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COD REDUCTION AND BIODEGRADATION OF TEXTILE DYE REACTIVE ORANGE M2R BY NEWLY ISOLATED BACTERIAL CONSORTIUM VSS

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ABSTRACT

Textile and Textile dyestuff industries discharge effluent having high Chemical Oxygen Demand (COD) and colour, making it difficult to treat the effluent completely. Several Physical, Chemical and Biological treatment methods are available to treat such an effluent. However, in recent years biological treatment with focus on bacteria have been drawing a tremendous attention due to their ability to degrade complex structured dyes and hence treat waste water. In the present study, Soil and Water samples collected from textile dye effluent contaminated sites of Vatva, (Ahmedabad, Gujarat) and Kadodara, (Surat, Gujarat) were studied for screening and isolation of bacteria capable of decolorizing and degrading textile dyes. A bacterial consortium VSS was selected on the basis of rapid dye decolorization. Bacterial consortium exhibited 93% decolorization ability within 30 hours under static conditions at 37 ^{0}C in presence of Glucose and Yeast Extract as cosubstrates. 16S rRNA gene amplification was carried out for the sequencing and identification of these strains. The degradation of dye was confirmed by HPTLC and FTIR analysis. Considerable decrease in COD of the dye (above 80 %) was indicative of conversion of complex dye into simple oxidizable products.

KEYWORDS: Bacterial Consortium, COD, Degradation, Textile Dyes, Decolorization

INTRODUCTION

Synthetic dyes are widely used in a number of industries, such as textile dyeing or printing, paper printing, cosmetics, pharmaceuticals and leather industries. Around 10,000 dyes exist commercially and more than 7×10^{5} tons of dyestuffs are produced annually. Among these 60% of dyes are azo dyes (Zollinger, 1987). Azo dyes can be classified as monoazo, diazo, triazo, and tetraazo dyes on the basis of chemical structure and acidic, basic, direct, reactive, disperse, azoic and pigments on the basis of application.

The textile dyeing and printing industries are major consumers of azo dyes. During the dyeing process approximately 10-15% of dyes are released into the wastewater (Clarke et al, 1980, Chudgar, 1985). The presence of these textile dyes in the natural water bodies are cause of serious environmental and health concerns because of their colour and high chemical oxygen demand (Fang et al, 2004, Asad et al, 2007, Dutta et al, 2002). Moreover it poses toxicity i.e. lethal effect, genotoxicity, mutagenicity and carcinogenicity to aquatic organisms as well as animals (Gunasekaran et al, 2006).

Several methods are used to treat textile effluents which include physico-chemical methods such as filtration, coagulation, activated carbon and chemical flocculation (Gogate, 2004). These methods are expensive and create a secondary disposal problem, whereas microbial decolorization and degradation is an environmentally friendly and cost competitive alternative to chemical treatment (Verma et al, 2003). Most studies on azo dye biodegradation have focused on bacteria and fungi. However a long growth cycle, requiring nitrogen limiting condition and long hydraulic retention time for complete decolorization still limit the performance of fungal decolorization system . In contrast bacterial decolorization is more efficient and faster. Individual bacterial strain usually cannot degrade azo dyes completely and intermediate

products are carcinogenic aromatic amines. Many bacteria are used in the dye decolorization like Brevibacillus sp., Klebsiella pneumoniae, Acetobacter liquefaciens, Pseudomonas desmolyticum, etc.(Alhassani et al, 2007, Kalme et al, 2007a, Kalme et al, 2007b, Wong et al, 1998). Microbial consortia are found to be efficient for environmental remediation (Senan et al, 2004). The complexicity of microbial consortium enables them to degrade variety of pollutants (Watnable, 2000).

In the present study, a consortium was selected from six consortiums isolated from soil and water samples collected from areas of textile dyeing and printing in Kadodara, Surat; Vatva, Ahmedabad and its ability to degrade Reactive Orange M2R (ROM2R) a monoazo dye was analysed. The consortium's ability to remove Chemical oxygen demand was measured to ensure the ability of the consortium to convert complex dye to simple non toxic compound. The degraded product after the treatment with consortium VSS was anaylsed by HPTLC and FTIR

MATERIALS AND METHOD

Dyes

The textile dyes that are manufactured and used mostly for dyeing and printing were selected for the study. The common name of all dyes has been used for convenience; the dyes were procured from Chemical Research and Development Centre (CRDC), Vatva, Ahmedabad. The dyes were Reactive Orange M2R, Remazol Brilliant Red 5, Procion Yellow, Reactive Green 19A, Reactive Red 195, Reactive Golden Yellow, Red HE8B, Reactive Blue 59, Remazol Brilliant Blue R, Remazol Black B, Reactive Black 8 and Turquoise Blue. A monoazo dye Reactive Orange M2R (ROM2R) was selected as model dye for all experiments (Figure 1).

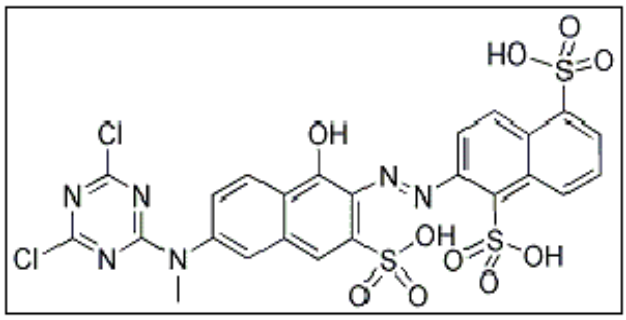

Figure 1: Reactive Orange M2R (Reactive Orange 4) C.I.No.18260 λ_{max}=488nm

Medium

Bushnell and Haas medium (BHM) (HiMedia) containing the following in gl^{-1}: $MgSO_4$, 0.2; K_2HPO4, 1.0; $CaCl_2$, 0.02; $FeCl_3$, 0.05; NH_4NO_3, 1.0 supplemented with Glucose (0.1% w/v) , Yeast extract(0.3% w/v) and 100ppm (mgl^{-1}) ROM2R was used for enrichment of organisms in soil and water sample.

Sample Collection for Isolation of Dye Degrading Organisms

Soil and water samples were collected from areas of textile dyeing and printing in Kadodara, Surat and areas around dye industries in Vatva, GIDC, Ahmedabad, Gujarat were used for screening of dye decolorizing bacteria .

Enrichment of Dye Degrading Organisms

BHM media containing dye (ROM2R 100 mgl^{-1}) as sole source of carbon and energy (without cosubstrate) (100 ml) was taken in 250 ml Erlenmeyer flasks and was inoculated with 10 ml of soil suspension (10% w/v) and incubated under static condition at 37 $^{\circ}$C. Repeated transfers were carried out in fresh dye containing media till stable dye

decolorizing cultures were obtained showing consistent growth and decolorization during successive transfer. Same was repeated for water samples. Co-substrates such as glucose and Yeast Extract were also used along with dye in BHM media.

Identification by Gram Staining and 16SrRNA Sequencing Method

The cultures that showed consistent decolorization were subjected to separation on dye containing agar plates and was submitted to Gujarat State Biotechnology Mission (GSBTM), Gandhinagar, Government of Gujarat, India for identification by 16S rRNA sequencing method.

Reduction of COD during Decolorization

The decolorization medium was observed for the change in Chemical Oxygen Demand at different time intervals. For determining change in COD, titrimetric procedure was followed in which supernatant refluxed with potassium dichromate, in presence of silver sulfate, mercury sulfate and concentrated H_2SO_4 was titrated with Ferrous Ammonium Sulfate (FAS) using ferroin indicator (APHA, 2005).

Dye Decolorization Experiments under Static and Shaking Conditions

Bushnell and Haas Broth (BHB) 100ml in 250ml Erlenmeyer flasks along with glucose (0.1%w/v) and Yeast Extract (0.3%w/v) amended with 100 ppm of ROM2R was inoculated with bacterial culture (5% w/v) and incubated at 37° C under static or shaking conditions. All the decolorization experiments were performed in triplicates.

Analysis of Growth and Decolorization of ROM2R

Culture broth of 5 ml was collected from each flask at different time intervals of incubation. These samples were centrifuged at 6000 rpm for 20 minutes and supernatant was analysed by UV-Vis spectrophotometer (Systronics 2203) at λ_{max} of respective dye (λ_{max} of ROM2R=488nm). The cell pellet obtained upon centrifugation of 5 ml culture was resuspended in 5 ml distilled water and its absorbance was studied at 660 nm. The uninoculated flask containing the dye (ROM2R) was used as reference to correct the abiotic colour disappearance and the uninoculated medium without dye was used as blank.

The efficiency of dye decolorization was determined based on the reduction of the absorbance in relation to the zero time absorbance expressed as percentage. The decolorization efficiency was expressed as follows:

$$\% \text{ decolorization} = [(A_i - A_f)/A_i] \times 100$$

Where A_i = Initial Absorbance,

A_f = Final Absorbance

HPTLC Analysis of the Degraded Product

Degradation of dyes was monitored on precoated TLC silica gel plates. The culture supernatants at different periods during decolorization were used for analysis. A 10µl of the sample was spotted on TLC plates using a micro syringe. The solvent system used was n-propanol, methyl ethyl ketone, Ammonium hydroxide (4:3:3 v/v).The dye chromatogram was observed in day light and in UV light (254nm).

Fourier Transform Infra Red Spectroscopy Analysis of Degraded Product

Metabolites produced by biodegradation of the ROM2R were extracted with equal volumes of ethyl acetate. The extracts were dried over anhydrous Na_2SO_4 evaporated to dryness in rotary evaporator and analysed. FTIR analysis was carried out on Thermo Scientific 6700 set up, frequency range 4000cm^{-1} to 400cm^{-1}.

Spectrum of Dyes Decolorized by the Culture

Mixture of various dyes is present in industrial effluents therefore ability of the consortium VSS to decolorize different dyes (as mentioned in Section II A.) was studied.

RESULTS AND DISCUSSIONS

Screening of Dye Decolorizing Bacterial Cultures

Isolation of bacteria was carried out from textile dye contaminated soil and water samples from various sites of Kadodara, Surat; Vatva, GIDC, Ahmedabad for decolorization and degradation of textile dyes. Six different bacterial consortium namely SAS, VCS, SWS, NAS, VWS and VSS were isolated by enrichment culture technique using dye ROM2R (100 ppm) and also by adding glucose and Yeast Extract as co-substrates. It was evident from the results that addition of co-substrate is essential for decolourisation. The same was reported by (Nigam et al, 1996). Of all the bacterial consortia obtained bacterial consortium VSS was found to be the most efficient with maximum decolorization efficiency under static conditions at 37 ^{0}C. Six different bacterial species were obtained upon isolation on dye agar plates from the mixed consortium VSS. When each pure culture was tested individually for its decolorizing ability in liquid medium and in combination with other pure cultures, none of the cultures showed decolorization even on extended incubation. But, when all the six bacterial cultures were mixed and inoculated together in a liquid medium, complete decolorization of ROM2R was observed, thus suggesting role of all six bacterial species in decolorization. Moosvi et al, 2005, Chen et al, 2006, Bafana et al, 2007, Yang et al, 2009, Patil et al, 2010, Phugare et al, 2011 reported synchronized action of microorganisms for the biodegradation of textile dye.

Change in COD during Decolorization

All six bacterial consortia isolated by enrichment culture technique were studied for change in COD. Mixed results were obtained with different consortia. Consortium VCS showed minimum COD reduction of 38% and 60% of ROM2R dye in 24 hours and 48 hours of inoculation respectively. Consortium VSS showed maximum COD reduction of 67% and 83 % of ROM2R dye in 24 hours and 48 hours of inoculation respectively. All the other consortia showed COD reductions in between the COD reduction by VCS and VSS (Figure 2). High rate in COD reduction is considered as an indicator of mineralization. (Hassan et al, 2002).

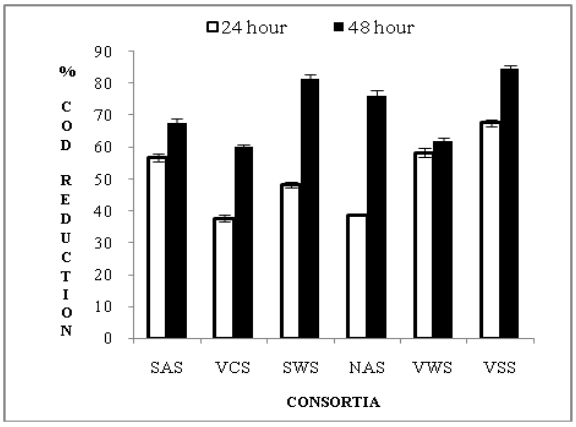

Figure 2: % COD Reduction by Various Consortia

Identification of Cultures Present in Consortium VSS by Gram Staining and 16S rRNA Technique

VSS consortium obtained after enrichment culture technique was subjected to gram staining. Of the six isolates obtained, three of them showed gram positive cocci, two of them showed gram negative short rods and one showed gram

positive bacilli. Organism present in consortium VSS was identified by 16SrRNA technique . The consortium contains Enterococcus casseliflavus, Bacillus subtilis, Vagococcus fluvialis, Stenotrophomonas sp., Enterococcus casseliflavus and Pseudomonas aeruginosa and deposited to National Centre for Biotechnology Information (NCBI) genebank with accession nos., JX081350, JX081351, JX081352, JX081353, JX081353, JX081354 and JX081355 respectively.

Decolorization Ability and Growth Pattern of Consortium VSS under Static/Shaking Conditions

Decolorization ability and growth of the bacterial cultures under static/shaking conditions was obtained by spectrophotometric method and the results are represented in Figure 3 and 4. The consortium VSS exhibited decolorization up to 93% within 30 hours under static condition, whereas under shaking condition, the culture showed 20% decolorization of ROM2R dye in 30 hours.

Under shaking condition, the culture though showed faster growth, it showed lesser decolorization. The results clearly indicated that decolorization was not depended on biomass concentration but was significantly correlated with dissolved oxygen. The azo reductase driven bacterial decolorization of azo dyes is normally inhibited by the presence of oxygen primarily due to competition in oxidation of reduced electron carriers (e.g. NADH) with either Oxygen or Azo group as the electron receptor (Chang et al, 2000).

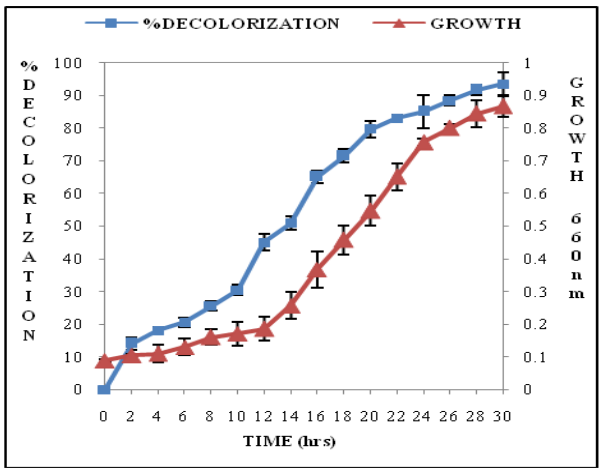

Figure 3: Decolorization of Reactive Orange M2R by Mixed Bacterial Consortium VSS under Static Conditions at 37 ^{0}C

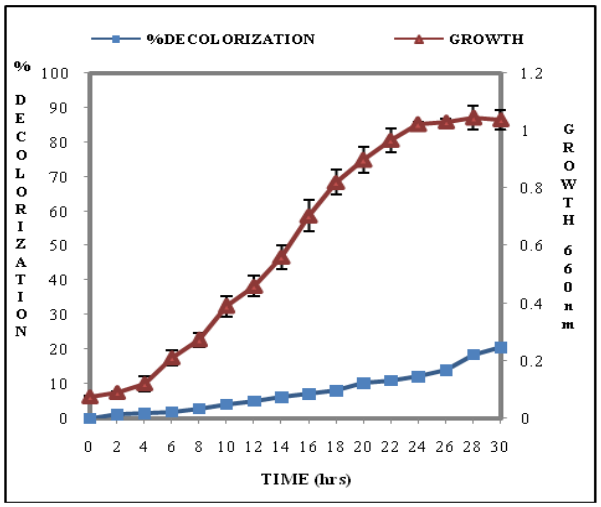

Figure 4: Decolorization of Reactive Orange M2R by Mixed Bacterial Consortium VSS under Shaking Conditions at 37 ^{0}C

HPTLC Analysis

Spectrophotometric analysis at 488 nm of uninoculated medium (Figure 5) containing ROM2R showed a simultaneous decrease in peak of samples withdrawn at various time intervals. Evidence of the removal of dye can be observed with absorbance at λ_{max} being virtually zero after 48 hours of inoculation with VSS consortium (b) and shift of peak towards the U.V. region as shown in Figure 5

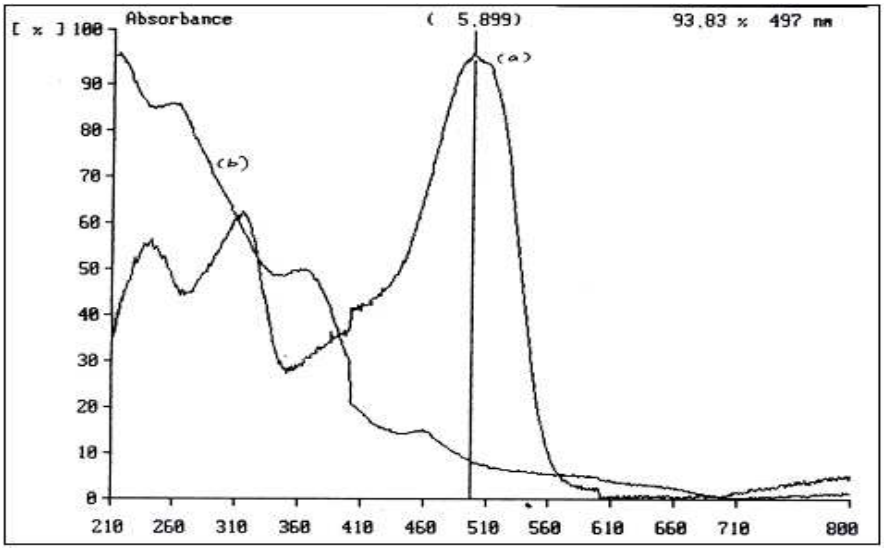

Figure 5: Spectrophotometric Analysis (a) Uninoculated Media Containing ROM2R (b) Media Containing ROM2R Inoculated with Consortium VSS

HPTLC results showed the formation of new peaks as observed and compared to control dye (Figure 6, 7, 8). The results obtained justify that the chromophoric groups were totally removed from the intact dye structure.

The multiple bands obtained in the metabolite lane confirmed the biodegradation of the azo dye as compared to single band of control dye (Figure 8). The difference in Rf values (data not shown) of control dye and metabolites supports the FTIR data which suggests biodegradation of ROM2R. The similar results were reported by Chaube et al, 2010, Waghmode et al, 2011.

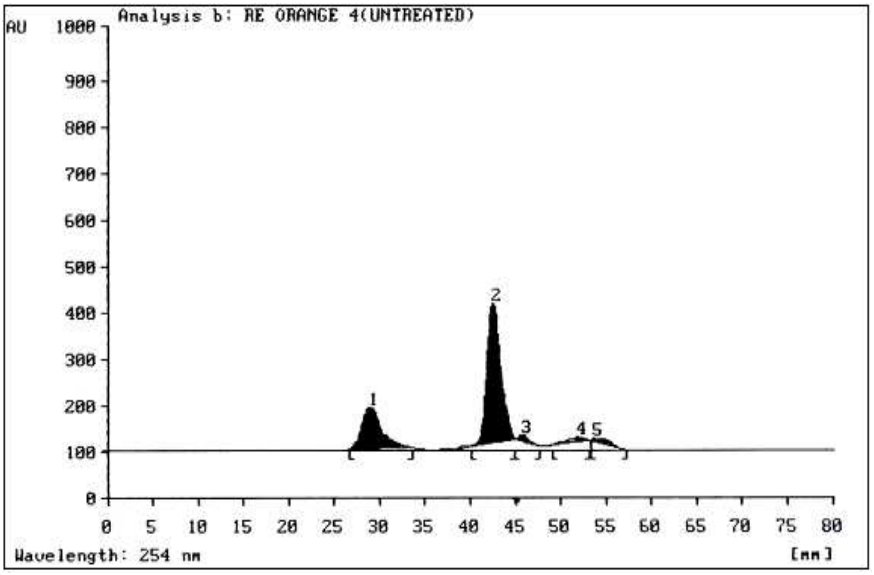

Figure 6: HPTLC Profiles of Reactive Orange M2R in Medium

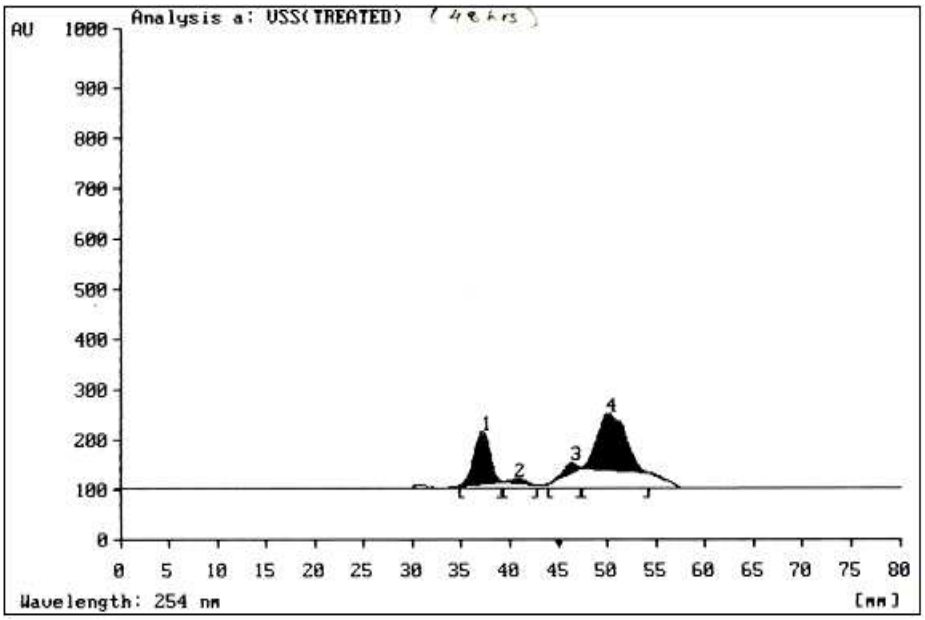

Figure 7: Metabolites of ROM2R Dye after Degradation

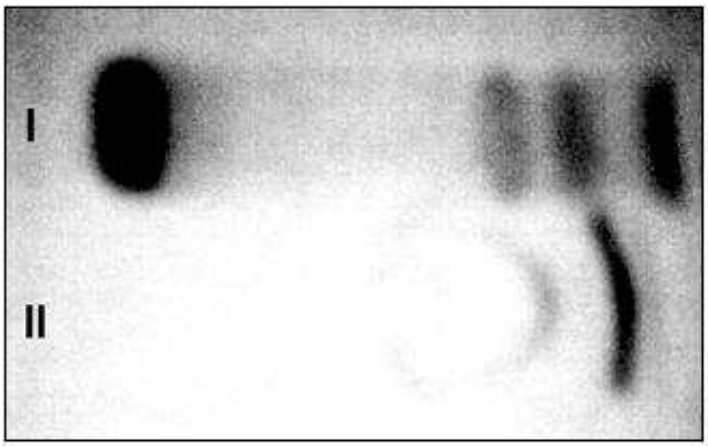

Figure 8: HPTLC Plate Exposed under UV Light, Control Dye Track (II) and Metabolites Track (I)

FTIR Analysis

Results of FTIR analysis of control and sample obtained after decolorization showed various peaks (Figure 9 and 10). The FTIR spectrum of control ROM2R displays peak at 3440.1 cm^{-1} for the intramolecular hydrogen bonding aromatic –OH and –O-H stretching, peak at 1622.7 $cm^{-1}$and 1555.4 cm^{-1} for –C=C stretching of aromatic rings, 1466.5 cm^{-1} and 1394.6 cm^{-1}–C-H stretching of alkyl acetals, peak at 1329.8 cm^{-1} and 1187.5 cm^{-1} for –C-N stretching due to amines, peak at 845.2 cm^{-1}, 799.9 cm^{-1}, 763.3 cm^{-1} for –C-H stretching, 617.8 cm^{-1} –C-Cl stretching.

The degradation metabolites of ROM2R using consortium VSS showed peak at 3442.1 cm^{-1} for –O-H- stretching, peak at 1644.8 cm^{-1} –C=C stretching of alkenes, 1400.1 cm^{-1} for –C-H stretching due to $–CH_3$, peak at 1110.4 cm^{-1} , 619.3 cm^{-1} , 476.9 cm^{-1} for –C-Cl stretching indicating presence of alkyl chloride.

Here peak at 1392.8 cm^{-1} is missing which confirms absence of amines (Figure 8) and formation of new peaks suggests the biotransformation of Reactive Orange M2R dye. The similar results were reported by Srivastava et al, 2012, Waghmode et al, 2011.

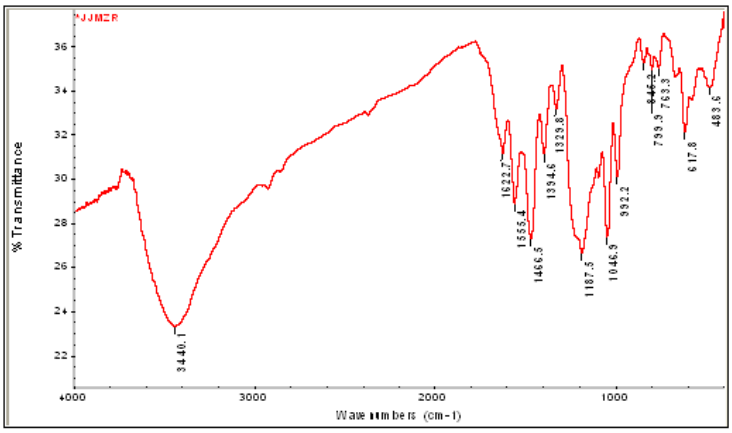

Figure 9: FTIR Spectra of Reactive Orange M2R

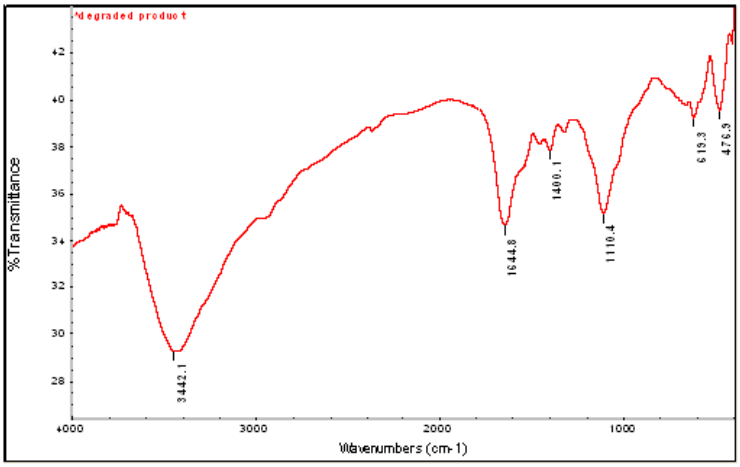

Figure 10: FTIR Spectra of Degradation Metabolites of Reactive Orange M2R

Spectrum of Dyes Decolorized by the Bacterial Consortium

Industrial effluents consist of a mixture of various dyes. Ability of consortium VSS to decolorize different dyes was studied (Figure 11). There was rapid degradation observed for all dyes (100 mgl^{-1}) used in the study within 24 hours. Reactive Orange M2R, Remazol Brilliant Red 5, Reactive Green 19A, Reactive Red 195, Reactive Golden Yellow, Red HE8B, Reactive Blue 59 showed decolorization in the range of 90-97 %. Remazol Brilliant Blue R and Remazol Black B showed decolorization in the range of 50- 86%. Procion Yellow and Reactive Black 8 showed decolorization in the range of 22-40%. Torquoise Blue showed negligible decolorization (3%). The chemical structure of the dye Turquoise Blue could be the reason for unsatisfactory decolorization. The percent decolorization varied which could be due to the structural differences of all dyes or due to the toxic effect of dyes (Khehra et al, 2005).

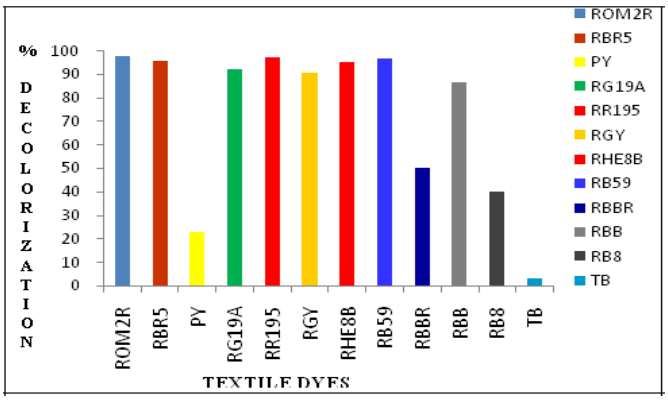

Figure 11: Spectrum of Dyes Decolorized by Consortium VSS

CONCLUSIONS

Our study demonstrates efficient decolorization of nine out of 12 dyes tested at pH 7 and 37 ^{0}C temperature by consortium VSS. The Consortium isolated grows best in static conditions, where in oxygen can be easily depleted, thus creating conditions favourable for decolorization of azo dyes. Reduction in COD of dye after treatment with VSS indicates degradation of the dye. HPTLC and FTIR results also confirm the dye degradation. High decolorization extent and complete mineralization of azo dye show the potential of this bacterial consortium to be used in the treatment of industrial effluents.

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