Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.913999

55 (2015) 241–251
June

Taylor & Francis
Taylor & Francis Group

Biodegradation studies on dye effluent and selective remazol dyes by indigenous bacterial species through spectral characterisation

E. Sanmuga Priya*, P. Senthamil Selvan, A.N. Umayal

Department of Pharmaceutical Technology, Anna University, BIT Campus, Tiruchirappalli 620 024, Tamil Nadu, India, Tel. +91 431 2407978; Fax: +91 431 2407333; email: sanmug77@gmail.com

Received 24 December 2013; Accepted 25 March 2014

ABSTRACT

Effluent collected from a dye industry in Tirupur, Tamilnadu, India, was screened for potential indigenous micro-organisms to treat remazol dyes and dye effluents. Out of sixty strains isolated, four strains were found to be potential in degrading the dye effluent and various remazol reactive dyes. The physicochemical characterisation of the dye effluent showed quite high values. Decolourisation studies were performed for two different effluents (I & II) and observed maximum decolourisation of 76.4% at 120 h (Effluent I) and 79.9% at 96 h (Effluent II) by the organism EI25. The synthetic dyes Remazol Red RGB (RR-RGB), Remazol Red RR (RR-RR), Remazol Yellow RGB (RY-RGB) and Remazol Yellow RR (RY-RR) showed complete decolourisation (100%) after 24–72 h by two potential strains (EI05 and EI25). EI25 have shown the maximum efficiency of 87.2, 95.1, 89.4 and 84.4% decolourisation for RR-RGB, RR-RR, RY-RGB and RY-RR, respectively, within a 24 h time period. Morphological characteristics and biochemical screening tests identified the organisms to be *Clostridium butyricum* and *Clostridium acetobutylicum*. *C. acetobutylicum* (EI25) was found to be the most potential strain isolated. Biodegradation of remazol dyes was characterised by spectral analysis using UV–Visible spectroscopy and FTIR spectroscopy. The peak disappearance in UV spectrum is at the λ_{max} 519 nm for RR-RGB and RR-RR; whereas, 410 nm for RY-RGB and 419 nm for RY-RR stands as the proof of decolourisation. In the FTIR spectrum, the shift of several important peaks responsible for the functional groups N=N, S=O and phenolic O–H corresponding to the structure of remazol group of dyes and appearance of new peaks indicating the degradation metabolites observed in the decolourised samples ensures biodegradation. Further, EI25 have also shown marked reduction in the basic wastewater characteristics like COD, BOD, TDS, EC and pH. Thus it is concluded that the *Clostridium* species on further studies can be well developed into a biological method of textile effluent treatment process.

Keywords: Tirupur; Dye effluent; Reactive dyes; Remazol dyes; Biodegradation

1. Introduction

Textile industry is one of the main pillars of Indian economy constituting approximately 14% of industrial production and 20% of total export earnings. Tirupur is a major source of foreign exchange in exporting textile clothing especially knitwear all over the world. Tirupur is a place traditionally famous for knitwear and now it has come to be known as "Knitwear

*Corresponding author.

Capital of India" and it is able to increase its share in the export market from about 15% in the year 1981 to more than 58% in 2008 [1].

Dyeing is the process of colouring materials like fibres, yarns, fabrics, etc. Many chemical dyes have been used excessively in textile and dyeing units because of their ease and cost effectiveness in synthesis, firmness and variety of attractive colours can be produced compared to that of natural dyes [2]. It has been reported that 10% of the dye stuffs used in the dyeing process remains unfixed from the fibres and are therefore present unaltered in the effluent. These dyeing units are water intensive, and generate a large quantity of wastewater. Typical water consumption in Tirupur is around 200–400 L/kg of finished product, compared with the international norm of 120–140 L/kg. The rapid growth of textile industries leads to serious environmental problems especially from bleaching and dyeing units. This top industrial city possesses the environmental hazards through its untreated dye effluent discharge into the Noyyal River. River Noyyal originates from Vellingiri hills (Coimbatore district, Tamilnadu, India) and it passes through Tirupur and is stored up in Orathapalayam Dam to be used in agriculture and drinking purposes from downstream Erode district, Tamilnadu, India [3]. The key environmental issues related with textile manufacturing in Tirupur and surrounding areas are as follows: pollution of the irrigation wells, disturbance in the ecosystem by the seepage and percolation in the Orathapalayam dam, agriculture, livestock, marine and fisheries of downstream around the Orathapalayam dam got affected, presence of heavy metals makes the river water unfit for domestic purpose. The TDS level of water in the Orathapalayam dam area was found to be above 9,000 ppm. Moreover, various human health hazards are reported in the population residing in the area [3]. In general, various forms of human toxicity were reported in the literature for dye substances [4–8]. The highest rates of toxicity have been found amongst the basic and diazo direct dyes [9]. Reactive dyes are typically azo-based chromophores combined with different types of reactive functional groups. Various kinds of toxicities have been reported for remazol dyes like teratogenicity in frog embryos [10], enzymic degradation metabolites toxicity [11], genotoxicity and phytotoxicity [12].

The conventional treatment systems like physicochemical treatment and physicochemical treatment followed by biological treatment system are installed in majority of the textile industries. There are numerous physical and chemical techniques that are capable of treating the effluents among which the development of low-cost adsorbents like industrial wastes is emerging nowadays [13–25] as physical method of treatment. All these methods have significant differences in capital cost, decolourisation, volume capacity and operating time. Complicated structure of the dyes renders difficulty to be treated with conventional physical/chemical processes [26]. The volume of wastes generated during the industrial treatment process also contributes towards the selection of decolourisation methods [27]. The objective of bioremediation is to immobilise contaminants or to transform them into chemical products no longer hazardous to the environment. Transformation and degradation processes differ depending on the physical environment, microbial communities and contaminants. The effectiveness of decolourisation depends on the organism which must have tolerability, adaptability and activity towards the effluent. Biological degradation techniques are advantageous on the basis of low-cost process and non-toxic end products. Moreover, it is an environment-friendly alternative for the chemical degradation techniques in the treatment of dye effluent [28].

Relying on the issues related to the effluent contamination in the Noyyal River, followed by human health hazards and complexity of the dye effluent treatment methods in Tirupur region, the aim of this research is focused on self-purification ability of the dye effluent-based indigenous microbes. Various studies on microbial degradation of the dye effluent on the specific region have been reported in the literature [29–33]. Thus the present study is designed in search of a potential microbe with higher efficiency to perform biodegradation. Based on the higher usage rate of the remazol dyes in the Tirupur industries for dyeing purposes, the objective is planned to isolate and characterise potential biodegrading indigenous bacteria from the effluent itself and to evaluate its efficacy in terms of decolourisation of the dye effluent and remazol reactive dyes used in the textile industries.

2. Materials and methods

2.1. Chemicals

Remazol Red RGB, Remazol Red RR, Remazol Yellow RGB and Remazol Yellow RR were obtained from Dystar India Pvt. Ltd., Mumbai. Two different samples of dye effluents (I & II) have been collected on different days from the same treatment plant located in a dyeing unit in Arulpuram, New Tirupur, Tamilnadu, India, during the period of January 2012. The media for the growth of microbes have been purchased from HiMedia Laboratories, Mumbai. All the

other chemicals used were of analytical grade. UV–Visible spectrophotometer (Shimadzu Pharmaspec UV-1700, Japan) was used for decolourisation studies. FTIR (Spectrum RX I, Perkin Elmer, US) was used for spectral characterisation.

2.2. Wastewater characteristics

The collected effluents (I & II) were tested for wastewater characteristics like pH, COD, BOD, Total dissolved solids, total suspended solids, sulphides, chlorides and electrical conductivity according to standard analytical procedures for water analysis [34].

2.3. Isolation of several bacterial strains from the effluent

The effluent samples (I & II) collected from the dye industry were inoculated on the Nutrient agar media with pH 7.4 and incubated at 30°C for 48 h [35]. The effluent was serially diluted with the distilled water, inoculated and incubated in the same conditions. Discrete bacterial colonies grown on the agar plates were noted and again streaked in separate agar plates, different strains were isolated. These purified strains are then inoculated into the liquid medium nutrient broth to study the decolourisation of the dye effluent.

2.4. Decolourisation assay

Using fixed concentration of medium in the effluent, decolourisation study has been carried out. Ten per cent of the freshly prepared inocula at the mid log phase was inoculated into the Erlenmeyer flask containing 50 mL of the effluent. Organisms showing significant decolourisation in the initial screening were inoculated. The inoculated effluent was incubated at 37°C for 4 d under static conditions and studied. The decolourisation assay was performed using Shimadzu Pharmaspec UV 1700. The effluent and the synthetic dyes were now screened for their λ_{max} and their spectra have been recorded. The maximum absorbance has been found. The initial optical density of the effluent and dye before inoculation was noted. Every 24 h, 5 mL of the decolourised sample was withdrawn and centrifuged at 7,830 rpm for 10 min. The optical density has been noted [36]. The percentage decolourisation was determined using the formula:

$$\% \text{ Decolourisation} = \frac{\text{Initial O.D.} - \text{Final O.D.}}{\text{Initial O.D.}} \times 100$$

2.5. Screening of potential strains against the effluent

Fixed concentration of medium in the effluent was taken to study the decolourisation. Ten per cent of the freshly prepared inocula at the mid log phase was inoculated into the Erlenmeyer flask containing 50 mL of the effluent. The inoculated effluent was incubated at 37°C for 4 d under static conditions and studied. Based on the visible decolourisation of the effluent in the four days, the potential organisms were noted and these organisms were carried for further studies [36]. The strains showing marked decolourisation have been identified by biochemical tests. The biochemical tests were performed using standard procedures and the strain was identified as prescribed in the text book Bergey's Determinative Bacteriology [37].

2.6. Identification of species

The bacterial colonies developed on agar plates were grouped on the basis of morphology. Selected bacterial isolates were further purified and sub-cultured. The pure cultures were identified based on their biochemical tests for the identification of species and by Bergey's manual of determinative Bacteriology [37].

2.7. Spectral analysis

2.7.1. UV–Visible spectroscopy

Decolourisation was quantitatively analysed by UV–Visible spectroscopy. The pure dye sample at 25 ppm concentration was analysed through UV–Visible spectrophotometer and the spectrum was recorded. Simultaneously, decolourised samples were also analysed. The samples previously prepared at the same concentration and the desired microbial sample was inoculated to perform decolourisation. The samples were collected after decolourisation. The decolourised sample was centrifuged at 7,830 rpm for 10 min. The supernatent sample was now analysed through Shimadzu UV–Visible spectrophotometer and the spectrum was overlaid with the control [36].

2.7.2. FTIR spectroscopy

The functional group characterisation of the dyes before and after decolourisation was done using FTIR Spectroscopy. The pure dye solid sample at 25 ppm concentration was mixed with potassium bromide and the pressed pellets were used for analysis. The liquid samples were directly analysed by placing a drop on

the thin film cell. IR spectrum was recorded. The samples prepared at the same concentration were inoculated with the desired microbial sample to perform decolourisation and the sample was centrifuged at 13,000 rpm for 30 min. The supernatant was analysed in the mid infrared region 400–4,000 cm^{-1} through FTIR spectroscopy and the spectrum was recorded [38]. FTIR spectrum was interpreted.

3. Results and discussion

3.1. Decolourisation studies with the dye effluent

Around 60 colonies have been isolated from the effluent and 12 organisms were chosen from the results of preliminary decolourisation studies. The decolourisation studies have been conducted with these 12 organisms against dye effluents I and II. UV–Vis spectrum was recorded at 0, 24, 72, 96 and 120 h. The results obtained were tabulated (Table 1). From the results obtained, the organisms showing more than 70% decolourisation in the effluents have been chosen. The chosen organisms were EI05, EI15, EI21 and EI25. Among which EI25 decolourised 76.4% of the dye effluent I and 79.9% of dye effluent II at 120 h. Organism EI25 showed a maximum decolourisation in both the effluents. The organism EI05 decolourised 75.1% of the effluent I and 74.1% of the effluent II at 120 h. EI15 and EI21 decolourised 72.1% of the effluent I as maximum decolourisation rate. The organism EI11 decolourised 76.2% of the effluent II at 120 h. Peaks observed in the effluent samples were decreased up to complete decolourisation of the medium (data not shown). From the results it is clear that the maximum decolourisation of the effluents by the micro-organisms could be achieved only after a long interval of 120 h, since the dye effluent is the complex mixture of several dyes the decolourisation takes long time. Control did not show any decolourisation when compared to the effluent sample which indicates that decolourisation occurred might be due to the metabolic activity of the microbes. Four organisms EI05, EI15, EI21 & EI25 have been chosen for further decolourisation studies with the pure synthetic dyes.

Table 1
Decolourisation of the dyeing effluents I & II at various time intervals

	% Decolourisation							
	24 h		72 h		96 h		120 h	
Organism	I	II	I	II	I	II	I	II
EI05	31.4	44.1	61.4	55.4	66.4	64.5	75.1	74.1
EI07	37.1	46.6	56.4	51.2	62.9	59.9	62.9	63.2
EI11	22.8	52.3	58.5	61.2	62.9	67.1	69.2	76.2
EI13	38.6	58.3	53.6	63.4	69.2	67.9	67.1	69.2
EI15	52.1	52.6	62.9	57.2	69.2	59.6	72.1	67.8
EI21	32.8	43.9	54.2	42.1	63.5	62.2	72.1	68.1
EI25	63.6	61.7	62.1	68.0	62.1	71.4	76.4	79.9
EI26	40	50.2	50.7	52.3	57.8	57.8	57.1	56.1
EI30	35.7	44.8	54.3	49.8	61.4	58.4	57.9	57.2
EI39	38.6	45.4	55.7	47.1	65	65	67.1	67.9
EI41	40.7	28.6	38.5	32.6	47.9	47.9	47.1	58.2
EI43	36.4	33.4	53.6	49.6	62.9	57.9	60.7	60.7

3.2. Wastewater characteristics

The wastewater characteristics of the dye effluents I & II before and after decolourisation were studied and results are tabulated in Table 2. On biological decolourisation, the colour and odour of the effluent had changed to pale yellow and odourless, respectively. Marked reduction in pH from alkaline side (10.2) to neutral pH (7.4) was observed. COD and BOD values were found to be higher than the permissible limits and these high values demands significant amount of dissolved oxygen for remediation of the wastewater. The percentage removal of COD was 53 and 58%, whereas the BOD removal was 68.3 and 75.8% for effluents I & II, respectively, after biological treatment. The other parameters like TDS, electrical conductivity, sulphides and chlorides were also found to be reduced on decolourisation, whereas TSS tends to show increment in values on biodegradation. Further studies are required to validate this result.

3.3. Decolourisation studies with the pure synthetic dyes

The synthetic dyes used in the study were Remazol Red RGB (RR-RGB), Remazol Red RR (RR-RR), Remazol Yellow RGB (RY-RGB) and Remazol Yellow RR (RY-RR). The λ_{max} of the synthetic dyes were found to be 519 nm for RR-RGB and RR-RR; whereas, 410 nm for RY-RGB and 419 nm for RY-RR. The percentage of decolourisation of the synthetic dyes by the selected four micro-organisms EI05, EI15, EI21 & EI25 are shown in Tables 3 and 4. Hundred per cent decolourisation was achieved after 48 h for RR-RGB and RR-RR by all the organisms. Within a 24 h time period, the organism EI25, have shown the maximum efficiency of 87.2 and 95.1% decolourisation for RR-RGB and RR-RR, respectively. From Table 4 it is clear that 100% decolourisation was achieved for RY-RGB at 48 h and RY-RR at 72 h by all the organisms. The maximum decolourisation (89.4% for RY-RGB and 84.4% for RR-RR) was shown at 24 h for RY-RGB and

Table 2
Wastewater characteristics of the textile effluents I & II before and after decolourisation with EI25

S. No.	Parameter	Before decolourisation		After decolourisation	
		Effluent I	Effluent II	Effluent I	Effluent II
1	Colour	Greenish	Bluish purple	Pale yellow	Pale yellow
2	Odour	Fishy	Pungent	–	–
3	pH	9.7	10.2	7.4	7.6
4	COD mg/L	526	742	247	311
5	BOD mg/L	193	219	61	53
6	TDS mg/L	7,500	9,500	1,500	1,500
7	TSS mg/L	1,211	1,567	2,543	2,601
8	Sulphides mg/L	16	11	2	4.5
9	Chlorides mg/L	80	95	8	11.1
10	EC μS/m	6.42	8.12	1.01	2.01

Table 3
Decolourisation studies of Remazol Red RGB & Remazol Red RR dyes

	% Decolourisation							
	24 h		48 h		72 h		96 h	
Organism	RR-RGB	RR-RR	RR-RGB	RR-RR	RR-RGB	RR-RR	RR-RGB	RR-RR
EI05	85.8	93	100	100	100	100	100	100
EI15	79.7	83.1	100	100	100	100	100	100
EI21	81.2	83.8	100	100	100	100	100	100
EI25	87.2	95.1	100	100	100	100	100	100

Table 4
Decolourisation studies of Remazol Yellow RGB & Remazol Yellow RR dyes

	% Decolourisation							
	24 h		48 h		72 h		96 h	
Organism	RY-RGB	RY-RR	RY-RGB	RY-RR	RY-RGB	RY-RR	RY-RGB	RY-RR
EI05	81.7	80.6	100	65.6	100	100	100	100
EI15	85.9	66.6	100	75.1	100	100	100	100
EI21	82.7	36.7	100	75.5	100	100	100	100
EI25	89.4	84.4	100	77.2	100	100	100	100

RR-RR. The decolourisation rate of Remazol RR decreased at 48 h by EI05 & EI25 followed by complete decolourisation at 72 h. This marked reduction in decolourisation at 48 h and followed by increase in decolourisation rate at 72 h needs further investigation on the decolourisation mechanism. Gül [39] observed 71.83% removal of RR in 8 d by *Rhizopus arrhizus*. Saratale et al. [40], reported 87% decolourisation of sulphonated azo dye Remazol Red by *Lipsibacillus* sp. at 48 h. Demir et al. [41] investigated the removal of aromatic groups in wastewater containing Remazol Yellow RR dye by white rot fungus *Phanerochaete chrysosporium*. Compared to the previous results, the effect of the microbe EI25 on RY-RGB, RY-RR, RR-RGB and RR-RR was found to be satisfactory, anyhow the detailed mechanism involved in the biodegradation and the effect of various factors on rate of decolourisation have to be studied in future.

Table 5
Morphological characterisation of selected micro-organisms

Tests	EI05	EI15	EI21	EI25
Cell morphology	Rods	Rods	Rods	Rods
Gram nature	+	+	+	+
Motility	Motile	Motile	Motile	Motile
Spore	+	+	+	+
Spore position	Sub terminal	Terminal	Sub terminal/central	Terminal
Spore shape	Oval	Oval	Oval	Oval

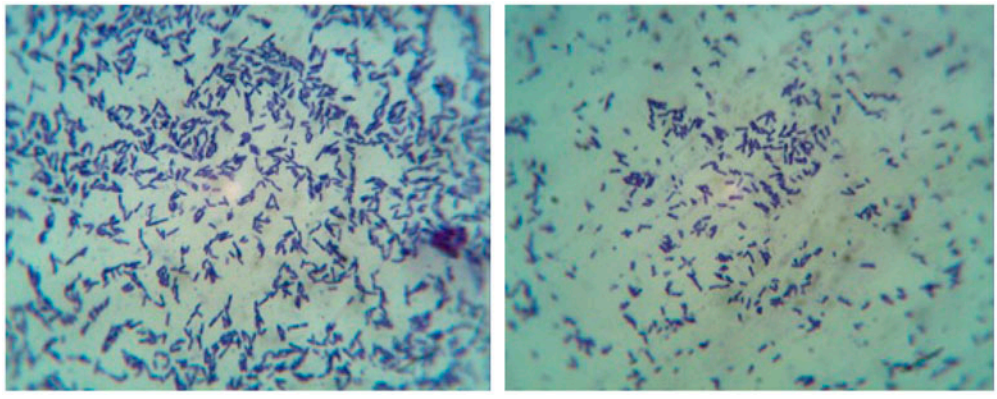

Fig. 1. Gram staining of EI05 and EI25.

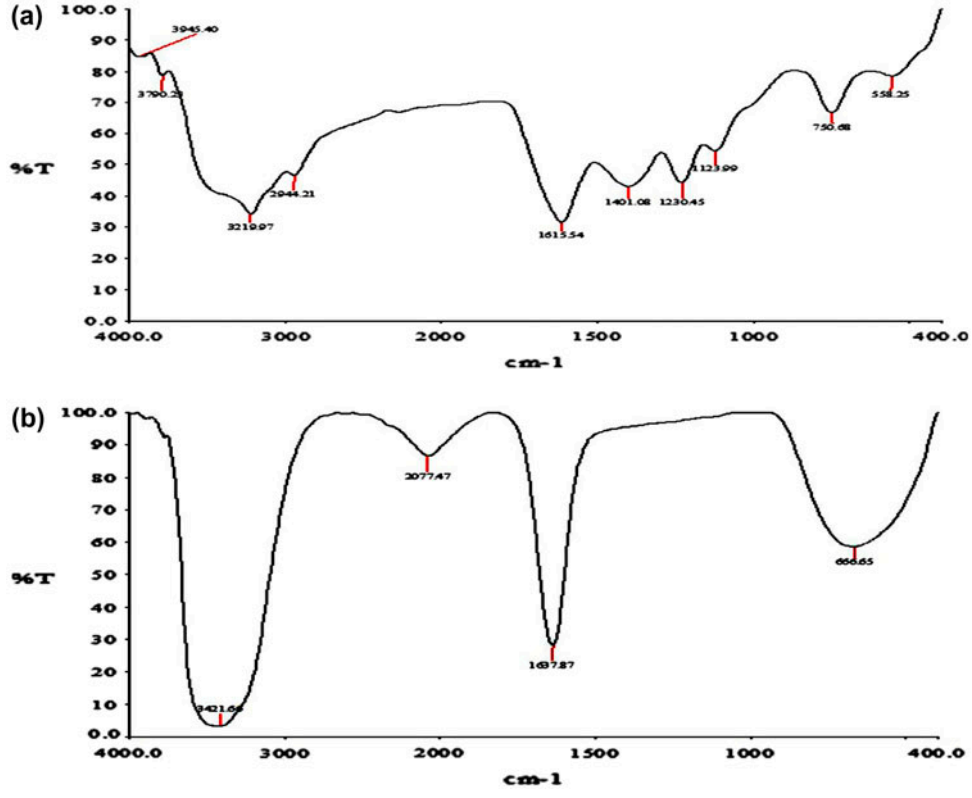

Fig. 2. FTIR spectrum of control dye and treated Remazol Red RGB Dye: (a) control RR-RGB and (b) treated RR-RGB.

3.4. Identification of species

Table 5 shows the morphological characteristics of selected micro-organisms. The gram test showed that all the isolates were gram positive, rod-shaped bacteria. The motility of the isolates was found to be motile. EI05 and EI21 had sub-terminal spore whereas EI15 and EI25 were found to have a terminal spore (Fig. 1) The spore shape was found to be oval in the isolates. From the above observations it was concluded that the micro-organisms may belong to *Bacillus* or *Clostridium* species. According to Cowan and Steel's Manual for Identification of Medical Bacteria [42] and Bergey's Manual of Determinative Bacteriology [37] from the series of biochemical tests, the species were confirmed to be *Clostridium butyricum* (EI05), *Clostridium chaouvei* (EI15), *Clostridium acetobutylicum* (EI25) and *Bacillus subtilis* (EI21) from the sugar fermentation tests.

3.5. Spectral analysis

From the spectral analysis by UV spectrophotometer, the peak present in the control dye was reduced drastically and the peak disappeared at its λ_{max} after decolourisation in all the dyes. Thus stands the proof of decolourisation. Further, biodegradation of remazol dyes was characterised by FTIR spectroscopy. The organism showing the top most decolourisation of the dye has been chosen (EI25) and FTIR spectrum was recorded. The spectrum of control dye and the spectrum of the decolourised sample were overlaid and recorded.

Figs. 2–5 show the spectrum recorded for the control and treated dyes of Remazol Red RGB, Remazol Red RR, Remazol Yellow RGB and Remazol Yellow RR, respectively. Fig. 2 shows the FT-IR spectrum of control dye and treated Remazol Red RGB dye. Compared to the control dye spectrum, the FTIR spectrum of the decolourised sample showed a significant change in the position of peaks. Peaks in the control dye spectrum (Fig. 2(a)) of untreated Remazol Red RGB represented major peaks indicating the stretching vibrations of O–H··· and ···N–H bond at 3,219 cm^{-1}, N=N stretching in azo compounds at 1,615 cm^{-1}, O–H deformation in phenols at 1,401 cm^{-1}, and S=O asymmetric stretching at 1,123 cm^{-1}. On degradation, the spectrum showed only four distinct peaks at 3430.69, 2076.16, 1639.53 and 689.70 cm^{-1}(Fig. 2(b)). Disappearance of various peaks

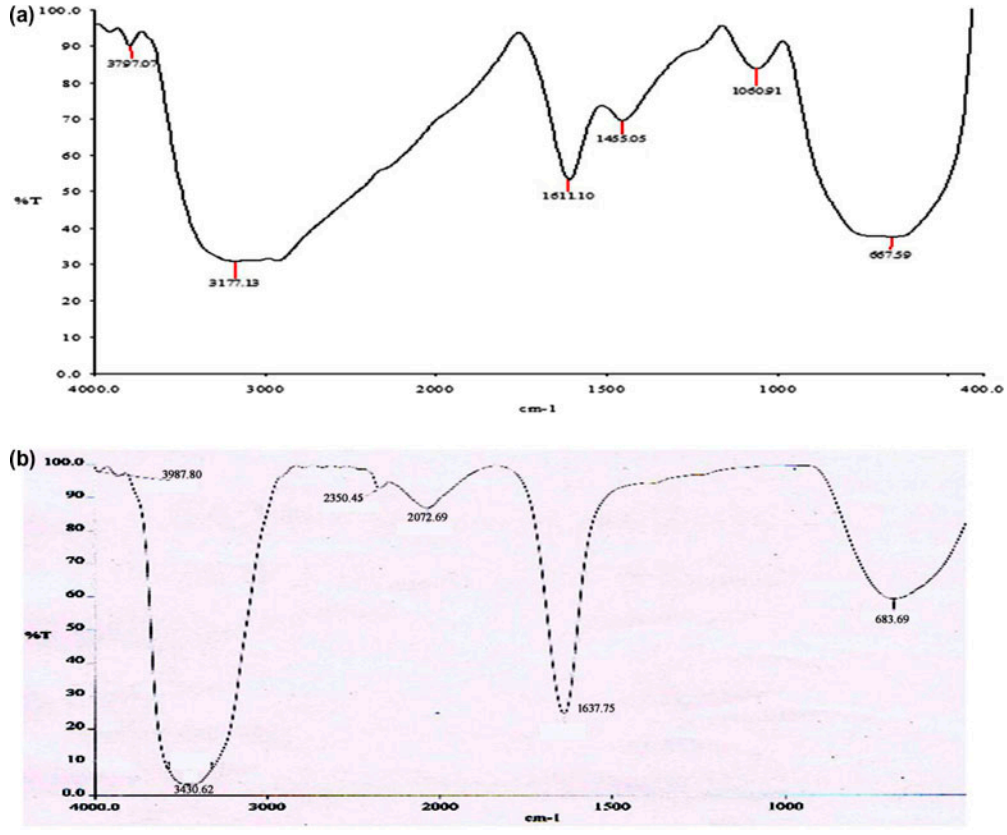

Fig. 3. FTIR spectrum of control and treated Remazol Red RR Dye: (a) control RR-RR and (b) treated RR-RR.

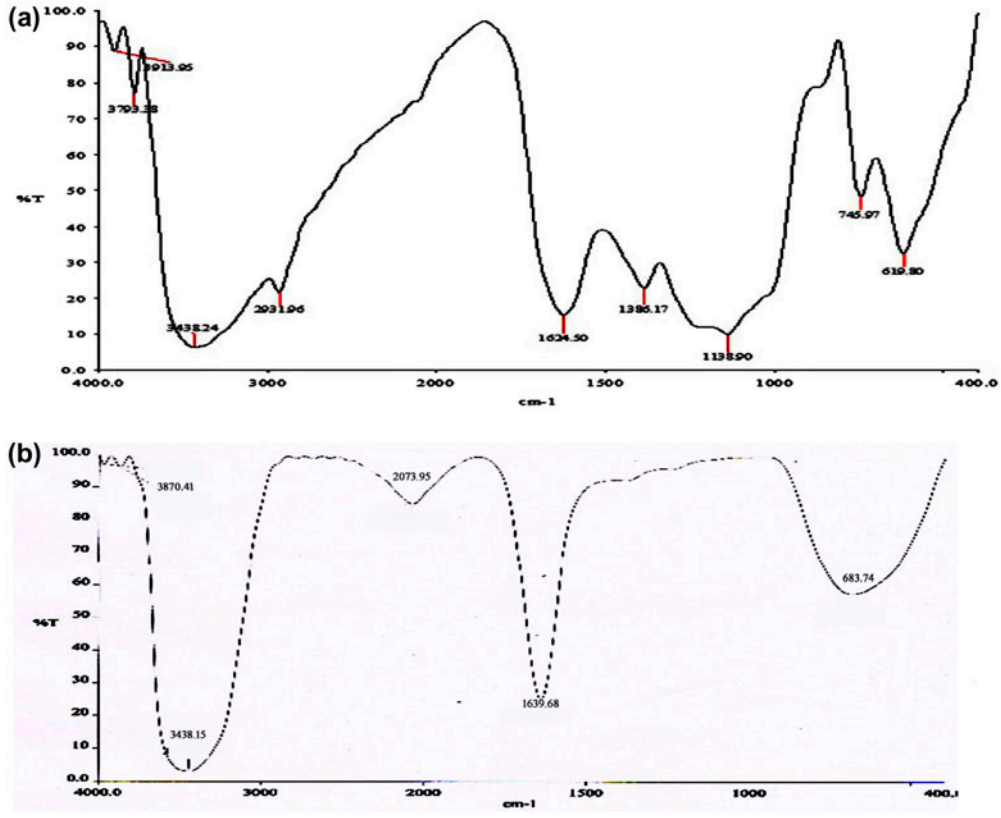

Fig. 4. FTIR spectrum of control and treated Remazol Yellow RGB Dye: (a) control RY-RGB and (b) treated RY-RGB.

and shifting of the main peaks were observed which indicates the biodegradation of the dye.

Fig. 3 shows the FTIR spectrum of control and treated Remazol Red RR dye. The peaks at 3,177 (stretching vibrations of O–H & N–H bond), 1611.10 (N=N azo stretching) and 667.59 (C–H out of plane bending) cm^{-1} in the control dye spectrum of Remazol Red RR (Fig. 3(a)) have been shifted to 3430.62, 1637.75 and 683.69 cm^{-1} in the degraded sample, respectively. The O–H deformation peak at 1453.05 (O–H deformation) in the control dye disappeared in the treated dye. Appearance of two more peaks at 2,396 and 2061 cm^{-1} was observed in the degraded dye sample (Fig. 3(b)).

Fig. 4 shows the FTIR spectrum of control and treated Remazol Yellow RGB Dye. The peaks at 3913.5, 3793.38, 3438.24 (stretching vibrations of O–H & N–H bond), 1624.50 (N=N azo stretching), 1385.17 (O–H deformation in phenols), 1138.90 (S=O asymmetric stretching), and 745.97, 619.30 (aromatic, and C–C bonds) in the control dye spectrum of Remazol Yellow RGB (Fig. 4(a)) were found to be altered in the treated sample. The treated dye sample showed peaks at 3438.15, 2075.95, 1639.68 and 683.74 cm^{-1}. The peaks at 1385.17 and 1138.90 cm^{-1} in the control dye disappeared in the spectrum of decolourised sample. (Fig. 4(b)). The peak at 2075.95 cm^{-1} appeared in the treated sample.

Fig. 5 shows the FTIR spectrum of control and treated Remazol Yellow RR Dye. The peaks at 3915.26, 3796.86, 3371.49 (stretching vibrations of O–H & N–H bond), 2177.98, 1625.25 (N=N azo stretching), 1494.02 (O–H deformation in phenols), 1193.89 (S=O asymmetric stretching) 749.96 and 613.47 (aromatic and C–C bond) cm^{-1} in the control dye spectrum of Remazol Yellow RR (Fig. 5(a)) have been shifted to 3985.80, 3833.50, 3442.21, 2078.90, 1637.75, 1392.70, 1042.94 and 642.04 cm^{-1} in the treated sample, respectively. New peaks appeared in the decolourised sample at 2858.49, 2783.64, 2573.20 and 2391.13 cm^{-1} (Fig. 5(b)). FTIR spectral comparison between the control and EI25-treated dye samples have shown variations like shifting, appearance of new peaks and disappearance of existing peaks. These alterations indicate the changes in the functional groups which may be due to the metabolic degradation of remazol dyes.

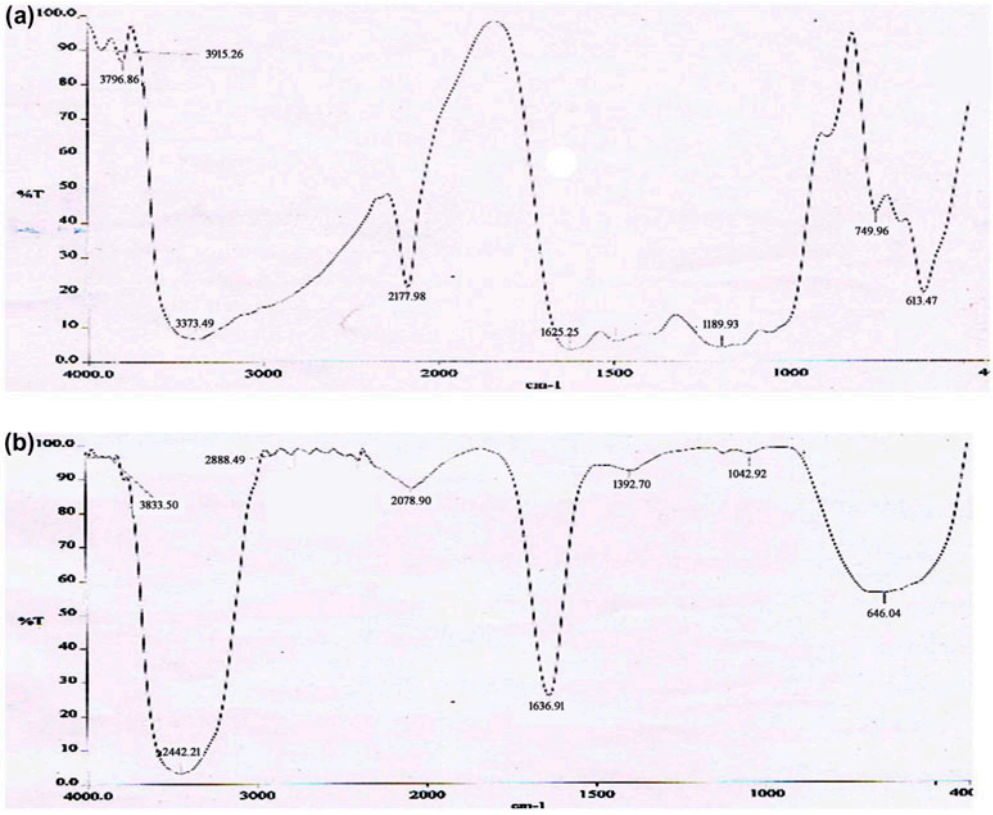

Fig. 5. FTIR spectrum of control and degraded Remazol Yellow RR Dye: (a) control RY-RR and (b) treated RY-RR.

4. Conclusion

The present study was conducted to identify the best indigenous decolourising microbe for the treatment of dye effluent in Tirupur region, Tamilnadu, India. Isolation of potential strains of bacteria based on its decolourising efficiency of the dye effluent (more than 70%) was done and the identified organisms were EI05, EI15, EI21 & EI25. Further investigation of the isolated micro-organisms on remazol reactive synthetic dyes have shown EI25 as the efficient strain in decolourising both effluent and individual dyes. The biodegradation of the dyes were characterised by UV–Visible and FTIR spectroscopy. In UV–Visible spectrum, the clear disappearance of the peak at its respective λ_{max} and significant variation in the peak pattern shown by FTIR spectrum of the remazol dyes by EI25 (*C. acetobutylicum*) indicates the biodegradation of the dyes. Thus the most effective decolourising organism was found to be *C. acetobutylicum* based on all decolourisation studies with the dye effluent and pure dyes. EI25 has also markedly reduced COD, BOD, pH, TDS, EC, etc. of the effluent sample after decolourisation. The species of *C. acetobutylicum* and *butyricum* also have the tendency to produce butanol, butyric acid and other industrially important solvents and gases. The present study concludes that species of *C. acetobutylicum* and *butyricum* along with its other industrial applications can also be used effectively as an economical and eco-friendly biological method of dye effluent treatment. Further investigations on the factors influencing the biodegradation along with its mechanism involved have to be studied.

References

[1] R. Karuppusamy, Tirupur exporters and their strength, Global Res. Anal. 1(5) (2012) 5–14.
[2] A.B.D. Santos, J. Francisco, B. Cervantes Jules, V. Lier, Review paper on current technologies for decolourisation of textile wastewaters: Perspectives for anaerobic biotechnology, Bioresour. Technol. 98 (2007) 2369–2385.
[3] K. Govindarajalu, Industrial effluent and health status – A case study of Noyyal River Basin, in: M.J. Bunch, V. Madha Suresh and T. Vasantha Kumaran (Eds.), Proceedings of the Third International Conference on Environment and Health, December 15–17, Chennai, India, 2003, Chennai, Department of Geography, University of Madras and Faculty of Environmental Studies, York University, 2003, pp. 150–157.

[4] K. Klemola, J. Pearson, P. Lindstrom-Seppä, Evaluating the toxicity of reactive dyes and dyed fabrics with the HaCat cytotoxicity test, AUTEX Res. J. 7(3) (2007) 217–223.
[5] A.J. De Roos, R.M. Ray, D.L. Gao, K.J. Wernli, E.D. Fitzgibbons, F. Ziding, G. Astrakianakis, D.B. Thoma, H. Checkoway, Colorectal cancer incidence among female textile workers in Shanghai, China: A case-cohort analysis of occupational exposures, Cancer Causes Contr. 16(10) (2005) 1177–1188.
[6] N. Mathur, P. Bathnagar, P. Nagar, M.K. Bijarnia, Mutagenicity assessment of effluents from textile/dye industries of Sanganer, Jaipur (India): A case study, Ecotoxicol. Environ. Saf. 61(1) (2005) 105–113.
[7] C. Wang, A. Yediler, D. Lienert, Z. Wang, A. Kettrup, Toxicity evaluation of reactive dyestuffs, auxiliaries and selected effluents in textile finishing industry to luminescent bacteria *Vibrio fischeri*, Chemosphere 46(2) (2002) 339–344.
[8] S.M. Wilkinson, K. McGechaen, Occupational allergic contact dermatitis from reactive dyes, Contact Derm. 35(6) (1996) 376–378.
[9] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247.
[10] A. Birhanl, M. Ozmen, Evaluation of the toxicity and teratogenity of six commercial textile dyes using the frog embryo teratogenesis assay–*Xenopus*, Drug Chem. Toxicol. 28(1) (2005) 51–65.
[11] M.C. Silva, J.A. Torres, L.R. Vasconcelos de Sá, P.M. Batista Chagas, V.S. Ferreira-Leitão, A.D. Corrêa, The use of soybean peroxidase in the decolourization of Remazol Brilliant Blue R and toxicological evaluation of its degradation products, J. Mol. Catal. B: Enzym. 89 (2013) 122–129.
[12] S.B. Jadhav, S.S. Phugare, P.S. Patil, J.P. Jadhav, Biochemical degradation pathway of textile dye Remazol Red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies, Int. Biodeterior. Biodegrad. 65(6) (2011) 733–743.
[13] A. Mittal, V. Thakur, V. Gajbe, Adsorptive removal of toxic azo dye Amido Black 10B by hen feather, Environ. Sci. Pollut. Res. 20(1) (2013) 260–269.
[14] A. Mittal, D. Jhare, J. Mittal, Adsorption of hazardous dye eosin yellow from aqueous solution onto waste material de-oiled soya: Isotherm, kinetics and bulk removal, J. Mol. Liq. 179 (2013) 133–140.
[15] H. Daraei, A. Mittal, J. Mittal, H. Kamali, Optimization of Cr (VI) removal onto biosorbent eggshell membrane: Experimental & theoretical approaches, Desalin. Water Treat. 52 (2014) 1307–1315.
[16] J. Mittal, V. Thakur, A. Mittal, Batch removal of hazardous azo dye Bismark Brown R using waste material hen feather, Ecol. Eng. 60 (2013) 249–253.
[17] H. Daraei, A. Mittal, M. Noorisepehr, F. Daraei, Kinetic and equilibrium studies of adsorptive removal of phenol onto eggshell waste, Environ. Sci. Pollut. Res. 20(7) (2013) 4603–4611.
[18] H. Daraei, A. Mittal, M. Noorisepehr, J. Mittal, Separation of chromium from water samples using eggshell powder as a low-cost sorbent: Kinetic and thermodynamic studies, Desalin. Water Treat. doi:10.1080/19443994.2013.837011.
[19] J. Mittal, D. Jhare, H. Vardhan, A. Mittal, Utilization of bottom ash as a low-cost sorbent for the removal and recovery of a toxic halogen containing dye eosin yellow, Desalin. Water Treat. doi:10.1080/19443994.2013.803265.
[20] A. Mittal, V. Thakur, J. Mittal, H. Vardhan, Process development for the removal of hazardous anionic azo dye Congo Red from wastewater by using hen feather as potential adsorbent, Desalin. Water Treat. 52 (2014) 227–237.
[21] A. Mittal, J. Rajiv, M. Jyoti, V. Shaily, S. Shalini, Removal of Yellow ME 7 GL from industrial effluent using electrochemical and adsorption techniques, Int. J. Environ. Pollut. 43(4) (2010) 308–323.
[22] V.K. Gupta, A. Mittal, D. Jhare, J. Mittal, Batch and bulk removal of hazardous colouring agent Rose Bengal by adsorption techniques using bottom ash as adsorbent, RSC Adv. 2 (2012) 8381–8389.
[23] A. Mittal, R. Jain, J. Mittal, M. Shrivastava, Adsorptive removal of hazardous dye Quinoline Yellow from waste water using coconut-husk as potential adsorbent, Fresenius Environ. Bull. 19(6) (2010) 1–9.
[24] A. Mittal, J. Mittal, L. Kurup, Utilization of hen feathers for the adsorption of Indigo Carmine from simulated effluents, J. Environ. Prot. Sci. 1 (2007) 92–100.
[25] A. Mittal, L. Kurup, Column operations for the removal & recovery of a hazardous dye "Acid Red – 27" from aqueous solutions, using waste materials – Bottom ash and de-oiled soya, Ecol. Environ. Conserv. 13(2) (2006) 181–186.
[26] C.H. Niebisch, A.K. Malinowski, F. Spina, R. Schadeck, A. David Mitchell, V.K. Cordeiro, J. Paba, Decolorisation and biodegradation of reactive blue 220 textile dye by Lentinus crinitus extracellular extract, J. Hazard Mater. 180 (2010) 316–322.
[27] A. Anastasi, B. Parato, F. Spina, V. Tigini, V. Prigione, G.C. Varese, Decolorisation and detoxification in the fungal treatment of textile wastewaters from dyeing processes, New Biotechnol. 29(1) (2011) 38–45.
[28] C.Z.A. Fahmi Abidin, N.R. Rahmat, Multi-stage ozonation and biological treatment for removal of azo dye industrial effluent, Int. J. Env. Sci. Dev. 1(2) (2010) 193–198.
[29] K. Selvam, K. Swaminathan, K.-S. Chae, Decolorisation of azo dyes and dye industry effluent by a white rot fungus *Thelephora* sp., Bioresour. Technol. 88 (2003) 115–119.
[30] C. Valli Nachiyar, S. Sunkar, G. Narendra Kumar, A. Karunya, P.B. Ananth, P. Prakash, S. Anuradha Jabasingh, Biodegradation of Acid Blue 113 containing textile effluent by constructed aerobic bacterial consortia: Optimization and mechanism, J. Biorem. Biodegrad. 3 (9) (2012) 162–171.
[31] P. Saranraj, V. Sumathi, D. Reetha, D. Stella, Fungal decolourization of direct azo dyes and biodegradation of textile dye effluent, J. Ecobiotechnol. 2(7) (2010) 12–16.
[32] R. Leena, D. Selva Raj, Bio-decolourization of textile effluent containing Reactive Black-B by effluent-adapted and non-adapted bacteria, Afr. J. Biotechnol. 7(18) (2008) 3309–3313.
[33] A.S. Arun Prasad, V. Bhaskara Rao, Physico chemical analysis of textile effluent and decolorisation of textile azo dye by Bacillus endophyticus strain VITABR 13, IIOAB J. 2(2) (2011) 55–62.

[34] Government of India and Government of The Netherlands, Hydrology Project, Water Quality Analysis, Operation Manual, 7 (2003) 1–87.
[35] J.P. Jadhav, S.S. Phugare, R.S. Dhanve, S.B. Jadhav, Rapid biodegradation and decolorisation of direct orange 39 (orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH, Biodegradation 21 (2010) 453–463.
[36] N. Ali, A. Hameed, S. Ahmed, Physicochemical characterization and bioremediation perspective of textile effluent, dyes and metals by indigenous bacteria, J. Hazard. Mater. 164 (2009) 322–328.
[37] J.G. Holt, N.R. Krieg, P.H.A. Sneath, J.T. Staley, S.T. Williams, Bergey's Manual of Determinative Bacteriology, 9th ed., Williams & Wilkins, Baltimore, MD, 1994.
[38] S.S. Phugare, D. Kalyani, S. Surwase, J. Jadhav, Ecofriendly degradation, decolorisation and detoxification of textile effluent by a developed bacterial consortium, Ecotoxicol. Environ. Saf. 74 (2011) 1288–1296.
[39] U.D. Gül, Treatment of dyeing wastewater including reactive dyes (Reactive Red RB, Reactive Black B, Remazol Blue) and Methylene Blue by fungal biomass, Water SA 39(5) (2013) 593–598.
[40] R.G. Saratale, S.S. Gandhi, M.V. Purankar, M.B. Kurade, S.P. Govindwar, S.E. Oh, G.D. Saratale, Decolorisation and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated Lysinibacillus sp. RGS, J. Biosci. Bioeng. 115(6) (2013) 658–67. doi:10.1016/j.jbiosc.2012.12.009.
[41] G. Demir, H. Kurtulus Ozcan, N. Tufekci, M. Borat, Decolorisation of Remazol Yellow RR Gran by white rot fungus *Phanerochaete chrysosporium*, J. Environ. Biol. 28(4) (2007) 813–817.
[42] G.I. Barrow, R.K.A. Feltham, Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed., Cambridge: Cambridge University Press, 2003.