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RESEARCH ARTICLE

# Surfactant-facilitated alginate-biochar beads embedded with PAH-degrading bacteria and their application in wastewater treatment

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## Abstract

Immobilized *Pseudomonas aeruginosa* beads with alginate and biochar as composite carriers and a nonionic surfactant (TX100) as degradation promoter were prepared by the gel embedding method. The optimal preparation parameters for the biochar addition amount and the concentrations of the bacterial suspension and TX100 were 1%, $OD_{600}$ = 1 and 200 mg/L, respectively. The addition of TX100 can simultaneously promote biochar sorption of PAHs and PAH degradation by *P. aeruginosa*. The removal ratio of acenaphthene was 24% higher for the TX100-facilitated immobilized bacterial beads than the beads in the absence of TX100. The surfactant-facilitated immobilized bacterial beads can thoroughly remove PAHs in wastewater under the conditions of 10~50 °C, pH 2.5~10.5, and less than 0.2 mol/L NaCl. The immobilized bacterial beads are suitable for continuous-flow reactors, and 2-mm-diameter beads will achieve better application results than larger beads. The new immobilized material can be widely used in various wastewater treatment reactors and in the in situ remediation of organic polluted water.

**Keywords** Alginate embedding · *P. aeruginosa* · Biochar · Triton X-100 · Acenaphthene · Wastewater treatment

## Introduction

With the rapid development of the economy, industrial wastewater and domestic sewage have caused serious pollution of water and soil. Persistent organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides, are considered a serious threat to human health and ecological balance (Chen and Liao 2006; Watts et al. 2010; Mitra et al. 2019; Portet-Koltalo et al. 2020). How to control and eliminate these organic pollutants is one of the research hotspots for environmental science workers. Several treatments, including chemical and electrochemical oxidation, photolysis, chemical and physical sorption, and biodegradation, were commonly used to remove organic pollutants from wastewater (Martínez-Huitle and Ferro 2006; Tran et al. 2015; Mota et al. 2020). Biotransformation is thought to be the principal and most promising mechanism. However, biodegradation treatments usually are time-consuming process. The immobilized-microorganism technique (IMT) combines the advantages of degradation and adsorption technologies by immobilizing microorganisms on the surface of carriers and creating a favorable degradation environment for the microorganisms through the effective adsorption of pollutants on the carrier to achieve the efficient removal of pollutants. The advantages of IMT are high microbial density and activity, a fast reaction speed, strong stability, better resistance to environmental impact, etc. Therefore, IMT is widely used in water and soil pollution remediation (Tao et al. 2009; Mohammadi et al. 2008; Cruz et al. 2013; Chuaphasuk and Prapagdee 2019).

The key factors affecting IMT are carrier material selection and microbial activity. An ideal immobilized-carrier material should be nontoxic, have good mass transfer, high stability and a large specific surface area, and be low cost and easy to obtain. Biochar has been recommended as a good carrier

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material for IMT (Chen et al. 2012; Lu et al. 2018; Chuaphasuk and Prapagdee 2019). One of the outstanding advantages of biochar is its strong adsorption capacity, which can enrich a large number of contaminants and microorganisms simultaneously, and the other is that it has an abundant porous structure and nutrient content, which are conducive to microbial growth. Another key problem is how to further improve the degradation activity of the immobilized microorganisms. Activators and promoters of microbial degradation activity have been employed in IMT. In our previous work, a surfactant-enhanced biochar-immobilized bacterial material was prepared using an adsorption method, and the immobilized bacterial material showed a good removal effect for PAHs in water (Lu et al. 2018). However, because of its powder properties, the application of the reported material in actual wastewater treatment, especially in the in situ remediation of polluted water, is greatly limited.

The gel embedding method is another way to immobilize microorganisms and is different from the adsorption method. Gel embedding can immobilize microbial cells in the limited space of microcells or microcapsules of gelatin. Immobilized materials prepared in this way enable substrates and metabolites to freely pass through the embedding carrier, while the microorganisms cannot leak out. However, the activity of microbial cells may be damaged during embedding. Adding biochar and surfactant on the basis of a general sodium alginate embedding agent may give rise to immobilized materials with higher microbial immobilization efficiency and degradation activity. On the other hand, compared with biochar powder-immobilized bacterial materials prepared by the adsorption method, immobilized bacterial beads prepared by the embedding method may have better settling performance and can also be applied to more types of reactors.

Based on the above hypothesis, immobilized *Pseudomonas aeruginosa* beads with biochar and alginate as the composite carriers and the nonionic surfactant TX100 as a promoter were prepared by the gel embedding method in this paper. The application of the beads in both batch and continuous reaction systems was conducted. This work may provide a new approach for efficient removal of organic pollutants in wastewater. The new immobilized material can be widely used in various wastewater treatment reactors and in the in situ remediation of organic polluted water.

## Materials and methods

### Experimental materials and chemicals

Biochar was prepared by oxygen-limited heating carbonization (Chun et al. 2004). Dried rice straw (RS), bamboo powder (BP), and rice bran (RB) were ground to a powdery consistency, placed in a quartz beaker after 0.1 mm sieving, compacted, capped, and placed at 500 °C for 4 h. Then, the carbonized products were treated with 1 mol/L hydrochloric acid for 12 h, filtered, washed with ultrapure water to neutral, and then dried (Lu et al. 2018). Three biochars derived from the different sources (RS500, BP500, and RB500) were obtained.

Acenaphthene was selected as a representative PAH and was purchased from Shanghai J&K with a purity > 98%. The nonionic surfactant Triton X-100 (TX100) was purchased from Sinopharm Chemical Reagent Co., Ltd. with a purity of analytical grade. The other chemical reagents were of analytical grade.

### Bacterial strains and culture conditions

The test bacterial strain *Pseudomonas aeruginosa* JXQ was isolated from PAH-contaminated soil. The *Pseudomonas* stain was usually recognized as important contributor for pollution removal (Zhang et al. 2019a, b; Wang et al. 2020) and was incubated in autoclaved mineral salt medium (MSM) (Yu et al. 2007) with acenaphthene as the sole carbon source. After 36 h of incubation on a shaker at 100 rpm at 28 °C, the cell suspensions were collected to prepare immobilized bacterial beads.

### Immobilization method

Surfactant-facilitated alginate-biochar-immobilized *P. aeruginosa* beads were prepared by gel embedding method. After centrifugation, bacterial suspensions were resuspended with MSM, and the $OD_{600}$ value was adjusted to 0.2~1. Then, TX100 at a concentration of 0~200 mg/L was added. The biochars and the abovementioned bacterial suspensions containing surfactants were mixed for 30 min, and the mixed solution was added to 10 mL of 3% (*w*/*v*) sodium alginate to form a mixture. The mixed suspensions were added dropwise to 2% (*w*/*v*) $CaCl_2$ solution using a 100–1000-μL (or 1000–5000-μL) pipetting gun to obtain immobilized beads with a spherical shape. The beads were kept for 12 h in the $CaCl_2$ solution for setting. The beads were harvested with filter paper and rinsed with sterile water. Immobilized beads without the TX100, biochar, or bacteria were also prepared as controls, namely raw beads, white beads, and blank beads, respectively.

### Removal of acenaphthene by immobilized bacterial beads in a batch system

The batch reaction was carried out in brown glass bottles with a volume of 1.5 L. Approximately 5 g alginate-biochar beads and 1000 mL of simulated wastewater solutions with a concentration of 3.5 mg/L acenaphthene were added into each

bottle. The bottles were put into an oscillator operated at 120 rpm at designated temperatures (10~50 °C). The initial pH value of the acenaphthene solution was adjusted by 1 mol/L HCl and NaOH, and the salinity in aqueous solution (0~0.2 mol/L) was controlled by the concentration of sodium chloride (NaCl).

At the end of the reaction, samples were taken at regular intervals and filtered through 0.22-μm hydrophilic PTFE needle filters, and the concentration of acenaphthene was determined by high-performance liquid chromatography.

### Removal of acenaphthene by immobilized bacterial beads in a continuous system

The removal of acenaphthene by the immobilized bacterial beads in a continuous system was conducted in a column reactor. The bottom of the glass column (length, 20 cm; inner diameter, 3 cm; and volume, 142 mL) was perforated with holes of approximately 1 mm. Immobilized bacteria beads were packed to the desired height in the glass column between two supporting layers of stainless-steel mesh. Below the beads was a 1-cm layer of quartz sand, and a 1-cm upper layer of quartz sand was placed in the column to provide a uniform inlet flow of solution into the column. The experiments were performed using a peristaltic pump to pump the solution in downward flow mode. The optimal bed height was 5 cm and the flow rate was 5 mL/min. The effluent solutions from the outlet of the column were successively collected for 1 min at a specific time.

### Determination of acenaphthene

The concentration of acenaphthene in the aqueous solution was determined by HPLC with a fluorescence detector (Agilent 1260, USA) using acetonitrile–water (80:20) as the mobile phase at a flow rate of 1 mL/min. The recovery and method detection limit of acenaphthene were 91.3% ($n = 10$, RSD = 1.95%) and 0.03 ng/L, respectively.

The removal ratio of acenaphthene in batch system was calculated by using the following formula:

$$R_t = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

where $C_0$ and $C_t$ were, respectively, the acenaphthene concentrations (mg/L) at the initial time and a particular moment, and $R_t$ was the removal ratio of acenaphthene at a particular moment.

For the continuous-flow reactor, $C_{eff}/C_{inf}$ was used to assess the removal performance of acenaphthene by immobilized bacterial beads, where $C_{eff}$ and $C_{inf}$ represented the effluent and influent concentration of acenaphthene (mg/L).

## Results and discussion

### Preparation and performance of alginate-biochar bacterial beads without surfactant addition

Previous studies have shown that biochar can play a role as a skeleton support in IMT, and adding suitable amount of biochar may not only help to improve the mechanical stability and the sorption capacity but also make the original dense gel become relatively loose, which increased the permeability of the immobilized microbial beads and benefited mass transfer (Bao et al. 2012; Sun et al. 2015). In this study, immobilized *P. aeruginosa* beads with alginate and different biochar as composite carriers, including rice straw (RS500), bamboo powder (BP500), and rice bran (RB500), were prepared. Figure 1 a shows that with the addition of the RS500 biochar, the removal of acenaphthene by the beads improved. When the biochar addition (1%) and degrading bacteria content ($OD_{600} = 1$) in the embedding system were the same, the beads with RS500 biochar showed higher acenaphthene removal than the beads with other biochars. Comparing the three biochars, the specific surface area of the rice straw biochar was 15.4 $m^2/g$, which was higher than those of the bamboo biochar (7.8 $m^2/g$) and rice bran biochar (9.2 $m^2/g$). In general, the biochar with a larger specific surface area and greater micropore number always shows stronger adsorption capacity for organic pollutants (Chen and Chen 2009; Chen et al. 2013). Therefore, when RS500 biochar with stronger adsorption capacity of acenaphthene was used as the carrier, the alginate-biochar beads showed better performance in removing acenaphthene. Figure 1 b shows the beads with 1% RS500 biochar had significantly higher acenaphthene removal ratios than the beads with 0.5% RS500 biochar. This study also found that when the biochar content exceeded 1.5%, the immobilized beads will exhibit a swelling phenomenon with biochar dissolution. Alginate and 1% RS500 were used as the composite carrier in the embedding system in the follow-up studies.

The concentration of the bacterial suspension will affect the actual bacterial loading in the beads. The results of the effects of bacterial concentration studies are depicted in Fig. 1c. The removal ratio of acenaphthene by the immobilized beads with a bacterial suspension concentration ($OD_{600}$) of 1.0 was greater than that of the bacterial suspensions with concentrations ($OD_{600}$) of 0.5 or 2.0. The main reason for this difference may be that as the concentration of bacteria increases, the bacteria sorbed on/in the biochar gradually approaches saturation. When the density of bacteria is too high, the internal space of the biochar carrier is limited; consequently, mass transfer of the substrate is suppressed, and the competition between microorganisms for nutrients and dissolved oxygen causes mutual inhibition, which leads to a decline in the overall treatment efficiency of the immobilized material for acenaphthene

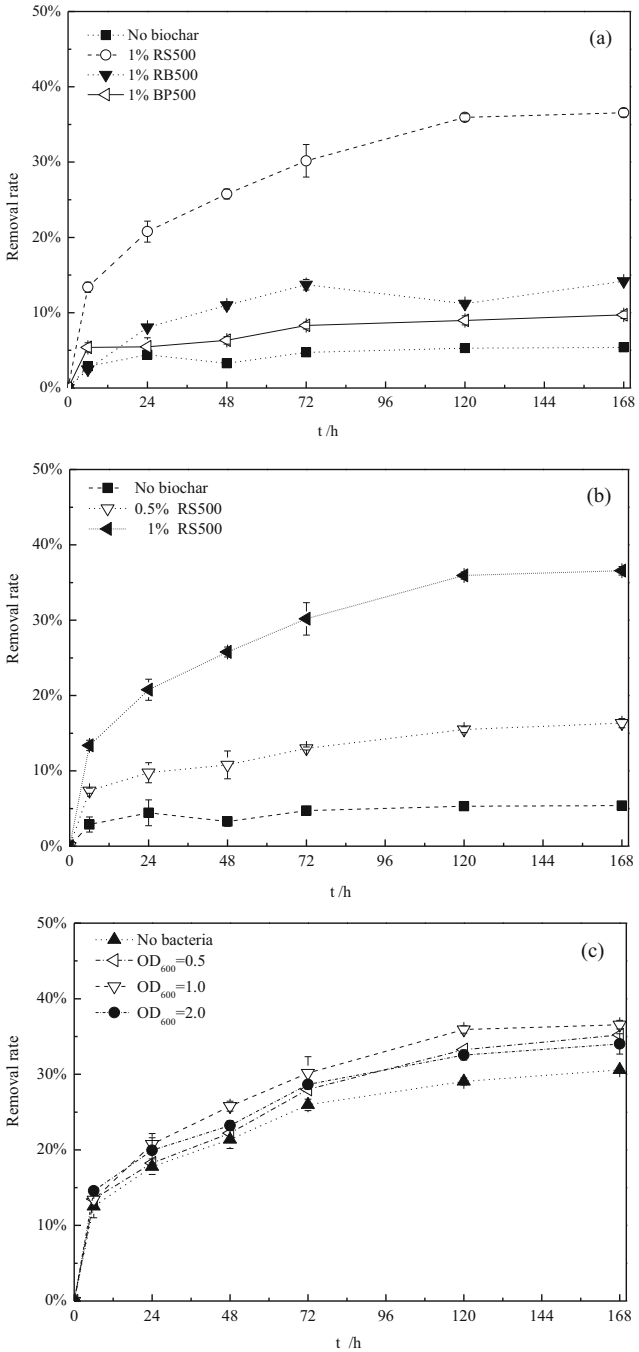


**Fig. 1** Effects of the addition of biochar and bacterial suspension on the performance of alginate-biochar-immobilized bacterial beads in removing acenaphthene. **a** Types of biochar; **b** biochar dosages; **c** concentration of biochar suspension

(Bao et al. 2009; Liu and Wang 2009; Chen et al. 2010). In addition, Bao et al. (2012) reported that the over proliferation of bacteria in beads has a certain destructive effect on calcium alginate gel when the $OD_{600}$ of the bacterial suspension was higher than 2.0 in the process of immobilization. Therefore, under the experimental conditions, the optimal concentration of bacterial ($OD_{600}$) is 1.0.

## Performance of TX100-facilitated alginate-biochar-immobilized bacterial beads

Alginate-biochar-immobilized *P. aeruginosa* beads was prepared by the gel embedding method with different concentrations of TX100, and the removal ratio of acenaphthene was determined in a batch experiment system. It can be seen from Fig. 2 that the removal of acenaphthene by the beads requires approximately 120 h to reach equilibrium. With an increase in TX100 concentration, the removal ratio of acenaphthene by the beads distinctly increased, and a TX100 concentration of 200 mg/L promoted the strongest removal; the corresponding removal ratio increased from 37.9 to 50.6%, an improvement of 24%. As the concentration of TX100 was further increased, the strengthening effect on the beads weakened, and at a TX100 concentration of 300 mg/L, the removal ratio was 32.6%, even lower than that without TX100. Consequently, adding 200 mg/L TX100 in the immobilization process is the most beneficial concentration to significantly improve the removal efficiency of acenaphthene in water.

In the practical application of immobilized bacterial beads, the complexity of environmental conditions will inevitably affect the removal efficiency of organic matter in water. The removal of acenaphthene by optimal TX100-facilitated alginate-biochar beads embedded with *P. aeruginosa* was investigated under different wastewater conditions. It can be observed from Fig. 3a and c that changes in pH and salinity had no obvious effect on the removal of acenaphthene by the beads at pH 2.5~10.5 and a NaCl concentration of 0~0.2 mol/L. Figure 3 b shows that an increase in water temperature (10~50 °C) was conducive to the removal of acenaphthene by the beads. It is notable that the increase of temperature improved the mass transfer, which was beneficial for the transport of acenaphthene within the bead matrix and for enzyme-degradation by the immobilized bacteria. The optimum conditions for *P. aeruginosa* JXQ growth in MSM with acenaphthene as the sole carbon source were 37 °C and

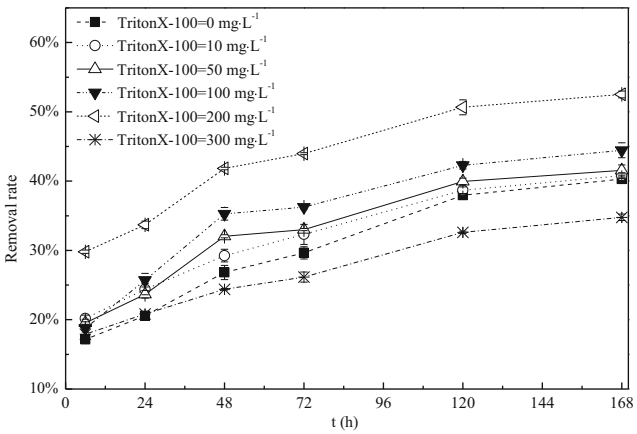


**Fig. 2** Effect of TX100 on acenaphthene removal by alginate-biochar-immobilized bacterial beads

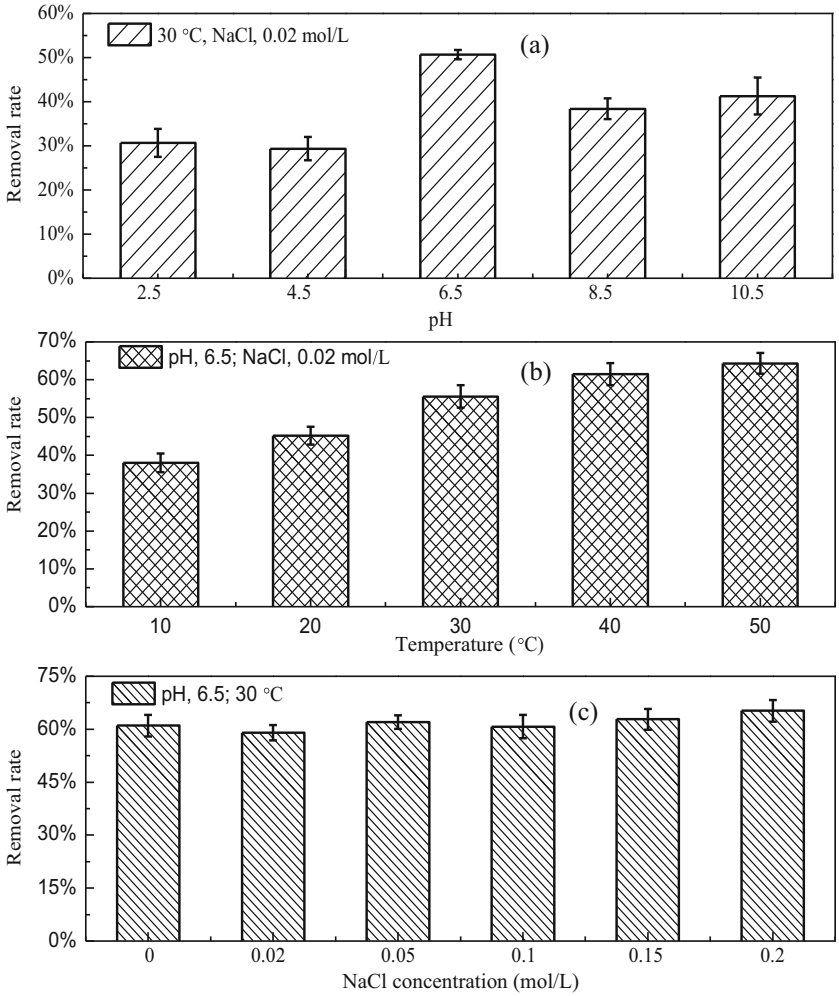


**Fig. 3** Effects of different environmental factors on the removal of acenaphthene by TX100-ficilitated alginate-biochar-immobilized bacterial beads

pH 7.0. In addition, the pH range conducive to growth was 5.0~9.0. It can be seen that the alginate-biochar-immobilized bacterial beads have a certain tolerance and a correspondingly broad scope of application. According to the test results, for the removal of PAHs in water by the TX100-ficilitated alginate-biochar-immobilized *P. aeruginosa* beads, the appropriate working conditions are a temperature of 10~50 °C, pH 2.5~10.5, and a salt concentration (NaCl) not higher than 0.2 mol/L.

It is universal that the immobilized bacteria could not be tolerant to various practical conditions such as sanity, pH, and temperature. Sonwani et al. (2020) reported high removal efficiency (97%) and high elimination capacity (34.3 mg/L/day) for naphthalene biodegradation by low-density polyethylene-immobilized *Exiguobacterium* sp. RKS3. However, the pH, sanity, and temperature dramatically affected the treatment efficiency. Gu and Chang (2001) found that the optimum temperature for PAH degradation by immobilized bacteria followed the optimum growth temperature for free bacteria but with no tolerance to temperature. In this study, the TX100-facilitated alginate-biochar-immobilized bacteria provided a pathway to form a degrader-based matrix with highly tolerance to these environmental conditions.

## The role and mechanism of TX100 in the immobilized bacterial beads

The effect of TX100 on biochar-immobilized bacterial materials may be reflected in two aspects: on the one hand, it affects the adsorption removal of biochar for acenaphthene, and on the other hand, it affects the utilization of nutrients and pollutants by the microorganisms immobilized on the biochar. To further explore the mechanism by which TX100 improves the performance of the biochar-immobilized bacteria beads, the effects of TX100 on acenaphthene removal by alginate-biochar beads without immobilized bacteria (blank beads) and by *P. aeruginosa* immobilized in alginate beads without biochar (white beads) were studied separately.

As shown in Fig. 4, when the concentration of TX100 was 50~200 mg/L, the removal of acenaphthene by the blank beads was obviously promoted. However, an increase in the concentration of TX100 up to 300 mg/L resulted in inhibition.

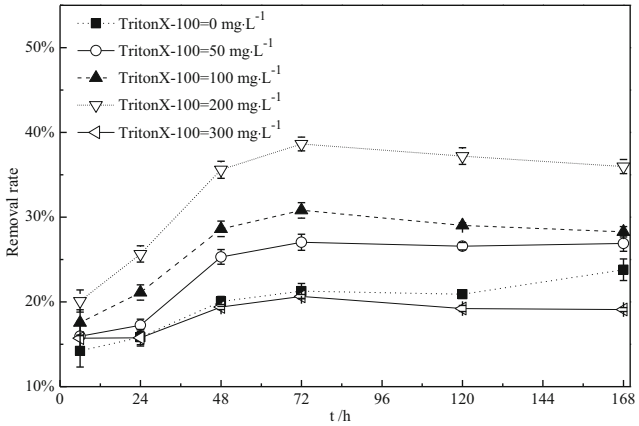


**Fig. 4** The removal of acenaphthene by alginate-biochar beads without bacteria (blank beads) prepared with different concentrations of TX100

The result is similar to the observations in previous research about the effect of surfactants on the soil adsorption of organic pollutants (Zhou and Zhu 2005; Zhu and Zhou 2008). A low concentration of surfactant is present in solution as a monomer and forms a semimicellar adsorbent surfactant on the solid surface. The hydrophobic organic compounds (HOCs) in aqueous solution can be effectively attracted by the adsorbed surfactants through the solid-phase partitioning, and the partition effect will increase over a limited range with an increasing amount of adsorbed surfactant. Moreover, when the concentration of surfactant increases to its critical micelle concentration (CMC) or higher, the monomers begin to form micelles and the micelles will promote the dissolution of organic pollutants into the solution (Ahn et al. 2008; Lu and Zhu 2012a, b). In this experiment, when the concentration of TX100 is below its CMC, which is about 194 mg/L, the sorbed TX100 monomer onto the biochar promoted the sorption remove of acenaphthene by the blank beads.

The effect of TX100 on *P. aeruginosa* was also studied by performing experiments with alginate beads embedded with *P. aeruginosa* but without biochar (white beads). Figure 5 shows that in the experimental range, all concentrations of TX100 could promote the removal of acenaphthene by the white beads, and the degree of promotion increased first and then decreased with increasing TX100 concentration. The removal of acenaphthene was higher than that of the control without the addition of TX100, and the removal ratio reached the maximum at a TX100 concentration of 200 mg/L (48 h), increasing from 6 to 15%. The micromechanism by which surfactants affect microorganisms with respect to the degradation of organic matter may be through a change in microbial cell surface hydrophobicity, which promotes the acquisition of hydrophobic organic matter by the microorganisms and facilitates the transmembrane and intracellular degradation of pollutants in the cells (Su et al. 2010; Zhao et al. 2011; Shoji et al. 2012). Zhang and Zhu 2012 and Zhang et al. 2013 pointed out that Tween series, which are another class of nonionic surfactants, can effectively promote the degradation of pyrene by *Klebsiella oxytoca* at concentrations below CMC due to facilitated pyrene sorption by sorbed surfactants. Similarly, the results of this experiment demonstrate that sorbed TX100 can promote the removal of acenaphthene by immobilized *P. aeruginosa* JXQ.

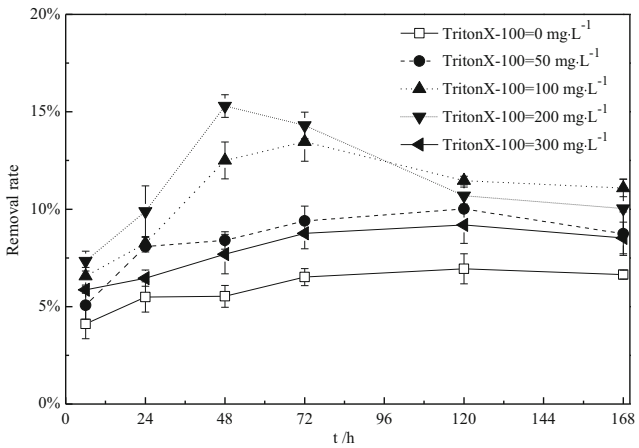


**Fig. 5** The removal of acenaphthene by *P. aeruginosa* immobilized in alginate beads without biochar (white beads) prepared with different concentrations of TX100

## Application of the TX100-facilitated alginate-biochar-immobilized bacterial beads in a continuous reaction system

Compared with biochar-immobilized bacterial powder prepared by the adsorption method (Lu et al. 2018), immobilized bacterial beads prepared by the gel embedding method will be more suitable for continuous reactors and in situ remediation cases. Therefore, the application of TX100-facilitated immobilized bacterial beads in a continuous reaction system was studied. Figure 6 shows that in a packed column reactor with a flow rate of 5 mL/min, the $C_{eff}/C_{inf}$ representing the residual acenaphthene in the flow decreased rapidly over time

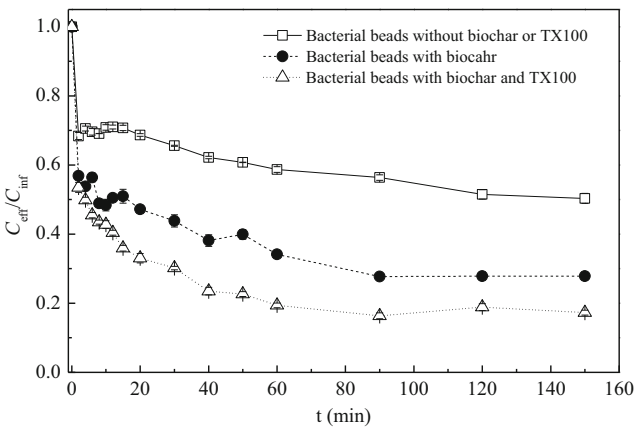


**Fig. 6** The removal of acenaphthene by immobilized bacterial beads in a continuous reaction system

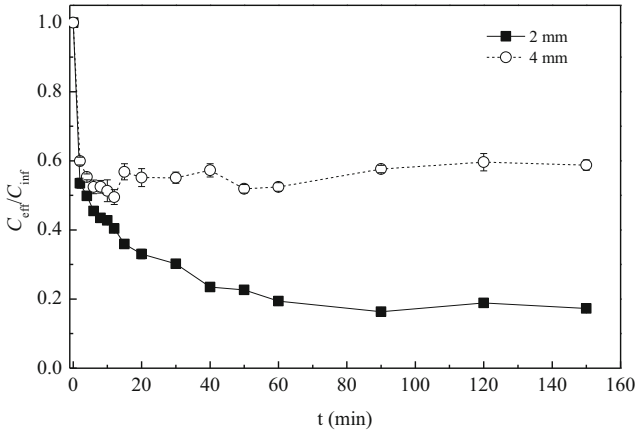


**Fig. 7** Effect of particle diameter on the removal of acenaphthene

during the first 60 min and then remained at a relatively stable level. Within the same reaction time, the residual acenaphthene was much smaller by the TX100-facilitated alginate-biochar bacterial beads than that of the bacterial beads without TX100, which meant that the removal ratio of acenaphthene by the TX100-facilitated alginate-biochar bacterial beads were significantly higher than that of the bacterial beads without TX100.

The size of the immobilized bacterial beads may have some impact on their application, and the influences of particle size are presented in Fig. 7. Acenaphthene residual was obviously lower with 2-mm-diameter beads than with 4-mm-diameter beads. This result may be due to the use of the same filling column height; the packed column would contain more small particles than large particles, resulting in a larger specific surface area (Jiu et al. 2011), which would be conducive to the adsorption and degradation of acenaphthene by TX100-facilitated biochar-immobilized bacterial beads. When the particle diameter of beads is large (4 mm), the mass transfer and mechanical strength will be poor (Becerra et al. 2001). On the other hand, during the experiment, beads with a larger diameter were more prone to deformation and adhesion. Therefore, in continuous reaction systems, smaller-diameter support materials will achieve better application results.

## Conclusions

TX100-facilitated alginate-biochar beads embedded with *P. aeruginosa* were prepared, and their removal performance for acenaphthene in water in both batch and continuous experimental systems was tested. The optimal embedding conditions were a biochar addition of 1% and concentrations of bacterial suspension and TX100 of $OD_{600} = 1.0$ and 200 mg/L, respectively. The addition of TX100 surfactant to the embedding system was demonstrated to promote the adsorption of acenaphthene by biochar as well as to facilitate the degradation of acenaphthene by *P. aeruginosa*, therefore, making the immobilized bacterial beads have better performance in removing acenaphthene. The optimized immobilized bacterial beads can efficiently remove acenaphthene from water at common wastewater conditions, such as 10~50 °C, pH 2.5~10.5, and salt concentrations (NaCl) of up to 0.2 mol/L. These immobilized bacterial beads are suitable for continuous-flow reactors, and 2-mm-diameter beads will achieve better application results than larger beads.

**Authors' contributions** LL was a major contributor in designing the experiment and writing the manuscript. AL prepared the surfactant-facilitated immobilized bacterial beads. XJ performed the examinations of bacterial isolation and cultivation. SH and CY revised the manuscript. All authors read and approved the final manuscript.

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**Data availability** All data generated or analyzed during this study are included in this published article.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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