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# DEVELOPMENT OF MICROBIAL CONSORTIUM FOR THE BIODEGRADATION AND BIODECOLORIZATION OF TEXTILE EFFLUENTS

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**Abstract:** In the current study three bacterial species (*Bacillus sp.*, *Pseudomonas sp.*, and *Alcaligenes sp.*) and two fungal species (*Aspergillus sp.*, and *Penicillium sp.*) screened from 265 bacterial isolates and 35 fungal isolates respectively, were used in 23 different combinations for the biotreatment of textile waste water collected from Karur, Tiruppur and Coimbatore districts under aerated conditions. The chemical oxygen demand (COD), total solids (TS) total dissolved solids (TDS) & total suspended solids (TSS), hardness, and color intensity of the textile effluent was found to be very high than the permissible limits before treatment. After treatment one particular combination was capable of reducing the COD of the effluent sample by 75%. About five combinations of microbes efficiently reduced the color of the effluent by more than 50%. Another combination was found to be the most effective in the reduction of TS and TDS by 90% and 69%, respectively. Though there was no drastic change in the pH of the sample, it was not of great concern as the pH of the sample was well within the permissible limits for the discharge of the wastewater in to natural sources after treatment.

**Keywords:** Microbial consortium; biodegradation; biodecolorization; textile effluents



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## INTRODUCTION

Our biosphere is under constant threat from continuing environmental pollution. Impact on its atmosphere, hydrosphere and lithosphere by anthropogenic activities on water, air and land have negative influence over biotic and abiotic components on different natural eco-systems. In recent years different approaches have been discussed to tackle man made environmental hazards. Clean technology, eco-mark and green chemistry are some of the most highlighted practices in preventing and or reducing the adverse effect on our surroundings.

Among many engineering disciplines, Textile Engineering has direct connection with environmental aspects to be explicitly and abundantly considered. The main reason is that the textile industry plays an important role in a country like India and it accounts for around one third of total export. Out of various activities in textile industry, chemical processing contributes about 70% of pollution. It is well known that cotton mills consume large volume of water for various processes such as sizing, desizing, scouring, bleaching, mercerization, dyeing, printing, finishing, and washing.

Various chemical processing of textiles, large volumes of wastewater with numerous pollutants are discharged. Since the stream of water affect the aquatic eco-system due to the nature of dyes and auxiliaries a number of ways such as depleting the dissolved oxygen content or settlement of suspended substances in anaerobic condition, a special attention needs to be demanded.

The control of water pollution has found increasing attention in the recent year by the governments especially after record findings of cancer cases of unidentified causes. A known case where azo dyes were found to be potential carcinogenic in the Cristais River of Brazil close to a textile azo dye processing plant where its effluent is disposed and has impacts on a drinking water of the surrounding area (Lima *et al.*, 2010).

The release of dyes into the environment constitutes only a small proportion of water pollution, but dyes are visible in small quantities due to their brilliance.

Coimbatore “a concentration of textile mills especially wet processing for a long time. It is called the Manchester of South India” is thriving mostly on the wealth generated by the textile industries at large. Though it brings in more investment, more jobs more profit, the processing and dyeing part of the industry brings in more organic content and color to the final effluent which when released to the natural water body creates havoc in the surrounding environment. Without suitable treatment, such wastewater would destroy the natural water environment (Banat *et al.*, 1996).

The azo-dyes, including reactive, acid, direct dyes and vat dyes are commonly used in the textile industry. The water consumption and wastewater generation from a textile industry depends upon the processing operations employed during the conversion of fiber to textile fabric. On the basis of waste and wastewater (or effluent) generation, the textile mills can be classified (ISPCH, 1995) into two main groups viz., Dry processing mill and Woven fabric finishing mills. These stages consume approximately 2400 to 2700 $m^3$/day of raw water (ISPCH, 1995).

The wastewater characteristics depend upon the processing stages. In general, the wastewater from a typical cotton textile industry is characterized by high values of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), color, and pH (ISPCH, 1995). Because of the high BOD, the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources causing a great damage to environment. The high color renders the water unfit for use at the downstream of the disposal point. The advanced oxidation processes are costly in terms of installation, operation and maintenance costs. Biological processes are cheaper than the others. Investment costs for biological processes are five to twenty times less than chemical ones such as ozone or hydrogen peroxide and the running costs are three to ten times less. In view of the above adverse effects, the textile industry effluent is to be treated and discharged according to the standards prescribed under Central Water (Prevention and Control of Pollution) Act, 1974.

This work aims to isolate indigenous predominant adapted bacterial and fungal strains from textile effluents which possess the ability to decolorize and degrade a variety of textile effluents. These isolates were used to develop a microbial consortium that can decolorize and biodegrade the organic load in the effluent at a faster time and can be used further to develop a continuous process of the treatment of textile effluents containing a wide variety of textile dyes including reactive dyes. Reactive dye a chromospheres contains a substituent that is activated and allowed to directly react to the surface of the substrate. Reactive dyes have good fastness properties owing to the bonding that occurs during dyeing. Reactive dyes are most commonly used in dyeing of cellulose like cotton or flax, but also wool is dyeable with reactive dyes. Acid Dyes, any dyes including acid dyes have the ability to induce sensitization in humans due to their complex molecular structure and the way in which they are metabolized in the body. Direct dyes, Direct or substantive dyeing is normally carried out in a neutral or slightly alkaline dyebath, at or near boiling point, with the addition of either sodium chloride (NaCl) or sodium sulfate ($Na_2SO_4$). Direct dyes are used on cotton, paper, leather, wool, silk and nylon.

Vat dyes are an ancient class of dyes, based on the natural dye, indigo, which is now produced synthetically. Plus Textile auxiliaries, such as caustic soda, acetic acid, sodium silicate and other more chemicals.

## MATERIALS AND METHODS

### Collection of effluent samples

The effluent samples were collected from the treatment plants located at various common effluent treatment plants located in the areas of Karur, Tiruppur, and Coimbatore districts Tamil Nadu, South India (GPS): N11 1.10448 E76 58.4826. The effluent samples were pooled together and used for the isolation of microbial strains and treatment trials.

### Isolation, screening and Identification of Indigenous adapted bacterial and fungal strains from textile effluents

The microbial source (pooled effluent sample) was enriched in culture media for bacteria and for fungi as cited in Khelifi *et al.* (2008). The enriched culture was then serially diluted and plated in the respective solid media. Those colonies on the isolation plates which were morphologically distinct and predominant were selected and screened were studied for their decolorization pattern on the three commonly used tough dyes Red BSID, Red yellow marl and Indigo carmine. About 1 mg of dye was incorporated in 100 ml of Nutrient broth (bacteria) and potato dextrose broth (fungi) separately with each isolate and the decolorization was studied for these three dyes by measuring the optical density of the sample in a UV-Visible spectrophotometer (Khelifi *et al.*, 2008). The efficient isolates of bacteria and fungi were selected and identified based on colony morphology, standard biochemical and Microbiological tests (Cappuccino & Sherman, 1999; Rafi & Sajjad-Ur-Rahman, 2002; Klich, 2002).

### Physic-Chemical characterization of pooled untreated effluent

Various physical and chemical parameters (Chemical Oxygen Demand [COD], Total Hardness, Total Solids (TS), Total Dissolved Solids (TDS), Total suspended solids (TSS), Color, Turbidity and pH) were assessed using **Table 2** for the pooled untreated effluent by standard methods (APHA, 1992).

### Design of microbial consortia and Treatment trials

Each bacterial isolates were given a number and the fungal isolates were designated with an alphabet (*Bacillus sp*., - 1; *Pseudomonas* sp., - 2; *Alcaligenes* sp., - 3; *Aspergillus* sp., - A; *Penicillium* sp., - B). Accordingly, A1, A2, A3, A12, A13, A23, A123, 123, B1, B2, B3, B12, B13, B23, AB, AB1, AB2, AB3, AB12, AB13, AB23, AB123 are the 23 different combinations which were used for treatment trials. An initial inoculums concentration of 5%, of 23 different combinations of bacteria and fungi were inoculated to 200 ml of the textile effluent sample in a 250 ml Erlenmeyer flask and were incubated at room temperature in a metabolic shaker at 120 rpm for a period of 7 days (Asgher *et al.*, 2007).

### Analytical procedures: Biodegradation and Biodecolorization assays

Various physical and chemical parameters of the effluent samples (COD, Hardness, TS, TDS, TSS, Color, Turbidity and pH) were studied from the first day onwards for a period of seven days for all the different combinations of organisms used (APHA, 1992) (Murugalatha *et al.*, 2010). The color intensity and the turbidity of the effluent sample were measured at 490 nm and 620 nm, respectively (Amar *et al.*, 2010). Reductions of these parameters were assayed by the % of reduction on comparison with the untreated effluent using **Eq. 1**:

$$A = 100(A_i - A_t)/A_i \qquad (1)$$

where $A$ is the percentage of reduction of that particular parameter, $A_i$ the initial concentration of the particular parameter and $A_t$, the concentration after specified time of the same parameter (Khelifi *et al.*, 2008).

## RESULTS AND DISCUSSION

### Isolation, Screening and Identification of Indigenous adapted bacterial and fungal strains from textile effluents

About 265 and 35 morphologically distinct bacterial and fungal isolates were obtained through dilution plating of textile effluents. Twelve organisms (seven bacteria and five fungi) which showed decolorization on all the three dyes were selected and further screened. Among these, five organisms (two fungus and three bacteria) capable of reducing all the three dyes, by more than 75% were found to be the efficient decolorizers. The selected bacterial isolates were identified as *Bacillus* sp., *Pseudomonas* sp., and *Alcaligenes* sp., and the results were given in the **Table 1** (Cappuccino & Sherman, 1999).

The two efficient fungal strains were identified as *Aspergillus* sp., and *Penicillium* sp., based on their microscopic observation. The first strain showed a septate and dichotomous hyphae, at $45^o$ angle branching. Conidial heads are radiate to loosely columnar. Conidiophores are coarsely roughened, uncolored, vesicles spherical, metulae covering nearly the entire vesicle in biseriate species.

**Table 1.** Identification of the Isolated Bacterial Isolates by Microscopic and Biochemical Tests C-Cocci; R-Rod; A-Acid production; 1-3 Isolates.

| Isolates | 1 | 2 | 3 |
|---|---|---|---|
| Gram Stain | - | - | - |
| Shape | R | R | R |
| Indole | - | - | - |
| MR | - | - | - |
| VP | + | - | - |
| Citrate | - | + | - |
| Dextrose | A | - | - |
| Lactose | - | - | - |
| Mannitol | - | - | - |
| Sucrose | A | - | - |
| H2S | - | - | - |
| Urease | - | - | - |
| Catalase | - | + | + |
| Oxidase | + | + | + |

Conidial heads radiate, uni- and biseriate; however, some isolates may remain uniseriate, producing only phialides covering the vesicle which are the characteristic features of *Aspergillus* sp., (Klich, 2002). For the second fungus, the hyphae were terverticillate and the conidia were spherical to elliptical in shape. Conidia were smooth and had a green color reflection in the mass. These microscopic features were found to be that of *Penicillium* sp., (Rafi & Sajjad-Ur-Rahman, 2002).

## Physico-Chemical characterization of pooled untreated effluent

The initial values of the estimated parameters for the pooled untreated effluent sample and that of the permissible limits for the safety release of the effluents in the natural water body has been provided in **Table 2**. The organic content in the effluent sample was found to be very high. The color of the sample also was found unacceptable to be discharged in to the natural water body.

## Treatment trials using microbial consortia

Samples were withdrawn at an interval of every 24 hours and all the physico-chemical parameters were recorded for all the different combinations. A pattern of reduction in parameters was observed in all the combinations, which showed a continuous reduction from the first day and a maximum reduction was observed at the end of five days of incubation and the parameters are given in **Table 3**. There is no change in the pH in the treatment trials and this parameter is negligible since it remained well under the permissible limits. A maximum of 75% reduction in the COD was observed in the combination B23. The capability of combination to reduce COD increased from day 1 to 5

**Table 2.** Physico-chemical characterization of pooled untreated effluent

| Parameters | Pollution Control Norms | Initial values |
|---|---|---|
| Hardness (mg/l) | Not objectionable | 325.125 |
| COD (mg/l) | 400 mg/l < | 1819.13 |
| TS (g/l) | 50 mg/l | 33.53 |
| TSS (g/l) | 3000 mg/l < | 12.1 |
| TDS | 3000 mg/l < | 21.43 |
| pH | 5-9 | 10.5 |
| Color | Nil | 1.096 |
| Turbidity | Not objectionable | 0.976 |

**Table 3**. Percentage of reduction of the observed parameters after five days of incubation

| Combi-nation | COD | Hardness | Color | TS | TSS | TDS | pH | Turbidity |
|---|---|---|---|---|---|---|---|---|
| A1 | 46.4 | 43.8 | 52.9 | 89.3 | 21.4 | 66.7 | 11.3 | 39 |
| A2 | 25 | 28.6 | 36.8 | 73.1 | 14.3 | 50 | 13.6 | 37.9 |
| A3 | 50 | 21.4 | 50.1 | 65.4 | 55.6 | 57.5 | 12.3 | 45.3 |
| A12 | 12.5 | 31.3 | 54.8 | 87.5 | 30 | 61.4 | 13.6 | 35 |
| A13 | 40 | 21.4 | 66.4 | 75.0 | 28.6 | 59.5 | 15.7 | 45.1 |
| A23 | 12.5 | 28.6 | 53.0 | 90.0 | 37.5 | 69.0 | 13.6 | 44.2 |
| A123 | 20.5 | 21.4 | 48.2 | 89.3 | 21.4 | 63.2 | 13.4 | 33.3 |
| 123 | 16.7 | 26.7 | 37.4 | 78.6 | 14.3 | 55 | 13.6 | 51.9 |
| B1 | 71.4 | 50.0 | 5.4 | 11 | 7.7 | 11.5 | 5.9 | 21.6 |
| B2 | 33.3 | 14.3 | 21.4 | 24.3 | 15.4 | 25.5 | 5.1 | 17.2 |
| B3 | 46.7 | 50 | 2.4 | 26.1 | 33.3 | 25.8 | 2.1 | 10.4 |
| B12 | 28.6 | 38.5 | 12.7 | 48.1 | 50 | 48.0 | 0.7 | 6.6 |
| B13 | 50 | 33.3 | 1.4 | 24.3 | 45.5 | 15.3 | 7.8 | 16.7 |
| B23 | 75 | 50.0 | 6.2 | 54.7 | 70.4 | 54.0 | 11.4 | 5.8 |
| B123 | 10.4 | 28.6 | 1.7 | 50.5 | 58.3 | 50.0 | 7.3 | 5.6 |
| AB | 71.4 | 21.4 | 1.5 | 51.8 | 84.6 | 51.6 | 7.4 | 5.4 |
| AB1 | 33.3 | 33.3 | 3.3 | 12.2 | 6.0 | 10.0 | 3.9 | 18.9 |
| AB2 | 15.9 | 35.7 | 8.7 | 32.5 | 55.7 | 10.1 | 5.8 | 22.9 |
| AB3 | 8.3 | 30.8 | 7.2 | 24.5 | 16.4 | 11.2 | 3.1 | 56.9 |
| AB123 | 15.3 | 25 | 17.2 | 5.0 | 26.9 | 9.5 | 4.9 | 30.8 |
| AB13 | 33.3 | 30.8 | 7.7 | 28.2 | 40 | 10.7 | 5.8 | 36 |
| AB23 | 18.8 | 9.1 | 3.0 | 29.6 | 20.6 | 14.4 | 3.1 | 38.2 |
| AB123 | 9.4 | 25.0 | 3.8 | 23.2 | 54.1 | 12.6 | 9.5 | 35.3 |

and a further increase in this COD was observed after five days. The increase in the COD value on the later days might be the evidence of inappropriate mixing ratio between microbial combinations and organic materials. About five different combinations (A3, B1, B13, B23, AB) gave more than 50% reduction in COD after 120 hours of incubation. The total organic content removal in case of combined electrochemical and activated carbon absorption for waste water treatment was about 59% (Rajkumar *et al.*, 2005), but in the present study consortium was able to reduce the organic content of up to 75%. A maximum of 66 % reduction in TDS was observed for the combination A1.

More number of combinations of microbes was found to be efficient in the reduction of TDS than other parameters. There is also about 56% of reduction in the turbidity of the sample by the combination AB3 which showed that there is a clear reduction in the percentage of the suspended solids in this combination. The combinations B1, B3 and B23 were able to reduce the calcium and magnesium content of the pooled effluent up to 50 %.

The combination A13 was able to efficiently decolorize the effluent sample up to 66.4%, apart from this there were four different combinations of microbes which were able to decolorize the pooled effluent of more than 50% (A1, A3, A12 and A23). The reason for the other combinations of microbes that are not so efficient might be because of the incompatible nature of the microbes used in the combination or because of the action the secondary metabolites produced by one on the other. Decolorization time taken by the cultures to achieve 66% decolorization compares favorably with reports on dye decolorization by most of the white rot fungi which require 7–20 days period for 90% decolorization of a diverse range of synthetic dyes (Kirby *et al*., 2000) and other mixed microbial cultures (Senan & Abraham 2004; Adedayo *et al*., 2004). Microbial components of mixed microbial cultures are capable of decolorizing dyes via biotransformation and biodegradation (Banat *et al*., 1996). The efficiency of the decolorization process depends on the survival, adaptability and activities of enzymes produced by microorganisms present in the mixed cultures (Cripps *et al*., 1990; Senan & Abraham, 2004). Normally the dye concentration in the effluent varies within a narrow range of 0.1–0.2 g/l (O'Neill *et al*., 1999).

## SUMMARY

The combinations A1 and A12 were found to be the most efficient as they have efficiently reduced all the parameters under study than other combinations. There were a few other combinations which were efficient on a few particular parameters. These two combinations could be made use of in treatment trials in the industries.

## CONCLUSION

Bacteria offers a cheaper and environment friendlier alternative for color removal in textile effluents. Biological treatment has been effective in reducing dye house effluents and when used properly has a lower operating cost than other remediation process.

Decolorization of Azo dyes during biological effluent treatment can involve both adsorption to cell biomass and degradation by azo-bond reduction during anaerobic digestion. Degradation is expected to form aromatic amines, which may be toxic and recalcitrant to anaerobic treatment but degradable aerobically. Methods for the quantitative detection of substituted aromatic amines arising from azo-dye cleavage are complex.

Microbial consortium has become a very good source for the textile industry in getting rid of their effluent problem by Biodegradation and Decolorization process. Since they are cost effective and efficient it is highly recommended for the industries in making use of the consortium for the proper disposal of textile effluents.

The process has many advantages of which:

- The treatment uses no chemicals.
- The consortium disposes no harmful matters into the environment.
- The process eliminates chemical processing and vapor.
- No energy used and consequently zero carbon process. Also worth carbon credit.
- No complicated plants needed and less capital investment.

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## REFERENCES

Adedayo, O., Javadpour, S., Taylor C., Anderson W.A. & Young, M. (2004). Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. *World J. Microbiol. Biotechnol*. **20**(6), 545–550.

Amar, A., Telke, S.M., Joshi, S., Jadhav, U., Dhawal, P.T., Sanjay, P. & Goivindwar, S. (2010) Decolorization and detoxification of Congo red and textile industry effluent by an isolated bacterium Pseudomonas sp. SU-EBT, *Bidegradation* **21**(2), 283–296. doi: 10.1007/s10532-009-9300-0

APHA, (1992) Standard methods for examination of water and wastewater APHA, AWWA, AWWA WPCF, Washington, DC.

Banat, I.M., Nigam, P., Singh, D. & Marchant, R. (1996) Microbial Decolorization of Textile Dye Containing Effluents: A Review. *Bioresour. Technol.* **58**(3), 217–227. doi: 10.1016/S0960-8524(96)00113-7

Cappuccino, J.G. & Sherman, N. (1999) *Microbiology: A Laboratory Manual.* 4th Ed., pp: 199–204. Addison Wesley Longman, Inc. Harlow, England

Cripps, C., Bumps, J.S. & Aust, S.D. (1990) Biodegradation of azo and heterocyclic dyes by Phanerochaete chrysosporium. *Appl. Environ. Microbiol*. **56**(4), 1114–1118.

Khelifi, E., Bouallagui, H., Touhami, Y., Godon, J.-J. & Hamdi, M. (2009) Enhancement of textile wastewater decolourization and biodegradation by isolated bacterial and fungal strains. *Desal. Water Treatm.* **2**, 310–316. doi: 10.5004/dwt.2009.294

Lima, G.M., Lins, Santa Cruz, W., Vieira, Z.M.C.L., Costa Neto, F.A. & Miranda, E.A.A. (2010) Determining indicators of urban household water consumption through multivariate statistical technique *J. Urban Environm. Engin.* **4**(2), 74–80. doi: 10.4090/juee.2010.v4n2.074080

ISPCH (1995) *Industrial safety and pollution control handbook*. A joint publication of National Safety Council and Associate (Data) Publishers Pvt. Ltd., Hyderabad, 2nd edition, 2nd reprint, 451-466.

Kirby, N., Marchant, R. & McMullan, G. (2000) Decolourisation of synthetic textile dyes by Phlebia tremellosa. FEMS Microbiol Lett **188**(1), 93–96. doi: 10.1016/S0378-1097(00)00216-0

Klich M. A. (2002). Identification of common Aspergillus sp., 1st ed. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands, 116p.

Asgher, M., Bhatti, H.N., Shah, S.A.H., Javaid Asad, M. & Legge, R.L. (2007) Decolorization potential of mixed microbial consortia for reactive and disperse textile dyestuffs. *Biodegradation* **18**(3), 311–316. doi: 10.1007/s10532-006-9065-7

Murugalatha, N., Mohankumar, A., Sankaravadivoo, A. & Rajesh, C. (2010) Textile effluent treatment by Bacillus species isolated from processed food. *African J. Microbiol. Res.* **4**(20), 2122–2126.

O'Neill, C., Hawkes, F.R., Hawkes, D.L., Lourenco, N.D., Pinheiro H.M. & Delee, W. (1999) Color in textile effluents: sources, measurement, discharge consents and simulation: a review. *J. Chem. Technol. Biotechnol.* **74**(11), 1009–1018. doi: 10.1002/(SICI)1097-4660(199911)74:11

Rafi, M. & Sajjad-Ur-Rahman (2002). Isolation and identification of indigenous Penicillium Chrysogenum series. *Int. J. Agri. Biol.* **4**(4), 553–558.

Rajkumar, D., Palanivelu, K. & Balasubramanian, N. (2005) Combined electrochemical degradation and activated carbon adsorption treatments for wastewater containing mixed phenolic compounds. *J. Environ. Eng. Sci.* **4**(1), 1–9. doi: 10.1139/s04-037

Senan R.C. & Abraham, T.E. (2004) Bioremediation of Textile Azo Dyes by Aerobic Bacterial Consortium Aerobic Degradation of Selected Azo Dyes by Bacterial Consortium. *Biodegradation* **15**(4), 275–280. doi: 10.1023/B:BIOD.0000043000.18427.0a