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ORIGINAL PAPER

# Decolorization and biodegradation of reactive Red 198 Azo dye by a new *Enterococcus faecalis–Klebsiella variicola* bacterial consortium isolated from textile wastewater sludge

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## Abstract

The present study investigated biodegradation and removal of Reactive Red 198 (RR198) dye from aqueous environments using a new bacterial consortium isolated from textile wastewater sludge on laboratory scale via batch study. Two bacterial species, *Enterococcus faecalis* (*EF*) and *Klebsiella variicola* (*KV*), were identified after isolation, through biochemical assays, Polymerase chain reaction (PCR), and 16S rRNA gene sequencing. To determine their ability to biodegrade RR198 dye, physicochemical parameters, including bacterial concentration, time, pH, and temperature, were tested; the results showed that the best conditions included a bacterial concentration of 3.5 mL × $10^5$ cells/mL and incubation time of 72 h. Under such conditions, the removal efficiency of RR198 dye at an initial concentration of 10–25 mg/L was more than 98%; however, for concentrations of 50, 75, and 100 mg/L, removal efficiency was reduced to 55.62%, 25.82%, and 15.42%, respectively ($p = 0.005$). The highest removal efficiency occurred at pH 8.0, reaching 99.26% after 72 h of incubation. With increasing the incubation temperature from 25 °C to 37 °C, removal efficiency increased from 71.71 to 99.26% after 72 h of incubation, and increasing the temperature from 37 to 45 °C, the removal efficiency was reduced ($p \leq 0.001$). Therefore, the *EF–KV* bacterial consortium can be used for efficient removal of RR198 dye from textile effluent.

**Keywords** Biodegradation · Decolorization · Rreactive red 198 · Bacterial consortium · *Enterococcus faecalis* · *Klebsiella variicola*

## Abbreviations

| | |
|---|---|
| RR198 | Reactive red 198 |
| *EF* | *Enterococcus faecalis* |
| *KV* | *Klebsiella variicola* |
| PCR | Polymerase chain reaction |
| TSB | Tryptic soy broth |
| SDS | Sodium dodecyl sulfate |
| PBS | Phosphate buffered saline |
| ANOVA | One-way analysis of variance |
| BC | Bacterial concentration |

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## Introduction

Industrial development has led to the entry of various chemical compounds and contaminants into the biosphere and water resources (Eslami et al. 2017a; Khosravi et al. 2018; Shahi et al. 2013). Industrial dyes are among these chemical contaminants, and they are divided into three main categories: (1) anionic dyes, including acidic and reactive dyes, (2) cationic dyes, and (3) non-ionic disperse dyes (Saadatjou et al. 2009). Azo dyes, such as reactive 198 dyes, are widely used in the cosmetics, leather, paper and cardboard,

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and textile industries due to their stability as well as their simpler and lower-cost production compared to that of other dye types (Singh et al. 2015; Yagub et al. 2014). Dyes are naturally visible at concentrations above 1 mg/L, while the average concentration of dye in textile wastewater is 300 mg/L (Tony et al. 2009); furthermore, the textile industry consumes a significant amount of water in the dyeing and finishing processes, and consequently produces a considerable volume of dyeing wastewater (Wu 2009; Yang et al. 2015). Reactive Red 198 dye, with the chemical formula ($C_{27}H_{18}CLN_7Na_4O_{16}S_5$), is one of the anionic dyes containing triazine and is widely used by the textile industry (Mahmoodi et al. 2006). Discharge of dyeing wastewater into untreated receiving waters can lead to reduced sunlight penetration, eutrophication, and interference in the ecology of the receptor waters (Kant 2012; Nilsson et al. 2006). Additionally, such discharging can negatively affect water quality for drinking and other uses, and can result in allergy, dermatitis, cutaneous stimulation, cancer, and gene mutation in humans (Kaur and Singh 2007).

Various physical, chemical, and biological methods, and their combinations have been used to treat wastewater from the dyeing industries via pre-treatment, conventional treatment, and advanced treatment processes (Crini and Badot 2008; Dos Santos et al. 2007; Eslami et al. 2018a, b). Examples include physical processes, such as adsorption (Velmurugan et al. 2011; Yagub et al. 2014); chemical processes, such as coagulation, flocculation (Wong et al. 2007), and ozonation (Inaloo et al. 2011; Tapalad et al. 2008); and advanced treatment processes, such as electrochemical treatment (Mahmoodi et al. 2011) and advanced oxidation, which includes fenton (Gutowska et al. 2007), photofenton (Khan et al. 2010), and photocatalytic processes (Moussavi and Mahmoudi 2009). These processes are relatively efficient in removal of various dyes from industrial wastewaters; however, their application is not cost-effective or energy-efficient (Brillas and Martínez-Huitle 2015; Eslami et al. 2017b, 2019). The use of one or more specific microorganisms for decomposition of a contaminant is a biological method that does not require chemicals, and it is highly efficient, environmentally friendly, and cost-effective than are other methods (Oller et al. 2011; Popli and Patel 2015; Solís et al. 2012); therefore, biodegradation by microorganisms has been highly regarded by researchers in recent years (Eslami et al. 2017c; Khan et al. 2013). To date, biodegradation methods have been used to remove various contaminants, such as pharmaceutical, petroleum, dyes and others compounds. In these methods, one or more microorganism species are used to biodegradation and break down contaminant molecules (Krishnan et al. 2017; Kurade et al. 2017). Many microorganisms, including bacteria, fungi, yeasts, and algae, are capable of removing various types of dyes, but their effectiveness depends on their compatibility and activity (Popli and Patel 2015). In previous studies, the *Aspergillus flavus* fungus, *Alcaligenes* species, and bacterial consortia have been used to remove azo dyes (Esmaeili and Kalantari 2012; Lalnunhlimi and Krishnaswamy 2016; Pandey et al. 2016).

On this basis, the present study was conducted to isolate a group of bacteria from textile industry wastewater sludge and to use this bacterial consortium specifically for biodegradation of RR198 dye under laboratory conditions. Additionally, this study investigated the effects of various parameters on biodegradation efficiency, and precisely identified the species and genera of the biodegrading bacteria via biochemical assays and PCR.

## Materials and methods

### Chemicals and culture medium

In the present study, RR198 azo dye with a $\lambda_{max}$ of 530 nm was obtained from a local textile company in Yazd, Iran. The chemical structure of RR198 is shown in Fig. 1. All materials, culture media, and indicators used in this study were obtained from Merck, Germany with > 99% purity. Solution pH was adjusted using 0.1 M HCl or 0.1 M NaOH. The mineral-salt culture medium contained 50 mg of KCl, 100 mg of $KH_2PO_4$, 50 mg of $MgSO_4.7H_2O$, and 300 mg of $K_2CO_3$ in a final volume of 1 L.

### Isolation and identification of bacteria

After sampling the wastewater sludge from the textile company, 1 g of sludge was added to 9 mL of BHI (brain–heart infusion) as an enrichment culture medium, and then placed in an incubator at 30 °C for 24 h. To isolate the bacteria, 3.5 mL × $10^5$ cells/mL of culture medium containing bacteria was added to an Erlenmeyer flask containing 50 mL of the mineral salt medium. Afterwards, 10 mL of RR198 dye at a concentration of 10 mg/L was added to the solution, and the Erlenmeyer flask was incubated at 30 °C for 24 h. In order to identify the bacteria grown in the Erlenmeyer flask containing the salt culture medium and RR198 dye, sampling was performed using a sterilized loop, and the isolates were cultured on EMB (eosin methylene blue) agar (for gram-negative bacteria) and blood agar culture medium (for gram-negative and gram-positive bacteria). Subsequently, the plates were incubated at 35 °C for 24 h, and the colonies were stained; furthermore, the catalase and oxidase tests were performed on the colonies. Then Indol, Methyl red, Voges-Proskauer and Citrate utilization (IMViC) tests and oxidative fermentative (OF) test were performed (Eslami et al. 2017c; Pandey et al. 2016).

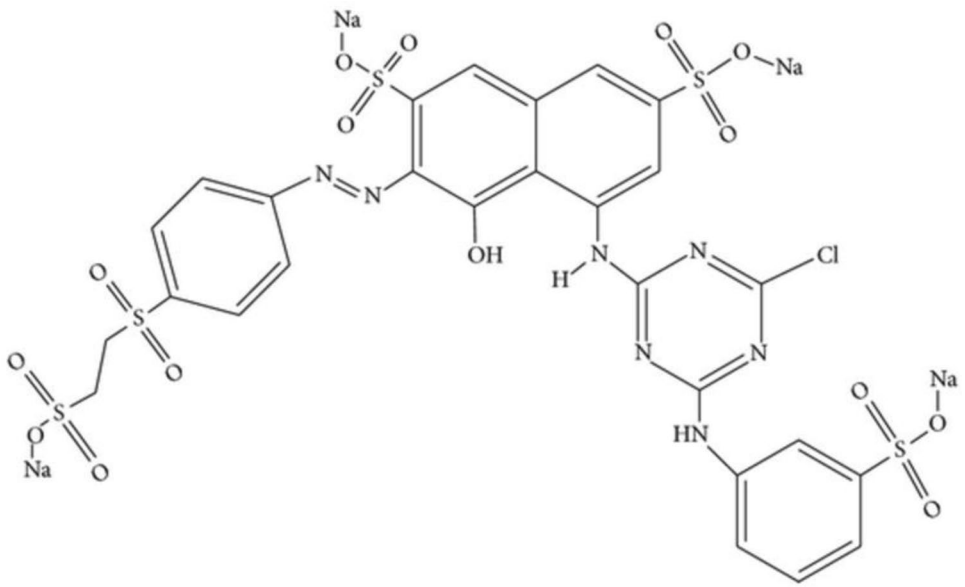


**Fig. 1** Molecular structure of RR198

## DNA extraction

The salting out method was used to obtain genomic DNA. A colony of each bacteria was cultured in 3 mL of sterilized Tryptic Soy Broth (TSB) medium in a screw cap microtube at 37 °C for 24 h to increase the number of bacteria. Then, 1 mL of the bacterial suspension was poured into a 1.5 mL sterilized microtube, which was then centrifuged at 1000×*g* for 10 min. Afterwards, 1 mL of sterilized Phosphate buffered saline (PBS) was added to the remaining sediment, and then centrifuged at 5232×*g* for 4 min; this process was repeated three times. After the final step, the supernatant was discarded, and 450 µL of NET buffer solution (NaCl 50 mM, EDTA 10 mM, Tris–HCL 50 mM, pH 7.6) along with 50 µL of sodium dodecyl sulfate (SDS) (10%) was added to each sample to lysis cells; the samples were then placed in a bain-marie at 65 °C for 20 min to obtain a completely transparent suspension. Afterwards, 300 µL of 6 M NaCl-saturated saline water solution, equivalent to half of the cell suspension's volume, was added to each microtube. Each sample was vortexed for 15 s. The sample-containing microtubes were centrifuged at 17,586×*g* for 10 min at ambient temperature. Afterwards, 500 µL of the solution was added to two new sterilized microtubes. Cold absolute ethanol was then added twice as the volume inside the microtube (at least 1 mL), and the solution was vigorously mixed for 3 s. The samples were placed in a freezer for at least 20 min. The microtubes were then centrifuged at 20,929×*g* for 10–15 min at 4 °C, and the supernatant was removed. 300 µL of cold 70% ethanol were added to each sample, and then each sample was mixed for 3 s followed by centrifugation at 24,562×*g* for 3 min. The supernatant in each microtube was discarded. The microtubes were inverted with caps open on filter paper for drying. 100 µL of cold distilled water were added to each sediment, which was then stored in a freezer at − 20 °C until further use.

## PCR and 16S rRNA gene sequencing

After DNA extraction, gene amplification for the two isolated bacterial species was performed using specific primers for *Ent2, Ent1, Mdh-f*, and *Mdh-r* via PCR.

*Forward (Ent 1):5′TACTGACAAACCATTCATGATG3′*
*Reverse (Ent 2):5′AACTTCGTCACCAACGCGAAC 3′*
*Forward (Mdh-f):5′GCGTGGCGGTAGATCTAAGTCATA 3′*
*Reverse (Mdh-r):5′TTCAGCTCCGCCACAAAGGTA3′*

Amplification was carried out at final volume of 20 µL, which contained 2X PCR master mix (contain 150 mM Tris–HCL pH 8.5, 40 mM $(NH4)_2SO_4$, 3 mM $MgCl_2$, 0.2% Tween20, 0.4 mM dNTPs, 0.05 units/µL Taq DNA Polymerase (Ampliqon, Denmark) and primers at a final concentration of 0.4 µM), 175 ng of the target DNA, and sterile distilled water. The PCR test conditions were as follows: initial denaturation at 98 °C for 5 min, denaturation with 35 cycles at 95 °C for 35 s, annealing at 54.6 °C for 30 s for *Enterococcus* and at 58.3 °C for 30 s for *Klebsiella*, extension at 72 °C for 90 s and the final extension at 72 °C for 300 s. The amplified products were examined using agarose gel electrophoresis. After observing the amplified products on the gel, the quality of the bands was ensured (the products should form single bands). Each of the amplified products was separately transferred to a 1.5 mL sterilized microtube, the cap of which was sealed with parafilm, and sent for 16S

rRNA gene sequencing. Finally, the gene sequencing results were investigated using the BLAST search tool in the NCBI gene database (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) (Pandey et al. 2016).

### Biodegradation and decolorization experiment

To determine the ability of the isolated bacteria to remove RR198 dye, 50 mL of mineral-salt culture medium and 10 mL of RR198 dye at a concentration of 10 mg/L were mixed at different bacterial concentrations (2.5, 3, 3.5, and 4 mL × $10^5$ cells/mL) in Erlenmeyer flasks. The flasks were then placed in a shaker-equipped incubator at 150 rpm and 30 °C to determine the optimal bacterial concentration for removal of RR198 dye. Next, RR198 dye at concentrations of 10, 15, 20, 25, 50, 75, and 100 mg/L, incubation time at 24, 48, 72, 96, and 120 h, pH (7, 8, and 9) and temperature (25, 30, 37, and 45 °C) were investigated. Dye removal results were assessed using a UV/Vis spectrophotometer (SP-3000 PLUS, Optima, Inc., Tokyo, Japan) at 530 nm. The dye removal efficiency was calculated by the following formula:

$$\text{Decolorization rate } (\%) = \left[\left(A_0 - A_1\right)/A_0\right] \times 100$$

where $A_0$ and $A_1$ represent initial and final absorbance of the dye, respectively (Esmaeili and Kalantari 2012).

### Statistical analysis

For statistical analysis, the collected data were imported into SPSS-18 (IBM, Armonk, NY, USA). One-way ANOVA was used to compare the removal efficiency at different bacterial concentrations, dye concentrations, incubation times, temperatures, and pH levels. Data are reported as mean of three independent experiments.

## Results

### Identification and characterization of isolated bacteria

Results of the biochemical experiments for identification of methylene blue-biodegrading bacteria initially showed that one type of colony grew on EMB culture medium, and two types of colonies on blood culture medium. Subsequently, through Gram staining, one of the colonies was found to be of gram-positive *cocci* and the other of gram-negative *bacilli*. The catalase test produced a negative result for the gram-positive bacterium, and thus it was cultured on 6.5% NaCl and bile esculin agar (BEA). The bacterium grew on both media, indicating that it belonged to the *Enterococcus* genus. The catalase and oxidase tests both produced positive results for the gram-negative bacterium; as a result, the IMViC test was performed. The bacterium exhibited positive results in urease; Voges-Proskauer; and citrate, glucose, and lactose metabolism tests; it exhibited negative results in methyl red, indole, and motility tests. Consequently, the gram-negative bacterium belonged to the *Enterobacteriaceae* family and *Klebsiella* genus. Finally, the two isolated bacterial species underwent a PCR test for more accurate identification of genus and species, and for DNA sequencing.

Figure 2a shows the product amplified by PCR and separated by agarose gel electrophoresis using the *Ent* primer; after sequencing, the gram-positive bacterium was found to be *Enterococcus faecalis* (*EF*). Figure 2b shows the product amplified by PCR and separated by agarose gel electrophoresis using the *Mdh* primer, indicating that the gram-negative bacterium was *Klebsiella variicola* (*KV*).

### Effect of bacterial concentration and incubation time

The effects of various bacterial concentrations added to the mineral-salt culture medium containing RR198 dye at a concentration of 10 mg/L under different incubation times was shown in Fig. 3. As shown, by increasing the bacterial concentration from 2.5 to 4mL × $10^5$ cells/mL, the removal efficiency of dye after 72 h of incubation was increased from 15.56 to 99.71%, but the removal efficiency of dye at bacterial concentrations of 3.5 and 4mL × $10^5$ cells/mL was not significantly different. The difference in the increase of removal efficiency between different bacterial concentrations was significant ($p = 0.007$). Furthermore, by increasing incubation time from 24 to 120 h, removal efficiency was increased, reaching above 99% after 72 h of incubation at bacterial concentrations of 3.5 and 4 mL × $10^5$ cells/mL. The difference in the increase of removal rate from 24 to 72 h and above 72 h was statistically significant ($p \leq 0.001$).

### Effect of RR198 concentration

Figure 4 shows the dye removal rate of the isolated bacteria at different initial dye concentrations and different incubation times under anoxic conditions. As shown, by increasing the incubation time in 0 and 24 h, the removal efficiency was increased at all initial concentrations of RR198 dye, such that at 10 and 100 mg/L of RR198, the removal efficiency was 99.77% and 4.92%, respectively. With 72 h incubation time, the dye removal efficiency at initial concentrations of 10–25 mg/L was more than 98%; however, for concentrations of 50, 75, and 100 mg/L, the removal efficiency was reduced to 55.62%, 25.82%, and 15.42%, respectively. The difference between dye removal efficiencies at different dye concentrations was significant ($p = 0.005$).

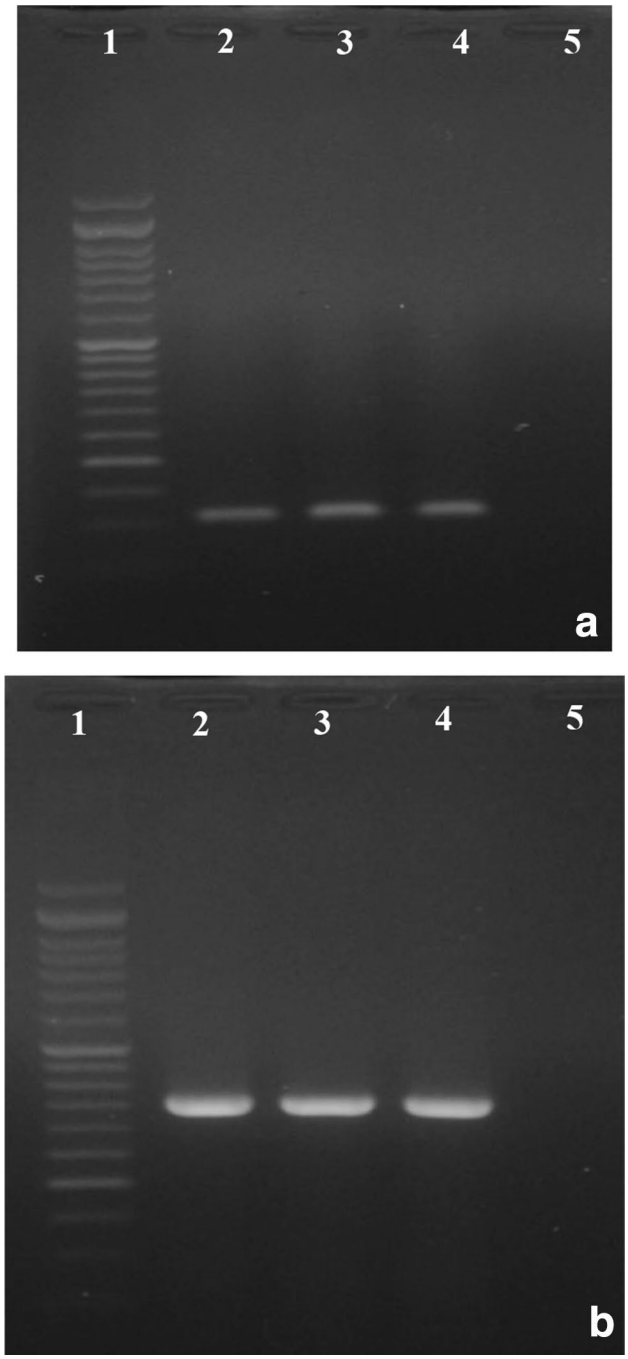


**Fig. 2** *Enterococcus faecalis* (*EF*) PCR amplification product from the *Ent* primer (**a**) and *Klebsiella variicola* (*KV*) PCR amplification product from the *Mdh* primer (**b**) shown by agarose gel electrophoresis (lanes: (1) 50 bp DNA ladder; (2) *EF* and *KV* positive control; (3, 4) approved strain of *EF* and *KV*; (5) negative control)

### Effect of initial pH

The effect of initial pH on RR198 removal efficiency was presented in Fig. 5. The highest removal efficiency occurred at pH 8, with 99.26% after 72 h of incubation; at pH 7 and 9, removal efficiency was 91.75% and 82.45%, respectively, after 72 h of incubation. Therefore, the optimal pH for removal of RR198 dye by the *EF–KV* bacterial consortium was at pH 8.0. The difference in removal efficiencies at different pH levels was statistically insignificant ($p = 0.131$).

### Effect of temperature

The effects of incubation temperature on removal efficiency of RR198 dye was shown in Fig. 6. At temperatures of 25, 30, and 37 °C, the removal efficiency after 72 h of incubation was 71.72%, 91.21%, and 99.26%, respectively, indicating an increasing trend; however, increasing the temperature from 37 to 45 °C reduced efficiency to 88.23%, indicating that the best temperature for growth of the isolated bacteria was 37 °C. This difference in the removal efficiency of dye at temperatures from 25 to 37 °C and above 37 °C was statistically significant ($p \leq 0.001$).

## Discussion

### Identification of bacteria

According to the PCR and 16S rRNA gene sequencing, *Klebsiella variicola* (*KV*) the gram-negative bacterium and *Enterococcus faecalis* (*EF*) the gram-positive bacterium were identified from textile wastewater sludge. In various studies, different strains of these two bacterial species have been used for biodegradation of many environmental contaminants. For instance, were used from the *EF YZ66* for biodegradation of RR195 dye (Mate and Pathade 2012). In another study, biodegradation and adsorption of acid orange 7 by *EF ZL* (Lim et al. 2013). Moreover, bioremoval of lead was conducted using *KV* (*VITMVCJ1*) along with a worm composting method (Das and Osborne 2017). Also in another study about biodegradation of crude oil using bacteria isolated from palm oil mill effluent, one of the isolated species with biodegradation capability was *KV* (Nwankwegu et al. 2016). Accordingly, it can be concluded that these two bacterial species isolated from the textile wastewater sludge were resistant to many of the contaminants present in the textile wastewater, including RR198 dye, and they could continue their growth and reproduction even in the presence of various dye concentrations; additionally, these bacteria together were identified as biodegraders of the dye.

### Optimization of RR198 decolorization

In this study, by increasing the bacterial concentration and incubation time, the RR198 removal efficiency was increased and the best bacterial concentration and incubation time for removal of RR198 dye were 3.5 mL × $10^5$

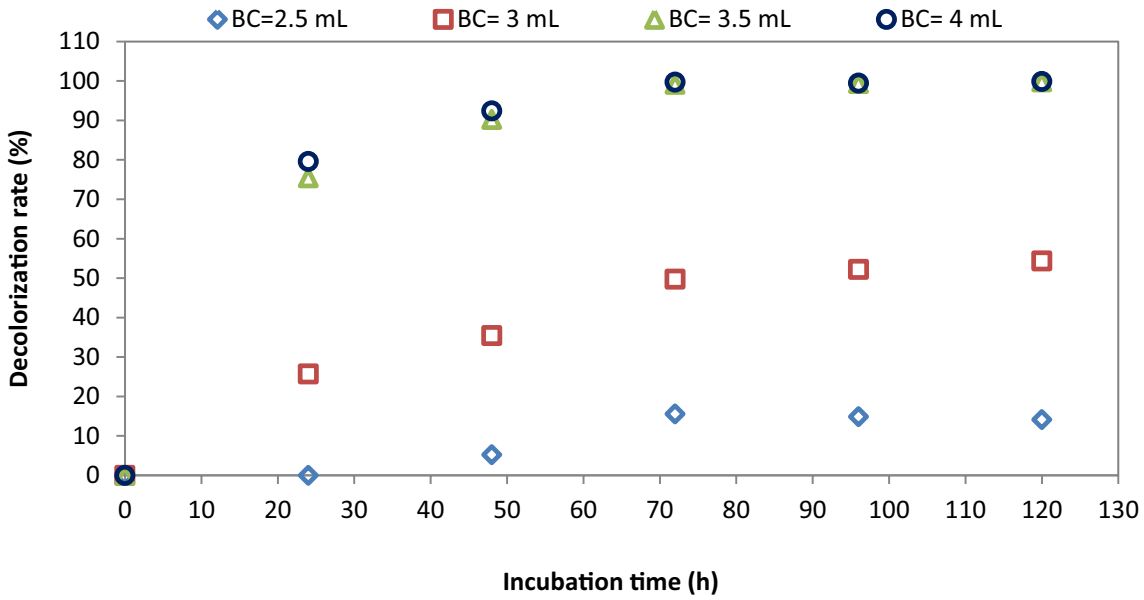


**Fig. 3** Decolorization rate of isolated bacteria at different bacterial concentrations (2.5–4 mL × $10^5$ cells/mL) and incubation times (pH 7, incubation temperature = 30 °C, and RR198 concentration = 10 mg/L)

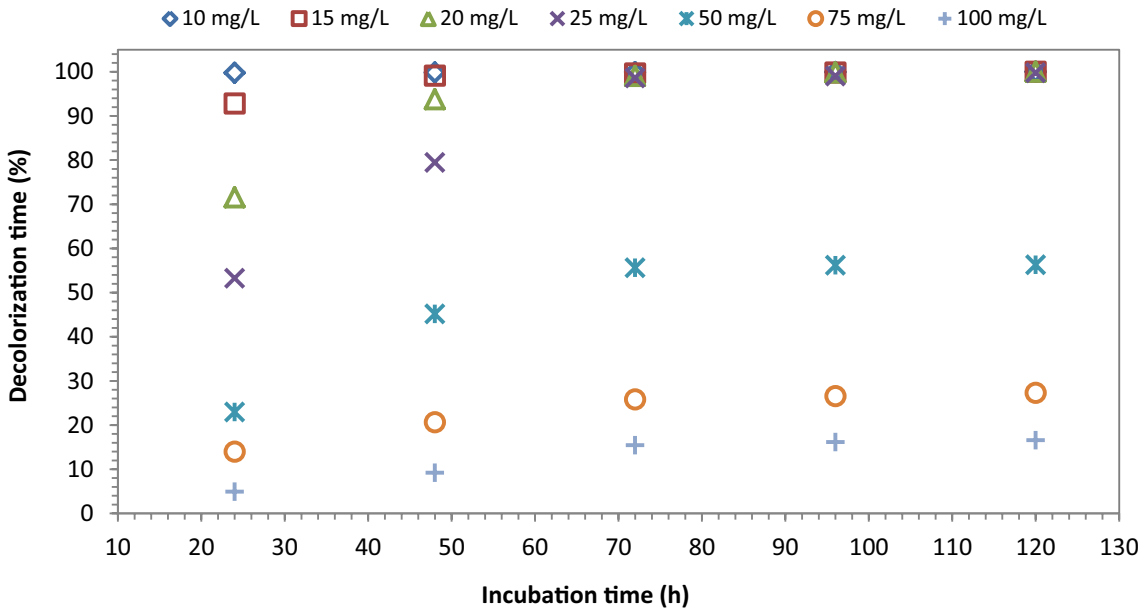


**Fig. 4** Decolorization rate of the isolated bacteria at different RR198 concentrations and incubation times (pH 7, incubation temperature = 30 °C, and bacterial concentration = 3.5 mL × $10^5$ cells/mL)

cells/mL and 72 h, respectively. Similar results have been reported in another study on bioremoval of RR198 dye by *Aspergillus flavus* (Esmaeili and Kalantari 2012). Moreover, two bacterial species were used simultaneously in the present study. The use of a microbial consortium, due to its intensifying effect on biodegradation, can also result in removal of reaction intermediates (Joshi et al. 2008). This was consistent with the results of Kurade et al. (2015) in a study on bioremoval of RR198 dye by a *bacteria-yeast consortium* (Kurade et al. 2017) and of Saratale et al. (2009) in a study on biodegradation of Scarlet R dye by a microbial consortium (Saratale et al. 2009). RR198 dye biodegradation by the *EF-KV* bacterial consortium entails deterioration of the (–N–N–) bond in the dye through three

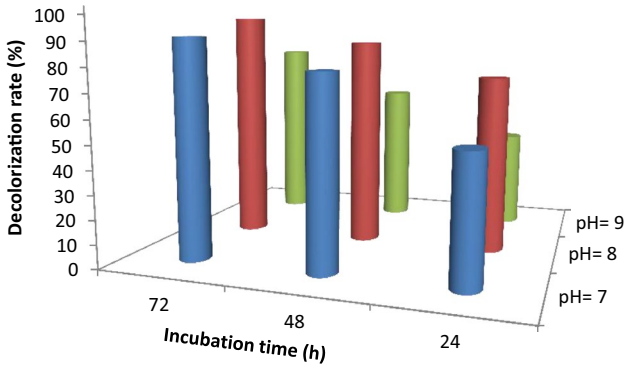


**Fig. 5** Decolorization rate of RR198 at different pHs (incubation temperature = 30 °C, bacterial concentration = 3.5 mL × $10^5$ cells/mL, and RR198 concentration = 25 mg/L)

mechanisms, including the release of azoreductase by the bacteria under anoxic conditions, low-molecular-weight mediators, and chemical reduction by biogenic reducing agents such as sulfide, which reduce the dye and decolorize the solution (Pandey et al. 2007; Saratale et al. 2011).

By increasing the initial concentrations of RR198 dye (until to 25 mg/L), the removal efficiency was increased; however, for concentrations above 25 mg/L, the removal efficiency was significantly reduced. The reduction in removal efficiency at concentrations above 25 mg/L could be due to the toxic effects of the dye at high concentrations or due to the low concentration of the bacterial consortium (Jadhav et al. 2008; Mate and Pathade 2012).

The highest removal efficiency and optimal pH for removal of RR198 dye by the *EF–KV* bacterial consortium was in the pH 8.0. In biodegradation processes, pH can affect the solubility of dye in solution and enzymatic activity of microorganisms (Sahasrabudhe et al. 2014). Multiple studies conducted on bioremoval of dye using microorganisms, especially bacteria, have shown that a pH in a neutral or slightly alkaline range was optimal for removal (Junnarkar et al. 2006; Krishnan et al. 2017; Kurade et al. 2015); additionally, the optimal pH for growth of *EF* and *Klebsiella* species is in the ranges of 5–9 (Mate and Pathade 2012) and 6–8 (Afzal et al. 2017), respectively. Similar results have been reported in studies conducted on biodegradation of RR198 dye by a *bacterial-yeast consortium* (Kurade et al. 2015), bioremoval of RR198 dye by *Alcaligenes* species (Pandey et al. 2016), removal of Direct red 81 dye by *EF* (Sahasrabudhe et al. 2014), and removal of Scarlet R azo dye by a *microbial consortium* (Saratale et al. 2009).

The best temperature for growth of the isolated bacteria was 37 °C. In general, temperature affects the growth of microorganisms, production of degrading enzymes, and consequently the biodegradation of dye (Sahasrabudhe et al. 2014). Therefore, it can be concluded that the increased production of degrading enzymes and optimal growth of the *EF–KV* bacterial consortium occurred at 37 °C, as the maximum removal efficiency was seen at this temperature. Furthermore, because biodegradation of dye by microorganisms via microbial reactions occurs at an optimal temperature of 37 °C, it can be concluded that the RR198 dye in the present study underwent biodegradation

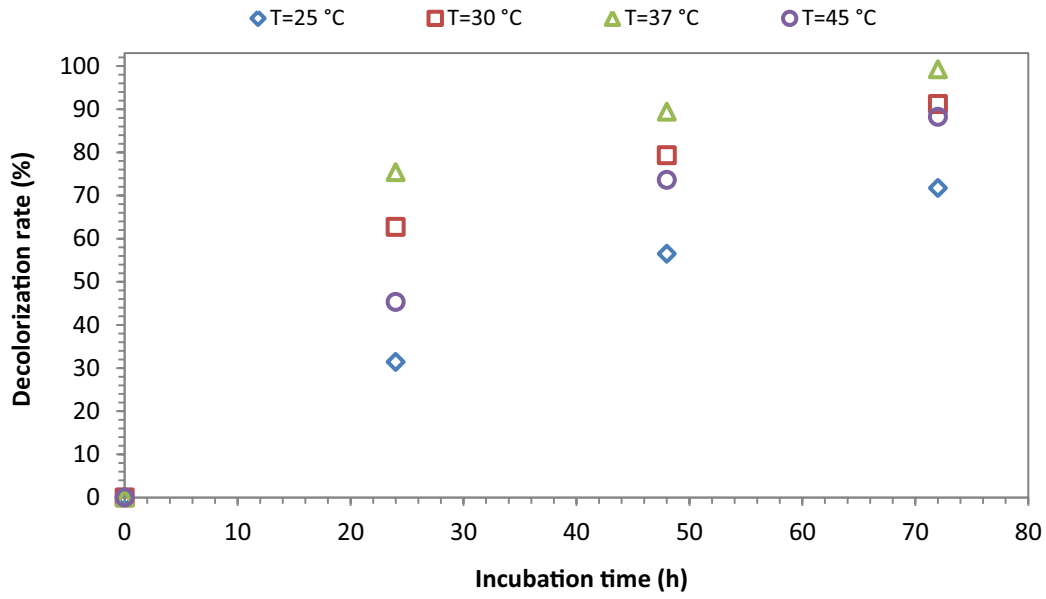


**Fig. 6** Decolorization rate of RR198 at different incubation temperatures (pH 8, RR198 concentration = 25 mg/L, and bacterial concentration = 3.5 mL × $10^5$ cells/mL)

**Table 1** Decolorization rate of RR198 reported in literature

| Species used | Decolonization rate (%) | Decolonization time (h) | References |
|---|---|---|---|
| *Bacillus* spp. | 96 | 36 | (Maulin et al. 2013) |
| *Alcaligenes* spp. | 90 | 24 | (Pandey et al. 2016) |
| *Brevibacterium* spp. | 97 | 120 | (Franciscon et al. 2012) |
| *Bacillus* spp. | 97 | 120 | (Maulin et al. 2016) |
| *Aspergillus flavus* | 84.96 | 24 | (Esmaeili and Kalantari 2012) |
| *Brevibacillus laterosporus* and *Galactomyces geotrichum* | 92 | 18 | (Kurade et al. 2015) |
| *Enterococcus faecalis* and *Klebsiella variicola* | 98.57 | 72 | Present study |

rather than bioadsorption (Mathew and Madamwar 2004). Similar results have been reported in studies conducted on biodegradation of RR195 dye (Mate and Pathade 2012) and of Acid orange 7 dye by *EF* (Lim et al. 2013). Table 1 summarizes the data of studies conducted on removal of RR198 dye and compares their results with those of the present study. Consequently, it can be concluded that the *EF–KV* bacterial consortium can be effectively used for removal of RR198 dye from textile wastewater.

## Conclusion

In this study, a bacterial consortium was isolated from textile wastewater and used for effective biodegradation of RR198 dye. Isolation, followed by PCR and gene sequencing, led to the identification of two bacterial species, *EF* and *KV*. Investigating the physicochemical parameters affecting the removal of RR198 dye indicated that the best removal rate occurred at a bacterial concentration of 3.5 mL × $10^5$ cells/mL and incubation time of 72 h. Furthermore, increasing incubation temperature from 25 to 37 °C increased the removal efficiency; however, increasing the temperature from 37 to 45 °C reduced the removal efficiency. Biodegradation of RR198 dye at more than 98% efficiency occurred at a pH of 8.0, incubation time of 72 h, and initial dye concentrations of 10–25 mg/L; therefore, the *EF–KV* bacterial consortium can be effectively used for removal of RR198 dye from textile wastewater.

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## Compliance with ethical standards

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

**Ethics approval** The ethical approval conducted in Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran with code IR.SSU.SPH.REC.1395.119.

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