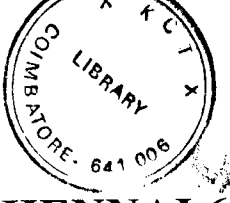

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BONAFIDE CERTIFICATE

Certified that this project report "**DECOLORIZATION AND DEGRADATION OF TEXTILE DYES USING MICROBIAL CONSORTIUM**" is the bonafide work of "**RAVISANKAR. V. B, SRIDHAR. S** and **VIGNESH. P** who carried out the project work under my supervision.

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The Report of the project work submitted by the above students in partial fulfilment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be their original work. It was submitted for evaluation and viva – voce held on 20/04/2010

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ABSTRACT

Textile dye effluents pose environmental hazards because of color and toxicity. The discharge of highly colored effluents affects water transparency and gas solubility in water bodies due to their toxicity. The present study deals with the decolorization and degradation of textile dyes using microbial consortium obtained from the microbial strains isolated from the effluent samples and soil samples from the dye contaminated sites of various textile industries. Reactive Red 120, Reactive Black 5 and Direct Red 81 were used as model dyes. About 5 bacterial colonies and 4 fungal strains which showed good synthetic dye decolorization efficiency were selected after initial screening. The various physical parameters such as pH, temperature and C:N ratio were optimized for the dye decolorization by the fungal strains. Four different fungal consortia were developed and they showed 55 to 80% of decolorization of three synthetic dyes. The four fungal strains were identified to be *Pleurotus ostreatus, Aspergillus niger, Penicillium simplicissimum* and *Penicillium chrysosporium.* Bacterial consortium containing 5 different bacterial strains was developed and it showed 50 to 80% of decolorization of the three synthetic dyes. The bacterial strains were identified to be *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Pseudomonas fluorescens*. The three synthetic dyes were decolorized and degraded by the microbial consortium efficiently.

Keywords: Toxicity, Decolorization, Degradation, Synthetic dyes.

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LIST OF ABBREVIATIONS

TSS	Total Suspended Solids
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
CETP	Common Effluent Treatment Plant
SIPCOT	State Industries Promotion Corporation of Tamilnadu
nm	nanometer
mg	milligram
^{o}C	Degree celsius
w/v	weight/volume
g/l	gram/litre
C:N	Carbon: Nitrogen
NA	Nutrient Agar
PDA	Potato Dextrose Agar
rpm	revolutions per minute
hrs	hours

INTRODUCTION

CHAPTER 1

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. Textile industries consume a considerable amount of water in their manufacturing processes. Considering both the volume and the effluent composition, the textile industry is rated as the most polluting among all industrial sectors. Wastewater from textile industries creates a great pollution problem due to the dye content. Textile dye effluents pose environmental hazards because of color and toxicity. All dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, many chemicals including oxidizing agents and microbial attack. During processing, up to 15% of the used dyestuffs are lost in industrial effluent. The effluents from these industries are complex; contain a wide variety of dyes and other products such as dispersants, acids, bases, salts, detergents, humectants, oxidants, etc. Among the wide spectrum of water pollutants, the synthetic dyes cause a severe problem. Synthetic dyes are colouring agents mainly used in textile industries which generate a huge amount of wastewater in the process of dyeing. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable. Colour is one of

the most obvious indicators of water pollution and discharge of highly coloured synthetic dye effluents can be damaging to the receiving water bodies (Nigam *et al*, 1996a)

Synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetics and other industries. Approximately 10,000 dyes and pigments are industrially used and over 0.7 million tons of synthetic dyes are produced annually worldwide. Approximately 10-15% of the dyes are released into the environment. Major classes of synthetic dyes used are azo, anthraquinone and triphenylmethane. In addition to their visual effect and adverse impact in terms of Chemical Oxygen Demand (COD), many synthetic dyes show their toxic, carcinogenic and genotoxic effects. Conventional wastewater treatment plants are unable to perform a complete dye removal, 90% of reactive textile dyes persist after activated sludge treatment. Since dyes are designed to be resistant to microbial and physico-chemical attack, most of them are not easily destroyed by conventional processes of wastewater treatment, including biological treatment by activated sludge. Azo dyes are the largest synthetic chemicals, which are used in textile, leather, printing, plastic, food, paint, cosmetics and pharmaceutical industries. Most of the azo dyes are either inert or non-toxic, but they become toxic, mutagenic and carcinogenic upon their biotransformation. Azo dyes are the main chemical class of dyes with the greatest variety of colors, therefore they have been extensively used by the industry. These dyes are characterized by one or more azo linkages (R_1-N=N-R_2) and by aromatic structures. Degradation of dyes, especially azo dyes, which comprise about 70% of all dyes used, is difficult due to their complex structure and synthetic nature.

Currently, various chemical, physical and biological treatment methods are used to remove color. The majority of physical, chemical and biological color removal

techniques work either by concentrating the color into sludge, solid supports, or by the complete destruction of the dye molecule. It is expected that decoloration systems involving destruction technologies will prevail, as the transfer of pollution from one part of the environment to another is prevented. Currently, the major methods of textile wastewater treatment involve physical and/or chemical processes as membrane filtration, coagulation, flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, ultrasonic mineralization, electrolysis, chemical reduction and advanced chemical. The advanced oxidation processes include chlorination, bleaching, ozonation, Fenton oxidation, photo catalytic oxidation and wet-air oxidation. Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater were not widely applied in the textile industries. There is also the possibility that a secondary pollution problem will arise due to excessive chemical use. There are many effective wastewater treatments; however these technologies are highly expensive. Biological treatments that have been conventionally applied present certain drawbacks.

Microbial communities are of primary importance in bioremediation of metal contaminated soil and water, because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization. Biological methods using various microbes like bacteria, fungi and algae of dye removal could be a viable option as a low-cost and eco-friendly decentralized wastewater treatment system for small- scale industries. The ability of bacteria to metabolize azo dyes has been investigated by a number or research groups. Under aerobic conditions azo dyes are not readily metabolized, although the ability of bacteria with specialized reducing enzymes to aerobically degrade certain azo dyes was reported. In contrast, under anaerobic conditions many bacteria reduce azo dyes by

the activity of unspecific, soluble, cytoplasmic reductase, known as azo reductases. The anaerobic reduction degrades the azo dyes that are converted into aromatic amines (Yaropolov *et al*, 1994) which may be toxic, mutagenic and possibly carcinogenic to mammalians. Therefore, to achieve complete degradation of azo dyes, another stage that involves aerobic biodegradation of the produced aromatic amines is necessary. Bacterial biodegradation of non-azo dyes has only recently been studied. It has been observed that several bacteria can degrade anthraquinone dyes. Aerobic decolourisation of triphenylmethane dyes has also been demonstrated. In phtalocyanine dyes, reversible reduction and decolorization under anaerobic conditions have been observed.

The most widely researched fungi in regard to dye degradation are the ligninolytic fungi. White-rot fungi in particular produced enzymes as lignin peroxidase, manganese peroxidase and laccase that degrade many aromatic compounds due to their non-specific activity. Large literature exists regarding the potential of these fungi to oxidize phenolic, non-phenolic, soluble and non-soluble dyes. In particular laccase from *Pleurotus ostreatus, Schizophyllum commune, Sclerotium rolfsii and Neurospora crassa,* seemed to increase up to 25% the degree of decolorization of individual commercial triarylmethane, anthraquinonic, and indigoid textile dyes using enzyme preparations (Barr and Aust, 1994). On the contrary, manganese peroxidase was reported as the main enzyme involved in dye decolorization by *Phanerochaete chrysosporium* (Pitter and Chudoba, 1990) and lignin peroxidase for *Bjerkandera adusta*. Some non-white-rot fungi that can successfully decolorize dyes have also been reported. Aerobic decolourization of azo dyes by bacterial as well as fungal cultures results in more complete degradation and avoids accumulation of carcinogenic intermediates.

The decolorization of the textile dyes using a microbial consortium using various with dye decolorizing bacterial and fungal strains is an effective method for treating the effluent dyes. The microbial consortium can produce a rapid decolorization due to concerted metabolism of the different microbes which are part of the consortium. The consortium developed with the bacterial and fungal strains produced a synergetic reaction of both strains leading to the complete mineralization of the textile wastewater. This study focuses on the biological decolourization of textile effluents through microbial isolates obtained from contaminated sites. The bacterial and fungal colonies were isolated from the effluent samples and the soil from the contaminated sites. Then the strains with the ability to decolorize the effluent dyes were selected and the microbial consortia are developed with the strains that decolorized the effluent dyes. The microbial consortia decolorized the dye with higher efficiency. The following are the different objectives of the current study,

1) Isolation and screening of bacterial and fungal strains from textile effluent samples and soil samples from the contaminated sites.

2) Decolorization and degradation of textile effluent dyes and commercial dyes using isolated bacterial and fungal strains and optimization of decolorization efficiency.

3) Development of Microbial consortium which can decolorize the dyes with a higher efficiency.

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 Dyes

A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fiber (Zollinger, 2002). A mordant is a substance used to set dyes. A mordant is either inherently colloidal or produces colloids and can be either acidic or basic. Mordants include tannic acid, alum, chrome alum, and certain salts of aluminum, chromium, copper, iron, potassium, and tin.

In other words, dye is defined as a substance, usually organic, which is designed to be absorbed or adsorbed by, made to react with, or deposited within a substrate in order to impart color to the substrate with some degree of permanence.

2.2 History of dyes

Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their clothes. The first evidence of the use of colorant materials by man goes as far as 15000-9000 BC, in the walls of the Altamira cave in Spain. The drawings were performed with inorganic pigments like soot, manganese oxide, hematite and ochre. Historically there is a dye, derived from animal sources (molluscs), that is very important, although presently has no relevance and it's not commercially available. It is Tyrian Purple and the pigment itself is not in the mollusc; however, when the precursor is extracted it can be converted to the dye by air or light. The presence of this dye goes as far as 1400 BC in the Late Bronze Age as found recently in

Lebanon. It has always been rare and costly being used by Roman emperors and high ranking ecclesiastics (Clark *et al.*, 1993).

Another ancient dye that is still in use, although not from natural origin nowadays, is indigo. It was extracted from *Indigofera tinctoria* by fermentation and had a characteristic blue colour. It was used as a pigment by the Romans because it had to be chemically reduced to become water soluble. It was firstly synthetically produced by Adolf von Baeyer in 1880, and actually is used to dye denim (Clark *et al.*, 1993). Up to the end of the nineteenth century natural dyes, obtained mainly from plants (roots, stems, leaves, flowers, fruits, seeds and lichens were the main colorants available for textile dyeing procedures. The main disadvantages of the use of natural dyes are the need for several steps in the dyeing process, the diversity of sources and related application procedures, the rapid change in trends and the demand for good fastness properties on different substrates that would require a complete database describing possible applications (Bechtold *et al.*, 2003).

The pioneering synthesis of mauveine by W. H. Perkins started the era of synthetic dyes, with chemical and physical properties better suited to contemporary demands, better level of quality and more reproducible techniques of application. It also allowed the development and extension of the use of particular products. For example, the development of synthetic fibres such as polyester and cellulose triacetate would have been severely hindered without the design and synthesis of dyes with appropriate properties.

Since then thousands of dyes have been synthesised, and dye manufacture has become a significant part of the chemical industry. Nowadays, when care of the

environment is a major issue, it is tempting to assume that the use of natural colours is an environmental friendly alternative to present-day practice. There are several groups studying the use of natural dyes in modern dyeing industry (Angelini *et al.,* 1997; Angelini *et al.,* 2003; Bermejo *et al.,* 2003; Kim *et al.,* 2004b; Paul *et al.,* 2004; Kamel *et al.,* 2005) Some of the advantages of the use of this type of compounds are the absence of toxicity upon humans, the use of sustainable sources and the fit into the natural pathways of biodegradation of the released dye baths.

2.3 Structures and uses of dyes

Dyes are compounds that absorb light with wavelengths in the visible range, i.e., 400 to 700 nm. The major structure element responsible for light absorption in dye molecules is the chromophore group, i.e. a delocalised electron system with conjugated double bonds. The absorption of UV/Vis radiation by an organic molecule is associated with electronic transitions between molecular orbitals. The energy of the absorbed radiation is given by:

$$\Delta E = E_1 - E_0 = h\nu = hc / \lambda$$

where E_0 is the energy corresponding to the fundamental state of the molecule (J), E_1 is the excited state energy (J), h is the Planck's constant ($6{,}626*10^{-34}$ Js), ν is the electromagnetic radiation frequency (Hz), c is the light velocity ($3*10^{8}$ $m.s^{-1}$) and λ is the wavelength (nm). The more extended the electronic delocalisation, the lower is the transition energy and the higher is the wavelength. To allow delocalization of the electrons double bonds must alternate with single bonds. In the case of synthetic dyes, delocalization is also promoted by benzene or naphthalene rings (Rocha Gomes 2001). Chromophores frequently contain heteroatoms as N, O and S, with non-bonding electrons. By incorporating these electrons into the

delocalised system in the aryl rings, the energy of the electron cloud is modified, the wavelength of the absorbed radiation will shift towards the visible range, and the compound will be coloured. In many cases dyes containadditional groups called auxochromes, which are electron withdrawing or electron donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. The most important auxochrome groups are: hydroxyl and derivates, -OH, -OR; amino and derivates, $-NH_2$, -NHR, $-NHR_2$; sulphonic, $-SO_3H$; carboxylic, -COOH; and sulphide, -SR. Some auxochromes also increase the dye affinity for the fibre (natural or synthetic). Natural fibres are based on cellulose (polymeric linear chains of glucose) – cotton and linen - or proteins – wool and silk. Synthetic fibres are for instance viscose, cellulose acetate, polyamide, polyester and acrylic.

According to the Colour Index dyes can be classified on the basis of colour and application method. Various attractive forces have the potential of binding dyes to fibres, and often more than one type of chemical bonding can operate with the same dye-fibre combination. The dominant force depends on the chemical character of the fibre and the chemical groups in the dye molecule. The types of bonds established between the dye and the fibre, by increasing relative strength of the bond, can be: Van der Waals, hydrogen, ionic or covalent (Gomes 2001) According to the application categories dyes can be classified as seen in Table 2.3.1.

Dyes are used in textile industry, leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair colouring and cosmetics. Moreover these compounds have been employed for the control of the efficacy of sewage and wastewater treatment, for

the determination of specific surface area of activated sludge and for ground water tracing (Forgacs *et al.,* 2004). Due to the large amounts used, the most significant industrial use is in textile dyeing.

Azo dyes are the most widely use among synthetic dyes, representing almost 70% of the textile dyestuffs produced. They are easy to synthesize, have low cost, are stable, can be used to colour several materials (Textile, leather, plastic and food) and allow a great variety of colours and shades. They have in their molecule one or more azo groups. They are obtained from the coupling of diazonium salts with aromatic amines, phenols, naphthols or aliphatic enols. Coupling usually takes place in the *para* position in respect to the amino or hydroxyl group or in the *orto* position if the latter is occupied. The diazonium salts are obtained from the reaction of sodium nitrite with an amine solution with a mineral acid, preferably HCl (Marias, 1976; Zollinger *et al.,* 2002). The structural class of azo dyes includes dyes from different application classes, namely, acid, basic, metal complex, reactive and mordant (Marias, 1976).

Table 2.3.1: Application categories of dyes (adapted from O'Neill *et al.* 1999 and Rocha Gomes 2001)

TYPE OF DYE	CHARACTERISTICS	SUBSTRATES
Acid	When in solution are negatively charged; bind to the cationic NH_3^+- groups present in fibres	Nylon, wool, polyamide, silk, modified acryl, paper, inks and leather
Reactive	Form covalent bonds with OH-, NH- or SH- groups	Cotton, wool, silk and nylon

Metal complex	Strong complexes of one metal ion (usually chromium, copper, cobalt or nickel) and one or two dye molecules (acid or reactive)	Silk, wool and polyamide
Direct	Large molecules bound by Van der Waals forces to the fibre	Cellulose fibres, cotton, viscose, paper, leather and nylon
Basic	Cationic compounds that bind to the acid groups of the fibre	Synthetic fibres, paper and inks
Mordant	Require the addition of a chemical that combines with the dye and the fibre, like tannic acid, alum, chrome alum, and other salts of aluminium, chromium, copper, iron, potassium, and tin	Wool, leather, silk, paper, modified cellulose fibres and anodised aluminium
Disperse	Scarcely soluble dyes that penetrate the fibre through fibres swelling	Polyester, polyamide, acetate, acrylic and plastics
Pigment	Insoluble, non-ionic compounds or insoluble salts that retain their crystalline or particulate structure throughout their application	Paints, inks, plastics and textiles
Vat	Insoluble coloured dyes which on reduction give soluble colourless forms (leuco form) with affinity for the fibre; on exposure to air are reoxidised	Cellulose fibres, cotton, viscose and wool
Azoic and Ingrain	Insoluble products of a reaction between a coupling component and	Cotton, viscose, cellulose acetate and polyester

	a diazotised aromatic amine that occurs in the fibre	
Sulphur	Complex polymeric aromatics with heterocyclic S-containing rings	Cellulose fibres, cotton and viscose
Solvent	Non ionic dyes that dissolve the substrate to which they bind	Plastics, gasoline, varnish, lacquer, stains, inks, oils, waxes and fats
Fluorescence brightners	Mask the yellowish tint of natural fibres	Soaps and detergents, all fibres, oils, paints and plastics
Food	Non-toxic and not used as textile dyes	Food
Natural	Obtained mainly from plants	Food, cotton, wool, silk, polyester, polyamide and polyacrylonitrile

2.4 Dyeing process

The dyeing of a textile fiber is carried out in a solution, generally aqueous, known as the dye liquor or dye bath. For true dyeing (as opposed to mere staining) to have taken place, the coloration must be relatively permanent; that is, not readily removed by rinsing in water or by normal washing procedures. Moreover, the dyeing must not fade rapidly on exposure to light. The process of attachment of the dye molecule to the fiber is one of absorption; that is, the dye molecules concentrate on the fiber surface.

There are four kinds of forces by which dye molecules are bound to fiber: (1) ionic forces, (2) hydrogen bonding, (3) van der Waals' forces, and (4) covalent chemical linkages. In the dyeing of wool, which is a complex protein containing about 20 different a-amino acids, the sulfuric acid added to the dye bath forms ionic linkages with the amino groups of the protein. In the process of dyeing, the sulfate

anion (negative ion) is replaced by a dye anion. In the dyeing of wool, silk, and synthetic fibers, hydrogen bonds are probably set up between the azo, amino, alkyl amino, and other groups, and the amido -CO-NH-, groups. Van der Waals' forces (the attractive forces between the atoms or molecules of all substances) are thought to act in the dyeing of cotton between the molecular units of the fiber and the linear, extended molecules of direct dyes. Covalent chemical links are brought about in the dye bath by chemical reaction between a fiber-reactive dye molecule, one containing a chemically reactive centre, and a hydroxyl group of a cotton fiber, in the presence of alkali.

2.5 Ecological aspects

The most problematic industries in terms of dye release to the environment in the form of wastewater are the production of dyes and the dyeing industry. The uncontrolled release of these compounds in the environment causes severe problems. Since they are designed to be chemically and photolytically stable they are highly persistent in natural environments. For instance, the half-life of hydrolised Reactive Blue 19 is about 46 years at pH 7 and 25°C. In fact they are xenobiotic compounds because they do not exist as natural products and therefore contain structural elements that cannot be synthesized biochemically (Stolz, 2001). During evolution of catabolic enzymes and pathways microorganisms were not exposed to these structures and have not developed the capability to use those compounds as sole sources of carbon and energy. Dyes, by decreasing light absorption, may significantly affect photosynthetic activity of aquatic life and may be toxic due to the presence of aromatics or heavy metals (Banat *et al.*, 1996; Slokar and Marechal, 1998). The possibility of bioaccumulation in fish was considered but the few studies on the subject did not confirm this hypothesis. In the case of azo dyes, their degradation under anaerobic conditions in sediments causes

the release of potentially carcinogenic and mutagenic aromatic amines, whose carcinogenicity and mutagenicity is further discussed in the next section.

2.6 Toxicological aspects

The acute toxicity of azo dyes, as defined by the EU criteria for the classification of dangerous substances, is rather low (Øllgaard *et al.,* 1998; Zee, 2002). Only a few azo dyes showed LD50 (lethal dose that kills half of the tested population) values below 250 mg / kg body weight (Zee, 2002). However, occupational sensitisation to azo dyes has been seen in the textile industry since 1930. Especially some disperse dyes with monoazo or anthraquinone structures have been found to cause allergic reactions, i.e. eczema or contact dermatitis. Nevertheless attributing an allergy to a particular azo dye is a complex and difficult process due to the large number of azo dyes that exist (2000), the different names that different fabricants attribute to the same azo dye and impurities that usually azo dyes carry along.

Following oral exposure azo dyes are metabolised to aromatic amines by intestinal microflora or liver azoreductases. The soluble aminosulphonates are usually quickly excreted, whereas those derived from aniline, toluene, benzidine and naphtalene have been shown to have carcinogenic properties. However these properties are attributed to further metabolism (oxidation to N-hydroxy-compounds) by mammalian cytochrome P-450 enzymes. For instance, methemoglobinemia results from the oxidation of iron (II) to iron (III) in haemoglobin, which prevents oxygen binding.

The biologically active dyes were mainly limited to those compounds containing *p*-phenylenediamine and benzidine moieties. It was found that for the

phenylenediamine component methylation or substitution of a nitro group for an amino group did not decrease mutagenicity. However sulphonation, carboxylation or diamination lead to a decrease in the mutagenicity.

2.7 Characterisation of textile wastewaters

Wet processing in textile industry generates large amounts of a wastewater whose pollution load arises not only from the removal of impurities from the raw materials themselves but also from the residual chemical reagents used for processing (Table 2.7.1). The extreme diversity of raw materials and production schemes employed poses problems in assessing effluent characteristics and subsequently defining pollution control technologies. Colour is one of the major problems of these types of wastewaters. During textile processing, inefficiencies in dyeing result in large amounts of dyestuff being directly lost to the wastewater, which ultimately finds its way into the environment. The amount of dye lost is dependent upon the dye application class, varying from only 2% loss when using basic dyes to a 50% loss when certain reactive dyes are used (O'Neill *et al.,* 1999; McMullan *et al.,* 2001).

Table 2.7.1: Major pollutant types in textile wastewater, chemical types and process of origin

POLLUTANTS	CHEMICAL TYPES	PROCESS OF ORIGIN
Organic load	Starches, enzymes, fats, greases, waxes, surfactants and acetic acid	Ds, S, W, Dy
Colour	Dyes, scoured wool impurities	Dy, S

Nutrients (N, P)	Ammonium salts, urea, phosphate-based buffers and sequestrants	Dy
pH and salts	NaOH, mineral/organic acids, sodium chloride, silicate, sulphate, carbonate	S, Ds, B, M, Dy, N
Sulphur	Sulphate, sulphite and hydrosulphite salts, sulphuric acid	Dy
Toxic compounds	Heavy metals, reducing agents (sulphide), oxidising agents (chlorite, peroxide, dichromate, persulphate), biocides, quaternary ammonium salts	Ds, B, Dy, F
Refractory organics	Surfactants, dyes, resins, synthetic sizes (PVA), chlorinated organic compounds, carrier organic solvents	S, Ds, B, Dy, W, F

Ds – desizing; **S** – scouring; **W** – washing; **Dy** – dyeing; **B** – bleaching; **M** – mercerising; **N** – neutralisation; **F** – finishing

2.8 Treatment of textile wastewaters

Colour is the first contaminant to be recognized in wastewater and has to be removed before discharging into water bodies. The presence of very small amounts of dyes in water (less than 1 ppm for some dyes) is highly visible and affects the aesthetic quality water transparency and gas solubility in lakes, rivers and other water bodies. The removal of colour is often more important than the removal of the soluble colourless organic substances, which usually contribute the major fraction of the Biochemical Oxygen Demand (BOD). Methods for the removal of BOD from most effluents are fairly well established. Dyes, however, are more difficult to treat because of their synthetic origin and complex aromatic molecular structures (Banat *et al.*, 1996).

The electron-withdrawal character of the azo-group generates electron deficiency. This makes the compounds less susceptible to oxidative catabolism and as a consequence many of these chemicals tend to persist under aerobic environmental conditions (Knackmuss, 1996). Dyes must have a high degree of chemical and photolytic stability in order to be useful, due to the harshness of the conditions to which they are submitted during and after the dyeing process (light, bases, peroxides, and others (Rocha Gomes, 2001). Therefore they will, in general, give negative results in short-term tests for aerobic biodegradability (Øllgaard *et al.*, 1998). Stability against microbial attack is also a required feature of azo dyes, because it will prolong the lifetime of the products in which azo dyes are applied.

When designing the solution for a particular wastewater there are several conditionings that should be considered in this choice. Due to the high variability of composition of this type of wastewater, not only in quantity but mainly in quality, usually there is the need of more than one type of treatment to achieve the necessary quality of treatment. There are two possible locations for any technology that could be used to remove the colour that is present in the wastewater. The first possibility is in the dyehouse, allowing the partial or full re-use of water. This possibility deals with a considerable smaller amount of wastewater than the next possibility which is the treatment on a municipal (or particular) wastewater treatment plant, usually with a biological step. In this case, the coloured wastewater is mixed with domestic wastewater to provide organic load for the biological treatment. And in this hypothesis it can be used before the current biological or chemical treatment or as a final polishing step. A wide range of methods has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their impact on the environment. They are divided in three major categories: physical, chemical or biological. They are described and discussed in detail in the next sections. Biological and chemical methods involve the destruction of the dye molecule, whilst physical methods usually transfer the pollutant to another phase. Destruction of azo dyes can be accomplished by reduction or by oxidation. The reduction of azo dyes generates aromatic amines.

2.8.1 Physical treatments

2.8.1.1 Sorption

Adsorption techniques have recently gained a considerable importance due to their efficiency in the removal of pollutants too stable for conventional methods (Robinson *et al.*, 2001). Remediation based on sorption phenomena involves

binding of soluble or suspended pollutants to a solid organic or inorganic matrix. Binding to the matrix, depending on its composition and on pollutant structure, may be due to several types of interactions, e.g. electrostatic, ionic exchange, van der Waals, complexation or chelation. Decolourisation of wastewater by this process is influenced by many factors such as sorbent surface area, particle size, contact time, temperature, pH and presence of salts, surfactants and metals (Robinson *et al.,* 2001). It must be emphasized, however, that sorption processes merely transfers pollutants from one phase to another and therefore invariably generate sludge that must be disposed off, or regenerated, by some other process. Most adsorbents are not equally effective towards different types of dyes (Zee, 2002). The efficiency of one sorbent it is frequently assessed using the Freundlich empirical equation:

$$X/M = KC^{1/n}$$

where:

X = amount of impurity adsorbed

M = mass of adsorbent

C = equilibrium concentration of impurity in solution

K, n = constants

From a logarithmic plot of data it is possible to determine the adsorbent capacity of the material at specified equilibrium concentrations of particular impurities (Reife and Freeman, 1996).

Adsorption methods have some drawbacks. Since adsorption processes are not selective, the other components of the wastewater can compete for the adsorbing sites reducing the dye binding capacity of the adsorbent. Moreover, an adsorption process removes the synthetic dyes from the wastewater by concentrating them on the surface retaining their structure practically unchanged. When the support is to

be regenerated, the fate of the resulting concentrated sludge of dyes presents a problem of correct disposal (Forgacs *et al.,* 2004).

Sorbents can be inorganic or organic materials, or biomass (living or dead). Inorganic sorbents have the advantage of good mechanical and chemical stability, high specific surface area and resistance to microbiological degradation (Forgacs *et al.,* 2004). Activated carbon is a versatile adsorbent because its superficial chemical groups can be modified according to the physical-chemical treatment to which it is submitted, e.g. HNO_3, H_2O_2, NH_3 or thermal treatments under H_2 or N_2. It is one of the most widely used adsorbent for the removal of different classes of dyes but, because it is expensive and the cost of its regeneration is also high, it is usually applied as a tertiary/polishing treatment.Recent works describe attempts to produce activated carbons from cheap and readily available sources, like sawdust and rice-husk (Malik, 2003; Malik, 2004). It is highly effective for adsorbing cationic, mordant and acid dyes and, to slightly lesser extent, disperse, direct, vat, reactive and pigment dyes (Robinson *et al.,* 2001).

In the last years there has been an effort towards the application of cheaper adsorbing materials for colour removal in wastewaters. There are studies describing the adsorbing capacities of peat and of several agricultural by-products, as wood chips (Nigam *et al.,* 2000), corn cobs (Robinson *et al.,* 2001), rice hulls, peanut hulls, and also aquatic plants. These materials are so cheap that regeneration is not necessary and the potential exists for dye-adsorbed materials to be used as substrates in solid-state fermentations for protein enrichment (Robinson *et al.,* 2001). There are also cheap materials that can be used like modified starch.

The term biosorption refers to metabolism-independent processes taking place essentially in the cell wall of non-growing or non-living microbial mass. The main advantages of biosorption are high selectivity and efficiency, cost effectiveness and good removal performance; raw materials which are either abundant or wastes from industrial operations (fermentation wastes, activated sludge process wastes) can be used as biosorbents presenting performances often comparable with those of ion exchange resins (Aksu, 2005). Both living and death (heat killed, dried, acid and/or otherwise chemically treated) biomass can be used to remove hazardous organics and is effective when conditions are not favourable for the growth and maintenance of the microbial population (Robinson *et al.,* 2001). Bacteria, yeasts and algae have been described as good adsorbents, with biosorption capacities up to 640mg dye/g biomass. Nevertheless since textile dyes vary greatly in their chemistries, their interaction with the microorganisms will depend on the chemical structure of the dye, the specific chemistry of the microbial biomass and the characteristics of the dye solution or wastewater (Aksu, 2005).

2.8.1.2 Membrane filtration

This technology has emerged as a feasible alternative to conventional treatment processes of dye wastewater and has proven to save operation costs and water consumptions by water recycling (Kim *et al,* 2004). Usually this technique is applied as a tertiary/final treatment after biological and/or physical-chemical treatments. It has also been used to concentrate and purify dyes in the manufacture of these compounds (Kim *et al.*, 2004). These techniques allow, when not applied as end-of-pipe solutions, the recovery and reuse of some reagents (Zee, 2002).

2.8.1.3 Ion exchange

Standard ion exchange systems have not been widely used for treatment of dye-containing effluents, mainly due to the opinion that ion exchangers cannot accommodate a wide range of dyes and dyeing conditions, and that their performance was greatly affected by the presence of additives in the wastewater (Slokar and Marechal, 1998). In this technique wastewater is passed over the ion exchanger resin until all available exchange sites are saturated. Both anionic and cationic dyes are efficiently removed by this method. A disadvantage of this method is the high cost of organic solvents to regenerate the ion-exchanger (Slokar and Le Marechal, 1998; Robinson *et al.,* 2001).

2.8.1.4 Coagulation/Floculation

This method is often applied in the treatment of different types of wastewaters and it is used to enhance the degree of removal of Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and colour (Semerjian and Ayoub,2003;Allegre *et al.,* 2004; Peres *et al.,* 2004; Aguilar *et al.,* 2005). The first step, coagulation, consists in the addition of a coagulant to the wastewater and mixing. This coagulant destabilizes the colloidal particles that exist in the suspension, allowing particle agglomeration. Flocculation is the physical process of bringing the destabilized particles in contact to form larger flocs that can be more easily removed from the solution (Zee, 2002). This is usually achieved by a slow mixing step. The most commonly used inorganic coagulants/flocculants are: Trivalent salts of iron [$FeCl_3$, $Fe_2(SO_4)_3$] and Aluminium [$Al(SO_4)_3$];Ferrous sulphate $Fe(SO_4)$; and Calcium Hydroxide/lime [$Ca(OH)_2$]. These are often used with various coagulant aids, such as synthetic

polyelectrolytes (anionic, cationic or non-ionic polymers), fly ash and clay (Zee, 2002). Inorganic compounds are, however, generally not very suitable to remove highly soluble (sulphonated) dyes from solution unless large quantities are dosed. The major disadvantage of the use of this process is the amount of useless and even toxic sludge that needs to be correctly disposed (Zee, 2002) and the possibility of a secondary pollution problem (due to excess use of chemicals). Recently some organic polymers have been developed with good dye coagulant properties and a relatively low sludge production (Zee, 2002).

2.8.2 Chemical treatments

2.8.2.1 Fenton's reagent

H_2O_2-Fe (II) salts are used when the wastewater is resistant to biological treatment or is toxic to the biomass (Slokar and Marechal, 1998). The reaction, that occurs at acidic pH, results in the formation of a strong oxidative hydroxyl radical ($HO^{\cdot}$) and ferric iron (from the oxidation of ferrous iron). Both forms of iron are coagulants thus conferring the dual function of oxidation and coagulation to this process. Therefore the mechanism of colour removal involves also sorption or bonding of the dissolved dyes to flocs that are formed. The formation of large amounts of sludge concentrated in dyes and iron is the main disadvantage of this process, carrying disposal problems (Slokar and Marechal, 1998; Robinson *et al.*, 2001). As far as colour removal is concerned, the method is suitable for different dye classes. Reactive, direct, metal-complex, pigment, disperse and vat dyes have good decolouration rates (Slokar and Marechal, 1998).

2.8.2.2 Ozone

This is a very good oxidizing agent due to its high instability (reduction potential 2.07V) when compared to chlorine (1.36V) and H_2O_2 (1.78V). It degrades a high number of pollutants like phenols, pesticides and aromatic hydrocarbons and it is used since the early 1970s in wastewater treatment (Robinson *et al.,* 2001). The major drawback of the use of this method is ozone short half-life – it decomposes in 20 min – requiring continuous ozonation and making this method expensive to apply (Slokar and Marechal, 1998; Robinson *et al,* 2001). Ozone stability is affected by the presence of dyes and salts, by pH and by temperature (Slokar and Le Marechal, 1998; Robinson *et al.,* 2001). Although thermodynamics for ozone-induced oxidation may be favourable (due to ozone's high reduction potential), kinetic factors will most often dictate whether ozone will oxidize a pollutant in a reasonable time frame. It is most useful as a tertiary treatment since the presence of reducing agents and foaming substances, among others, reduces colour removal. Ozone can also be used as a hydrogen peroxide activator (Slokar and Marechal, 1998). Advantages of its use over the ones referred above are: no residue or sludge formation and no toxic metabolites are formed, and the application in gaseous state not increasing the volume of the wastewater (Robinson *et al.,* 2001).

2.8.2.3 Photochemical

Processes like UV/H_2O_2, UV/TiO_2, UV/Fenton's reagent, UV/O_3 and others are photochemical methods based on the formation of free radicals due to UV irradiation. Degradation is caused by the production of high concentrations of hydroxyl radicals and the dye molecule is degraded to CO_2 and H_2O (Robinson *et al,* 2001). The rate of dye removal is influenced by the intensity of the UV

radiation, pH, dye structure and the dye bath composition (Slokar and Le Marechal 1998). When H_2O_2 is used as oxidizing agent, the UV light activates the decomposition of H_2O_2 into two hydroxyl radicals ($H_2O_2+h\nu \rightarrow 2OH^{\cdot}$). This method does not produce sludge and greatly reduces foul odours (Robinson *et al.* 2001). There is also the possibility of effectively using sunlight or near UV light for irradiation, which would result in considerable economic savings, especially for large-scale operations. Faster, cheaper and more effective photocatalytic processes are based on catalysis by solid semiconductor materials, mostly TiO_2. With TiO_2 catalysed UV treatment a wide range of dyes can be mineralised (Gonçalves *et al.*, 1999). The photodegradation of dyes by this method depends considerably on the chemical structure of the dye (Forgacs *et al.*, 2004). The rate of dye removal is influenced by the intensity of the UV radiation, pH, dye structure and the dye bath composition (Slokar and Marechal, 1998). When H_2O_2 is used as oxidizing agent, the UV light activates the decomposition of H_2O_2 into two hydroxyl radicals ($H_2O_2+h\nu \rightarrow 2OH^{\cdot}$). This method does not produce sludge and greatly reduces foul odours (Robinson *et al.*, 2001). There is also the possibility of effectively using sunlight or near UV light for irradiation, which would result in considerable economic savings, especially for large-scale operations. Faster, cheaper and more effective photocatalytic processes are based on catalysis by solid semiconductor materials, mostly TiO_2 (Zee, 2002). With TiO_2 catalysed UV treatment a wide range of dyes can be mineralised (Gonçalves *et al.*, 1999). The photodegradation of dyes by this method depends considerably on the chemical structure of the dye (Forgacs *et al.*, 2004).

2.8.2.4 Sodium hypochlorite (NaOCl)

Coloured wastewaters can be chemically oxidized by chlorine compounds although, for environmental reasons (release of toxic organochlorinated compounds), its use is becoming less frequent (Slokar and Marechal 1998). Electrophilic attack at the amino group by Cl^+ initiates and accelerates the subsequent azo bound cleavage. The decolourisation is affected by pH and by NaOCl concentration (Slokar and Le Marechal 1998; Robinson *et al.,* 2001). This method is not efficient for the decolourisation of disperse dyes, and longer times are required to decolourise reactive and metal-complex dyes (Slokar and Marechal, 1998).

2.8.2.5 Electrolysis

Electrolysis is based on applying an electric current through to the wastewater by using electrodes. Organic compounds like dyes react through a combination of electrochemical oxidation, electrochemical reduction, electrocoagulation and electroflotation reactions. For instance, when iron is the sacrificial anode, Fe (II)-ions are released to the bulk solution, and acid dyes are sorbed on the precipitated $Fe(OH)_2$. Moreover Fe (II) can reduce azo dyes to arylamines (Vandevivere *et al.,* 1998; Wilcock *et al.,*1996). Moreover, water can also be oxidized resulting in the formation of O_2 and O_3 and, if chloride is present, there is also formation of Cl_2 and oxychloride anions. In the cathode occurs reduction of water to H_2 and OH^-. In order to improve the performance of the system different materials have been tested in the electrodes like carbon-fibre, Ti/Pt and aluminium. The main disadvantage of these types of methods is the cost, both initial capital costs, energy and of electrode replacement (Vandevivere *et al.,*1998; van der Zee, 2002; Kobya

et al., 2003; Cerón-Rivera *et al.*, 2004). The formation of unwanted breakdown products and foam are also drawbacks of this method (Vandevivere *et al.*, 1998; van der Zee, 2002). The main advantages are compact size of equipment, simplicity in operation, fast rate of pollutant removal and decrease amount of sludge generated. The method is efficient for colour, BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), TOC (Total Organic Carbon), TDS (Total Dissolved Solids), TSS (Total Suspended Solids) and heavy metals removal (Wilcock *et al.*, 1996).

2.8.2.6 Wet Air Oxidation (WAO)

In this process the primary oxidant species is oxygen and it is used to produce OH-radicals which actually react with organic and inorganic species under sub-critical conditions, i.e. temperature between 175 and 320°C and pressure between 60 and 200 bar. These operation conditions result in high capital investments and high maintenance costs. Economic operation of WAO processes requires wastewaters with COD contents above $20g.L^{-1}$ to allow autothermic operation.

2.8.2.7 Ultrasound

This technology can also be used in textile dye remediation but is still a very recent technology in phase of development (Marechal 2005).

2.8.3 Biological treatments

The fate of environment pollutants is largely determined by abiotic processes, such as photooxidation, and by the metabolic activities of microorganisms. Since catabolic enzymes are more or less specific, they can act on more than their natural

substrate. This explains why the majority of xenobiotics are subject to fortuitous metabolism (cometabolism) and several groups explore these microbial capacities for the bioremediation of dyes. The limitations of biological processes are mainly caused by limited biodegradability of primarily xenobiotic compounds like dyes, by toxic or inhibitory effects of pollutants for the microbial population and by the slow rate of biodegradation of particular pollutants.

2.8.3.1 Bacteria

Actinomycetes, particularly *Streptomyces* species are known to produce extracellular peroxidases that have a role in the degradation of lignin and were also found effective in the degradation of dyes (Zhou and Zimmerman, 1982; McMullan *et al.,* 2001). In these studies were used azo dyes with exception for Zhou and Zimmerman that tried reactive dyes (anthraquinone, phthalocyanine, azo and metal complex dyes) (Zhou and Zimmerman, 1982). With the notable exception of actinomycetes, the isolation of bacteria capable of aerobic decolourisation and mineralization of dyes, specially sulfonated azo dyes, has proven difficult (McMullan *et al.* 2001). For aerobic bacteria to be significant in the reductive process they must be specifically adapted. Using this methodology, and testing some analogues of sulfonated azo dyes as sole source of carbon and energy, several groups manage to isolate and purify "azoreductases" from *Pseudomonas* strains KF46 (Zimmermann *et al.,*1982) and K24 and from *Xenophilus azovorans* KF46F. The ability of bacteria to aerobically metabolise other dye classes was described for *Kurthia* sp. and for *Pseudomonas mendocina* MCM B-402. The initial step in bacterial azo dye metabolism under anaerobic conditions involves the reductive cleavage of the azo linkage. This process is catalysed by a variety of soluble cytoplasmic enzymes with low-substrate

pecificity, which is known as "azoreductases" (Robinson *et al.,* 2001; Stolz, 001). Under anoxic conditions, these enzymes facilitate the transfer of electrons ia soluble flavins to the azo dye, which is then reduced. The role that such ytoplasmic enzymes have *in vivo* is, however, uncertain (McMullan *et al.,* 2001). he work of Russ and co-workers showed however that the cytoplasmic azoreductases" are presumably flavin reductases and that they have insignificant nportance in the *in vivo* reduction of sulfonated azo compounds. The possibility f non-cytoplasmic azoreductases is then reinforced since it is highly improbable hat highly charged sulfonated azo dyes or polymeric azo dyes pass through the acterial cell wall. A membrane bound "azoreductase" was found by Kudlich and o-workers in the cell wall of a *Sphingomonas* sp. This strain possessed both ytoplasmic and membrane-bound azoreductase activities (Kudlich *et al.,* 1997). A ifferent model for the unspecific reduction of azo dyes by bacteria was proposed ased on studies with *Sphingomonas xenophaga* BN6. They observed the increase n the reduction rate when quinones, like anthraquinone-2-sulfonate or 2-hydroxy-1, 4-naphtoquinone, were added to the culture medium. It was suggested then that quinones added to the medium or some decomposition products released by the cells to the medium, acted as redox mediators which were enzimatically reduced by the bacteria cells and that the hydroquinones formed reduced the azo dye in a purely chemical redox reaction. Rafii suggests an extracellular azoreductase activity in studies done with bacteria isolated from human intestine, mainly *Eubacterium* sp. and *Clostridium* sp. (Rafii *et al.,* 1999). Another possibility for the extracellular reduction of azo compounds under anaerobic conditions is the action of reduced inorganic compounds (e.g. Fe^{2+}, H_2S), that are formed as end products of certain strictly anaerobic bacterial metabolic reactions (Stolz, 2001; Zee *et al.,* 2001). The further mineralization of the formed amines is not possible under

naerobic conditions. This is why there are several studies that propose a combined naerobic-aerobic system for the removal of dyes from wastewaters with a onsortium/sludge (Seshadri *et al.,* 1994; O'Neill *et al.,* 2000; Lourenço *et al.,* 001; Sponza and Isik, 2002; Libra *et al.,* 2004; Sponza and Isik, 2005). The tilization of consortia offers considerable advantages over the use of pure cultures n the degradation of synthetic dyes. The individual strains may attack the dye nolecule at different positions or may use the decomposition products produced by nother strain for further decomposition. However, the composition of mixed ultures may change during the decomposition process interfering with the control of the system. The most used consortium is activated sludge system, mainly onstituted by bacteria, but also with the usual presence of fungi and protozoa.

2.8.3.2 Fungi

The most widely studied dye-decolourising microorganisms are the white-rot fungi ike *Phanerochaete chrysosporium*, *Trametes versicolor*, *Coriolus versicolor* and *Bjerkandera adusta* (Heinfling-Weidtmann *et al.,* 2001). Several other non-white-ot fungi can also successfully decolorize dyes like *Aspergillus niger*, *Geotrichum candidum* (Kim *et al.,* 1995; Kim and Shoda., 1999), *Pleurotus ostreatus* and *Cunninghamella elegans* among others. White-rot fungi constitute a diverse ecophysiological group comprising mostly basidiomycetous fungi capable of aerobic lignin depolymerization and mineralization, playing a central role in the global C-cycle (McMullan *et al.,* 2001). This ability is correlated to the capacity of these organisms to synthesise lignin-degrading extracellular enzymes such as lignin peroxidases (LiP) and manganese peroxidases (MnP), or laccases (Lac) (Robinson *et al.,* 2001; Stolz, 2001; Forgacs *et al.*, 2004) which, thanks to their lack of substrate specificity, are also capable of degrading a wide range of

enobiotics (McMullan *et al.,* 2001; Zee, 2002). Among these are dioxins, Polychlorinated Biphenyls (PCBs), Chlorophenols, polycyclic Aromatic Hydrocarbons (PAHs) and Nitroaromatics, including dyes (Robinson *et al.,* 2001; Forgacs *et al.,* 2004). LiP catalyses the oxidation of non-phenolic aromatic compounds such as veratryl alcohol. MnP oxidizes preferably Mn^{2+} to Mn^{3+} which is able to oxidise many phenolic compounds. Laccase is a copper-containing enzyme that catalyses the oxidation of phenolic substrates by coupling it to the reduction of oxygen to water (McMullan *et al.,* 2001). Whilst it is clear that these enzymes play a significant role in dye metabolism, care must be taken not to exclude the possibility of the existence of other degradative mechanism (McMullan *et al.,* 2001). Recently a third group of peroxidases, versatile peroxidase (VP), has been recognized in species of *Pleurotus* and *Bjerkandera,* (Heinfling *et al.,* 1998). A number of other enzymes are produced in parallel including H_2O_2 producing enzymes required by other peroxidases (glyoxal oxidase and superoxide dismutase), and enzymes linked to lignocellulose degradation pathways (glucose oxidase and aryl alcohol oxidase) (Wesenberg *et al.,* 2003; Novotný *et al.,* 2004). Although these fungi have been shown to decolourise dyes in liquid fermentations, enzyme production has also shown to be unreliable mainly due the unfamiliar water environment (Robinson *et al.,* 2001). Their performance is also closely related to the operation conditions (concentration of dye, pH and temperature), which is a serious drawback for this type of wastewaters. Nevertheless they have the potential to oxidise substrates that have low solubility which is an advantage for the treatment of non-soluble dyes (like vat for instance). Another advantage of these systems is that the constitutive nature of the enzymes obviates the need for the adaptation. In process design and optimization of fungal treatment there are some features that should be considered. As the decolourization

of dyes by *P. chrysosporium* and other white-rot fungi occurs in secondary metabolic conditions, the important enzyme system is released by the fungal cells under either carbon or nitrogen limitation. Production of LiP and MnP is generally optimal at high oxygen tension but is repressed by agitation in submerged liquid culture, while laccase production is often enhanced by agitation. Usually more than one isoform of the enzyme system is expressed by different taxa and culture conditions. Due to the complexity of these systems, both dye structures and enzymatic transformations involved, there is a gap in the knowledge of the degradation and mineralization of dyes by these microorganisms.

2.8.3.3 Yeasts

In literature the ability to degrade azo dyes by yeasts was only described in a few reports. The first two reports use the ascomycete yeast *Candida zeylanoides* isolated from contaminated soil to reduce model azo dyes. The characterisation of an enzymatic activity is described in further studies with the yeast *Issatchenkia occidentalis*, and the enzymatic system involved is presented in a work with *Saccharomyces cerevisiae*.

2.8.3.4 Algae

The use of algae for the degradation of dyes is mentioned in only few reports and is achieved by *Chlorella*, *Oscillateria* and *Spirogy* species. All the reports which used azo dyes mention the reduction of the azo bond as the decolourising mechanism. Mohan attributes the decolourisation to biosorption followed by bioconvertion and biocoagulation. Furthermore they all state that the formed amines can be totally mineralized. It also state that some of the tested azo compounds could be used as sole sources of carbon and nitrogen by the algae. This

ould mean that algae can play an important role in the removal of azo dyes and romatic amines in stabilization ponds (Banat *et al.,* 1996).

2.8.3.5 Plants

There are two recent studies that describe the use of plants (Phytoremediation) for the dye removal from wastewaters. The first one mentions a good removal capacity of sulphonated anthraquinones with *Rheum rabarbarum* (Aubert and Schwitzguébel, 2004), although only shows preliminary results that need to be further investigated. This plant possesses enzymes that accept anthraquinones as substrates and in cell culture were able to remove up to 700-800 $mg.L^{-1}$ of anthraquinones with sulphonate groups in different positions. Also Mbuligwe, 2005, describes a reduction in colour of 72-77% in wetlands vegetated with coco yam plants (Mbuligwe, 2005). These systems have the serious disadvantage of requiring big areas to implant the treatment.

2.8.3.6 Enzymes

In the studies of biological degradation of dyes an effort as been made in order to identify, isolate and test the enzymes responsible for the decolourisation. In the case of extracellular fungal enzymes, like manganese and lignin peroxidases and laccases or cytosolic azoreductases from bacteria, this has been achieved by several groups (Heinfling *et al.,* 1998; Rafii and Coleman, 1999; Nyanhongo *et al.,* 2002; Blümel and Stolz, 2003). The application of enzyme preparations shows considerable benefits over the use of microorganisms. Commercial preparations can be easily standardized, facilitating accurate dosage. The application is simple and can be rapidly modified according to the character of the dye or dyes to be removed (Forgacs *et al.,* 2004). Nevertheless the use of whole cells rather than

solated enzymes is advantageous, because costs of purification are extremely high and the cell offers protection from the harsh process environment to the enzymes. Also, degrading is often carried out by a number of enzymes working sequentially and not by one single enzyme.

2.9 Decolorization of textile dyes by using microbial isolates obtained from the dye contaminated sites

The decolorization of the textile dyes with the microbial isolates from the dye contaminated sites is widely studied. The bacterial and fungal strains isolated from the contaminated sites can easily adapt to the adverse environment and thus can be used for the decolorizing the textile dyes. An azo dye reducing bacteria *Paenibacillus azoreducens* was isolated from textile industry waste water (Colette *et al*, 2001). The bacterial species such as *Acinetobacter, Legionella* and *Bacillus* isolated from textile industries wastewater and drains decolorized the textile dyes (Olukanni *et al.*, 2006). Decolorization of the textile dyes namely Orange 3R, Blue 3R, Yellow GR, Black RL and T blue was carried out with indigenous soil fungi isolated from the soil samples around textile distillery industries (Raju *et al.*, 2007). *Aspergillus* sp. isolated from the soil sample near textile industry effectively decolorized Reactive Blue and other structurally different synthetic dyes (Mohandass *et al.*, 2007). Microbial isolates both bacterial and fungal obtained from the contaminated sites were used to decolorize Three azo dyes [Acid Navy Blue (Acid Blue 120), Fast Red A (Acid Red 88) and Acid Sulphone Blue (Acid Blue 89)] and one triarylmethane dye [Acid Magenta (Acid Violet 19)] (Prachi Kaushik and Anushree Malik, 2009). A fungus, *Penicillium simplicissimum* isolated from the sediment collected from the industrial contaminated sources decolorized three reactive dyes very effectively (Bergsten *et al.*, 2009).

2.10 Decolorization of the textile dyes using microbial consortia:

There are many reports regarding the use of microbial consortia for the decolorization of the textile dyes. The microbial consortium can produce a rapid decolorization due to concerted metabolism of the different microbes which are part of the consortium. The consortium developed with the bacterial and fungal strains produced a synergetic reaction of both strains leading to the complete mineralization of the textile wastewater. The decolorization, COD removal yields were remarkably improved with the microbial consortium compared with individual and/ or bacterial and fungal strains. The advantage of the mixed culture is apparent as some strains can collectively carry out such biodegradation tasks that no individual pure strain can achieve and the complexity of such microbial consortium enables them to act on variety of pollutants. Four bacterial isolates identified as *Bacillus cereus* (BN-7), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5) and *Stenotrophomonas acidaminiphila* were used to develop a consortium which decolorized Acid Red 88, Acid Red 119, Acid Red 97, Acid Blue 113 and 82% of Reactive Red 120 dyes with high efficiency (Manjinder *et al.*, 2004). An aerobic bacterial consortium consisting of two isolated strains and a strain of *Pseudomonas putida*(MTCC1194) was developed for the aerobic degradation of a mixture of textile azodyes and individual azodyes (Resmi *et al.*, 2004). A bacterial consortium developed from the organisms isolated from the soil samples of the contaminated sites decolorized Reactive Violet 5 and 10 other dyes (Safia *et al.*, 2005).

Four different aerobic mixed consortia collected from basins of wastewater streams coming out of dying plants showed decolorization of Drimarene Orange K-GL, Drimarene Brilliant Red K-4BL, Foron Yellow SE4G and Foron Blue RDGLN dyes (Muhammad *et al.*, 2006). The consortium-GB (*Galactomyces geotrichum*

and *Bacillus* sp.) exhibited 100% decolorization ability with the dye Brown 3REL. (Jadhav *et al.*, 2008). Microbial consortia developed with *Bacillus cereus* (KEB-7) and *Bacillus pumilus* (KEB-10) for bacteria and *Aspergillus alliaceus* (KF-3) for fungi showed 100% decolorization of textile wastewater (Eltaief *et al.*, 2009). The bacterial consortium consisting of five different bacterial species *Bacillus vallismortis*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* were efficient in decolorizing individual as well as mixture of dyes (Bella *et al.*, 2009). Local bacterial consortia developed from the bacterial strains isolated from the effluent, soil and sludge samples from the industrial areas is used to decolorize various azo dyes (Khadiah *et al.*, 2009).

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample collection

The dye effluent samples and soil sample from the dye contaminated sites were collected from various textile industries such as CETP in SIPCOT, Perundurai, Towin dyeing and Popular textiles in Tirupur, Kasiraja textiles and Seethalakshmi textiles in Salem. About 3 effluent samples (2 untreated samples and 1 treated sample) and 1 soil sample were collected from CETP, Perundurai. 2 effluent samples and 1 soil sample were collected from Towin dyeing and Popular textiles in Tirupur. 2 effluent samples and 4 soil samples were collected from Kasiraja textiles and Seethalakshmi textiles in Salem. Totally about 13 different effluent and soil samples were collected. The isolation of Bacterial and Fungal colonies from these samples was then carried out.

3.2 Isolation of bacterial and fungal colonies from the collected effluent and samples

The bacterial and fungal colonies are isolated from the effluent and soil samples collected from the various textile industries. These are then studied for their ability to decolorize the effluent dyes.

3.2.1 Isolation of bacterial colonies from effluent and soil samples

1. The effluent dye samples were serially diluted to a dilution factor of 10^{-5} with sterile distilled water while in the case of soil sample about 0.1 g of the soil sample is dissolved in 1ml of sterile distilled water and is then serially diluted to a dilution factor of 10^{-5}.

2. The Nutrient Agar (Himedia) plates were then prepared.
3. 100 µl of the diluted effluent sample is added to the center of the Nutrient Agar plates and using the L-Rod spread the sample to the whole surface of the Agar plate.
4. The plates were then incubated at 37°C for 24 hrs.
5. A mixture of Bacterial colonies was obtained on these plates after incubation. From these distinct colonies were chosen and are then pure cultured through streak plate method.
6. The pure cultured bacterial colonies were then maintained as slant for further studies.

3.2.2 Isolation of fungal colonies from effluent and soil samples

1. The effluent dye samples were serially diluted to a dilution factor of 10^{-4} with sterile distilled water while in the case of soil samples about 0.1 g of the soil sample is dissolved in 1ml of sterile distilled water and is then serially diluted to a dilution factor of 10^{-4}.
2. The Potato Dextrose Agar (Himedia) plates were then prepared.
3. 100 µl of the diluted effluent sample is added to the center of the Potato Dextrose Agar plates and using the L-Rod spread the sample to the whole surface of the Agar plate.
4. The plates were then incubated at 30°C for 48 hrs.
5. A mixture of fungal colonies was obtained on these plates after incubation. From these distinct colonies were chosen and are then pure cultured through streak plate method.
6. The pure cultured fungal colonies were then maintained as slant for further studies.

3.3 Decolorization studies

The isolated bacterial and fungal colonies were then studied for their ability to decolorize the effluent dyes. The following is the procedure used for the decolorization assay,

3.3.1 Decolorization studies on the isolated bacterial colonies

1. The decolorization studies were carried out for the isolated Bacterial colonies on a specialized media called Bushnell & Haas medium of the following composition (g/l): NH_4NO_3, 1; $CaCl_2$, 0.02; $FeCl_3$, 0.05; $MgSO_4.7H_2O$, 0.2; K_2HPO_4,1; Glucose, 0.1% w/v; Yeast extract, 0.05% w/v and pH 7.
2. The isolated bacterial colonies maintained as slant were then subcultured in Eppendorf tubes containing 1 ml sterilized Nutrient Broth (Himedia) and are incubated at 37°C for 24 hrs.
3. About 1ml of the inoculum is then added to a test tube containing 8 ml of the Bushnell & Hass medium and 1ml of sterilized effluent dye and is then maintained at 37°C.
4. The samples were withdrawn at 24 hr interval for 2 days and were centrifuged at 10,000 rpm for 15 mins.
5. The Absorbance of the supernatant was measured spectrophotometrically at 572 nm.
6. Dye removal (%) was calculated as

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

7. The dye removal % after 48 hrs was calculated and the bacterial colonies with high decolorization efficiency were selected.

3.3.2 Decolorization studies on the isolated fungal colonies

1. The decolorization studies were carried out for the isolated fungal colonies on a specialized media called Bushnell & Haas medium of the following composition (g/l): NH_4NO_3, 0.5; $MgSO_4.7H_2O$, 0.1; K_2HPO_4. 0.5; NaCl, 1g/l; glucose, 10; yeast extract, 2% and pH 6.
2. About a loopful of the inoculum of the fungal colony from the PDA plates is then added to a test tube containing 8 ml of the Bushnell & Hass medium and 1ml of sterilized effluent dye and is then maintained at 37°C.
3. The samples were withdrawn at 24 hr interval for 7 days and were centrifuged at 10,000 rpm for 15 mins.
4. The Absorbance of the supernatant was measured spectrophotometrically at 572 nm.
5. Dye removal (%) was calculated as

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

6. The dye removal % after 7 days was calculated and the fungal colonies with high decolorization efficiency were selected.

3.3.3 Decolorization studies with Synthetic dyes

The decolorization studies were carried out for the selected colonies with three synthetic dyes namely Reactive Red 120, Reactive Black 5 and Direct Red 81. These dyes were prepared at a concentration of 50 mg/ 100ml and the decolorization assay mixture consists of 10% of dye. The decolorization studies were then carried out following the same procedure used for the decolorization of the effluent dyes. These studies were done for the selected bacterial and fungal colonies.

3.4 Identification of the bacterial and fungal strains

The bacterial colonies which were used to develop the consortia were identified using the biochemical characterization process involving 11 different tests as follows: Gram staining, Indole test, Methyl Red (MR) test, Voges-Proskauer (VP) test, Citrate test, Triple Sugar Iron Agar (TSI) test, Mannitol Motility (MM) test, Urea Hydrolysis test, Nitrate Reduction test, Oxidase test, Sugar Fermentation test. The fungal colonies were also identified based various tests.

3.5 Optimization of decolorization of the synthetic dyes by fungal strains

The optimization of the decolorization efficiency was then carried out for the fungal colonies. The effect of pH, Temperature, C: N ratio and shaking conditions were studied and these parameters are optimized to obtain maximum decolorization of the commercial dyes using the fungal colonies.

3.5.1 Effect of Temperature on the decolorization efficiency

The effect of temperature on the decolorization of the commercial dyes was analyzed at three different incubation temperatures 30°C, 37°C and 45°C. The decolorization % was calculated for the samples incubated at different temperatures and the optimum temperature for the decolorization was determined.

3.5.2 Effect of pH on the decolorization efficiency

The effect of pH on the decolorization of commercial dyes was analyzed. The decolorization media was prepared at different pH 3, 4, 5 and 6 and the decolorization % was calculated and the optimum pH for the decolorization of the textile dyes by the fungi was determined.

3.5.3 Effect of C:N ratio on the decolorization efficiency

The effect of the C:N ratio on the decolorization efficiency was determined by using the Bushnell and Haas media with different Carbon: Nitrogen source ratios such as 1:2, 1:1, 2:1. The optimum C:N ratio for the effective dye decolorization was determined from these studies.

3.5.4 Effect of shaking and static conditions on the decolorization efficiency

The effect of the shaking and stationary conditions on the dye decolorization was studied. The decolorization was carried out by incubating the decolorization assay mixture in the orbital shaker. Then the effect of static and shaking condition on the decolorization efficiency compared and the optimum condition for decolorization was determined.

3.6 Development of the Microbial Consortia and determination of the decolorization efficiency

The microbial consortia were developed by using mixed culture of the microorganisms. The bacterial and fungal consortia were developed and their decolorization efficiency of the commercial dyes was determined.

3.6.1 Development of fungal consortia and evaluation of its dye decolorizing efficiency

Four different types of fungal consortium were developed and their commercial dye decolorizing efficiency was studied. These four consortia consist of different sets of fungi and they were named as FC1, FC2, FC3 and FC4. The following is the composition of the consortia

FC1 – F1, F2, F3, F4

FC2 – F2, F3, F4

FC3 – F1, F2, F4

FC 4 – F1, F3, F4

where F1, F2, F3 & F4 are the four fungal cultures that possessed high commercial dye decolorization efficiency.

The following is the procedure followed for the development of the fungal consortia and evaluation of the decolorization efficiency,

1. A mixed culture consisting of the fungal colonies required for developing the consortium is prepared in Potato Dextrose Broth (Himedia). The mixed culture was prepared for the four different consortia with different sets of fungal colonies.
2. The Bushnell & Haas medium which is used for the decolorization studies was prepared and is sterilized.
3. The decolorization assay mixture was formed with 80ml of Bushnell& Haas medium, 10% inoculum of the mixed culture and 10% of the synthetic dye prepared at a concentration of 50 mg/100ml under study. The mixture is then incubated at 30°C at static condition. The decolorization study was carried out for three commercial dyes Reactive Red 120, Reactive Black 5 and Direct Red 81.
4. The samples were withdrawn at 48 hr interval for 7 days and were centrifuged at 10,000 rpm for 15 mins.
5. The Absorbance of the supernatant was measured spectrophotometrically at the wavelength at which maximum absorbance spectra was obtained for each dye.

6. Dye removal (%) was calculated as

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

7. The dye decolorization efficiency of the different consortia was compared.

3.6.2 Development of a Bacterial consortium and evaluation of its dye decolorizing efficiency

A bacterial consortium was developed by using five bacterial strains which were capable of decolorizing the synthetic dyes. The bacterial consortium was named BC and it composed of bacterial strains B1, B2, B3, B4 and B5. The decolorization efficiency of this consortium was evaluated on three commercial dyes Reactive Red 120, Reactive Black 5, and Direct Red 81. The following is the procedure for the development of the consortium and evaluation of the dye decolorization efficiency of the same,

1. A mixed culture consisting of the five Bacterial colonies required for developing the consortium is prepared in Nutrient Broth.
2. The Bushnell & Haas medium which is used for the decolorization studies was prepared and is sterilized.
3. The decolorization assay mixture was formed with 80ml of Bushnell& Haas medium, 10% inoculum of the mixed culture and 10% of the synthetic dye prepared at a concentration of 50 mg/100ml under study. The mixture is then incubated at 37°C at static condition. The decolorization study was carried out for three synthetic dyes Reactive Red 120, Reactive Black 5 and Direct Red 81.
4. The samples were withdrawn at 24 hr interval for 2 days and were centrifuged at 10,000 rpm for 15 mins.

5. The Absorbance of the supernatant was measured spectrophotometrically at the wavelength at which maximum absorbance spectra was obtained for each dye.
6. Dye removal (%) was calculated as

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

7. The dye removal % of the consortium was thus determined.

3.7 Dye degradation studies for the microbial consortia

The decolorization of the synthetic dyes by the microbial consortia can be due to adsorption or degradation. The mechanism by which the decolorization of the dyes occurs can be determined by the following procedure,

1. The decolorization assay mixture is withdrawn at 24 hr interval and the sample is centrifuged at 10,000 rpm for 15 mins.
2. The supernatant is discarded and the pellet is dissolved in the Bushnell and Haas medium.
3. Then the absorbance of the sample is measured spectrophotometrically to determine the presence or absence of dye in the pellet.
4. Once there is no dye in the pellet, then it can be deduced that there is degradation of the dye by the consortia. Otherwise, the decolorization is due to the adsorption by the microbial cells.
5. This procedure is carried out for both the fungal consortia and the bacterial consortium with three synthetic dyes and the mechanism of decolorization was determined.

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

The decolorization and degradation of the textile effluent dyes and commercial dyes was carried out using a microbial consortium which was developed from the combination of microbial strains isolated from the effluent samples and soil samples from the effluent dye contaminated sites. The microbial strains isolated from the contaminated sites can easily adapt to the adverse environment and thus can be used for the decolorization of the dyes. The advantage of the mixed culture is apparent as some strains can collectively carry out such biodegradation tasks that no individual pure strain can achieve and the complexity of such microbial consortium enables them to act on variety of pollutants. Four fungal consortia and one bacterial consortium were developed with strains isolated from the samples from the contaminated sites. The decolorization and degradation studies were performed with these five consortia.

4.1 Isolation of bacterial and fungal colonies from the effluent and soil samples

The dye effluent samples and soil samples from the dye contaminated sites were collected from various textile industries such as CETP in SIPCOT, Perundurai, Towin dyeing and Popular textiles in Tirupur, Kasiraja textiles and Seethalakshmi textiles in Salem. About 3 effluent samples (2 untreated samples and 1 treated sample) and 1 soil sample were collected from CETP, Perundurai. 2 effluent samples and 1 soil sample were collected from Towin dyeing and Popular textiles in Tirupur. 2 effluent samples and 4 soil samples were collected from Kasiraja textiles and Seethalakshmi textiles in Salem. Totally about 13 different effluent and soil samples were collected. Then isolation of bacterial and fungal colonies

was proceeded. About 32 different bacterial colonies and 8 different fungal colonies were obtained from the samples obtained from CETP, Perundurai. About 50 different bacterial colonies and 11 different fungal colonies were isolated from the Salem and Tirupur samples. These colonies were named based on the source from which it was isolated. The bacterial and fungal isolates were obtained from the soil and effluent samples from the small scale dyeing and printing units in Baddi (Himachal Pradesh, India) and Pali (Rajasthan, India) by this method (Prachi and Anushree, 2009). The following tables give the name and the source of the bacterial and fungal colonies.

Table: 4.1.1 Name and source of the bacterial colonies isolated from CETP (Common Effluent Treatment Plant) samples

Source of sample for isolation of bacterial strains	Source and Culture Name
CETPE1	CETPB1, CETPB2, CETPB3 and CETPB4
CETPE2	CETPB5, CETPB6, CETPB7 and CETPB8
CETPE1	CETPB9
CETPS	CETPB10, CETPB11, CETPB12, CETPB13, CETPB14, CETPB15, CETPB16, CETPB17, CETPB18, CETPB19, CETPB20, CETP21 and CETPB22
CETPE3	CETPB23, CETPB24, CETPB25, CETPB26, CETPB27 and CETPB28
CETPS	CETPB29, CETPB30, CETPB31 and CETPB32

CETPE1 – CETP Effluent sample 1

CETPE2 – CETP Effluent sample 2

CETPE3 – CETP Effluent sample 3

CETPS – CETP Soil sample

CETPB – CETP sample Bacteria

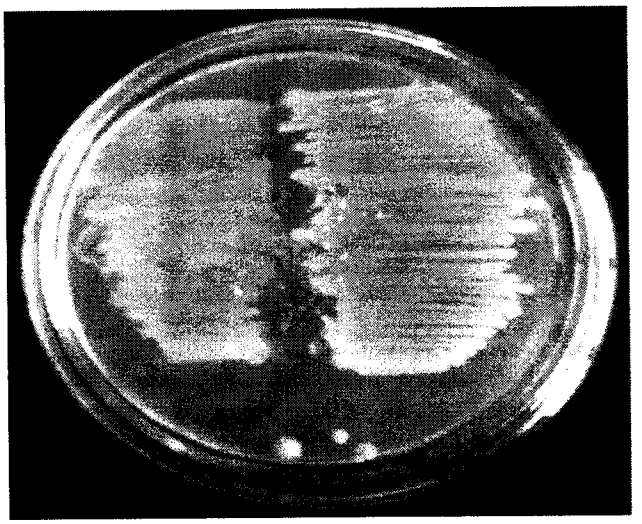

Fig 4.1.1 Pure culture of Bacterial colonies from the source of CETP Soil sample

4.1.2 Name and source of the fungal colonies isolated from CETP samples

Source of sample for isolation of fungal strains	Source and Culture Name
CETPS	CETPF1 and CETPF2
CETPE1	CETPF3
CETPE2	CETPF4
CETPE3	CETPF5 and CETPF6
CETPS	CETPF7 and CETPF8

CETPE1 – CETP Effluent sample 1

CETPE2 – CETP Effluent sample 2

CETPE3 – CETP Effluent sample 3

CETPS – CETP Soil sample

CETPF – CETP sample Fungi

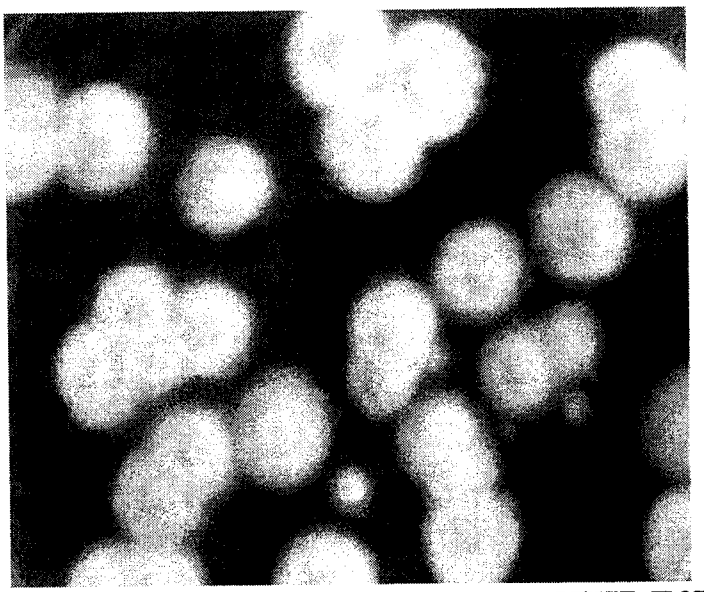

Fig 4.1.2 Fungal colony CETPF6 from the source CETP Effluent Sample 3

Table: 4.1.3 Name and source of the bacterial colonies isolated from Salem and Tirupur samples

Source of sample for isolation of bacterial strains	Source and Culture Name
TES1	TSB1 and TSB2
TES2	TSB3
TSS	TSB4, TSB5, TSB6, TSB7, TSB8, TSB9, TSB10 and TSB11
SSS1	SSB1, SSB2 and SSB3

SSS2	SSB4, SSB5, SSB6 and SSB7
SSS3	SSB8, SSB9, SSB10 and SSB11
SSS3	SSB12, SSB13, SSB14 and SSB15
SSS4	SSB16, SSB17, SSB18, SSB19, SSB20, SSB21 and SSB22
SES1	SSB23, SSB24, SSB25, SSB26 and SSB27
SES2	SSB28, SSB29, SSB30, SSB30, SSB31, SSB32, SSB33, SSB34 and SSB35
SSS4	SSB36
SSS3	SSB37
SSS2	SSB38
SSS1	SSB39

TES1- Tirupur Effluent Sample 1

TES2 – Tirupur Effluent Sample 2

TSS – Tirupur Soil Sample

SSS1 - Salem Soil Sample 1

SSS2 - Salem Soil Sample 2

SSS3 - Salem Effluent Sample 3

SSS4 – Salem Soil Sample 4

SES1 – Salem Effluent Sample 1

SES2 – Salem Effluent Sample 2

SSB - Salem Sample Bacteria

TSB – Tirupur Sample Bacteria

Table: 4.1.4 Name and source of the fungal colonies isolated from Salem and Tirupur samples

Source of sample for isolation of fungal strains	Source and Culture Name
SSS2	SSF 1 and SSF 2
SSS4	SSF 3
SSS3	SSF 4 and SSF 5
SES2	SSF 6 and SSF 7
SSS1	SSF 8
SSS2	SSF 9
SES1	SSF 10
TSS	TSF 1

TSS – Tirupur Soil Sample

SSS1 – Salem Soil Sample 1

SSS2 - Salem Soil Sample 2

SSS3 - Salem Soil Sample 3

SSS4 - Salem Soil Sample 4

SES1 - Salem Effluent Sample 1

SES2 - Salem Effluent Sample 2

SSF – Salem Sample Fungi

TSF – Tirupur Sample Fungi

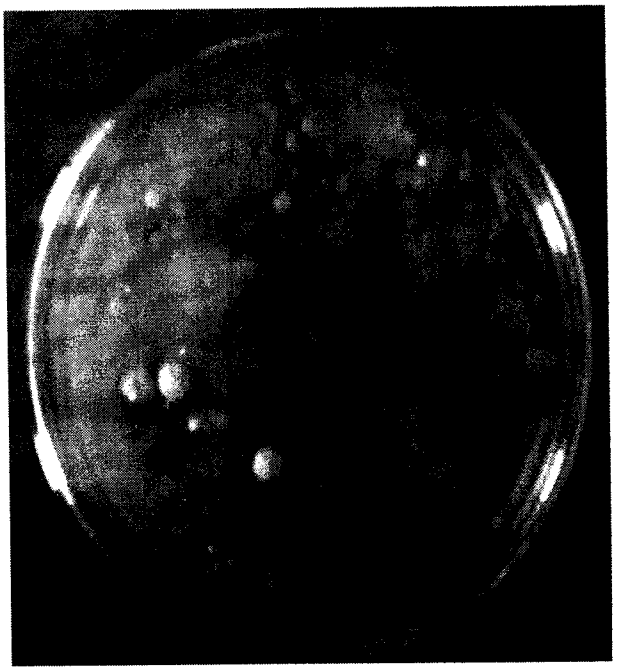

Fig 4.1.3 Fungal colonies from the source Salem Soil Sample 1

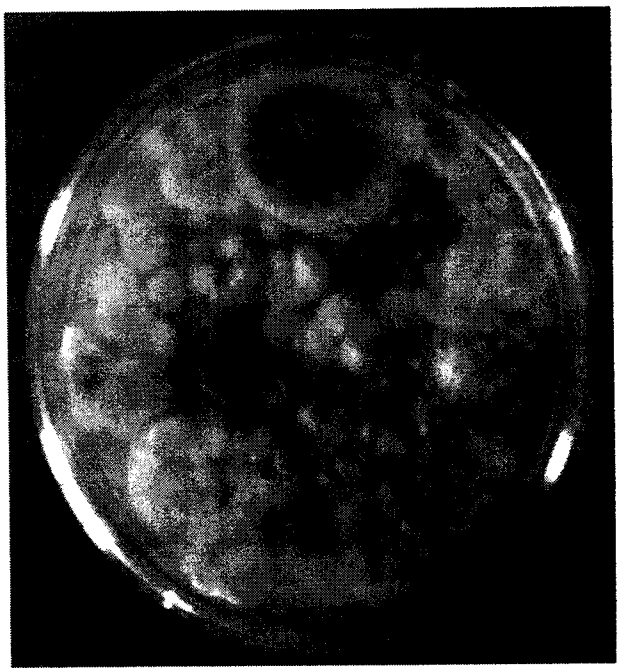

Fig 4.1.4 Fungal colonies from the source Salem Soil Sample 2

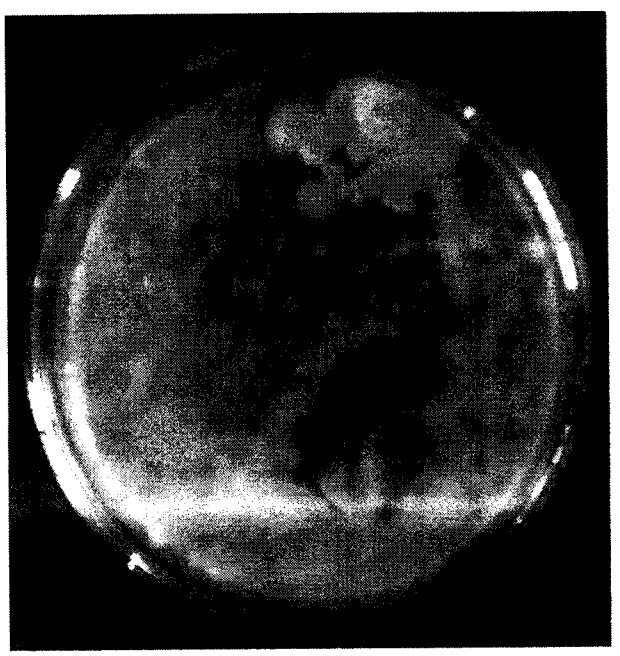

Fig 4.1.5 Fungal colonies from the source Salem Soil Sample

Fig 4.1.6 Fungal colonies from the source Salem Soil Sample 4

4.2 Decolorization of the effluent dyes using isolated microbial strains

The isolated bacterial and fungal colonies were then studied for their ability to decolorize effluent dyes. The effluent dye from the CETP in SIPCOT, Perundurai was used for this study. All the 82 bacterial colonies and 19 Fungal colonies were screened for their ability to decolorize the effluent dyes. The dye removal % was calculated for all the colonies and the colonies with high decolorization efficiency were then selected. The following table gives the dye removal % for the isolated bacterial and fungal colonies.

Table: 4.2.1 Effluent dye removal % of the Bacterial colonies isolated from CETP samples

Culture Name	Effluent dye removal (%) (after 48 hrs of incubation)
CETPB1	-
CETPB2	53.33
CETPB3	72.04
CETPB4	54.28
CETPB5	47.85
CETPB6	40.4
CETPB7	-
CETPB8	47.71
CETPB9	52.6
CETPB10	28.3
CETPB11	41.88
CETPB12	46.29

CETPB13	45.39
CETPB14	-
CETPB15	39.47
CETPB16	48.14
CETPB17	44.4
CETPB18	-
CETPB19	-
CETPB20	18.49
CETPB21	38.29
CETPB22	47.51
CETPB23	-
CETPB24	40
CETPB25	17.35
CETPB26	75.9
CETPB27	18.79
CETPB28	40.42
CETPB29	53.89
CETPB30	29.70

CETPB31	17.17
CETPB32	19.04

Table: 4.2.2 Effluent dye removal % of the fungal colonies isolated from CETP samples

Culture name	Effluent dye removal (%) (after 48 hrs of incubation)
CETPF1	56.14
CETPF2	44.64
CETPF3	48.29
CETPF4	62.67
CETPF5	51.29
CETPF6	44.37
CETPF7	49.74
CETPF8	68.98

Table 4.2.3 Effluent dye removal % of bacterial colonies isolated from Tirupur and Salem samples

Culture Name	Effluent dye removal (%) (after 48 hrs of incubation)
TEB 1	37.3
TEB 2	70.9
TEB 3	59.3
TEB 4	80
TEB 5	76.1
TEB 6	34.2
TEB 7	53.7
TEB 8	50.8
TEB 9	76.5
TEB10	50.9
TEB11	74.5
SEB 1	49.6
SEB 2	39.6
SEB 3	75.6
SEB 4	85
SEB 5	57.8
SEB 6	52.6

SEB 7	40.1
SEB 8	36.3
SEB 9	33.2
SEB10	42.5
SEB11	36.7
SEB12	23.3
SEB13	21.5
SEB14	50
SEB15	34.7
SEB16	49.5
SEB17	37.6
SEB18	35.9
SEB19	29.3
SEB20	28.7
SEB21	21.9
SEB22	31.1
SEB23	20
SEB24	52.7
SEB25	51.4

SEB26	53.5
SEB27	53.6
SEB28	53.06
SEB29	49.3
SEB30	57.5
SEB31	46.9
SEB32	44.9
SEB33	78.4
SEB34	38.3
SEB35	47
SEB36	48.4
SEB37	38.3
SEB38	50
SEB39	34.6

Table 4.2.4 Effluent dye removal % of fungal colonies isolated from Tirupur and Salem samples

Culture name	Effluent dye removal (%) (after 48 hrs of incubation)
SSF 1	62.6
SSF 2	57.5
SSF 3	37.5
SSF 4	45.5
SSF 5	40.12
SSF 6	43.4
SSF 7	41.06
SSF 8	46.7
SSF 9	44.8
SSF 10	53.35
TSF 1	50.09

Based on the decolorization studies, totally 10 bacterial colonies were selected for further studies based on their high dye removal %. Of these 2 bacterial cultures (CETPB3 & CETPB26) is from the CETP sample and the remaining 8 (TSB2, TSB4, TSB5, TSB9, TSB11, SSB3, SSB4 & SSB33) are from Salem and Tirupur samples. Since all the fungal colonies showed considerably high effluent dye removal %, all the 19 cultures (8 from CETP samples and 11 from Tirupur and Salem samples) were tested for their ability to decolorize the synthetic dyes.

4.3 Decolorization of the synthetic dyes using the selected bacterial and fungal strains

The screened bacterial and fungal strains were then tested for their ability to decolorize the synthetic dyes. Three synthetic dyes namely Reactive Red 120, Reactive Black 5 and Direct Red 81 were used for these studies. The synthetic dye removal % of the screened bacterial and fungal strains is used for further screening. The synthetic dye decolorization studies were made for 10 bacterial and 19 fungal strains.

Table 4.3.1 Synthetic dye removal% of screened bacterial cultures

Culture Name	Dye removal % Reactive Red 120 (520nm) (after 48 hrs of incubation)	Dye removal % Reactive Black 5 (574nm) (after 48 hrs of incubation)	Dye removal % Direct Red 81 (510nm) (after 48 hrs of incubation)
CETPB3	12.9	18.9	36.9
CETPB26	36.7	44.8	59.1
TSB2	37.8	48.4	48.08
TSB4	39.6	40.6	52.7
TSB5	41.9	48	50.5
TSB9	12.3	17.3	29.1
TSB11	19.5	15.2	27.7
SSB3	25.6	19.8	19.1
SSB4	23.3	14.4	28.7
SSB33	38.6	43.4	59.05

Table 4.3.2 Synthetic dye removal% of the fungal cultures

Culture Name	Dye removal % Reactive Red 120 (520nm) (after 7 days of incubation)	Dye removal % Reactive Black 5 (574nm) (after 7 days of incubation)	Dye removal % Direct Red 81 (510nm) (after 7 days of incubation)
CETPF1	55.7	44.4	42.5
CETPF2	34.8	33.5	15.6
CETPF3	24.3	27.4	30.7
CETPF4	23.6	17.8	26.7
CETPF5	33.6	25.8	30.6
CETPF6	65.6	75.8	57.3
CETPF7	37.8	28.1	32.4
CETPF8	65.4	66.1	54.2
SSF 1	69.6	56.2	70.4
SSF 2	67.4	62	52.6
SSF 3	24.1	33.6	23.1
SSF 4	21.05	42.3	20
SSF 5	18.8	10.3	37.4
SSF 6	35.7	15.5	41.7
SSF 7	28.6	17.3	9.9
SSF 8	18.7	10.5	14.6
SSF 9	8.9	19.8	8.2
SSF 10	36.1	49.5	45.1
TSF 1	49.5	35.4	44.6

Based on these studies on the synthetic dye removal by the microbial strains, final screening of the bacterial and fungal cultures was done. Four different fungal cultures and five different bacterial cultures were selected for the development of the consortia on the basis of their high dye removal efficiency of the three synthetic dyes. The following are the different bacterial and fungal strains that were selected and were renamed as follows,

Table 4.3.3 Selected bacterial cultures

Culture Name	Bacterial strain
CETPB26	B1
TSB2	B2
TSB4	B3
TSB5	B4
SEB33	B5

Table 4.3.4 Selected fungal cultures

Culture Name	Fungal Strain
CETPF6	F1
CETPF8	F2
SSF1	F3
SSF2	F4

4.4 Identification of the bacterial and fungal strains

The bacterial and fungal strains were identified as follows

F1 - *Pleurotus ostreatus*

F2 - *Aspergillus niger*

F3 - Penicillium simplicissimum

F4- *Penicillium chrysosporium*

B1 - *Bacillus subtilis*

B2 - *Bacillus cereus*

B3 - *Bacillus cereus*

B4 - *Bacillus megaterium*

B5 - *Pseudomonas fluorescens*

4.5 Optimization of the decolorization efficiency for the selected fungal strains

The optimization of the decolorization efficiency was then carried out for the screened fungal strains. The effect of various physical parameters such as pH, Temperature, C: N ratio and shaking conditions were studied. The optimum conditions for the decolorization of the synthetic dyes were determined.

4.5.1 Effect of Temperature on the decolorization efficiency

The effect of temperature on the decolorization of the commercial dyes was analyzed at three different incubation temperatures 30°C, 37°C and 45°C. The decolorization % was calculated for the samples incubated at different temperatures and the following table gives the decolorization % after 7 days for the four fungal strains,

Table 4.5.1.1 Effect of Temperature on the decolorization efficiency

Culture Name	% Dye removal Reactive Red 120 (520nm)	%Dye removal Reactive Black 5 (574nm)	%Dye Removal Direct Red 81 (510nm)
	Incubation Temperature - 30°C		
Pleurotus ostreatus (F1)	64	77	62
Aspergillus niger (F2)	78	40	56
Penicillium simplicissimum (F3)	87	92	64
Penicillium chrysosporium (F4)	73	88	88
Culture Name	**Incubation Temperature - 37°C**		
Pleurotus ostreatus (F1)	34	55	32
Aspergillus niger (F2)	51	35	46
Penicillium simplicissimum (F3)	42	28	30
Penicillium chrysosporium (F4)	39	41	43
Culture Name	**Incubation Temperature - 45°C**		
Pleurotus ostreatus (F1)	21	17	32
Aspergillus niger (F2)	16	22	23

Penicillium simplicissimum (F3)	27	19	14
Penicillium chrysosporium (F4)	13	50	22

The maximum dye removal was found to occur at the incubation temperature of 30°C. Thus, the optimum temperature for decolorization for the three synthetic dyes by the fungal strains was found to be 30°C.

4.5.2 Effect of pH on the decolorization efficiency

The effect of pH on the decolorization of synthetic dyes by the fungal strains was analyzed. The decolorization media was prepared at different pH 3, 4, 5 and 6 and the assay mixture decolorization % was calculated. The following table gives the synthetic dye removal % of the four fungal strains at different pH,

Table 4.5.2.1 Effect of pH on the decolorization efficiency

Culture Name	**% Dye removal Reactive Red 120 (520nm)**	**%Dye removal Reactive Black 5 (574nm)**	**%Dye Removal Direct Red 81 (510nm)**
	pH 3		
Pleurotus ostreatus (F1)	22	10	39
Aspergillus niger (F2)	17	5.5	42
Penicillium simplicissimum (F3)	23	15	41
Penicillium chrysosporium (F4)	11	21	38

Culture Name	**pH 4**		
Pleurotus ostreatus (F1)	31	48	39
Aspergillus niger (F2)	36	45	42
Penicillium simplicissimum (F3)	29	24	31
Penicillium chrysosporium (F4)	42	32	29
Culture Name	**pH 5**		
Pleurotus ostreatus (F1)	38	48	33
Aspergillus niger (F2)	56	45	51
Penicillium simplicissimum (F3)	48	31	49
Penicillium chrysosporium (F4)	46	37	49
Culture Name	**pH 6**		
Pleurotus ostreatus (F1)	64	77	62
Aspergillus niger (F2)	78	61	56
Penicillium simplicissimum (F3)	87	92	64
Penicillium chrysosporium (F4)	73	88	88

The maximum dye removal was found to occur at pH 6. Thus, the optimum pH for the decolorization of the synthetic dye by the fungal strains was determined to be 6.

4.5.3 Effect of C:N ratio on the decolorization Efficiency

The effect of the C:N ratio on the decolorization efficiency was determined by using the decoloriization media with different Carbon:Nitrogen source ratios such as 1:2, 1:1, 2:1. The following table gives the dye removal % of the fungal strains at different C:N ratios,

Table 4.5.3.1 Effect of C:N ratio on the decolorization Efficiency

Culture Name	**% Dye removal Reactive Red 120 (520nm)**	**%Dye removal Reactive Black 5 (574nm)**	**%Dye Removal Direct Red 81 (510nm)**
	C:N ratio - 1:1		
Pleurotus ostreatus (F1)	12	22	30
Aspergillus niger (F2)	11	14	22
Penicillium simplicissimum (F3)	34	64	12
Penicillium chrysosporium (F4)	12	31	23
Culture Name	**C:N ratio - 2:1**		
Pleurotus ostreatus (F1)	36	21	33
Aspergillus niger (F2)	29	52	60

Penicillium simplicissimum (F3)	14	26	31
Penicillium chrysosporium (F4)	31	22	12
Culture Name	**C:N ratio - 1:2**		
Pleurotus ostreatus (F1)	48	51	55
Aspergillus niger (F2)	56	85	69
Penicillium simplicissimum (F3)	29	77	57
Penicillium chrysosporium (F4)	69	75	59

The maximum dye removal % occurred at the C:N ratio of 1:2. Thus, the optimum C:N ratio for synthetic dye decolorization was determined to be 1:2 for the fungal strains.

4.5.4 Effect of shaking and static conditions on the Decolorization Efficiency

The effect of the shaking and stationary conditions on the dye decolorization was studied. The decolorization was carried out by incubating the decolorization assay mixture in the orbital shaker at 180rpm at 30°C. Then the effect of static and shaking condition on the decolorization efficiency was studied. The following table gives the dye removal% of samples incubated at shaking and static conditions,

Table 4.5.4 Effect of shaking and static conditions on the Decolorization Efficiency

Culture Name	% Dye removal Reactive Red 120 (520nm)	%Dye removal Reactive Black 5 (574nm)	%Dye Removal Direct Red 81 (510nm)
	Incubated at 180rpm		
Pleurotus ostreatus (F1)	22	32	30
Aspergillus niger (F2)	44	33	24
Penicillium simplicissimum (F3)	31	14	25
Penicillium chrysosporium (F4)	32	22	24
Culture Name	**Static condition**		
Pleurotus ostreatus (F1)	64	77	62
Aspergillus niger (F2)	78	61	56
Penicillium simplicissimum (F3)	87	92	64
Penicillium chrysosporium (F4)	73	88	88

The maximum dye removal % occurred at the static. Thus, the optimum condition for synthetic dye decolorization by the fungal strains was under the static condition.

Thus, the various physical parameters were optimized for the decolorization of the synthetic dyes by the fungal strains. The optimum conditions were incubation temperature of 30°C, pH 6, C:N ratio 1:2 and incubation under static condition. This was in accordance with the literature studies, the fungal isolates from the dye contaminated sites showed high decolorization of the synthetic dyes at the incubation temperature of 30°C, at pH 6 and C:N ratio of 1:2 of the decolorization media (Prachi and Anushree, 2009). The fungal strains secrete lignolytic enzymes extracellularly which produce high decolorization under static condition (Prachi and Anushree, 2009).

4.6 Development of the microbial consortia and determination of the decolorization efficiency

The microbial consortia were developed by using mixed culture of the microorganisms. One bacterial consortium and four fungal consortia were developed and their decolorization efficiency of the commercial dyes was determined.

4.6.1 Development of fungal consortia

Four different types of fungal consortium were developed. These four consortia consist of different sets of fungi and they were named as FC1, FC2, FC3 and FC4. The following is the composition of the consortia

FC1 – *Pleurotus ostreatus* (F1), *Aspergillus niger* (F2), *Penicillium simplicissimum* (F3) & *Penicillium chrysosporium* (F4)

FC2 – Aspergillus *niger* (F2), *Penicillium simplicissimum* (F3) & *Penicillium chrysosporium* (F4)

FC3 – *Pleurotus ostreatus* (F1), *Aspergillus niger* (F2) & *Penicillium chrysosporium* (F4)

FC 4 – *Pleurotus ostreatus* (F1), *Penicillium simplicissimum* (F3) & *Penicillium chrysosporium* (F4)

where F1, F2, F3 & F4 are the four fungal cultures that possessed high commercial dye decolorization efficiency.

4.6.2 Development of a Bacterial consortium

A bacterial consortium was developed by using five bacterial strains which were capable of decolorizing the commercial dyes. The bacterial consortium was named BC and it composed of bacterial strains *Bacillus subtilis* (B1), *Bacillus cereus* (B2), *Bacillus cereus* (B3), *Bacillus megaterium* (B4) and *Pseudomonas fluorescens* (B5).

4.6.3 Determination of decolorization efficiency of the microbial consortia

The decolorization of the three commercial dyes by the fungal and microbial consortium was tested. The following tables and figures give the dye removal % for the three synthetic dyes by the four fungal consortia and one bacterial consortium.

Table 4.6.1 Synthetic dye removal % of the fungal consortia

Fungal Consortium	% Dye removal Reactive Red 120 (520nm)			%Dye removal Reactive Black 5 (574nm)			%Dye Removal Direct Red 81 (510nm)		
	2nd day	5th day	7th day	2nd day	5th day	7th day	2nd day	5th day	7th day
FC1	23	49	73	22	39	77.2	27	43	62
FC2	29	53	73	16.4	37	65.5	23	42	54
FC3	31	55	76	21.5	46.7	74.5	22	46.1	56.3
FC4	7.3	11	19	14.3	28.7	51.8	12.2	23	45

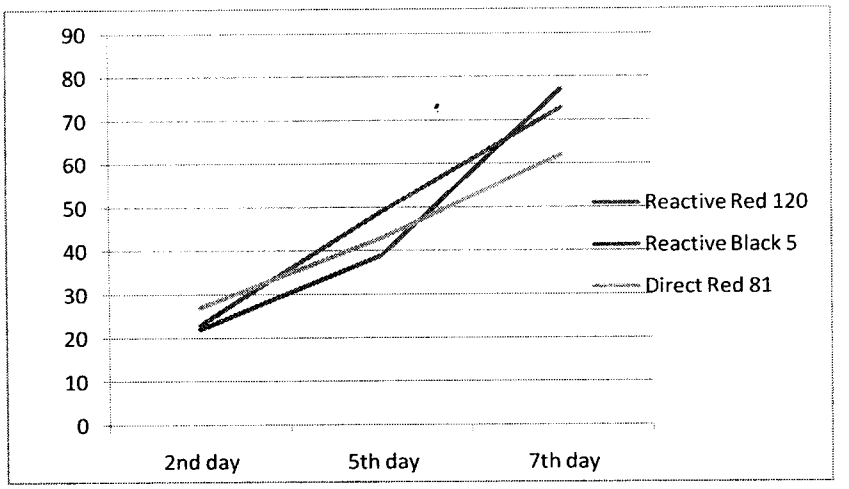

Fig 4.6.1 Synthetic Dye Removal by Fungal Consortium FC1

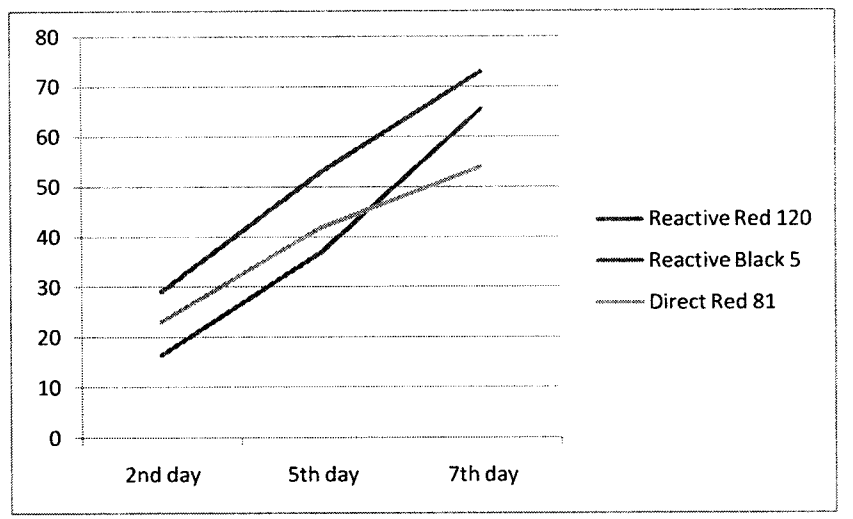

Fig 4.6.2 Synthetic Dye Removal by Fungal Consortium FC2

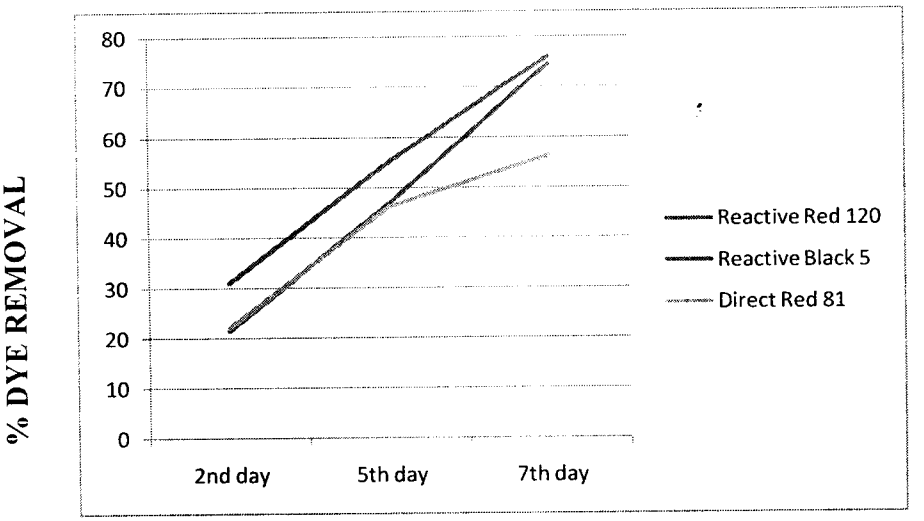

Fig 4.6.3 Synthetic Dye Removal by Fungal Consortium FC3

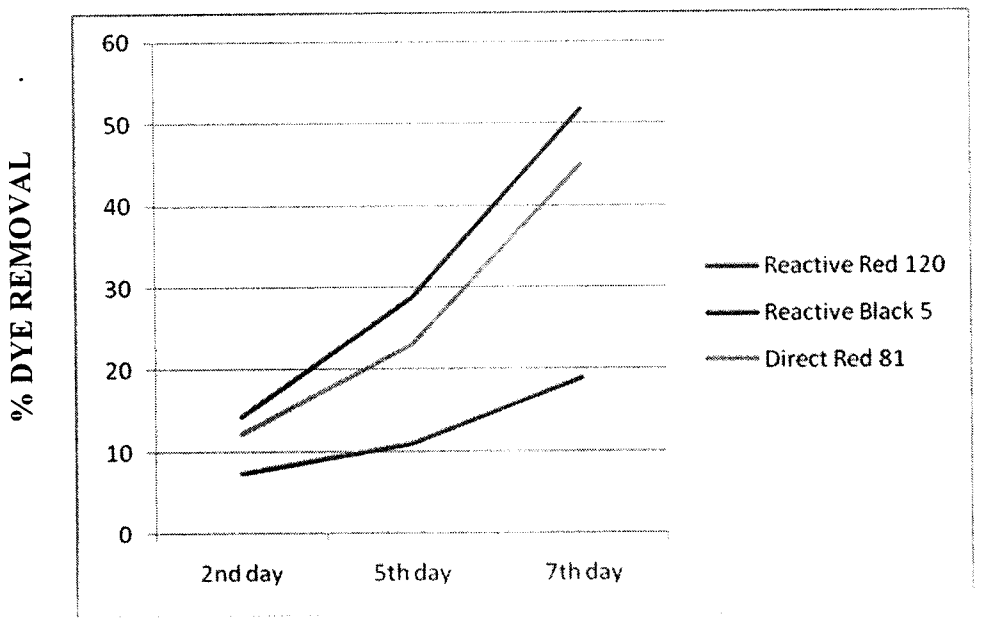

Fig 4.6.4 Synthetic Dye Removal by Fungal Consortium FC4

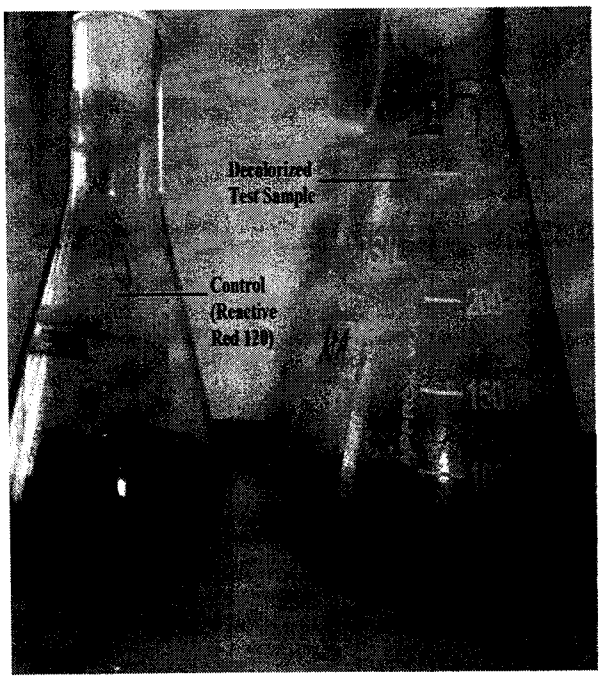

Fig 4.6.5 Decolorization of Reactive Red 120 by FC1

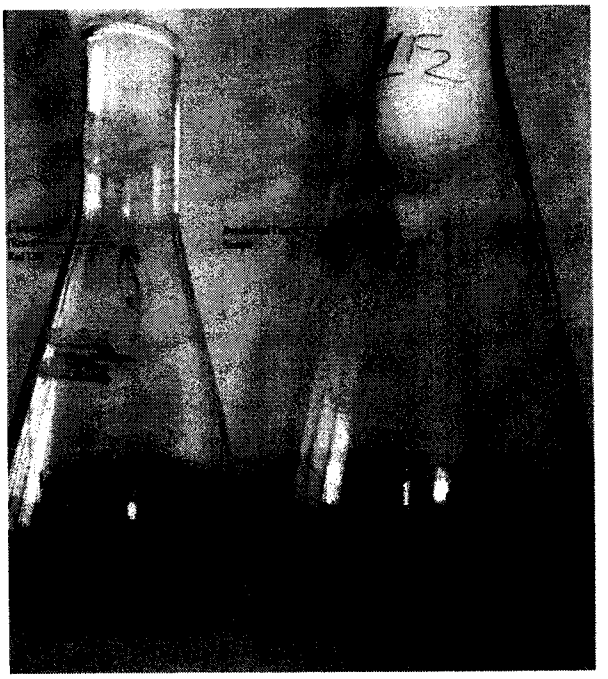

Fig 4.6.6 Decolorization of Reactive Red 120 by FC2

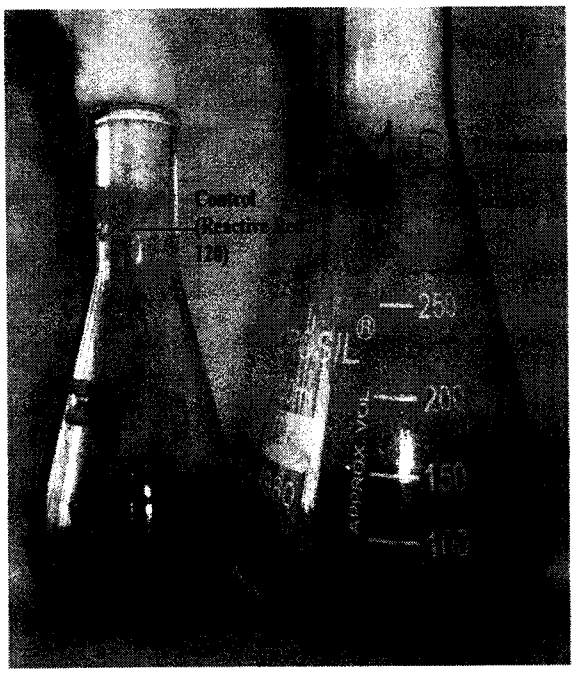

Fig 4.6.7 Decolorization of Reactive Red 120 by FC3

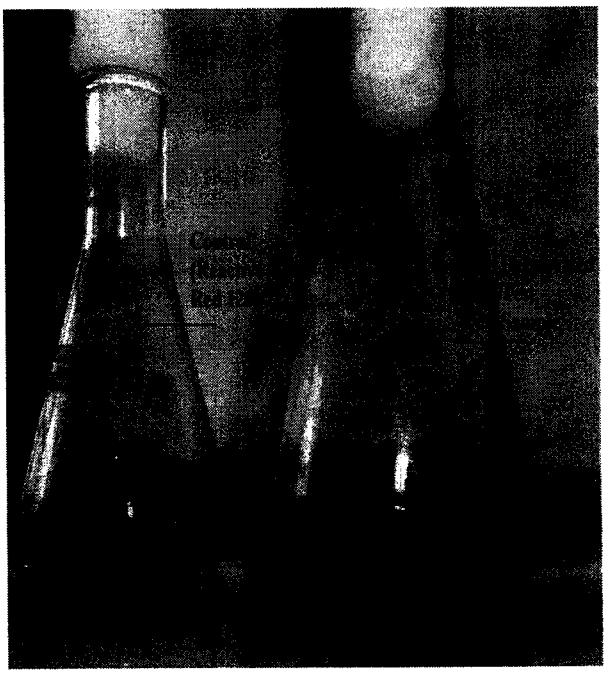

Fig 4.6.8 Decolorization of Reactive Red 120 by FC4

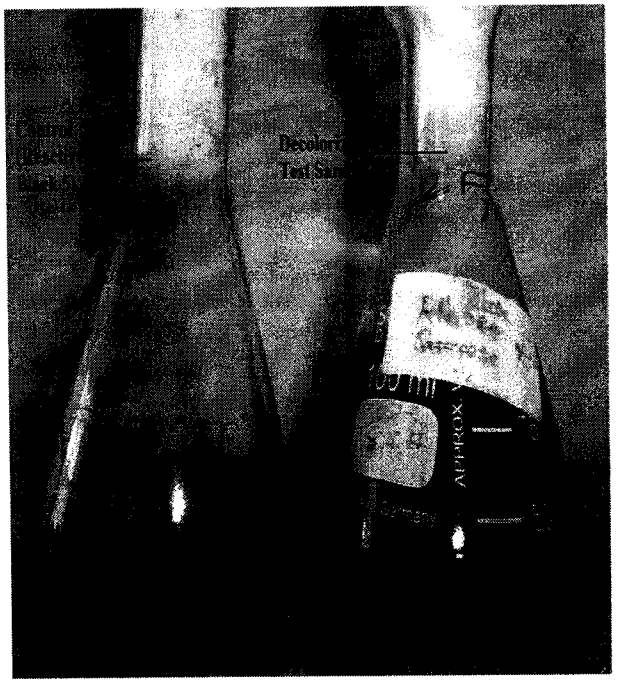

Fig 4.6.9 Decolorization of Reactive Black 5 by FC1

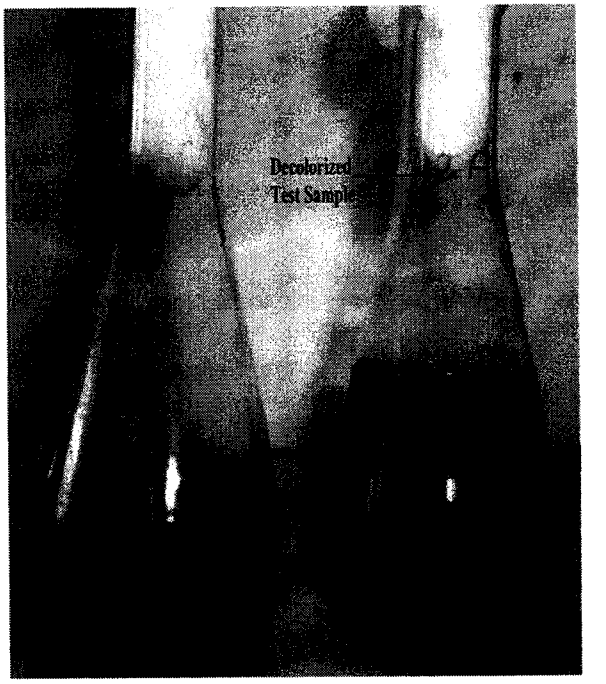

Fig 4.6.10 Decolorization of Reactive Black 5 by FC2

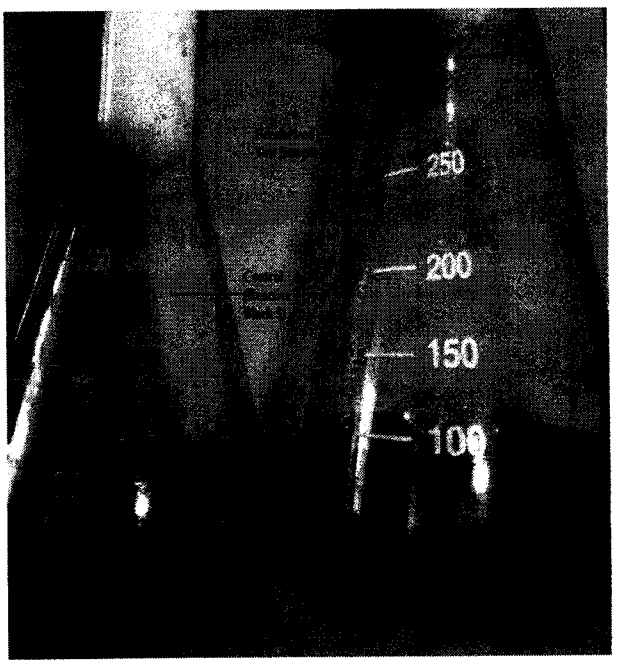

Fig 4.6.11 Decolorization of Reactive Black 5 by FC3

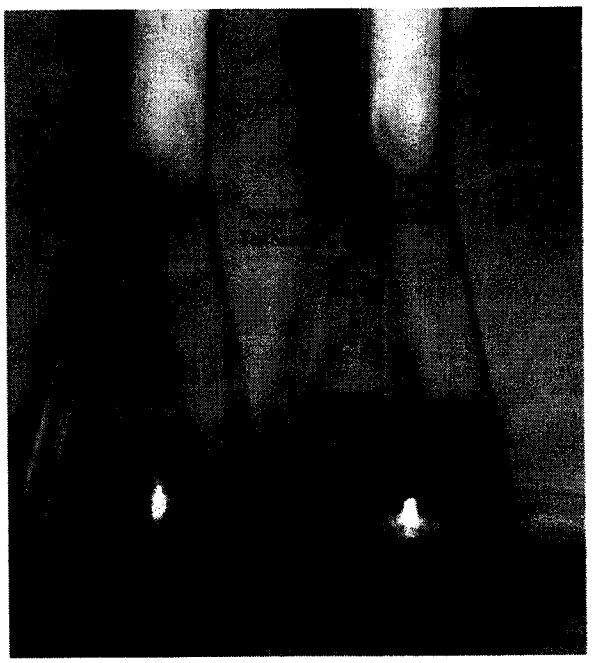

Fig 4.6.12 Decolorization of Reactive Black 5 by FC4

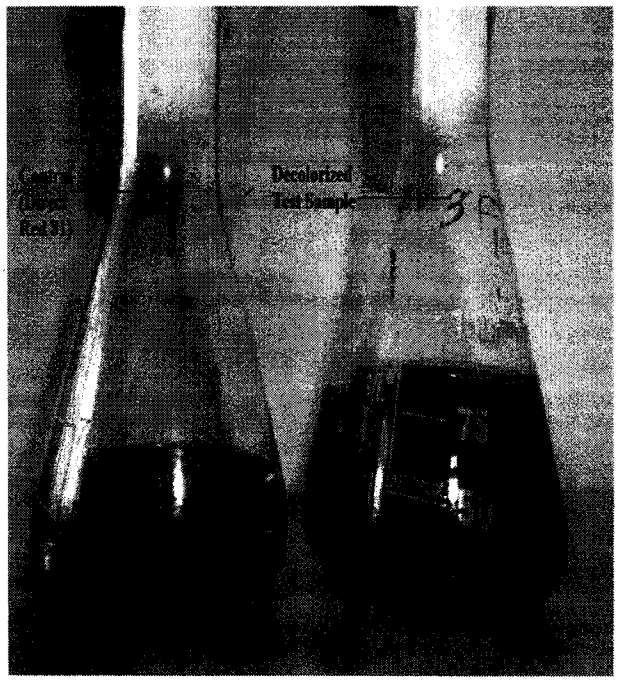

Fig 4.6.13 Decolorization of Direct Red 81 by FC1

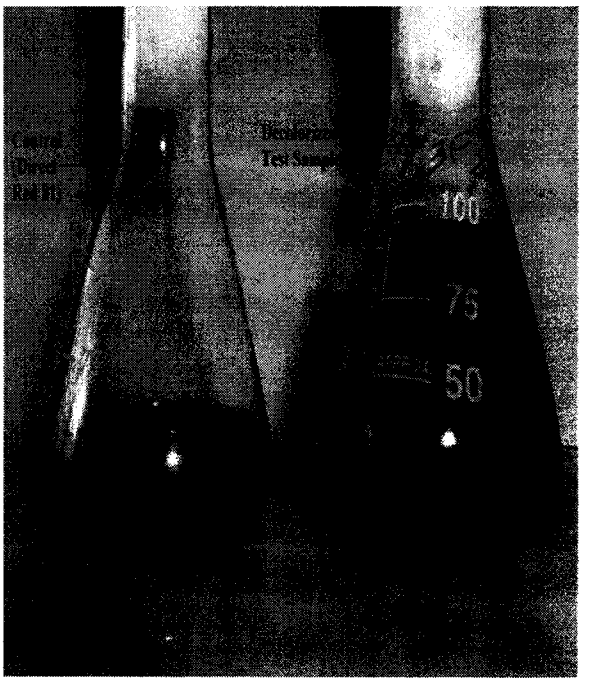

Fig 4.6.14 Decolorization of Direct Red 81 by FC2

Fig 4.6.15 Decolorization of Direct Red 81 by FC3

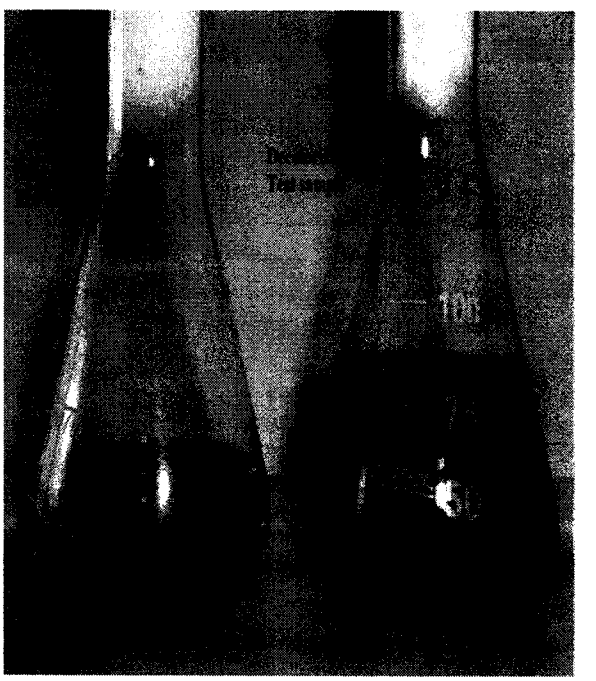

Fig 4.6.16 Decolorization of Direct Red 81 by FC4

Thus the fungal consortia showed high efficiency of synthetic dye decolorization. The consortia FC1, FC2 and FC3 showed high dye removal of the three synthetic dyes. These consortia exhibited about 55 – 80% dye removal.

Table 4.6.2 Synthetic dye removal % of the bacterial consortium

Bacterial Consortium	% Dye removal Reactive Red 120 (520nm)		%Dye removal Reactive Black 5 (574nm)		%Dye Removal Direct Red 81 (510nm)	
	1st Day	2nd Day	1st Day	2nd Day	1st Day	2nd Day
BC	13.26	23.2	31	53	45	78

Thus, the bacterial consortium showed high decolorization of Direct Red dye while it showed moderate removal of Reactive black and very low removal of Reactive Red. The consortium-GB (*Galactomyces geotrichum* and *Bacillus* sp.) exhibited 100% decolorization ability with the dye Brown 3REL (Jadhav *etal.*, 2008).). Microbial consortia developed with *Bacillus cereus* and *Bacillus pumilus* for bacteria and *Aspergillus alliaceus* for fungi showed 100% decolorization of textile wastewater (Eltaief *etal.*, 2009). The bacterial consortium consisting of five different bacterial species *Bacillus vallismortis*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* were efficient in decolorizing individual as well as mixture of dyes (Bella *etal.*, 2009).

4.7 Dye degradation studies of the microbial consortia

The decolorization of the synthetic dyes by the microbial consortia can be due to adsorption or degradation. The samples from the decolorization assay mixture were withdrawn at different time interval for decolorization studies. These samples were

centrifuged at 10,000 rpm for 15 mins. The supernatant formed was discarded and the pellet was dissolved in the Bushneel & Haas medium and the absorbance reading was measured spectrophotometrically. It was found that there was no dye in the pellet obtained from the fungal samples. Thus, there is degradation of the synthetic dyes. The bacterial sample showed considerable amount of dye in the pellet. Thus, the decolorization by bacterial consortium was due to adsorption.

CONCLUSION

CHAPTER 5

CONCLUSION

Environmental pollution caused by the release of a wide range of compounds as a consequence of industrial progress has now assumed serious proportions. Textile dyes are one of the most prevalent chemicals in use today. With increasing usage of the wide variety of dyes in the industries pollution from the effluents has become increasingly alarming. Microbial decolorization and degradation is and environmentally friendly and cost – effective process.

The microbial strains including the bacterial and fungal strains were isolated from the effluent and soil samples from the contaminated sites. These strains were subjected to initial screening through the decolorization studies on effluent dyes. The screened strains were then tested for their ability to decolorize three synthetic dyes Reactive Red 120, Reactive Black 5 and Direct Red 81 and then final screening was done based on these studies. After final screening, 5 bacterial strains and 4 fungal strains were selected. The fungal strains were identified as *Pleurotus ostreatus, Aspergillus niger, Penicillium simplicissimum* and *Penicillium chrysosporium.* and the bacterial strains were identified to be *Bacillus subtilis, Bacillus cereus, Bacillus megaterium* and *Pseudomonas fluorescens.*

The various physical parameters such as temperature, pH, C:N ratio, shaking and stationary conditions were optimized for the decolorization of the three synthetic dyes by the fungal strains. About 4 different fungal consortia and 1 bacterial consortium were developed with the selected strains. These consortia were tested for the ability to decolorize the synthetic dye and it was found that they showed about 55 – 80% dye removal. The fungal consortia were found to degrade the

synthetic dyes while the dye removal by the bacterial consortium was predominantly due to the adsorption.

It was concluded that the fungal consortia and bacterial consortium obtained by grouping the fungal and bacterial strains respectively isolated from the samples of the dye contaminated sites showed higher synthetic dye removal % than the individual strains. Thus, the microbial consortia can be used for the decolorization of textile dyes efficiently.

APPENDIX

APPENDIX

NUTRIENT AGAR

Composition per litre

Meat extract	- 3.0g
Peptic digest of animal tissue	- 5.0g
Agar	- 15.0g
pH	- 6.8 ± 0.2

NUTRIENT BROTH

Composition per litre

Peptic digest of animal tissue	- 5.0g
Sodium chloride	- 5.0g
Beef extract	- 1.50g
Yeast extract	- 1.50g
pH	- 7.4 ± 0.2

BUSHNELL & HAAS MEDIUM FOR BACTERIA

Ammonium Nitrate,	- 1g/l
Calcium Chloride	- 0.02g/l
Ferric Chloride	- 0.05g/l
Magnesium Sulphate	- 0.2g/l
Dipotassium Hydrogen Phosphate	- 1g/l
Glucose,	- 0.1% w/v
Yeast extract,	- 0.05% w/v
pH	- 7

BUSHNELL & HAAS MEDIUM FOR FUNGI

Ammonium Nitrate	- 0.5g/l
Magnesium Sulphate	- 0.1g/l
Dipotassium Hydrogen Phosphate	- 0.5g/l
Glucose,	- 10g/l
Yeast extract,	- 2% w/v
Sodium Chloride	- 1g/l
pH	- 6

POTATO DEXTROSE AGAR

Potatoes, infusion from	- 200g/l
Dextrose	- 20g/l
Agar	- 15g/l
pH	- 5.6 ±0.2

POTATO DEXTROSE BROTH

Potatoes, infusion from	- 200g/l
Dextrose	- 20g/l
pH	- 5.1 ±0.2

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