5% and incubation temperature-27°c and the treated samples were not found to have any toxic compounds. Hence, the consortium of non-adapted fungal strains were found to have good dye reduction ability than the individual non-adapted fungal strains.

Keywords: non-adapted fungal strains, Azo dye degradation, bioremediation, GC-MS, Physico-chemical parameters, consortium.

INTRODUCTION

Textile industries play a vital role in the economic increases in India. Water is one of the major products of nature used enormously by human beings and it is not unnatural that any growing community generates enormous waste water or sewage (Deviram *et al.*, 2011).The textile industries dispose the waste water during the process of dyeing. The textile waste water generates the effluent system (Tariq *et al.*, 1996). dyes are organic colours having less water solubility(Malik and Zadafiya, 2010). Azo dyes are frequently used in the textile industries because of the cheapest material and wide spectral color range in that (Chung and Stevens, 1993). Azo dyes represent about half of the dyes used in the textile industry and, as a consequence, a relevant problem of pollution related to the release of these products in the environment is taking place (Vinod Shrivastava, 2011). Traditional methods for the cleanup of azo dyes in the textile waste water usually involve the removal of unwanted materials through sedimentation, filtration, and subsequent chemical treatments such as flocculation, neutralization and electro-dialysis before disposal. These processes may not guarantee the treatment of toxic dye in the effluent. Moreover, considering the volume of wastes released during the industrial production process these are often laborious and expensive. The past two decades have seen a tremendous upsurge in the search for cost effective and environmentally sound alternatives to the

conventional methods for dealing with dye wastes (Quezada *et al*., 2000). Natural systems that have restored environments to their original status following undesirable perturbations, but yet no cost effective treatments have been reported in non biological systems. In fact the self-restoring process in nature is what has actually given birth to the concept that the self- cleansing ability of nature is infinite. Of all the technologies that have been investigated, bioremediation has emerged as the most desirable approach for cleaning up many environmental pollutants in effluents. Although a wide variety of microbes are capable to degrade the textile effluent, fungi are considered to be the best than bacteria because of two reasons. 1) They can able to produce heat-shock proteins 2) Ability to produce different kinds of enzymes like lacases, manganese peroxidase, lignin peroxidase etc. Recently, many studies have shown that fungi are able to degrade dyes by extracellular, nonspecific and non-stereo selective enzyme systems [Reddy, 1995]. Until recently various investigations have been focused on either the decolorization of various dyes by a single fungal strain, or consortium fungal strains (Arora *et al*., 2002). Degradation of a dye involves aromatic ring substituent with the presence of phenolic, acetamido, amino or other easily biodegradable functional groups resulting in a greater amount of degradation (Spadaro *et al*., 1992). Azo dyes are recalcitrant compounds and these dyes are discharged in the water systems. These dyes are mainly degraded by the fungal strains (Cooper, 1995). Their number and species composition in the habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Thennarasu *et al*., 2011).

However, enzyme consortium based decolorization is an efficient approach and of current interest in industrial effluent treatment. Among microorganisms, fungi are widely known for their superior capacities to produce a wide variety of extracellular enzymes, organic acids various metabolites, and for their capabilities to adapt severe environmental constraints than other microorganisms (Wainwright, 1992). The fungal consortium could prove the more effective dye reduction than individual fungal strains by producing wide spectrum of enzymes (Robinson *et al.,* 2001).This study investigated the potential of fungi isolated from a environment (effluent non-adapted fungi).

In the present study fungal strains were isolated from non-adapted sources. The efficiency of the individual and consortium non-adapted fungal strains were studied. The efficient organisms were then optimized under different cultural conditions to study the optimal bioremediative capacity.

MATERIALS AND METHODS

2.1 Sample collection

Soil samples, water samples and fruiting bodies were collected from the different natural locations. These were used to isolate the non-adapted fungal strains. The samples were brought to the laboratory and stored at 4ºC in a refrigerator.

2.2 Isolation of non- adapted fungal strains from different environmental samples

Fungi were isolated through spore suspension technique, enrichment and dilution plating technique for the fruiting bodies and other environmental samples respectively (Harley and Prescott, 1993).

2.3 Screening of isolated fungal strains for dye decolorization using synthetic textile dyes

2.3.1 Plate decolorization assay of non-adapted fungal strains

Primary screening it was done on potato dextrose agar amended with a mixture of 0.01mg six different dyes (reactive red 120, amido black, anthraquinone violet R, indigo, indigo carmine, remazol brilliant violet 5R). It was inoculated through radial streaking with the isolated individual cultures and incubated at 27°c for 48 hours. The plates were checked for the zone of clearance after incubation.

2.3.2 Broth decolorization assay of non-adapted fungal strains

Broth decolourization assay (secondary screening) was done on potato dextrose broth amended with 0.01g of six different dyes individually. The colonies which showed a zone of clearance around them in primary screening were selected and inoculated in individual tubes for decolorization efficiency. The tubes were incubated at 27°c for 48 hours. The broth was checked for the color reduction in UV-Vis spectrophotometer after incubation (Olukanni *et al*., 2006).

$$\text{Percentage of decolorization} = \frac{\text{Initial absorbance - final absorbance}}{\text{Initial absorbance}} \times 100$$

2.4 Identification of screened non-adapted fungal strains

The selected efficient non-adapted fungal strains were subjected for microscopic identification. The non-adapted fungal strains were numbered from 1-4 respectively. These fungal strains were identified by using lacto phenol cotton blue staining technique (Cappuccino and Sherman, 1999).

2.5 Compatibility analysis of the screened adapted and non adapted fungal strains

Compatibility analysis was done for checking the antagonistic effect of each organism on other fungal strains by standard well-cut method. The culture is said to be compatible with each other if no zone of clearance was observed.(Rajendran *et al*., 2011).

2.6 Treatment trials of textile effluents using Non-adapted fungal strains

The screened efficient Non-adapted fungal strains were numbered from 1-4. About 5ml of the each fungal broth culture was inoculated in 95ml of the azo dye containing textile effluent individually. One set of the effluent was inoculated with a combination of all the 4 Non-adapted fungal strains (in equal concentration making a volume of 5 ml) in 95ml of textile effluent sample. The flasks were incubated at 27°c in a metabolic shaker at 120rpm for 7 days. Samples were retrieved from the flasks at the end of 7 days of incubation and analyzed for the bioremediation efficiency of the fungal cultures involved.

2.7 Bioremediation assays

2.7.1 Physico- chemical parameters analysis for untreated textile effluent

The azo dye containing effluent was characterized by various parameters such as pH, color, turbidity, Chemical oxygen demand (COD),Biological oxygen demand (BOD), Total solid (TS),Total dissolved solids (TDS), resistivity, Total suspended solids (TDS), alkalinity, electrical conductivity, hardness. The color and turbidity were measured by using UV spectra at 435nm and 620 nm respectively. The remaining parameters were done by standard procedures (APHA, 1992).

2.8 Optimization of cultural conditions for maximum bioremediation ability

The efficient adapted fungal consortium was optimized under different cultural conditions such as retention time, initial pH, incubation temperature, initial inoculum concentration and initial organic loading rate.

2.8.1 Effect of Retention time on bioremediation

Five percent of the fungal consortium was inoculated in 95ml of azo textile effluent. The sample was incubated in room temperature in a metabolic shaker at 120rpm for a period of 7 days. At the end of each day, samples were retrieved and the bioremediation ability of the consortium was studied by measuring the various physico- chemical parameters. The retention time which shows the maximum reduction was taken as one complete cycle for studying the optimization of other cultural characters.

2.8.2 Effect of initial pH on bioremediation

Five percent of the fungal consortium was inoculated in 95ml of azo textile effluent at varying pH ranges (5, 6, 7, 8, and 9) and incubated in room temperature under shaking conditions (120rpm). At the end of the five days of incubation, sample was retrieved and the bioremediation ability of the consortium was studied by measuring the various physico- chemical parameters. The optimum pH for the consortium could be found out by measuring the reduction in the parameters after five days of incubation (Madhuri Sahasrabudhe and Girish Pathade 2011).

2.8.3 Effect of initial organic loading rate on bioremediation

Five percent of the fungal consortium was inoculated in different initial organic loading rate (OLR) of azo textile effluent (20%, 40%, 60%, 80%, and 100%) and incubated in room temperature under shaking conditions (120rpm). At the end of the five days of incubation, sample was retrieved and the bioremediation ability of the consortium was studied by measuring the various physico- chemical parameters. The optimum initial substrate concentration for the consortium could be finding out by measuring the reduction in the parameters after five days of incubation

2.8.4 Effect of initial incubation temperature on bioremediation
Five percent of the fungal consortium was inoculated in 95ml of azo textile effluent at varying temperature ranges (7°c, 17°c, 27°c, 37°c, and 47°c) and incubated. At the end of the five days of incubation, sample was retrieved and the bioremediation ability of the consortium was studied by measuring the various physico- chemical parameters. The optimum incubation temperature for the consortium could be found out by measuring the reduction in the parameters after five days of incubation.

2.8.5 Effect of initial inoculum concentration on bioremediation
Different concentrations of the efficient fungal consortium (1%, 2%, 3%, 4%, and 5%) was inoculated in azo textile effluent making a total volume of 100 ml and was incubated at room temperature under shaking conditions (120rpm). At the end of the five days of incubation, sample was retrieved and the bioremediation ability of the consortium was studied by measuring the various physico- chemical parameters. The optimum inoculum concentrations for the consortium could be found out by measuring the reduction in the parameters after five days of incubation.

2.9 Characterization of treated textile effluent under optimized cultural conditions
About 5% concentration of the adapted fungal consortium was inoculated in 95 ml of 100% textile effluent at pH 5. The sample was incubated at 27ºc for a period of 5 days. The bioremediation of the selected combination under optimized condition was calculated by analyzing the reduction in the values of all the 11 different parameters from its initial values. The sample was also checked qualitatively for the presence of toxic intermediates using GC-MS analysis.

3. GC-MS analysis
GC-MS is particularly useful for identification of products from disperse azo dyes, which are relatively small molecules that lack sulfonic acid groups. GC-MS has been widely used to identify products of dyes degraded with fungi. The major limitation of this technique is that the sample must be volatile and thermally stable at the temperature of analysis. Treated and untreated effluents were centrifuged. Equal volumes of the supernatant collected were mixed with diethyl ether separately in order to retrieve the organic content of the treated and untreated effluent samples in the ether layer. The organic layer was then collected separately from the aqueous layer. The organic layer was allowed to evaporate at room temperature on Petri dish which was cleaned with chromic acid. The residue that remains in the Petri dish is then suspended in 5ml 100% methanol solution. The methanol solution is then used for GC-MS analysis for both the treated and untreated effluent samples.

GC-MS was performed using a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. Gas chromatography was conducted in the temperature-programming mode with a 100 - 250°c, RATE: 8/Min, HOLDING TIME: 10 Min @250. The initial column temperature was held at 100 °C for 8 min, then increased linearly to 250°C at 10 °C/min, and held for 4 min at 270°C. The temperature of the injection port was 275 °C and the GC/MS interface was maintained at 300°C. Helium was used as carrier gas with a flow rate of 1.0 ml/min. Injection was split less to increase sensitivity. Identification of degradation products was made by comparison of retention time and fragmentation pattern with known reference compounds as well as with mass spectra in the library search results stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS (Poole and Poole, 1992).

RESULTS AND DISCUSSION

4.1 Isolation of non-adapted fungal Strains from different environmental samples
From the dilution plates 10 non-adapted fungal strains were selected. In that five strains were isolated from water sample two from soil sample and three isolates from fruiting bodies which were different in their colony morphology were chosen and used for further screening. Fungal strains were isolated from the soil samples were characterized by standard microbiological methods.

4.2 Screening of isolated fungal strains for dye decolorization using synthetic textile dyes.

4.2.1 Plate Decolorization Assay of Non-Adapted fungal strains

Among the 10 fungal isolates, 4 fungal isolates were able to degrade the synthetic azo dye in the medium. The fungi which were capable of reducing the chromophore showed a zone of clearance around their colonies and were chosen for further screening.

4.2.2 Broth decolorization assay of non-adapted fungal strains

Among the 10 fungal isolates, 4 fungi isolates (1, 5, 8, and 9) were able to reduce the chromophore of the synthetic textile dyes by more than 50% (Table 1). These 4 fungal isolates were identified and used for treatment trials. Mostly the Non-adapted fungal isolates reduced all the synthetic dyes used in the study. The consortium were degrade the azo dyes more than individual strains (Coughlin *et al*., 1997)

Table 1: Broth decolourization assay of the selected non-adapted fungal strains

Fungal Isolates	Percentage of dye decolourization on different synthetic Textile dyes					
	Anthraquinone violet R	Reactive red 120	Indigo carmine	Indigo	Remazol brilliant violet 5R	Amido black
1	59.25	49.31	50.98	57.21	62.98	**74.23**
2	21.69	44.81	54.22	20.37	33.26	19.87
3	27.32	33.87	44.26	39.11	25.59	33.20
4	40.32	22.02	31.25	44.02	36.61	22.36
5	62.52	51.25	56.32	53.26	65.36	**62.36**
6	17.23	21.74	29.36	23.51	33.44	25.76
7	22.39	54.01	41.38	36.54	45.23	33.69
8	61.54	59.37	65.23	54.52	53.69	**66.32**
9	50.48	52.31	65.63	58.25	68.85	**56.37**
10	27.34	31.32	27.64	40.81	34.11	37.99

4.3 Identification of screened non adapted fungal isolates

The screened non-adapted fungal strains 1, 5, 8, and 9 were found to be *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp., based on the Microscopic appearance of fungi through lactophenol cotton blue staining (Opasols and Adewoye , 2010).

4.4 Compatibility analysis of the screened non adapted fungal strains

The 4 non-adapted fungal strains it was found that 3 were compatible with the other fungal strains but one fungus was found to be incompatible with that of the other three fungi in the PDA medium. The zone for clearance around the fungal colony (*Fusarium* sp.,) indicated that biocidal products produced by the fungi, that restricted the growth of the other fungi. In building a consortium all the fungi used in the system should be compatible with each other and since *Fusarium* sp., was found to be incompatible with the other isolates. This species was not included in the consortium using the non-adapted fungal strains (Aslim *et al*., 2002).

4.5 Treatment trials using Non-Adapted fungal strains

The reduction in the physico-chemical parameters of the effluent treated using the non-adapted fungal strains were observed (Ali *et al*., 2009) Since one of the fungi was found to be incompatible with the rest of the screened non-adapted fungus, a consortium was built using the 3 compatible fungi. The consortium of fungi consisting of the three compatible fungi was able to exert a significant reduction in the parameters observed (Table 2) than that of the individual selected strains.

4.6 Optimization of cultural conditions for maximum bioremediation ability

4.6.1 Effect of retention time

A reduction was observed from the first day onwards till the fifth day of incubation. Maximum reductions in all the parameters were observed on the fifth day and there was no significant reduction after 5 days of incubation. It should be noted that there was a steady increase in the reduction of parameters from the second day onwards which was because of the long lag phase followed by the individual fungal cultures. The maximum percentage of reduction after five days of incubation were found to be colour- 77.1, turbitity-31.8, resistivity-375.4, COD- 73.1, TS-77.3, TSS-61.6, TDS-59.8, hardness-56.9, Conductivity-79, alkalinity-7.31, pH- 6.02 (fig 1). Kilic *et al*., (2007) states that the period of time in bioremediation was one of the major conditions which play a vital role.

Table 2: Treatment of textile effluent by using Non-adapted fungi

Parameters	Initial values	Non- adapted fungal isolates				Consortium	Percentage of dye reduction
		Aspergillus sp.,	*Penicillium* sp.,	*Fusarium* sp.,	*Rhizopus* sp.,		
Colour (435 nm)	0.8578	0.683	0.721	0.538	0.693	**0.281**	**67.24%**
Turbidity (620 nm)	0.5713	0.613	0.618	0.641	0.711	**0.508**	**11.12%**
TS(mg/L)	13600	12100	11030	9090	10081	**8517**	**37.37%**
TSS(mg/L)	3330	3183	2220	1073	1948	**1497**	**55.04%**
TDS(mg/L)	10270	8917	8810	8017	8133	**7020**	**32%**
COD(mg/L)	1120	843	927	857	915	**830**	**26%**
Conductivity(mS)	13.74	13.89	13.78	13.91	13.84	**12.21**	**11.13%**
pH	6.9	5.62	5.34	5.21	5.34	**5.11**	**23.62%**
Alkalinity (ppt)	7.958	8.213	8.102	8.229	8.310	**6.952**	**12.34%**
Resistivity(Ω)	33.9	34.17	34.03	36.24	33.95	**33.13**	**2.65%**
Hardness(mg/L)	260	223	241	219	234	**189**	**27%**

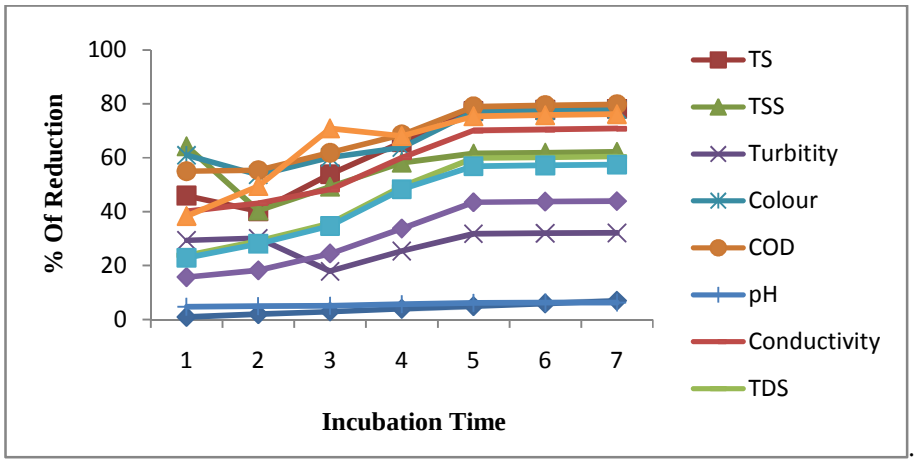

Figure 1: Effect of retention time by Non-adapted fungal consortium on bioremediation

4.6.2 Effect of initial pH

An efficient reduction in the physico-chemical parameters were observed at pH 6. At other pH there was no significant reduction in the physico-chemical parameters. The maximum reduction percentage values for colour-49.6, turbitity-22.9, resistivity-60.8, conductivity- 60.50, TS-49.5, TSS-45.6, TDS-33.8, hardness-40.1, COD-40.3, alkalinity-55, pH- 6.1(fig 2). Levin *et al.*, 2004 states that pH might have an effect on the activities of the fungal enzymes for different dyes and biomass production.

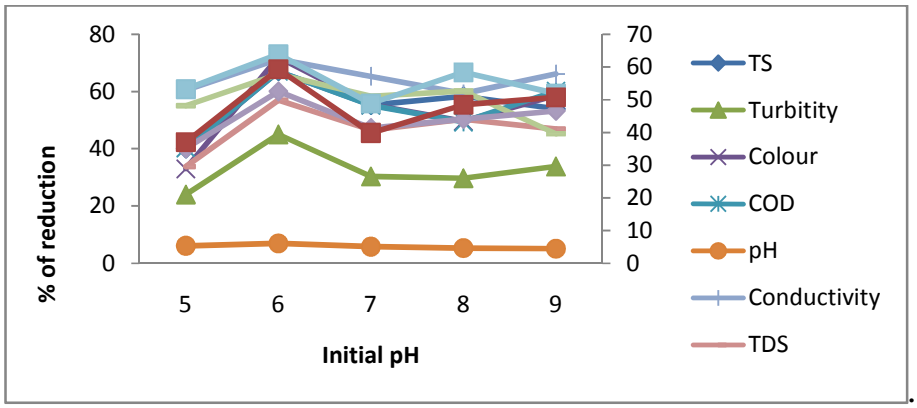

Figure 2: Effect of initial pH by Non-adapted fungal consortium on bioremediation

4.6.3 Effect of initial incubation temperature

The efficient reduction in the physico-chemical parameters were observed at 27°c. At other incubation temperature there was no significant reduction in the physico-chemical parameters. The maximum reduction percentage values for various incubation temperatures were found to be colour- 65.4, turbitity-38.1, resistivity-73.2, conductivity-75.30, TS-75.1, TSS-53.50, TDS-63.10, hardness-50.70, COD-59.70, alkalinity-54.40, pH- 7.1(fig 3). An optimal temperature of 27°c was best for the maximum decolorization (Yesilada *et al.*, 1998).

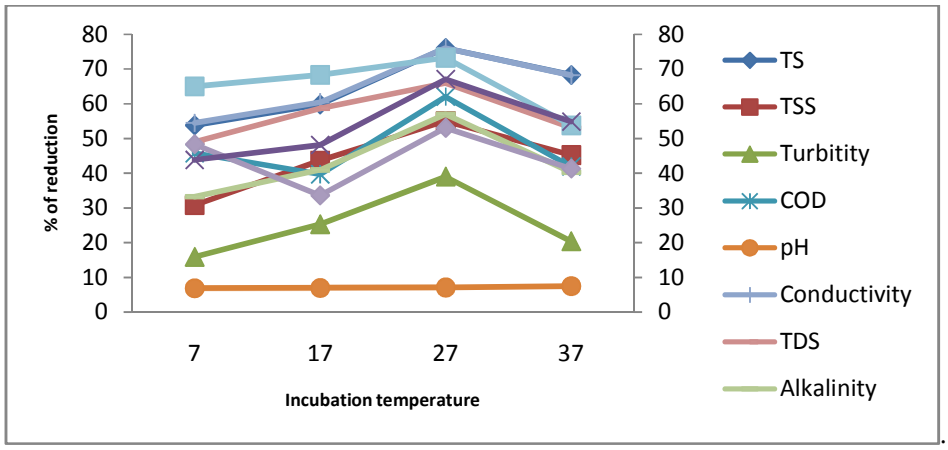

Figure 3: Effect of incubation temperature by Non-adapted fungal consortium on bioremediation

4.6.4 Effect of initial organic loading rate concentration

The efficient reduction in the physico-chemical parameters were observed at 100% organic loading rate (OLR). At other OLR there was no significant reduction in the physico-chemical parameters. The maximum reduction percentage values for colour- 77.1, turbitity-31.8, resistivity-375.4, COD- 73.1, TS-77.3, TSS-61.6, TDS-59.8, hardness-56.9, Conductivity-79, alkalinity-7.31, pH- 6.02(fig 4). Chen *et al.*, (2003) reported that increased amount of organic matter in the azo textile effluent increases the biomass content and hence the enzymes. This increased the reduction of the dye chromophore as well as the organic load.

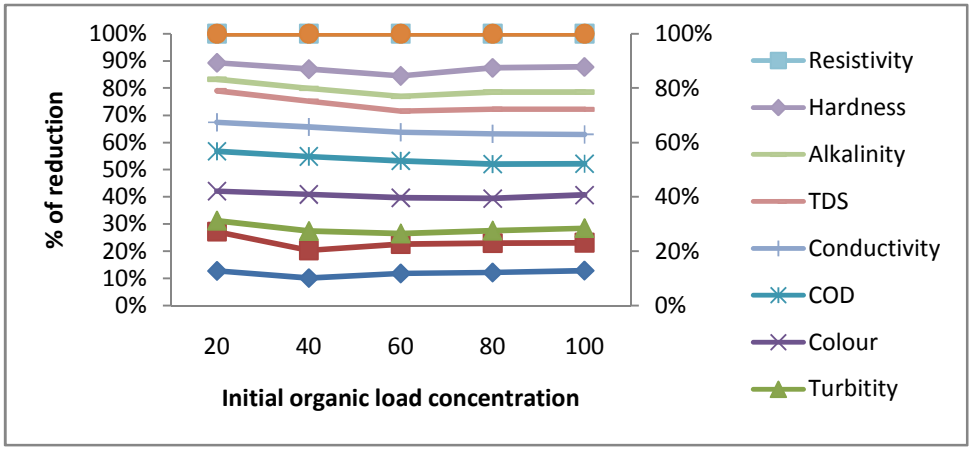

Figure 4: Effect of initial organic load concentration by Non-adapted fungal consortium on bioremediation

4.6.5 Effect of initial Inoculum concentration

The maximum reduction percentage values were found to be colour- 29.80, turbitity-60.10, resistivity-68.80, conductivity- 78.3, TS-59.8, TSS-63.2, TDS-53.00, hardness-45.7, COD-56.40, alkalinity-45.3, pH- 7.08. A very high inoculum concentration leads to the development of more biomass racing for the limited supply of nutrients in the form of organic load. Thus when a high initial inoculum load was added to the system of effluent for bioremediation there will be an immediate increase in the biomass concentration and a complete utilization of the

organic load in the effluent. As a result of the depletion of the organic load the biomass developed will not be further supported for the synthesis of enzymes for bioremediation (fig 5).

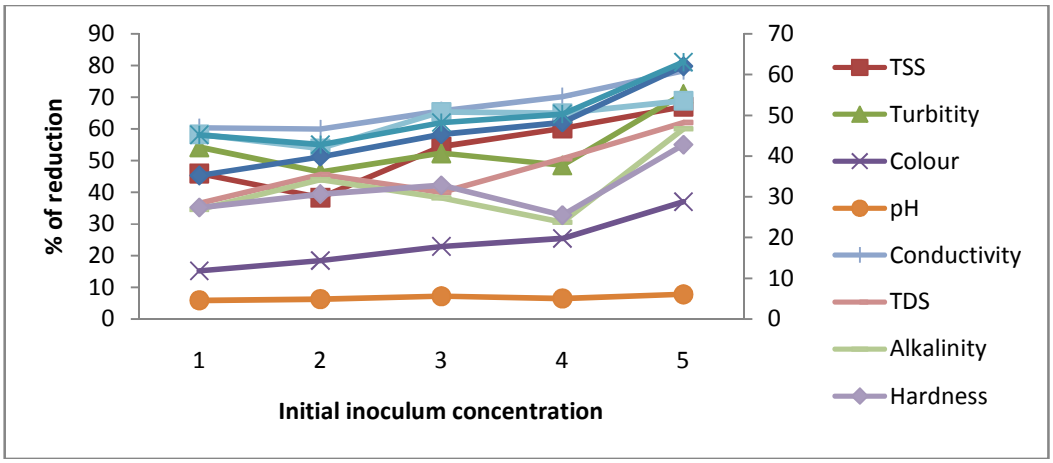

Figure 5: Effect of initial organic load concentration by Non- adapted fungal consortium on bioremediation

In optimized conditions the efficient bioremediation occurs about 5% concentration of the adapted fungal consortium was inoculated in 95 ml of 100% textile effluent at pH 5. The sample was incubated at 27ºc for a period of 5 days (Lu *et al*., 2009).

4.7 GC-MS analysis

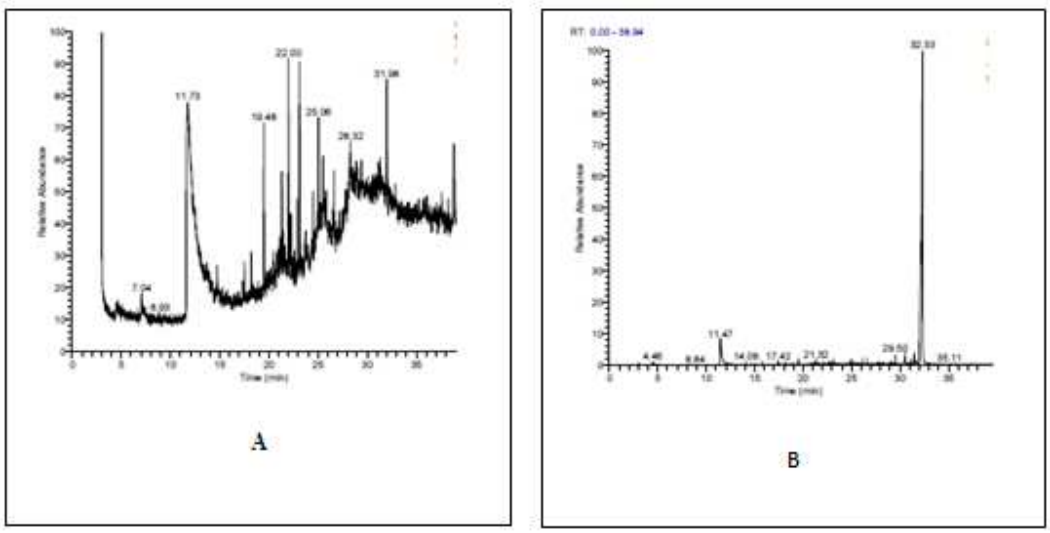

Figure 6: GC-MS chromatogram of untreated azo effluent (A) and effluent sample treated by the Non-adapted fungal consortium (B)

The untreated textile effluent showed a number of peaks in its chromatogram with a few peaks that were predominant and which were not found to be a contaminant of the column were studied. The compounds analyzed for these peaks were found to be the toxic product present in the untreated raw effluent sample. The treated effluent showed a major reduction in all the organic contents and the number of peaks that were observed was reduced to significant extent. The treated effluent were analyzed and found to be not toxic.

CONCLUSION

It could be ascertained from analyzing the chromatogram of the Non-adapted treated and untreated effluent sample that the toxicity and organic load was reduced by the action of the Non-adapted fungal consortium as compared to the individual fungi. This is mainly because of the wide spectral range and enzymes produced by the Non-adapted fungal consortium.

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