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Biodegradation of dye-containing wastewater by fusant strains using a sequential anaerobic–aerobic process

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ABSTRACT

A fusant strain T1, which was constructed by protoplast fusion between *Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68 and *Acinetobacter* sp. RC10, could simultaneously decolorize three different dyes, namely reactive brilliant blue XBR, disperse orange SRL, and red base RC. Maximum decolorization of initial 30 mg L^{-1} was achieved at pH 7.0, 35°C and 3% inoculum (v/v) under anaerobic condition during 24 h incubation. The decolorization ratio for XBR, SRL, and RC by the strain T1 could reach 91.5, 87.6, and 65.7%, respectively. Supplementation with appropriate concentration of glucose (0.2%, w/v) in the decolorization reaction system could further enhance decolorization percent. The strain T1 was highly tolerant to three different dyes up to 400 mg L^{-1}. Biological decolorization of the three different dyes by the strain T1 was investigated in a sequential anaerobic/aerobic process. The reaction system was operated at room temperature. The results indicated that over 95% color removal was obtained in anaerobic process and 97.6% COD removal occurred in the aerobic process.

Keywords: Microbial decolorization; Protoplast fusion; Anthraquinone dye; Azo dye; Biodegradation kinetics

1. Introduction

Synthetic dyes are widely produced and applied in paper, food, leather, textile, dyeing, and printing industries. It was reported that there are more than 100,000 commercially different dyes with over 700,000 tons of dyestuff produced annually worldwide [1,2]. The production of dyes in China is 150,000 tons annually. The dyes productive processes lead to a large amount of wastewater production, which contains many kinds of dyes, such as azo dye, anthraquinone dye, and azoic dyes. It is estimated that 10–15% of dyes per year is discharged into the industrial wastewater. Untreated dye wastewater is usually highly colored and these industrial dye wastes are toxic and carcinogenic to organism [3–6]. Industrial effluents containing dyes are discharged particularly in developing countries, leading to gross pollution of environment [7,8].

The dye-containing wastewaters are very difficult to treat for its higher chroma and COD concentration. Decolorization process is a great challenge for dye-containing wastewater treatment industry. Physicochemical treatment methods, such as catalytic

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ozonation, photocatalysis, ultrasonic irradiation, and electrochemical oxidation are often used in dyes wastewater treatment. However, many of these methods are cost prohibitive and therefore are not viable options for treating large dye-containing wastewater streams. Microbial decolorization techniques are more attractive and economic because micro-organism could cleave the color bond of dyes to generate much simpler compounds. Ramya et al. reported that there is a great potential for developing microbial decolorization technique for its total color removal in 24–72 h, in some cases within a few hours [9]. Many bacteria and fungus can degrade the dyes and decolorize. *Pseudomonas putida*, *Bacillus* sp., *Lysinibacillus* sp., *Aspergillus niger*, *Staphylococcus arlettae*, *Bjerkandera adusta*, *Trameters* sp., *Irpex lacteus*, etc. were isolated and used for azo dyes decolorization [1,2,10–12]. *Rhodopseudomonas* sp., *Flavobacterium* sp., *Shewanella* sp., *Desudomonas* sp., *Escherichia* sp., *Klebslella* sp., *Phanerochaete* sp., *Trametes versicolor*, *Penicillium* sp., *Rhodotorula harrison* sp. etc. were reported for anthraquinone dye decolorization [13–17]. Prokaryotic cells had capability of decolorization through break the color bond of dyes, while fungi perform decolorization process through absorbing dyes [10,18,19]. Biological treatment offers a cost-effective and friendly way for decolorization and degradation of dyes in industrial effluents and contaminated soil [20].

In our previous study, we have isolated the strains of *Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68 and *Acinetobacter* sp. RC10, which have decolorization ability for anthraquinone dye- reactive brilliant blue XBR, azo dye disperse orange SRL and azoic dye red base RC, respectively. The various nutritional and environmental factors influencing dye degradation have also been studied [21]. In this study, in order to improve dye decolorization capabilities, a functional strain T1 was constructed using *Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68 and *Acinetobacter* sp. RC10 by protoplast fusion technology and was employed to decolorize three different dyes.

2. Materials and methods

2.1. Chemicals and micro-organisms

Reactive brilliant blue XBR, azo dye disperse orange SRL, and azoic dye red base RC (Fig. 1) were obtained from Light chemical engineering laboratory, Changzhou University. All other chemicals were purchased from Sinopharm Chemical Reagent Company (Shanghai, China) and were of analytical grade and of highest purity available.

The strains of *Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68, *Acinetobacter* sp. RC10, degrading fusant strain J8 and its parent strains (aniline-degrading strain An7 and naphthalene-degrading strain Nai 8) used in this study were previously isolated from dyes-contaminated soil by authors [21].

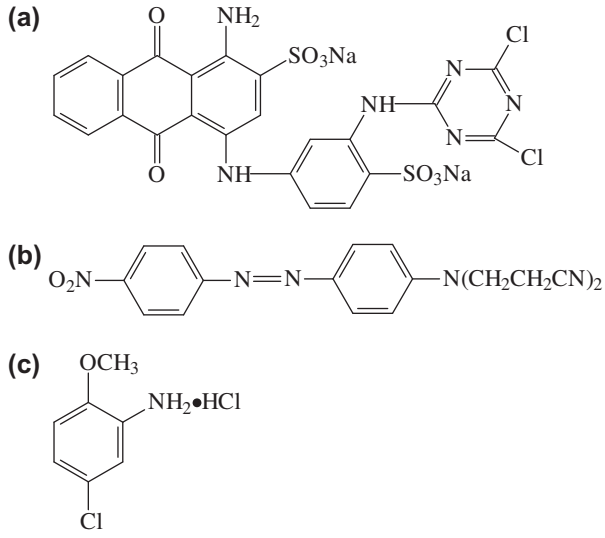

Fig. 1. The chemical structure of anthraquinone dye reactive brilliant blue XBR (a), azo dye disperse orange SRL (b), and azoic dye red base RC (c).

2.2. Protoplast formation, fusion, and regeneration

Each strain was harvested and centrifuged at 5,000 r/min at 4℃ for 5 min, washed three times with 0.55 M NaCl solution. Then the cells were resuspended in 1 mL NSM solution buffer (0.55 M NaCl, 0.2 M sodium succinate, 20 mM $MgCl_2{\cdot}6H_2O$, pH 6.7) containing lysozyme (1 mg mL^{-1}) and EDTA (0.12 M) and were shaken 40 min at 35℃ for the strain XBR8, at 30℃ for SRL8 and at 25℃ for RC10 to protoplast formation. Protoplasts were fused by suspension in NSM buffer containing 0.3 mg L^{-1} PEG6000 and 60 mM $CaCl_2$ at 30℃ for 3 min, then 2 mL of NSM buffer was added in the fusion solution and centrifuged at 3,000 rpm at 4℃ for 15 min. The cells were resuspended in 1 mL of 0.55 M NaCl solution. The suspension was spread onto completed regeneration medium (10 g L^{-1} beef extract, 20 g L^{-1} peptone, 5 g L^{-1} NaCl, 170 g L^{-1} sucrose, pH 7.0). The plates were incubated for 7 d at 30℃.

2.3. Screening of the fusant Strains

Twenty-six fusant strains were selected and inoculated in solid mineral medium containing dyes. The fusants were selected based on their capability of utilizing and decoloring three different dyes, XBR,

SRL, and RC simultaneously. The selected strains with obvious clear zone were inoculated in liquid mineral medium containing 30 mg L^{-1} of XBR, SRL, and RC, respectively [8,10]. Three fusant strains (T1, T2, and T3) with higher decolorization ability were selected for the further studies.

2.4. Decolorization experiments

Synthetic wastewater composed of BMM medium and dyes stuff. One liter of BMM contained 5.17 g K_2HPO_4, 1.70 g KH_2PO_4, 1.63 g NH_4Cl, and 10 mL of a salt solution. One liter of the salt solution contained 8.5 g $MgSO_4$, 5 g $MnSO_4$, 5 g $FeSO_4$, 0.3 g $CaCl_2$. The initial pH value of media was 7.0 [10]. XBR, SRL, and RC were added in the BMM medium to give a final concentration of 30 mg L^{-1}, respectively.

In order to study the fusant strain decolorization capability, the decolorization factors, such as dye concentration (10–400 mg L^{-1}), glucose concentration (0–0.5%), and dissolved oxygen concentration were investigated.

Sequential decolorization and biodegradation experiments in this study were performed with 4-L feed tank, an anaerobic tank (2 L), two aerobic tanks (2 L), and one sedimentation tank (2 L) (Fig. 2). Two variable-speed peristaltic pumps were used, one for delivering the water to the anaerobic tank, and one for recycling the waste activated sludge to the aerobic tank. The fusant strain and its parent strains mixture (*Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68 and *Acinetobacter* sp. RC10) were inoculated in the batch decolorization equipment, the inoculum was 3 g L^{-1}. The blank experiment (the control experiment, without inoculation of decoloring-bacteria) was carried out at the same time.

2.5. Kinetic analysis

In order to estimate the decolorization characteristics of the fusant strain T1, Michaelis–Menten kinetics Eq. (1) was used to calculate the v_{max} and Km.

$$v = \frac{v_{max} S}{S + Km} \tag{1}$$

where v is specific degradation rate of substrate (h^{-1}), S is the substrate concentration (mg L^{-1}), v_{max} is the maximum specific degradation rate (h^{-1}), Km is the Michaelis constant (i.e. substrate concentration at half $v_{max,}$ mg L^{-1}).

$$\frac{1}{v} = \frac{Km}{v_{max}} \times \frac{1}{s} + \frac{1}{v_{max}} \tag{2}$$

Eq. (2) is the linear equation, in order to estimate the decolorization characteristics of the fusant strain T1, v_{max} and Km could be calculated using Eq. (2).

2.6. Sequencing batch decolorization experiment start up and operation

The fusant strain T1, *Acinetobacter* sp. SRL8, *Escherichia* sp. XBR68, and *Acinetobacter* sp. RC10 were activated with LB medium for 24–36 h, then centrifuged at 6,000 rpm for 10 min at 4℃. The cells were washed twice with the sterile NaCl solution (0.85%). Cells were inoculated and the inoculum was 3 g L^{-1} in the anaerobic tank. The fusant strain T1 and mixed original strains were separately inoculated at the same time. The decolorization system was incubated for 12 h at room temperature and then was fed by synthetic dyestuff wastewater through variable-speed peristaltic pumps. Synthetic dyestuffs wastewater contained 90 mg L^{-1} of dyes stuff and 0.2 g L^{-1} of glucose, its COD and color were 2,243 mg L^{-1} and 300 times, respectively. After 48–72 h, the cells grew up and the anaerobic reactor showed signs of stability, i.e. constant color and COD concentration in the treated effluent. In this way the facultative bacteria were acclimatized. When anaerobic decolorization process was finished, the wastewater was spilled into the

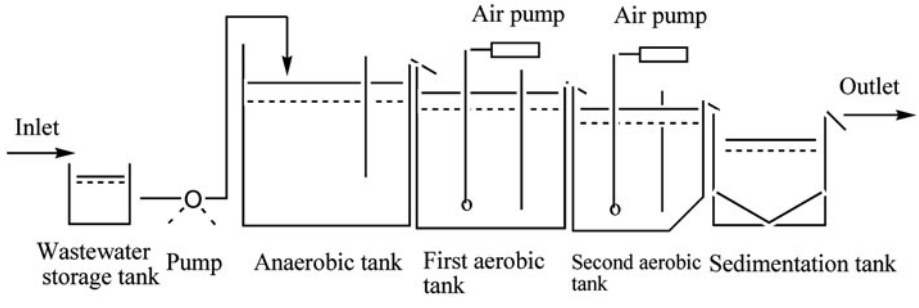

Fig. 2. Schematic layout of sequential anaerobic–aerobic process.

aerobic tanks one by one. Aeration intensity was 10 mL min^{-1} in the aerobic tank. Degrading fusant strain J8 and original strains (aniline-degrading strain An7 and naphthalene-degrading strain Nai 8) were inoculated in the aerobic tank with inocula of 3 g L^{-1}, respectively. The method of startup aerobic reaction was the same as for that of anaerobic reaction. The two reactors were joined up after the bacterial population had been able to degrade the dye wastewater. Last step was sedimentation tank and the effluent from anaerobic tank and sedimentation tank was collected to measure the value of chroma and COD concentration.

2.7. Analytical methods

All analyses were carried out in accordance with Analysis Methods for Examination of Water and Wastewater [22]. COD, pH, chroma, decolorization ratio and dye concentrations were monitored.

Chemical oxygen demand (COD) was measured with potassium dichromate method. The method consists of the following steps [22,23]: (1) Boiling a mixture of 20 mL of undiluted or diluted water sample in the 250-mL conical flask, 10 mL of Solution *A* (0.25 mol L^{-1} $K_2Cr_2O_7$) and 30 mL of solution *B* (10.0 g L^{-1} $AgSO_4$; 18.0 mol L^{-1} H_2SO_4) for 2 h, under open reflux conditions. (2) Dilution of the digested solution, by adding approximately 90 mL of water, after reaching room temperature. Titration of the diluted solution with a standard solution *C* [FAS solution: 0.12 mol L^{-1} $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$, 0.360 mol L^{-1} H_2SO_4]. Solution *C* is standardized daily with Solution *A* for FAS concentration. (3) Blank test performance by running steps 1–2 for the analysis of 20 mL of deionized water.

UV–vis spectrophotometer (Gold Spectrumlab 53, Shanghai, China) was used to determine the absorbance (*A*) and transmittance (*T*) of the solution before and after reaction, measuring the absorbance of the solution at the maximum absorption wavelength. The decolorization rate was calculated as the formula:

$$\text{decolorization rate}\,(\%) = \frac{A_0 - A_t}{A_0} \times 100 \quad (3)$$

where A_0: initial absorbance of the solution, A_t: absorbance of the solution at any time interval after reaction. Each decolorization experiment was performed in triplicate and mean of decolorization rate were reported.

3. Results and discussion

3.1. Stability and decolorization tests of the fusants

The isolates T1, T2, and T3 had larger decolorized zone (10, 8, and 7 mm). Their colony sizes were 5, 4, and 3.2 mm, respectively. They were selected with sixteenth transfer on the plates containing 30 mg L^{-1} of dyes (XBR, SRL, and RC, respectively). The fusant strains T1, T2, and T3 grew well with obvious clear zone, which showed that T1, T2, and T3 could simultaneously degrade XBR, SRL, and RC.

In order to test the fusant strains' decolorization capability, three fusant strains were inoculated in the BMM medium containing XBR, SRL, or RC, respectively. Under the conditions of 30 mg L^{-1} of dye, 1 g L^{-1} of inoculum (w/w), 35℃ and standing for 24 h, decolorization percent was measured and showed in Fig. 3(a). The results illustrated that the strain T1 had highest decolorization ability. The decolorization ratio for T1 for XBR, SRL, and RC reached 82.5, 80.9, and 51.7%, respectively, which was higher than that of parent strains. Substrate-utilization experiments were performed and Fig. 3(b) showed that the strain T1 had the broad decolorizing spectrum and could decolorize a variety of dyes, including reactive blue BES, reactive yellow BES, reactive red BES, reactive brown KAG, reactive red M2B, Reactive Yellow 3RS, Argazol black CBD, C-D red, CER yellow, and reactive deep blue BIGCN (Fig. 3(b)). Decolorization percent of T1 for these dyes was more than 77.3%. Decolorization tests of strain T1 for these dyes were carried out under the condition of 30 mg L^{-1} of dye, 1 g L^{-1} of inoculum (w/w), 35℃, and standing for 24 h. Fig. 3(c) also showed the decolorization percent of strain T1 remained unchanging after 20 transfers, which illustrated the strain T1 had good stability. The strain T1 was selected for further study.

3.2. Effect of glucose concentration on decolorization by strain T1

To test the effect of glucose concentration on decolorization by T1, temperature and pH were kept constant at 35℃ and pH 7.0 for 24 h, the glucose concentration varied from 0 to 0.5% (w/w). T1 grew slowly when it used XBR, RC, and SRL as the sole carbon source. When glucose was added in the medium, T1 growth rate increased greatly, which lead to higher decolorization rate. Fig. 4(a) showed that decolorization rate of XBR, SRL, and RC by T1 was both higher when glucose concentration ranged 0.2–0.3%. The highest decolorization rate of XBR, SRL, and RC by T1 was 91.5, 87.6, and 65.7%, respectively, when glucose

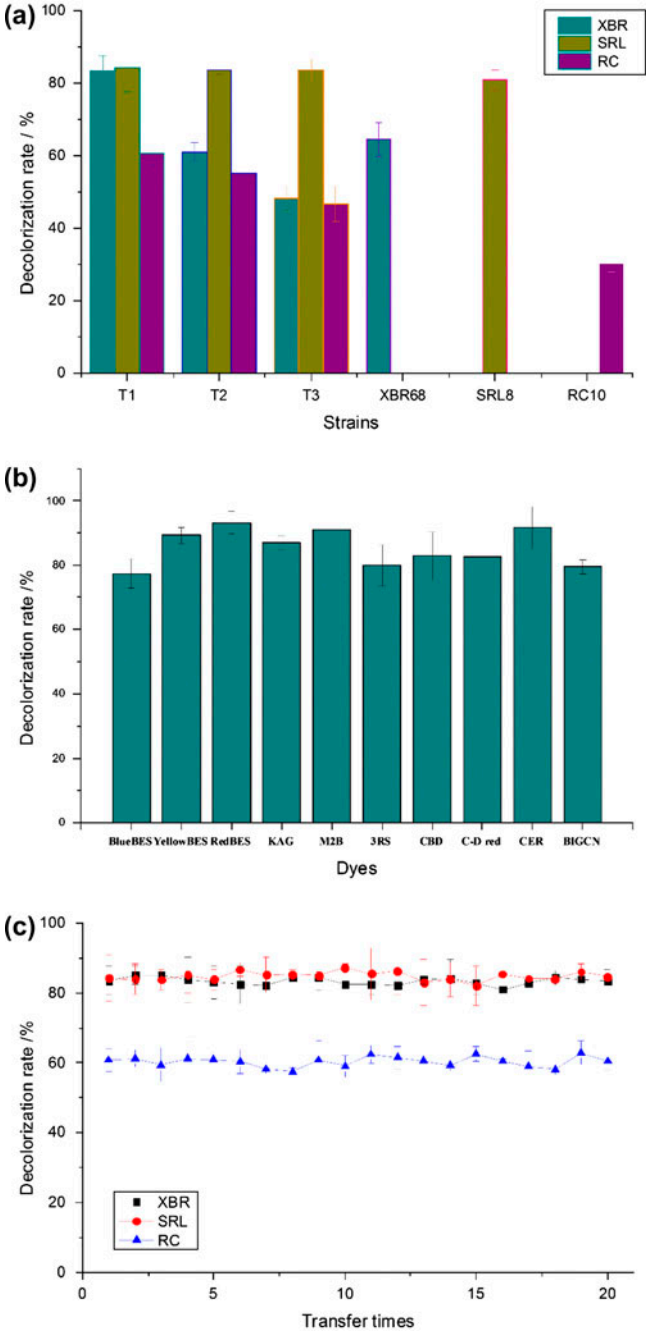

Fig. 3. The decolorization rate of the fusant strains: (a) the strains T1, T2, and T3, (b) the decolorization substrate spectrum of strain T1, and (c) the stability of the strain T1.

concentration was 0.2%. The decolorization percent rapidly decreased when glucose content was over 0.3%. Bacteria densities varied greatly in the range of 0–0.5%, the highest value reached 10^9 cfu mL^{-1} at 0.5%. The results are similar with the previous studies [8,10,21]. T1 growth was faster when it used glucose as carbon and energy source, thus provided sufficient

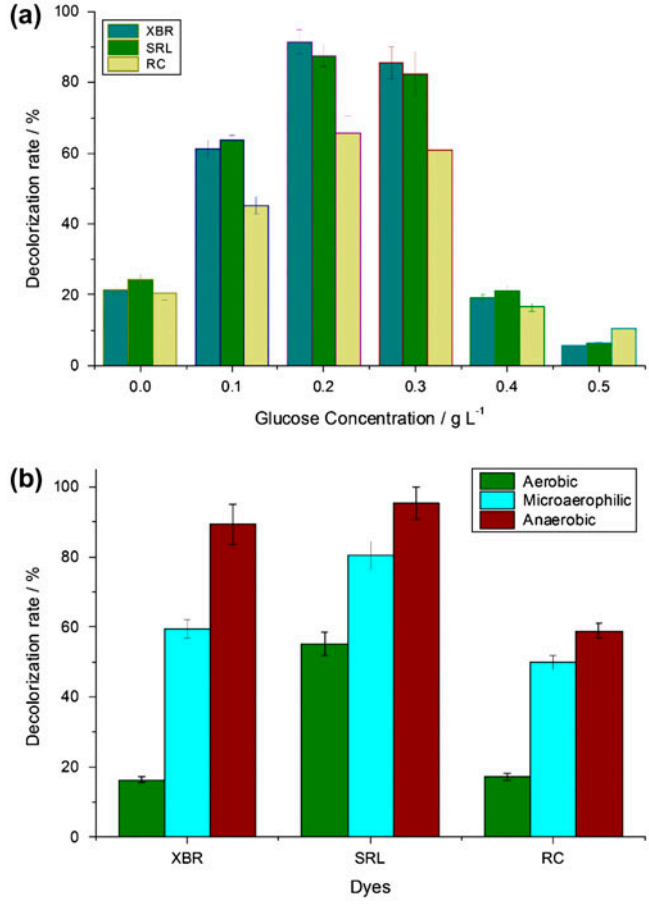

Fig. 4. Effect of glucose concentration (a) and O_2 (b) on dyes decolorization by the fusant strain T1.

electrons for reductive conditions through the cleavage of the dye bond. The glucose concentration increased while the decolorization rate decreased, which could be assumed that when there is enough glucose in the medium, T1 prefer to use glucose instead of the dyes.

3.3. *Effect of dissolved O_2 on decolorization*

Fig. 4(b) illustrated color removal efficiencies throughout 48 h of incubation under anaerobic, aerobic, and microaerophilic conditions. More than 80% of the color was removed within 24 h under anaerobic culture, while 18 and 70% of decolorization was observed under aerobic and microaerophilic incubation, respectively, which showed that the color removal percent of XBR, SRL, and RC by T1 in anaerobiosis was faster than that of in aerobiosis. Color removal percent increased with the decreasing dissolved O_2, which indicated that O_2 could inhibit decolorization rate and O_2 could inhibit the activity of decolorization enzyme in the cell. The results were in

accordance with the previous reports [10,24]. Kudlich et al. found that decolorization rate were greatly enhanced under anaerobic conditions and the color reductase activity was found to be catalyzed predominantly by NADPH- and NADH-dependent components of the microsomal monooxygenase system [24]. In the presence of O_2, NADH or NADPH will be bound with O_2, which inhibit decolorize rate.

3.4. Effect of dyeing concentration on decolorization

The dyes concentration is one of the critical factors influencing microbial dye decolorization rate. In order to study the effect of dyes concentration by fusant strain T1 on decolorization rate, temperature, pH, and glucose concentration were kept constant at 35°C, 7.0, and 0.2%, respectively, for 24 h, while dyes concentration varied from 10 to 400 mg L^{-1}. Fig. 5(a) and (b) depicted that as the concentration of dyes (ranged from 10 to 400 mg L^{-1}) increased in the culture medium, the decolorization percent decreased. With increase in initial dye concentration, decolorization rate decreased over the same time interval. The cell growth rate was relative slowly at 100–400 mg L^{-1} on 24 h of incubation (data not shown). Previous reports showed that dye decolorization percent was strongly inhibited when high concentration dye was used, and it was due to toxic effect of the dye on the degrading micro-organisms [25,26]. Similar observations have been recorded earlier for decolorization of synthetic dyes using a batch decolorization assay [27]. Strain T1 could effectively decolorize XBR with decolorization percentage of 91.5% for 30 mg L^{-1}, 83.9% for 50 mg L^{-1}, 47.6% for 200 mg L^{-1}, and 28.7% for 400 mg L^{-1}, while the parent strains mixture decolorize XBR of 79.9% for 30 mg L^{-1}, 72.9% for 50 mg L^{-1}, 40.4% for 200 mg L^{-1}, and 20.4% for 400 mg L^{-1}. The percentage of decolorization for SRL and RC by T1 was also higher than decolorization percent by the strains mixture. The cells of T1 growth response were also relatively faster than the parent strains over the same time interval (data not shown). These results indicated the decolorization percent and resistance to high dye concentration of the strain T1 are far greater than that of parent strains. The results showed that after gene recombination, the fusant strain T1 could enhance the decolorization capability and rate [28].

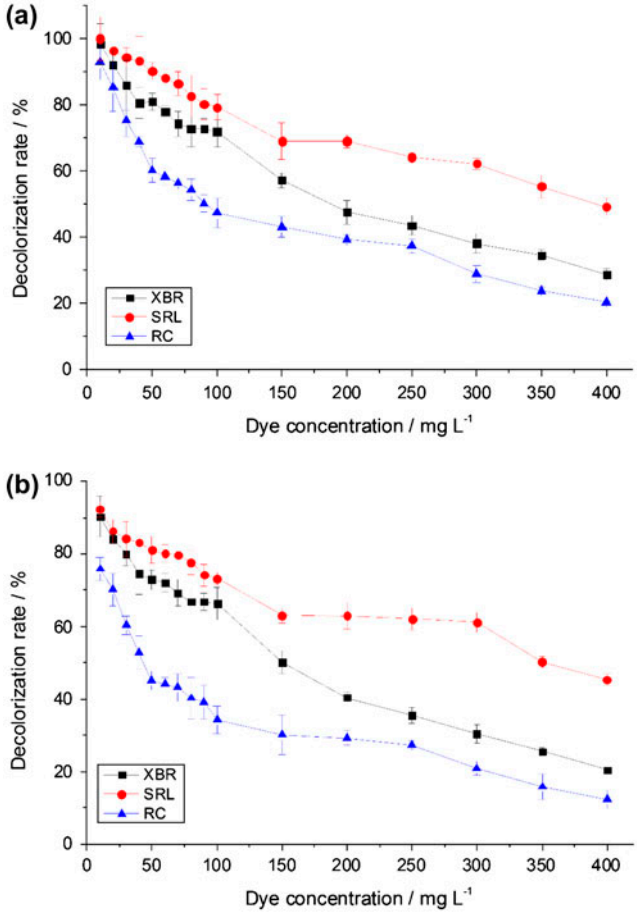

Fig. 5. Effect of dye concentration on decolorization: (a) fusant strain T1 and (b) parent strains mixture.

3.5. Kinetics analysis

From the values obtained for decolorization removal, kinetic analysis was performed using Michaelis–Menten kinetics models. Decolorization removal and dyes concentration decreased are the main indicators, which were used to assess the efficiency of the decolorizing process. Fig. 5(a) and (b) depicted that decolorize removal by parent strains for each concentration of dyes compared with decolorize percent by the fusant train T1. Decolorization rate of T1 in each initial concentration of dye increased up to values of 5.6–9.1% for XRB, 1.0–10.2% for SRL, and 8.0–17.1% for RC than that of parent strains XRB68, SRL8, and RC10, respectively. According Michaelis–Menten kinetics Eq. (2), *Km*, v_{max}, and Michaelis–Menten kinetics equation were calculated as in Table 1. It is clear that the Michaelis–Menten kinetics model gives a good correlation and the kinetic analysis was performed used Michaelis–Menten kinetics equation. The Table 1 demonstrated that the *Km* and v_{max} of fusant strain T1 were both greater than that of its parent strains, which showed decolorization rate of strain T1 increased. The *Km* of fusant strain T1 for XRB, SRL,

Table 1
Kinetic parameters evaluated for decolorization by T1 and parent strains

Strains	Dyes	*Km* (mg L^{-1})	v_{max} (h^{-1})	R^2	Michaelis–Menten equation
Fusant Strain T1	XRB	24.26	370.37	0.9982	$v = \frac{370.37 \times S}{S + 24.26}$
	SRL	11.63	196.08	0.9989	$v = \frac{196.08 \times S}{S + 11.63}$
	RC	18.13	333.33	0.9923	$v = \frac{333.33 \times S}{S + 18.13}$
XRB68	XRB	21.36	357.14	0.9987	$v = \frac{357.14 \times S}{S + 21.36}$
SRL8	SRL	9.96	188.68	0.9995	$v = \frac{188.68 \times S}{S + 9.96}$
RC10	RC	8.85	212.77	0.9659	$v = \frac{212.77 \times S}{S + 8.85}$

and RC increased 13.6, 16.8, and 104.9% than that of parent strains, respectively. Fig. 5(a) and (b) also showed the resistance to high dye concentration of the strain T1 is far greater than that of parent strains. All these results indicated that the fusant strain T1 had the broad decolorizing spectrum, increase degradation rate and be resistance to higher substrate concentration.

3.6. Sequencing batch decolorization reactor system

In anaerobic process, the pH value ranged from 6.8 to 7.5 and remained almost unchangeable. The overall color removal ratio could reach 91.7% by strain T1 and 80.00% by original strain mixture, respectively. Table 2 showed the color and COD reduction in anaerobic/aerobic process. The color removal by fusant strain T1 was far greater than the value achieved by original strain mixture, moreover the strain T1 can decolorize three different dyes simultaneously. They found ~65% of color removal in a two-stage anaerobic/aerobic process, 80.5% removal at the anaerobic unit and 38.2–58.7% an UASB reactor, respectively. The color removal in this study was less than these previous reports [2,29–31]. Only 11.3 and 12.9% of dye wastewater COD reduction was observed by fusant strain T1 and decoloring-bacteria mixture in anaerobic tank, respectively, which showed decolorization capability of the fusant strain T1 and original decoloring-bacteria is greater than COD reduction capability. Both of them could cleave the dye bond and their metabolites-degrading capability was poor. The results of UV–vis spectra of dye wastewater also showed the color bond was broken (Fig. 6).

In the first aerobic biochemical process, the outlet COD value decreased from 1,990 to 379.2 mg L^{-1}, 1,953.7 to 251.8 mg L^{-1}, COD removal reached 80.95 and 87.11% by degrading-bacteria mixture and fusant strain J8, respectively. The capability of COD removal by fusant strain J8 was greater than that by parent-bacteria mixture. Color in aerobic tank dropped

Table 2
The results of dye-containing wastewater sequential anaerobic/aerobic treatment

Treatment process	Strain	Inlet chroma (times)	Outlet chroma (times)	Color removal (%)	Inlet COD (mg L^{-1})	Outlet COD (mg L^{-1})	COD removal (%)
Anaerobic process	Decoloring-Mixture	300 ± 5	60 ± 3	80.00	2243.13 ± 125.67	1989.87 ± 103.23	11.29
	Strain T1	300 ± 6	25 ± 2	91.67	2243.13 ± 83.46	1953.69 ± 112.85	12.90
The first aerobic biochemical process	Degrading-Mixture	60 ± 3	50 ± 2	16.67	1989.87 ± 103.23	379.16 ± 15.65	80.95
	Strain J8	25 ± 2	20 ± 1	20.00	1953.69 ± 112.85	251.81 ± 13.72	87.11
The second aerobic biochemical process	Degrading-Mixture	50 ± 2	45 ± 2	10.00	379.16 ± 15.65	151.95 ± 11.46	59.92
	Strain J8	20 ± 1	15 ± 1	25.00	251.81 ± 13.72	53.55 ± 4.35	78.73

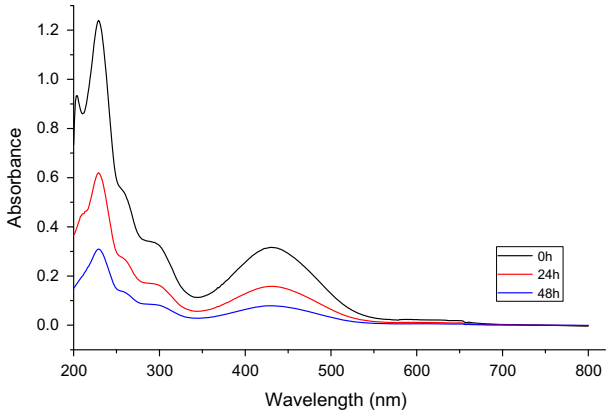

Fig. 6. The UV–vis spectrophotometric analysis of dyes wastewater before and after anaerobic treatment by the strain T1.

weakly, from 25 to 20 and from 60 to 50. Higher redox potential in the aerobic tanks may lead to the weak drop of wastewater color.

The overall color removal was 95% through anaerobic/aerobic process by the fusant strains T1. Most color removal occurred in the anaerobic process. A maximum color removal of 91.7% was obtained at 2 mL L^{-1} of wastewater influent rate and 30 mg L^{-1} of dyes concentration in this study. The COD removal percent was 97.61% and most COD removal occurred in the aerobic process. Almost 12.90% COD removal was determined in the anaerobic process, about 84.71% COD removal was achieved in the aerobic process. The outlet color and COD value reached 15 and 53.6 mg L^{-1} after the second biochemical process by strain J8, and pH value of outlet water was 6.8–7.3. The result of dying wastewater sequencing anaerobic/aerobic treatment by fusant strain T1 and J8 was sufficient meet China standards for discharge (a fixed emission standard of 60 mg L^{-1} COD and 20 color is required in south of Jiangsu Province, China). However, the color and COD value of outlet wastewater treated by decolorizing and degrading strain mixture only reached 45 and 152 mg L^{-1}, which did not meet the discharge standards in China. In the same equipment and same condition, the color and COD value of outlet wastewater treated by fusant strain was lower than that treated by their parent strains.

4. Conclusions

This study is focused on the construction of the fusant strains, decolorization and degradation of three different dyes by fusant strain and its application for the dyes wastewater treatment through a sequential anaerobic/aerobic reaction. The fusant strain T1 could simultaneously decolorize a variety of dyes. The result of sequential anaerobic/aerobic reaction showed that the color and COD removal are superior over those of parent strain mixture. In this study, the fusants strains T1 and J8 were employed for decolorization and degradation, which may open new possibilities of gene engineering bacterium construction for its applications in industrial effluents.

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