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Degradation characteristics of two *Bacillus* strains on the *Microcystis aeruginosa*

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Abstract: The degradation kinetics of strains P05 and P07 and the degradation effects of mixed strain on *Microcystis aeruginosa* were studied. The results showed that: (1) The degradation processes of strains P05 and P07 on *Microcystis aeruginosa* accorded with the first-order reaction model when the range of Chl-*a* concentration was from 0 to 1500 µg/L. (2) The initial bacterium densities had a strong influence on the degradation velocity. The greater the initial bacterium density was, the faster the degradation was. The degradation velocity constants of P05 were 0.1913, 0.2175 and 0.3092 respectively, when bacterium densities were 4.8×10^5 , 4.8×10^6 , 2.4×10^7 cells/ml. For strain P07, they were 0.1509, 0.1647 and 0.2708. The degradation velocity constant of strain P05 was higher than that of P07 when the bacterium density was under 4.8×10^5 cells/ml, but the constant increasing of P07 was quicker than that of P05. (3) The degradation effects of P05 and P07 strains did not antagonize. When the concentration of Chl-*a* was high, the degradation effects of mixed strain excelled that of any single strains. But with the decrease of the Chl-*a* concentration, this advantage was not clear. When the concentration was less than 180 µg/L, the degradation effects of mixed were consistent with that of strain P07.

Keywords: *Bacillus* sp.; *Microcystis aeruginosa*; characteristic; degradation kinetics; velocity constant

Introduction

Many kinds of algae can lead to water bloom and red tide in the seas, lakes and reservoirs. This phenomenon influences or changes the physical and chemical character of water and then results in many trouble in the drinking water production process (Hargensheimer, 1996; Graham, 1997; MD, 2001). Especially, cyanobacterial blooms are the most harmful because they not only occur frequently, lasting out for a long time, but also produce toxins and bring many hazards (Cormichael, 1996). It was reported that 70% of the blue-green algae could produce microcystins (MCYST; Kunimitsu, 1994), which harm animal and human bodies all over the world (Falconer, 1989; Mez, 1997). For instance, after contacting with, or ingestion of water containing cyanobacteria, a number of human illnesses such as skin rashes, irritation, blistering, vomiting and pulmonary consolidation have been reported. It was also verified by the epidemiological study that drinking the water polluted by the blue-green algae could beget liver tumour (Frederic, 2000). *Microcystis aeruginosa* is the main strain producing toxins among cyanobacteria, which causes severe damage to human health. Thus, it has very important scientific and practical significance to study the removal of *Microcystis aeruginosa*. During the traditional drinking water production with the process of flocculation-sedimentation-filtration-chloride disinfection, considered those removal of cyanobacterial cells using physical and chemical methods, some were not ideal, and some resulted in the majority of microcystins entering into the surrounding water after lysis and cell death (Lawton, 1998). This would threaten human health. Therefore, it has important theoretical and practical significance to research on the economic and feasible approaches of algae removal in drinking water production.

Our research group had studied the removal of algae and microcystin through the immobilized biosystem of sponge. The results showed that when HRT was 5 h, the removal rates of algae and microcystin were 90%, 94.17% and there were large numbers of bacilliform bacterium in this biosystem. Five strains of bacteria were separated from this system, which are highly effective in degradation of algae and microcystin. Among them, the bacteria numbered P05 and P07 were identified to be *Bacillus* sp. by the sequence analysis of 16S rDNA. In this paper, the degradation kinetics of P05 and P07 strains and the degradation effects of mixed strain on *Microcystis aeruginosa* were studied on emphasis.

1 Materials and methods

1.1 Materials

1.1.1 Tested strains

The tested strains numbered P05 and P07 were isolated from the

immobilized biosystem of sponge, whose removal effects on algae and microcystin were favourable. In this test, the strains were put into liquid LB culture medium and cultured in the constant temperature table, which was 190 r/min and 30°C, for two days and the tested bacterium densities of P05 and P07 were 3.5×10^8 and 3.8×10^8 cells/ml, respectively.

1.1.2 Tested algae

The *Microcystis aeruginosa* was from Institute of Hydrobiology, Chinese Academy of Sciences. It was cultivated at 25°C and 3000 lx of illumination intensity after being activated.

1.1.3 Culture medium

Bacterial culture medium: The content of solid and liquid LB culture medium was referred to the reference (Shen, 2002).

Cyanobacteria culture medium (BG11 medium): NaNO₃ 150 mg, K₂HPO₄ 4 mg, MgSO₄·7H₂O 7.5 mg, CaCl₂·2H₂O 3.6 mg, Na₂SiO₃·9H₂O 5.8 mg, citric acid 0.6 mg, ferric ammonium citrate 0.6 mg, EDTA 0.1 mg, Na₂CO₃ 2 mg, A₅ solution + Co 0.1 ml, distilled water 99.9 ml.

A₅ solution: H₃BO₃ 286 mg, MnCl₂·4H₂O 181 mg, ZnSO₄·7H₂O 22 mg, CuSO₄·5H₂O 7.9 mg, Na₂MoO₄·2H₂O 3.9 mg, distilled water 100 ml.

1.2 Methods

1.2.1 Degradation test of the *Microcystis aeruginosa* of different concentrations by the single strains

The *Microcystis aeruginosa* was confected into different densities, which were 133.872 (C1), 273.912 (C2), 523.948 (C3), 682.568 (C4), 1218.86 (C5), 1444.996 (C6) µg/L and 200 ml of each was put into 6 Erlenmeyer flasks respectively. Then 30 ml of P05 bacterium was inoculated into each flask. These flasks were cultured in the constant temperature table, which was 190 r/min and 30°C, and the chlorophyll-*a* (Chl-*a*) was measured every 24 h. The degradation test of the *Microcystis aeruginosa* of different concentrations by P07 bacterium was made by the same method as the above.

1.2.2 Degradation test of bacteria of different concentrations

Each 30 ml of P05 bacteria liquid, whose concentration was 0, 2.4×10^7 , 4.8×10^6 , 4.8×10^5 cells/ml respectively, was put into each 200 ml tested algal samples. They were cultured in the constant temperature table, which was 190 r/min and 30°C, and the chlorophyll-*a* (Chl-*a*) was measured every 24 h. The degradation test on *Microcystis aeruginosa* by the P07 bacteria of different concentrations was made, the same as the above.

1.2.3 Degradation test on *Microcystis aeruginosa* by the mixed strain

Each 30 ml P05 bacteria liquid, whose concentration was 3.5×10^8 cells/ml, and P07, whose concentration was 3.8×10^8 cells/ml,

was put into 200 ml tested algal samples respectively. The 30 ml mixed bacterium liquid, which was made of 15 ml P05 and 15 ml P07 bacterium liquid whose concentrations were as the same as the above, was put into 200 ml tested algal samples at the same time. All the tested algal samples were cultured in the constant temperature table, which was 190 r/min and 30°C and the chlorophyll-*a* (Chl-*a*), was measured every 24 h.

2 Results and discussion

2.1 Degradation effects of P05 and P07 strains on *Microcystis aeruginosa* of different concentrations

The degradation effects of P05 and P07 strains on *Microcystis aeruginosa* of different initial concentrations are shown in Fig.1 and Fig. 2. The Chl-*a* concentration of the *Microcystis aeruginosa* was decreased in the exponential form from each initial concentration. At the start, the Chl-*a* concentration decreased very quickly and the degradation curves were more precipitous. Then these curves tended smoothly. From these two figures, we also could find that the degradation rules of P05 and P07 bacteria on the *Microcystis aeruginosa* of the same initial concentrations were almost coincident and their degradation effects almost corresponded. There was a noticeable phenomenon that the degradation capacity of P07 on the *Microcystis aeruginosa* of low initial concentrations became weak from the third day, but that of P05 was still well. For example, at the concentration of 133.872(C1) µg/L, the Chl-*a* of *Microcystis aeruginosa* was degraded by P07 bacteria from 50.013 µg/L of the third day to 46.432 µg/L of the seventh day. On the contrary, the P05 bacteria could degrade the Chl-*a* from 50.808 µg/L to 25.302 µg/L.

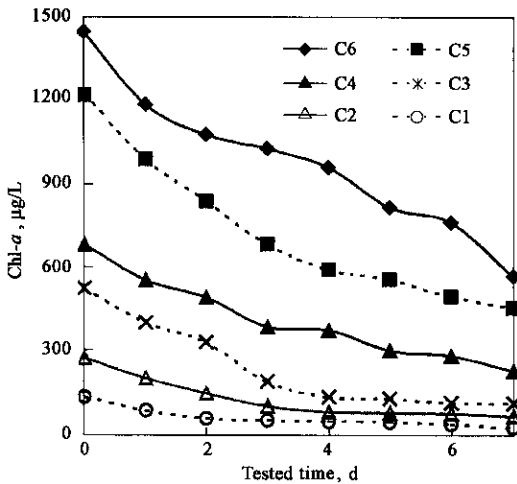


Fig. 1 Degradation effects of strain P05 on *Microcystis aeruginosa* of different concentrations

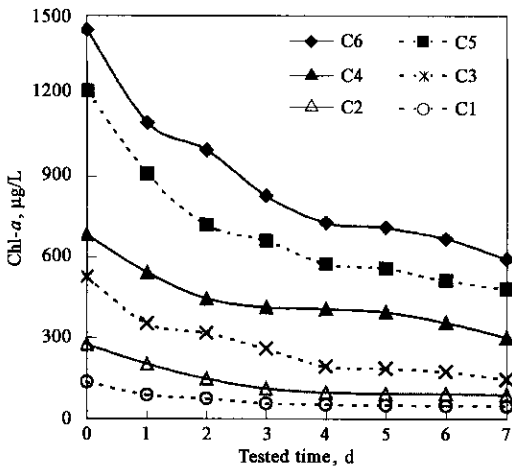


Fig. 2 Degradation effects of strain P07 on *Microcystis aeruginosa* of different concentrations

2.2 Degradation kinetics of P05 and P07 strains on *Microcystis aeruginosa*

Commonly, the process of biochemistry reaction can be described as the formula below:

$$v = v_m \frac{[s]}{k_s + [s]} \tag{1}$$

Where, *v* is the reaction velocity and *v_m* is the maximum. In addition, *s* and *k_s* stand for the concentration of medium and the half saturation rate constant.

During the degradation process whose medium was algae, there was not only the degradation by bacteria, but also the algae's perish due to the competition with the bacteria and the lack of nutrition. Therefore, this degradation process should be described by the formula hereinafter.

$$v = v_m \frac{[s - \Delta s]}{k_s + [s - \Delta s]} \tag{2}$$

where, Δs is the decreasing amount of Chl-*a* as a result of algae perish.

The relationships between degradation velocity of P05 and P07 strains and the concentration of *Microcystis aeruginosa* in this test are shown in Fig.3. They were two straight lines when the range of Chl-*a* concentration was from 0 to 1500 µg/L. In other words, the degradation process of these two strains on *Microcystis aeruginosa* accorded with the first-order reaction model. Therefore, $[s - \Delta s] \ll k_s$ and the Equation (2) could be described as follows:

$$v = v_m \frac{[s - \Delta s]}{k_s} \tag{3}$$

The relationship between degradation rate constant (*k₀*) and *v_m* and *k_s* was described as $k_0 = v_m/k_s$. Therefore, from Formula (3), the relationship formula of concentration via reaction time could be attained and it was written as $[s - \Delta s] = [s_0 - \Delta s] e^{-k_0 t}$. When the degradation results on the six different initial concentrations of *Microcystis aeruginosa* were regressed on the linearity, six equations of reaction velocity and six first-order velocity constants were gained. The degradation kinetics parameters of strains P05 and P07 on *Microcystis aeruginosa* are given in Table 1. When the algae blooms in the polluted source water, the concentration of Chl-*a* is no more than 1000 µg/L. Therefore, the degradation processes were all accorded with the first-order kinetics when the strains P05 and P07 were used to treat the polluted source water.

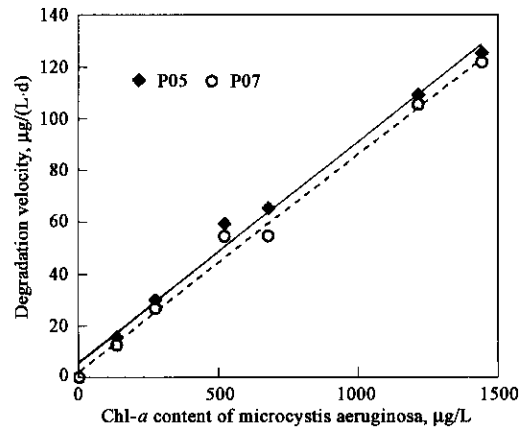


Fig. 3 The relationships between degradation velocity of strains P05 and P07 and the concentration of *Microcystis aeruginosa*

2.3 Degradation test of bacteria of different concentrations on *Microcystis aeruginosa*

Under the conditions that the Chl-*a* concentration of *Microcystis aeruginosa* was 408.036 µg/L and the bacterium densities were 2.4×10^7 , 4.8×10^6 , 4.8×10^5 cells/ml, the degradation velocity of P05 and P07 on *Microcystis aeruginosa* increased with the extension of reaction time. The degradation kinetics parameters are shown in Table 2. From this table, we could draw the conclusion that the initial bacterium densities had a strong influence on the degradation velocity. The greater the initial bacterium density, the quicker the degradation. The data made it clear that the degradation velocity constant of strain P05 was higher than that of P07 at the same initial bacterium density and the density was under 2.4×10^7 cells/ml, but the constant increasing of strain P07 was quicker than that of strain P05.

Table 1 Degradation kinetics of P05 and P07 strains on *Microcystis aeruginosa*

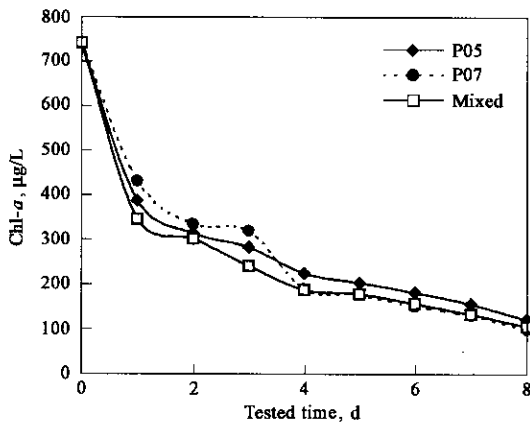
Initial Chl- <i>a</i> concentration, $\mu\text{g/L}$	Strain P05			Strain P07		
	Kinetic equation	Velocity constant k_0 , 1/d	Correlation coefficient, r	Kinetic equation	Velocity constant k_0 , 1/d	Correlation coefficient, r
133.872	$[s - \Delta s] = 106.04e^{-0.1956t}$	0.1956	0.9013	$[s - \Delta s] = 102.39e^{-0.1375t}$	0.1375	0.8143
273.912	$[s - \Delta s] = 227.39e^{-0.2007t}$	0.2007	0.9024	$[s - \Delta s] = 219.51e^{-0.1581t}$	0.1581	0.8418
523.948	$[s - \Delta s] = 472.65e^{-0.243t}$	0.243	0.9157	$[s - \Delta s] = 448.02e^{-0.1716t}$	0.1716	0.9454
682.568	$[s - \Delta s] = 655.57e^{-0.1498t}$	0.1498	0.9848	$[s - \Delta s] = 603.63e^{-0.097t}$	0.097	0.9002
1218.86	$[s - \Delta s] = 1123.4e^{-0.139t}$	0.139	0.9694	$[s - \Delta s] = 1031.9e^{-0.1222t}$	0.1222	0.9041
1444.996	$[s - \Delta s] = 1412.0e^{-0.1144t}$	0.1144	0.9494	$[s - \Delta s] = 1281.6e^{-0.1173t}$	0.1173	0.9389

Table 2 Degradation results of bacterium of different concentrations on *Microcystis aeruginosa*

Initial bacterium density, cells/ml	Strain P05			Strain P07		
	Kinetic equation	Velocity constant k_0 , 1/d	Correlation coefficient, r	Kinetic equation	Velocity constant k_0 , 1/d	Correlation coefficient, r
4.8×10^5	$[s - \Delta s] = 383.84e^{-0.1913t}$	0.1913	0.9413	$[s - \Delta s] = 329.43e^{-0.1509t}$	0.1509	0.8899
4.8×10^6	$[s - \Delta s] = 332.35e^{-0.2157t}$	0.2157	0.9439	$[s - \Delta s] = 299.26e^{-0.1647t}$	0.1647	0.8409
2.4×10^7	$[s - \Delta s] = 294.33e^{-0.3092t}$	0.3092	0.9376	$[s - \Delta s] = 285.75e^{-0.2708t}$	0.2708	0.8909

2.4 Degradation of mixed strain on *Microcystis aeruginosa*

The degradation effects of mixed strain on *Microcystis aeruginosa* are shown in Fig. 4. At the first four days, the degradation effects of mixed strain excelled that of any single strains, but from the fourth day, they were consistent with that of strain P07. It made out that the degradation effects of P05 and P07 strains did not antagonize. When the concentration of Chl-*a* was high, the degradation effects of mixed strain excelled that of any single strains, even if the densities of these two strains in the mixed bacterium liquid were half of that of the single strains. But with the decrease of the Chl-*a* concentration, this advantage was not clear. When the concentration was less than 180 $\mu\text{g/L}$, the degradation effects of mixed strain were consistent with that of strain P07.

Fig. 4 Degradation of mixed strain on *Microcystis aeruginosa*

3 Conclusions

The relationships between the degradation velocity of strains P05 and P07 and the concentration of *Microcystis aeruginosa* in this test were two straight lines when the range of Chl-*a* concentration was from 0 to 1500 $\mu\text{g/L}$. In other words, the degradation processes of these two strains on *Microcystis aeruginosa* accorded with the first-order reaction model.

The initial bacterium densities had a strong influence on the

degradation velocity. The greater the initial bacterium density, the faster the degradation. The degradation velocity constants of P05 were 0.1913, 0.2175 and 0.3092 respectively, under the condition that the bacterium densities were 4.8×10^5 , 4.8×10^6 , 2.4×10^7 cells/ml. For strain P07, they were 0.1509, 0.1647 and 0.2708. The degradation velocity constant of strain P05 was higher than that of P07 when the bacterium density was under 4.8×10^5 cells/ml, but the constant increasing of strain P07 was quicker than that of P05.

The degradation effects of P05 and P07 strains did not antagonize. When the concentration of Chl-*a* was high, the degradation effects of mixed strain excelled that of any single strains, even if the densities of these two strains in the mixed bacterium liquid were half of that of the single strain. But with the decrease of the Chl-*a* concentration, this advantage was not clear. When the concentration was less than 180 $\mu\text{g/L}$, the degradation effects of mixed strain were consistent with that of strain P07.

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