

## Decolorization of methyl red using *Bacillus thuringiensis* RI16 strain: Enhanced bacterial treatment by SPB1 biosurfactant addition

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### ABSTRACT

In this work, we evaluate the ability of a novel isolated bacteria identified as *Bacillus thuringiensis* RI16 (KM111604) to decolorize methyl red. Results showed its efficiency for the treatment of azo-dye-contaminated wastewaters under static conditions at pH values ranging from 7 to 9. The isolated bacteria could tolerate higher doses of dyes as it was able to decolorize up to 1000 mg/L. For better methyl red decolorization, we evaluate the potential use of *Bacillus subtilis* SPB1-derived lipopeptide biosurfactant. The study showed its ability to accelerate the decolorization rate and slightly maximized the decolorization efficiency at an optimal concentration of 0.025%. Compared with synthetic surfactants, the bacterial surfactant was more efficient as we observe a drastic decrease and an inhibition of methyl red decolorization by cetyl trimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) additions. Also, studies ensured that methyl red removal by this strain could be due to extracellular enzymatic activities. SPB1 biosurfactant could improve enzyme activities and/or enzyme synthesis and diffusion.

**Key words:** *Bacillus thuringiensis*, biodecolorization, biosurfactants, methyl red

### HIGHLIGHTS

- *B. thuringiensis* RI16 for methyl red biodecolorization under static conditions and neutral and alkaline pH values.
- *B. subtilis* SPB1-derived lipopeptide-enhanced methyl red decolorization.
- Inhibition of methyl red decolorization by CTAB and SDS additions.
- Tween 80 enhanced methyl red decolorization.
- Improvement of color removal-related enzymes' activities and/or enzymes' synthesis and diffusion by SPB1 biosurfactant.

### ABBREVIATION

BioS biosurfactant

### INTRODUCTION

A wide variety of compounds can be found in textile effluent. In the Color Index, there are more than 8000 chemical products associated with the dyeing process ranging from inorganic compounds and elements to polymers and organic products (Priyanka *et al.* 2022). The most abundant are non-biodegradable synthetic dyes, pigments, hydrocarbons and heavy metals (Ardila-Leal *et al.* 2021; Al-Tohamy *et al.* 2022). Synthetic dyes include several structural varieties of dyes, such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal-complex dyes (Ardila-Leal *et al.* 2021; Al-Tohamy *et al.* 2022). These chemicals are used for desizing, scouring, bleaching, dyeing, printing and finishing (Al-Tohamy *et al.* 2022). Also, in textile industries, during the dyeing process, up to 15% of the total textile dye remains unreacted and is directly lost in the effluents generating huge amounts of wastewaters (Ardila-Leal *et al.* 2021; Al-Tohamy *et al.* 2022).

Azo dyes are the largest synthetic chemicals extensively used in textile, food, plastic, printing, leather, cosmetics and pharmaceutical industries (Benkhaya *et al.* 2020). They are highly used in the industry for dyeing fabrics (Ngo & Tischler 2022). Generally, they are characterized by a nitrogen-to-nitrogen double bond  $-N=N-$

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(Benkhaya *et al.* 2020). As they have poor exhaustion properties, nearly 10–15% of the dyestuff used remains unbound to the fiber, synthetic dyes are released into the environment leading to ecotoxicity risk, direct and indirect toxic effects and potential danger of bioaccumulation (Lellis *et al.* 2019; Ngo & Tischler 2022). First, the presence of dyes in water is highly visible and affects its transparency and esthetics impeding light penetration and reducing dissolved oxygen concentration (Lellis *et al.* 2019; Al-Tohamy *et al.* 2022; Ngo & Tischler 2022). Second, synthetic dyes can lead to the formation of tumors, cancers and allergies besides growth inhibition of bacteria, protozoan, algae, plants and different animals including human beings (Ardila-Leal *et al.* 2021). Having the ability to cause great damage to the environment and living organisms, an urgent need for their detoxification and/or elimination from the environment was developed recently (Ardila-Leal *et al.* 2021).

Numerous studies were developed including physical methods such as adsorption, ion exchange and membrane filtration and chemical methods such as coagulation-flocculation, electrochemical and advanced oxidation processes are used to treat dye-containing wastewater (Al-Tohamy *et al.* 2022). However, they are quite expensive and unable to completely remove the recalcitrant azo dyes and/or their organic metabolites (Al-Tohamy *et al.* 2022). Also, they generate significant amounts of sludge and toxic byproducts and metabolites during the treatment process that may cause secondary pollution problems and involve complicated procedures for their elimination and discharge (Al-Tohamy *et al.* 2022). So, biological treatment methods using microorganisms appear to be the best alternative to physicochemical methods since they are eco-friendly and cost-competitive (Lellis *et al.* 2019; Ngo & Tischler 2022). They involve the use of bacteria or fungi capable of dye decolorization either in pure culture or in consortia (Lellis *et al.* 2019; Abd El-Rahim *et al.* 2021). Recent studies reported the use of pure bacterial culture (Kalme *et al.* 2007; Dawkar *et al.* 2008; Ayed *et al.* 2010, 2011; Cai *et al.* 2012; Garg *et al.* 2012; Ng *et al.* 2014; Thakur *et al.* 2014; Nair *et al.* 2017; Chittal *et al.* 2019; Khairun *et al.* 2019; Al-Tohamy *et al.* 2020), mixed bacterial culture (Fang *et al.* 2004; Moosvi *et al.* 2005; Joshi *et al.* 2008; Lalnunhlimi & Krishnaswamy 2016; Kamal *et al.* 2022), pure fungi (Chakraborty *et al.* 2013; Hadibarata *et al.* 2013; Zuleta-Correa *et al.* 2016), fungal consortium (Almeida & Corso 2019), yeast (Jadhav *et al.* 2007; Jafari *et al.* 2014) and algae (El-Sheekh *et al.* 2009; Ishchi & Sibi 2020) for dyes decolorization.

The ability of microorganisms to decolorize and metabolize azo dyes has long been known, and the use of bioremediation-based technologies for treating textile wastewater has attracted interest (Ngo & Tischler 2022). Being cost-effective, environmentally friendly and producing low sludge, biological approaches are very advantageous (Ngo & Tischler 2022). Methyl red (2-(N,N-dimethyl-4-aminophenyl)azobenzenecarboxylic acid), also called C.I. Acid Red 2, is one of the most frequently used secondary diazo dyes well known by their ecotoxicity (Benkhaya *et al.* 2020; Ngo & Tischler 2022). Numerous microbial strains were reported to have the ability to degrade azo dyes especially methyl red, including bacteria (Ayed *et al.* 2011; Ng *et al.* 2014; Abd El-Rahim *et al.* 2021; Ngo & Tischler 2022), fungi (Zuleta-Correa *et al.* 2016; Abd El-Rahim *et al.* 2021), cyanobacteria (Dellamatrice *et al.* 2017), yeast (Al-Tohamy *et al.* 2020) and algae (El-Sheekh *et al.* 2009; Ishchi & Sibi 2020). The increasing interest in bacteria is a result of their potential to degrade and mineralize most textile dyes effectively; bacteria also have a rapid growth rate and are straightforward to culture (Ayed *et al.* 2011). In addition, biological treatment methods involving the use of bacteria belonging to *Bacillus* strains were highly discussed to treat azo dyes (Thakur *et al.* 2014; Nair *et al.* 2017; Khairun *et al.* 2019; Ngo & Tischler 2022; Pinheiro *et al.* 2022). In this aim, this study will discuss the ability of a *Bacillus thuringiensis* RI16 (KM111604) strain to decolorize the azo-dye methyl red. In this regard, the present study aims to investigate, primarily, the potential of a hydrocarbon-degrading bacterium *B. thuringiensis* RI16 (KM111604) to decolorize the azo-dye methyl red. Subsequently, we studied the efficiency of *Bacillus subtilis* SPB1 lipopeptide biosurfactant (BioS) in the improvement of the dye bioremoval process.

## MATERIALS AND METHODS

### Microorganism strain

A *B. thuringiensis* RI16 (KM111604) strain was used for the study of the biodecolorization of the azo-dye methyl red. It was isolated and identified in our previous study for its ability to degrade diesel oil (Mnif *et al.* 2015a). A wild-type strain of *B. subtilis* SPB1 (HQ392822) was used to produce BioS (Ghribi *et al.* 2012). As presented in our previous work, the lipopeptide BioS was composed mainly of different Surfactin isoforms with molecular weights of 1007, 1021 and 1035 Da, Iturin isoforms with molecular weights of 1028, 1042 and 1056 Da and Fengycin isoforms with molecular weights of 1432 and 1446 Da along with two new clusters of lipopeptide isoforms

having molecular weights of 1410 and 1424 Da and 973 and 987 Da (Mnif *et al.* 2016). Mass spectroscopic analysis served to identify the lipopeptides BioS after acid precipitation and purification (Mnif *et al.* 2016).

### Culture conditions and preparation of the purified lipopeptide preparation

Culture conditions for BioS production were carried out as described by Mnif *et al.* (2021a, 2021b, 2021c). They were incubated for 2 days at 37 °C and 150 rpm. At the end of the cultivation, the culture was centrifuged at 10,000 rpm and 4 °C for 20 min to remove bacterial cells. Hence, BioS were extracted from the supernatant-free cells as described in the next section. Lipopeptide BioS were partially purified during three consecutive cycles of acid precipitation–dissolution as described in our previous work (Mnif *et al.* 2021a, 2021b, 2021c). In fact, each time, the pellet formed by acid precipitation was suspended in alkaline water at a concentration of 4 mg/mL and the pH was readjusted to 8 with NaOH 1 N. The supernatant was collected by centrifugation at 10,000 rpm and 4 °C for 20 min followed by second acid precipitation. The final pellet formed was washed three times with acid water (pH = 2), dissolved in distilled water at a concentration of 10 mg/mL, pH adjusted to 8 with NaOH 1 N and lyophilized. This serves as crude lipopeptide preparation to enhance the decolorization efficiency (Mnif *et al.* 2015a, 2015b, 2015c).

### Growth medium and decolorization of dye solution

The decolorization of methyl red was studied at different concentrations of the mentioned dyes (50, 200, 500, 750 and 1000 mg/L) in 250-mL Erlenmeyer flasks containing 50-mL mineral salt medium (MSM). The pH of the medium was adjusted to 7 as described by Ayed *et al.* (2011). The inoculum was prepared in an LB medium. *B. thuringiensis* RI16 strain was streaked on a nutrient agar slant and incubated overnight at 37 °C. After that, one loop of cells was dispensed in 50-mL LB medium (250-mL Erlenmeyer flasks) and incubated in a shaker at 150 rpm and 37 °C ( $\pm 0.5$ ) overnight. Flasks were inoculated by the newly isolated strain at a final optical density of 600 nm corresponding to 0.2. The culture was incubated under shaking conditions (150 rpm) and at static conditions at 37 °C for 24 h. Color removal was quantified after 4, 8 and 24 h of incubation. The resultant culture was centrifuged for 10 min at 10,000 rpm and the cell pellet was washed twice in sterile saline solution (9‰) and re-suspended in the liquid culture medium (Mnif *et al.* 2015a).

In order to study the effect of pH on biodecolorization efficiency, methyl red decolorization was studied at different pH values ranging from 5 to 9. pH of the MSM was adjusted before dye addition and inoculation. Results were expressed as residual activity toward the biodecolorization at a neutral pH value. It is calculated according to this formula:

$$\text{Residual activity (\%)} = \frac{\text{Biodecolorization at different pH value}}{\text{Biodecolorization at neutral pH value}} \times 100 \quad (1)$$

### Effect of SPB1 biosurfactant addition on methyl red biodecolorization

In order to study the effect of SPB1 lipopeptide addition on methyl red removal efficiency by the respective strain, various concentrations (0.025, 0.05, 0.075 and 0.1%) were added in the nutrient growth before incubation. The effect of SPB1 lipopeptide was compared to those of three different chemical surfactants, the anionic surfactant sodium dodecyl sulfate (SDS) (8.2 mM at 25 °C = 0.2364%) (Mukerjee & Mysels 1971); the cationic surfactant cetyl trimethylammonium bromide (CTAB) (0.89–0.93 mM at 25 °C = 0.0324–0.0338%) (Yaofeng 2009) and the nonionic surfactant polyoxyethylene-sorbitan monooleate; polysorbate 80 (Tween 80) (0.012 Mm at 25 °C = 0.00157%) (Chou *et al.* 2005).

### Mechanism of microbial decolorization

In order to elucidate the mechanism of microbial decolorization of methyl red, decolorization tests were realized using living cells, dead cells and two enzymatic preparations of the respective decolorizing strain corresponding to extracellular enzymes in the supernatant and the intracellular enzymes present in the sonication product. Similar experiments were conducted by Jadhav *et al.* (2011), and Mohanty & Kumar (2021). In fact, *B. thuringiensis* RI16 was grown in LB medium overnight at 37 °C and 150 rpm. The supernatant was filter-sterilized using 0.2- $\mu$ m membranes to remove residual cells and serve as extracellular enzyme preparation to measure enzymatic dye biodegradation. The resulting biomass was harvested by centrifugation (10,000 rpm, 20 min) and suspended in 50-mM potassium phosphate buffer (pH 7.4). The first prize of the biomass suspension was sonicated (30 s, 60

amplitude, 10 strokes) at 4 °C (Jadhav *et al.* 2011). After centrifugation, this extract was used as an intracellular enzyme source to quantify intracellular enzymatic dye biodegradation. A second prize of the biomass suspension was autoclaved and the resultant cellular debris was used as biosorbent material to quantify methyl red removal. A third part serves as active cells to quantify color removal resulting from decolorization and biodegradation. In total, four tests were realized in parallel; one is using living beings' cells, one using dead cells, one using bacterial supernatant and one using intracellular bacterial content. They were carried out in duplicate with and without the addition of SPB1 lipopeptide BioS at its optimal concentration determined in the result part. Biodecolorization efficiencies were measured for each trial.

### Analytical methods

To measure decolorization, sampling was carried out at different time intervals from the inoculated decolorizing media. Withdrawn samples were clarified before absorbance measurements in order to prevent absorbance interference from the cellular or other suspended debris. Sample clarification was carried out by centrifugation at 10,000 rpm for 10 min and the absorbance of supernatants obtained was determined at the maximum absorption wavelength ( $\lambda_{\max}$ ) of methyl red (442 nm) using UV-vis spectrophotometer (Spectro UV-Vis Double Beam PC Scanning spectrophotomètre UVD-2960). Uninoculated culture media with and without added dyes were used as negative controls. The decolorization efficiency of different isolates was calculated according to Equation (2) (Ayed *et al.* 2010; Mnif *et al.* 2015b, 2015c):

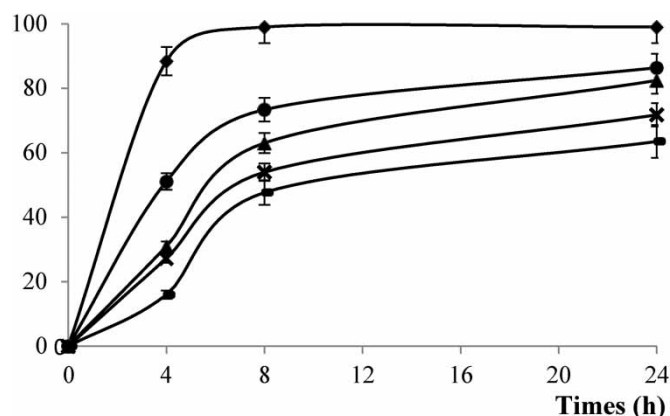
$$\% \text{Decolorization (MR)} = \frac{(\text{Initial Absorbance (0 h)} - \text{Observed Absorbance (t)})}{\text{Initial Absorbance (0 h)}} \times 100 \quad (2)$$

## RESULTS AND DISCUSSION

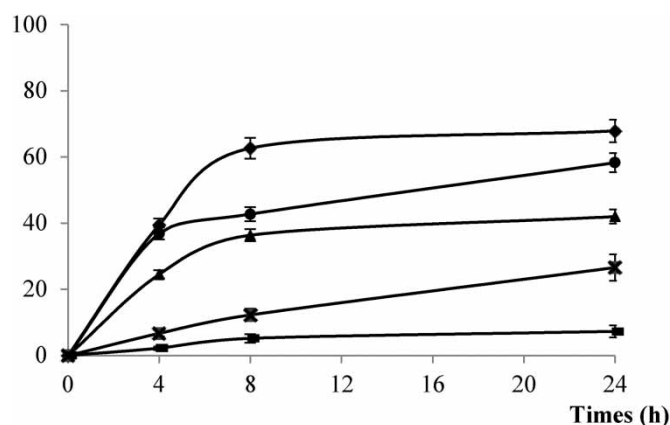
### Methyl red decolorization under different culture conditions

#### Effect of initial dye concentration

The decolorization of methyl red was studied at various increasing concentrations of dye, i.e. from 100, 200, 300, 500, 750 and 1000 mg/L at both static and shaking conditions. A progressive increase of dye decolorization was observed during the first 24 h of incubation. After, no significant variation occurs (data not shown). It is well observed that the rate of decolorization decreased with increasing concentration of dye (Figures 1 and 2). Moreover, the time required for decolorization increase with increasing dyes' concentrations. Additionally, decolorization was more efficient at static conditions with more than 90% after 8 h of incubation in contrast to 60% at shaking conditions. Optimal decolorization efficiency was obtained at 200 mg/L methyl red under static conditions. Similar observations were also reported during the decolorization of reactive violet 5 (at 200 mg/L after 37 h) by a newly isolated bacterial consortium (Moosvi *et al.* 2005), the decolorization of the azo-dye acid orange 7 (at 200 mg/L after 16 h) by newly isolated consortium TJ-1 (Joshi *et al.* 2008), the decolorization of Brown 3REL (100% at static conditions after 8 h) by *Bacillus* sp. (Dawkar *et al.* 2008) and the



**Figure 1** | Effect of dye concentration on the biodecolorization efficiency of methyl red at static conditions. —◆—: 200 mg/L; —●—: 300 mg/L; —▲—: 500 mg/L; —×—: 750 mg/L; —■—: 1000 mg/L.



**Figure 2** | Effect of dye concentration on the biodecolorization efficiency of methyl red at shaking conditions. ◆: 200 mg/L; ●: 300 mg/L; ▲: 500 mg/L; ✕: 750 mg/L; ■: 1000 mg/L.

decolorization of Congo Red (97% of 100 mg/L after 8 h of incubation) by *Pseudomonas* sp. SU-EBT (Telke *et al.* 2010). Uninoculated negative controls realized at the same conditions indicate that there was no biotic loss of methyl red within the increase of incubation time. In addition, the strain can tolerate higher doses of dyes as it can decolorize up to 1000 mg/L of dye and could be considered an efficient decolorizer of methyl red. Results are similar to those published by Ayed *et al.* (2011), Jafari *et al.* (2014) and Kamal *et al.* (2022) reporting an efficient biodecolorization of higher doses of azo dyes by selected bacterial strains and fungi. In contradiction, other studies reported the biodecolorization of lower concentrations of azo dyes (Jadhav *et al.* 2007; Chittal *et al.* 2019; Al-Tohamy *et al.* 2020). Generally, higher doses of azo dyes higher than 500 mg/L can cause toxicity to living cells and decrease decolorization efficiency (Shi *et al.* 2021; Ngo & Tischler 2022). In some cases, even moderate concentration of the azo-dye caused a drastic decrease of decolorization efficiency. For example, only 15% decolorization percentage of Direct Blue-6 by *Pseudomonas desmolyticum* NCIM 2112 was observed at 250-mg/L dye concentration (Kalme *et al.* 2007).

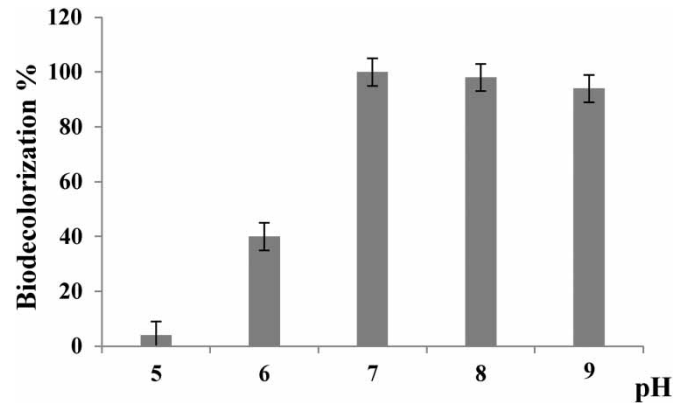
#### Effect of static and shaking conditions

Results presented in Figures 1 and 2 indicated that the strain could decolorize methyl red under both static and shaking conditions, but the decolorization under static conditions appeared to be significantly higher. In fact, as shown in Figures 1 and 2, the decolorization of methyl red (100–1000 mg/L) under shaking condition by *B. thuringiensis* RI12 cannot exceed 76% with very weak biodecolorization at high dye concentration. However, under static conditions, a complete biodecolorization was obtained for doses between 50 and 200 mg/L with 82.41% when working in the presence of 500 mg/L. Results are similar to many literature studies reporting high microbial decolorization of azo dyes under static conditions (Fang *et al.* 2004; Telke *et al.* 2010; Garg *et al.* 2012). Also, Cai *et al.* (2012) reported efficient azo-dye biodegradation under static conditions. However, several studies reported the efficient biodecolorization of methyl red under shaking conditions (Ayed *et al.* 2011).

#### Effect of pH on methyl red decolorization

Having a great influence on dye structure and microbial growth, pH could affect biodecolorization and biodegradation efficiency. Therefore, it was recognized as an important factor for the biodecolorization process (Shah *et al.* 2013; Nair *et al.* 2017; Lalnunhlimi & Krishnaswamy 2016). As shown in Figure 3, the optimal pH for methyl red biodecolorization range from 7 to 9. Therefore, it was obvious that neutral and basic conditions higher than 7 were favorable for the decolorization of methyl red. A drastic decrease in biodecolorization efficiency was observed when operating at acidic pH conditions lower than 7. Results are similar to those published by Shah *et al.* (2013) and Nair *et al.* (2017) showing preferential azo dyes biodegradation at neutral pH values. However, Lalnunhlimi & Krishnaswamy (2016) and Kamal *et al.* (2022) show favorable decolorization of azo dyes (Direct Blue 151 and Direct Red 31) at alkaline conditions. Gou *et al.* (2009) reported efficient azo-dye biosorption at acidic pH values of 4 or 5 by fungal-bacterial co-cultures. *Bacillus* spp. shows optimum decolorization of Remazol Black-B from pH 5 to 7 (Shah *et al.* 2013). Also, Chakraborty *et al.* (2013) and Senthilkumar *et al.* (2014) reported a maximal azo dyes biotreatment at pH 5.

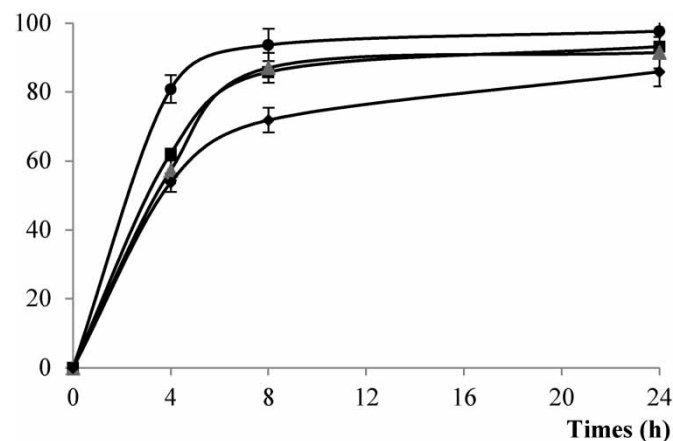




**Figure 3** | Effect of initial pH values on biodecolorization efficiency.

### Effect of SPB1 lipopeptide biosurfactant addition on dye biodecolorization

*B. thuringiensis* RI16 strain could decolorize the textile azo-dye methyl red efficiently under static conditions. Surfactant-enhanced decolorization technology is one of the feasible approaches to improve textile dye removal from wastewater. So, in order to upgrade the biodecolorization efficiency in the course of time, different concentrations of SPB1 lipopeptide BioS (0.025; 0.05; 0.075; 0.1 and 0.15%) were added before culture inoculation (Figure 4). In fact, SPB1 BioS was characterized by its high emulsification activity (Ghribi *et al.* 2012) and was demonstrated to enhance *in situ* (Mnif *et al.* 2014) and *ex situ* hydrocarbon biodegradation (Mnif *et al.* 2015a). Moreover, the described BioS stimulate methyl orange and malachite green biotreatment by *Aeromonas veroni* and *Citrobacter sedlakii*, respectively (Mnif *et al.* 2015b, 2015c). Results in Figure 3 show a significant enhancement of decolorization percentage when using 0.025% BioS. A gradual decrease of decolorization proportion was observed. It rose from 62 to 81%, from 78 to 93.69% and from 91.47 to 97.69% after 4, 8 and 24 h, respectively. When adding 0.05% SPB1 BioS, biodecolorization percentages were similar to those without BioS addition. For superior doses, an inhibition of biodecolorization was observed and SPB1 BioS addition seems to have a deleterious effect on decolorization efficiency. The dose-dependent effect on biodecolorization efficiency was also observed by Kristensen *et al.* (2007), Champagne *et al.* (2010), Gül & Dönmez (2012) and Ruta & Juozas (2013). Therefore, we can assume that an optimal concentration of BioS of about 0.025% accelerates the decolorization rate and maximizes the decolorization efficiency. Thus, it could save energy and lower costs are required for the treatment technique by shortening the incubation period. These findings are similar to those described by Jadhav *et al.* (2011) and Gül & Dönmez (2012). Furthermore, as dye biodecolorization efficiency enhancement by chemical surfactants addition was highly described in many literature reviews (Gül & Dönmez 2011, 2012); the effect of SPB1 BioS was compared to those of synthetic surfactants Tween 80,



**Figure 4** | Effect of the addition of SPB1 BioS on methyl red biodecolorization. —■—: Without BioS; —●—: +0.025% BioS; —▲—: +0.05% BioS; —◆—: +0.15% BioS.

CTAB and SDS at its optimal concentration (0.025%). In fact, many authors described that they could improve synthetic dyes solubilization through their incorporation within their micelle structure and therefore their microbial treatment (Tehrani-Bagha & Holmberg 2013). When added at a concentration of 0.025%, results showed a drastic decrease and a total inhibition of methyl red decolorization by CTAB and SDS additions. These results remembered those published by Liu *et al.* (2012). In fact, due to their chemical nature and toxic effect, we can suppose that they can disturb membrane cells integrity inhibiting; therefore, their growth and biological activities and consequently lead to the inhibition of decolorization rate (Kapadia Sanket & Yagnik 2013), whereas, the nonionic surfactant Tween 80 did not have any negative effect on methyl red biodecolorization. Results are similar to those published by Hadibarata *et al.* (2013), reporting the enhancement of microbial decolorization of an Azo Dye Reactive Black 5 using white-rot fungus *Pleurotus eryngii* by Tween 80 addition. Also, Liu *et al.* (2006) and Zenz Zeng *et al.* (2006) reported an increase of certain enzyme activities by Tween 80 addition. Remember, due to their natural origin, biodegradability and higher efficiency in extreme conditions could be the best alternative to chemical surfactant addition in promoting the treatment and eliminating contaminants. Actual results show the higher efficiency of SPB1 lipopeptide BioS as an enhancer of methyl red biotreatment compared to the reported chemical emulsifiers.

### Mechanism of bacterial decolorization of methyl red

Decolorization of the dye solution may take place in two ways: either adsorption on the microbial biomass or enzymatic biotreatment of the dyes (Pinheiro *et al.* 2022). When conducting biodecolorization using different preparation, efficient decolorization was observed using living cells of *B. thuringiensis* RI12 and extracellular supernatant of the strain (data not presented). However, no decolorization was observed by the application of the dead cells and intracellular enzymatic preparation. Therefore, these results indicate that methyl red removal by this strain can be due to extracellular enzymatic activities. Other studies reported the enzymatic biotreatment of certain azo dyes (Liu *et al.* 2020; Singh *et al.* 2020; Ardila-Leal *et al.* 2021; Ngo & Tischler 2022; Vishani & Shrivastav 2022). However, many reports described the adsorptive removal of azo-dye using bacteria, fungi and yeast (Almeida & Corso 2019; Öztürk *et al.* 2020; Ngo & Tischler 2022; Pinheiro *et al.* 2022). Studies with and without BioS addition were also conducted when treating methyl red with living cells and extracellular enzymatic preparation. Results indicated the enhancement of methyl red biodecolorization in both cases due to the addition of 0.025% BioS. Regarding the preliminary results showing the enhancement of methyl red biodecolorization by the addition of SPB1 bioemulsifier, we can propose an enhancement of bacterial growth and, therefore, methyl orange biodecolorization due to the fact that this BioS is biodegradable and could be used as a primary and more accessible carbon source to initiate microorganisms' growth (Bautista *et al.* 2009; Mnif *et al.* 2015b). Also, we can propose that the BioS improve enzyme production and, therefore, their biological activities. Similar results are described by Liu *et al.* (2017) and Oliva-Taravilla *et al.* (2020) and reporting the improvement of enzyme production by the addition of lipopeptides and rhamnolipids, respectively. Moreover, we can assume that the addition of SPB1 BioS may increase bacterial membrane permeability as reported in other studies (Vaidyanathan *et al.* 2014; Kaczorek *et al.* 2018) facilitating the enzymes diffusion. Regarding the enhancement of methyl red biodecolorization when using extracellular enzymatic preparation, we can assume that the bioemulsifier can activate decolorizing enzymes. In fact, recent studies reported the promotion of enzyme activities by chemical surfactants addition. As reported by Singh & Singh (2017), laccase activity was improved by Triton X-100 and Tween 20. Similarly, as described by Bento *et al.* (2020), surfactant-based ionic liquids improve the efficiency of laccase to degrade indigo carmine. Additionally, Britos *et al.* (2018) proved the improvement of laccase activity by ionic surfactant addition. Also, Tween 80 increased the cellobiohydrolase and endoglucanase activities from 63.0 and 76.7% to 123.2 and 103.1%, respectively (Ying *et al.* 2021), and Tween 20 enhanced cellulose activity (Wang *et al.* 2020). Similarly, Jadhav *et al.* (2011) showed the intensified effects of rhamnolipid on the enzymes involved in the dye degradation process and the stimulatory effect on lignin peroxidase (increased by 324.52%) and Veratryl alcohol oxidase (increased by 100%) were much eminent. Also, Liu *et al.* (2008) and Liang *et al.* (2010) reported 161.98 and 86% increase in the activity of lignin peroxidase in the presence of dirhamnolipid, respectively. Moreover, Zhou *et al.* (2011) and Liu *et al.* (2012) reported the improvement of laccase production and phenol biodegradation by rhamnolipid addition. Similar findings describing the improvement of diverse enzymes activities through dirhamnolipid addition were reported (Shao *et al.* 2017; Oliva-Taravilla *et al.* 2020; Gayathiri *et al.* 2022).

Also, we can assume a stabilizing effect on enzymes enhancing, therefore, their activities. Similarly, Ji *et al.* (2009) reported the stabilizing effect of laccase by Triton X-100 addition. Also, *P. aeruginosa* BSZ-07 rhamnolipid BioS was demonstrated to stimulate rice straw hydrolysis bioprocess by *Trichoderma reesei* ZM4-F3, a cellulose decomposing fungus, through increasing the activity of  $\beta$ -glucosidase and stabilizing Cel7A activity (Zhang *et al.* 2009).

## CONCLUSION

*B. thuringiensis* RI16 showed excellent potential for bioremediation application as it can decolorize up to 1000 mg/L of methyl red dye at neutral and basic pH values. Results were better under static conditions and for lower dye concentrations. When elucidating the mechanism of dye removal, biodecolorization of methyl red can result from enzymatic activities. The addition of SPB1 BioS at 0.025% increased the biotreatment yields significantly. It seems to activate the extracellular enzymes involved in dye biodecolorization. However, chemical surfactants addition seems to have a deleterious effect on biodecolorization efficiency. The addition of SPB1 bioemulsifier reduced energy costs by reducing effective decolorization time, and BioS-stimulated bacterial decolorization method may provide a highly-efficient, inexpensive, and time-saving procedure in the treatment of textile effluents.

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## CONSENT FOR PUBLICATION

All authors read the final manuscript and approved its submission to Water Practice and Technology.

## AVAILABILITY OF DATA AND MATERIALS

The data sets supporting the conclusions of this article are included in the article.

## AUTHORS CONTRIBUTIONS

All authors directly participated in the planning, execution, or analysis of this study. All authors read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

The authors declare there is no conflict.

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