



# Biodegradation of reactive yellow EXF dye: optimization of physicochemical parameters and analysis of degradation products

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## Abstract

Biological decolourization of textile dyes is getting more attention as a cost-effective and environmentally friendly technique. Bacterial strain *Proteus mirabilis* has been identified as an effective decolourizer of Reactive Yellow EXF dye. Laboratory-scale experiments were carried out to study effects of physicochemical parameters (pH, temperature, concentration of dye, agitation and sources of carbon) for the maximum decolourization, and the structural changes caused in the dye due to biological treatments were investigated. Maximum decolourization of the dye was observed at 40 °C and pH 7–8. The highest percentage colour removal of more than 95% at 48 h of incubation was observed in the medium containing 5 g/l yeast extract as the carbon source. Further, this bacterial strain could tolerate high concentrations of dye and decolourize dye solutions of 500 mg/l. Ultraviolet–visible spectra and high-performance liquid chromatography analyses clearly indicated changes in dye structures due to treatment. Compounds formed due to degradation of the dye under static and shaking conditions were analysed using gas chromatography–mass spectrophotometry and found to be non-toxic and benign.

**Keywords** Decolourization · *Proteus mirabilis* · Reactive dyes · Textile effluent

## Introduction

Textile dyes used in industrial dyeing processes can be mainly classified into two categories based on their structure and application. Based on application, they can be classified as reactive, acid, direct, basic, mordant, disperse, sulphur and vat dyes (Popli and Patel 2015) and based on structure they can be classified as azo, anthraquinone, sulphur, indigoid, triphenylmethyl (trityl) and phthalocyanine derivatives (Forgacs et al. 2004). Some dyes are banned in textile dyeing processes because of their toxicity. In particular, azo dyes which can produce any of the 22 regulated carcinogenic aromatic amines are banned in dyeing of clothing textiles in the European Union (Brüschweiler and Merlot 2017).

Approximately 10% (Singh and Arora 2011) of dyes use in textile dyeing process may remain unfixed and mix with process water creating coloured effluent. Release of such

dye-containing effluent into water bodies without proper treatments may reduce light penetration through water affecting photosynthetic activities of aquatic flora. This results in the reduction of food sources for aquatic organisms and ultimately destroy aquatic flora and fauna (Pereira and Alves 2012). In order to reduce possible harmful effects due to coloured effluents and to meet discharge standards, industries use different chemical and physical methods to decolourize textile effluents (Singh and Arora 2011). However, those treatment methods have a number of drawbacks such as production of secondary sludge, incomplete removal of dyes and high cost (Saratale et al. 2011). Biological dye decolourization techniques currently capture more attention over chemical and physical treatment methods due to their environmental friendly and cost-effective nature.

Biosorption and biodegradation are the two main mechanisms of biological dye decolourization. Different microbial species such as bacteria, fungi and algae with dye decolourization potential have been reported in the literature (Olukanni et al. 2010; El-Sheekh et al. 2009; Bafana et al. 2008; Deng et al. 2008; Jadhav et al. 2007). In biosorption, the original structure of the dye particle may not be destroyed but the dye will be entrapped in living or dead microbial cells (Pereira and Alves 2012). However, in biodegradation,

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complex dye structures are broken down to simple molecules destroying the chromophore groups of the dyes resulting colour removal. Microbial enzymes such as azoreductases, peroxidases and phenoloxidases (lignin peroxidase, manganese peroxidase, tyrosinase, laccase and N-demethylase) are reported to be responsible for degradation of azo dyes (Solís et al. 2012). Bacterial degradation of azo dyes under anaerobic or oxygen-limited conditions is due to reductive cleavage of azo bond producing their respective aromatic amines in the presence of azoreductase enzymes (Solís et al. 2012; Saratale et al. 2011).

Most of these biological dye decolourization studies have been conducted for model dyes with known structures such as Methyl red (Jadhav et al. 2007), Indigo and Congo red (Khelifi et al. 2009) and Malachite Green (Deng et al. 2008). Application of such dyes in current industrial dyeing processes is limited, and recently developed dyes with enhanced properties are mostly used instead. Biological decolourization of these dyes and analysis of their degraded compounds are rarely reported. The main reason for this may be the unavailability of dye structures due to trade secrets. However, bi-functional Yellow EXF dye (Iqbal 2008) was selected for this study considering its enhanced dyeing properties and current usage in textile industries. The locally isolated bacterial strain identified as *Proteus mirabilis* was able to decolorize this dye effectively (Madhushika et al. 2019a, 2019b). Physicochemical parameter optimization for decolourization and identification of products formed due to degradation are aimed in this study in view of applying these findings in industrial dye effluent treatment.

This research was conducted in the microbiology laboratory in the Department of Chemical and Process Engineering, University of Moratuwa, from February- October 2018.

## Materials and methods

### Bacterial strain

*Proteus mirabilis* (GenBank accession number MT138732.1), isolated from a textile processing industry wastewater treatment plant, was used in the study. Isolation and identification procedures of this microorganism are reported elsewhere (Madhushika et al. 2019a).

### Dyes and reagents

Commercial grade Reactive Yellow EXF (Sumifix supra) dye was kindly provided by a local textile processing industry located in Colombo, Sri Lanka. Reactive Yellow EXF is a mixture of reactive dyes and its structure is not available

due to trade secrets. All chemical reagents used for media preparation were of analytical grade.

### Dye decolourization

Dye decolourization was conducted in 250-ml Erlenmeyer flasks containing decolourization medium (100 ml) and 50 mg/l of Yellow EXF dye. Mineral salt medium (MSM) consisting of 12.8 g/l  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 3 g/l  $\text{KH}_2\text{PO}_4$ , 0.5 g/l NaCl and 1.0 g/l  $\text{NH}_4\text{Cl}$  and 5.0 g/l of yeast extract was used as the decolourization medium. pH of the medium was adjusted to 7. *Proteus mirabilis* was precultured in Luria–Bertani medium at 35 °C under static conditions for 24 h to be used as the inoculum. Then, the flasks containing decolourization medium were inoculated after adding 2% (v/v) of inoculum and incubated at 35 °C under static (oxygen-limited) conditions. 5 ml samples were withdrawn from the decolourization medium at 24 h intervals, centrifuged at 12,000 rpm for 10 min, and the supernatant was scanned at the maximum absorbance wavelength of the dye (422 nm) under visible light of the spectrophotometer (UV-1800 Shimadzu spectrophotometer). An uninoculated dye-free medium was used as the blank. Dye decolourization percentages were calculated using Eq. 1.

$$\text{Percentage decolourization} = \frac{(A_{\text{initial}} - A_{\text{final}}) \times 100\%}{A_{\text{initial}}} \quad (1)$$

where  $A_{\text{initial}}$  is the absorbance before decolourization and  $A_{\text{final}}$  is the absorbance after a certain time of decolourization. Cell concentration was calculated using optical density values of samples at 600 nm wavelength ( $\text{OD}_{600}$ ) as reported in (Madhushika et al. 2019a, b; Silveira et al. 2009; Chen 2002).

Temperature, pH, effect of agitation, carbon source and dye concentration on colour removal were investigated, and each parameter was optimized by changing the parameter in interest while keeping all the other parameters constant. All investigations were done in duplicate.

### Effect of agitation

The effect of agitation on dye decolourization was investigated by maintaining inoculated dye and nutrients containing flasks under both static and shaking conditions. Shaking speed was set at 120 rpm based on literature (Saratale et al. 2013). Decolourization was conducted as in "Dye decolourization", and the percentage decolourizations were calculated by measuring the absorbance of samples (Eq. 1).

### Effect of pH

Decolourization of Yellow EXF dye at various initial pH values (5–10) was studied by adjusting the pH of the media using 0.1 N NaOH and HCl before sterilization of the media. Each sample was inoculated with 24 h grown cultures of *Proteus mirabilis* and incubated as described in "Dye decolourization". Samples collected after 18, 26 and 42 h of incubation were tested, and the percentage decolourizations were calculated (Eq. 1).

### Effect of temperature

Biological dye decolourization is mainly attributed to various enzymes produced by microorganisms. Enzymes are highly temperature-sensitive and high temperature conditions may denature these enzymes resulting inefficient decolourization. Therefore, the ability of this bacterial strain to decolourize Yellow EXF dye under different temperature conditions was studied by incubating flasks at different temperatures; 25, 30, 35, 40 and 45 °C and the percentage decolourizations were calculated.

### Effect of carbon sources

The effect of different carbon sources in biological decolourization of the dye was investigated using 5 g/l of glucose, corn starch and yeast extract in the decolourization medium. Yeast extract (Anjaneya et al. 2011; Vijaykumar et al. 2007) and glucose (Bafana et al. 2008; Khalid et al. 2008) are commonly used carbon sources in dye decolourization studies. Moreover, corn starch was selected in this study considering its cost-effectiveness in industrial-scale applications. An additional study was carried out with 2 g/l yeast extract in the medium. All these decolourization studies were conducted as in "Dye decolourization" and the percentage removal of colour was calculated.

### Effect of initial dye concentration

The ability of *Proteus mirabilis* to tolerate and decolourize Yellow EXF dye at initial dye concentrations of 50, 100, 200, 300, 400 and 500 mg/l was investigated. In typical industrial effluents, the concentration of the dye does not exceed 500 mg/l and hence this dye concentration range was selected. Dye decolourization with each of the above initial dye concentrations was conducted (as in "Dye decolourization") and the percentage decolourization was calculated.

## Degraded product analysis

### High-performance liquid chromatography (HPLC) analysis

Yellow EXF dye sample was decolourized as described in Sect. "Dye decolourization". After 72 h of decolourization, the contents in the flask were centrifuged at 12,000 rpm for 10 min and the resulting supernatant was clarified by passing through a 0.45 µm filter (Bafana et al. 2008).

Filtered sample was analysed using HPLC. Prior to analysis, filtered sample was extracted using t-butyl methyl ether (MTBE) based on the ISO 14,362 standard (which is used for the determination of certain aromatic amines derived from azo colourants) and concentrated. Then, the extracted metabolites were analysed in an HPLC (1260 infinity series using C18 reverse-phase column, at 32 °C using eluent methanol and potassium dihydrogen phosphate in water) to identify spectral changes of compounds due to biodegradation. The injection volume was 5 µl and the flow rate was 0.6–2.0 ml/min (gradient flow).

### Gas chromatography–mass spectroscopy (GCMS) analysis

Two Yellow EXF dye samples were decolourized (in static conditions) as described in Sect. "Dye decolourization". After 72 h of decolourization, the contents in one flask were centrifuged at 12,000 rpm for 10 min and the resulting supernatants were clarified by passing through a 0.45 µm filter.

Contents in the other flask were centrifuged at 12,000 rpm for 10 min, and the supernatant was transferred to a sterile fresh flask under aseptic conditions. This flask was inoculated once again with 2% (v/v) of 24 h grown culture of *Proteus mirabilis* and placed in a shaking incubator at 35 °C, and 120 rpm rotational speed. After 7 days of incubation, contents in that flask were centrifuged and filtered through a 0.45 µm filter.

Both filtrates were extracted (as done for HPLC analysis) and concentrated metabolites were analysed in a GCMS (5975C inert MSD, 7890A GC system) and the compounds were identified using national institute of standards and technology (NIST) library.

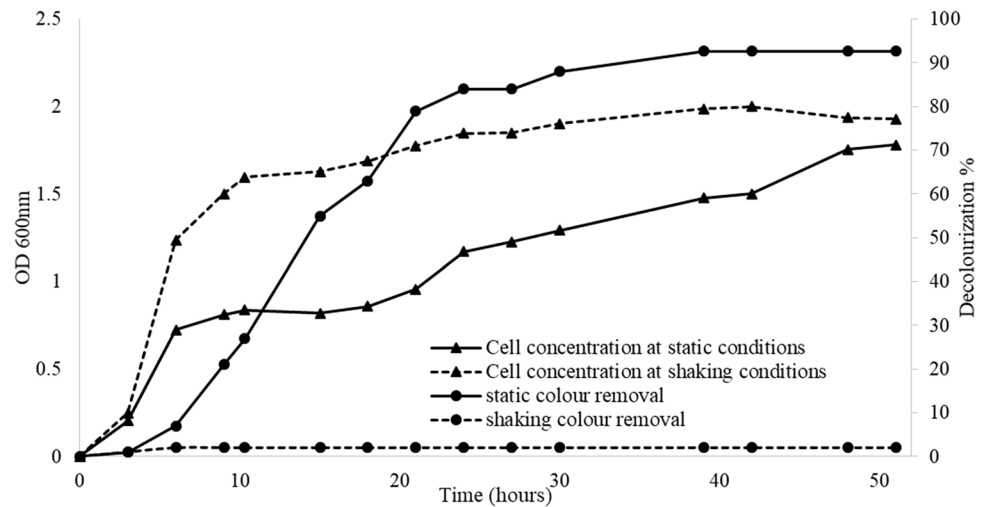
## Results and discussion

### Effect of agitation

Yellow EXF dye decolourization by *Proteus mirabilis* under static conditions showed significant differences to that under shaking conditions. In static conditions, more than 80% colour removal was observed within 24 h, whereas under



**Fig. 1** Time-dependent cell growth and decolourization of Yellow EXF dye under static and shaking conditions



shaking conditions colour removal was only 2% (Fig. 1). This shows that agitation has a negative effect on colour removal.

Contrarily, biomass concentrations were high under shaking conditions compared to static conditions and this is because shaking increases mass and oxygen transfer between cells and the medium (Solís et al. 2012) which is favourable for the growth of this facultative anaerobic bacteria. Furthermore, there is no effect of the amount of biomass on colour removal in oxygen-rich media. According to (Chang and Kuo 2000), dissolved oxygen (DO) level in the media dropped nearly to zero at static incubation of cultures of *E. coli* NO3 strain while DO level greater than 0.5 mg/l was detected in cultures agitated at 200 rpm.

Electrons required for azo bond cleavage are not readily available under shaking conditions, because oxygen take up the electrons available in the medium as it is a better

electron acceptor than the azo dye (Erkurt 2010; dos Santos et al. 2007). Hence, dye decolourization is more effective under static conditions than under shaking conditions. Similar results reported for biological decolourization of different textile dyes at static and shaking conditions are given in Table 1.

### Effect of pH

Figure 2a shows that pH value of the decolourization medium has a significant effect on biological decolourization of Yellow EXF dye. After 18 h of incubation, the highest dye decolourization was observed in the culture maintained at pH 7.5. However, the highest final decolourization was exhibited by the culture at pH 8 (Fig. 2a). It is clearly observed that the pH range of 7–8 is more appropriate for decolourization

**Table 1** Decolourization of different dyes by various bacteria

Microorganism	Dye	Colour removal	Reference
<i>Aeromonas hydrophila</i>	Red RBN	Dye concentration reduced from 50 mg/l to $\approx$ 38 mg/l within 1.5 days under shaking condition Dye concentration reduced from 50 mg/l to $\approx$ 4 mg/l within 1.5 days under anaerobic <sup>a</sup> and static <sup>b</sup> conditions	(Chen et al. 2003)
<i>Citrobacter sp.</i> CK3	Reactive red 180	13% decolourization within $\approx$ 72 h under shaking condition 96% decolourization within $\approx$ 72 h under anaerobic condition	(Wang et al. 2009)
<i>Proteus vulgaris</i> and <i>Micrococcus glutamicus</i>	Scarlet R	30–35% decolourization under shaking condition 100% decolourization under static condition	(Saratale et al. 2009)
Mixed culture of bacteria	Reactive violet 5R	30% decolourization within 18 h under shaking condition 100% decolourization within 18 h under static condition	(Jain et al. 2012)
<i>Proteus mirabilis</i>	Reactive blue 13	23% decolourization within 5 h under shaking condition 88% decolourization within 5 h, under static anoxic condition	(Olukanni et al. 2010)

<sup>a</sup> At the beginning of the experiment, culture had bubbled with pure nitrogen until the DO became zero

<sup>b</sup> Culture had not bubbled with nitrogen but kept at static conditions without shaking



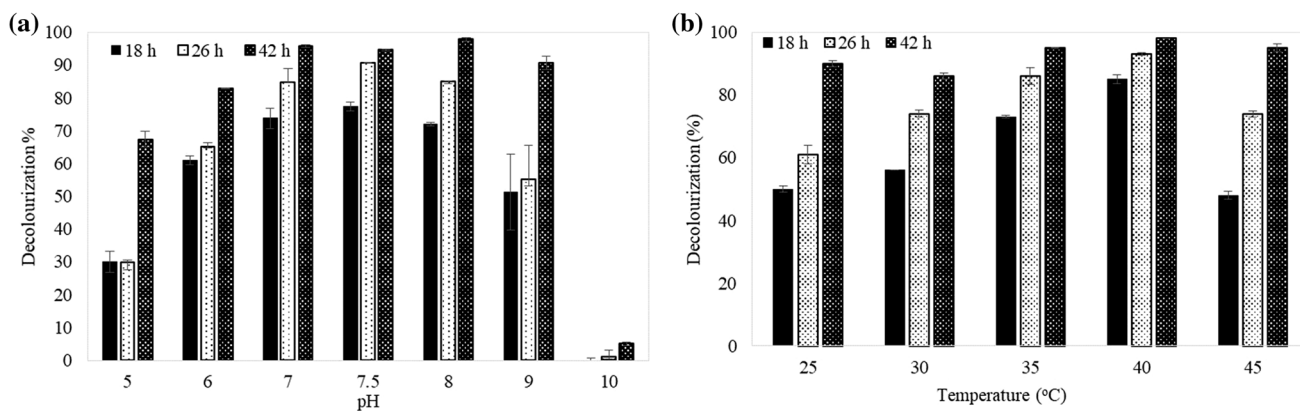


Fig. 2 Effect of a pH and b temperature on Yellow EXF decolourization by *Proteus mirabilis*

of Yellow EXF dye. Olukanni et al. (2010) reported pH 7 as the optimum pH for decolourization of Reactive Blue 13 dye by *Proteus mirabilis*. According to the literature, *Proteus* species have the ability to grow in 5–10 pH range and optimum growth has been observed at pH 7–8 (Hamilton et al. 2018). Favourable cell growth of *Proteus mirabilis* around neutral pH conditions enhances dye decolourization, indicating that the dye degradation is associated with cell growth. Further, it is reported that pH has an effect on transporting dye molecules through the cell membrane, which is a rate-limiting step for decolourization (Saratale et al. 2011). When compared to other pH values tested in this study, negligible dye decolourization was observed at pH 10. Similar behaviour has been exhibited in the pH optimization studies conducted for decolourization of Red EXF dye by *Proteus mirabilis* (Madhushika et al. 2018).

### Effect of temperature

As shown in Fig. 2b, the maximum percentage dye decolourization was observed at 40 °C. At this optimum temperature, 85% of colour was removed within 18 h of incubation. Even though samples incubated at temperatures 25, 30 and 45 °C indicated low percentage decolourization at 18 h, colour removal at all temperatures considered was more than 85% after 42 h. Generally, the temperature of textile dyeing industrial effluent is above ambient, since it gets mixed with hot water released from dyeing baths. The increased temperature of the industrial effluent provides favourable conditions for effective colour removal by *Proteus mirabilis*.

### Effect of carbon sources

The majority of previous studies reported the requirement of external carbon sources for the decolourization of azo dyes (Silveira et al. 2009). Carbon has the ability to behave as an electron donor and donates electrons required for the

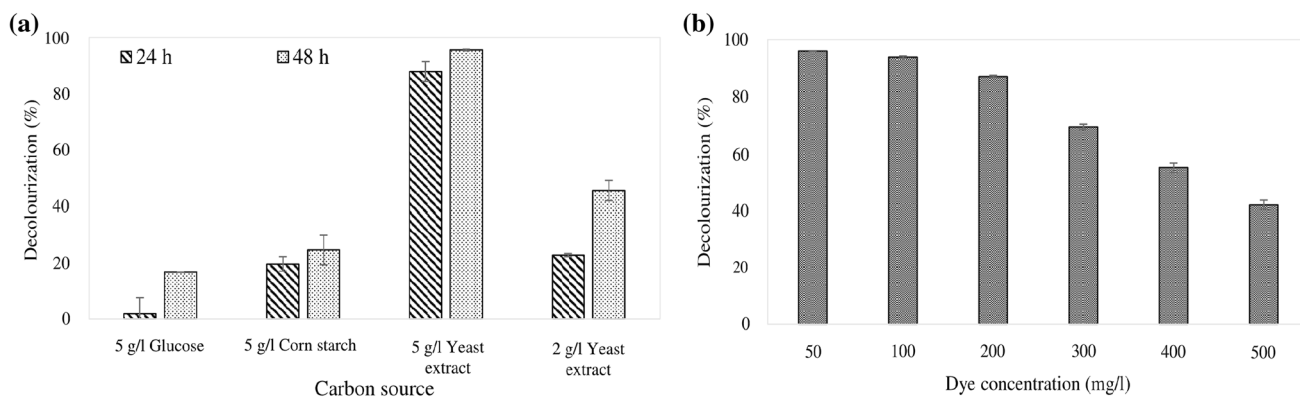


Fig. 3 Effect of a carbon sources and b initial dye concentration on Yellow EXF decolourization by *Proteus mirabilis*

reductive cleavage of azo bond under anaerobic conditions (Solís et al. 2012). Hence, the quantity and the type of carbon source are major parameters that determine the effectiveness of bacterial dye degradation.

Yellow EXF dye solution inoculated with *Proteus mirabilis* did not indicate considerable cell growth or colour removal without an external carbon source (data not shown) in the decolourization medium. Out of the three carbon sources used, yeast extract containing samples indicated the highest colour removal after 24 h and 48 h of incubation (Fig. 3a). However, when the concentration of yeast extract was reduced from 5 g/l to 2 g/l, a reduction in decolourization was observed. Even though low colour reductions were observed in starch and glucose-containing samples at 24 h and 48 h incubation, after 8 days of incubation decolourization percentages increased to 53 and 71%, respectively (data not shown).

Starch is a commonly used additive in textile finishing processes, as well as a frequently used carbon source for microbial growth in biological studies. Hence, the addition of external carbon sources may not be required since starch has the potential to enhance colour removal. However, a significant enhancement of dye decolourization was not observed with starch in the current study. Similarly, Joshi et al. (2008) reported yeast extract as a more effective co-substrate than glucose or starch for decolourization of azo dyes by a bacterial consortium containing *Proteus mirabilis*. According to the literature, carbohydrates such as glucose and starch can effectively be utilized as co-substrates for dye decolourization under methanogenic conditions, although yeast extract is preferred under static (microaerophilic/anoxic) conditions (Joshi et al. 2008; Pandey et al. 2007).

Utilization of different carbon sources such as glucose, starch, acetate, yeast extract, ethanol whey and tapioca in various dye degradation experiments with different microbial strains are reported in the literature (Singh 2014; Deng et al. 2008; Moosvi et al. 2007). Type and quantity of the carbon source required for optimum decolourization of a particular dye will vary depending on the microbial species utilized for the experiment.

Besides being the source of carbon, yeast extract involves in the regeneration of NADH (reduced form of nicotinamide adenine dinucleotide) that acts as an electron donor for the degradation of azo bonds (Moosvi et al. 2007). The ability of yeast extract on providing carbon, vitamins and other compounds which may serve as electron shuttle (Imran et al. 2016) could be the reason for observed enhanced dye decolourization in yeast extract containing medium.

## Effect of dye concentration

In this study, dye concentrations up to 500 mg/l were used to investigate the dye tolerance of this bacteria. Textile effluents contain only unfixed dyes and hence dye concentrations in industrial effluent are generally much lower than 500 mg/l. According to the literature (Madhushika et al. 2019b; Jadhav et al. 2015; Santhanam et al. 2015), absorption spectra of textile effluents indicate absorbance values lower than 1.5 at 422 nm wavelength whereas 100 mg/l Yellow EXF dye has more than 1.5 absorbance.

With the increase of initial dye concentration, biodegradable organic load also increases and the percentage decolourization is expected to drop when the same volume of inoculum (2% (v/v)) is used, as observed in Fig. 3b.

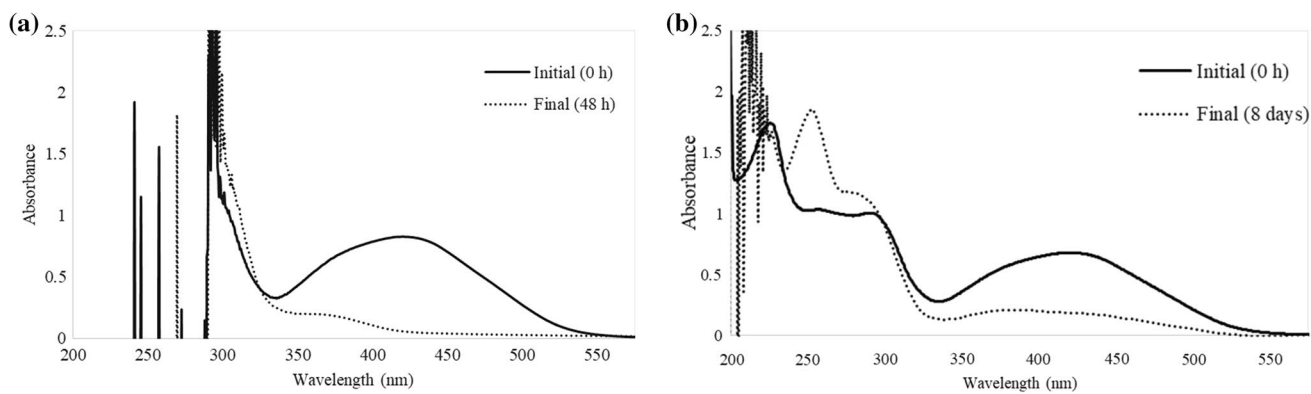
Even though it is reported that the toxic nature of highly concentrated textile dyes may inhibit the growth of most microorganisms in dye containing environments (Silveira et al. 2009), Joshi et al. (2008) effectively decolourized Acid Orange 7 dye by a bacterial consortium composed with *Proteus mirabilis* up to 200 mg/l. However, in this work, *Proteus mirabilis* was able to grow in flasks containing up to 500 mg/l of Yellow EXF and resulted in more than 40% decolourization. The ability of this bacterial strain to tolerate and decolourize highly concentrated dye solutions could be beneficial when implementing biological decolourization techniques in textile industries with high loads of residual dyes.

Nevertheless, textile industry effluent contains various quantities of different chemical substances such as salts, detergents and organic acids and these may negatively affect the microbial growth and consequently on the biological dye decolourization (Khalid et al. 2008; Hessel et al. 2007). Hence, further studies should be conducted to evaluate the ability of this bacterial strain to survive and decolourize textile industry effluents.

## Degraded product analysis

### UV-visible spectrophotometric analysis

UV-visible spectra of Yellow EXF dye and decolourized samples (dye degraded products) in media containing yeast extract and glucose as the carbon source are shown in Fig. 4. Changes in spectra prior to and after the biological treatment are an indication of the disappearance of initial substances from the medium and formation of new substances. In both figures, maximum absorbance peak of Yellow EXF dye is observed at 422 nm wavelength and it is due to the absorption of light by the chromophore group of the dye (Pereira and Alves 2012). This peak in the UV visible spectrum of the biologically treated samples has significantly decreased



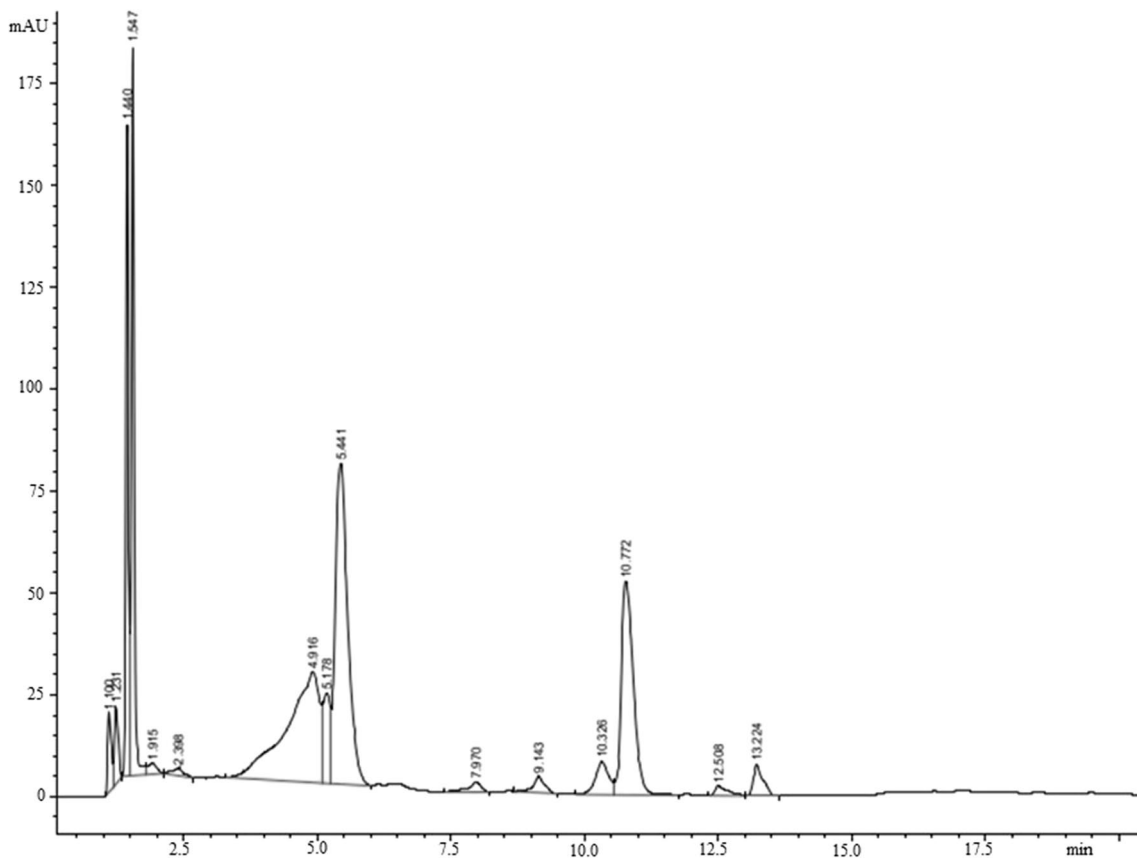
**Fig. 4** UV-visible spectrums of Yellow EXF dye before and after the biological treatments in **a** yeast extract and **b** glucose containing media

indicating the removal of the chromophore group. Due to the large conjugated system around the chromophore group, it can absorb light at low frequencies indicating maximum absorbance peaks at the visible range of the spectrum. Other conjugated systems such as benzene and naphthalene are possible candidates present in dye molecules indicate absorbance peaks at the UV range of the spectrum (Feng et al. 2000). As the dye is degraded, dye structure will be broken down into simpler compounds, and the strength of

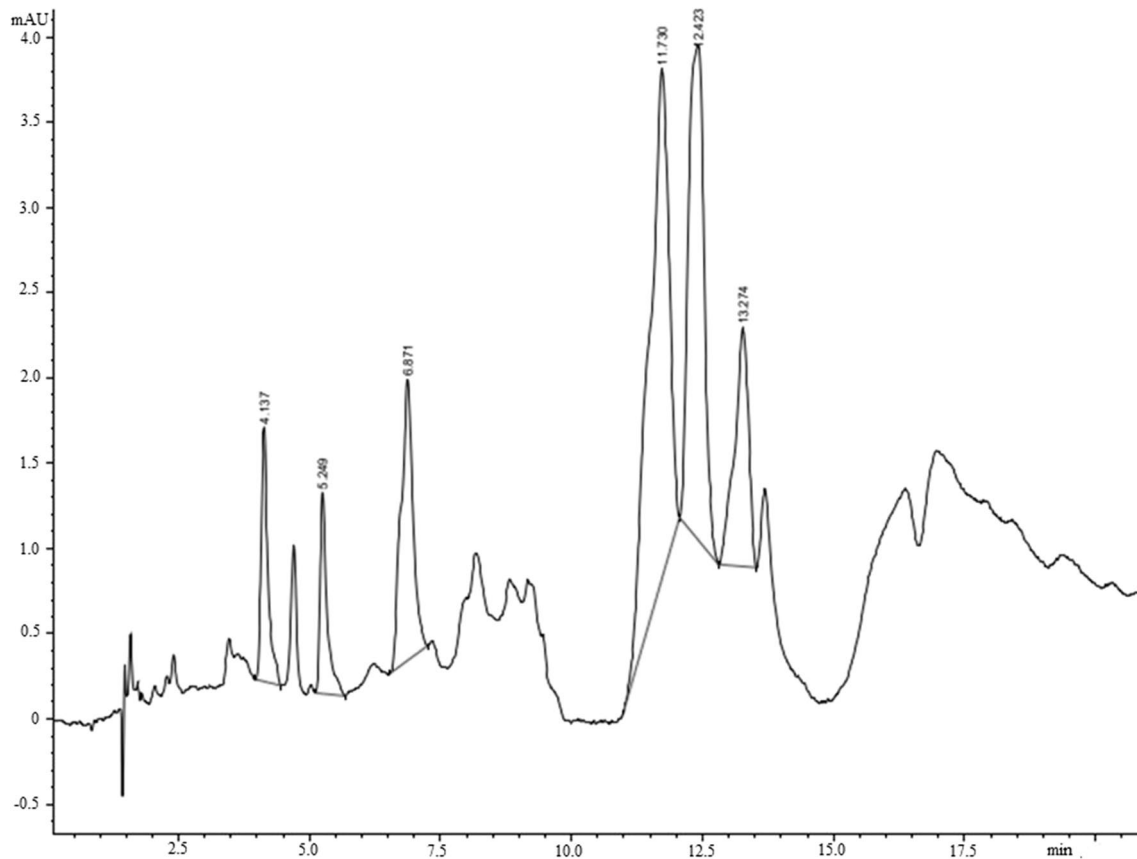
the conjugated system will be reduced, resulting in the formation of peaks in the UV range.

In this study, a peak was observed at 225 nm wavelength for the glucose-containing medium prior to biological treatment; however, this peak was not visible in yeast extract containing medium due to noises appeared in the UV range of the spectrum (Fig. 4a).

This peak at 225 nm was not observed after biological treatments but a new peak is visible at 252 nm



**Fig. 5** HPLC chromatogram of Yellow EXF dye



**Fig. 6** HPLC chromatogram of dye degraded products

wavelength indicating dye degradation and formation of degraded products (Fig. 4b). Formation of aromatic amines during the degradation of azo dyes in oxygen-limited conditions has been reported in the literature (Anjaneya et al. 2011; Bafana et al. 2008). Peaks relevant to such aromatic amines can mostly be observed in the range of 190 nm–380 nm wavelength in the UV–visible spectrum (Pinheiro et al. 2004). Hence, it can be expected that the peak at 252 nm as an indication of the formation of an aromatic amine.

#### HPLC analysis

HPLC chromatogram of Yellow EXF dye and dye degraded products are shown in Fig. 5 and Fig. 6, respectively. Major peaks appeared in original dye chromatogram at retention times 1.44, 1.547, 4.916, 5.441 and 10.772 min with peak areas of 557, 666, 1158, 1283 and 830 mAU.s, respectively, were not observed in the chromatogram of degraded products. Few minor peaks were observed at retention times 11.730, 12.423 and 26.753 min with peak areas of 81, 60 and 148 mAU.s, respectively, in the HPLC chromatogram of degraded

products. The absence of prominent peaks observed in the HPLC chromatogram of the dye after the biological treatments can be attributed to degradation of original dye structure and minor peaks observed at new retention times demonstrated the formation of degraded products in Yellow EXF dye due to biological treatments.

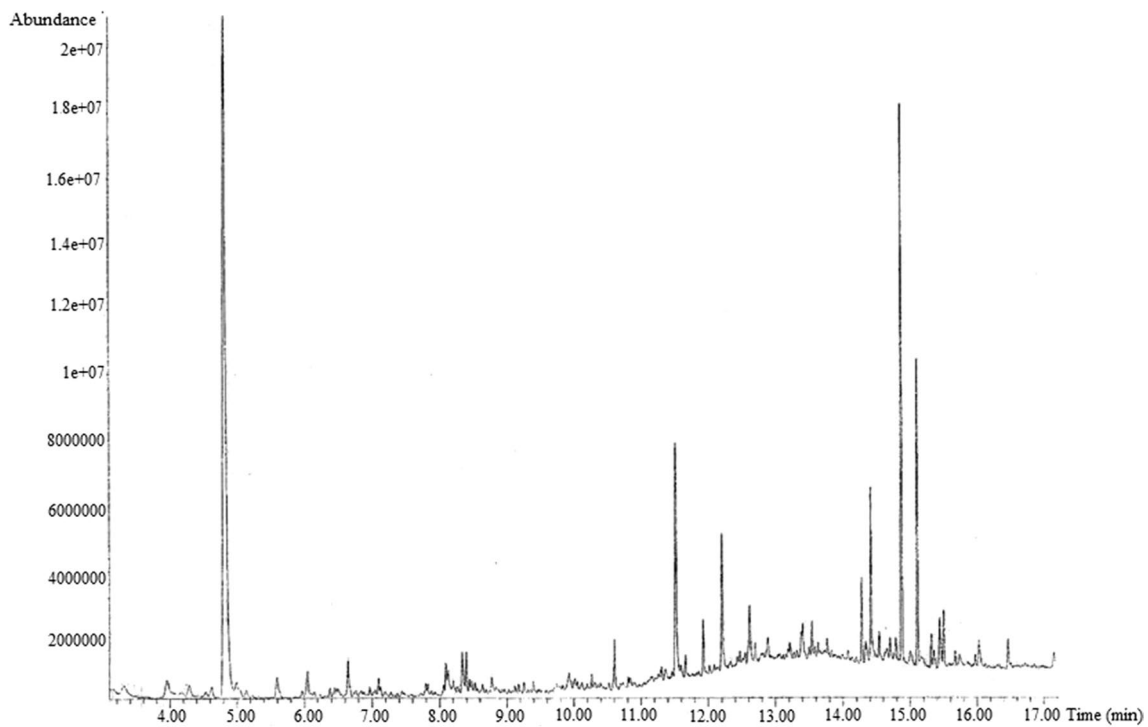
#### GCMS analysis

Gas chromatograms of the Yellow EXF dye after static treatment and after both static and shaking culture treatments are shown in Figs. 7 and 8, respectively.

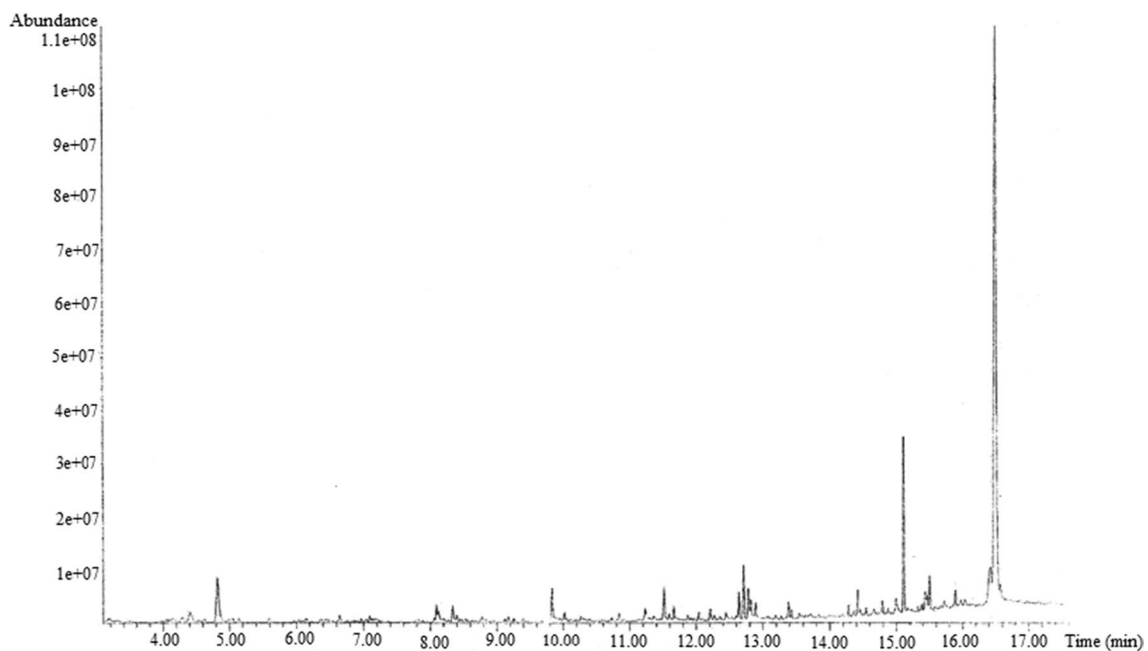
As shown in Fig. 7, gas chromatogram of the sample treated under static condition indicated prominent peaks at 4.825, 10.604, 11.521, 12.208, 14.875 and 15.108 min retention times. However, the compounds that indicated more than 89% matching quality in library search were only considered in the analysis of degraded products. Based on the library search, peaks observed at retention times 4.825, 11.521, 12.208, 10.604 and 3.954 min were identified as Phenylethyl alcohol, 1H-Indole-3-ethanol, 1H-Indole-3-carboxaldehyde, Benzenemethanamine N-(phenylmethyl)- and Benzyl alcohol (Fig. 7). According to gas chromatogram







**Fig. 7** Gas chromatogram of the dye degraded products



**Fig. 8** Gas chromatogram of the mineralized degraded products under shaking conditions

(Fig. 7), Phenylethyl alcohol is the major compound present in degraded products and contributed to 41.98% peak area while Benzenemethanamine, N-(phenylmethyl)- contributed to 1.53%.

Formation of aromatic amines as a result of azo bond cleavage by bacteria under oxygen-limited (static) conditions is reported in the literature (Anjaneya et al. 2011; Bafana et al. 2008). However, in this analysis, only

Benzenemethanamine, N-(phenylmethyl)- has been detected as an aromatic amine and this compound could either originally be present in the parent dye or formed due to degradation of the original dye by *Proteus mirabilis*.

Cleavage of azo bond in dye structure is the main mechanism of biological decolourization and further degradation and mineralization of degraded products can be achieved under aerobic conditions (Popli and Patel 2015). In the presence of oxygen, aromatic amines can be further degraded into simpler compounds by the insertion of oxygen into those molecules. Some aromatic amines are reported to undergo oxidative deamination producing different intermediate products under shaking culture conditions (Karunya et al. 2014), whereas some microbial species have the ability to completely mineralize aromatic amines into CO<sub>2</sub>, H<sub>2</sub>O and NH<sub>3</sub> (Pereira and Alves 2012).

Peaks observed in gas chromatogram of the sample treated under static conditions (Fig. 7) have almost disappeared after incubation in shaking conditions. However, few major peaks have been formed at 15.114 and 16.516 min retention times (Fig. 8) indicating formation of new compounds. Furthermore, out of the previously detected compounds, only Phenylethyl alcohol was detected after incubation in shaking conditions (Fig. 8) but, a significant (87%) reduction of gas chromatogram peak area was observed, compared to the sample treated under static conditions.

Hence, it can be assumed that this bacterial strain could decolourize Yellow EXF dye under static conditions and further breakdown degraded products under shaking conditions. Even though a large number of studies reported on the dye decolourization ability of different bacteria, only few reported mineralization of dye degraded products (Karunya et al. 2014; Elisangela et al. 2009; Bafana et al. 2008). Hence, this bacterial strain can be considered as a favourable and eco-friendly solution for textile dye decolourization applications.

## Conclusion

The isolated bacterial strain *Proteus mirabilis*, used in this study, was able to decolourize Yellow EXF dye effectively under static conditions and further breakdown the degraded products under shaking conditions. Optimum temperature and pH for the dye decolourization in the medium containing yeast extract as the carbon source was observed at 40 °C and pH 7–8. Dye decolourization ability at a broad range of temperatures, ability to survive at high dye concentrations and potential to mineralize dyes into non-toxic compounds

indicate the appropriateness of this bacterial strain as a potential candidate in industrial effluent decolourization applications.

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**Data availability and material** All data generated or analysed during this study are included in this published article.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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