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Preparation of Seeding Type Immobilized Microorganisms and Their Degradation Characteristics on Di-n-Butyl Phthalate[1]

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Objective To study the preparation of seeding type immobilized microorganisms and their degradation characteristics on di-n-butyl phthalate (DBP). **Methods** Diatomite, clinoptilolite, silk zeolite, and coal fly ash were chosen as reserved materials and modified. Their adsorption capacity and intensity in the bacteria were determined and the best carrier was picked out. The seeding type immobilized microorganisms were prepared by the best carrier and then it degraded DBP under different primary concentration, vibration rate, pH, temperature in the presence of metal compounds. **Results** The adsorption capacity of the modified coal fly ash, silk zeolite, clinoptilolite and zeolite was 44.2%, 71.6%, 84.0%, and 94.4%, respectively, which was 1.66, 1.49, 1.37, and 1.16 times as high as that of their natural state. Their adsorption intensity was 72.1%, 90.5%, 90.1%, and 91.1% in turn. The modified diatomite was selected to prepare the seeding type immobilized microorganisms. When the primary DBP concentration was 100 to 500 mg/L, the DBP-degraded rate of the immobilized microorganisms could be above 80%. The degradation activity of both the dissociative and immobilized microorganisms was higher in vibration than in stillness. When pH was 6.0 to 9.0, the DBP-degraded rate of the immobilized microorganisms was above 82%, which was higher than the dissociative microorganisms. When the temperature was between 20℃ and 40℃, the DBP-degraded rate could reach 84.5% in 24 h. The metal compounds could inhibit the degradation activity of both the dissociative and immobilized microorganisms. The degradation process of the immobilized microorganisms could be described by the first-order model. **Conclusion** The adsorption capacity of the diatomite, clinoptilolite, silk zeolite and coal fly ash on DBP-degrading bacteria can be improved obviously after they are modified. The modified diatomite is best in terms of its adsorption capacity and intensity. Its seeding type immobilized microorganisms could degrade DBP effectively and is more adaptable to DBP load, temperature, pH than the dissociative microorganisms. The metal compounds could inhibit the activity of both the immobilized and dissociative microorganisms. The degradation reaction of the immobilized microorganisms on DBP is consistent with the first-order model.

Key words: Carrier; Modify; Seeding type immobilized microorganism; Biodegradation; DBP

INTRODUCTION

Bacteria could be immobilized on water-dissolvable carriers through physical adsorption, chemical or ionic combination. The advantages of this immobilization method are broad choices for adsorption carriers, lower price, simple immobilization process and less effects on the bacteria activity.

DBP is one of the environmental endocrine disruptors and toxic to the procreation system of animals and human beings[1-2]. It has been defined as a priority pollutant in drinking water and water source. After obtaining a kind of DBP-degrading bacteria[3], the carrier with preferable adsorptive capacity and intensity in the bacteria selected from diatomite, clinoptilolite, silk zeolite, and coal fly ash was used to prepare the seeding type immobilized microorganisms. The effects of DBP primary concentration, vibration rate, pH, temperature and metal compounds on DBP degradation by the immobilized microorganisms were studied. The degradation kinetics model was analyzed.

MATERIALS AND METHODS

Experimental Materials

Diatomite was donated by the Mineral Resources Development Corporation of Hu Bei Province. Clinoptilolite and silk zeolite were donated by the

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Magical Stone Mining Limited Company of Jinyun County, Zhejiang Province. Coal fly ash was collected from stokeholders in Tongji Medical College, Huazhong University of Science & Technology.

Major Reagents

All chemicals used in this study were analytical grade reagents, including pancreas peptone, nutrient agar, yeast-extracted solution, sodium chloride, hydrochloric acid, sodium hydroxide, DBP, bluestone, managnous sulphate, zinc nitrate, and caesium chloride.

Experimental Methods

Preparation of the DBP-degrading bacteria suspension In order to gain the DBP-degrading bacterial suspension, a loop of the bacteria was added to 50 mL of sterilized enrichment culture medium (pancreas peptone 10 g/L, yeast-extracted solution 5 g/L, sodium chloride 10 g/L) and incubated at 37℃ with vibration for 12 h.

Modified-management of adsorption materials Diatomite, clinoptilolite, silk zeolite, and coal fly ash were crushed down and passed the boult of 100 orders. Then the carriers were modified. The coal fly ash was modified by the way of making fire[4]. The zeolite and diatomite were by the way of combining acid reaction and heat treatment[5-6].

Preparation of seeding type immobilized microorganisms The natural and modified carriers (5 g each) were contacted with 50 mL of DBP-degrading bacterial suspension adequately under vibration in order to prepare the seeding type immobilized microorganisms. The major techniques were as follows:

●The carriers were dipped in 5% hydrochloric acid for 2 h, and washed with distilled water to their neutral state;

●The carriers were dipped in 5% sodium hydroxide for 2 h, and washed with distilled water to their neutral state;

●The carriers were sterilized by high pressure steam at 121℃ for 20 min;

●The sterilized carriers were added to DBP-degrading dominant bacterial suspension, and vibrated at 30℃ in the pulsator (30 r/min) for 30 min;

●After the grain was held still for 10 min, it was leached and washed with 0.9% Nacl solution to wipe off the non-adsorbed bacteria, then cryodesiccated to gain the seeding type immobilized microorganisms.

DBP degraded tests The seeding type immobilized microorganism (1 g) or dissociative bacterial solution with an equal amount of microorganisms was added to 50 mL of sample DBP water. Then DBP-degraded tests were carried out under different conditions. In order to get more comparable results, the bacteria added to the sample water should be as equal as possible.

RESULTS

Capacity of Various Carriers of Adsorbing DBP-degrading Bacteria Before and After Modification

Five g of natural or modified sterilized carrier was added to DBP-degrading dominant bacterial suspension. The solution was vibrated with at a moderate rate at normal temperature for 30 min, held still for 10min, and then filtrated. The living bacteria in the solution were determined before and after the above process (Table 1).

TABLE 1

Capacity of Various Carriers of Adsorbing DBP-degrading Bacteria Before and After Modification

Carriers	Bacteria Amount in Suspension (10^9 mL $^{-1}$)		Adsorption Capacity (%)*
	Before Adsorption	After Adsorption	
Natural Coal Fly Ash	64	47	26.6
Modified Coal Fly Ash	77	43	44.2
Natural Silk Zeolite	73	38	47.9
Modified Silk Zeolite	67	19	71.6
Natural Clinoptilolite	78	30	61.5
Modified Clinoptilolite	75	12	84.0
Natural Diatomite	74	14	81.1
Modified Diatomite	72	4	94.4

Note. *Adsorption capacity = (bacteria amount adsorbed by carrier/bacteria amount in the suspension)×100%.

Table 1 shows that the capacity of the modified carriers of adsorbing DBP-degrading bacteria improved obviously. The adsorption capacity of the modified coal fly ash, silk zeolite, clinoptilolite, and zeolite was 1.66, 1.49, 1.37, and 1.16 times as high as that of their natural state respectively. The diatomite got the highest adsorption capacity of 94.4% in the modified carriers, followed by clinoptilolite and silk zeolite, which had an adsorption capacity of 84.0% and 71.6% respectively. The coal fly ash was the weakest, and its adsorption capacity was only 44.2%. So the adsorption capacity of the modified carriers in order of their strength was diatomite, clinoptilolite, silk zeolite, and coal fly ash.

Comparison of Various Modified Carriers for Their Adsorption Intensity

Two grams of immobilized microorganisms prepared separately by modified zeolite, clinoptilolite, silk zeolite and coal fly ash was put in 0.9% sterilized Nacl solution, vibrated at the frequency of 3 Hz for 20 min in a pulsator, and held still for 10 min. After that, the bacterial amount separated from the immobilized microorganisms was determined (Table 2).

Table 2 shows that the bacterial amount separated from the immobilized microorganisms was 8.9% in zeolite, 9.2% in clinoptilolite, 9.5% in silk zeolite and 27.9% in coal fly ash. The results showed that zeolite, clinoptilolite, and silk zeolite had a higher adsorption intensity of DBP-degrading bacteria. Less bacteria were separated from them. Their adsorption intensity was 91.1%, 90.8%, and 90.5% respectively. At the same time, the adsorption intensity of the coal fly ash was only 72.1%.

TABLE 2

Comparison of Adsorptive Intensity of Various Carriers to DBP-degrading Bacteria

Carriers	Bacteria Amount Adsorbed by Carriers (g^{-1})	Bacteria Amount Separated out From Immobilized Microorganism	Adsorption Intensity* (%)
Diatomite	0.68×10^{12}	0.60×10^{11}	91.1
Clinoptilolite	0.63×10^{12}	0.58×10^{11}	90.8
Silk Zeolite	4.80×10^{11}	4.56×10^{10}	90.5
Coal Fly Ash	3.40×10^{11}	0.95×10^{11}	72.1

Note. *Adsorption intensity=(bacterial amount adsorbed by carriers−bacterial amount separated from carriers)×100%/bacterial amount adsorbed by carriers.

Degradation Characteristics of Immobilized Microorganisms on DBP

The modified diatomite was selected to prepare the seeding type immobilized microorganisms for degrading DBP according to the above results of carriers' adsorption capacity and intensity.

DBP-degraded rate under different vibration rates The rates of the shaking incubator were 0, 50, 100 and 150 r/min. The initial DBP concentration of the sample water was 100 mg/L. The pH was 7.0 and the temperature was 30℃ (Table 3).

Table 3 shows that the DBP-degraded rates of immobilized and dissociative microorganisms were 90% and 85% respectively when the shaking rates of the incubator were 100 r/min and 150 r/min, and were 74.3% and 65.2% respectively when the shaking incubator was still. The degrading rate was in the midst when the shaking incubator rate was 50 r/min.

TABLE 3

Results of DBP-degraded Rate Under Different Vibration Rates

Incubator Rate (r/min)	Dissociative Microorganism		Immobilized Microorganism	
	Concentration After 24 h (mg/L)	Degraded Rate (%)	Concentration After 24 h (mg/L)	Degraded Rate (%)
0	34.8	65.2	25.7	74.3
50	23.3	76.7	19.3	80.7
100	15.7	84.3	9.8	90.2
150	14.4	85.6	11.6	89.4

Effects of DBP primary concentration on degradation The DBP initial concentrations of the sample water were 100, 200, 300, 400, and 500 mg/L. The pH was 7.0. The tests were done on the shaking incubator (30℃, 100 r/min). The retention time was 24 h. Table 4 shows the degradation results.

TABLE 4

Effects of DBP Primary Concentration on Its Degraded Rate

DBP Primary Concentration (mg/L)	Dissociative Microorganism		Immobilized Microorganism	
	Concentration After 24 h (mg/L)	Degraded Rate (%)	Concentration After 24 h (mg/L)	Degraded Rate (%)
100	15.7	84.3	9.8	90.2
200	32.3	83.8	23.3	88.3
300	51.6	82.8	38.4	87.2
400	124.3	68.9	77.1	80.7
500	216.7	56.7	108.2	78.4

Table 4 shows that both the dissociative and immobilized microorganisms could got higher degradation activities when the DBP primary concentration were 100 mg/L, 200 mg/L, and 300 mg/L. When the DBP primary concentrations increased to 400 mg/L and 500 mg/L, the DBP-degraded rate was all depressed, being more obvious in dissociative microorganisms than in immobilized microorganisms. The facts indicate that the immobilized microorganisms were more tolerant to DBP loading than the dissociative microorganisms.

Effects of pH on degradation The pH values of the sample water were 5.0, 6.0, 7.0, 8.0, and 9.0 adjusted by 10% NaOH and HCl. The DBP primary concentration was 100 mg/L. The tests were carried out on the shaking incubator (30℃, 100 r/min). The results are shown in Table 5.

TABLE 5

Effects of pH on DBP Degradation

pH	Dissociative Microorganism		Immobilized Microorganism	
	Concentration After 24 h (mg/L)	Degraded Rate (%)	Concentration After 24 h (mg/L)	Degraded Rate (%)
5.0	28.6	71.4	25.7	74.5
6.0	19.4	80.6	17.7	82.3
7.0	15.7	84.3	9.8	90.2
8.0	17.5	82.5	11.6	88.4
9.0	26.9	73.1	14.5	85.5

Table 5 shows that the degradation activities were higher in dissociative and immobilized microorganisms when the pH was between 6.0 and 8.0. If pH <6.0 or >8.0, the DBP-degraded rate in the dissociative microorganisms was depressed by about 10%. The immobilized microorganisms could maintain a higher degradation rate in the partial alkaline condition. If the pH was declined to 5.0, the DBP-degraded rate of the immobilized microorganisms was also depressed. However it was higher than that of the dissociative microorganisms, indicating that the immobilized microorganisms were more adaptable to pH.

Effects of temperature on degradation The initial DBP concentration of the sample water was 100 mg/L, pH was 7.0. The rate of the shaking incubator was 100 r/min. The DBP-degraded rates at 20℃, 25℃, 30℃, 35℃, and 40℃ are shown in Table 6.

TABLE 6

DBP Degradation Results Under Different Temperatures

Temperature (℃)	Dissociative Microorganism		Immobilized Microorganism	
	Concentration After 24 h (mg/L)	Degraded Rate (%)	Concentration After 24 h (mg/L)	Degraded Rate (%)
20	24.6	76.4	15.5	84.5
25	19.3	80.7	12.7	87.3
30	15.7	84.3	9.8	90.2
35	17.5	82.5	8.7	91.3
40	25.2	70.8	13.7	86.3

Table 6 shows that the immobilized microorganisms maintained higher activities and the DBP-degraded rate was above 80% when the temperature was between 20℃-40℃. At the same time the DBP-degraded rate of the dissociative microorganisms was declined to 70.8% at 40℃, indicating that the immobilized microorganisms were more adaptable to the change of environmental temperature.

Effects of metal compounds on degradation Metal compounds of a certain concentration were added to 50 mL of sample water (DBP concentration 100 mg/L, pH 7). The rate of shaking incubator was 100 r/min. The temperature was 30℃. The DBP-degraded rates were determined after 24 h. Table 7 shows the results.

TABLE 7

Effects of Metal Compounds on DBP Degradation

Metal Compounds	Concentration (mmol/L)	Degraded Rate (%)	
		Dissociative Microorganism	Immobilized Microorganism
$CuSO_4$	1.0	68.4	75.4
$MnSO_4$	1.0	67.7	73.6
$Zn(NO_3)_2$	1.0	70.3	76.2
$SrCl_2$	1.0	71.2	78.6
Control	0.0	84.3	90.2

Table 7 shows that the metal compounds could inhibit the activities of both dissociative and immobilized microorganisms.

Degradation kinetics In order to study the degradation kinetics of the immobilized microorganisms on DBP, the sample water was prepared under the following conditions: DBP primary concentrations 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, 500 mg/L; pH 7.0; temperature 30 ℃. The rate of shaking incubator was 100 r/min. The DBP-degraded rates of different concentrations at different time points were determined (Table 8). Then the data were analyzed by linear regression as shown in Table 9.

The process of DBP biodegradation could be described preferably by the first-order reaction according to the above kinetics equations and their correlation coefficients. From Table 7, it could also be seen that the degradation rate constants were 0.0758, 0.0805, 0.0774, 0.0720, and 0.0731 when the

TABLE 8

Results of DBP Degradation Under Different Primary Concentrations

DBP Primary Concentration (mg/L)	DBP Concentration (mg/L)			
	8 h	16 h	24 h	32 h
100	30.1	15.3	9.8	4.6
200	89.4	31.2	23.3	11.5
300	168.5	79.6	38.4	27.3
400	261.3	154.7	77.1	48.6
500	348.2	221.7	108.2	63.1

TABLE 9

DBP Degradation Kinetics Equation

Primary DBP Concentration (mg/L)	Kinetics Equation	Pearson Correlation Coefficients (r)
100	lnC=4.000-0.0758t	0.996
200	lnC=4.990-0.0805t	0.977
300	lnC=5.665-0.0774t	0.987
400	lnC=6.150-0.0720t	0.998
500	lnC=6.480-0.0731t	0.996

DBP primary concentrations were 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, showing that the immobilized microorganisms could keep a high activity and tolerate DBP loading effectively when the DBP concentration was from 100 mg/L to 500 mg/L.

DISCUSSION

Tables 1 and 2 show that the adsorption capacity of the carriers improved obviously after they were modified. The reason lies in the fact that modification can improve carriers' specific surface and porosity [4-6]. Although diatomite, clinoptilolite, and silk zeolite had a higher adsorption capacity in DBP-degrading bacteria, the coal fly ash, a kind of waste residue, could also improve its adsorption capacity after modification, suggesting that coal fly ash can be used as a carrier for immobilizing the bacteria.

Tables 3 to 6 show that the seeding type immobilized microorganisms were more adaptable to DBP loading, temperature and pH than the dissociative microorganisms. The reason is that the dissociative microorganisms are limited in a certain space through adsorption. The stability of cell membranes was improved through the adsorptive action between the carrier and the bacteria. In addition, the micro-environment of the immobilized microorganisms was changed, which could change the microorganism' shape, structure, physiological characteristics, and metabolic activities. Thereby the immobilized, microorganisms can enhance their tolerance to the environment attacks and toxic substance[7-8].

Table 7 shows that diatomite could not protect the DBP-degrading bacteria in the presence of metal compounds. This might be explained by the fact that diatomite, a kind of sorbent, could also adsorb metal compounds. In this way, the metal compounds could inhibit bacteria within the special structure of diatomite.

Compared with the traditional biological techniques, the seeding type immobilized has prominent virtues such as high work efficiency, little sludge output, better adaptability, simple preparation methods, low cost, convenient application, and popularization. But further research is needed before the seeding type immobilized microorganisms are used practically to remove trace DBP in drinking water sources.

CONCLUSIONS

The capacity of the reserved carriers of adsorbing the DBP-degrading dominant bacteria can be improved obviously after they are modified. Their adsorption capacity and intensity in order of their strength are diatomite, clinoptilolite, silk zeolite, and coal fly ash.

The seeding type immobilized microorganisms made by the modified diatomite could be more adaptable to DBP loading, temperature and pH than the dissociative microorganisms.

The degradation ability of both the dissociative microorganisms and the seeding type immobilized microorganisms with the diatomite used as a carrier is inhibited by the metal compounds, showing that the metal compounds with high concentration should be avoided in actual applications.

The degradation kinetics of the seeding type immobilized microorganisms on DBP is in accord with the first-order model.

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