Biosorption of Congo Red from aqueous solution by *Bacillus weihenstephanensis* RI12; effect of SPB1 biosurfactant addition on biodecolorization potency

Inès Mnif, Raouia Fendri and Dhouha Ghribi

ABSTRACT

Bacillus weihenstephanensis RI12, isolated from hydrocarbon contaminated soil, was assessed for Congo Red bio-treatment potency. Results suggested the potential of this bacterium for use in effective treatment of Congo Red contaminated wastewaters under shaking conditions at acidic and neutral pH value. The strain could tolerate higher doses of dyes as it could decolorize up to 1,000 mg/l of Congo Red. When used as microbial surfactant to enhance Congo Red biodecolorization, *Bacillus subtilis* SPB1-derived lipopeptide accelerated the decolorization rate and maximized the decolorization efficiency at an optimal concentration of biosurfactant of about 0.075%. Studies ensured that Congo Red removal by this strain could be due to an adsorption phenomena. Germination potencies of tomato seeds using the treated dyes under different conditions showed the efficient biotreatment of the azo dye Congo Red especially with the addition of SPB1 biosurfactant. To conclude, the addition of SPB1 bioemulsifier reduced energy costs by reducing the effective decolorization period; the biosurfactant stimulated bacterial decolorization method may provide a highly efficient, inexpensive and time-saving procedure in the treatment of textile effluents.

Key words | *Bacillus weihenstephanensis*, biosorption, biosurfactants, Congo Red, phytotoxicity treatment

Inès Mnif (corresponding author)
Raouia Fendri
Dhouha Ghribi
National School of Engineers of Sfax,
Unité "Enzymes et Bioconversion", ENIS,
Université de Sfax,
BP W 3038 Sfax,
Tunisia
and
Higher Institute of Biotechnology of Sfax,
Université de Sfax,
Sfax,
Tunisia
E-mail: *inesmnif2011@gmail.com*

INTRODUCTION

A wide variety of compounds can be found in textile effluent. In the Color Index, there are more than 8,000 chemical products associated with the dyeing process. They range from inorganic compounds and elements to polymers and organic products: mainly synthetic dyes, non-biodegradable pigments, hydrocarbons and heavy metals (Wang *et al.* 2011). Synthetic dyes include several structural varieties of dyes, such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal-complex dyes (Wang *et al.* 2011). These chemicals are used for desizing, scouring, bleaching, dyeing, printing and finishing (Wang *et al.* 2011). In addition, in textile industries, during the dyeing process, up to 15% of the total textile dye remains un-reacted and is directly lost in the effluent, generating huge amounts of wastewater (Lal & Srivastava 2011).

Azo dyes are by far the largest type of synthetic chemicals extensively used in the textile, food, plastic, printing, leather, cosmetics and pharmaceutical industries. They are mainly used in industry for the purpose of dyeing fabrics. Generally, they are characterized by a nitrogen to nitrogen double bond –N = N– (Prasad & Rao 2012). As they have poor exhaustion properties, nearly 10–15% of the dyestuff used remains unbound to the fiber and is therefore released as color into the environment, leading to ecotoxicity and the potential danger of bioaccumulation (Prasad & Rao 2012; Gupte *et al.* 2013). In addition, the presence of dye in water is highly visible and affects its transparency and aesthetics, impeding light penetration and reducing the dissolved oxygen concentration (Prasad & Rao 2012). The direct and indirect toxic effects of dyes and metals are mentioned in many reports. In fact, they can lead to the formation of tumors, cancers, and allergies besides growth inhibition of bacteria, protozoa, algae, plants and different animals including human beings (Sponza 2006). So, an urgent need for their detoxification and/or elimination from the environment was developed recently. Numerous studies were developed including physiochemical methods such as filtration, specific coagulation, use of activated

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carbon and chemical flocculation and precipitation, ion exchange, adsorption, electrochemical processes and membrane processes for heavy metals (Wang *et al.* 2011). They are effective but quite expensive (Ramachandran *et al.* 2013). So, biological treatment methods using microorganisms appears to be the best alternative to physico-chemical methods. They involve the use of bacteria or fungi capable of dye decolorization and/or heavy metal adsorption either in pure culture or in consortia (Wang *et al.* 2011; Ramachandran *et al.* 2013). The ability of microorganisms to decolorize and metabolize dyes has long been known, and the use of bioremediation-based technologies for treating textile wastewater has attracted interest (Ramachandran *et al.* 2013). In fact, they have several advantages over physico-chemical techniques as they are cost effective, produce low sludge, and they are environmentally friendly (Prasad & Rao 2012). To date, many physical and chemical methods exist for the treatment of azo dyes, namely adsorption using activated carbon (Belhachemi & Addoun, 2014; Dawood *et al.* 2014), coagulation (Patel & Vashi 2012; Verma *et al.* 2012), oxidation process (Kiran *et al.* 2013; Castro *et al.* 2014), ozonation (Khadhraoui *et al.* 2009), photocatalytic degradation (Rastegar *et al.* 2012; Khenniche *et al.* 2014) and sonochemical processes (Giwa *et al.* 2012; Dükkanci *et al.* 2014) and photo-electrochemical techniques (Sala *et al.* 2014). However, several biological methods are applied for the treatment of azo dyes. Many microorganisms are capable of treating Congo Red, including bacteria (Ayed *et al.* 2010; Telke *et al.* 2010), fungi (Bhattacharya *et al.* 2011; Selvam & Shanmuga 2012; Shinde & Thorat 2013; Senthilkumar *et al.* 2014), yeast (Jafari *et al.* 2012; Tan *et al.* 2014) and algae (Mahajan & Kaushal 2013; Hernández-Zamora *et al.* 2014). Combined physico-chemical and biological methods are also used for the treatment of azo dyes (Lu *et al.* 2009; de Arruda *et al.* 2010).

The ability of bacteria to metabolize azo dyes has been investigated by a number of research groups. Anaerobic conditions are found to be favorable for the reduction of azo dyes and are well documented (Mendez-Paz *et al.* 2005; Baêta *et al.* 2012; Cai *et al.* 2012). Nevertheless, the resulting colorless compounds (amines) are biorecalcitrant under anaerobic conditions (Prasad & Rao 2012) and can be toxic and carcinogenic (Platzek *et al.* 1999; Sanfins *et al.* 2011). Hence, an aerobic treatment step is required to degrade aromatic amines. Generally, two steps of anaerobic-aerobic conditions are found to provide a complete mineralization of azo dyes (Koupaie *et al.* 2013; Liu *et al.* 2013; Murali *et al.* 2013). However, certain bacteria are able to completely decolorize azo compounds under aerobic conditions (Adedayo *et al.* 2004; Mabrouk & Yusef 2008; Ayed *et al.* 2011; Kolekar *et al.* 2012; AL Ahmed 2014). Several studies reported the biodecolorization of Congo Red under shaking conditions by bacteria (Ayed *et al.* 2010; Bhattacharya *et al.* 2011; Chakraborty *et al.* 2013), fungi (Bhattacharya *et al.* 2011; Shinde & Thorat 2013; Senthilkumar *et al.* 2014) and yeast (Tan *et al.* 2014). In addition to biodegradation, several microorganisms are able to eliminate azo dyes through adsorption including bacteria (Yu *et al.* 2011; Das *et al.* 2012) and fungi (Erden *et al.* 2011; Selvam & Shanmuga 2012).

The present study aims to investigate, on the one hand, the potential of a newly isolated strain for decolorizing a solution containing the azo dye, Congo Red, and on the other hand, the efficiency of *Bacillus subtilis* SPB1 lipopeptide biosurfactant in the improvement of the dye bioremoval process.

MATERIALS AND METHODS

Dyes and chemicals

The Congo Red (sodium 3,3-(1*E*,1*E*)-biphenyl-4,4-diylbis (diazene-2,1-diyl) bis(4-aminonaphthalene-1-sulfonate)) used in this study was purchased from Sigma-Aldrich, St Louis, MO, USA. The sodium dodecyl sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB), and Tween 80 were purchased from Sigma-Aldrich and dissolved in distilled water. They were assayed as synthetic emulsifiers to compare the efficiency of *B. subtilis* SPB1 biosurfactant with those of chemical surfactants.

Growth media used

The mineral salt medium (MSM) was prepared by mixing the following composition (g/l): [K_2HPO_4 (1), ammonium sulfate (1), NaCl (0.5), $MgSO_4$ (0.2), KH_2PO_4 (0.5), $MnSO_4$ (0.001), $FeSO_4$ (0.001), $ZnSO_4$ (0.001)] in 1,000 ml of distilled water as described by Mnif *et al.* (2013). For biosurfactant production, the mineral salt medium was supplemented with glucose as described by Mnif *et al.* (2012a, b).

Luria Bertani (LB) medium was prepared by mixing the following composition (g/l): [Tryptone (10), yeast extract (5), NaCl (5)] in 1,000 ml of distilled water (Bertani 1952). It was used for inocula preparation as described by Mnif *et al.* (2012a, b). It was solidified with agar (15 g/l) and used for bacteria streaking and maintenance on agar plates.

Microorganism strain

Bacillus weihenstephanensis RI12 (KM094930), screened in our previous work for its ability to degrade diesel oil (Mnif *et al.* in press), was used for the study of the biodecolorization of Congo Red. It was isolated and identified in our previous study for its ability to degrade hydrocarbons. A wild-type strain of *B. subtilis* SPB1 (HQ392822) was used to produce biosurfactants (Ghribi *et al.* 2012).

Culture conditions and preparation of the purified lipopeptide preparation

Culture conditions for biosurfactant production were carried out as described by Mnif *et al.* (2013). 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 *g* and 4 °C for 20 min to remove bacterial cells. Hence, biosurfactants were extracted from the supernatant-free cells as described in the next section. Lipopeptide biosurfactants were partially purified during three consecutive cycles of acid precipitation–dissolution as described in our previous work (Mnif *et al.* 2013). 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 a 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 served as a crude lipopeptide preparation to enhance the decolorization efficiency.

Growth medium and decolorization of dye solution

The pH of the medium was adjusted to 7. The decolorization of Congo Red was studied at different concentrations (50, 200, 500, 750 and 1,000 mg/l) in 250 ml Erlenmeyer flasks containing 50 ml MSM. The flasks were inoculated with 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 of 37 °C. The inoculum was prepared in LB medium. *B. weihenstephanensis* RI12 strain was streaked on a nutrient agar slant and incubated overnight at 37 °C. After that, one loop of cells was dispersed in 50 ml LB medium prepared in 250 ml Erlenmeyer flasks and incubated in a rotatory shaker at 150 rpm and 37 °C (± 0.5) overnight. 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 MSM mentioned in 'Growth media used' (Ganesh & Lin 2009).

Effect of SPB1 biosurfactant on Congo Red biodecolorization

In order to study the effect of the SPB1 lipopeptide addition on Congo Red removal efficiency by the respective strain, various concentrations (0.025, 0.05, 0.075 and 0.1%) were added to the growth nutrient before incubation. The effect of SPB1 lipopeptide was compared to those of three different chemical surfactants: SDS (anionic); CTAB (cationic) and Tween 80 (non-ionic) at the optimal concentration.

Mechanism of microbial decolorization

In order to elucidate the mechanism of microbial decolorization of Congo Red, decolorization tests were realized using living cells and dead cells of *B. weihenstephanensis* RI12, 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. In fact, *B. weihenstephanensis* RI12 was grown in LB medium overnight at 37 °C and 150 rpm. The supernatant was filter sterilized using 0.2 μm membranes to remove residual cells and serve as an 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). A first quantity of the resulting biomass was sonicated (30 seconds, 60 amplitude and 10 strokes) at 4 °C (Jadhav *et al.* 2011). This extract was used as an intracellular enzyme source after centrifugation to quantify intracellular enzymatic dye biodegradation. A second quantity of the resulting biomass was autoclaved and the resultant cellular debris was used as biosorbent material to quantify Congo Red removal. A third part serves as active cells to quantify color removal resulting from decolorization and biodegradation. In total, four decolorization trials were performed in parallel: one using living 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 the SPB1 lipopeptide preparation.

Phytotoxicity studies

Phytotoxicity tests were performed to assess the toxicity of the untreated and treated dye (Ayed *et al.* 2010). The seeds were germinated in sterile 10 cm Petri dishes, layered with sterile filter paper. The phytotoxicity study was carried out at room temperature ($32 \pm 2\,^{\circ}C$) in relation to tomato seeds (10 seeds per plate) by watering separately 5 ml samples of textile effluent in its different cases (untreated and treated). Experiments were carried out in duplicate. Seeds germinated in a water irrigated Petri dish were used as a control. Length of radicle (root) and germination (%) were recorded at the end of the incubation until the negative control germinated completely after approximately four days. Hence, the germination index was calculated according to this formula:

$$\mathbf{IG}(\%) = \frac{\begin{array}{c}(\text{Number of germinated seeds}) \\ \times(\text{Total length of root})\end{array}}{\begin{array}{c}(\text{Number of germinated seeds in the negative control}) \\ \times(\text{Total length of root in the negative control})\end{array}} \times 100$$

RESULTS AND DISCUSSION

Congo Red (sodium 3,3-(1*E*,1*E*)-biphenyl-4,4-diylbis (diazene-2,1-diyl) bis(4-aminonaphthalene-1-sulfonate)) is one of the most frequently used secondary diazo dyes (Sakkas *et al.* 2010). Benzidine is a toxic metabolite of Congo Red, which causes cancer of the bladder in humans (Boeniger 1980). Congo Red effluents are highly colored, have low biological oxygen demand and high chemical oxygen demand while they contain high amounts of dissolved solids (Maiti *et al.* 2008). Biological treatment methods involving the use of bacteria belonging to *Bacillus* strains have been frequently discussed for the treatment of azo dyes (Mabrouk & Yusef 2008; Dawkar *et al.* 2009; Thakur *et al.* 2014). In this study, we discuss the ability of *B. weihenstephanensis* RI12 to decolorize the azo dye Congo Red.

Congo Red decolorization under different culture conditions

Effect of initial dye concentration

The decolorization of Congo Red was studied at increasing concentrations of dye, i.e., from 100, 200, 300, 500, 750 and 1,000 mg/l at both static and shaking conditions. A progressive increase of dye decolorization was observed during the first 4 days of incubation. No significant variation occurred on the 6th day of incubation (data not shown). In addition, we found that the rate of decolorization decreased with increasing concentration of dye and the time required for decolorization was increased (Figures 1 and 2). The time required for decolorization was proportional to the dye concentration. Similar observations were also reported during decolorization of reactive violet 5 by newly isolated bacterial consortium (Moosvi *et al.* 2005), the decolorization of the azo dye acid orange 7 by newly isolated consortium TJ-1 (Joshi *et al.* 2008), the decolorization of Brown 3REL by *Bacillus* sp. (Dawkar *et al.* 2008) and the decolorization of Congo Red by *Pseudomonas* sp. SU-EBT (Telke *et al.* 2010). Un-inoculated negative controls realized at the same conditions indicate that there was no

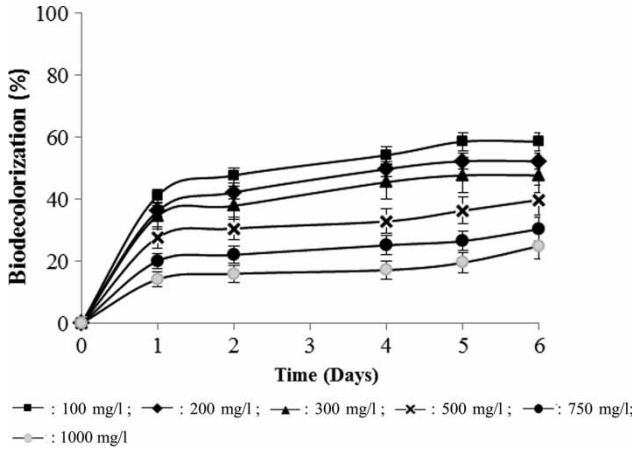

Figure 1 | Effect of dye concentration on the biodecolorization efficiency of Congo Red under static conditions.

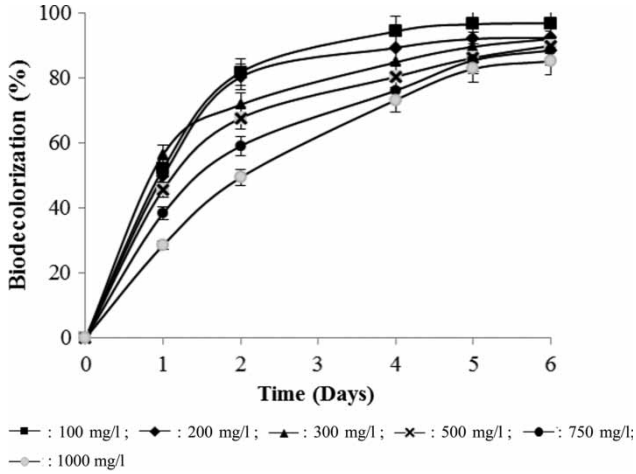

Figure 2 | Effect of dye concentration on the biodecolorization efficiency of Congo Red under agitated conditions.

abiotic loss of Congo Red within the increase of incubation time. However, the strain can tolerate higher doses of dyes as it can decolorize up to 1,000 mg/l of dye. Results are similar to those published by Telke *et al.* (2010) reporting the ability to biodecolorize up to 1,000 mg/l of Congo Red by *Pseudomonas* sp. SU-EBT and by Ayed *et al.* (2010) reporting the ability to decolorize up to 750 mg/l of Congo Red by bacterial consortium. Therefore, the strain *B. weihenstephanensis* RI12 could be considered as an efficient decolorizer of Congo Red. The results are similar to those published by Telke *et al.* (2010) and Chakraborty *et al.* (2013) reporting an efficient biodecolorization of higher doses of Congo Red by *Pseudomonas* sp. and *Alternaria alternate*, respectively. However, other studies reported the biodecolorization of lower doses of Congo Red (Bhattacharya *et al.* 2011; Senthilkumar *et al.* 2011, 2014; Babu *et al.* 2015). Generally, higher doses of azo dye can cause toxicity to living cells and a drastic decrease of decolorization efficiency (Hsueh & Chen 2007; Garg *et al.* 2012).

Effect of static and shaking conditions

The results presented in Figures 1 and 2 indicated that the strain could decolorize Congo Red under both static and shaking conditions, but the decolorization under shaking conditions appeared to be significantly higher than that under static conditions. In fact, as shown in Figures 1 and 2, the decolorization of Congo Red (100–1,000 mg/l) under shaking conditions by *B. weihenstephanensis* RI12 reached 94.29–73.18% after 4 days of incubation. However, values were decreased up to 17.16–54.24% at static conditions after incubating for 4 days. The results are similar to those published by Sani *et al.* (1998) and An *et al.* (2002) reporting the decolorization of diverse textile dyes by *Phanerochœte chrysosporium* and crystal violet by *Citrobacter* sp., respectively. Regarding azo dye biodecolorization, most of the literature has reported high microbial decolorization under static conditions (Fang *et al.* 2004; Telke *et al.* 2010; Garg *et al.* 2012). However, several studies have reported the biodecolorization of Congo Red under shaking conditions (Ayed *et al.* 2010; Bhattacharya *et al.* 2011; Chakraborty *et al.* 2013).

Effect of pH on Congo Red decolorization

The pH was an important factor for the bioadsorption process, because it could affect the dye structure and electric charges related to bioadsorption on the surface of cells and can also affect cellular growth and therefore biodecolorization and biodegradation efficiency (Gou *et al.* 2009). As

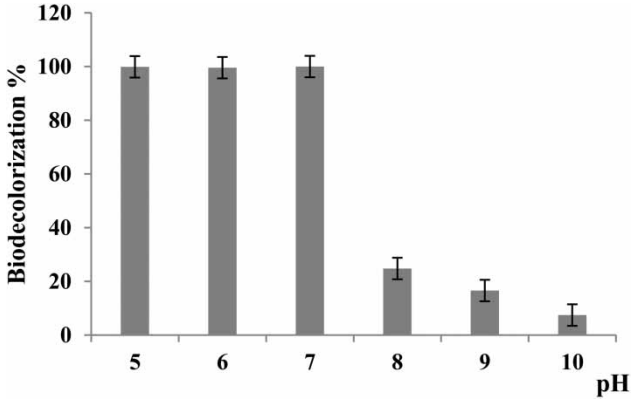

Figure 3 | Effect of initial pH values on Congo Red biodecolorization efficiency.

shown in Figure 3, the optimal pH for Congo Red biodecolorization ranges from 5 to 7. Therefore, it was obvious that a weak acid condition was profitable for decolorization of Congo Red. A drastic decrease of biodecolorization efficiency was observed when operating at basic pH conditions. The results are similar to those presented by Gou *et al.* (2009) reporting efficient azo-dye biosorption at acidic pH values by fungal–bacterial co-cultures. In addition, Chakraborty *et al.* (2013) and Senthilkumar *et al.* (2014) reported a maximal Congo Red biotreatment at pH 5. However, Congo Red adsorptive removal was optimal at pH 7–9 by *Citrobacter* sp. cells (An *et al.* 2002). Furthermore, Hsueh & Chen (2007) and Telke *et al.* (2010) show a preferential Congo Red biodegradation at pH 8.

Effect of SPB1 lipopeptide biosurfactant addition

The newly isolated strain could decolorize the textile dye Congo Red efficiently under shaking conditions. The percentages of decolorization of 1,000 mg/l were about 97.12% after 6 days of incubation whereas they are near to 31.7% in the 1st day of incubation. Surfactant enhanced decolorization technology is one of the feasible approaches to remove textile dye from wastewater. So, in order to ameliorate the biodecolorization efficiency in the course of time, different concentrations of SPB1 lipopeptide biosurfactant (0.05; 0.075 and 0.1%) were added before culture incubation. In fact, SPB1 biosurfactant was characterized by its high emulsification activity (Ghribi *et al.* 2012) and was demonstrated to enhance *in situ* hydrocarbon biodegradation (Mnif *et al.* 2014). Different concentrations of SPB1 lipopeptide biosurfactant (0.025; 0.05 and 0.075%) were added before culture incubation. The results presented in Figure 4 show a significant enhancement of decolorization percentages when using 0.05 and 0.075% biosurfactant. When increasing the

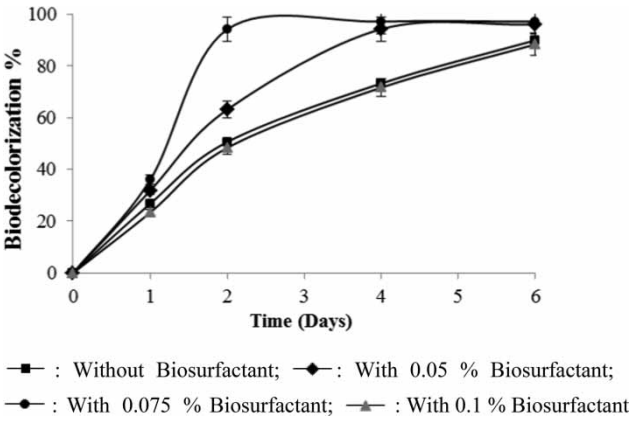

Figure 4 | Effect of the addition of SPB1 biosurfactant on Congo Red biodecolorization.

concentration to 0.1%, biodecolorization efficiency decreases below the negative control without biosurfactant addition. A greater increase of biodecolorization of about double was observed at the 2nd day of incubation with the addition of 0.075% biosurfactant when we reached a maximal decolorization. So, it can save energy and lower energy costs (such as the required energy for treatment techniques) by shortening the incubation period. The findings are similar to those described by Gül & Dönmez (2012) and Jadhav *et al.* (2011) reporting the enhancement of color removal of Remazol Blue by a Living *Aspergillus versicolor* through the addition of dodecyl trimethyl ammonium bromide surfactant and of Brown 3REL by *Bacillus* sp. through the addition of a rhamnolipid biosurfactant, respectively. It is worth noting that, owing to their natural origin, biodegradability and higher efficiency at extreme conditions, biosurfactants have been widely discussed as an enhancer of hydrocarbon solubility and degradation (Owsianiak *et al.* 2009; Saeki *et al.* 2009). Here, they were demonstrated as being efficient to enhance synthetic dye biodecolorization.

The effect of SPB1 biosurfactant was compared to those of Tween 80, CTAB and SDS at optimal concentration. The results presented below show that synthetic surfactant additions decrease the decolorization percentage in comparison to a negative control with no surface active compound supplementation. In fact, a maximal decolorization rate was equal to 70.79% when adding Tween 80; 79.01% when adding CTAB and 58.87% when adding SDS. The slight decrease of biodecolorization efficiency when adding chemical surfactants can be due to their chemical nature and toxic effect so that they can disturb membrane cell integrity, therefore inhibiting their growth and biological activities leading to the decrease of decolorization rate (Kapadia Sanket & Yagnik 2013).

Mechanism of bacterial decolorization of Congo Red

Decolorization of the dye solution may take place in two ways: either adsorption on the microbial biomass or biodegradation of the dyes by the cells (An *et al.* 2002). When conducting biodecolorization using living and dead cells of *B. weihenstephanensis* RI12, efficient decolorizations were observed in the two cases. However, no decolorization was observed by the application of the extracellular and intracellular supernatant. In addition, cells are deeply colored, suggesting the occurrence of an adsorption mechanism. In fact, dye adsorption may be evident from inspection of the bacterial growth; those adsorbing dyes will be colored, whereas those causing degradation will remain colorless.

Therefore, these results indicate that Congo Red removal by this strain can be due to adsorption. Fu & Viraraghavan (2002), Yang *et al.* (2011) and Si *et al.* (2014) reported the efficient removal of Congo Red through adsorption on dead fungus *Aspergillus niger*, *Penicillium* YW 01 and *Trametes pubescens*, respectively. However, many previous studies reported Congo Red mineralization as described by Telke *et al.* (2010) and Bhattacharya *et al.* (2011). Other studies reported the biodegradation of certain azo dyes (Fang *et al.* 2004; Garg *et al.* 2012; Saroj *et al.* 2014). However, Chakraborty *et al.* (2013) reported the occurrence of decolorization of Congo Red dye by a novel white rot fungus *Alternaria alternata* CMERI F6 through biosorption and biodegradation. Moreover, SPB1 lipopeptide was demonstrated to enhance dye removal by adsorption. Previous studies reported the efficiency of certain surfactant-modified supports to adsorb dyes more than unmodified support (Jin *et al.* 2008; Chatterjee *et al.* 2009; Anirudhan & Ramachandran 2015).

Evaluation of the germination potency after biotreatment

After biotreatment, phytotoxicity of the treated dye was measured as the reduction of dye toxicity that represents the main aim of the process (Prasad & Rao 2012). In fact, the increase of germination potency suggesting the abolishment of the toxic effect of dye after treatment could be an efficient tool to evaluate biotreatment efficiency. The germination indicated a lower toxicity of the treated effluent to the plants. Hence, phytotoxicity studies using tomato seeds revealed that the biodecolorization of Congo Red dye by *B. weihenstephanensis* RI12 under different culture conditions resulted in a reduction of toxicity of the dye. In fact, germination potency is about 7% for the non-treated dye, which became about 60.8% for the dye treated by

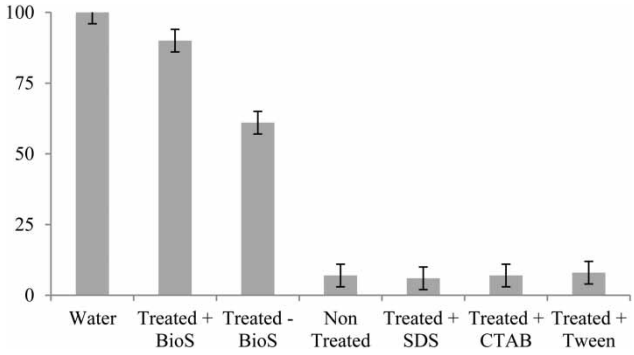

Figure 5 | Germination potencies of tomato seeds before and after treatment of Congo Red.

the strain alone and 90% for the dye treated in the presence of the biosurfactant (Figure 5). The results are similar to those published by Ayed *et al.* (2010, 2011) suggesting the abolishment of dye toxicity after biotreatment. The results showed the great beneficial effect of the SPB1 biosurfactant addition. We observe a great enhancement of the germination potency with the dye treated in the presence of the biosurfactant. So, these treated effluents could be used for ferti-irrigation and do not present a high threat to the environment. In spite of the percentage of biodecolorization not being low when adding chemical surfactants, no germination was observed. These can be due to their higher toxicity to the environment and living organisms, which limits their application in an environmental field (Dayeh *et al.* 2004; Pavlic *et al.* 2005; Kapadia Sanket & Yagnik 2013).

Generally, microbial derived surfactants could be the best alternative to chemical surfactant addition in promoting treatment and eliminating contaminants. It is worth noting that, owing to their natural origin, biodegradability and higher efficiency at extreme conditions, biosurfactants were widely discussed as an enhancer of hydrocarbon solubility and degradation (Owsianiak *et al.* 2009; Saeki *et al.* 2009). In addition, the actual results show the higher efficiency of SPB1 lipopeptide biosurfactant in comparison to the reported chemical emulsifiers. This suggests the competence of *B. subtilis* biosurfactant in promoting Congo Red treatment by *B. weinhenstephensis* RI12. The SPB1 derived biosurfactant has been previously described as an enhancer of diesel solubility and mobility (Mnif *et al.* 2012b, 2013). In another study, *in situ* SPB1 biosurfactant production (Mnif *et al.* 2014) and *ex situ* SPB1 biosurfactant addition (Mnif *et al.* in press) were reported to enhance hydrocarbon assimilation. Hence, SPB1 bioemulsifier could be considered as an exciting candidate in environmental technology to increase contaminant bioavailability and biodegradation. Here, it was described as an effective enhancer of Congo Red biotreatment.

CONCLUSION

In the present investigation, the azo dye Congo Red was decolorized by *B. weihenstephanensis* RI12 isolated from hydrocarbon contaminated soil. The influence of various parameters such as the effect of initial dye concentration and pH on biodegradation was studied. *B. weihenstephanensis* RI12 showed great potential for bioremediation application as it can decolorize up to 1,000 mg/L of Congo Red dye at acidic and neutral pH values. Results were better under shaking conditions. The study of the mechanism of bacterial decolorization suggested the adsorption of Congo Red to the strain. The addition of SPB1 biosurfactant at 0.075% increased significantly the biosorption capacity of Congo Red. The germination potencies of tomato seeds using the treated and non-treated dyes confirmed the efficient biodecolorization of Congo Red. In addition, biosurfactant addition was in favor of a better treatment along with a better germination.

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