

Methodology



3.0 EXPERIMENTAL PROCEDURE

Industrial effluent is the main source of dye to the soil environment. In India, due to technical and economical reasons untreated or partially treated industrial effluents containing dyes are released into nearby water bodies. Most of the industries do not have adequate provision for treating the effluents. Considerable amounts of hazardous pollutants including heavy metals find their way to river basins and also tend to percolate into ground water (Krishnasamy *et al.*, 2002).

The waste water carries significant levels of chlorides, sulphates, phenolic compounds and heavy metals. Some of these compounds are released into the environment. The ever increasing demand for water of high quality has caused considerable attention to be focussed towards recovery and reuse of wastewater (Maleekabegum *et al.*, 2005).

Removal of colour from wastewater is most challenging and perplexing problem. It also affects the ecosystem by affecting the photosynthetic activity and dissolved oxygen concentration. Thus, effluents containing dyes must be treated before they are discharged into the environment. Synthetic dyes have antimicrobial effects and are relatively resistant to biodegradation, especially to conventional biological wastewater treatment system.

Decolourization of textile factory effluent is usually based on physicochemical methods, while physicochemical treatments of dyes have several disadvantages, like low decolourization efficiency. So, there is a need for development of new environment friendly methods for dye decolourization. Microbial treatment may be a suitable alternative for decolourization by proper selection of the process and microorganism (Yesilada *et al.*, 2010).

The present study has been undertaken to isolate a bacterial culture, which has the capacity to decolourize the textile dyes. Decolourization capacities of the isolated culture were studied against various dyes. Finally the isolated efficient bacterial cultures were studied to decolourize the textile industry effluent. The study was carried out in five phases.

3.1 PHASE I

3.1.1. Collection of the textile effluent and soil

3.1.2. Characterization of the effluent

3.2 PHASE II

3.2.1. Collection of dyes

3.2.2. Enrichment of microorganism from the soil collected from effluent area

3.2.3. Isolation of bacterial strain

3.2.4. Decolourization of selected azo dyes by isolated bacteria

3.3 PHASE III

3.3.1. Characterization of isolated microorganism by various biochemical tests

3.4 PHASE IV

3.4.1. Effect of culture conditions on decolourization of individual dye

3.4.2. Effect of pH on dye decolourization

3.4.3. Effect of initial dye concentration on decolourization

3.4.4 Effect of temperature on dye decolourization

3.5 PHASE V

3.5.1. Treatment of textile effluent with the free cells.

3.5.2. Treatment of textile effluent with immobilized microbial cells

3.5.3. Decolourization of various textile dyes

3.5.4. Determination of BOD and COD on treated effluent

3.1. PHASE I

3.1.1. COLLECTION OF THE TEXTILE EFFLUENT

The effluent was collected at regular intervals and soil samples were collected from effluent disposal site of selected textile industry Perur, Coimbatore and stored at 4°C for analysis

3.1.2. CHARACTERIZATION OF TEXTILE EFFLUENT

The collected textile industry effluent was analyzed for selected physicochemical parameters. The parameters analyzed, method of analysis, references and appendices of the parameters analyzed are listed in Table 2.

TABLE 2
PHYSICOCHEMICAL CHARACTERISTICS OF THE
TEXTILE DYEING EFFLUENT (METHOD OF ANALYSIS)

Characteristics		Method of analysis	References	Appendix No
Colour		Visual	-	-
Odour		Sense	-	-
Turbidity		Visual	-	-
pH		Using digital meter	-	-
Total solids	suspended	Filtration method	APHA,1998	(I)
Total solids	dissolved	Filtration method	APHA,1998	(II)

3.2. PHASE II

3.2.1. COLLECTION OF DYES

Commercially available azodyes like Reactive Red, Reactive Yellow, Reactive Blue, Congo Red, Acid Red, Navy Blue and Malachite Green were purchased from a local market Coimbatore, and used for this study without any further purification

3.2.2. ENRICHMENT OF MICROORGANISMS FROM THE SOIL COLLECTED FROM EFFLUENT AREA

For isolation, first the microorganism present in the soil sample was enriched by inoculating one gram of soil into the nutrient broth containing mixture of textile dyes and incubated for 48hrs under static condition.

3.2.3. ISOLATION OF BACTERIAL STRAIN

After 48hrs of incubation 1ml of cell suspension was serially diluted upto 10^{-8} , and $10\mu\text{l}$ of each dilution was spread on the nutrient agar plates containing a mixture of dyes and incubated for 24hrs.

3.2.4. DECOLOURIZATION OF SELECTED AZODYES BY ISOLATED BACTERIA.

Overnight culture of the isolated bacteria was prepared. 1ml of this overnight culture was inoculated into 50ml nutrient broth containing individual dyes at 50mg/l concentration and incubated at 35°C . Then 2ml was aseptically withdrawn from each flask and centrifuged at 6000 rpm for 10min. The cell free supernatant was used to measure decolourization at the absorbance maxima of the dye. An abiotic control (with no microorganism) was always included. The decolourization percentage was measured at various time intervals

3.3. PHASE III

3.3.2. BIOCHEMICAL ANALYSIS OF THE ISOLATED MICROORGANISM

Biochemical tests were carried out to identify the isolated microorganism. The test, method of analysis, references and appendices of the tests are listed in Table 3

TABLE 3
BIOCHEMICAL TESTS

S.No	Biochemical tests	References	Appendix No
1	Gram stain	Sundarajan,1995	III
2	Glucose fermentation	Dubey &Maheswari (2002)	IV
3	Starch hydrolysis	Dubey &Maheswari (2002)	V
4	Indole production	Dubey &Maheswari (2002)	VI
5	MR Reaction	Dubey &Maheswari (2002)	VII
6	VP Reaction	Dubey &Maheswari (2002)	VIII
7	Citrate use	Dubey &Maheswari (2002)	IX
8	Urease Activity	Dubey &Maheswari (2002)	X
9	Oxidase Activity	Dubey &Maheswari (2002)	XI
10	Catalase Activity	Dubey &Maheswari (2002)	XII

3.4. PHASE IV

3.4.1 EFFECT OF CULTURE CONDITION ON DECOLOURIZATION OF INDIVIDUAL DYE.

The isolated culture was grown for 24h at 35⁰c in 100ml nutrient broth containing dyes at 50mg/l concentration to study the effect of the static and shaking condition on the decolourization of reactive red. This culture flask was incubated at static as well as shaking condition at 35⁰c for 120rpm on orbital shaker. The aliquot (3ml) of the culture media was withdrawn at different time intervals and centrifuged at 6000 rpm for 10 min. Decolourization was monitored by measuring the absorbance of culture supernatant at absorbance maxima of the respective dye.

3.4.2. EFFECT OF pH ON DYE DECOLOURIZATION

To examine the effect of pH on decolourization, nutrient broth containing 50 mg/l reactive red dye was adjusted to pH from 5-10 using HCL or NaOH and a loop full of isolated culture was inoculated. The decolourization rate was determined at 12, 24, 36 and 48 hrs after incubation.

3.4.3. EFFECT OF INITIAL DYE CONCENTRATION ON DECOLOURIZATION

In order to examine the effect of initial dye concentration on the decolourization in static condition various concentration of reactive red dye (25, 50, 75,100,125 and 125 mg/l) were prepared in nutrient broth and inoculated with isolated culture. After incubation at 35⁰c, the decolourization rates were determined at 12, 24, 36 and 48 hrs. Abiotic controls (without micro organism) were always included.

3.4.4 EFFECT OF TEMPERATURE ON DECOLOURIZATION

To examine the effect of temperature, nutrient broth containing 50 mg/l concentration of reactive red dye was inoculated with isolated culture. This was incubated at various temperatures (25, 35, 45 and 55 °C). The decolourization rate was determined at 12, 24, 36 and 48 hrs.

3.5 PHASE V

3.5.1. TREATMENT OF TEXTILE EFFLUENT WITH FREE BACTERIA CELL.

The original textile effluent was filtered using filter paper. 100ml of filtered effluent was taken in to 250ml flask and sterilized and inoculated with 50ml of the inoculum and incubated at static condition. Samples were withdrawn at regular interval and analyzed for colour removal. The intensity of colour was measured at its maximum absorbance (490nm).

3.5.2. TREATMENT OF TEXTILE EFFLUENT WITH IMMOBILIZED CELLS

The isolated microbial were immobilized by sodium alginate. The immobilized cells were used as a inoculums to treat the textile effluent. The sterilized effluent was inoculated with the immobilized cells at 35° in static condition. Samples were withdrawn at regular interval and analyzed for colour removal. The intensity of colour was measured at its maximum absorbance (490nm).

3.5.3. DETERMINATION OF BOD AND COD ON TREATED EFFLUENT

Chemical oxygen demand was determined by Titrimetric method (APHA, 1998) and Biological oxygen demand was measured by Winklers iodometric method (APHA, 1998).

3.5.4. DECOLOURIZATION OF VARIOUS TEXTILE DYES.

Other textile dyes like Congored, Malachite green, Navyblue and Reactive red were examined for decolourization by the isolated organism. The dyes at 50mg/l in nutrient broth were prepared and inoculated with the isolated organism. The decolourization of these dyes was measured at the maximum absorbance of the dyes after 48 hrs.