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Effective decolorization of anthraquinone dye reactive blue 19 using immobilized *Bacillus* sp. JF4 isolated by resuscitation-promoting factor strategy

Jiafang Cai, Jiale Liu, Aodong Pan, Jinfu Liu, Yuyang Wang, Jianbo Liu, Faqian Sun, Hongjun Lin, Jianrong Chen and Xiaomei Su

ABSTRACT

Given the highly complex recalcitrant nature of synthetic dyes, biological treatment of textile wastewater using efficient bacterial species is still considered as an environmentally friendly manner. In this study, a reactive blue 19 (RB19)-degrading strain, *Bacillus* sp. JF4, which was isolated by resuscitation-promoting factor (Rpf) strategy, was immobilized into polyvinyl alcohol–calcium alginate–activated carbon beads (JF4-immobilized beads) for RB19 decolorization. Results suggest that the JF4-immobilized beads, which were capable of simultaneous adsorption and biodegradation, showed a high decolorization activity, while they exhibited better tolerability towards high RB19 concentrations. The JF4-immobilized beads could almost completely decolorize 100 mg/L RB19 within 10 d, while only 92.1% was decolorized by free bacteria within 12 d. Further investigation on the equilibrium and kinetics of the adsorption process suggests that the pseudo-second-order model best fit the adsorption kinetics data, and the Freundlich isotherm was the most suitable for the description of the equilibrium data. Notably, the repeated batch cycles indicated that complete decolorization of 100 mg/L RB19 by JF4-immobilized beads can be maintained for at least three cycles without much reduction in efficiency. These findings suggest that immobilizing Rpf-resuscitated strain into beads was an effective strategy for textile wastewater treatment.

Key words | adsorption, *Bacillus* sp. JF4, decolorization, immobilization, reactive blue 19

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HIGHLIGHTS

- Efficient decolorization of RB19 by a resuscitated strain, *Bacillus* sp. JF4.
- JF4-immobilized beads have a high capacity for RB19 decolorization.
- JF4-immobilized beads exhibited a tolerability towards high RB19 concentration.
- Pseudo-second-order and Freundlich models well described the adsorption of beads.

doi: 10.2166/wst.2020.201

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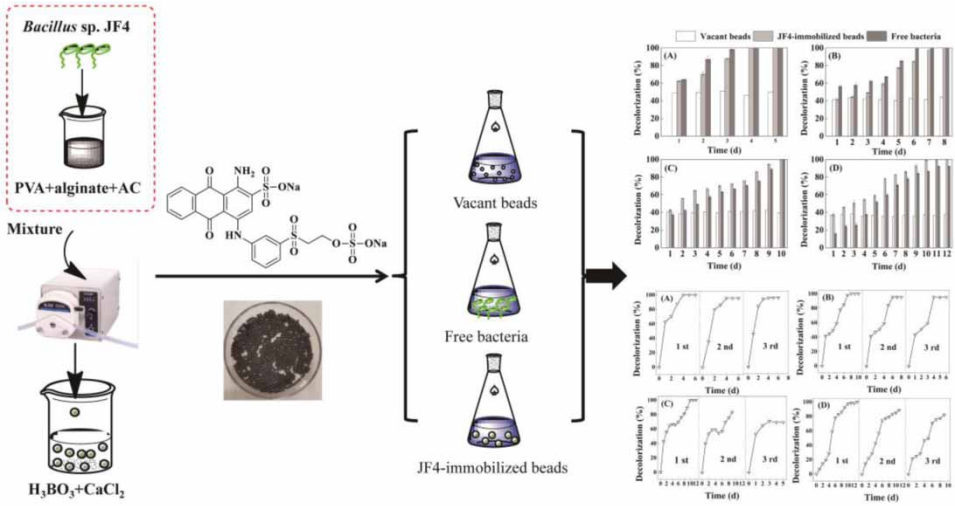
GRAPHICAL ABSTRACT



INTRODUCTION

Dye-consuming industries, especially textile industries, produce a considerable amount of highly polluted wastewater containing various dyes, which are, most often, toxic and persistent (Farshid & Mahsa 2016). Reactive blue 19 (RB19) is an anthraquinone dye, which has been widely used in textiles, food, paper, pharmaceutics and cosmetics industries. It was estimated that 10–15% of unused dye was eventually discharged into water bodies during the dyeing process (Holkar *et al.* 2018). The development of methods for RB19 removal from wastewater attracted lots of interest, because RB19 with high toxicity and stability causes serious environmental pollution (Holkar *et al.* 2018). Compared with physico-chemical techniques, bioremediation is considered a cost-effective and environmentally sustainable technique (Lu *et al.* 2019; Zhang *et al.* 2019). Although RB19-degrading bacteria in the genera of *Ganoderma*, *Bacillus*, *Enterobacter*, *Klebsiella* and *Staphylococcus* have been isolated and characterized (Holkar *et al.* 2018), the understanding of RB19 biodegradation is still limited, because most of the microbial diversity on the earth cannot be cultivated and remains inaccessible (Epstein 2013).

It is well accepted that most bacteria enter into a viable but non-culturable (VBNC) state under unfavorable conditions (Fida *et al.* 2017). In this state, bacteria cannot be cultured on bacteriological media but still survive and keep cell viability by decreasing metabolic activities (Su *et al.* 2019b). Several VBNC bacteria including *Rhodococcus*, *Alcaligenes*, *Pseudomonas*, *Microbacterium* and *Bacillus*, which are known for degradation of refractory organic pollutants, have already been explored in polychlorinated biphenyl contaminated sites, and saline phenolic and dyeing wastewater (Su *et al.* 2018; Su *et al.* 2019a). Also, it has been demonstrated that due to entry into the VBNC state, many high-efficiency degraders exhibited much lower activity when applied in the bioaugmentation process (Fida *et al.* 2017). Undoubtedly, resuscitation of VBNC bacteria in pollutant-contaminated environments is of great importance for isolating new anthraquinone-dye degraders. It was found that a resuscitation-promoting factor (Rpf) secreted by *Micrococcus luteus* could resuscitate and stimulate the growth of most VBNC bacteria (Mukamolova *et al.* 1998). Especially, the Rpf at picomolar concentrations has been successfully applied to recover the culturability of several Gram-positive and Gram-negative bacteria which contained well-recognized pollutant-degrading bacterial populations (Su *et al.* 2019b). For instance, Jin *et al.* (2017) obtained the Congo red-degrading strain *Ochrobactrum anthropi* YRJ1

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from printing and dying wastewater by addition of Rpf. Therefore, Rpf could be employed as a useful method for exploring high-efficiency strains for RB19 dye degradation.

Another problem of RB19 dye biodegradation is the limited capacities of free microbial cells. Dye removal efficiency by free bacteria was highly dependent on the environmental parameters, including pH, temperature and toxic substances concentration (Bharti *et al.* 2019). Moreover, the toxicity of RB19 directly affected the decolorization efficiency of bacteria. To overcome these drawbacks, immobilized technologies, which can maintain bacterial cells' high stability, increase cell metabolism, prevent cell wash-out, and shield from the stress of high toxicity, have been developed (Liu *et al.* 2018). It has been well established that the usage of immobilized technologies could obviously accelerate dye biodecolorization (Su *et al.* 2009). To date, the most preferred immobilization technology for dyes degradation was entrapping cells in calcium alginate beads (Cheng *et al.* 2012). However, long-term application of calcium alginate beads was restricted due to its poor mechanical strength. To address these shortcomings, a variety of supporting materials such as polyvinyl alcohol (PVA) and activated carbon (AC) were used for immobilization (Wang *et al.* 2020). PVA is an immobilization matrix and always cross-linked with alginate. The porous structure of PVA makes the oxygen and contaminants easily enter into the particles (Al-Zuhair & El-Naas 2011). Meanwhile, a high-efficiency AC adsorbent, with microporous structure and good mechanical properties, has been widely utilized to remove synthetic dyes (Benhouria *et al.* 2015). Thus, calcium alginate beads immobilized with PVA and AC could provide a suitable environment for bacterial growth, enhanced mechanical strength and the reusability of immobilized beads.

In this study, a resuscitated strain, *Bacillus* sp. JF4, which was isolated from activated sludge by addition of Rpf, was selected for investigating RB19-degrading capability. The RB19 decolorization performance of the immobilized strain JF4 with PVA and AC as the carrier was evaluated. The specific aims of this study were to: (1) obtain high-efficiency RB19-degrading strain by Rpf addition; (2) compare the dye degradation capability of free bacteria and immobilized bacteria; (3) investigate the dye adsorption capacity of vacant beads; and (4) evaluate the reusability of immobilized bacteria. To the best of our knowledge, this is the first study to immobilize a resuscitated strain which was isolated by Rpf addition for textile wastewater treatment.

METHODS

Screening for high-efficiency RB19-degrading strains

The activated sludge samples were collected from Shibali landfill leachate treatment plant in Jinhua city, China, and was then acclimated with synthetic wastewater containing RB19 (100–150 mg/L) for about 30 days. After that, the potential RB19-degrading bacteria were isolated by a series of selective enrichments. Briefly, the enrichment was divided into two groups, named as Rpf treatment group and control group. In the Rpf treatment group, recombinant Rpf protein (0–1.6%, v/v), which was prepared as described before (Su *et al.* 2018, 2019c), was added. Correspondingly, the control group was run without Rpf addition. Then, individual strains were isolated from these groups using MSM medium with 50 mg/L RB19 and 250 mg/L peptone as carbon sources, and identified by 16S rRNA gene sequencing (Su *et al.* 2019b). The RB19-degrading strains unique to Rpf treatment were individually tested for the RB19 decolorization capabilities. The strain *Bacillus* sp. JF4 (GenBank accession number MK542825) with the highest decolorization capability of RB19 was selected for further experiments.

Immobilization

The strain *Bacillus* sp. JF4 was inoculated (5%, v/v) into the Luria-Bertani (LB) medium and cultured overnight at 30 °C and 150 r/min. The cells were harvested by centrifugation and resuspended in NaCl solution. The JF4-immobilized beads were prepared using the dripping method modified from Liu *et al.* (2019). Briefly, a mixture of sodium alginate, PVA, powdered AC and JF4 suspension (OD_{600} about 1.0) was dripped into $CaCl_2$-H_3BO_3 solution to form beads. Then, the beads were washed and stored in water at 4 °C. Non-cell-immobilized beads, that is, vacant beads without *Bacillus* sp. JF4, were also prepared by the same method as for JF4-immobilized beads. The size of beads can be adjusted by the size of drip head. Morphological characteristics of free bacteria, vacant beads and JF4-immobilized beads were observed using scanning electron microscopy (SEM, Hitachi S-4800) (Ke *et al.* 2018).

Dye decolorization capabilities of free and immobilized bacteria

The free bacteria, vacant beads and JF4-immobilized beads were inoculated at the ratio of 5% (v/v) into MSM medium with

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25–100 mg/L RB19 and 250 mg/L peptone as carbon sources. Each culture was incubated at pH 7.0, 30 °C and 130 r/min. Samples were collected every 24 h until the RB19 was completely decolorized by immobilized bacteria. Each sample was centrifuged, and then the absorbance of supernatant at 592 nm (OD_{592}) was measured with a spectrophotometer (TU-1810, Purkinje, China). The decolorization efficiency was calculated as $[(A_0 - A_t)/A_0] \times 100\%$, where A_0 and A_t refer to the initial and final absorbance values, respectively.

Adsorption capability of vacant beads

The effect of bead size (1, 2, 4 and 5 mm) on the RB19 adsorption by vacant beads was investigated at RB19 concentration of 100 mg/L. Specifically, the vacant beads were inoculated at the ratio of 5% (v/v) into MSM medium with 100 mg/L RB19 and 250 mg/L peptone as carbon sources. The residual concentration of RB19 was measured every 1 h, and then the amount of RB19 adsorption and decolorization rate were calculated. After determining the optimum bead size, the adsorption capacity of vacant beads was further investigated at various initial RB19 concentrations from 25 to 200 mg/L. The adsorption capacity (q_t) of vacant beads was calculated as $V \times (C_0 - C_t)/m$, where V (L) is the volume of medium, m (g) is the wet mass of the beads, and C_0 and C_t (mg/L) are the initial and final concentration of RB19, respectively.

Reusability of JF4-immobilized bacteria

The JF4-immobilized beads were firstly washed and then transferred into fresh MSM with initial RB19 concentrations of 20, 50, 75 and 100 mg/L, respectively. Under the same incubation conditions, the residual concentrations of dye in each culture were measured at an interval of 1 h. After complete decolorization, the beads were collected, washed three times and transferred into a new fresh MSM containing the same dye concentration for the next cycle.

RESULTS AND DISCUSSION

RB19-degrading strain JF4 and immobilization

Six RB19-degrading strains, which were unique to Rpf treatment, were identified (Figure S1, Supplementary Material). Meanwhile, the RB19 decolorization capabilities of the six strains are shown in Figure S2 (Supplementary Material). The *Bacillus* sp. JF4 exhibited the highest decolorization efficiency, degrading 90% of 50 mg/L RB19 within 5 days. The degrading capacity of *Bacillus* sp. JF4 was comparable with reported strains of *Pseudomonas aeruginosa* which can decolorize an initial 50 mg/L of RB19 by 94.8 ± 0.4% in 96 h (Mishra *et al.* 2019). Thus, the results suggest that Rpf addition could be an effective strategy to explore efficient bacterial strains for pollutant degradation. To explore the potential application of *Bacillus* sp. JF4, the strain was immobilized on the PVA–alginate–AC beads to determine whether it could achieve better decolorization efficiency than free bacteria. The morphological characterization of free bacteria, vacant beads and JF4-immobilized beads is shown in Figure 1. The free cells possessed a large surface area of biomass (Figure 1(a) middle and right), which allowed the dye to access the biomass materials (Binupriya *et al.* 2010). A heterogeneous and rough surface caused by numerous bulges on the vacant beads (Figure 1(b) middle and right) and JF4-immobilized beads (Figure 1(c) middle and right) were found. Moreover, more bulges with folds were found in JF4-immobilized beads than in vacant beads, which was consistent with the morphological characteristics of calcium alginate–bentonite–AC composite beads (Benhouria *et al.* 2015).

Comparison of free and immobilized bacteria for dye decolorization

Decolorization of RB19 by free bacteria, JF4-immobilized beads and vacant beads was investigated at different initial dye concentrations of 25, 50, 75 and 100 mg/L. As indicated in Figure 2, the adsorption of vacant beads was obvious, and the decolorization efficiency reached 49.9%, 43.9%, 39.7% and 37.9% at concentrations of 25, 50, 75 and 100 mg/L, respectively. At low concentration of RB19 (25 and 50 mg/L), the decolorization efficiencies of JF4-immobilized beads were lower than those of free bacteria. Free bacteria could completely degrade 25 and 50 mg/L RB19 within 3 and 6 days, respectively, while JF4-immobilized beads needed 4 and 8 days, respectively (Figure 2(a) and 2(b)). The results were inconsistent with previous studies which suggested the activity of immobilized microorganisms was higher than free bacteria (Cheng *et al.* 2012; Liu *et al.* 2018). However, under certain concentration, the lower activity of immobilized bacteria was also observed (Ke *et al.* 2018). Similarly, Sharma *et al.* (2016) found that compared with suspended bacteria, immobilized bacterial beads required longer incubation time for complete decolorizaiton of methyl red when equal amount of bacterial cells was utilized. The lower activity of immobilized bacteria may be attributed to the decreased permeability of porous materials after immobilization.

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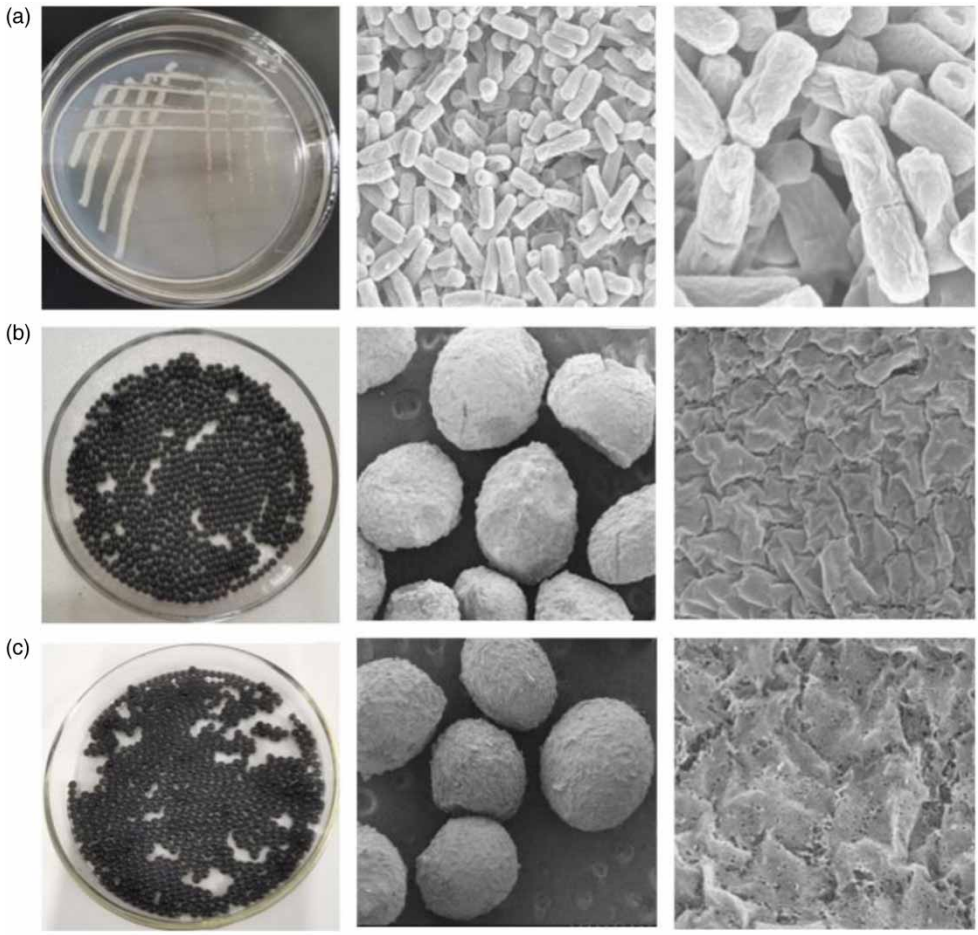

Figure 1 | Morphological characterization of free bacteria (a), vacant beads (b) and JF4-immobilized beads (c). SEM images of free bacteria ((a) middle and right), vacant beads ((b) middle and right) and JF4-immobilized beads ((c) middle and right).

At high RB19 concentrations of 75 and 100 mg/L (Figure 2(c) and 2(d)), free bacteria exhibited lower decolorization efficiencies than immobilized bacteria. JF4-immobilized beads could almost completely decolorize 100 mg/L RB19 within 10 days, while only 92.1% of RB19 was decolorized by free bacteria within 12 days. The finding suggests that immobilized bacteria could be tolerant of high concentration of RB19, and could be used for treatment of high concentration of anthraquinone dye wastewater. Similarly, Hameed & Ismail (2018) found that the immobilized bacteria were capable of effectively decolorizing azo dye reactive red 2 (RR2) at high concentration, while the activity of free bacteria was inhibited at RR2 concentration of 100 mg/L. Indeed, based on the advantages of immobilization, previous studies have focused on immobilization of bacterial consortia for removal of synthetic dyes. For instance, Sharma *et al.* (2016) found that the immobilized *Aeromonas jandaei* strain could resist the toxic effect of methyl red at higher concentrations (>1,000 mg/L). Therefore, immobilization of bacterium *Bacillus* sp. JF4 could be an effective approach for decolorization of RB19 at high concentrations (>75 mg/L).

RB19 adsorption by vacant beads

To obtain the maximum adsorption capacity, the effect of bead size on the RB19 adsorption by vacant beads was investigated. As shown in Figure S3 (Supplementary Material), the dye decolorization rates increased with decreasing bead size, and reached the maximum decolorization rate of 0.02 mg/(g·h) with 1–2 mm particle size. This could be attributed to the larger surface area with the decrease in bead size, which shortened the equilibrium time. Similarly, Guerrero-Coronilla *et al.* (2015) indicated that biosorption of anionic dye increased with decreasing water hyacinth leaves particle size. However, the beads of 1 mm in diameter were easily washed out during application; thus bead size of 2 mm in diameter was chosen for further adsorption experiments.

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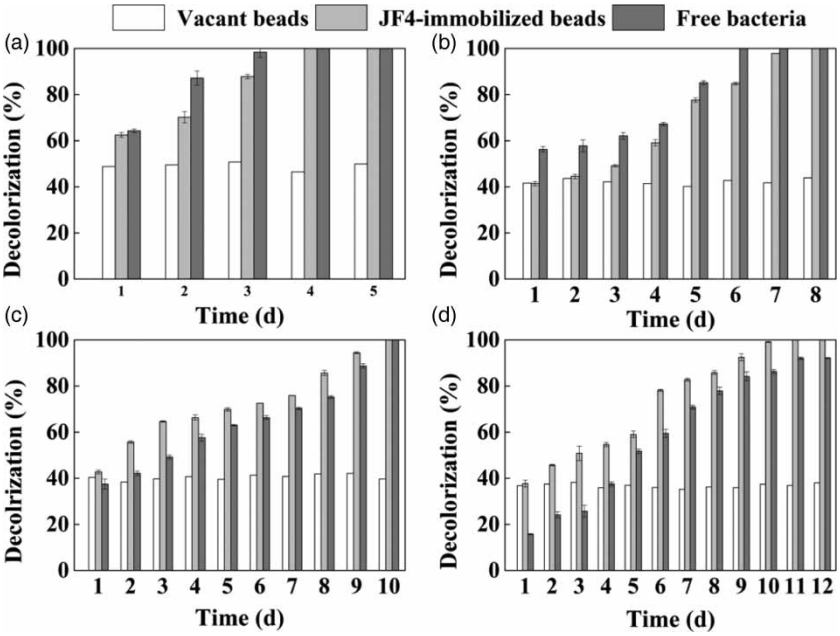

Figure 2 | Decolorization of RB19 by free bacteria, vacant beads and JF4-immobilized beads at different initial RB19 concentrations of 25 mg/L (a), 50 mg/L (b), 75 mg/L (c) and 100 mg/L (d).

The adsorption capacity of vacant bacteria was evaluated at various RB19 concentrations. As illustrated in Figure 3, the amount of RB19 adsorption increased with time, and a significant increased adsorption occurred from 0 to 60 min. The rate of adsorption declined with time, and the adsorption of RB19 onto the beads reached equilibrium within 540 min. The adsorption sites on the beads were gradually occupied with time (Liang *et al.* 2010), which led to the declining adsorption rate of RB19. Meanwhile, the amount of adsorption at equilibrium also increased with the elevated concentration, and was 0.05, 0.10, 0.14, 0.16, 0.22 and 0.30 mg/g at RB19 concentrations of 25, 50, 75, 100, 150 and 200 mg/L, respectively. The results were consistent with the decolorization efficiency of vacant beads described in Figure 2, which also demonstrated the increased amount of adsorption with elevated RB19 concentrations. This phenomenon may be attributed to the increased amounts of RB19 in solution, which facilitates the interactions between dye and beads (Guerrero-Coronilla *et al.* 2015).

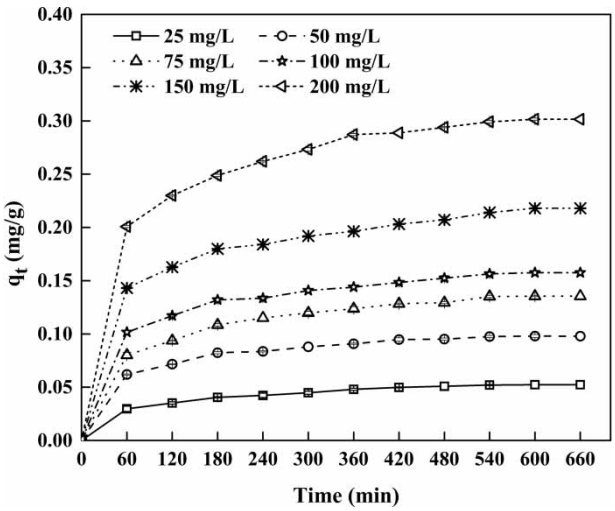

Figure 3 | Effect of incubation time and initial RB19 concentrations on the adsorption capacity of vacant beads.

RB19 adsorption kinetics

To investigate the adsorption kinetics of RB19 onto vacant beads, the experimental data were fitted into the pseudo-first-order (1), pseudo-second-order (2), intra-particle diffusion (3) and Elovich models (4), respectively (Maneerung *et al.* 2016).

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{1}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) t \tag{2}$$

$$q_t = k_i t^{0.5} \tag{3}$$

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln t \tag{4}$$

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where q_t (mg/g) and q_e (mg/g) are the amount of the dye adsorbed at time t (min) and at the equilibrium, respectively; k_1 (1/min), k_2 (g/(mg·min)) and k_i (mg/(g·min$^{1/2}$)) are the rate constants of pseudo-first-order adsorption, pseudo-second-order adsorption and intra-particle diffusion, respectively; α (mg/(g·min)) is the initial adsorption rate and β (g/mg) is a desorption constant.

The plots of pseudo-first-order, pseudo-second-order, intra-particle diffusion and Elovich adsorption kinetic models are shown in Figure 4. The correlation coefficient (R^2), constant values and q_e were calculated and are presented in Table 1. It can be observed that the pseudo-first-order model is insufficient in describing the adsorption process. For the other three models, only the R^2 values for the pseudo-second-order model were higher than 0.99. The calculated q_e values were also almost the same as the experimental q_e values. The results suggest that the pseudo-second-order well described the adsorption of RB19 onto the beads. Interestingly, the rate constant k_2 decreased from 0.44 to 0.10 g/(mg·min) with the adsorption capacity $q_{e,\mathrm{cal}}$ values increasing from 0.05 to 0.31. This can be attributed to the fact that high concentration of RB19 will enhance the competition for the active adsorption site of beads, resulting in the slower adsorption process (Nandi *et al.* 2009). This kinetics result was consistent with the previous result which demonstrated crystal violet was adsorbed onto PVA–alginate–kaolin beads (Cheng *et al.* 2012).

RB19 adsorption isotherm

To investigate the characteristics of the adsorption process, four adsorption isotherm models, Langmuir (Equation (5)), Freundlich (Equation (6)), Temkin (Equation (7)) and Dubinin–Radushkevich (Equations (8) and (9)) (Maneerung *et al.* 2016), were chosen to fit the adsorption equilibrium data obtained from batch experiments with varying

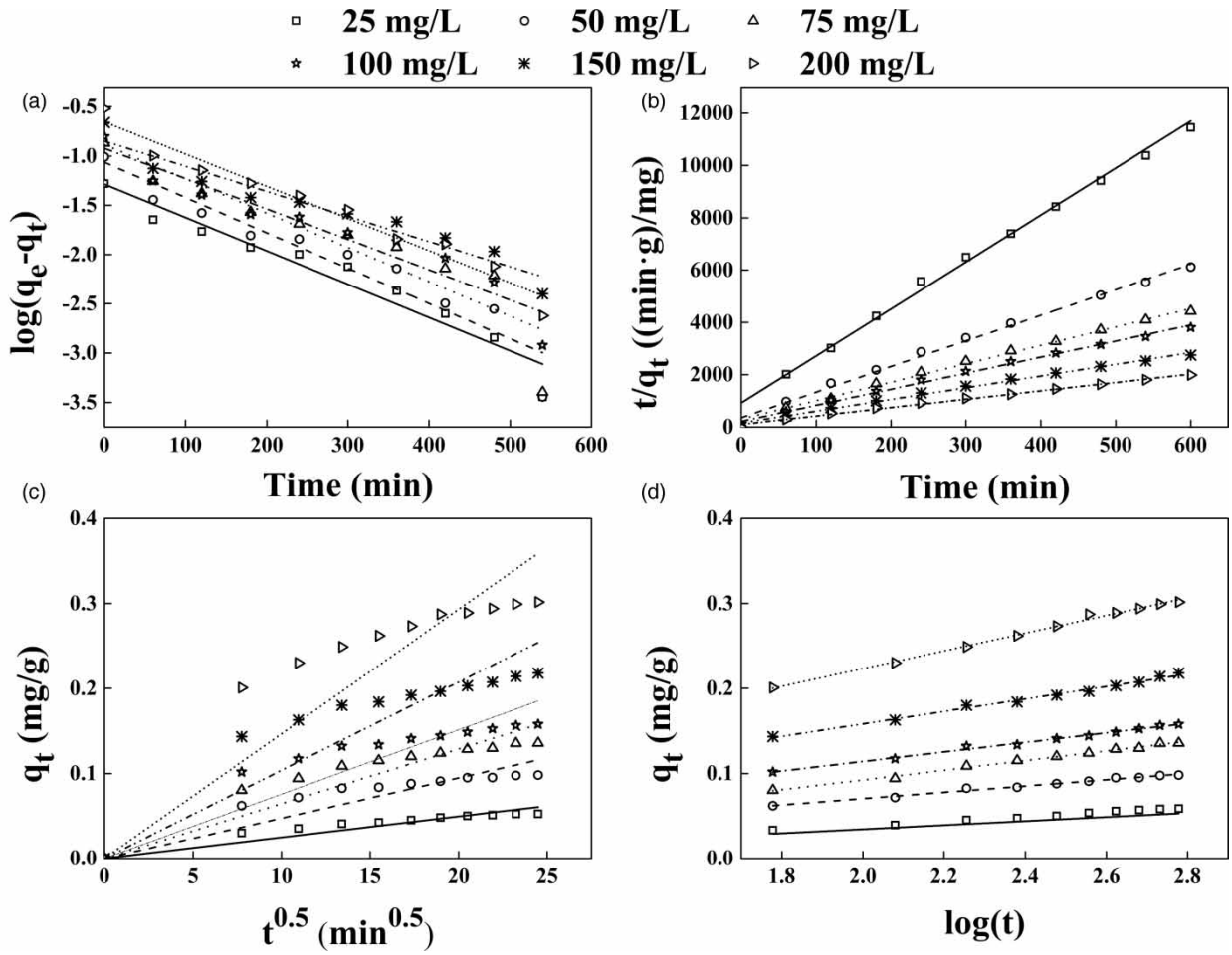

Figure 4 | Pseudo-first-order (a), pseudo-second-order (b), intra-particle diffusion (c) and Elovich (d) kinetic models for the adsorption of RB19 onto vacant beads.

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Table 1 | Parameters of the four different kinetic models for the adsorption of non-immobilized beads at 30 °C

			Pseudo-first-order kinetic			Pseudo-second-order kinetic			Intra-particle diffusion		Elovich		
C_0 (mg/L)	C_e (mg/L)	$q_{e,exp}$ (mg/g)	K_1 (1/min)	$q_{e,cal}$ (mg/g)	R^2	K_2 (g/(mg·min))	$q_{e,cal}$ (mg/g)	R^2	K_3 (mg/(g·min$^{1/2}$))	R^2	α (mg/(g·min))	β (g/mg)	R^2
25	14.37	0.06	0.0088	0.05	0.93	0.44	0.05	0.99	0.0025	0.98	0.01	41.85	0.99
50	34.09	0.10	0.0082	0.09	0.90	0.27	0.10	0.99	0.0047	0.97	0.03	26.97	0.99
75	48.54	0.15	0.0080	0.13	0.82	0.21	0.14	0.99	0.0064	0.97	0.02	17.56	0.99
100	62.89	0.16	0.0071	0.12	0.91	0.17	0.16	0.99	0.0076	0.97	0.06	17.82	0.99
150	102.42	0.22	0.0059	0.14	0.93	0.13	0.22	0.99	0.0104	0.97	0.11	13.64	0.99
200	137.52	0.30	0.0075	0.22	0.96	0.10	0.31	0.99	0.0147	0.97	0.14	9.59	0.99

concentrations (25–200 mg/L) of RB19.

$$\frac{1}{q_e} = \frac{1}{q_{\max}} + \frac{1}{K_L q_{\max} C_e} \tag{5}$$

$$\ln q_e = \ln \mathrm{K}_F + \frac{1}{n} \ln \mathrm{C}_e \tag{6}$$

$$q_e = \frac{RT}{b_t} \ln a_t + \frac{RT}{b_t} \ln C_e \tag{7}$$

$$\ln q = \ln Q_m - B\varepsilon^2 \tag{8}$$

$$\varepsilon = \mathrm{RT} \ln\left(1 + \frac{1}{C_e}\right) \tag{9}$$

where K_L is the Langmuir constant (L/mg); q_e is RB19 concentration at equilibrium (mg/g); C_e is the liquid phase concentration of RB19 at equilibrium (mg/L); $q_{\max}$ is the maximum adsorption capacity of beads (mg/g). K_F ((mg$^{1-(1/n)}$·L$^{1/n}$)/g) and n are the Freundlich constants; R is ideal gas constant (8.314 J/(mol·K)), T (K) is the absolute temperature during the adsorption process; a_t (L/g) and b_t (J/mol) are Temkin isotherm constants; B is the activity coefficient (mol^2/kJ^2) related to the mean sorption energy, ε is the Polanyi potential and Q_m is the theoretical saturation capacity of the adsorbent (mg/g).

The plots of Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherm models are presented in Figure 5. The adsorption process of dyes on vacant beads fits well with Langmuir and Freundlich models ($R^2 > 0.95$) (Table S1, Supplementary Material), indicating that the adsorption mechanism is not limited to single layer

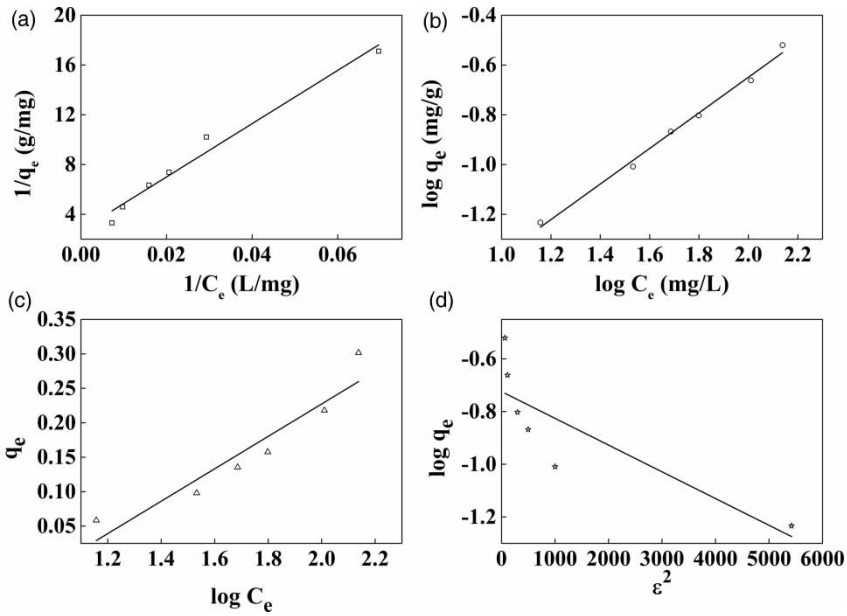

Figure 5 | Linearized plots of Langmuir (a), Freundlich (b), Temkin (c) and Dubinin–Radushkevich (d) isotherm models for the adsorption of RB19 onto the vacant beads.

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adsorption, but chemical adsorption mechanism also exists. Furthermore, based on R^2 value, the Freundlich model shows the better fit with equilibrium adsorption data than does the Langmuir model. In addition, the Freundlich constant ($n = 1.398$) was greater than 1.0, indicating favorable adsorption conditions (Turabik 2008).

Reusability of JF4-immobilized bacteria

As known, long-term operation is crucial for immobilized technology in practical applications. Therefore, to confirm the performance of repeated utilization of JF4-immobilized beads, several reusability experiments of JF4-immobilized beads was investigated at different initial RB19 concentrations of 25, 50, 75 and 100 mg/L. Based on the amount of adsorption at equilibrium (Figure 3), adsorption only existed in the first cycle, accounting for 37.9–49.9% of the decolorization efficiency. As depicted in Figure 6, the JF4-immobilized beads can be efficiently reused for at least three cycles, and maintained the decolorization ability without much reduction in efficiency. It should be noted that the decolorization rate was increased in the second cycle, which completely decolorize 25 and 50 mg/L RB19 in 4 and 6 days, respectively. For the RB19 concentrations of 75 and 100 mg/L, the time for complete decolorization was prolonged to about 10 days due to high dye concentration. Meanwhile, in the third cycle, when the RB19 concentration was 25 and 50 mg/L, complete removal of RB19 was observed within 4 days, and the decolorization efficiency after 3 days was up to 98.1% and 62.1%, respectively, which were much higher than those in the first and second cycles. At high concentrations of 75 and 100 mg/L, the maximum decolorization efficiency in the second and third cycle reached over 90% after 10 days.

The reusability of JF4-immobilized beads showed a high decolorization efficiency could be maintained in different batch operations. The RB19-tolerant ability of immobilized beads was clearly higher than that of free bacteria. Notably, although the majority of studies uncovered the advantage of an immobilized system for dye removal (Cheng *et al.* 2012; Sharma *et al.* 2016), few studies were conducted with regard to the decolorization of anthraquinone dyes by immobilized bacteria. Thus, immobilization of *Bacillus* sp. JF4 could be employed as an efficient bio-additive for anthraquinone dye removal from wastewater. However, it should be noted that, in this study, the concentrations of *Bacillus* sp. JF4 entrapped in beads were very low and only the preliminary results were described. Further efforts should be devoted to optimization of *Bacillus* sp. JF4 dosage for immobilization and long-term operation evaluation in future. Moreover, to fully evaluate the potential economic feasibility of the immobilized bacteria technology, the benefits and costs based on the continuous operation need to be assessed.

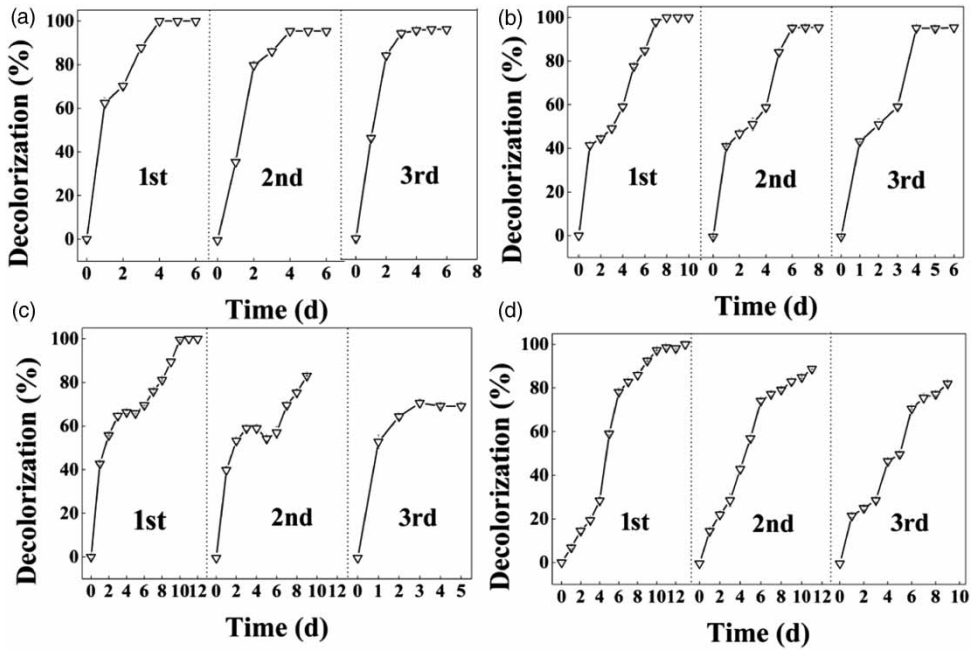

Figure 6 | Reusability of JF4-immobilized beads in a series of repeated batch experiments with different initial RB19 concentrations of 25 mg/L (a), 50 mg/L (b), 75 mg/L (c) and 100 mg/L (d).

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CONCLUSIONS

The strain *Bacillus* sp. JF4 resuscitated by Rpf addition could effectively decolorize RB19. JF4-immobilized beads, which were capable of simultaneous adsorption and biodegradation, showed a tolerability towards high RB19 concentrations and higher decolorization efficiency for RB19 than free bacteria. The JF4-immobilized beads could almost completely decolorize 100 mg/L RB19 within 10 d, while only 92.1% was decolorized by free bacteria within 12 d. The adsorption process can be well described by the pseudo-second-order model, and the most suitable adsorption isotherm was Freundlich. Especially, the repeated-batch experiments indicated that complete decolorization of 100 mg/L RB19 by JF4-immobilized beads can be maintained for at least three cycles without much reduction in efficiency. These results demonstrated that immobilizing Rpf-resuscitated strain into beads was an effective strategy for textile wastewater treatment.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2020.201.

ACKNOWLEDGEMENTS

This study was financially supported by the National Natural Science Foundation of China (41701354 and 51808501), the Natural Science Foundation of Zhejiang Province, China (LQ17D010002), and the Project of Jinhua Science and Technology Department, China (2018-4-007).

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First received 24 February 2020; accepted in revised form 15 April 2020. Available online 27 April 2020

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