Ecofriendly decolorisation of Cr-complex dye Acid Black 172 by a newly isolated *Pseudomonas* sp. strain DY1

Lin-Na Du, Sheng Wang, Gang Li, Yu-Yi Yang, Xiao-Ming Jia and Yu-Hua Zhao

**ABSTRACT**

*Pseudomonas* sp. strain DY1 was newly isolated from soil with rotten wood and identified as a member of the genus *Pseudomonas* based on 16S rDNA and biochemical tests. Acid Black 172, a water soluble Cr-complex dye, was then selected as a model dye to investigate the decolorisation ability of the strain. It was observed that the growth of the strain was not inhibited by high dose of metal ions (10 mM), and efficient decolorisation was still observed when high concentrations of Fe$^{2+}$, Fe$^{3+}$ and Ca$^{2+}$ existed. The optimal decolorising conditions obtained from Taguchi design were as follows: temperature 37°C, pH 7.0, Fe$^{3+}$ and proline concentrations of 7 mM and 3.0 g/L, respectively. Under the optimal conditions, 94.5% of Acid Black 172 (100 mg/L) could be decolorised by the strain in 24 h. The kinetics of the decolorisation best fitted the first order kinetic model ($R^2 = 0.981$). Besides, the phytotoxicity study demonstrated a good detoxification by the strain. This work has a certain practical value in microbial decolorisation of textile wastewater.

**Key words** | heavy metal complex dye, kinetic model, microbial decolorisation, phytotoxicity, Taguchi design

**INTRODUCTION**

Large quantities of dyes are produced (more than $7 \times 10^5$ tonnes) and widely used in many fields all through the world per year. With the extensive usage of various dyes, it has been found that serious environmental and health problems have been caused owing to their toxicity, since about 10–15% of dyes are released to the environment during the processes of production and usage (Van der Zee & Villaverde 2005). Although traditional technologies, such as zero-valent iron reduction, adsorption on carbon and coagulation, have made certain progress in solving the problem, the limitations of these conventional technologies, including high cost, low efficiency and disposal and recontamination problems, still exist, especially when dealing with water soluble acid dyes and complex aromatic molecular structure of dyes (Fu & Viraraghavan 2002; Ghoreishi & Haghhighi 2003; Xin et al. 2010). Therefore, removal of dyes from wastewater has become a challenge to the environmental scientists. Recently, many new methods involving adsorption on low-cost inorganic or organic matrices, photocatalysis and oxidation processes, electrocoagulation, ozonation and microbiological or enzymatic decomposition have been developed, in which biological techniques were thought to be a valuable removal method in dye wastewater treatment since the biological techniques were ecofriendly to the environment (Mohey El-Dein et al. 2001; Wu & Wang 2001).

Biological techniques in dye wastewater treatment mainly include the biodegradation, biosorption and bioaccumulation of dyes using various microorganisms (Xin et al. 2010), in which biosorption and bioaccumulation are thought to be the prospect for the practical application (Chojnacka 2010). In recent years, a few investigations have focused on bioaccumulation of dyes by various growing biomass (Wang & Hu 2008; Das et al. 2010). Compared to biosorption, bioaccumulation dyes from dye wastewater by growing biomass can avoid certain disadvantages of the traditional techniques, make the system simpler, and lower the operational cost (Xin et al. 2010).

Although a large number of microorganisms were tested for decolourisation of various dyes (Fu & Viraraghavan 2001), few studies have focused on the decolourisation of the heavy metal complex dyes or dye wastewater containing heavy metals. It is well known that various heavy metals are usually contained in dye wastewater because of the usage of the metal complex dyes in textile industry and high dose of metals in the process of physicochemical pretreatment of dye wastewater (Hao et al. 2000; Chang et al. 2009). Therefore, more attention should be paid to the decolourisation of the heavy metal complex dyes or dye wastewater containing heavy metals, since heavy metals are also hazardous to humans and organisms (Sari et al. 2007).

The present study aims at: (1) isolating and identifying of bacteria to decolourise water soluble Cr-complex dye Acid Black 172; (2) investigating the effect of operational parameters on decolourisation by the isolated strain, and optimising the parameters of decolourisation by the strain; (3) investigating the kinetics of decolourisation by the strain and the phytotoxicity of the dye before and after treatment.

Materials and methods

Chemicals and media

Acid Black 172 was obtained from Hangzhou Xiasha Hengsheng (Sunshine) Chemicals Company, China. All other chemicals used in this study were reagent/analytical grade.

Mineral salts medium (MSM) pH 7.0 consists of 15.13 g/L Na₂HPO₄, 5.0 g/L KH₂PO₄, 0.5 g/L NaCl, 1.0 g/L NH₄Cl, 0.491 g/L MgSO₄·7H₂O, 0.026 g/L CaCl₂·2H₂O. Luria-Bertani medium (LB) pH 7.0 consists of 10.0 g/L Tryptone, 5.0 g/L Yeast Extract, 10.0 g/L NaCl. Dye solution I pH 7.0 consists of 15.13 g/L Na₂HPO₄, 3.0 g/L KH₂PO₄, 0.5 g/L NaCl and 100 mg/L dye.

Isolation and identification of dye-decolorising bacteria

The dye-decolorising strains were isolated from soil with rotten wood at Shandong, China. These isolates were capable of growth in MSM amended with Acid Black 172 and 2.0 g/L yeast extract. The DY1 strain which has an efficient decolorising performance was chosen for the further study. Identification of the decolorising strain DY1 was based on morphological and biochemical tests according to Bergey’s manual of determinative bacteriology and 16S rDNA analysis (Holt et al. 1994). To do the 16S rDNA analysis, genomic DNA of the strain DY1 was firstly extracted according to Ausubel et al. (2005) with modifications as the template of polymerase chain reaction (PCR). Then two universal primers for 16S rRNA gene, BSF8/20: 5’-AGAGTTTGATCCTGGCTCAG-3’ and BSR1541/20: 5’-AAGGAGGTAGCCCCGGA-3’, were used. The sequences of the amplified 16S rDNA gene were determined by Shanghai Invitrogen Biotechnology Company, China. Finally, the sequence with similarity was searched by using BLASTN program based on web NCBI server (www.ncbi.nlm.nih.gov/BLAST).

Decolourisation experiments

The “one-factor-at-a-time approach” was firstly applied to investigate the effect of pH, temperature, different carbon and nitrogen sources, and metal ions on decolourisation of Acid Black 172 by strain DY1.

The initial pH of the medium was adjusted to 6.0, 7.0, 8.0, 9.0, 10.0 or 11.0 with 1 M HCl or NaOH, and then 1 mL of inoculum was used to inoculate 100 mL Erlenmeyer flasks containing 20 mL of LB medium which contained 100 mg/L Acid Black 172. The cultures were then incubated at 30°C in a rotary shaker running at 200 rpm for 24 h. Control experiments without inoculation were set up simultaneously.

To determine the effect of temperature on decolourisation of Acid Black 172 by strain DY1, we conducted the decolourisation tests at 25.0, 28.0, 30.0, 33.5, 37.0 and 40.0°C. The tests were carried out in the 100 mL flasks containing 20 mL of dye solution I which also contained 100 mg/L Acid Black 172 and 2.0 g/L yeast extract. Each flask was inoculated with an equal cell density of bacteria. All flasks were then incubated at controlled temperature in a rotary shaker running at 200 rpm for 24 h. Control experiments without inoculation were set up simultaneously.

In order to investigate the effect of carbon source and nitrogen source on decolourisation, 2.0 g/L carbon source or nitrogen source were added to the dye solution I, respectively. All cultures were incubated at 30°C and 200 rpm in a rotary shaker for 24 h. Control experiments without carbon or nitrogen source were set up simultaneously.

Effects of different metal ions on decolourisation, including CuCl₂, PbCl₂, MgCl₂, FeCl₂, CoCl₂, CdCl₂, MnCl₂, LiCl, CaCl₂, ZnCl₂ and FeSO₄, were investigated by adjusting the concentration of metal ions to 10 mM. All cultures were then incubated at 30°C in a rotary shaker running at 200 rpm for 24 h. Control experiments without additional metal ions were set up simultaneously.
Optimisation of decolorisation using Taguchi design and kinetics study under the optimal conditions

Based on the “one-factor-at-a-time approach” experiments, Taguchi design, which is very efficient in generating useful information on key variables, was used to optimise the variables of the decolorisation. The variables employed were pH of the analytical system, concentration of Fe$^{3+}$, concentration of proline and temperature. The factors and their levels are presented in Table 2. Taguchi L9 ($2^3 \times 3^3$) orthogonal array which was constructed using Quasi-level method is also presented in Table 2. Meanwhile, the order of experiments was randomised to avoid noise sources.

Thereafter, the cultures were incubated under the optimal conditions to study the kinetics of the decolorisation. At several time intervals, 1 mL samples were collected and centrifuged at 10,000 rpm for 10 min. The supernatants were analysed separately using a spectrophotometer at $\lambda_{\text{max}}$ of Acid Black 172.

Phytotoxicity tests

In order to determine the toxicity of the dye-containing solution before and after inoculation of strain DY1, phytotoxicity tests were performed using the seeds of Lucerne (Medicago sativa Linn.) and Chinese cabbage (B. chinensis Linn.). The dye-containing wastewater, before and after inoculation, was sterilised at 121°C for 20 min. The seeds of Lucerne and Chinese cabbage were sterilised with 3–5% hydrogen peroxide solution for 5 min, and washed with sterile water 3–5 times. Thirty seeds of Lucerne or Chinese cabbage were put into individual plates containing 7 mL of dye-containing solution, treated or untreated. The plates were then cultivated at 22°C with 65% humidity. After a week, the growth state of the plants was checked.

Computational and analytical methods

The absorbance peak ($\lambda_{\text{max}}$) of the dye was firstly scanned by a UV-3100PC spectrophotometer (Shanghai MAPADA Instruments Co., Ltd.). Then 4 mL samples were centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant for each sample was determined at $\lambda_{\text{max}}$ of the dye. The decolorisation was calculated by the following formula:

\[
\text{Decolorisation percentage(%) } = \frac{A_i - A_f}{A_i} \times 100
\]

$A_i$ initial absorbance

$A_f$ final absorbance

Each data point represented the mean values of at least three replicates.

RESULTS AND DISCUSSION

Isolation and preliminary identification

The mechanism of decolorisation by growing cells was due to either biodegradation or bioaccumulation (Wang & Hu 2008). If the decolorisation is due to biodegradation, either the absorbance peak will disappear or a new absorbance peak will appear; while, if the decolorisation is due to bioaccumulation, all peaks will decrease approximately in proportion (Parshetti et al. 2010). It was observed that the cells of strain DY1 after decolorisation were highly coloured and all peaks of the UV-visible spectra of the dye solution after decolorisation decreased approximately in proportion (data not shown). Therefore, the mechanism of the decolorisation by the strain was mainly due to bioaccumulation. The strain was then identified according to Bergey’s manual of determinative bacteriology (Holt et al. 1994). It was Gram negative, aerobic and rod-shaped. The biochemical tests indicated that the strain was esterase negative, arginine hydrodase and oxidase positive. The results indicated that the strain might belong to the Pseudomonas sp. Our further analysis of the G + C content of the genomic DNA of the strain indicated that there was a similar percentage between the strain (with 67.7%) and other previous reported Pseudomonas sp. strains (range from 58% to 70%) (Holt et al. 1994). Meanwhile, the 16S rDNA sequence (Accession No. GU136489) analysis of strain DY1 at BLASTN site at NCBI server showed 98% similarity with Pseudomonas putida strain ATCC 17390. Multiple alignment with the close species was carried out by CLUSTAL X 1.8.3 software for the neighbour-joining analysis. A phylogenetic tree was then constructed using the neighbour-joining method with bootstrap in the MEGA4.1. The phylogenetic relationship between the strain DY1 and other related bacteria in the GenBank database is shown in Figure 1. The phylogenetic tree also indicated that this strain was in the phylogenetic branch of the Pseudomonas genus.

Effect of various operational parameters on decolorisation

The effects of various parameters on decolorisation by the strain are summarised in Table 1. The suitable initial pH for decolorisation ranged from 8.0 to 11.0. The decolorisation
percentage was increased in the range pH 6.0 to 10.0, and it reached the maximum at the initial pH 10.0. However, the dried biomass was decreased from 1.47 g/L to 0.75 g/L in the range pH 6.0 to 11.0, indicating that pH does not only affect the population of the strain but also affects some physical and chemical properties of the strain and further affects decolourisation. The decolourisation had no significant change in the temperature range of 25 to 33.5°C, while a greater decolourisation was induced when the temperature was beyond 33.5°C, and the performance of decolourisation was greatest at 37°C. The dried biomass exhibited the same change trend with decolourisation at the tested temperature, and the highest biomass (1.92 g/L) was observed at 37°C. It was noteworthy that 70.9% decolourisation, which was insignificantly decreased compared to that at 37°C (p > 0.05), was still observed at 40°C (Table 1). The actual working experience in the wastewater treatment factory indicated that the decolourisation could be compromised by high temperatures, and this strain which still has a higher decolourising efficiency at 40°C will have an applicable future prospect in solving this problem.

Among the tested carbon sources, glucose obviously inhibited decolourisation, while the other carbon sources had no significant influence. Compared to the carbon source, most tested nitrogen sources could significantly enhance decolourisation. The observation was in accordance with other reports (Saratale et al. 2009a, b). The addition of inorganic nitrogen sources (NH₄Cl, NaNO₃) slightly enhanced decolourisation, while nitrogen sources with complex composition (beef extract, peptone and yeast extract) were better than inorganic nitrogen sources. Besides, proline, glycine and glutamate also strongly enhanced decolourisation (Table 1).

Various metal ions which may affect the microbial decolourisation usually exist in the textile wastewater. It has been reported that Mg²⁺, Mn²⁺, Ca²⁺ and Zn²⁺ appeared to have almost no effect on decolourisation, and Fe²⁺ nearly completely inhibited decolourisation, and Cu²⁺ and Hg²⁺ moderately (Nachiyar & Rajakumar 2005). However, in the present study, it was observed that decolourisation was significantly enhanced by Fe²⁺ > Fe³⁺ > Ca²⁺, but inhibited by Ca²⁺ > Mg²⁺ > Co²⁺ > Cd²⁺. Besides, Zn²⁺, Li⁺, Pb²⁺ and Mn²⁺ had no significant effect on decolourisation (Table 1). Similar to the report of Nachiyar et al. (2005), Ca²⁺ slightly affected decolourisation, but, differently, Fe²⁺ and Fe³⁺ strongly enhanced decolourisation. Xu et al. (2007) also reported that Fe³⁺ enhanced decolourisation by Shewanella decolorationis S12 under anaerobic condition, but Fe²⁺ did not. In this investigation, both additional Fe³⁺ and Fe²⁺ could enhance decolourisation by the strain DY1 under aerobic conditions.

Since the metal complex dyes are used in textile industry and a high dose of Fe⁰ is also widely used in pretreatment of dye wastewater, variable concentrations of heavy metals, especially Fe³⁺ and Fe²⁺, are usually contained in the dye wastewater (Aksu 2005; Chang et al. 2009; Tastan et al. 2010). Therefore, the effects of different concentrations of Fe³⁺ on decolourisation by the strain were also investigated. The data showed that decolourisation by strain DY1 was enhanced and remained steady when the concentration of Fe³⁺ lingered between 5 mM and 17 mM, but declined when the amounts of Fe³⁺ increased beyond 17 mM (data not shown). Heavy metals often have unique biotoxities to microorganisms (Wei et al. 2009); therefore, there are few reports on microbial decolourisation of dyes at the existence of high concentration of heavy metals, and the concentration of heavy metals reported previously was usually below 1 mM even in the studies focused on the bioremediation of heavy metals (Wei et al. 2009; Tastan et al. 2010). In the present study, concentrations of different metal ions were as high as 10 mM; however, decolourisation of Acid Black 172 and biomass of the strain were still higher than that of control experiments when Fe²⁺, Fe³⁺ and Ca²⁺ existed (data not shown). This discovery will be practical in solving the problem that functional microorganisms are often inhibited by the heavy
Table 1 | Effect of various parameters on decolorisation by strain DY1

<table>
<thead>
<tr>
<th>Various parameters</th>
<th>pH</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
<th>9.0</th>
<th>10.0</th>
<th>11.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decolorisation (%)</td>
<td></td>
<td>25.3</td>
<td>26.5</td>
<td>38.0</td>
<td>45.0</td>
<td>45.8</td>
<td>26.6</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>1.3</td>
<td>2.1</td>
<td>5.8</td>
<td>2.3</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td>25</td>
<td>28</td>
<td>30</td>
<td>33.5</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>Decolorisation (%)</td>
<td></td>
<td>32.3</td>
<td>36.3</td>
<td>28.8</td>
<td>27.7</td>
<td>72.6</td>
<td>70.9</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.9</td>
<td>3.4</td>
<td>0.3</td>
<td>1.1</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Carbon source</td>
<td>Glucose</td>
<td>Maltose</td>
<td>Lactose</td>
<td>D-galactose</td>
<td>D-mannose</td>
<td>Starch</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Decolorisation (%)</td>
<td></td>
<td>18.8***</td>
<td>40.9</td>
<td>46.2</td>
<td>39.2</td>
<td>52.3</td>
<td>40.2</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>3.8</td>
<td>3.9</td>
<td>2.6</td>
<td>4.6</td>
<td>2.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Nitrogen source</td>
<td>NH₄Cl</td>
<td>NaNO₃</td>
<td>Beef extract</td>
<td>Peptone</td>
<td>Yeast extract</td>
<td>Proline</td>
<td>Glycine</td>
</tr>
<tr>
<td>Decolorisation (%)</td>
<td></td>
<td>41.1</td>
<td>46.7*</td>
<td>55.5***</td>
<td>50.8***</td>
<td>57.4***</td>
<td>71.0***</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.1</td>
<td>1.9</td>
<td>1.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Metal ions</td>
<td>Cu²⁺</td>
<td>Pb²⁺</td>
<td>Mg²⁺</td>
<td>Fe³⁺</td>
<td>Co²⁺</td>
<td>Cd²⁺</td>
<td>Mn²⁺</td>
</tr>
<tr>
<td>Decolorisation (%)</td>
<td></td>
<td>0**</td>
<td>10.4</td>
<td>2.4**</td>
<td>25.2**</td>
<td>5.7***</td>
<td>9.7*</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0</td>
<td>0.9</td>
<td>1.1</td>
<td>1.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The data are means of triplicate experiments ± SE. Significantly different from control cells at *

\( p \leq 0.05 \),

** \( p \leq 0.01 \),

*** \( p \leq 0.001 \) by two-tailed \( p \)-values comparison.
metals in the textile wastewater. Therefore, this strain will be applicable especially when high concentrations of Fe$^{3+}$ and Fe$^{2+}$ exist in the textile wastewater. To further enhance the decolorisation performance by the strain, in the next step, we optimised the operational parameters using Taguchi design.

**Optimisation of decolorisation efficiency using Taguchi design**

Based on the above “one-factor-at-a-time approach” experiments, four key variables, including pH, temperature, Fe$^{3+}$ and proline, were investigated to obtain the optimal conditions using Taguchi design (L9). The results are presented in Table 2. Based on the data of Table 2, the sum and average at each level of a factor and range of response were shown in Table 3. It is clear from Table 3 that the factor A (pH) is more prominent than other factors. Data of Table 3 also indicated that the maximum sum and average values were at level 1 for the factor A, since these values at each level of the factor A are K1 > K3 > K2 and k1 > k3 > k2, respectively. Therefore, the optimal pH for decolorisation is at level 1 (pH 7.0). Similarly, the optimal concentration of Fe$^{3+}$ for decolourisation is at level 1 (7 mM). Besides, the optimal concentration of proline for decolourisation is at level 3 (3.0 g/L) and optimal temperature is at level 2 (37°C). Therefore, the optimal conditions are as follows: temperature 37°C, pH 7.0, Fe$^{3+}$ and proline concentrations of 7 mM and 3.0 g/L, respectively. Subsequently, three replicated confirmation tests under the optimal conditions obtained from the Taguchi design were carried out to verify the accuracy of the experiments. The results showed that 94.5% decolourisation (higher than any decolourisation percentage value in Taguchi design tests) was observed in the verification experiments. Hence, Taguchi design was suitable in the present research and successfully produced desired results.

**Kinetics modelling**

It has been reported that decolourisation obviously changed with incubation time, and various kinetic models of decolourisation which will provide useful information to the practical application have been investigated (Wei et al. 2009; Tastan et al. 2010). The kinetics tests were also performed in the present study under the optimal conditions. The data were plotted in forms C$_t$ versus time, lnC$_t$ versus time and 1/C$_t$ versus time according to the Equations (1–4) described by Das et al. (2010) and depicted in Figure 2.

\[ C_t = C_0 - r_0 t \]  
\[ C_t = C_0 e^{-k_1 t} \]  
\[ 1/C_t = 1/C_0 + k_2 t \]  
\[ \ln C_t = -k_1 t + \ln C_0 \]

where \( C_t (\text{mg/L}) \) is the concentration of Acid Black 172 at time \( t \), \( C_0 (\text{mg/L}) \) is the initial dye concentration, \( t (\text{h}) \) is time of incubation, \( k_0, k_1 \) and \( k_2 \) are the zero order, first order and second order rate constants, respectively. Equation (2) also can be written as:

\[ \ln C_t = -k_1 t + \ln C_0 \]

As indicated in Figure 2, the rate constants for the zero order, first order and second order model were as follows: \( k_0 = 0.041, \ k_1 = 0.123, \ k_2 = 0.454 \). It was observed that the

**Table 3 | The sum (K) and average (k) at each level of a factor and range of response**

<table>
<thead>
<tr>
<th>Level</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>267.1</td>
<td>266.4</td>
<td>256.8</td>
<td>243.6</td>
</tr>
<tr>
<td>K2</td>
<td>246.9</td>
<td>249.3</td>
<td>249.9</td>
<td>260.4</td>
</tr>
<tr>
<td>K3</td>
<td>250.7</td>
<td>249.3</td>
<td>257.7</td>
<td></td>
</tr>
<tr>
<td>k1</td>
<td>89.0</td>
<td>88.8</td>
<td>85.6</td>
<td>81.2</td>
</tr>
<tr>
<td>k2</td>
<td>82.3</td>
<td>83.1</td>
<td>83.3</td>
<td>86.8</td>
</tr>
<tr>
<td>k3</td>
<td>83.6</td>
<td>83.1</td>
<td>85.9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.7</td>
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</tr>
<tr>
<td>Rank</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

---

**Table 2 | Taguchi L$_{9}$ (3$^4$) orthogonal array and results for decolourisation**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>A (pH)</th>
<th>B (Fe$^{3+}$ (mM))</th>
<th>C (Proline (g/L))</th>
<th>D (Temperature (°C))</th>
<th>Decolourisation percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.0</td>
<td>7</td>
<td>1</td>
<td>30</td>
<td>89.8</td>
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<td>2</td>
<td>7.0</td>
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<td>2</td>
<td>37</td>
<td>87.6</td>
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<td>2</td>
<td>37</td>
<td>86.1</td>
</tr>
<tr>
<td>5</td>
<td>8.0</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>77.6</td>
</tr>
<tr>
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<td>1</td>
<td>37</td>
<td>83.2</td>
</tr>
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<td>7</td>
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<td>7</td>
<td>3</td>
<td>37</td>
<td>90.5</td>
</tr>
<tr>
<td>8</td>
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<td>10</td>
<td>1</td>
<td>37</td>
<td>84.0</td>
</tr>
<tr>
<td>9</td>
<td>9.0</td>
<td>13</td>
<td>2</td>
<td>30</td>
<td>76.2</td>
</tr>
</tbody>
</table>
determination coefficients of the zero ($R^2 = 0.791$) and second order ($R^2 = 0.963$) kinetic model were lower than that of the first order kinetic model. Therefore, the data was best fitted in the first order kinetic model: $\ln C_t = \frac{1}{k_1}t + \ln C_0$ ($k_1 = 0.123$, $R^2 = 0.981$), since the determination coefficient $R^2$ was 0.981. In other words, the relationship between dye decolorisation by the strain DY1 and time can be well described by the first order kinetic model.

Phytotoxicity analysis

Considering that untreated dye-containing wastewater is hazardous to the environment, and treated dye-containing wastewater can be reused in the agriculture, it is necessary to assess the toxicity of the treated dye-containing solution (Jadhav et al. 2008). Therefore, a phytotoxicity study was performed to assess the toxicity of the dye-containing wastewater before and after being treated by strain DY1. The results revealed that the leaves of the Lucerne and Chinese cabbage in untreated dye-containing wastewater were yellow and the germination percentage was very low, while they both grew better in treated dye-containing wastewater (Figure 3), suggesting that the toxicity of the dye-containing solution was reduced after being treated by the strain DY1. Compared to the seeds in water, the growth of the seeds in dye-containing wastewater either treated or not was inhibited, although the toxicity of the treated dye-containing solution was reduced. Besides, the seeds of Chinese cabbage were more sensitive to the toxicity of the dye-containing solution, since the growth state of the seeds was really bad in dye-containing solution. Therefore, the phytotoxicity study demonstrated a good detoxification of Acid Black 172 by the strain DY1 and the decolorisation of Acid Black 172 by the strain is environmentally friendly.

CONCLUSIONS

The present study focused on the decolorisation of water soluble Cr-complex dye by a newly isolated Pseudomonas sp. strain DY1. Effects of various operational parameters on decolorisation by the strain were investigated systematically.
Based on these results, Taguchi design was successfully used to obtain the optimal conditions for the decolorisation. It was observed that the kinetics of the decolorisation best fitted the first order kinetic model. Besides, the phytotoxicity study demonstrated a good detoxification of Acid Black 172 by the strain. This study presented some valuable information on microbial decolorisation of water soluble Cr-complex dye and had a certain value in bioremediation of textile wastewater.

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**REFERENCES**


