Biodegradation of omethoate by *Bacillus* sp. YB-10: optimization of culture conditions and degradation characteristics

Riqiang Li^{1†}, Jianxing Wang^{1†}, Songhui Li²

¹College of Environmental & Resources Sciences, Shanxi University, Taiyuan 030006, China

²Weihai Water Group Co., Ltd., Weihai 264200, China

ABSTRACT

Omethoate is an acute organophosphorus pesticide which is widely used. It presents a high pollution potential and health risk nowadays. In this study, *Bacillus* sp. YB-10, which was isolated from the activated sludge of a pharmaceutical wastewater treatment plant, was used for degrading omethoate. The culture conditions and degradation characteristics were investigated. Results showed that the *Bacillus* sp. YB-10 could degrade omethoate through co-metabolism. The optimal carbon and nitrogen sources were glucose and NH_4NO_3 , respectively. Response surface methodology (RSM) results showed that the most appropriate nutrition ratio of C:N:P was 3:1:1 when degrading 1,000 mg/L of omethoate. Omethoate degradation kinetics could be described by a first-order rate equation. The optimal degradation conditions were pH of 7.0, temperature of 30°C, initial bacteria concentration of 0.25%, rotation speed of 150 r/min. Under the optimal conditions, the degradation rate reached 77.24% within 5 d by the *Bacillus* sp. YB-10 when the initial omethoate concentration was 1,000 mg/L.

Keywords: *Bacillus* sp. YB-10, Biodegradation, Co-metabolism, Omethoate, Organophosphorus pesticide

1. Introduction

Omethoate (O,O-dimethyl-S-methylcarbamoylmethylthiophosphate, $\text{C}_5\text{H}_{12}\text{NO}_4\text{PS}$) is one of the acute organophosphorus pesticides that are widely used to prevent, destroy, repel, or mitigate agricultural and domestic insects in developing countries [1-4]. For example, 8,000 tons of omethoate are applied to agricultural crops annually in China [5]. Actually, only a small percent of them reaches the target pest; the rest spreads to the ecosystem through water, soil, and food [6]. Therefore, omethoate is found throughout the environment nowadays [7]. It was reported that omethoate had clear mutagenic potential and allergic disorder [5], and was hardly biodegradable by the environment naturally in short time. Therefore, omethoate presents a serious pollution potential and health risk to the environment and human [8, 9]. It is very important to find a feasible way to alleviate the contamination of omethoate in wastewater, soil and groundwater.

In view of the bioresistant of omethoate, some researchers tried to remove it through physicochemical methods, such as adsorption [10], catalytic ozonation [11, 12], phase surface discharge plasma [13] and photocatalytic [14]. Xiao et al. [15] reported that omethoate

removal efficiency could achieve 72.1% using a new adsorbent of PBPC (which rice bran protein was cross-linked with eerie ammonium nitrate). Some researchers developed novel photocatalyst for efficient destruction of organic contaminations in water [16, 17], such as $\text{Dy}_2\text{Ce}_2\text{O}_7$ ceramic nanostructures [18] and silver tungstate nanostructures [19]. All these nanomaterials revealed excellent photocatalytic efficiency for the environmental remediation. The physicochemical methods had perfect removal efficiency; however, most of them were limited by application, operation or cost.

Biodegradation of pesticides by microorganisms has excellent advantages such as simpler operation and less cost than physical and chemical approach [20]. Thus it was considered to be one of the most effective ways for remediation of pesticide contamination [3]. Cheng et al. [21] reported that multifunctional constructed wetland could remove omethoate completely; however, the omethoate concentration in the influent was much low (60 $\mu\text{g/L}$). The key of pesticides biodegradation was isolating the bacteria strains which have high degradation ability. In recent year, various microbes were isolated for biodegradation of organophosphorus pesticides, including the species of *Arthrobacter*, *Burkholderia*,

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[†] Corresponding author

Email: liriqiang@sxu.edu.cn, wangjx23@163.com

Tel: +86 351 7010600 Fax: +86 351 7010600

Enterobacter, *Flavobacterium*, *Pseudomonas*, *Serratia*, *Sphingobium*, *Bacillus*, *Aspergillus*, *Trichoderma*, *Streptomyces*, *Brevibacterium* [7, 22-25]. However, the researches of omethoate biodegradation were very limited.

The microbes which could degrade omethoate in literatures were also lacking. The reported omethoate-degrading microbes mainly belonged to fungal species. Tao *et al.* [3] had isolated an *Aspergillus niger* strain to degrade the omethoate. Meng *et al.* [8] screened out a strain of *Aspergillus* spp. F1 which could degrade omethoate effectively. Zhao *et al.* [26] showed fungus strain of *Penicillium oxalicum* could degrade omethoate using glucose as carbon source. As lots of bacteria could degrade organophosphorus pesticides, there might be certain bacteria strains which had high degradation ability of omethoate. Li *et al.* [6] found six *Lactobacillus plantatum* strains possessing high capacity of degrading omethoate. Zhang *et al.* [27] isolated a *Paracoccus* sp. strain FLN-7 which could hydrolyze omethoate through amide bond cleavage. In addition to these researches, omethoate degradation bacteria were rarely reported. Furthermore, various studies showed that the ability for organophosphorus pesticides biodegradation was affected by external conditions including nutrients, temperature, pH, etc [26, 28]. Therefore, the culture conditions and degradation characteristics should also be defined in order to achieve perfect degradation effectivity.

We have isolated a strain of *Bacillus* sp. YB-10 from the activated sludge of omethoate wastewater treatment plant and used it for biodegrading omethoate. The objective of this study was to investigate the optimal culture conditions through single-factor experiments and response surface methodology (RSM); and determine the omethoate degradation characteristics and conditions of *Bacillus* sp. YB-10. In addition, kinetics of omethoate degradation would also be investigated.

2. Materials and Methods

2.1. Chemicals and Media

40% omethoate (molecular formula: $C_5H_{12}NO_4PS$) emulsifiable concentrate was used in this study. The chemical structure of omethoate was described as Fig. S1.

The enrichment medium (EM) comprised (g/L) [29]: peptone, 10.0; NaCl, 1.0; KH_2PO_4 , 1.0; glucose, 1.0. The mineral salt medium (MSM) was composed of (g/L): $NaNO_3$, 2.0; KCl, 0.5; $MgSO_4 \cdot 7 H_2O$, 0.5; $MnSO_4$, 0.02; $CaCl_2$, 0.04. The mineral salt medium with glucose was composed of MSM and 1.5 g of glucose. The beef extract peptone medium was composed of (g/L) [30]: beef extract, 3.0; peptone, 10.0; NaCl, 5.0. To prepare solid medium, 2% of agar was added in the liquid medium. All media had their pH maintained at 7.0-7.5 and were sterilized under 0.1 MPa for 30 min. When the media were cooled to 50°C, certain concentration of omethoate were added in and mixed.

2.2. Microorganism

A *Bacillus* sp. strain was isolated in our laboratory from the activated sludge sample of the wastewater treatment plant in a pharmaceutical industry located in Henan Province, China. During enrichment

cultures, 10 mL of activated sludge suspension (20%) was added in 90 mL of EM which contained 200 mg/L of omethoate, and then incubated at 30°C in a rotary shaker at 150 rpm for 5 d. Then 10% of the enriched activated sludge was added to EM with certain concentration of omethoate and incubated under the same conditions. The enrichment step was repeated for 4 times and omethoate concentration was 500, (1,000), (2,000) and 5,000 mg/L, respectively. Then the organisms were separated on beef extract peptone medium using plate smearing method. The strain was isolated and purified by inoculating on beef extract peptone medium and incubated for 2 d. The bacterial strain was preliminarily characterized and identified as *Bacillus* sp. YB-10 on the basis of morphological, physiological, biochemical and carbon source utilization characteristics.

Before use, the selected strains were incubated in beef extract peptone medium and agitated at 30°C and 150 rpm for 24 h, followed by centrifugation at 5,000 r/min for 20 min. The precipitates were washed with sterile water for 3 times. The cells were diluted with sterile water to obtain a bacterial suspension with 0.25 g/mL (which 0.25 g wet bacteria suspended in 1 mL of sterile distilled water) for the omethoate degradation test.

2.3. Optimization of Culture Conditions

2.3.1. Single-factor experiments

Effect of different carbon and nitrogen sources, and the nutrition concentrations on *Bacillus* sp. YB-10 growth and omethoate degradation were carried out through single-factor experiments. The details and steps of the tests were described as follows:

- ① Effect of different carbon sources: The carbon sources included starch, glucose, mannitol, sorbitol, maltose, sucrose, lactose, fructose, methionine, and threonine. 1.5 g/L of each carbon source was added in 100 mL of MSM which contained 1,000 mg/L of omethoate.
- ② Effects of carbon source concentration: Certain concentration of optimal carbon source determined by step ① was added in the same medium to confirm the optimal concentration.
- ③ Effect of different nitrogen sources: The nitrogen sources included NH_4Cl , NH_4NO_3 , KNO_3 , $(NH_4)_2SO_4$, beef extract, peptone and yeast. 0.5 g/L of each nitrogen source was added in 100 mL of MSM to replace the original nitrogen sources of $NaNO_3$. In addition, the used MSM contained 1,000 mg/L of omethoate and optimal carbon source according to the results of above tests.
- ④ Effects of nitrogen source concentration: Certain concentration of optimal nitrogen source determined by step ③ was added in the same medium to investigate the optimal concentration.
- ⑤ Effects of phosphorus source concentration: The phosphorus source used in this study was KH_2PO_4 . Certain concentration of KH_2PO_4 (0-3 g/L) was added in 100 mL of MSM. The MSM contained 1,000 mg/L of omethoate and optimal carbon and nitrogen source according to the results of above tests.

During each experiment, 0.25 mL of bacterial suspension was inoculated to 100 mL of medium at 30°C and 150 r/min for 5 d. Then the biomass and omethoate concentration were measured. All the experiments were carried out in triplicate.

2.3.2. Response surface methodology (RSM)

The concentrations of carbon, nitrogen and phosphorous sources were identified by experiment as variables with significant effects on omethoate degradation by the *Bacillus* sp. YB-10. The experimental design chosen for this study was that of Box- Behnken, which needed relatively a few experimental combinations of the variables [31]. The experimental design and statistical analysis were done with SAS software (SAS Institute, Cary, NC, USA). In this study, the variable factors with the coded and actual values were given in Table 1 according to the single-factor experiments results.

Table 1. Levels and Codes of the Box-Behnken Experimental Design

Code	X ₁ (Glucose/g·L ⁻¹)	X ₂ (NH ₄ NO ₃ /g·L ⁻¹)	X ₃ (KH ₂ PO ₄ /g·L ⁻¹)
-1	1.2	0.25	0.5
0	1.5	0.5	1.0
1	1.8	0.75	1.5

2.4. Effect of Omethoate Concentration and Omethoate Degradation Modeling

To investigate the effect of omethoate concentration, 0.25 mL of the *Bacillus* sp. YB-10 bacterial suspension was incubated in 100 mL of the optimal medium with omethoate concentration of 200-5,000 mg/L and reacted at 30°C, 150 r/min for 7 d. The omethoate degradation rate was measured every day.

First order kinetics was used to describe the biodegradation of omethoate under concentration of 200-5,000 mg/L. The first-order rate equation could be described as Eq. (1) [3]:

$$[C] = [C_0]e^{-Kt} \quad (1)$$

Where C is the concentration of omethoate, C_0 is the initial concentration of omethoate, K is the degradation constant, and t is time.

When $[C] = 0.5 [C_0]$, the half-time for omethoate degradation is $t_{1/2} = \ln 2/K$.

2.5. Effect of Degradation Conditions

To investigate the effect of degradation conditions, different pH (5.0-8.0), temperature (15°C-35°C), initial bacteria concentration (0.1%-0.5%, which 0.1-0.5 mL of bacterial suspension were added in 100 mL of medium), and rotation speed (100-250 r/min) were chosen in this study. For each experiment, the bacterial suspension was incubated in the optimal medium and degraded for 5 d. The initial omethoate concentrations of all the experiments were 1,000 mg/L. Each experiment was repeated 3 times.

2.6. Analysis Methods

Gas chromatograph (GC-7900 Techcomp, China) was used to measure the concentration of omethoate. The chromatography analysis was performed using HP-1 30 m × 0.53 mm × 1.5 μm chromatographic column. The temperature programming comprised a starting temperature of 160°C for 3 min that increased from 160°C to 250°C at a rate of 15°C/min. The temperature was held for 12

min at 250°C to complete the run. The sample size used for analysis was 1 μL. The carrier flow was N₂.

The bacteria biomass was measured by determining the optical density at 600 nm (OD₆₀₀) using a spectrophotometer (TU-1800S, PERSEE, China) [32].

3. Results and Discussion

3.1. Optimization of Culture Condition for the *Bacillus* sp. YB-10 Strain

3.1.1. Effects of carbon source and concentration

Carbon source is an essential energy material for microbial growth, reproduction and movement [33]. It could also form the cells of microbial. The effects of different carbon sources on strain growth and omethoate degradation were shown in Fig. 1(a).

As shown in Fig. 1(a), glucose was the optimal carbon source for *Bacillus* sp. YB-10, which the OD₆₀₀ reached 0.884, and the omethoate degradation rate reached 75.81%. This result was consistent with Meyer et al.'s study [33], which stated glucose was the preferred carbon source for *Bacillus subtilis*. Maltose and sucrose were the second proper carbon sources in this study. There were poor strain growth and omethoate degradation of fructose and lactose. Ren et al. [34] also reported that fructose exhibited lower biomass than glucose for *Scenedesmus* sp. strain R-16, which was in agreement with this study. It also showed that starch had no promotes for strain growth. In this work, glucose is a kind of monosaccharides; therefore, it can be used by microbes easily for its simple structures. However, disaccharides such as maltose, sucrose and lactose have complex structures comparing to monosaccharides; hence, they cannot convert as energy efficiently by bacteria [35].

Fig. 1(a) also shows that *Bacillus* sp. YB-10 could grow with sugar alcohols such as mannitol and sorbitol; however, the omethoate degradation efficiency was poor. Methionine and threonine could neither be used by *Bacillus* sp. YB-10, nor promoted omethoate degradation. This was because that some amino acids could only be used as energy sources by anaerobic microbes when lacking of carbon source [23]. Consequently, glucose was chosen as the optimal carbon source for the *Bacillus* sp. YB-10.

Then the effects of glucose concentration were investigated. Results were shown in Fig. 1(b). It was clear that both the OD₆₀₀ and omethoate degradation rate were 0 when no glucose was added. This implied that stain of *Bacillus* sp. YB-10 could not grow and degrade omethoate when the omethoate was used as sole carbon source. Therefore, it suggested that the *Bacillus* sp. YB-10 degraded omethoate through co-metabolism. When the glucose concentration increase as 0, 0.3, 0.9, 1.2, 1.5, 1.8, 2.5 and 3 g/L, the biomass increased obviously and then achieved a stable level. The omethoate degradation rate increased when the glucose concentration increased from 0 to 1.5 g/L; however, when the glucose concentration was > 1.5 g/L, the degradation rate decreased. This result showed that when the omethoate concentration was constant, it has a proper ratio of glucose concentration in the co-metabolism system. When the glucose concentration was low, there were not enough carbon sources for the bacteria. However, high concentration of glucose would induce the bacteria to give up using omethoate. In this

Fig. 1. The effect of carbon source and concentration on *Bacillus* sp. YB-10 growth and omethoate degradation. (omethoate concentration: 1,000 mg/L, pH = 7.0, 30°C, initial bacteria concentration: 0.25%, rotation speed: 150 r/min, degradation time: 5 d)

study, the glucose concentration was determined at 1.5 g/L when the omethoate concentration was 1,000 mg/L.

3.1.2. Effects of nitrogen source and concentration

The nitrogen source material in medium was mainly used to synthesize the nitrogenous substances such as protein, nucleic acid, enzymes, and kinds of primary and secondary metabolites in cell. Lacks of nitrogen could restrict the growth of microbes [35]. The effects of different nitrogen sources on strain growth and omethoate degradation were shown in Fig. 2(a).

As shown in Fig. 2(a), the *Bacillus* sp. YB-10 could grow in medium contained different nitrogen sources; however, the utilization effectivities were much different. The strain growth and omethoate degradation revealed the best effectivities when NH_4NO_3 was used as nitrogen source. The following were $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl , and KNO_3 , which the degradation rates were 74.55%, 66.84% and 62.13%, respectively. There were poor utilizations of yeast, peptone, and beef extract, which the degradation rates were about 50%. These results illustrated that inorganic nitrogen were more con-

ducive than organic nitrogen for bacteria growth and omethoate degradation. Abundant organic nitrogen might restrict the use of omethoate, which correspondingly decreased the degradation rate [36]. Among the four inorganic nitrogen sources, NH_4NO_3 has the best degradation effectivity. This result was consistent with previous study [37]. The reason for this phenomenon might be that both NH_4^+ and NO_3^- could provide nitrogen for using. In addition, the NH_4^+ in $(\text{NH}_4)_2\text{SO}_4$ and NH_4Cl was more helpful than the NO_3^- in KNO_3 , which was due to the NO_3^- could not be used unless it was reduced to ammonia. In this study, NH_4NO_3 was chosen as the most appropriate nitrogen source.

Effects of NH_4NO_3 concentration were investigated subsequently and results were shown in Fig. 2(b). It was clear that the effects of NH_4NO_3 concentration were obvious. When the NH_4NO_3 concentration was 0, the bacteria could still grow and degrade omethoate. This phenomenon suggested that *Bacillus* sp. YB-10 could use the omethoate as the sole nitrogen source. The OD₆₀₀ of the *Bacillus* sp. YB-10 and omethoate degradation rate increased evidently when NH_4NO_3 concentration was < 0.5 g/L. Then the growth of *Bacillus*

Fig. 2. The effect of nitrogen source and concentration on *Bacillus* sp. YB-10 growth and omethoate degradation. (omethoate concentration: 1,000 mg/L, pH = 7.0, 30°C, initial bacteria concentration: 0.25%, rotation speed: 150 r/min, degradation time: 5 d)

Table 3. Analysis of Variance (ANOVA) for the Regressive Model

Error Source	DF	SS	MS	F	Pr > F	Significant
X ₁	1	14.1778	14.1778	24.1955	0.0044	**
X ₂	1	14.6341	14.6341	24.9741	0.0041	**
X ₃	1	7.9800	7.9800	13.6185	0.0141	*
X ₁ X ₁	1	70.9830	70.9830	121.1380	0.0001	**
X ₁ X ₂	1	0.0256	0.0256	0.0437	0.8427	
X ₁ X ₃	1	1.7292	1.7292	2.9511	0.1465	
X ₂ X ₂	1	78.0302	78.0302	133.1645	0.0001	**
X ₂ X ₃	1	1.4641	1.4641	2.4986	0.1748	
X ₃ X ₃	1	112.8971	112.8971	192.6675	0.0001	**
Linear	9	36.7919	12.2640	20.9294	0.0029	
Quadratic	3	227.5825	75.8608	129.4623	0.0001	
Cross Product	3	3.2189	1.0730	1.8311	0.2586	
Model	3	267.5933	29.7326	50.7410	0.0002	
Error	5	2.9298	0.5860			
Lack of fit	3	2.9234	0.9745	301.3789	0.0033	
Pure Error	2	0.0065	0.0032			
Total	14	270.5231				

SS, sum of squares; DF, degree of freedom; MS, mean square.

*Significant ($P < 0.05$), **Highly significant ($P < 0.01$).

sp. YB-10 and omethoate degradation rate decreased as the NH_4NO_3 concentration keeping increase. This was because that the ratio of C:N is an important factor during the biodegradation of pesticides by bacteria. High concentration of NH_4NO_3 would decrease C:N ratio, which correspondingly restricted the growth of bacteria [36, 37].

3.1.3. Effects of KH_2PO_4 concentration

Phosphorous could improve the growth of microbial cells. However, when the concentration was too high, it could restrain the secondary metabolism of some microbes. The effects of KH_2PO_4 concentration on strain growth and omethoate degradation were shown in Fig. 3.

As shown in Fig. 3, the effect of KH_2PO_4 concentration was similar to NH_4NO_3 concentration. When the KH_2PO_4 concentration was 0, the bacteria could also grow and degrade omethoate. Thus it implied that *Bacillus* sp. YB-10 could use the omethoate as the sole phosphorous source. The OD_{600} of the *Bacillus* sp. YB-10 and omethoate degradation rate increased with KH_2PO_4 concentration when KH_2PO_4 concentration was < 1.0 g/L. When KH_2PO_4 concentration was 1.0 g/L, the OD_{600} of the YB-10 and omethoate degradation rate reached the highest lever, i.e. 0.896 and 77.38% after 5 d, respectively. In addition, the OD_{600} of the YB-10 and omethoate degradation rate declined when KH_2PO_4 concentration was > 1.0 g/L. Prakash et al. [37] also reported that higher concentration of phosphate would decrease the curdlan production by *Bacillus cereus* PR3.

As mentioned above, *Bacillus* sp. YB-10 could use the omethoate as the sole nitrogen source and phosphorous source, whereas not as sole carbon source. Adding certain amount of nitrogen and phosphorous sources could promote bacteria growth and omethoate degradation; however, higher content of nitrogen and phosphorous sources could decrease the degradation rates.

Fig. 3. The effect of KH_2PO_4 concentration on *Bacillus* sp. YB-10 growth and omethoate degradation. (omethoate concentration: 1,000 mg/L, pH = 7.0, 30°C, initial bacteria concentration: 0.25%, rotation speed: 150 r/min, degradation time: 5 d)

3.1.4. Response surface methodology (RSM) results

The Box-behnken experimental design was used to determine the composition of medium on the basis of single-factor experiments above. Concentrations of glucose, NH_4NO_3 and KH_2PO_4 were defined as variables according to Table 1, and results were shown in Table 2. The second-order response surface model fitting results were given in Table 3 in the form of ANOVA.

Table 2. Experimental Values of the Box-Behnken Design

Runs	X ₁	X ₂	X ₃	Omethoate degradation rate (%)
1	-1	-1	0	68.45
2	-1	1	0	70.34
3	1	-1	0	66.65
4	1	1	0	68.22
5	0	-1	-1	67.10
6	0	-1	1	63.76
7	0	1	-1	69.57
8	0	1	1	68.65
9	-1	0	-1	69.44
10	1	0	-1	67.39
11	-1	0	1	68.89
12	1	0	1	64.21
13	0	0	0	77.35
14	0	0	0	77.46
15	0	0	0	77.38

By applying multiple regression analysis of the experimental data, the results were fit with a second-order polynomial equation. Thus, a mathematical regression model for omethoate degradation using the coded factors was given as:

$$Y = 77.3966 - 1.33125X_1 + 1.3525X_2 - 0.99875X_3 - 4.384583X_1X_1 - 4.597083X_2X_2 - 5.529583X_3X_3 \quad (2)$$

The determination coefficient (R^2) was 0.9892, which indicating the model had extremely good fitting degree. ANOVA results showed that the linear effects (X_1 and X_2) and the quadratic effects (X_1^2 , X_2^2 and X_3^2) were highly significant ($P < 0.01$), the linear effects of X_3 were significant ($P < 0.05$), while the interaction effects (X_1X_2 , X_1X_3 , and X_2X_3) were insignificant.

The optimum conditions for omethoate degradation were calcu-

lated with the SAS software as $X_1 = -0.15$, $X_2 = 0.14$, $X_3 = -0.07$, which accordingly the optimum composition of nutrition in medium were glucose of 1.455 g/L, NH_4NO_3 of 0.535 g/L, KH_2PO_4 of 0.965 g/L. In this condition, the mass ratio of C:N:P was 3:1:1. The omethoate degradation rate was predicted as 77.40%. To confirm the validity of the statistical experimental strategies, additional confirmation experiments were done under the optimal culture condition of glucose 1.46 g/L, NH_4NO_3 0.54 g/L, KH_2PO_4 0.97 g/L. Result showed that omethoate degradation rate reached 77.16%, which was much close to the predicted value.

3.2. Effect of Omethoate Concentration and Degradation Kinetics

The effect of omethoate concentration on degradation effectivity by *Bacillus* sp. YB-10 was shown in Fig. 4(a). It is clear that high concentration of the omethoate would restrain the degradation process. This might be because that high concentration of omethoate could have toxicity to the *Bacillus* sp. YB-10, which subsequently restrain the bacteria growth and affect the metabolic activity. Also, the intermediate which was produced during the omethoate degradation process might restrain the activity of degrading enzymes. As the omethoate concentration rose, the intermediate increased and more degrading enzymes would be disabled; therefore, the degradation ability was decreased. These were also the reasons of slight increase in omethoate degradation after 5 d. In addition, Fig. 4(a) revealed that the degradation rate increased slowly with time at high omethoate concentration, which might also due to the toxicity of omethoate. This was consistent with Meng et al.'s [8] study. Therefore, the degradation time was determined as 5 d.

As the results of omethoate degradation kinetics shown in Fig. 4(b), it was clear that the first-order model gave a good fit under different initial omethoate concentrations. The regression coefficients R^2 were larger than 0.95. Therefore, it concluded that the kinetics of omethoate biodegradation by *Bacillus* sp. YB-10 could be described by a first-order reaction model under test conditions. When the initial omethoate concentrations were 200, 500, (1,000), (2,000) and 5,000 mg/L, the biodegradation half-lives

Fig. 4. The effect of omethoate concentrations on degradation rate and kinetics of omethoate degradation by *Bacillus* sp. YB-10. (pH = 7.0, 30°C, initial bacteria concentration: 0.25%, rotation speed: 150 r/min)

Fig. 5. The effect of different conditions on omethoate degradation by *Bacillus* sp. YB-10. (a) pH (30°C, initial bacteria concentration: 0.25%, rotation speed: 150 r/min); (b) temperature (pH = 7.0, initial bacteria concentration: 0.25%, rotation speed: 150 r/min); (c) initial bacteria concentration (pH = 7.0, 30°C, rotation speed: 150 r/min); (d) rotation speed (pH = 7.0, 30°C, initial bacteria concentration: 0.25%).

($t_{1/2}$) were 1.86, 2.15, 2.38, 4.65 and 15.40 d, respectively. This result also showed that high concentration of omethoate could constraint the biodegradation ability. Tao et al. [3] reported that the biodegradation half-life was 5.24 d by *Aspergillus niger* when degrading 1,000 mg/L of omethoate. In contrast, the biodegradation half-life in this study was much lower under same initial omethoate concentration. This demonstrated that the biodegradation ability of *Bacillus* sp. YB-10 was much stronger than *Aspergillus niger*. In addition, in view of the strong omethoate degradation ability *Bacillus* sp. YB-10, we inferred that the strain could be induced to produce an enzyme which was able to cleave the P-S bond in omethoate. However, further study is needed.

3.3. Biodegradation Conditions of Omethoate

3.3.1. Effect of initial pH

As omethoate could be hydrolyzed and lost efficacy under alkaline condition, we investigated the effect of pH 5.0-8.0 at a temperature of 30°C, initial bacteria concentration of 0.25%, rotation speed of 150 r/min. Results were shown in Fig. 5(a). As revealed in Fig. 5(a), *Bacillus* sp. YB-10 could degrade omethoate at pH of 5-8. The best degradation effectivity was obtained at pH 7.0, under which the degradation rate increased fastly with time (77.11% at 5 d). Both the acid and alkaline conditions could restrain the growth of *Bacillus* sp. YB-10. Omethoate degradation rates were less than 40% when pH was 5.0 during the degradation process. When the pH was 8.0, the degradation rates were also decreased

(56.11% at 5 d). In this study, the best pH for degrading omethoate was determined as 7.0.

3.3.2. Effect of temperature

The effects of temperature were investigated at a pH of 7.0, initial bacteria concentration of 0.25%, rotation speed of 150 r/min. As revealed in Fig. 5(b), there was obvious effect of temperature on omethoate degradation. When the temperature increased from 15°C to 30°C, the degradation rate increased. The highest degradation rate obtained at temperature of 30°C. Actually, it was biochemical reactions by enzymes during the organic biodegradation process. Higher temperature could induce the inactivation of enzymatic; whereas lower temperature could restrain the effectivity of enzymatic. Also, the growth of bacteria was affected by temperature directly. The bacterial grew well at optimal temperature of 30°C, which accordingly increased the activity of organophosphates degrading enzymes.

3.3.3. Effect of initial bacteria concentration

The effects of the initial bacteria concentrations of *Bacillus* sp. YB-10 were investigated at a pH of 7.0, temperature of 30°C, rotation speed of 150 r/min. Results were shown in Fig. 5(c). The degradation rate increased with initial bacteria concentration when it was < 0.25%. Fig. 5(c) also reveals that the higher of initial bacteria concentration, the better of degradation rate at the first day. However, the degradation rate increased slowly in the following days when the initial bacteria was > 0.25%. These results suggested that the

degradation ability did not increase with initial bacteria concentration. At lower bacteria concentrations, there were not enough biomass for omethoate degrading. However, as the initial bacteria concentration was too high, the bacteria were in poor nutrient. Accordingly there were competition between bacteria, which could also restrain the bacteria growth [38]. In this study, the optimal initial bacteria concentration was 0.25%.

3.3.4. Effect of rotation speed

Fig. 5(d) shows the effects of different rotation speeds on the omethoate degradation rate at a pH of 7.0, temperature of 30°C, initial bacteria concentration of 0.25%. The degradation rate increased obviously as the rotation speed increased from 100 to 150 r/min. When the rotation speeds were higher than 150 r/min, all the degradation rates became lower except the first day. Lower rotation speed could not provide enough dissolved oxygen for the bacteria growth. On the other hand, the bacteria could gather together at lower rotation speed, which restrained the contact with medium. All these factors restrained the growth of bacterial. Under higher rotation speed, the mechanical impact could destroy the normal growth of bacteria. In the current study, the optimal rotation speed was 150 r/min, under which the maximum degradation rate reached 77.24% at 5 d. The degradation effectivity was consistent with other's study, in which the omethoate degradation rate was 77.59% by *Aspergillus niger* [3].

In this study, the degradation ability of *Bacillus* sp. YB-10 was based on a pure microbia culture. However, the conditions of environment were very complex. When the bacteria ware used for bioremediation or bioaugmentation, the influence of nature conditions must be considered. Nonetheless, the results of this study could provide useful information for the biodegradation of omethoate in the environment.

4. Conclusions

Bacillus sp. YB-10 was used for degrading omethoate in this study. The culture conditions and degradation characteristics were investigated. It was conclude that the *Bacillus* sp. YB-10 could degrade omethoate through co-metabolism. The most appropriate nutrition ration was glucose of 1.455 g/L, NH_4NO_3 of 0.535 g/L, and KH_2PO_4 of 0.965 g/L when degrading 1,000 mg/L of omethoate. The kinetics for omethoate degradation could be described by a first-order rate equation. The optimal degradation conditions were pH of 7.0, temperature of 30°C, initial bacteria concentration of 0.25%, rotational speed of 150 r/min. Under the optimal conditions, the degradation rate by the *Bacillus* sp. YB-10 within 5 d reached 77.24% when the omethoate concentration was 1,000 mg/L. The results of this work may provide useful information for the biodegradation of omethoate in wastewater, soil and groundwater.

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Author Contributions

R.L. (Associate professor) planned the study, wrote and revised the manuscript. J.W. (Associate professor) and S.L. (Engineer) conducted the experiments.

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