Research article

Optimization of complete RB-5 azo dye decolorization using novel cold-adapted and mesophilic bacterial consortia

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A B S T R A C T

Azo dyes are an important group of recalcitrant xenobiotics, which are difficult to degrade and deteriorate in cold environments. In this study, two microbial consortia consisting of cold-adapted and mesophilic bacteria were developed for effective decolorization of Reactive Black-5 azo dye. These bacteria were isolated from textile wastewater and soil of a cold region. Identification of bacterial isolates using 16s rRNA gene analysis revealed that they belong to genus Pseudoarthrobacter, Gordonia, Stenotrophomonas, and Sphingomonas. Decolorization assay was performed for every strain at dye concentrations of 25, 50 and 100 mg/L and the consortia PsGo consisting of mesophilic bacteria and StSp consisting of cold-adapted bacteria were constructed accordingly. Results showed that the consortia PsGo and StSp were able to decolorize 54 and 34 percent of RB-5 (50 mg/L) during 7 days. To improve the dye removal efficiency of the consortia, several parameters including temperature, pH, carbon and nitrogen sources were optimized. Over longer periods, StSp consortium managed to completely decolorize RB-5 (50 mg/L) at optimized conditions of 25–30 °C, pH 9, and using glucose and NH₄H₂PO₄ as carbon and nitrogen source respectively, whereas PsGo consortium decolorized RB-5 (50 mg/mL) completely at 37 °C, pH 11, and with lactose and NH₄H₂PO₄ used as carbon and nitrogen sources. kinetic reactions for StSp and PsGo consortia were found to be 0.05 and 0.13 day⁻¹ respectively, but became 0.71 and 0.9 day⁻¹ after optimization. In general, cold ecosystems are good sources for the isolation of novel bacterial strains with a potential application, especially when used as consortia, in environmental biotechnology such as decolorization of RB-5.

1. Introduction

Azo dyes are a group of colorants well known for their variety and their extensive use in the textile industry. Azo dyes exhibit high resistant to degradation and deterioration, as their chemical structure contains aromatic compounds with one or more –N=N– groups.

There are several physical and chemical techniques for dye removal from wastewater, including adsorption by activated carbon/peat/silica gel, membrane filtration, electrokinetic coagulation, flocculation, and flotation. However, most of these methods are expensive, time-consuming, generate by-products, or produce dense sludge, which needs to be further processed (Robinson et al., 2001). Recently, growing interest has been shown in the microbial treatment of azo dye contaminated areas, as it is relatively eco-friendly, inexpensive, produces lower amounts of sludge and fewer toxic metabolites, and has lower water consumption than many of its alternatives (Saratale et al., 2009).

In this approach, the type of microbial species could affect the efficacy of degradation. Numerous studies have demonstrated the ability of bacterial strains such as Comamonas sp. (Jadhav et al., 2009), Providencia sp. (Agrawal et al., 2014), Pseudomonas sp. (Lin and Leu, 2008), Bacillus sp. (Misal et al., 2011) and deep sea Dermacoccus abyssi (Lang et al., 2014) to remove azo dye (Reactive Black 5). However, the use of a single bacterium seldom leads to complete dye degradation, as no one strain contains all the enzymes necessary for the biodegradation process.

A more efficient approach is to use a synergistic bacterial-bacterial metabolism for this purpose (Jain et al., 2012; Lade et al., 2012; Kadam et al., 2011). In microbial consortia, each strain may attack dye molecule at various positions or consume intermediate degradation metabolites generated by others microorganisms (Keck et al., 2002).

In microbial consortia, the enzyme activity of each individual microorganism is influenced by the presence of other microorganisms,
hence their synergistic effects may lead to enhanced decolorization activity and complete dye degradation.

If the catabolic activities of microorganisms in a consortium are complementary, synthetic interactions between microbial communities can indeed result in complete dye mineralization. For example, Moosvi et al. have shown that a microbial consortium of *Paenibacillus polymyxa*, *Micrococcus luteus*, and *Micrococcus sp.* can completely remove Reactive Violet 5R in 36 h, although none of these bacteria can undertake this task by itself (Moosvi et al., 2007). Another example is the consortium of *Pseudomonas* sp. LBC2, *Pseudomonas* sp. LBC3, and *Pseudomonas* sp. SUK1, which is effective in removing Reactive Orange 16, although the two first bacteria have no such decolorization capability (Jadhav et al., 2010).

It should be noted that the enzyme activity in a consortium will not be necessarily equal to the total enzyme activity in each of the individual member strains (Solis et al., 2012). The degradation capability of microbial consortia is also influenced by other factors such as pH, temperature, aerobic and anaerobic condition, carbon and nitrogen availability, and dye structure, which need to be optimized as well. Most of the past studies on the biodegradation of azo dyes by mesophilic microorganisms have been carried out at 30 °C, but the real temperature in many environments is lower than what is considered optimum for the growth of these microorganisms. Indeed, at some temperatures, degradation by mesophilic bacteria remains extremely limited.

The importance of biodegradation of contaminants at low temperatures in cold soils (alpine) and surface or underground waters in temperate climates with temperatures below 20 °C cannot be ignored. In cold ecosystems, biodegradation is often carried out by psychrophilic or cold-tolerant microorganisms, whose metabolism is adapted to function at low temperatures (Margarin et al., 2002). For example, *Pseudomonas putida*, *Arthrobacter* sp. and *Rhodococcus* sp. isolated from Alpine or Antarctic soils are able to degrade phenol or phenolic compounds (Kotturi et al., 1991; Margerin et al., 2004, 2005; Lee et al., 2017), and cold-tolerant marine bacteria *Pseudoalteromonas* sp., *Marinobacter* sp., *Oleispira* sp., *Alcanivorax* sp., *Sphingopyxis* sp., *Rhodobacter* sp., and *Hyphomonas* sp. isolated from Arctic are effective in the biodegradation of oil/PAH (Crisafi et al., 2016). Considering the composition and condition of textile wastewaters, extremophiles can be a good candidate for the biodegradation of such contaminants. For example, thermophilic bacteria *Anoxybacillus flavithermus*, *Anoxybacillus kamchatkensis* and *Anoxybacillus pushchinoensis* have been shown capable of removing more than 70% of Reactive Black 5 in 24 h at 65 °C (Deive et al., 2010). As another example, it has been reported that the alkaliphilic bacteria *Bacillus badius* can produce azoreductase, which can degrade some azo dyes. Thermal stability of purified azoreductase was up to 85 °C (Misal et al., 2011). However, to the best of authors’ knowledge, this is the first study on the azo dye removal capability of consortia of cold-tolerant bacteria.

RB5 was selected as a model compound because it is highly soluble and contains reactive chemical groups that form covalent bonds between dye and fiber (Vijaykumar et al., 2007). The study aimed to find a novel microbial procedure for the removal of azo dye Reactive Black 5 by designing and optimizing two cold tolerant and mesophilic bacterial consortia for maximum decolorization in the shortest possible time.

### 2. Material and methods

#### 2.1. Isolation of Reactive Black-5 dye decolorizing bacteria

Two types of samples were considered for the construction of microbial consortia. The samples of the first type were collected from the drain of a textile factory located near the city of Mashhad, Iran. Bacterial strains were isolated from the wastewater samples. 10 mL of the sample was mixed with 90 mL of mineral salt medium (MSM) containing (per liter) 5 g of glucose, 1 g of KH₂PO₄; 0.05 g of NH₄NO₃; 0.005 g of MgSO₄·H₂O; 0.05 g of FeSO₄·H₂O; 1.22 g of Na₂HPO₄·12H₂O; 0.013 g of CaCl₂·H₂O; 0.05 g of NaCl and 50 mg/L of azo dye Reactive Black-5. The medium was incubated at 25 °C for 7 days under static condition. After incubation, serial dilutions from each flask were plated on MSM agar containing azo dye (50 mg/L) and incubated at 25 °C. The bacterial colonies that appeared on the agar medium were purified for subsequent experiments.

The samples of the second type, which were the soil samples needed for the isolation of cold-tolerant azo dye decolorizing bacteria were collected from the Zagros Mountains, Iran (33.11N, 48.58E) from an altitude of 2500 m. Ten grams of the soil sample was mixed with 90 mL of MSM supplemented with 50 mg/L of azo dye Reactive Black-5. The growth condition was set up as mentioned above. After the turbidity of the medium, serial dilutions were plated on MSM agar containing RB-5 by the spread plate method and incubated at 15 °C.

Then, to identify the most effective azo dye decolorizing bacterial isolates (from two samples), 2% of pure bacterial suspension in logarithmic phase (OD = 0.65 at λ = 550 nm) was inoculated to the MSM containing RB-5 (50 mg/L). The flasks were incubated at 20–25 °C for 7 days under anaerobic and aerobic conditions.

Azo dye removal was measured at 24 h intervals by spectrophotometry at 597 nm. The cell suspension was centrifuged at 1000 rpm for 15 min. Percentage of decolorization was measured using Equation (1).

\[
\text{Decolorization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100
\]

#### 2.2. Identification of azo dye-decolorizing bacterial strains

DNA genome was extracted using the Genomic DNA isolation kit VI (DenaZist, Mashhad) as per the manufacturer’s instructions. The 16S rRNA amplification was performed using 27F and 1492 R primers. The PCR mix contained 1.5 mM MgCl₂, 30 mM KCl, 10 mM Tris-HCl, 2.5 mM of each dNTP, 5–10 pmol of each primer, and 1U of Top DNA polymerase. The PCR profiling was carried out with the following conditions: initial denaturation at 95 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 54 °C for 30 s, elongation at 72 °C for 1.5 min, and the final elongation for 7 min at 72 °C. Sequencing was carried out at Macrogen, South Korea.

The partial 16S rRNA sequences of the isolates were compared with Ez-Taxon. Multiple sequence alignments were performed using ClustalW (Thompson et al., 1994). The phylogenetic tree was constructed using the Neighbor-Joining method and was evaluated by bootstrap analysis of 1000 data sets using MEGA 7 (Kumar et al., 2016).

#### 2.3. Preparation of bacterial co-culture cell suspension

In order to improve the decolorization ability of RB5 decolorizing strains, two bacterial consortia were defined and studied. The first consortium, named PsGo, was made with the strains obtained from wastewater (Ps and Go stand for molecularly identified bacterial species *Pseudoarthrobacter* and *Gordonia*, respectively), while the second consortium, StSp, was created using the strains from wastewater and soil (St and Sp stands for molecularly identified bacterial species *Stenotrophomonas* and *Sphingomonas*, respectively). A cell suspension at an OD₅₅₀ of 0.65 from 24 h cultivation of each bacterial strain was prepared. The bacterial consortia PsGo and StSp were obtained by mixing the cell suspensions at 1:1 rate.

#### 2.4. RB-5 decolorization

Different initial concentrations of RB-5 (25, 50 and 100 mg/L) in 25 mL of MSM was inoculated by 2% of StSp or PsGo consortia and
incubated under the aerobic and anaerobic condition at 20 °C for StSp and 30 °C for PsGo. Then, the RB-5 residue was measured at 24 h intervals by spectrophotometry at 597 nm.

In order to achieve the best RB-5 decolorization, bacterial consortia were optimized using the one-factor-at-a-time experimental design method. Accordingly, the effects of temperature level (20, 25, 30, 37 °C), pH (6, 7, 9 and 11), carbon source (glucose, sucrose, mannitol, lactose, and starch) at 1% w/v, and nitrogen source (ammonium sulfate, yeast extract, and peptone) at 0.1% w/v on the performance of each bacterial consortium in the decolorization of RB-5 at concentration of 50 mg/L were investigated.

2.5. Determination of the kinetic constant

In the kinetics of biochemical reactions, the change in the contaminant concentration from the start of the reaction (t = 0) and from any time such as t is represented by $[C]_0$ and $[C]_t$ respectively. Since biochemical reactions are usually assumed to be the first order, the kinetic constant may be determined by the Integral Method using the following equations:

$$\int_{[C]_k}^{[C]_0} \frac{d[C]}{[C]} = -k \int_0^t dt$$

$$\ln \frac{[C]_0}{[C]} = kt$$

$$\ln [C] = -kt + \ln [C]_0$$

where $[C]_0$ is the initial concentrations of the azo dye (mg/L), t is the time (day), and k is the kinetic constant (l/day). In the diagram of ln [C] vs. time, the slope of the line is the kinetic constant. The half-life of azo dye decolorization by bacterial combination was calculated using the equation:

$$t_{1/2} = \ln 2/k$$

2.6. Statistical analysis

All experiments were performed in triplicate. One-way analysis of variance (ANOVA) at the 95% confidence level was performed using R software.

3. Results

3.1. Sequence analyses of the RB-5 decolorizing bacterial isolates

Four isolates, including one obtained from soil sample (OSR) and three from wastewater (EF6, EF10, and EF11), were able to grow at the RB-5 concentrations of 25, 50 and 100 mg/L. These strains were identified by their 16S rRNA gene sequences. The strains represented diverse genera including Stenotrophomonas (γ-proteobacteria), Sphingomonas (α-proteobacteria), Gordonia and Pseudarthrobacter (Actinobacteria). Analysis of 16S rRNA sequences revealed that the strains EF11 and OSR shared the highest similarity (100%) with Stenotrophomonas rhizophila and Sphingomonas echinoides. The strain EF6 had the highest similarity (99.33%) with Pseudarthrobacter oxydans. The strain EF10 showed 99% similarity with Gordonia westfalica (Fig. 1).

The MH734610, MH734611, KX839160 and KX839161 are GenBank accesses of sequences of EF6, EF10, EF11 and OSR, respectively.

The cold-tolerant Stenotrophomonas sp. EF11 and Sphingomonas sp. OSR exhibited the properties of a facultative psychrophile (Morita, 1975), showing growth at 4 °C and 20 °C, but not at 35 °C. Finally, two bacterial consortia were prepared based on the growth temperature of the strains: the consortium StSp consisting of psychrotolerant isolates (EF11 and OSR) and the consortium PsGo (EF6 and EF10) consisting of mesophilic strains. Decolorization of the RB-5 dye with pure and co-culture of these bacteria under anaerobic conditions showed that both bacterial consortia exhibited a better decolorization capability than pure bacteria (Fig. 2). However, in comparison, PsGo decolorized RB-5 azo dye (50 mg/L) in a higher percentage (54%) (Fig. 2B) than StSp (34%) did at the same period (7 days) (Fig. 2A).

3.2. Kinetics of RB-5 removal

RB-5 removal was fitted with the first order kinetic constant as given in Equation (4). The kinetic parameters for the decolorization of RB-5 at different initial concentrations are listed in Table 1. For both bacterial consortia, as the RB-5 concentration increased from 25 to 100 mg/L, the rate constant and $t_{1/2}$ both decreased. With the increase in RB-5 concentration from 25 to 100, k of the consortium StSp changed from 0.12 to 0.03 (day $^{-1}$) and that of the consortium PsGo k changed from 0.17 to 0.05 (day $^{-1}$). The results showed that at the initial RB-5 concentration (25 mg/L), both consortia decolorized the dye quickly within 4–6 days of incubation. But at higher RB-5 concentrations, there were significant differences between the rate constant and $t_{1/2}$ of the two consortia, as the mesophilic consortium PsGo managed to decolorize RB-5 about twice as much as the consortium StSp did. At the highest tested RB-5 concentration (100 mg/L), residue RB-5 concentration of PsGo and StSp reached to 64 and 75 mg/L, respectively (Fig. 3).

3.3. Optimization of azo dye decolorization by bacterial consortia

Both bacterial consortia were able to metabolize RB-5 at all temperatures examined. The cold-tolerant consortium StSp exhibited significantly higher removal capability at 25–30 °C (Fig. 4A), while the mesophilic co-culture PsGo decolorized RB-5 better at 37 °C (Fig. 4B).

The changes observed in the kinetic constant of the reaction also support these results. For the consortium StSp, as the temperature increased from 20 to 25 °C and then to 30 °C, the kinetic constant initially increased from 0.07 to 0.24 but then remained constant at 0.24. For the consortium PsGo, however, the highest kinetic constant was obtained at 37 °C (Table 2).

For both StSp and PsGo, increase in pH from 6 to 7 increased the RB-5 decolorization. Decolorization of RB-5 by the bacterial consortia was almost inhibited by the relatively acidic condition (pH 6) and the reduced kinetic constant of the reaction (Table 2). The optimal pH for the decolorization of RB-5 by the consortia StSp and PsGo were 9 and 11, respectively (Fig. 5-AB).

The bacterial consortia were able to grow in the presence of all carbon sources. The RB-5 decolorization ratios achieved after 7 days of incubation by StSp in the presence of glucose, sucrose, mannitol, lactose and starch in the culture medium were 71.5, 52.7, 32.5, 40 and 48.7% respectively (Fig. 6A). In contrast, the data showed that the most effective carbon source for the consortium PsGo was lactose. In the presence of lactose, the concentration of the residual RB-5 reached down to 7.5 mg/L after 168 h (85% decolorization enhancement) (Fig. 6B).

Adding organic and mineral nitrogen sources considerably reduced the RB-5 decolorization time and increased the kinetic constant (Fig. 7). The results showed that, for both consortia, using NH4H2PO4 reduced the residual RB-5 to about 1 mg/L or lower in 5 days and increased the kinetic constant to 0.71 day $^{-1}$ for StSp and 0.9 day$^{-1}$ for PsGo.

4. Discussion

Although many bacterial strains are capable of degrading azo dyes, most of them may produce metabolites that are actually harder to degrade. Furthermore, because of the complex chemical composition of dye-containing wastewaters, the enzymatic reactions caused by microbial consortia will be more effective in the degradation of dye compounds (Joshi et al., 2010; Phugare et al., 2011). In microbial consortia, different strains may attack the dye molecules in different
ways, and one strain may facilitate the activity of others by triggering
an initial degradation.

This study investigated the ability of two consortia, namely StSp (Stenotrophomonas sp.EF11 and Sphingomonas sp.OSR) and PsGo (Pseudarthrobacter sp.EF6 and Gordonia sp.EF10) to decolorize a specific type of dye called Reactive Black-5. Although several researchers have studied the decolorization of azo dyes and contaminants by Stenotrophomonas, Sphingomonas, and Pseudarthrobacter, so far no research has been conducted on the decolorization of RB-5 by Gordonia (Khehra et al., 2005; Ayed et al., 2009; Lin et al., 2012). In this study, the ability of the investigated bacterial consortia to decolorize RB-5 at 50 mg/L was 17% better than that of the individual strains. The removal of RG-5 by the two microbial consortia under anaerobic conditions showed that azo dyes are resistant to bacterial attack in aerobic conditions.

The azo degrading bacteria trigger the enzymatic reduction of azo compounds. In the presence of oxygen, the electron released from the oxidation of electron donor cells goes to the oxygen rather than the azo dye (Jadhav et al., 2010). Under anaerobic conditions, azo dye degradation is a random process, where reductive intermediates resulting

![Fig. 1. Neighbor-joining phylogenetic relationship between the 16S rRNA sequence of the selected strains and the sequences from the database. The sequences of the Halopenitus persicus (JF979130) was used as the outgroup. Bootstrap values (%) are based on 1000 replicates.](image)

![Fig. 2. Decolorization curves for the pure strains and the bacterial consortia StSp and PsGo in MSM medium supplemented with 50 mg/L RB-5. The error bars represent the mean ± standard deviation for three replicates.](image)

<table>
<thead>
<tr>
<th>Initial concentration of RB-5 (mg/L)</th>
<th>k (day^{-1})</th>
<th>R^2</th>
<th>t_{1/2} (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>StSp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.12</td>
<td>0.94</td>
<td>5.75</td>
</tr>
<tr>
<td>50</td>
<td>0.05</td>
<td>0.97</td>
<td>13.8</td>
</tr>
<tr>
<td>100</td>
<td>0.03</td>
<td>0.96</td>
<td>23</td>
</tr>
<tr>
<td>PsGo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.17</td>
<td>0.95</td>
<td>4.05</td>
</tr>
<tr>
<td>50</td>
<td>0.13</td>
<td>0.95</td>
<td>5.3</td>
</tr>
<tr>
<td>100</td>
<td>0.05</td>
<td>0.97</td>
<td>13.8</td>
</tr>
</tbody>
</table>
from the initial substrate degradation are transferred to the azo dye. Nevertheless, the effective degradation of azo dyes under aerobic, anaerobic, and anoxic conditions is well reported (Nachiyar and Rajkumar, 2003; Chen et al., 2003; Khehra et al., 2005; Pourbabaee et al., 2006).

Several studies have examined the degradation of azo dyes by pure strains or a combination of bacteria isolated from active sludge, wastewater or contaminated environments (Yang et al., 2009; Telke et al., 2011; Liu et al., 2013). To the best of our knowledge, this is the first study to investigate the RB-5 decolorization capability of bacterial consortia with cold-tolerance. The importance of this investigation stems from the role of temperature as one of the key factors that affect the rate of degradation of contaminants. The results of this investigation showed that using the consortium StSp, 79% decolorization can be achieved at the temperatures of 25 °C and 30 °C, and it is even possible to achieve 35% dye removal at 20 °C. The consortium PsGo can also acclimate to a relatively wide range of temperatures. This is important because many aquatic and terrestrial environments, such as aquifers and surface waters, have a lower temperature than what is optimal for the growth and activity of mesophilic bacteria, and this can affect the rate of degradation.

A wide-ranging pH tolerance is also very important because it makes the consortium suitable for the biodegradation of dye-containing effluents, including textile industry wastes. Both of the investigated bacterial consortia exhibited adequate decolorization capability at pHs of 6–11. With the consortium StSp, as the pH increased from 6 to 9, decolorization underwent a three times increase (71.5%). But with the consortium PsGo, increasing the pH from 6 to 11 led to a seven times increase in decolorization (85%). This is consistent with the findings of Lalnunhlimi et al., who have reported that in an alkaline pH, a moderately alkaliphilic bacterial consortium was able to remove 87% of Direct Blue 151 and Direct Red 31 at the end of the fifth day (Lalnunhlimi and Krishnaswamy, 2016).

Typically, dyes have a low carbon content, which makes the biodegradation extremely difficult (Khelfi et al., 2009; Asgher et al., 2008). Adding carbon and nitrogen sources can play an effective role in boosting decolorization. Since microorganisms exhibit different metabolic characteristics in using nutrients, they can have different effects on dye biodegradation. Several studies have shown that adding carbon sources increases the azo dye removal capability of mesophilic microorganisms. There is evidence showing that adding sucrose improves the removal of reactive dyes by *Aspergillus fumigatus* (Jin et al., 2007), or adding glucose to the consortium of *Paenibacillus polymyxa*, *Micrococcus luteus* and *Micrococcus sp.* increases the decolorization of Reactive Violet 5 (Moosvi et al., 2007). However, there is no existing report (on the removal of RB5 with cold-tolerant bacteria) that the present study could use as the point of comparison or reference.

In this study, the consortium StSp exhibited a stronger decolorization capability in the presence of any carbon source than in its absence (control). The highest decolorization ratio, 75%, was achieved in the presence of glucose. This is because glucose is a carbon source that can be easily metabolized, accelerates the bacterial growth and metabolism, and acts as a reductive agent for the dye. The differences between the metabolic characteristics of bacteria lead to the consumption of different carbon sources. The preferred carbon source for the consortium PsGo was lactose, which increased the decolorization ratio to 76.5%. In contrast, some researchers have reported reduced decolorization in the presence of some other carbon sources (Kurade et al., 2011; Wagmode et al., 2011).

Organic nitrogen metabolism is important for the regeneration of NADH, as it plays the role of an electron donor (Saratale et al., 2009). The use of organic nitrogen sources such as yeast extract, peptone, and beef extract has been found effective in improving the dye removal of mesophilic microorganisms (Moosvi et al., 2007; Ponraj et al., 2011). For some cold-tolerant bacteria such as *Rhodococcus* sp. and *Arthrobacter* spp., adding inorganic nitrogen sources such as ammonium sulfate and ammonium chloride increases the phenol biodegradation. Ammonium sulfate is abundant in nature and is easily consumed by bacteria as a nitrogen source (Lee et al., 2017).

In this study, although yeast extract and peptone were both effective in enhancing the dye decolorization of the bacterial consortia, the inorganic nitrogen source NH4H2PO4 was found to be a better nitrogen substrate, as using it led to complete dye removal and shortened the decolorization time from 7 to 5 days. This finding is consistent with the
The mesophilic and cold-tolerant bacterial consortia strain investigated in this study were able to decolorize RB-5 at the concentration of 50 mg/L within 5 days at 25 °C (cold tolerant) or 37 °C (mesophile). At the beginning of the experiment, the cold-tolerant bacterial consortium was able to reduce the RB-5 concentration from 50 mg/L to 34 mg/L. The optimization of physicochemical conditions including carbon and nitrogen source, pH, and temperature effectively increased the rate of RB-5 decolorization. After using glucose and NH₄H₂PO₄ as carbon and nitrogen sources and adjusting the pH to 9 and the temperature to 25 °C, the bacterial consortia managed to completely decolorize the RB-5 in 5 days. Biodegradation in cold climates depends on the ability of cold-adapted microorganisms to degrade contaminants under cold conditions. These results demonstrate the efficiency of cold-tolerant bacteria in biodegradation at low temperatures. Moreover, the investigated bacterial consortia could be economically viable for the treatment of industrial effluents.

### Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Consortium-StSp</th>
<th>Consortium-PsGo</th>
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<tr>
<td></td>
<td>kinetic constant (day⁻¹)</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>(R²)</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>0.07 (0.95)</td>
<td>7</td>
</tr>
<tr>
<td>25</td>
<td>0.24 (0.94)</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>0.24 (0.94)</td>
<td>11</td>
</tr>
<tr>
<td>37</td>
<td>0.28 (0.98)</td>
<td>11</td>
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</table>

Fig. 5. Effect of pH on RB-5 decolorization by A) StSp and B) PsGo consortia. pH of the MSM was adjusted to 6, 7, 9 and 11 using 0.1 mol/L HCl or 0.1 mol/L NaOH. The error bars represent the mean ± standard deviation for three replicates.

Fig. 6. Effects of different carbon sources on RB-5 decolorization by A) StSp and B) PsGo consortia. The error bars represent the mean ± standard deviation for three replicates.

results of Saratale, who has reported the improved removal of Reactive Black RC and Reactive Black B-150 by Aspergillus fumigatus in the presence of ammonium sulfate and ammonium chloride as nitrogen sources (2009).

### 5. Conclusion

The mesophilic and cold-tolerant bacterial consortia strain investigated in this study were able to decolorize RB-5 at the concentration of 50 mg/L within 5 days at 25 °C (cold tolerant) or 37 °C (mesophile). At the beginning of the experiment, the cold-tolerant bacterial consortium was able to reduce the RB-5 concentration from 50 mg/L to 34 mg/L. The optimization of physicochemical conditions...


Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. ClustalW- Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-
Decolourization of naphthalene-containing sulfonated azo dyes by Kerstersia sp strain
aerobic/microaerophilic decolorization of sulfonated monoazo dye Golden Yellow
Yang, Q., Li, C., Li, H., Li, Y., Yu, N., 2009. Degradation of synthetic reactiveazo dyes and
treatment of textile wastewater by a fungi consortium reactor. Biochem. Eng. J. 43,
225–230.