Microbial degradation and decolorization of textile dye effluent collected from Amritsar area.

A report presented to

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In partial fulfillment

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(2013-2015)

Project supervisor

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LOVELY PROFESSIONAL UNIVERSITY

School of: Biotechnology and Biosciences

DISSERTATION TOPIC APPROVAL PERFORMA

Name of the Student: Gayatri Mahajan Registration No: 11301793

Batch: 2013 Roll No. 05

Session: 2013-2015 Parent Section: B1304

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Research Experience: 07

SPECIALIZATION AREA: _____ (pick from list of provided specialization areas by DAA)

PROPOSED TOPICS

1. Microbial decolorization and degradation of textile dye effluent collected from Jalandhar area.
2. Production of ethanol from cellulosic biomass.
3. Production of bioethanol biobutanol from feed stock.

Ajay Kumar

Signature of Supervisor

OR

PAC Remarks:

- Revise the topic.
- Topic not clear

12.3.15

13.3.15

APPROVAL OF PAC CHAIRPERSON: **Signature:** **Date:**

*Supervisor should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)

*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation final report.

*One copy to be submitted to Supervisor.

CERTIFICATE

This is to certify that Gayatri Mahajan bearing registration no. 11301793 has completed dissertation project report, entitled "Microbial degradation and decolorization of textile dye effluent collected from Amritsar area" under my guidance and supervision. To the best of my knowledge, the present work is the result of thorough study. No part of the report has ever been submitted for any other degree at any university. The report is apt for fulfilling the conditions for the award of degree of Masters in Technology in Biotechnology.

Er. Ajay Kumar
Assistant Professor
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India

DECLARATION

I, Gayatri Mahajan, hereby certify that the work which is being presented in this report entitled "Microbial degradation and decolorization of textile dye effluent collected from Amritsar area" for partial fulfillment of degree of Masters of technology in Biotechnology submitted to Lovely Professional University is the outcome of my own efforts.

Gayatri Mahajan

Registration no. 11301793

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LIST OF ABBREVIATIONS:

HPLC	High performance liquid chromatoFigy
HPTLC	High performance thin layer chromatoFigy
GC	Gas chromatoFigy
MS	Mass Spectrophotometry
USA	United States of America
FTIR	Fourier Transformed Infra Red
RNA	Ribonucleic Acid
BMM	Basal Mineral Medium
SDS-PAGE	Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
UV-VIS	Ultra Violet- Visible
PPM	Parts per million
$(NH_4)_2SO_4$	Ammonium Sulfate
KDa	Kilo Daltons
$Na_2HPO_4.2H_2O$	Hydrated sodium hydrogen phosphate
H_2PO_4	Potassium hydrogen phosphate
NaCl	Sodium Chloride
PCR	Polymerase Chain Reaction
FDA	Food and Drug Administration
W/V	Weight by Volume

ABSTRACT

Textile dyeing industry is one of the industries making exhaustive use of synthetic chemicals and the chief adulterant of palatable water. It yields colossal amounts of toxic and complex chemical substances as effluent wastewater during almost every stage of textile fabrication. The direct release of this wastewater effluent into surrounding waterbodies affects its ecological parameters by imposing a vast number of unacceptable changes. Amongst all dyes being used to dye fabric, azo dyes are fractious and unyielding to multifarious tactics of degradation and are supreme contaminants in environment. Water pollution because of elevated use of dyestuffs is one of the dominating cosmic dilemmas. Present work is focused on degradation and decolorization of three dyes; Disperse Orange, Disperse Blue and Scarlet RR by microbes isolated from textile effluent of a textile dyeing industry located at Amritsar. Two isolates were preferred for degradation studies based on % decolorization achieved. W2 isolate yielded 97.51% decolorization of Scarlet RR dye at pH 7 and 37 °C. Decolorization was observed at a wide range of pH and at vacillating dye concentrations.

Keywords: Degradation, decolorization, azo dyes, textile effluent, carcinogenic.

CHAPTER 1
INTRODUCTION

Dyes are being used by almost every industry in the world, be it, cosmetic industry, paper industry, food industry, textile industry, pharmaceutical industry, electronics industry, biotechnology industry, real estate etc. We are basically surrounded by dyes, wherever we look around, we will find different colors, majority of these colors come from artificial dyeing, our clothes, paints on walls, colored electronic products(fans, mobile phones etc), colored medicines, colored toys, colored papers, colored cosmetics, colored plastics etc. We are using dyes to such an extent that we cannot think to live without colors. The textile industry alone constitutes 66% of the dye consumption. Approximately, 10% of the dye stuff reaches water bodies during production and dying process (Elisangela *et al*., 2009). And, these dyes are complex organic compounds which are harmful when released in environment through any disposal method. These dyes have been found to be carcinogenic by many of the researchers (Faison *et al*., 1985). The number of cancer patients worldwide is increasing annually. So there must be certain changes that have been taken place in the environment in last few decades that is causing mutations to occur more frequently. The major reason can be certain chemicals which we are exploiting in our industries and then releasing in environment without following strict parameters of environmental policies. One of these chemicals are dyes, innumerable types of dyes are being used in various industries, for example, azo dyes, indigo dyes etc. These dyes when released cause a huge disturbance in environment as the production and release of these dyes is quite substantial. Much of the studies have been done on dyes used in the industries because of recalcitrant nature of dyes, carcinogenic and mutagenic properties and so many other toxic effects. These dyes never die, these stay inside the environment only. Sometimes, these dyes undergo incomplete degradation biotically or abiotically, which results in end products even more toxic than the native dye itself. Due to incomplete degradation, these dyes appear somewhat colorless visually, but pollution problem is not solved. So, to ensure complete degradation, we need novel methods that must be promising enough to solve the environmental problems incurred by these dyes (Brown and De vito, 1993).

Earlier physical and chemical methods have been used to treat this waste but that was bit expensive. Moreover, in the chemical methods, we are using more chemicals to degrade the harmful ones. So, overall consumption of chemicals increases further. So, some biological treatment needs to be developed and employed. As the microorganisms are being employed for bioremediation of wastes of many industries, be it heavy metals, antibiotic degradation etc., this microbial degradation has been used for dye degradation as well. In presented report, we are going to isolate bacteria which have the capability to degrade a broad spectrum of dyes used in industries. Three dyes are being used in the study. These are Scarlet red and Disperse blue, disperse orange. Scarlet dye is a type of azo dye, which has been reported to be carcinogenic by many of the research workers. Azo dyes contain a functional group R-N=N-R, where R and R' can be alkyl group or aryl group. The N=N group is the azo group. Mostly all the textile stuff is dyed using azo dyes. Next, disperse orange is a disperse dye which are used to dye polyester and acetate fabric and are water insoluble. They have the smallest molecular size among all the dyes currently being used. Disperse dyes can be azobenzene or anthraquinone based dyes. Disperse orange is a type of azobenzene dye containing 25% of dye by weight and 75% salts like NaCl etc. Molecular formula of disperse orange is $C_{18}H_{14}N_4O_2$. Molecular mass is 318 g/mol and melting point is 160°C.

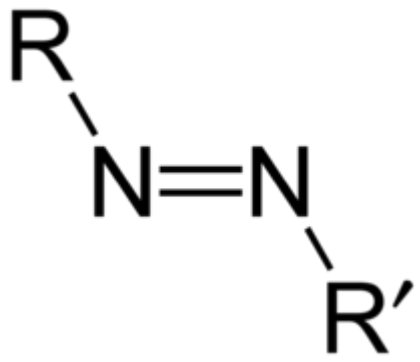

Fig 1.1: Basic structure of an azo dye

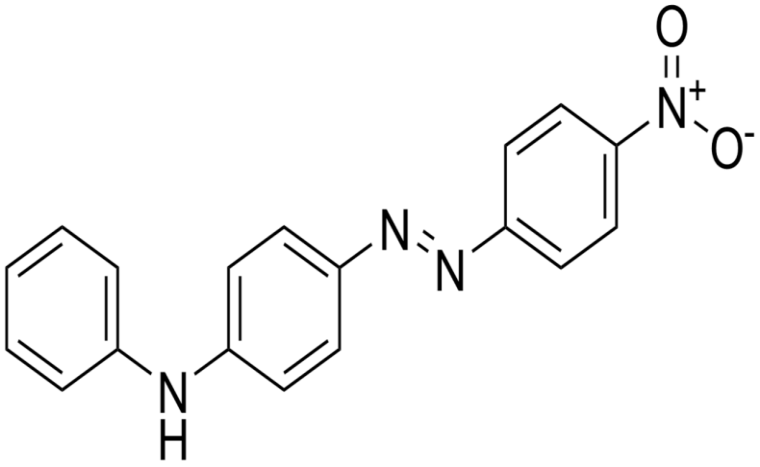

Fig 1.2: Chemical structure of disperse orange

CHAPTER 2

REVIEW OF LITERATURE

2.1 Dyes

According to Dykes *et al*., 1994, dye is a colored substance which absorbs a particular wavelength and gives color corresponding to that wavelength. There are two types of dyes- natural dyes (obtained from leaves, wood, and bark) and synthetic dyes. Synthetic dyes when discovered, they readily replaced the natural dyes as the former are less expensive and available in wide range of colors. Dyeing has emerged as the most used practice in almost every industry in this whole wide world. Food, pharmaceutical, biotechnology, textile, electronics, home appliances, hair coloring, paper dyeing, construction, paints, automobile industries, all of these use dyeing in some or the other way. Out of these textile and dyeing industries are the major players, and effluent of these can alter the aquatic environment by imparting changes in color, turbidity of marine bodies, rendering it harmful to public health, fish and other life forms. Many water borne diseases are being reported at a higher frequency like dermatitis, allergic reactions of respiratory tract. So, one can imagine the production and disposal rates of these dyes combined together. And as carcinogenicity of these dyes has been reported by many research fellows, so anyone can feel the need for a remedy to this hazard.

History of Dyes:

From the beginning of humanism, there have been endeavors to add color to the world. With the development of agriculture around 7,000-2,000 B.C., people began to synthesize and use textiles, and would therefore color them as well (Xing *et al*., 2010). Organic natural colors have a huge history of application, especially as textile dyes. The first man made dye was discovered by William Henry Perkin, a student of Chemistry. He tried to make the drug quinine using aniline. The experiment resulted in the production of a thick dark sludge. Perkin diluted that sludge with alcohol and found a purple color, this solution was found able to be used to dye silk and other fabrics and it was water resistant and light resistant. This concept was soon followed by many

and there began an era or a kind of revolution in the history of dyes. Numerous new dyes began to appear in the market.

Textile Dyes

Synthetic dyes are extensively used in textile coloring, paper printing, color photoFigy, pharmaceutical, food, cosmetics, electronics, candle industry and many other industries (Rafi, Franklin and Cerniglia *et al*., 1990). Approximately, 10,000 different dyes are used industrially, and several thousand tons of synthetic dyes are produced annually. In 1991, the world production of dyes was estimated 6,68,000 tons, out of which azo dyes contributed 70% (ETAD *et al*., 1997). During dying process, a substantial amount of dye is lost in water effluent. Zollinger *et al* in 1987, reported that about 10-15% of dyes were lost in effluent after dyeing process. Major classes of synthetic dyes are azo, anthraquinon and triaryl-methane dyes, and many of them are toxic or even carcinogenic. With the increased use of a wide variety of dyes, pollution of wastewater is becoming increasingly alarming issue.

2.1.2 Classification of Dyes

All aromatic compounds absorb electromagnetic energy but only those that absorb light having wavelengths in the visible range are colored. Dyes contain chromophores (delocalized electron systems with conjugated double bonds) as well as auxochromes (electron-withdrawing or electron-donating substituents that intensify the color of the chromophore by altering the overall energy of the electron system). Common chromophores are -C=C-, -C=N-, -C=O, -N=N-, -NO2 and quinoid rings and common auxochromes are -NH3, -COOH, -SO3H and –OH (Zee *et al*., 2002).

Classes of synthetic dyes: Following classes have been reported according to wesenberg *et al.,* 2003):

Code	Chemical Class	Code	Chemical Class	Code	Chemical Class
10000	Nitroso	42000	Triarylmethane	53000	Sulfur
10300	Nitro	45000	Xanthene	55000	Lactone
11000	Monoazo	46000	Acridine	56000	Aminoketone
20000	Disazo	47000	Quinoline	57000	Hydroxyketone
30000	Trisazo	48000	Methine	58000	Anthraquinone
35000	Polyazo	49000	Thiazole	73000	Indigoid
37000	Azoic	49400	Indamine/Indophenol	74000	Phthalocyanine
40000	Stilbene	50000	Azine	75000	Natural
40800	Carotenoid	51000	Oxazine	76000	Oxidation Base
41000	Diphenylmethane	52000	Thaizine	77000	Inorganic

Table 2.1: Classes of synthetic dyes according to Color index (Anliker., & Moser *et al*., 1981)

2.1.3 Global market of dyes:

Major dyes manufacturing companies include Tronox Ltd (U.S.), CPS Color AG (Switzerland), Kiri Industries Ltd (India), Clariant International Ltd (Switzerland), BASF (Germany), Cabot Corp. (U.S.), DIC Corp. (Japan), E.I. Du Pont De Nemours & Co. (U.S.), Heubach GmbH (Germany), Huntsman Corporation (U.S.), Kronos Worldwide Inc. (U.S.), CRISTAL (Saudi Arabia), Rockwood Holdings Inc. (U.S.), Zhejiang Longsheng Group Co. Ltd (China) and Sudarshan Chemicals (India) etc. Recently, it has been seen that USA and Europe is mostly shifting all the dye manufacturing units to China, Taiwan and India, reason may be former are developed countries and they do not want to contaminate their water bodies, so the former are outsourcing the coloring operations from developing countries or the countries thriving for a better economy.

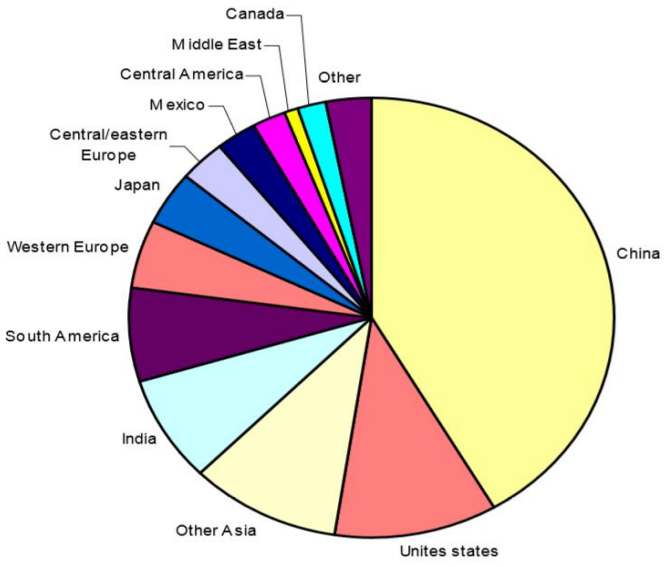

Fig 2.1: Cosmic consumption of dyes (Bhimani., & Singh, 2011)

The Indian dyeing industry, which was initially set up to meet the needs of domestic dyeing business, now caters to more than 95% of the domestic business, and also dumps substantial amounts of dyestuff in the global market. Today, Indian dye manufacturers convey dyes to many countries which used to export dye intermediates to us till a decade ago (Brown., & Hamburger et al., 1987). Gross range of disperse, acid, reactive, azo, triarylmethane and other dyes are now being produced in India.

2.2 Impact of Dyes used in textile industries:

The textile effluent is considered as one of the most polluting effluent among all other industries. The environmental and toxic health effects of textile effluent have been a subject of scientific research for a long time. The release of reactive dyes into the environment causes serious damage as they intensely affect the photosynthetic activity of hydrophytes by limiting the light penetration. Also the breakdown products of these dyes are toxic to some aquatic organisms (Wang *et al*., 2009). It also affects water bodies, ecosystem, soil fertility and plant growth and human beings. The azo dyes have come up with huge protesting voices due to their visible color, biorecalcitrance and toxicity to animals and humans (Senan and Abraham *et al*., 2004). The

incomplete microbial metabolic cleavage of azo linkages in the azo dyes results in free aromatic amines that are toxic, mutagenic or carcinogenic and cause serious health hazards.(He *et al*., 2004; Khadijah *et al*., 2009). The colored effluents that enter into the water bodies can also cause water- borne disorders such as nausea, ulceration of skin and mucous membrane, renal damage, cramps, dermatitis, haemorrhage, hypertension, severe allergies of respiratory tract or cancer.

In the year 1991, FDA gave approval to the use of 3,000 tons of azo dyes in Food, Drugs and Cosmetics. Some azo dyes cause liver nodules in laboratory animals. The dye workers are at higher risk of being affected by bladder cancer if exposed to large amounts of azo dyes. Benzidine based azo dyes are widely used by industries. In 1980, The National Institute for Occupational Safety and Health (NIOSH) in US published data on Benzidine based dyes on laboratory animals and epidemiological studies on dye workers. Benzidine is known to be associated with human urinary bladder cancer and is tumorigenic to many animals. Administration of benzidine dyes or their derivatives to experimental animals (rats and dogs) showed presence of carcinogenic aromatic amines and their N-acetylated derivative in their urine (Puvaneswari *et al*., 2006).

2.3 Need of degradation:

The developments in fashion industries have started exploiting increasing use of dyes. It is estimated that over 2, 80,000 tons of textile dyes are released as effluent annually worldwide. Therefore, pollution from this effluent contaminated with dyes is alarming to the society (Pandey *et al*., 2007; Jin *et al*., 2007). Paper industries, molasses based-alcohol distilleries, tanneries, dyeing units of textiles are some of the major industries that produce and release highly colored effluents. Each of these industrial effluents creates problems in environment. These colored effluent block the passage of light to the aquatic system resulting in reduction in the rate of photosynthesis, leading to anaerobic conditions, which results in the death of aquatic life causing bad smelling toxic water bodies(Mansour *et al*., 2007).

2.3 .1 Methods of degradation:

2.3.1 .1 Physical and chemical methods:

Industrial wastewater has been treated by various physico-chemical processes. These processes can be flocculation, membrane-filtration, ion exchange, irradiation, precipitation and adsorption using activated carbon or biological adsorption using bacteria, fungi, algae or plant biomass (Robinson *et al*.,2001).

2.3.1.2 Demerits of using physical and chemical methods:

These chemical or physico-chemical treatment methods are inefficient, expensive, have limited applicability, these cannot be applied to a large scale effluent treatment process (Banat *et al*., 1996; Verma *et al*., 2003; Zhang *et al*., 2004). The effluent treatment process cannot decolorize the azo dyes, because of their strong electron-withdrawing group, which protects them against attack by the oxygenases (Nigam *et al*., 1996). Moreover, it is like using more chemicals to treat released chemicals, so again it is not eco-friendly.

2.3.2 Other methods of dye degradation:

Detoxification of textile effluent has also been reported using apple pomace and wheat straw by Robinson *et al* in 2001. Rate of absorption of dyes was higher in apple pomace than wheat straw. This method is cheaper one and use locally available raw material but the flaw with this method is it is not applicable to a large scale bioremediation process.

Table 2.2: Methods of dye degradation other than biological methods. (Robinson *et al*., 2002)

Physical/chemical methods	Advantages	Disadvantages
Activated carbon	Sufficient removal of dyes	High cost involved
Photochemical	Less amount of sludge	By products are harmful
Membrane filteration	Effective for all types of dyes	High quantity of sludge
Ion exchange	Absorbent can be used repeatedly	Not applicable for all types of dyes
Irradiation	Oxidation at lab scale is possible	Requires high quantity of dissolved oxygen
Electrokinetic coagulation	Economically feasible	High quantity of sludge

2.3.4 Alternative to these methods:

The alternative is Bioremediation as it is cheaper, less chemicals used so is eco-friendly (Robinson *et al*., 2001; Chen *et al*., 2003). Much of the research work has been done for the bioremediation of certain dyes and many fungal and bacterial species, including gram-positive and gram-negative have been reported to be capable of degrading textile dyes (Kodam *et al*., 2005). Bioremediation can be achieved using naturally occurring or genetically modified organisms. This bioremediation has to be safe and comparatively cheaper than treatments employed earlier. Many bacteria and their enzymes are capable of degrading these colored products (Liao *et al*., 2001).

2.4 Microbial degradation of dyes:

Albeit a few physicochemical measures have delineated effective dye removal, their appositeness is restricted on the grounds that plethora of toxic chemicals were used and immense expenditure for installation and operating parameters were incurred (Robinson *et al*., 2001). With heightened federal acknowledgement of biotechnological measures, many researchers attempted to degrade stubborn dyes using biological methods like microbial cells, competency of which depends upon process design, operational cost reduction and optimization of parameters (Georgiou *et al*.,

2006). A research was carried out by Khehra *et al*., 2005, to isolate micro-organism capable of degrading textile dye Brown 3 REL. Microorganisms were isolated from soil contaminated with textile effluent. Then, those microorganisms were incubated in presence of different dyes on solid media and decolorization zones were observed. Colonies with maximum decolorization zones were picked up and further cultured to obtain pure cultures. After pure cultures were obtained, 16s RNA sequencing revealed that it was Bacillus sp. VUS. This bacterium has the ability to degrade 100% of Brown 3REL dye within 8 hours of incubation. Optimum temperature and pH was found to be 40º C and 6.5-12.0. Enzymes involved in degradation activity were lignin peroxidase and laccase. Then results were analysed by UV-VIS, FTIR and HPLC. The final product was 6, 8-dichloro-quinazoline-4-ol and cyclopentanone and was analysed by GC. This strain Bacillus sp. VUS decolorized 16 textile dyes. Another research was carried out by Khan *et al*, in which he utilized halotolerant microbe Bacillus megaterium (0.5-2.5M NaCl tolerant) to degrade dye. 64.89% degradation was reported by him. Enzyme azoreductase responsible for this degradation was also isolated and estimated by Lowry method and from standard Fig; concentration of the isolated enzyme was interpolated. Enzyme was run on SDS PAGE to know the molecular weight and was purified with ion exchange chromatoFigy. Total activity of the enzyme that was purified with ion exchange chromatoFigy was 36.03U and molecular weight was 65 KDa.

2.5 Azo dyes:

Azo dyes are most commonly used dyes in industries. So, we are discussing about these separately. Azo dyes are aromatic compounds with one or more azo linkages (R_1-N=N-R_2). Monoazo dyes have single N=N group, whereas diazo have two N=N bonds. Azo dyes are made up of aromatic amines. Azo group, N=N is generally attached to benzene and naphthalene rings. This reactive azo group is responsible for imparting color to the dye, color being vary in intensity (Zollinger *et al*., 1991). Several Textile industries worldwide mainly use azo dyes. According to Brown, D *et al*., 1981, azo dyes constitute more than 50% of the dyes used in industries. Azo dyes contaminate the ground water and rivers. Out of different types of dyes, azo dyes are more widely used in textile industries worldwide. These dyes are resistant to degradation as they are resistant to light, temperature conditions so remain in the environment for a long time.(Xu *et al*.,

2006). The toxicity of these azo dyes is a serious environmental concern as these are found to be carcinogenic and mutagenic as well (Dong *et al*., 2003, Wang *et al*., 2009). Substituted benzene and naphthalene rings of azo dye have been found to be carcinogenic (IARC, 1982). Further, azo dyes themselves are not carcinogenic significantly but their metabolites are carcinogenic (Growther *et al*., 2009). It has been postulated by Brown and DeVito in 2003 that:

- Azo dyes may be toxic only when reduced and cleaved at the azo linkage, and thereby producing aromatic amines.
- Azo dyes containing free aromatic amine groups that can be metabolically oxidized may cause toxicity.

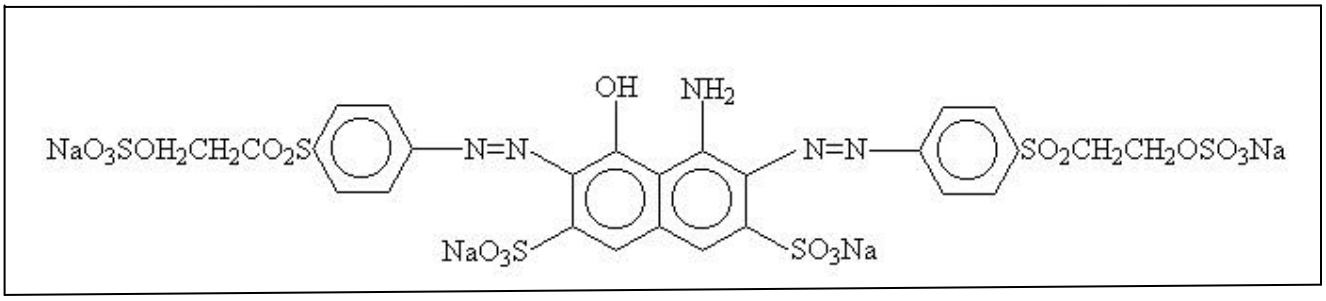

Fig 2.5.1: Structure of Remazol black

2.5.1 Degradation of azo dyes:

A research was carried out on biodegradation and detoxification of Rubine GFL which is an azo dye by Lade; waghmode; Kadam; Govindwar in 2012. They isolated a fungi Aspergillus ochraceus and a bacterium Pseudomonas sp. They checked the decolorization by individual strain as well as in consortium. Consortium was found to have 95% decolorization efficiency. A. ochraceus individually showed only 46% decolorization efficiency. Pseudomonas was found to be 63% efficient in decolorization.

Decolorization was calculated by following formula:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

After the incubation period, fungus was removed by filtration and bacterium was removed by centrifugation at 7500 rpm for 15 minutes, whereas consortium was removed by filtration followed by centrifugation. Supernatant was used to study all the metabolites by HPLC, GC-MS and FTIR. Degradation of azo dye Rubine GFL was confirmed by HPLC, FTIR and GC.

HPTLC analysis of culture obtained after incubation with consortium of fungus and bacterium showed the absence of control dye band which means complete degradation, whereas it was present in cultures incubated with *A. ochraceus* and *Pseudomonas sp*. Individually. That indicated its partial dye degradation when incubated individually.

Enzyme activites was studied using filtrate of fungus and supernatant of bacterium. For this, the individual cultures and consortium was separately suspended in 50 mM potassium phosphate buffer (pH 7.4), homogenized and sonicated at 4ºC. The sonicated cultures were centrifuged in cold condition and resulting supernatant was used as the source to check the activity of intracellular enzymes. Activities of dye degrading enzymes such as laccase, veratryl alcohol oxidase, tyrosinase and azoreductase were also assayed spectrophotometrically. The results of toxicity studies also suggested that this consortium of fungus and bacterium can be effectively used for complete detoxification of dye.

Another research was done by Nigam *et al*, (1996), wherein, he added 0.5% W/V soil to the mixture of nine different dyes(2g l^{-1}) and medium. Composition of media used was (g l^{-1}): $(NH_4)_2SO_4$; 0.5, KH_2PO_4; 2.66, $Na_2HPO_4.2H_2O$; 4.32, Yeast Extract; 5.0, pH 7.0. *Alcaligenes faecalis* and *Commamonas acidivorans* strains were isolated that were capable of degrading azo dyes. *Streptomyces chromofuscus* was also reported to degrade dyes. Only exception was Remazol Turqoise Blue that was not degraded out of nine dyes because of presence of copper in the aromatic structure. One research on determining the rate of microbial reduction was carried out by Wuhrmann *et al* in1980. Degradation was found to follow first order kinetics. Permeation of dyes in the cell wall was the rate determining step. Reduction was an intracellular, non enzymatic process.

$$R_1\text{-}N{=}N\text{-}R_2 + 4e^- + 4H^+ \text{ ---------- } R_1\text{-}NH_2 + R_2\text{-}NH_2$$

Amino moieties produced after the reduction of azo dyes have found to be carcinogenic. Dyes present in foodstuff are reduced inside the human because of reducing environment in the liver cells. Whereas in anaerobic microorganisms, these are the intestinal cells, where the azo dyes get reduced. The azo dyes are characterized by the presence of a sulfonic acid group in their aromatic structure. This sulfonic acid group is responsible for resistance to the microbial attack.

2.5.2 Mechanism of azo dye degradation:

The primordial step in the degradation of azo dyes, in both aerobic and anaerobic ambience, is the reduction of the –N=N– bond. This reduction can follow different mechanisms, it can be enzyme mediated, low molecular weight redox mediated or it can be chemical reduction by reductants such as sulphide (Fig 2.5.). Also, these reduction reactions can be intracellular or extracellular. Direct enzyme mediated azo dye reduction involves enzyme intervened transfer of reducing agents, spawned from the oxidation of the substrate or coenzyme to azo dyes. These enzymes involved may be specific or nonspecific. Nonspecific ones catalyze the reduction of a vast range of substrates. Owing to their nonspecific nature, these enzymes spontaneously reduce azo dyes.

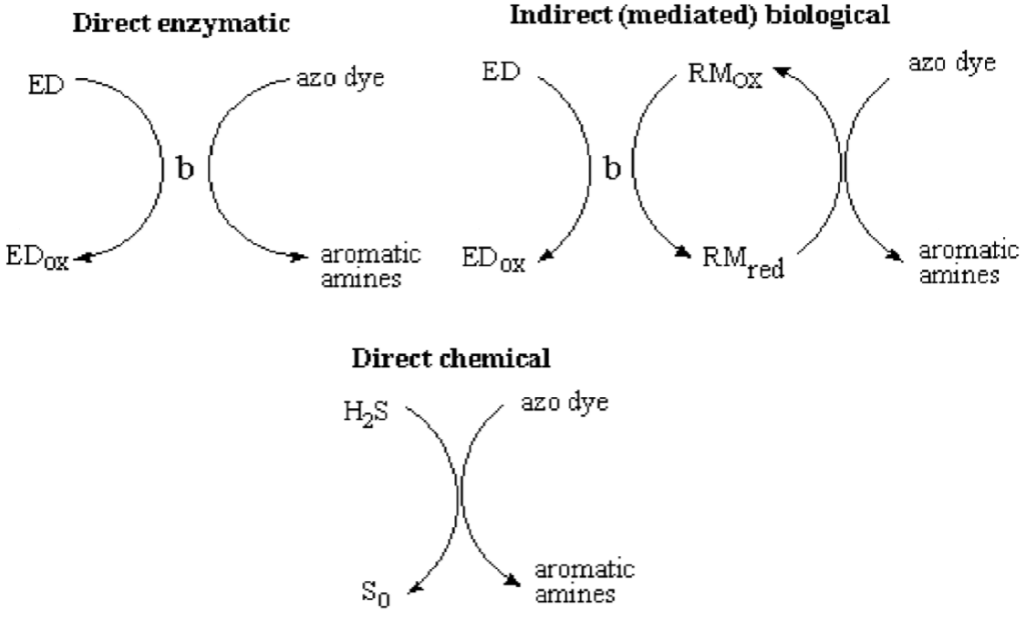

Fig 2.5. Diverse Approaches of azo dye reduction.
Where RM is Redox mediator, ED is electron donor and b is bacteria. (Pandey *et al*., 2007)

2.5.3 Degradation of azo dyes by bacterial peroxidases

It has been discovered that some peroxidase producing bacterial strains like Streptomyces species decolorize azo dyes (Cao *et al*. 1993; Paszczynski *et al*. 1992). The oxidation of azo dyes by *Streptomyces chromofuscus* A11 involves an peroxidase that is present in extracellular fluid, shows a restricted substrate specificity.

2.5.3 Aerobic growth of bacteria with azo dyes as sole source of carbon and energy

There are several claims in the literature that bacteria having the ability to reduce azo dyes aerobically can use these dyes as sole source of carbon and energy (Dykes *et al*., 1994). The ability of bacteria to grow with azo compounds as sole source of carbon and energy was first shown by Stolz (1979), who isolated a *Flavobacterium* that was able to grow aerobically with the compound 4,4′-dicarboxyazobenzene. In a later study it was demonstrated that a wide range of bacterial strains could be readily isolated from different inocula. These strains were found to belong to different genera, such as *Sphingomonas, Comamonas, Pseudomonas, Xanthomonas, or Alcaligenes.*

2.6 Factors affecting degradation

2.6.1 Temperature, pH, biomass concentration, dye concentration:

In a research carried out by Sinoy *et al*.,2011, delecolorization pattern of Malachite Green, Crystal Violet, Carbol Fuchsin, Brilliant Green by *Pseudomonas sp.* and *E.coli* was studied at different pH, temperature, inoculums size and initial dye concentration. They found that maximum decolorization was achieved at pH 7.0, temperature 37°C, Inoculum concentration of 20% W/V and initial dye concentration of 50 ppm. So, these parameters or factors need to be taken care of.

2.6.2 Role of salts in process of dyeing:

The fabric to be dyed and the dye itself, not necessarily have the compatible characteristics to combine with each other. So, we need to add a catalyst that help in fixing the dye with the fabric, this catalyst is “salt”. As the water retention capacities of salts are high, it acts as electrolyte for the reactions occurring in dyeing processes which leads to fixation of color to the cellulosic fabric. Glauber’s salt or common salt can be used but presence of chlorine in common salt may result in corrosion of the equipment, so Glauber’s salt (sodium sulfate decahydrate, $Na_2SO_4.10H_2O$) is much more commonly used salt in textile industries than common salt.

CHAPTER 3

RATIONALE AND SCOPE OF STUDY

Dyeing is an indispensable process, we are surrounded by different colors all around us. We cannot stop using colored products. So, when it comes to worldwide production of dyes, one can just imagine the amount being released in the environment. This study has been undertaken to check the newer possibilities of dye degradation which has been an alarming issue for the environmental well being worldwide. Bacterial degradation of textile dyes have been studied by many researchers till now (Pandey *et al*.,2007). In this work, we isolated few isolates of bacteria which will be able to degrade the most commonly used dyes at a large scale. These bacteria can utilize textile dye effluent as their energy source. Dyes have been found to be carcinogenic and harmful to the water dwelling orgainsms. On these grounds, much of the research work has been carried out worldwide. Here, bacteria mediated cleavage of toxic azo bonds are reported, we can recommend this microbial degradation of dye effluent before the discharge of effluent from the industry into surrounding waterbodies.

OBJECTIVES

Our objective for this study is to find a remedy for the dyes which we are using in almost every industry.

I. Isolate bacteria capable of degrading commonly used textile azo dyes.

II. Checking the decolorizing efficiency of microorganisms on various dyes.

III. Spectrophotometric analysis of degraded samples.

IV. GC-MS, FTIR analysis of pure dye solution(control) and decolorized cultures.

CHAPTER 4

MATERIALS AND RESEARCH METHODOLOGY

4.1 Materials: Following materials were used for the project work. Dyes, Scarlet RR, Disperse blue, Disperse orange were obtained from Jai Ambey Textiles, and are manufactured by Colortex dye stuff company, Surat. Nutrient agar (Hi Media), Nutrient broth (Hi Media), Silica Gel, Ferric chloride, Ethyl Acetate.

4.1.1 Sampling collection: Effluent water samples were taken from "Jai ambey textile mill" at Amritsar, Punjab, India. Soil samples were taken nearby this textile mill. These samples were immediately put into ice until taking into laboratory. In laboratory, samples were stored in refrigerator.

4.1.2 Dyes: Three dyes were obtained from the same textile mill. These two dyes are the most commonly used dyes by this mill. These three dyes were: Scarlet RR, Disperse blue and Disperse Orange. Scarlet RR is a type of azo dye, which has been reported to be carcinogenic by many of the research workers (Blanquez *et al.,* 2008). Disperse dyes are one kind of dyes that are used to dye polyester and acetate fabric and are water insoluble. They have the smallest molecular size among all the dyes currently being used. Disperse dyes can be azobenzene or anthraquinone based dyes. Disperse orange is a type of azobenzene dye containing 25% of dye by weight and 75% salts like NaCl etc.

4.2 Isolation of bacteria from samples:

The bacterial isolates were withdrawn from textile effluent facility of a local dyeing house. Culture media used was nutrient agar. Serial dilution was performed from 10^{-1} to 10^{-6} dilutions. From each of these dilutions, 100 microlitres of waste water sample was spread on different petri-plates labeled with the exact dilution. Colonies obtained were subcultured on fresh nutrient agar plates, single streak was done (Fang *et al.,* 2004).

4.2.1 Culturing and maintenance of cultures:

Bacterial colonies obtained on agar plates were then subcultured to a fresh nutrient agar medium to obtain pure colonies.

4.3 Decolorization analysis:

4.3.1 Spectrophotometer analysis:

Bacterial culture was prepared by inoculating colony (obtained on agar plates) in nutrient broth. This culture was kept in shaker rotating at 200 rpm for 24 hours. Then, 1ml of this suspension culture was taken and added to a fresh medium containing dye which is to be decolorized. Then at the intervals of 24, 48, 72, 96 hours, culture was withdrawn and centrifuged at 10,000 rpm for 15-20 minutes. 4 ml of the supernatant was used to measure the decolorization.

Decolorization was calculated by following formula (Saratale *et al*., 2009):

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

4.4 Degradation analysis:

4.4.1 FTIR analysis:

FTIR analysis was carried out for the treated samples, to check whether harmful functional groups have been removed or not. Comparative analysis of dyes before and after incubation in presence of bacteria can also be done (Asad *et al*., 2006). For this, we centrifuged the control and degraded dye samples at 5000 rpm for 30 minutes. Extraction was done by adding equal amount of ethyl acetate in supernatant. Samples dried over Na_2SO_4 and kept at 60°C in oven. Spectrum was compared in the mid-IR region from 500 to 4000 cm^{-1}.

4.4.2 GC-MS analysis:

Centrifugation of samples at 5000 rpm for 30 minutes and use of supernatant to extract the degraded metabolites with an equal volume of solvent ethyl acetate. And then samples were dried using Na_2SO_4. GC-MS analysis was done by adding HPLC grade methanol to the dried samples (Kurade *et al.,* 2011). The chromatoFigy column was VF-5 ms, 30 m long and 0.250 mm internal diameter with a film thickness of 0.25 mm and oven temperature varied from 70 to 300 °C with an accrual of 10 °C per minute and injection temperature was 240 °C. M/Z ratio of range 10-610 was used for MS analysis (Karunya *et al*., 2014).

4.5 Decolorization experiment and optimizing physicochemical parameters:

Decolorization of azo dyes Disperse Orange, Disperse Blue, Scarlet RR which are thought to be recalcitrant, was observed using UV-Vis Spectrophotometer at different physicochemical conditions. There is a pronounced effect of pH on decolorization reaction because transport of dye molecule into microbial cell is pH dependent and considered as the rate limiting step for decolorization of dyes (Lourenco *et al*., 2000).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Isolation of bacterium

It has been proclaimed repeatedly that bacteria dwell in textile effluents to utilize its ingredients for their survival. Similarly, the textile dye effluent examined in this work was found to accomodate an assorted turf of microbes. These bacteria are native inhabitants of the textile effluent as the latter serves as their supply of essential nutrients. This finding is in compliance with certain previous studies (Mihir *et al*., 2006). Isolation of bacteria from soil samples collected from Jai ambey textiles situated in Amritsar district of Punjab was done. It yielded no growth of any microorganism, even in the 10^{-1} dilution. The reason may be that the soil sample was taken from the closest vicinity to textile industry, and that too was the site of release of the dye effluent by the textile industry. So, the dye concentration was above the maximum tolerance by bacteria. Again soil samples from few kilometres to this industry were taken and we were finally able to isolate few bacterial isolates. Then after testing for decolorization efficiency, we selected two isolates(corresponding to maximum decolorization).

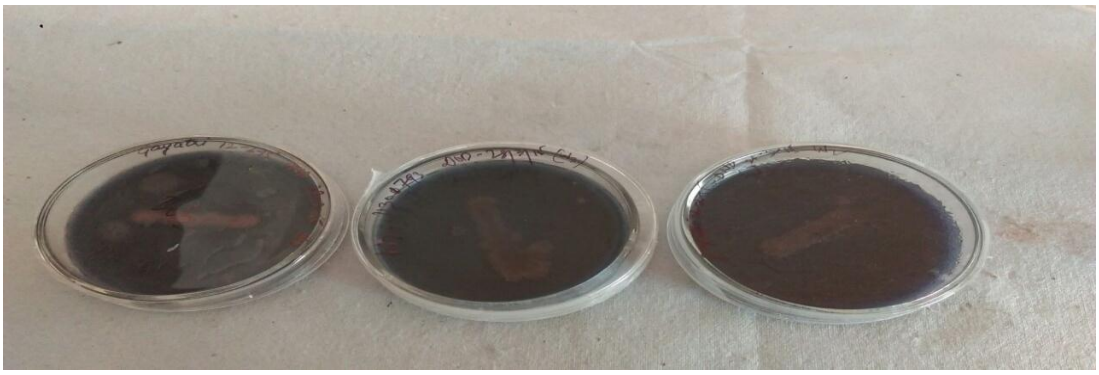

Fig 5.1.1: Bacterial Isolates (Y1)

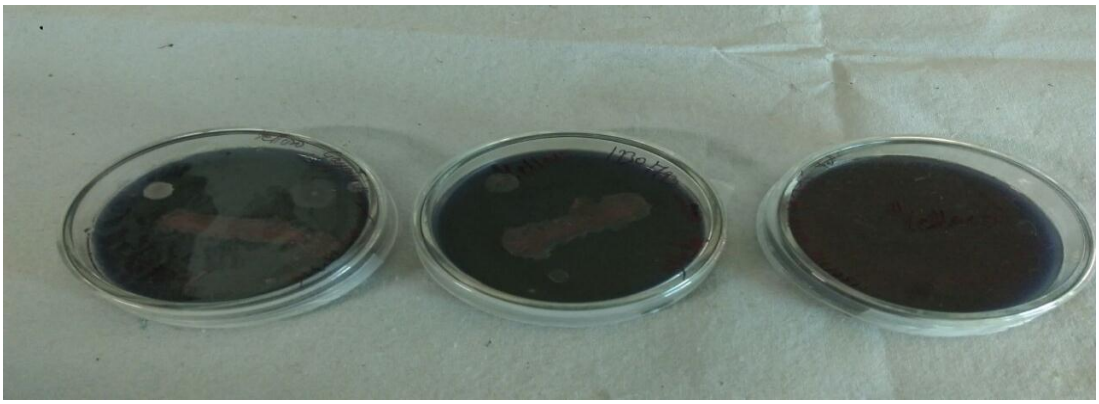

Fig 5.1.2: Bacterial Isolates (W2)

5.2 Decolorization studies:

Decolorization studies were carried out by noting down the absorbance value for the samples after inoculation with the bacterium using spectrophotometer readings. Media used was nutrient broth, pH was 7 and temperature used was 37 °C at static condition (no agitation provided). Scarlet RR was decolorized to light pink color, color variation was seen after 2 days of incubation. Disperse orange turned into light orange and disperse blue turned almost colorless after 12 days of incubation time. Similar results have been reported earlier by Barragan., et al in 2007. They proposed that dye degradation follows second order reaction kinetics.

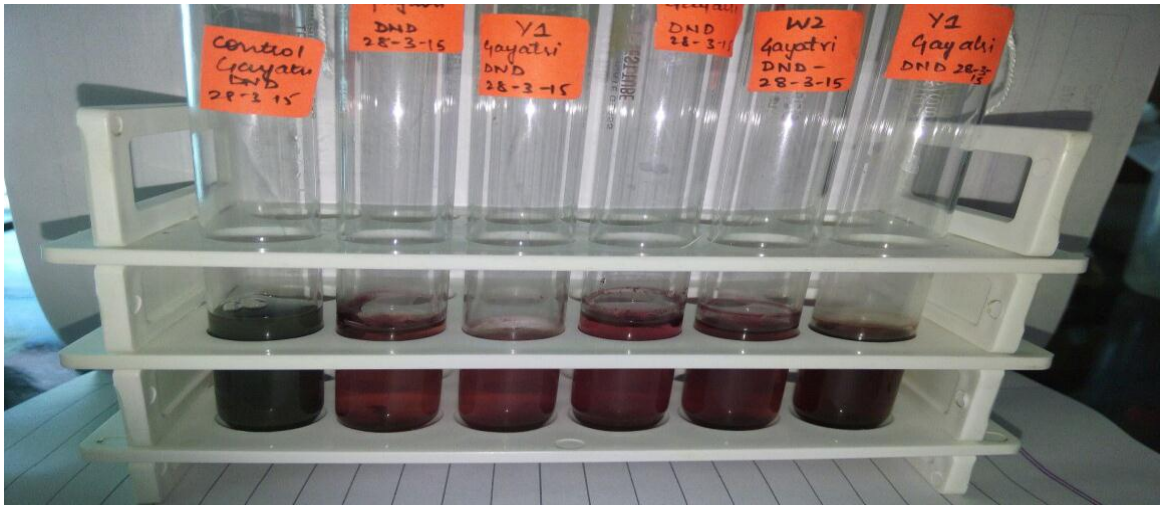

Fig 5.2.1: Decolorization of Scarlet RR

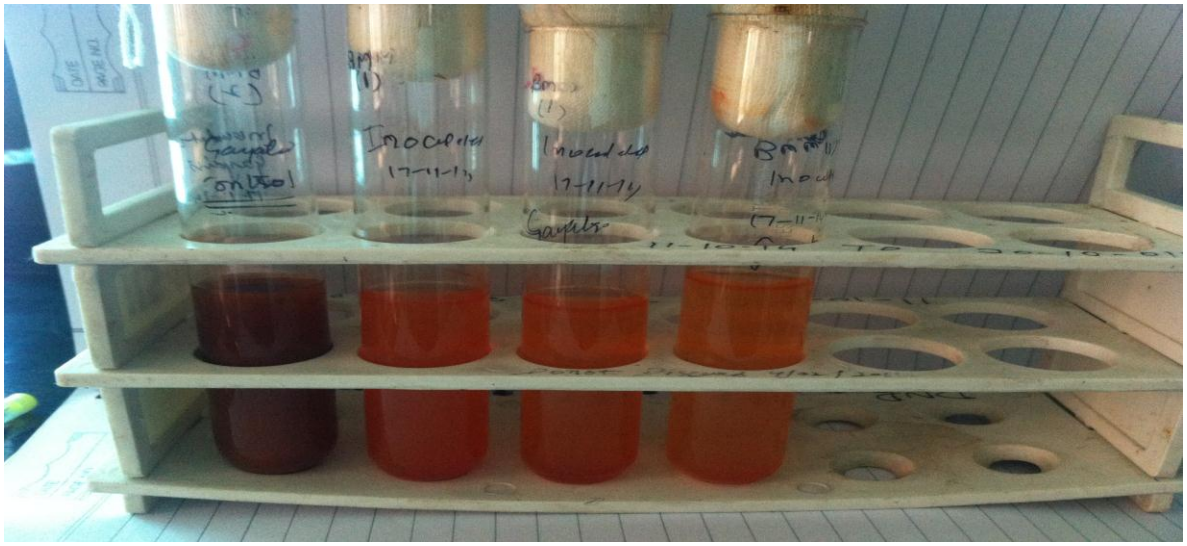

Fig 5.2.2: Decolorization of Disperse orange

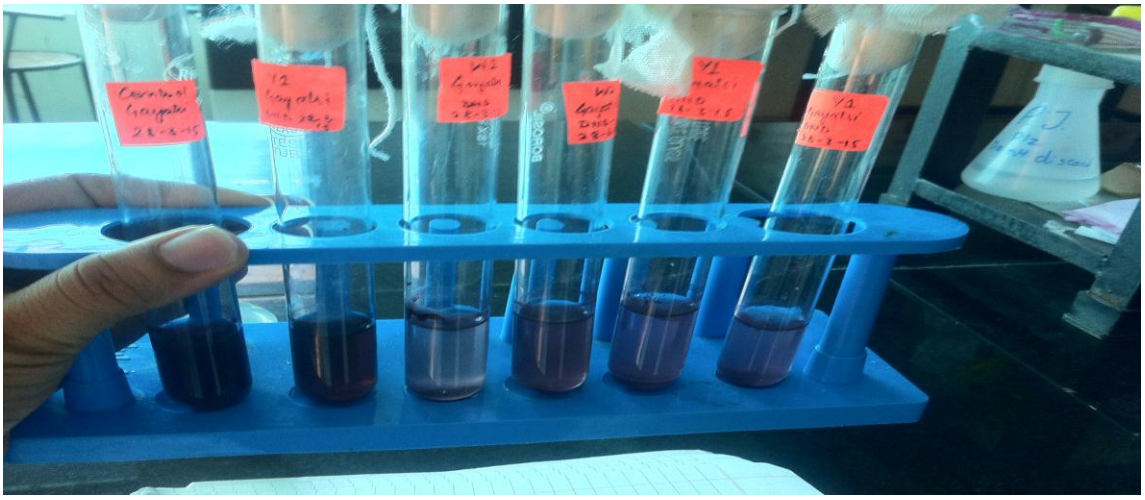

Fig 5.2.3: Degradation of Disperse blue.

5.3 Decolorization experiment and optimizing physicochemical parameters: Decolorization studies were done under different physicochemical culture conditions by changing one parameter at a time. Effect of pH was studied over decolorization percentage. Decolorization increased above pH 5 and decreased below pH 5 in most of the cases. All experiments were done in triplicates along with the uninoculated control. Decolorization and degradation was achieved at a wide range of pH from 3 to 10.

Following graphs (Fig 5.6.1 and 5.6.2) were obtained when decolorization was studied from pH 3 to 10. It was found that pH 3 was the most unacceptable pH for decolorization of dyes. Maximum decolorization was observed at pH 7 and temperature 37 °C by W2 isolate. Decolorization in both the isolates follows polynomial trends when plotted against pH.

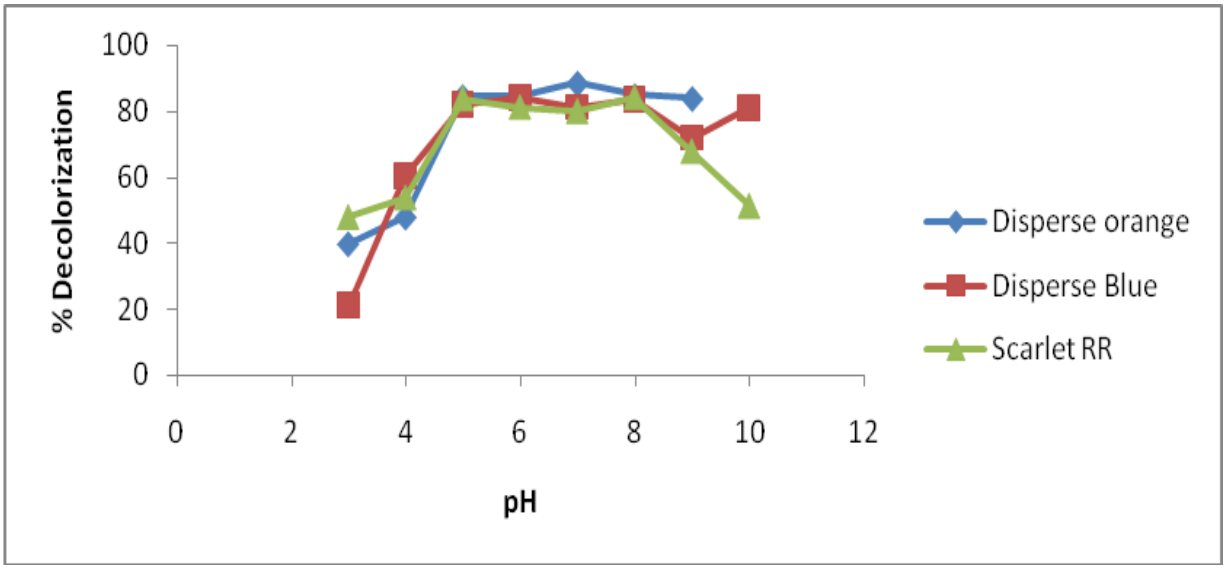

Fig 5.3.1 Effect of pH on decolorization (Y1 Isolate)

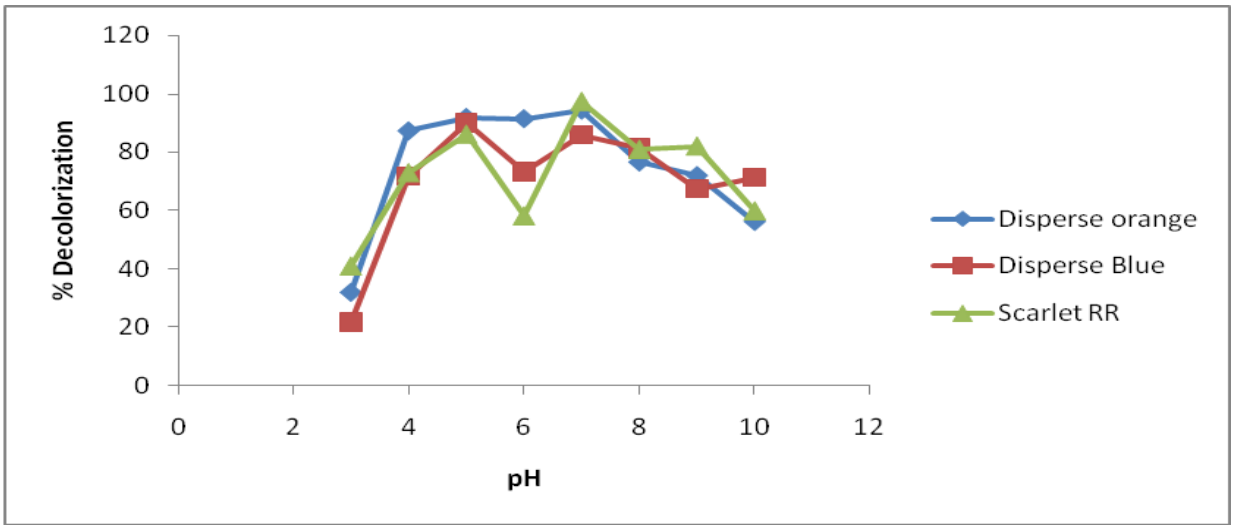

Fig 5.3.2 Effect of pH on decolorization (W2 Isolate)

5.4. Spectrophotometric analysis:

The isolated strains Y1 and W2 grew in the medium having 500 mg/l of dyes. Decolorized medium was taken and centrifuged at 5000 rpm for 20 minutes and supernatant was used to measure OD. We obtained maximum decolorization of Scarlet RR dye by W2 isolate at pH 7 and temperature 37 °C. Marked decrease in the absorbance of degraded samples was observed at ƛmax of three dyes. This can be an outcome of cleavage of azo bond. Change in absorbance readings for all three dyes indicates a conspicuous change in the structure these dyes.

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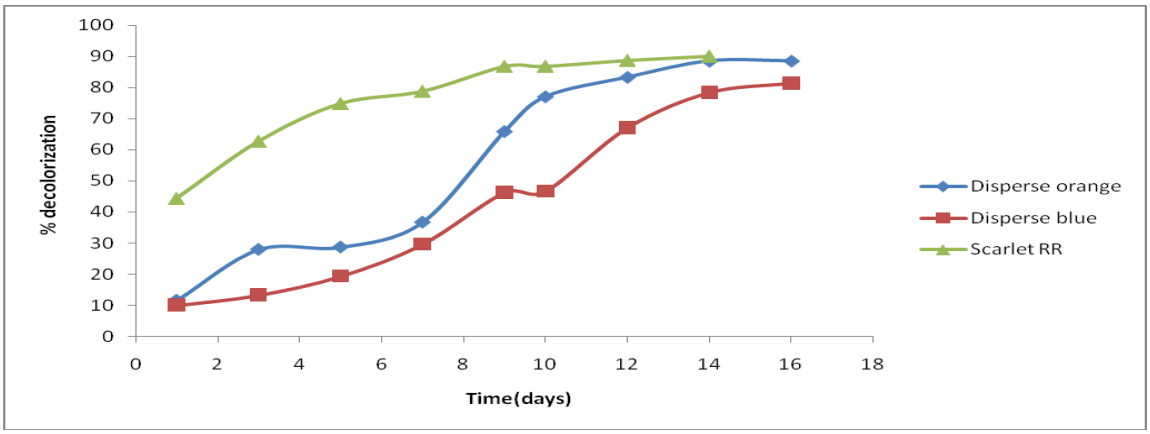

Fig 5.4.1. Decolorization by Y1 isolate at pH 7

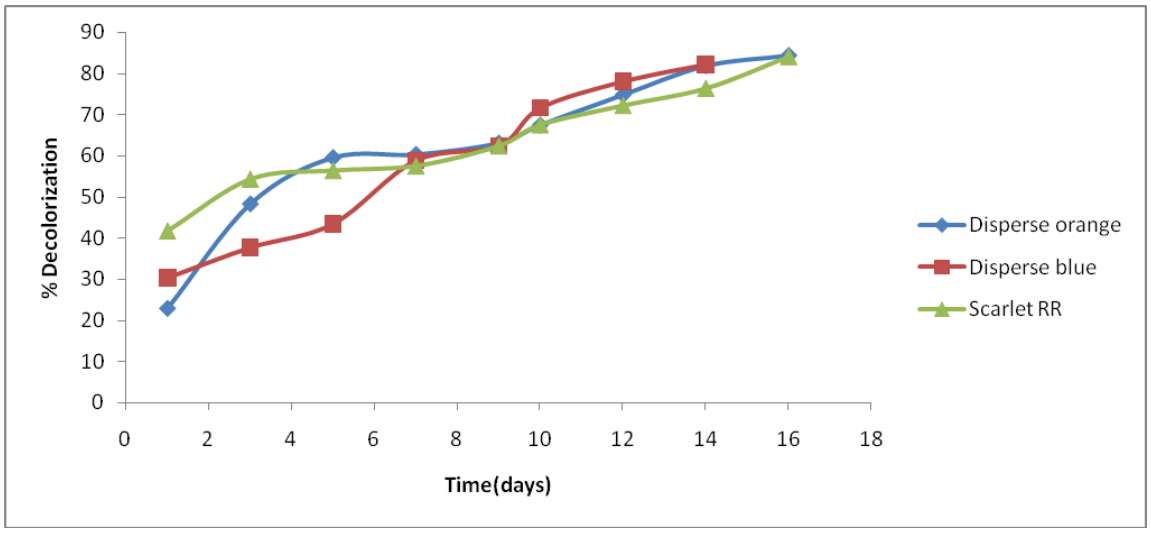

Fig 5.4.2. Decolorization by Y1 isolate at pH 5

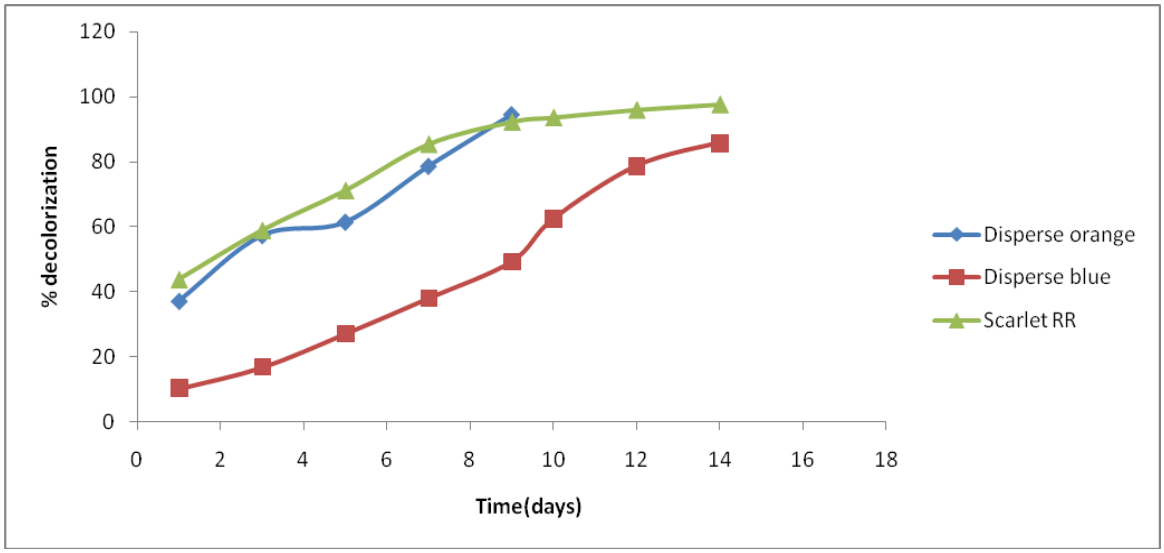

Fig 5.4.3. Decolorization by W2 isolate at pH 7

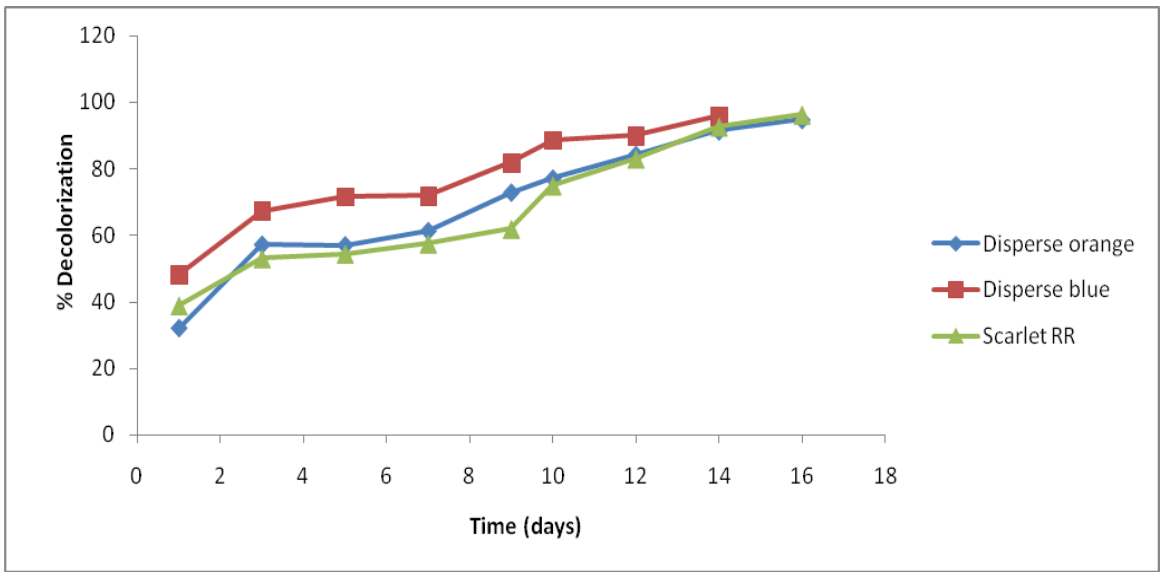

Fig 5.3.4 Decolorization by W2 isolate at pH 5

5.5 FTIR analysis:

FTIR spectrum of both the control and degraded samples were compared. FTIR results confirmed the biodegradation of Scarlet RR by Y1 isolate. O-H Stretching, C-N stretching, N-H stretching, C-C stretching and –C ≡ C–H: C–H bend appeared at 3450.77, 1932.74, 1645.33, 1614.47, 1437.02, 1215.19, 1138.04, 630.74, 495.72 cm-1. Greater number of peaks was found to shift in the metabolized samples. Intensity of peaks was greatly reduced in the degraded metabolites(Fig. 5.5.2). Similar results have been produced by Patel *et al* in 2012. They reported metabolization of Acid Maroon dye by bacterial consortium EDPA. FTIR results indicated the wreckage of azo bond and main chromophore was destroyed.

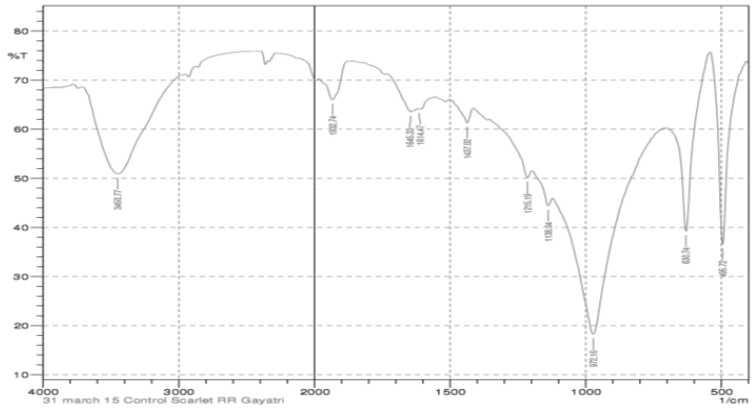

Fig 5.5.1: FTIR spectra of Scarlet RR (control)

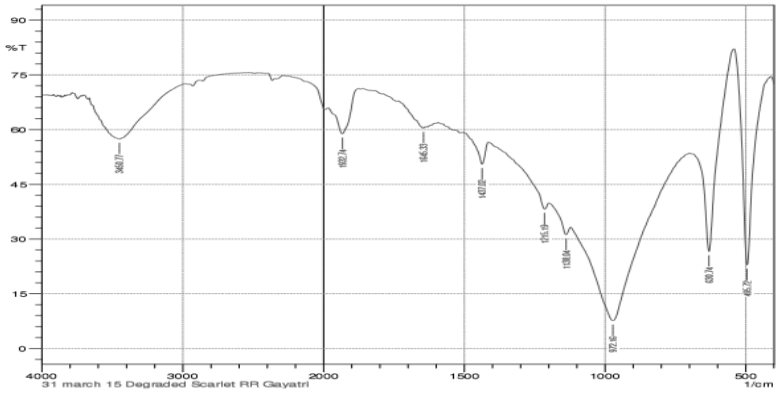

Fig 5.5.2: FTIR spectra of Scarlet RR (degraded)

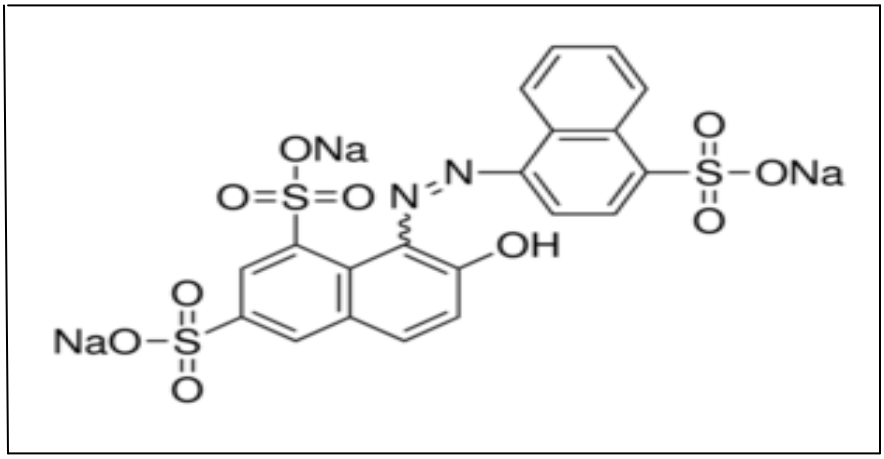

Fig 5.5.3: Structure of Scarlet RR dye

5.6. GC-MS analysis:

Further confirmation of biodegradation was done by GC-MS analysis. Mass spectra of control and degraded samples of Scarlet RR are shown in fig 5.6.1 and 2. We used GC-MS of agilent technologies for the recognition of degraded metabolites. Different mass spectra were obtained when control and degraded samples were analysed in GC-MS. M/Z ratio obtained were significantly different in control and degraded samples. Peaks at 503, 399, 415 were completely eradicated in case of degraded scarlet RR dye. This again shows complete eradication of toxic azo bond. This result is in compliance with the FTIR results obtained for Scarlet RR dye.

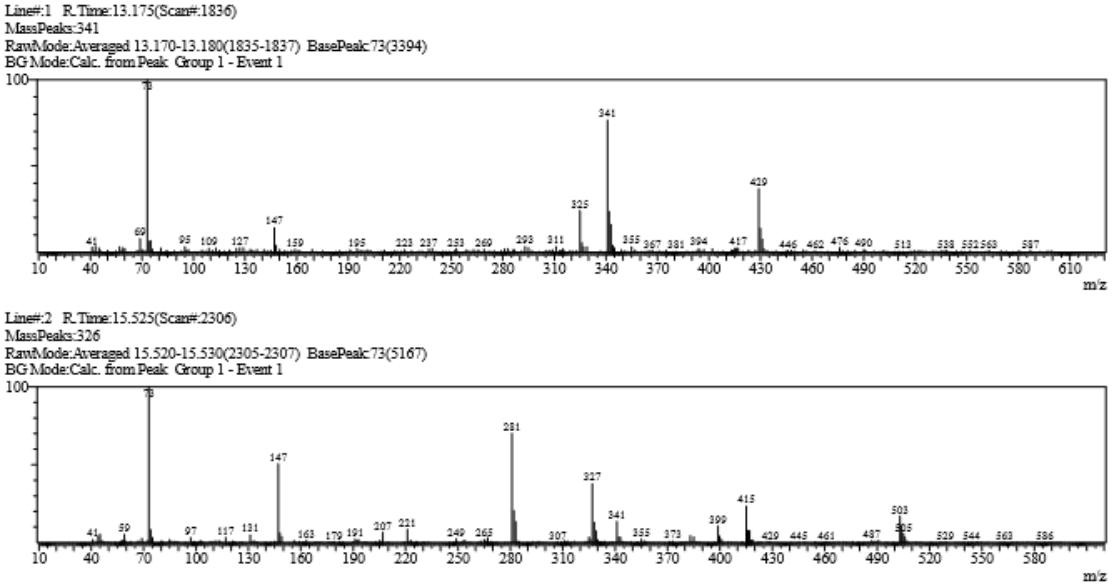

Fig 5.6.1 Mass spectra of Scarlet RR (control)

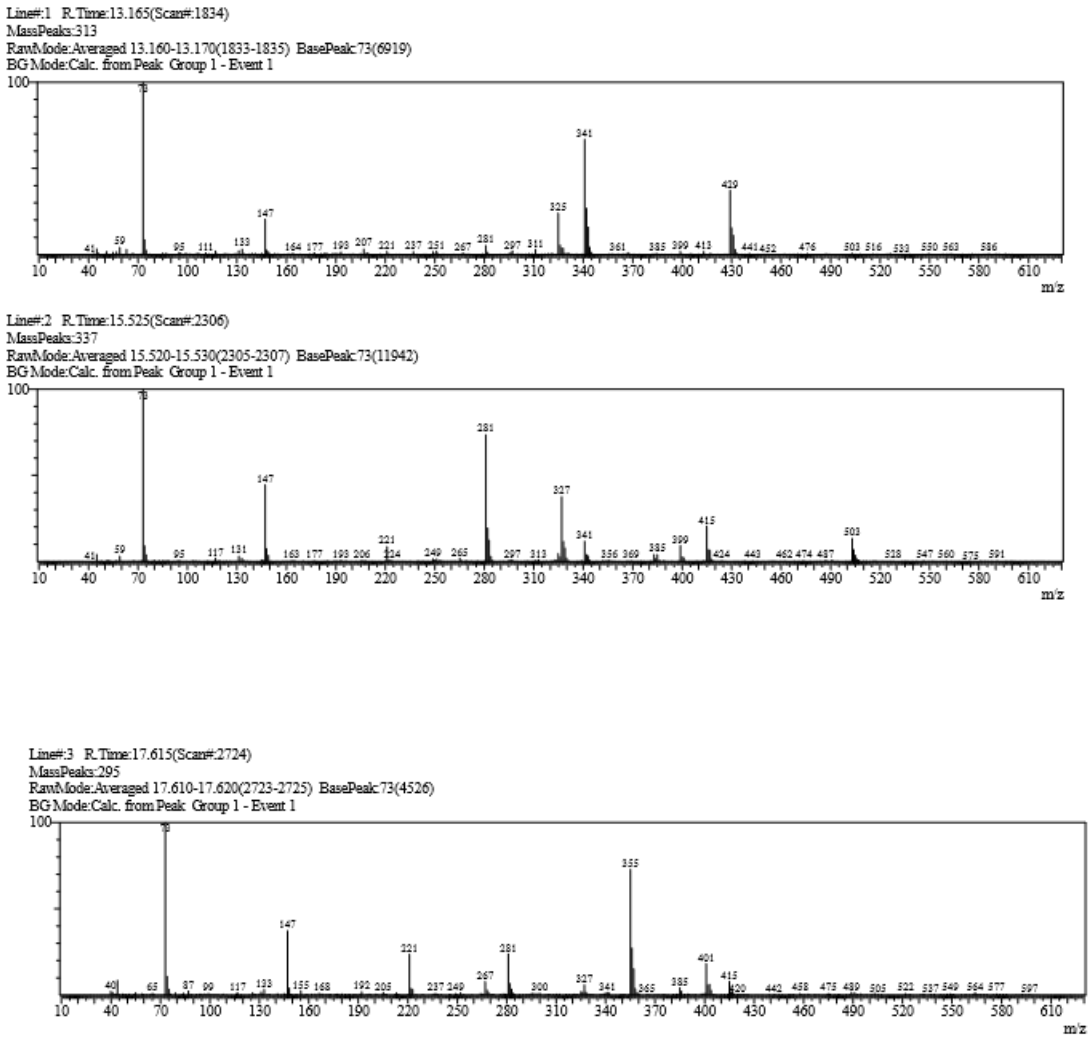

Fig 5.6.2 Mass spectra of Scarlet RR (Degraded)

CHAPTER 6

CONCLUSION AND FUTURE SCOPE

Industrial dye effluent and sludge generated by effluent treatment plant is loaded with dye degrading and decolorizing microbial populace. Two plausible isolates were selected on the basis of ability to decolorize dyes and were inoculated in the presence of Disperse Orange, Disperse blue and Scarlett RR dyes. Massive idiosyncrasies in chemical structures of three dyes led to the fluctuation in % decolorization of these three different dyes by same isolates even. At static conditions, successful decolorization was achieved and decolorization percentage varied from 81% to 97%. Maximum decolorization was observed at pH 7 and temperature 37 °C. But, one thing was observed that prolonged incubation time is required for complete degradation. Spectrophotometric analysis done after 5 days did not yielded the complete degradation, so with the prolonged incubation, newly formed metabolites were also degraded. Biodegradation of dyes was confirmed by FTIR, GC-MS analysis. FTIR results confirmed the biodegradation of Scarlet RR by Y1 isolate. O-H Stretching, C-N stretching, N-H stretching, C-C stretching and –C ≡ C– H: C–H bend appeared at 3450.77, 1932.74, 1645.33, 1614.47, 1437.02, 1215.19, 1138.04, 630.74, 495.72 $cm^{-}1$. Greater number of peaks was found to shift in the metabolized samples. Intensity of peaks was greatly reduced in the degraded metabolites. Similar results have been produced by Patel *et al* in 2012. They reported metabolization of Acid Maroon dye by bacterial consortium EDPA. FTIR results indicated the wreckage of azo bond and main chromophore was destroyed. Further confirmation of biodegradation was done by GC-MS analysis. Mass spectra of control and degraded samples of Scarlet RR are shown in fig 5.6.1 and 2. We used GC-MS of agilent technologies for the recognition of degraded metabolites. Different mass spectra were obtained when control and degraded samples were analysed in GC-MS. M/Z ratio obtained were significantly different in control and degraded samples. Peaks at 503, 399, 415 were completely eradicated in case of degraded scarlet RR dye. According to a literature by lade et al., 2012, they also found certain metabolites by comparing the m/z ratios. Similar results have been reported earlier by many of the research workers. We can recommend further research to develop a customized alternative treatment for textile dye degradation. This can resolve leading problems touching contamination of the water bodies because of textile effluent discharge.

CHAPTER 7

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CHAPTER 8
APPENDIX

8.1 Equipments used for the project were as follows:

Material/Equipment	**Company**
Autoclave	Nsw pvt ltd India
Glass ware	Borosil glass
Hot air oven	Nsw pvt ltd. India
Incubator	Yorco incubator bacteriological
Microwave	Inalsa
Microtips	Tarson
Micropippetes	P' fact A
Laminar air flow	Rescholar equipment
Refrigerator	LG
UV-Vis Spectrophotometer	Shimazdu
FTIR Spectrophotometer	Shimazdu
GC-MS	Agilent Technologies
Weighing balance	Adventurer, DHAVS

Table 8.1. List of equipments

8.2 Chemicals used:

Chemicals	**Company**
Ethanol	Changshu yanguan chemical
Glucose	Loba Chemie
Ferric Chloride	Loba Chemie
Agar	Loba Chemie
Nutrient Agar	Himedia Laboratories pvt. Ltd.
Nutrient Broth	Himedia Laboratories pvt. Ltd.
NaCl	Loba Chemie
Peptone	Loba Chemie
Beef Extract	Loba Chemie

Table 8.2 List of chemicals

8.3 Data corresponding to section 5.3: Optimization of physico-chemical parameters.

pH	Disperse Orange	Disperse Blue	Scarlet RR
3	39.671	20.93	47.8
4	47.834	60.382	53.792
5	84.43	82.22	84.002
6	84.21	84.325	81.35
7	88.57	81.43	80.17
8	84.761	83.67	84.32
9	83.901	71.923	67.88
10		81.21	51.26

Table 8.3.1: Effect of pH on decolorization (Y1 isolate).

pH	Disperse Orange	Disperse Blue	Scarlet RR
3	31.916	21.54	41.09
4	87.213	71.621	73.1
5	91.83	89.96	86.29
6	91.371	73.21	58.31
7	94.3	85.81	97.51
8	76.525	81.264	81.12
9	71.934	67.263	82.21
10	56.184	71.182	60

Table 8.3.2: Effect of pH on decolorization (W2 isolate).

8.4. Spectrophotometer readings: Corresponding to section 5.4

Following absorbance readings were obtained for three dyes:

Type of dye	**Mean decolorization (in %) at dye concentration 500mg/l (Y1), pH 7, Temp 37 °C**								
	Incubation time (days)								
	1	3	5	7	9	10	12	14	16
Disperse orange	11.56	27.91	28.67	36.68	65.87	77.09	83.34	88.57	88.57
Disperse blue	9.98	13.28	19.36	29.65	46.24	46.65	67.06	78.365	81.43
Scarlet red	44.35	62.78	74.93	78.87	86.89	86.93	88.81	90.17	----

Table 8.4.1 : Decolorization by Isolate Y1 at pH 7, Temp 37 °C

Type of Dye	**Mean decolorization (in %) at dye concentration 500mg/l (Y1), pH 5, temp 37 °C**								
	Incubation time(days)								
	1	3	5	7	9	10	12	14	16
Disperse orange	23	48.382	59.621	60.38	63.13	67.510	74.87	81.93	84.43
Disperse blue	30.26	37.638	43.427	58.85	62.39	71.61	79.06	82.22	-------
Scarlet red	41.68	54.25	56.34	57.46	62.33	67.42	72.14	76.29	84.002

Table 8.4.2: Decolorization by Isolate Y1 at pH 5, Temp 37 °C

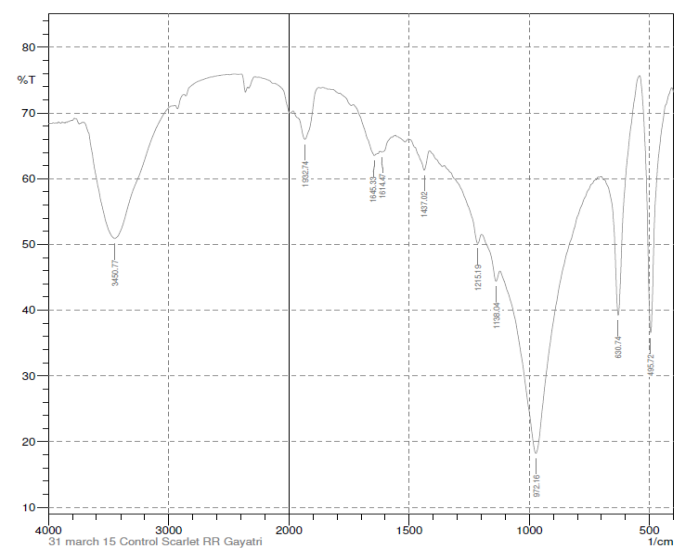
Type of dye	**Mean decolorization (in %) at dye concentration 500mg/l (W2), pH 7, Temp 37 °C**								
	Incubation time(days)								
	1	3	5	7	9	10	12	14	16
Disperse orange	37	57.20	61.3	78.45	94.30	------	-------	-------	-------
Disperse blue	10.17	16.73	27.03	37.98	49.3	62.42	78.70	85.81	-------
Scarlet RR	43.67	58.81	71.05	85.29	92.16	93.51	95.84	97.51	------

Table 8.4.3: Decolorization by Isolate W2 at pH 7, Temp 37 °C

Type of Dye	**Mean decolorization (in %) at dye concentration 500mg/l (W2), pH 5, Temp 37 °C**								
	Incubation time(days)								
	1	3	5	7	9	10	12	14	16
Disperse orange	32	57.2	57	61.299	72.91	77.10	84.27	91.39	91.83
Disperse blue	48.156	67.43	71.67	71.89	81.92	88.701	89.96	89.96	-------
Scarlet red	38.76	52.985	54.364	57.438	61.83	74.92	83.015	84.298	86.29

Table 8.4.4: Decolorization by Isolate Y1 at pH 5, Temp 37 °C

8.5 FTIR data: Corresponding to section 5.5



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	495.72	36.564	38.524	540.09	406.99	28.543	11.74
2	630.74	39.212	27.799	700.18	540.09	37.269	10.037
3	972.16	18.23	33.009	1122.61	727.19	163.155	52.297
4	1138.04	44.397	2.717	1197.83	1122.61	24.043	0.52
5	1215.19	50.157	2.546	1354.07	1197.83	38.363	-0.328
6	1437.02	61.287	3.433	1502.6	1415.8	16.955	0.816
7	1614.47	64.088	0.384	1620.26	1573.97	8.665	0.084
8	1645.33	63.525	1.316	1732.13	1631.83	17.382	0.238
9	1932.74	65.995	6.003	1982.89	1876.8	16.96	1.871
10	3450.77	50.91	18.567	3707.31	2970.48	160.844	45.997

Comment;
31 march 15 Control Scarlet RR Gayatri

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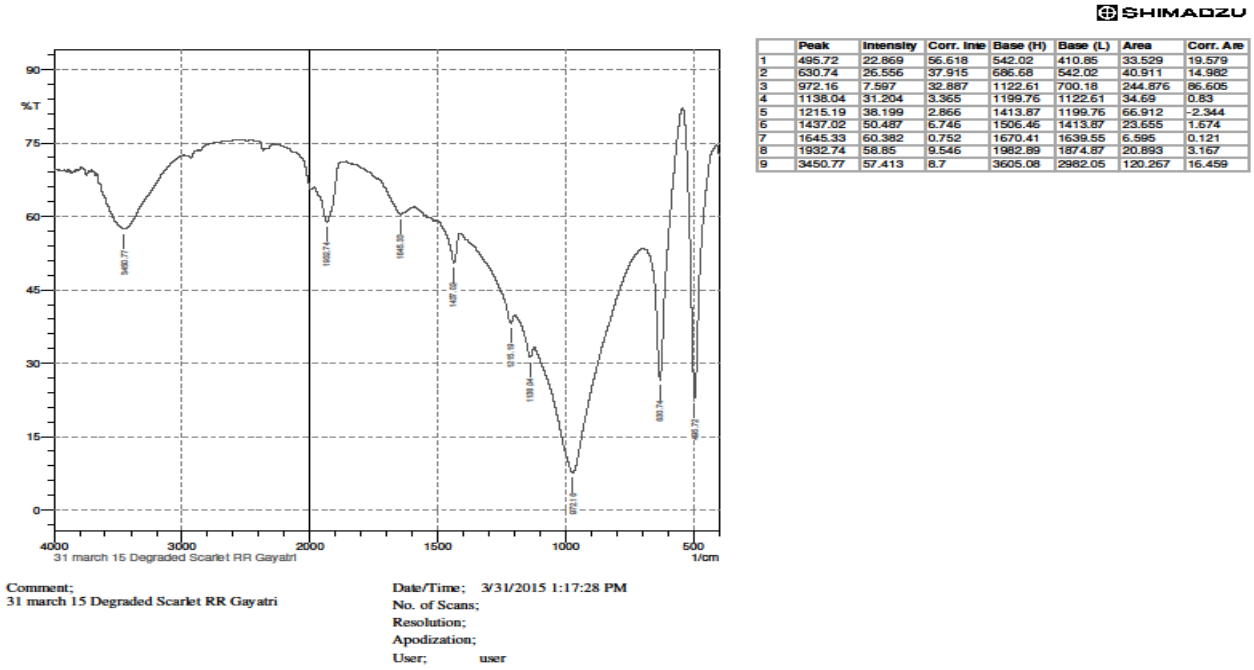
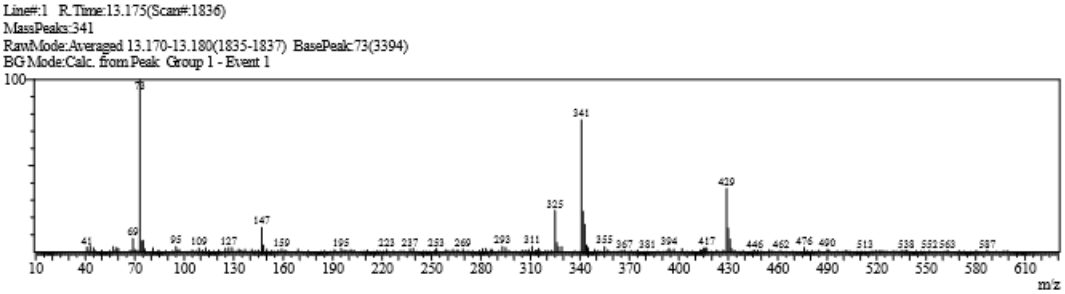
Fig 8.5.1: FTIR Spectra of Scarlet RR (Control)

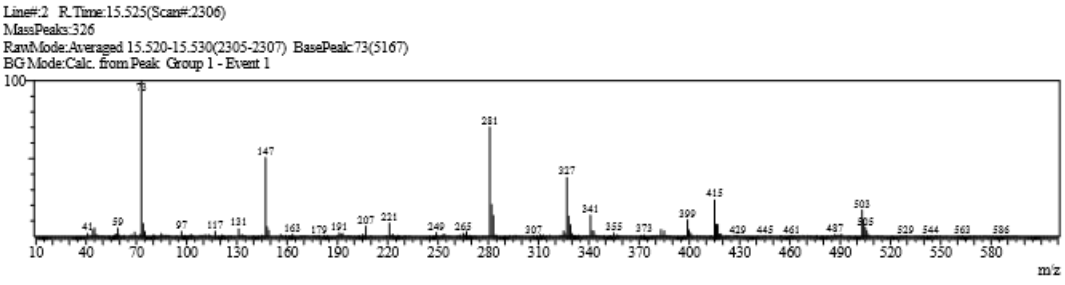


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	495.72	22.869	56.618	542.02	410.85	33.529	19.579
2	630.74	26.556	37.915	686.68	542.02	40.911	14.982
3	972.16	7.597	32.887	1122.61	700.18	244.876	86.605
4	1138.04	31.204	3.365	1199.76	1122.61	34.69	0.83
5	1215.19	38.199	2.866	1413.87	1199.76	66.912	-2.344
6	1437.02	50.487	6.746	1506.46	1413.87	23.655	1.674
7	1645.33	60.382	0.752	1670.41	1639.55	6.595	0.121
8	1932.74	58.85	9.546	1982.89	1874.87	20.893	3.167
9	3450.77	57.413	8.7	3605.08	2982.05	120.267	16.459

Fig 8.5.2: FTIR Spectra of Scarlet RR (Degraded)

8.6: GC-MS results: Corresponding to section 5.7

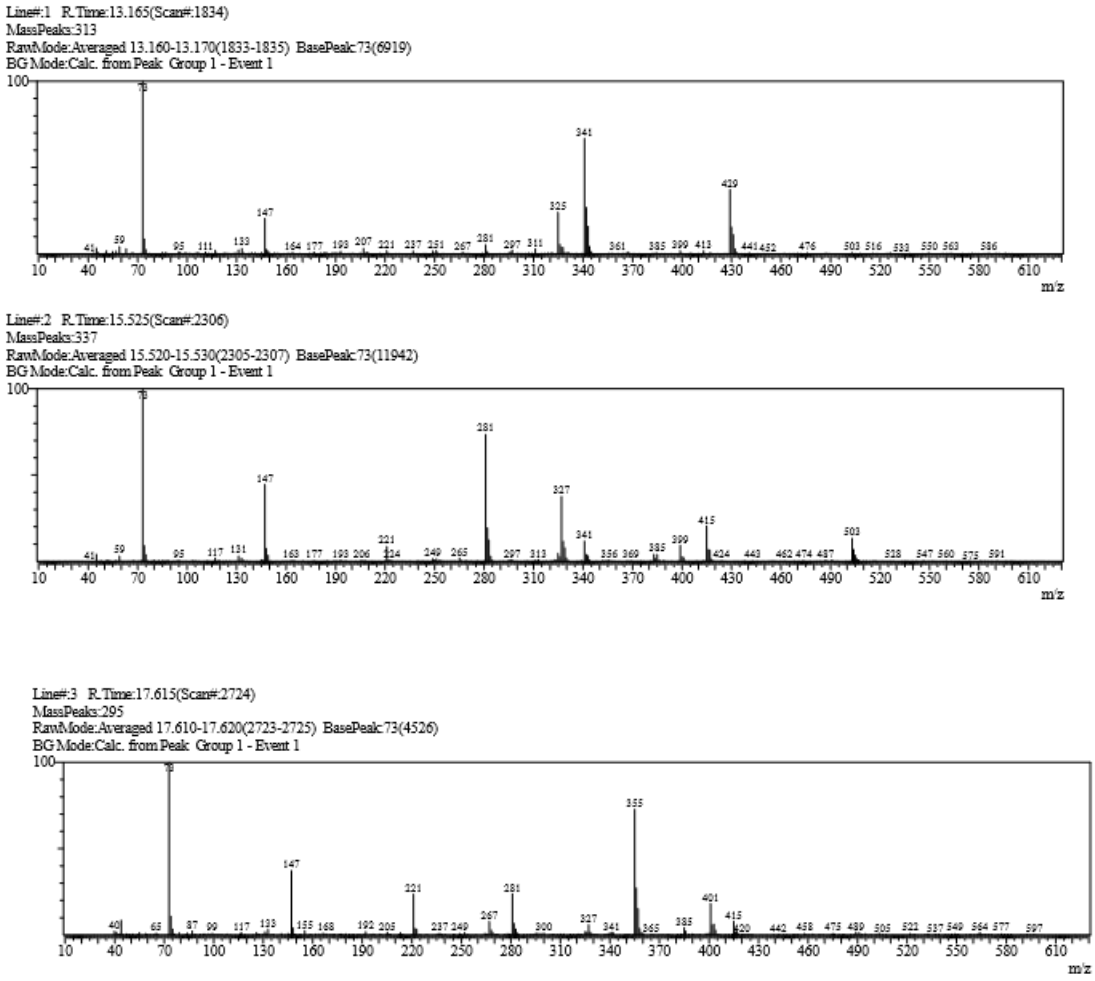




Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name	Base m/z
1	13.175	56034	41.58	19352		73.05
2	15.524	78740	58.42	33420		73.05
		134774	100.00	52772		

Fig 8.6.1: Mass spectra of Scarlett RR (control).



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name	Base m/z
1	13.164	117157	37.46	30918		73.05
2	15.524	157249	50.28	66588		73.05
3	17.615	38342	12.26	22172		73.05
		312748	100.00	119678		

Fig 8.6.2: Mass spectra of Scarlett RR (degraded).

8.7. Table of Characteristic IR Absorptions

m=medium, w=weak, s=strong, n=narrow, b=broad, sh=sharp

Frequency(/cm)	Bond	Functional Group
3640–3610 (s, sh)	O-H stretch	Free hydroxyl alcohols, phenols
3500–3200 (s,b)	O-H stretch	H–bonded alcohols, phenols
3400–3250 (m)	N–H stretch	1˚, 2˚ amines, amides
3300–2500 (m)	O–H stretch	Carboxylic acids
3100-3270(n,s)	-C=C-H: stretch	Alkynes(terminal)
3100-3000(s)	C-H stretch	Aromatics
3100-3000(m)	=C-H stretch	Alkenes
3000-2850(m)	C-H stretch	Alkanes
2830-2695(m)	H-C=O:C-H stretch	Aldehydes
2260-2100(w)	-C=N- stretch	Nitriles
2260-2100(w)	-C=C- stretch	Alkynes
1760-1665(s)	C=O stretch	Carbonyls
1760-1690(s)	C=O stretch	Carboxylic acids
1750–1735 (s)	C=O stretch	esters, saturated aliphatic
1740–1720 (s)	C=O stretch	aldehydes, saturated aliphatic
1730–1715 (s)	C=O stretch	α , β –unsaturated esters
1715 (s)	C=O stretch	ketones, saturated aliphatic
1710–1665 (s)	C=O stretch	α , β –unsaturated aldehydes, ketones
1680–1640 (m)	–C=C– stretch	Alkenes
1650–1580 (m)	N–H bend	1˚ amines
1600–1585 (m)	C–C stretch	(in–ring) aromatics
1550–1475 (s)	N–O asymmetric stretch	nitro compounds
1500–1400 (m)	C–C stretch	(in–ring) aromatics
1470–1450 (m)	C–H bend	Alkanes
1370–1350 (m)	C–H rock	Alkanes
1360–1290 (m)	N–O symmetric stretch	nitro compounds
1335–1250 (s)	C–N stretch	aromatic amines
1320–1000 (s)	C–O stretch	alcohols, carboxylic acids, esters, ethers
1300–1150 (m)	C–H wag (–CH 2 X)	alkyl halides
1250–1020 (m)	C–N stretch	aliphatic amines
1000–650 (s)	=C–H bend	Alkenes
950–910 (m)	O–H bend	carboxylic acids
910–665 (s, b)	N–H stretch	1˚, 2˚ amines
900–675 (s)	C–H stretch	Aromatics

850–550 (m)	C–Cl stretch	alkyl halides
725–720 (m)	C–H	Alkanes
700–610 (b, s)	–C ≡ C–H: C–H bend	Alkynes
690–515 (m)	C–Br stretch	alkyl halides