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Research Article

Al_2O_3 Nanoparticles Promote the Removal of Carbamazepine in Water by *Chlorella vulgaris* Immobilized in Sodium Alginate Gel Beads

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The roles of Al_2O_3 nanoparticles on the removal of carbamazepine (CBZ) by *Chlorella vulgaris* immobilized in sodium alginate gel beads were for the first time investigated. The optimum conditions to prepare immobilized *C. vulgaris* beads with addition of Al_2O_3 nanoparticles were determined as follows: *C. vulgaris* density was 3.0×10^6 cells for 1 mL sodium alginate solution, Al_2O_3 nanoparticle concentration was 0.5 g/L, and concentrations of sodium alginate and $CaCl_2$ were 1.6% and 1%, respectively. The results showed that the proposed algae beads achieved the highest CBZ removal rate of 89.6% after 4 days of treatment, relative to 68.84%, 48.56%, and 17.76% in sodium alginate-immobilized *C. vulgaris*, free microalgae, and Al_2O_3 nanoparticle alginate beads, respectively. The results also showed that the CBZ removal rate increased with more proposed algae beads, while decreased with increased bead diameter. The algae beads exhibited excellent CBZ removal ability even after three recycles. This work provided an economical and effective approach to remove CBZ from water.

1. Introduction

The presence of pharmaceutical and personal care products (PPCPs) in surface water has been well documented in recent years [1–4]. Carbamazepine (CBZ) as an important species of PPCPs is widely used for the treatment of epilepsy and neuralgia. As early as in 1997, the consumption of CBZ had reached 6.33 tons per year in Austria [4]. After being consumed, CBZ is predominantly metabolized in the liver, and 2-3% of the given dose would be excreted via urine in its original form [5]. The excreted CBZ was suspected to enter rivers, streams, and surface waters through the effluent of waste water treatment plants due to its low removal efficiency [6–8]. The accumulation of CBZ in surface wasters was found to pose a great threat for aquatic environment and human health [9–11].

Owing to high photosynthesis efficiency and removal efficiency towards CBZ, microalgae are considered to be one of the most promising ways to polish CBZ-contaminated water [12–14]. It was revealed that immobilized microalgae demonstrated higher resistance to hazardous materials and faster reaction kinetics relative to free microalgae because of the higher cell density [15,16]. However, immobilization with traditional carriers such as sodium alginate has the disadvantage of poor mass transfer process [17–19]. Because of the large specific surface area and perfect biocompatibility, addition of nanoparticles to a sodium alginate-immobilized microalgae system demonstrated promising advantage to promote the contact between immobilized microorganisms and pollutants [20–22].

Al_2O_3 nanoparticles possess high specific surface area and surface energy, which provided sufficient space for the immobilized microorganism and promoted the contact of the microorganism and the pollutant and thus enhanced the removal efficiency of pollutants [23]. In the current study,

Al_2O_3 nanoparticles were adopted as additives into sodium alginate-immobilized *C. vulgaris* (termed as ANFICV) for the purpose of promoting the removal of CBZ.

2. Materials and Methods

2.1. Chemicals and Reagents. CBZ (purity ≥ 98%) and Al_2O_3 nanoparticles (purity ≥ 99.99%) were purchased from Aladdin Industrial Corporation (California, USA), and methanol (HPLC grade) was provided by Fisher Chemical (USA). All the other chemicals used in this study were of analytical grade.

2.2. Microalgae Cultures. The *C. vulgaris* was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. The microalga was cultivated with the autoclaved BG11 medium in an Erlenmeyer flask under illumination at 28°C and with a light/dark period of 18 h/6 h [24]. Fluorescent lamps (36 W; Lifemax Super 80, Philips, Shanghai, China) were employed as the light source.

2.3. Immobilization of C. vulgaris. ANFICV was prepared according to previous studies [25, 26]. *C. vulgaris* in the logarithmic growth phase was harvested by centrifuging at 4500 rpm for 5 min. The cell residues were washed with distilled water and then resuspended in the BG11 medium to form a microalgae solution with high cell density. Both Al_2O_3 nanoparticles and the resuspended *C. vulgaris* were added to the sodium alginate solution and then mixed evenly in 100 ml Erlenmeyer flasks, resulting in a volume of 30 mL. After that, the BG11 medium was added to the resulted mixture. The mixture was then added to the $CaCl_2$ solution drop by drop with a syringe to form immobilized beads. Beads without *C. vulgaris* cells or Al_2O_3 nanoparticles were prepared to carry out control experiments. The prepared beads were washed with sterile distilled water and stored at 4°C for further experiments.

To gain optimal immobilization condition, the microalgae density and concentration of Al_2O_3 nanoparticles, $CaCl_2$, and sodium alginate were adopted as factors to design a four-factor and three-level orthogonal experiment $L_9(3^4)$ (Table 1).

2.4. Determination of Cell Density in ANFICV. The cell density of suspended *C. vulgaris* was determined at the wavelength of 680 nm by an F-4600 spectrophotometer (Hitachi, Japan). Optical density was converted to cell density using a previously prepared calibration curve. Cell density in ANFICV was measured as follows: ANFICV beads were dissolved in 50 g/L sodium citrate solution and then centrifuged at 4500 rpm for 10 min, and the cell residues were resuspended in BG11 for the subsequent optical density determination [27].

2.5. Determination of CBZ. The concentration of CBZ was determined by high pressure liquid chromatography (HPLC) equipped with a UV-VIS detector (LC-20, Shimadzu, Japan) [28, 29]. Prior to analysis, the samples were filtrated through a 0.22 μm membrane filter (Bandao, China) and then injected into a C18 column (250 mm × 4.6 mm, 5 μm, Shimadzu, Japan), maintaining a column temperature of 30°C and a pressure of 25 MPa. The mobile phase was the mixture of HPLC grade methanol and water (65 : 35 v/v) and was introduced into the C18 column at a flow rate of 1 mL/min. The CBZ in the column effluent was detected at 285 nm.

2.6. Statistical Analysis. The experiments were carried out in triplicates, and data were processed by OriginPro8.0 and the statistical software package of SPSS (Ver.19.0). One-way analysis of variance was performed to determine the significant difference between means ($n = 3$) at a significant level of $P < 0.05$.

3. Results and Discussion

3.1. Optimization of ANFICV Preparation. An orthogonal experiment was carried out in order to determine the optimal factors to prepare ANFICV (Table 2). It was demonstrated that $A_3B_2C_3D_1$ achieved the best results towards CBZ removal, for which Al_2O_3 nanoparticle concentration was determined as 0.5 g/L, sodium alginate concentration as 1.6%, the density of microalga cell as 3.0×10^6 cell/mL, and the $CaCl_2$ concentration as 1%. Under such conditions, the removal efficiency of CBZ reached 94.68 ± 0.81% after 5 days; meanwhile, the mechanical strength of the ANFICV bead was the best. According to the range value (R), the concentration of $CaCl_2$ was the most important factor for the removal of CBZ, followed by microalgae density, the concentration of Al_2O_3 nanoparticles, and sodium alginate concentration.

3.2. Al_2O_3 Nanoparticles Promoted the Growth of Immobilized Microalgae. The specific surface area was measured by an automatic specific surface area and porosity analyzer (ASAP2020, Micromeritics Instrument Corporation, USA). The results showed that the specific surface area of the beads prepared by the sodium alginate alone was 2.64 m^2/g, which was increased to 3.57 m^2/g with the addition of Al_2O_3 nanoparticles. The effect of addition of Al_2O_3 nanoparticles into sodium alginate-immobilized beads on the growth of microalgae was evaluated through determining the cell density of the microalgae. It was demonstrated that the difference in cell density was enlarged over inoculation time ($P < 0.05$). After 5 days inoculation, the cell density with Al_2O_3 nanoparticles was determined as 4.80×10^6 cells/mL, larger than 4.21×10^6 cells/mL with no addition of Al_2O_3 nanoparticles (Figure 1). The results confirmed that Al_2O_3 nanoparticles could effectively promote the metabolism of *C. vulgaris*.

3.3. ANFICV Enhanced the Removal Efficiency of CBZ. The removal of CBZ from water by ANFICV and three control treatments (sodium alginate-immobilized *C. vulgaris*, free algae, and Al_2O_3 nanoparticles alginate bead) was

Table 1: Factors and levels of the orthogonal experiment design.

Levels	Factor A Al_2O_3 nanoparticles (g/L)	Factor B Sodium alginate concentration (%)	Factor C Microalgae density (cell/ml)	Factor D $CaCl_2$ concentration (%)
1	0.5	1	1×10^6	1
2	1.0	1.6	2×10^6	2
3	1.5	2.4	3×10^6	3

Table 2: Orthogonal experimental results.

Group number	Factor level				Removal efficiency (%)	Strength
	A	B	C	D		
1	0.5	1.0	1	1	82.43 ± 1.49	+
2	0.5	1.6	2	2	82.66 ± 2.99	++
3	0.5	2.4	3	3	78.53 ± 0.80	+++
4	1.0	1.0	3	2	90.28 ± 0.51	+
5	1.0	1.6	1	3	80.97 ± 0.28	++
6	1.0	2.4	2	1	89.78 ± 5.18	+++
7	1.5	1.0	2	3	76.36 ± 0.78	+
8	1.5	1.6	3	13	94.68 ± 0.81	+++
9	1.5	2.4	1	2	78.58 ± 0.93	++
K_1	78.79	89.29	89.92	90.92		
K_2	79.45	81.63	77.35	80.39		
K_3	86.34	83.28	69.41	65.31		
R	5.800	3.806	7.168	9.215		

The number of "+" indicates the level of mechanical strength of the prepared microalgae beads. More "+" means higher level. K_1, K_2, and K_3 represent the value of CBZ removal under different factors at leve1, 2, and 3, respectively. R represents the range of CBZ removal under different factors.

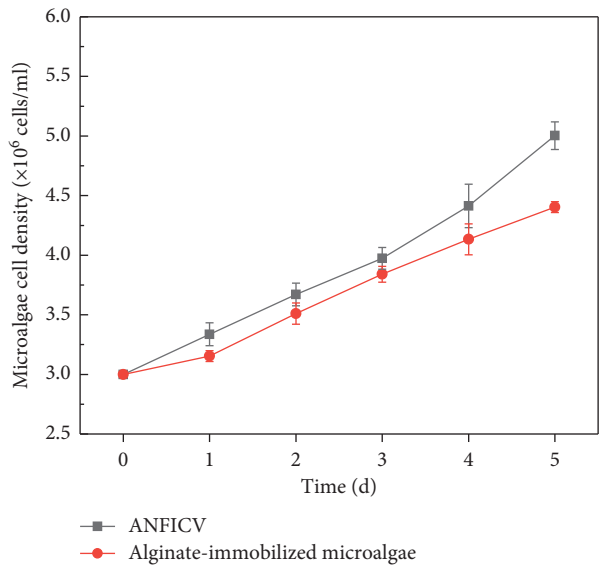

Figure 1: The growth curves of *C. vulgaris* immobilized in beads with and without Al_2O_3 nanoparticles.

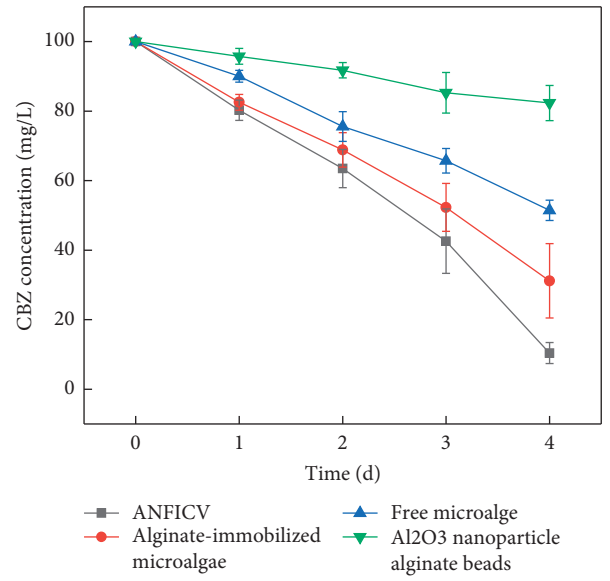

Figure 2: The concentration of CBZ with time under different treatment proposals.

compared. It was illustrated that the concentration of CBZ decreased rapidly on the first day for ANFICV and sodium alginate-immobilized microalgae beads, followed by free microalgae and Al_2O_3 nanoparticle alginate beads (Figure 2). It might be related to the strong adsorption of CBZ by the alginate bead and *C. vulgaris*. The concentration of CBZ in water decreased slightly in Al_2O_3 nanoparticle alginate beads over time probably due to the saturated adsorption of CZB.

With prolonged treatment, the concentration of CBZ decreased continuously and the treatment efficiency for ANFICV, sodium alginate-immobilized *C. vulgaris*, and free *C. vulgaris* became larger and larger ($P < 0.05$). After 4 days of treatment, the CBZ concentration decreased from 100 to 10.40 ± 3.01 mg/L treated by ANFICV, followed by 31.16 ± 10.69 mg/L and 51.44 ± 2.91 mg/L for sodium alginate-immobilized *C. vulgaris* and free algae, respectively,

while it was decreased to 82.34 ± 5.08 mg/L for Al_2O_3 nanoparticle alginate beads. The largest removal efficiency of CBZ by ANFICV might be because of the promoted metabolism of *C. vulgaris* caused by the addition of Al_2O_3 nanoparticles, as well as the adsorption of CBZ by Al_2O_3 nanoparticles.

3.4. Effect of Initial Concentration on CBZ Removal. The effect of initial concentration of CBZ on the removal efficiency by ANFICV was evaluated. It was observed that the removal efficiency of CBZ decreased with increased CBZ concentration (Figure 3). The removal efficiency of CBZ for 4 days of treatment by ANFICV reached $86.73 \pm 3.06\%$ for an initial concentration of 40 mg/L, which was reduced to $72.83 \pm 3.46\%$, $65.29 \pm 3.57\%$, and $65.25 \pm 2.75\%$ for 80, 120, and 160 mg/L CBZ, respectively. The result showed the removal efficiency of CBZ decreases with the increase in initial concentration ($P < 0.05$). The reason might be that the increase in CBZ concentration inhibited the growth and the physiological characteristics of C. *vulgaris*, especially when the concentration of CBZ was increased to 160 mg/L.

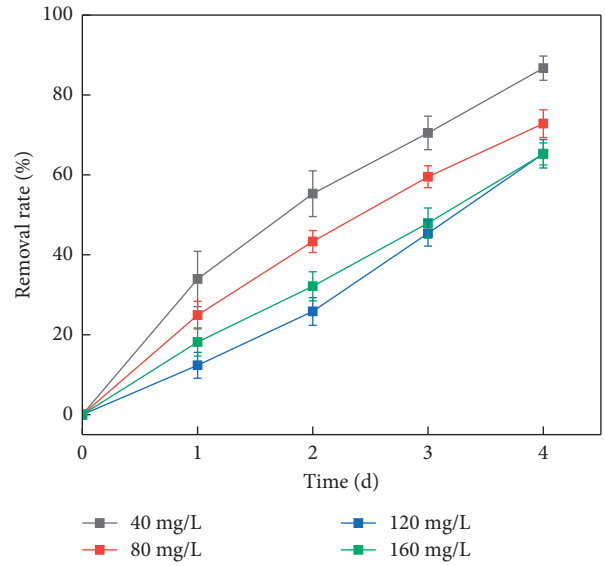

Figure 3: Removal efficiency of CBZ with different initial concentrations by ANFICV.

3.5. Effect of ANFICV Bead Diameter on CBZ Removal. Bead diameter could affect the mass transfer performance of the bead, which in turn affects pollutant absorption by the microorganism immobilized within the bead [30]. In order to investigate the effect of bead size on CBZ removal by ANFICV, three bead diameters of 6 mm (big bead), 4 mm (middle bead), and 2 mm (small bead) were prepared with the same Al_2O_3 nanoparticles concentration, C. *vulgaris* cell density, and sodium alginate. It was shown that the bead diameter significantly affected the removal of CBZ, and its effects were enlarged over time ($P < 0.05$). After 4 days of inoculation, the concentration of CBZ reduced from 100 to 60.02 ± 7.93 mg/L with big beads, which was 16.89 mg/L higher than that for middle beads and was 5.91 times of that for small beads (Figure 4). The larger removal efficiency of CBZ by the smaller bead might be because the smaller bead could result in a larger surface/volume ratio, which was beneficial for microalgae to obtain light and pollutant.

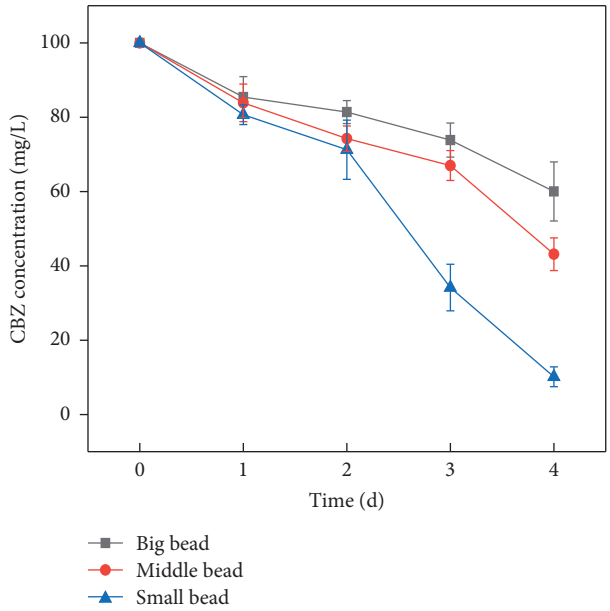

Figure 4: The effect of bead size of ANFICV on CBZ removal.

3.6. Effect of ANFICV Bead Dosage on CBZ Removal. Moreover, the present research evaluated the effects of the ANFICV bead dosage on the removal of CBZ. The results showed that the concentration of CBZ was reduced from 100 mg/L to 50.14 ± 3.54 mg/L when 100 ANFICV beads were adopted (Figure 5). It was further decreased to 38.77 ± 4.40 mg/L, 21.71 ± 5.43 mg/L, and 7.44 ± 2.42 mg/L when the dosage of ANFICV bead was increased to 300, 500, and 700, respectively. The promoted removal performance of CBZ with increased ANFICV dosage might be mainly because more ANFICV beads could increase the number of microalgae cells to remove CBZ, and it could also increase the quantity of ANFICV to absorb CBZ.

3.7. Effect of Recycling Times on the Performance of CBZ Removal. The recycling of ANFICV bead can significantly reduce the cost and promote its practical application. The effect of recycling times of the ANFICV bead on the removal efficiency of CBZ was evaluated. As shown in Figure 6, the removal efficiency of CBZ was 87.67% for the first cycle, while decreased to 52.86% for the third cycle. The removal efficiency of CBZ decreased with the increased recycling

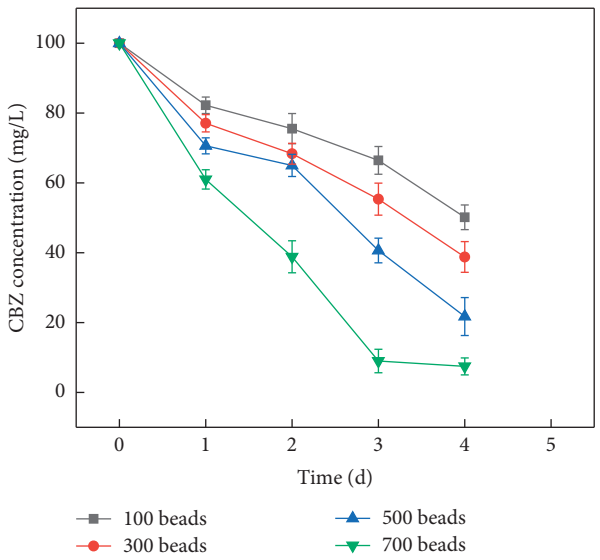

Figure 5: Effect of the dosage of the ANFICV bead on the removal of CBZ.

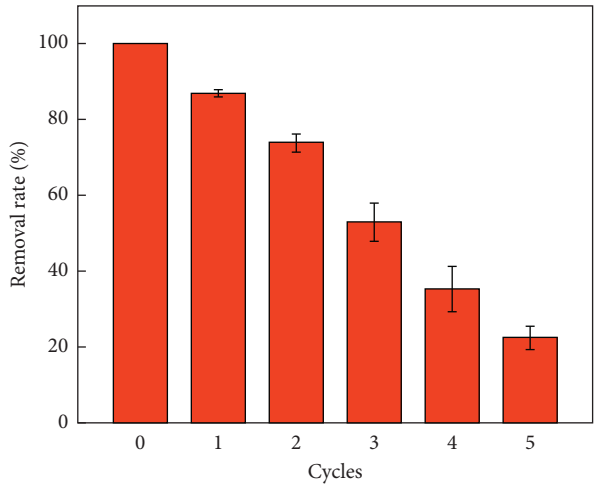

Figure 6: Effect of recycling times on the performance of CBZ removal.

times, which was mainly due to the expansion and pyrolysis of partial ANFICV beads during operation.

4. Conclusions

The present research confirmed that ANFICV with Al_2O_3 nanoparticles as additives significantly promoted the growth of immobilized *C. vulgaris* and improved the performance of CBZ removal by immobilized *C. vulgaris*. The $CaCl_2$ concentration was revealed with the most significant impact on ANFICV preparation for CBZ removal. The results demonstrated that both dosage and diameter of the ANFICV bead could affect the removal efficiency of CBZ. Relative smaller ANFICV beads benefited CBZ removal, and CBZ removal efficiency was increased with the increased dosage of ANFICV beads. This study provided a low-cost and efficient method for the treatment of CBZ-contaminated water.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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