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Kinetic Studies on the Effects of Organophosphorus Pesticides on the Growth of *Microcystis aeruginosa* and Uptake of the Phosphorus Forms

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Organophosphorus pesticides (OPs) have been widely used in agricultural production in China because most of them are higher effective of killing insect. The pesticides do not only kill target organisms but also extend to affect some nontarget organisms. They can contaminate in aquatic environment through surface runoff. In the recent years the *Cyanobacteria* blooms break out in many lakes in China. The *Microcystis aeruginosa* belongs to a kind of the *Cyanobacteria*, it is a common algal in harmful algal blooms in many fresh-water lakes in China, such as Taihu lake, Chaohu lake, Donghu lake and Dianchi lake and so on (Jin et al. 1990). As all known that the causes of the algal blooms are very complex, the nitrogen (N) and phosphorus (P) are two important factors of eutrophication in aquatic environment. A lot of investigations indicated that P is naturally present in more limiting amounts than that of other essential elements in aquatic ecosystem (Correll 1998), P is an essential element for algal growth, and the orthophosphate is the only phosphorus forms utilized by algal directly, but the content of orthophosphorus is less than 5% of the concentration of total phosphorus in lakes. Organic phosphorus is primary component of total phosphorus, it is over than 90% of the concentration of total phosphorus in lakes (Wetzel 1983). It is very important to study the regularity of uptake the phosphorus forms by algal in order to investigate the mechanism of the algal blooms. Some toxic studies on the higher concentration of OPs to the growth of algal have been reported (Sabater et al. 2001, Zou et al. 1999), the studied results showed that the algal was sensitive to many OPs and the growth of algal have been inhibited by OPs. There are few reports about effects of OPs on the growth of the *Microcystis aeruginosa* at lower concentration of the OPs. In fact, the OPs are usually at lower concentration in aquatic environment.

In this paper, the effects of the methamidophs (MAP) and phoxime on the growth of the *Microcystis aeruginosa* and the kinetic mechanism of uptake of three kinds of phosphorus forms-total soluble phosphorus (TSP), soluble reaction phosphorus (SRP) and dissolved organic phosphorus (DOP) by the *Microcystis aeruginosa* were studied, the results showed that the growth of the *Microcystis aeruginosa* stimulated by the MAP and phoxime at lower concentrations, and the MAP and

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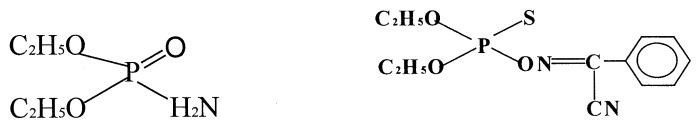

methamidophs phoxime

Figure 1. Chemical structures of the methamidophs and phoxime.

phoxime cannot directly utilized by the algal cells. The cause of the stimulation might be that the rate of the uptake SRP of the algal cells could be accelerated at lower concentration of the MAP and phoxime.

MATERIALS AND METHODS

A culture of freshwater blue green algae, *Microcystics aerugionosa* Kütz, was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China. The algal were grown in sterile HGZ medium at a temperature of 28℃±1℃, and a light intensity of 2000 lux on a 12 hr light-dark cycle, the solution was adjusted to pH 8 and shaken manually fourth a day. The algae pure cultured 2 or 3 times, then centrifugalized at 5000 rpm and hunger cultured for three days in order to consume nutriment cumulated in the algal cells. The algal after hunger culture for 3 d can be used for the experiments (Zhou et al. 2001).

Methamidophs (active ingredient >80%) and phoxime (active ingredient >90%) were obtained from Examination Center of Agrochemical, Hubei Province, China. The chemical structures of the compounds were shown as the Figure1. The concentration of total phosphorus was 6.95 mg / L in each HGZ medium. methamidophs was diluted with sterile distilled water and added into the HGZ medium at various concentration ratios of methamidophs to K_2HPO_4 (1 to 9, 1 to 7, 1 to 5) and no K_2HPO_4. Phoxime was diluted with sterile distilled water and added into the HGZ medium at various concentration ratios of phoxime to K_2HPO_4 (1 to 264, 1 to 87, 1 to 60) and no K_2HPO_4.

The concentration of the algal cells were measured by spectrophotometer at 663 nm. The relationship between the algal cell density (y) and its absorbance at 663nm (A) was a linear correlation ($y = 0.284+31.5A$, $r = 0.9992$, $P < 0.0001$). *M. aeruginosa* cell density were measured by spectrophotometer every 24 hr after inoculation. Adopted the ammonium molybdate spectrophotometric method (Zhou et al. 2001) to determine the concentration of the phosphorus forms (TSP and SRP) in the solution, and the value of DOP is the difference of the TSP and SRP.

RESULTS AND DISCUSSION

The kinetic curves of the growth of the *M. aeruginosa* at different concentrations of methamidophs and phoxime were shown in Figures 2 and 3. Using the kinetic equation (1)

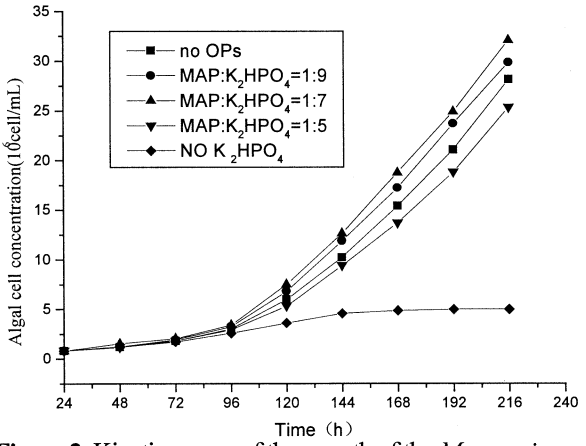

Figure 2. Kinetic curves of the growth of the *M. aeruginosa.* in the methamidophs treated medium.

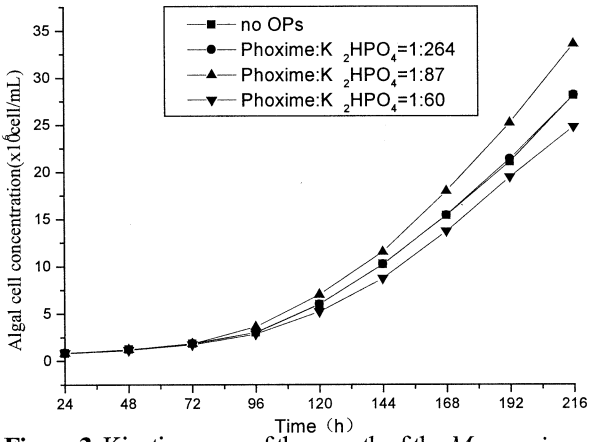

Figure 3. Kinetic curves of the growth of the *M. aeruginosa.* in the phoxime treated medium.

to fit the curves of the growth of the algal cells.

$$N_t = N_0 e^{kt} \quad (1)$$

Where, N_t is the concentration of the algal cells at time t ($\times 10^6$cell / mL); N_0 is the extrapolate initial concentration of the algal cells ($\times 10^6$cell / mL); k is the growth rate constant; t is time (hr).The equation (1) can be expressed as

$$\ln N_t = \ln N_0 + kt \quad (2)$$

The kinetic parameters of growth of the *M. aeruginosa* cells were listed in Tabal 1. Compared with the control (no OPs in the medium), when the concentration ratios of the MAP to K_2HPO_4 in the medium was1 to 9 and 1 to 7 , the growth of the *M. aeruginosa* cells was stimulated, and the algal cells grew faster at the concentration ratio of 1 to 7 than that of at 1 to 9 of the concentration ratio of MAP to K_2HPO_4 in the medium. The growth of the *M. aeruginosa* is inhibited by MAP significantly at no K_2HPO_4 in the medium. The growth of algal cells was stimulated at the concentration ratio of the phoxime to K_2HPO_4

Table 1. Kinetic parameters of the *M. aeruginosa* growth in different OPs treated medium at 28℃.

Concentration ratio of the OPs to K_2HPO_4	k (/h)	N_0 ($\times 10^6$cell / mL)	r
no OPs	0.01961	13.1182	0.9947
MAP to K_2HPO_4 is 1 to 9	0.02013	13.1347	0.9922
MAP to K_2HPO_4 is 1 to 7	0.02045	13.1512	0.9915
MAP to K_2HPO_4 is 1 to 5	0.01891	13.1371	0.9957
no K_2HPO_4	0.00990	13.6315	0.9437
Phoxime to K_2HPO_4 is 1 to 264	0.01970	13.1075	0.9946
Phoxime to K_2HPO_4 is 1 to 87	0.02062	13.1053	0.9938
Phoxime to K_2HPO_4 is 1 to 60	0.01913	13.0685	0.9958

was 1 to 87, and inhibited at the concentration ratio of the phoxime to K_2HPO_4 was 1 to 60. The results indicated that the MAP and phoxime could stimulate growth of the *M. aeruginosa* at the certain concentration ratio of the OPs to inorganic phosphorus, but the certain concentration ratio for the MAP to K_2HPO_4 is higher than that of for the phoxime, and when MAP as unique P source for the algal growth, the *Microcystis* cells grew very slow. The certain concentration ratio for the MAP to K_2HPO_4 different from that of for the phoxime because the chemical structure and physical chemical characters of the MAP different from that of the phoxime. The phoxime is esterophilic compounds and the MAP is hydrophilic compounds. The stronger of the close ability to ester of Ops the higher of the toxicity to ester of OPs (Zou et al 1999). Because there is a phenyl in the phoxime, have higher electron density, and the –CN group connected with phenyl, could pull electron strongly. The structure of the phoxime is steadier than that of the MAP, the toxicity of the phoxime is higher than that of the MAP.

The kinetic curves of the concentration change of the phosphorus forms in each medium were shown as the Figures 4 to 7. These figures showed that the kinetic curves of the concentration change of the phosphorus forms reversed sigmoid curves, and fitted to logistic model, they can be expressed as

$$dM_t/dt = -k_s M_t(1 - sM_t) \quad (3)$$

Where, M_t is assumed to the decreased qualities of the phosphorus forms at time t in the medium (mg); s is the restricted factor of the uptake phosphorus forms by the *M. aeruginosa*; k_s is rate constants of uptake of the phosphorus forms by the *M. aeruginosa* (h^{-1}). Supposed C_N is the concentration of the phosphorus forms of the decrement of the unit phosphorus forms; C_t is the concentration of the phosphorus forms at time t (mg / L), it can be gained as

$$C_t = C_N M_t \quad (4)$$

Differential coefficient on equation (4) and yields as

$$dM_t/dt = (1/C_N)(dC_t/dt) \quad (5)$$

Took equations (4) and (5) into equation (3) and yields as

$$\frac{dC_t}{dt} = -k_s \frac{C_t - sC_t^2}{C_N} \quad (6)$$

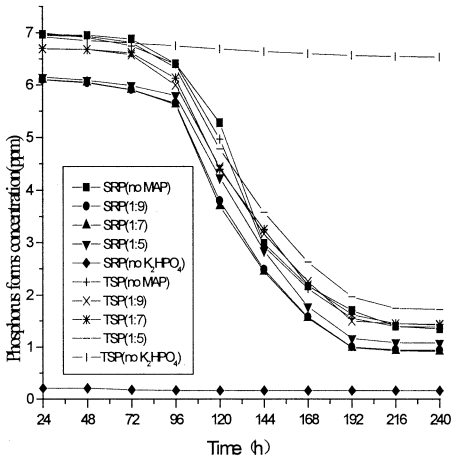

Figure 4. Kinetic curves of change of SRP and TSP concentration in methamidophs treated medium.

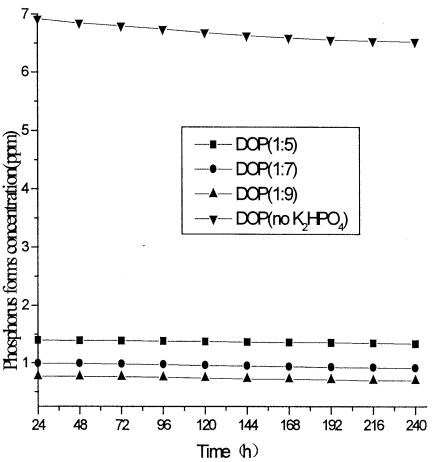

Figure 5. Kinetic curves of change of DOP concentration in methamidophs treated medium.

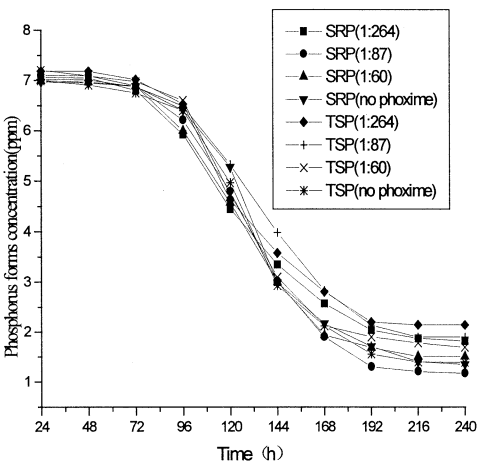

Figure 6. Kinetic curves of change of SRP and TSP concentration in phoxime treated medium.

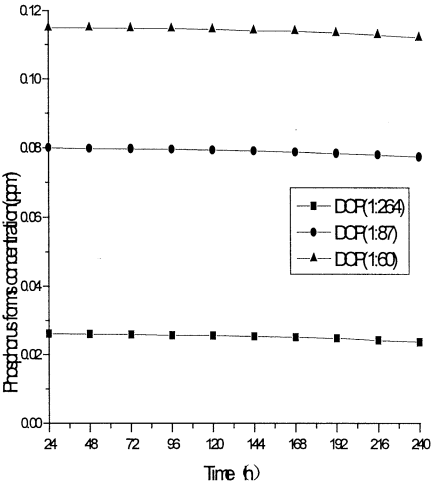

Figure 7. Kinetic curves of change of DOP concentration in phoxime treated medium.

Integrating equation (6) and yields as

$$\ln \frac{C_t(1-s)}{C_N(1-\frac{sC_N}{C_t})} = -k_s t \tag{7}$$

Equation (7) can be rewritten as

$$\ln(\frac{C_N/s}{C_t} - 1) = \ln\left(\frac{1-s}{s}\right) - k_s t \tag{8}$$

Table 2. Kinetic parameters of change of phosphorus forms concentration in OPs treated medium at 28℃.

Concentration ratio of the OPs to K_2HPO_4	P forms	k_s (/h)	r
no OPs	SRP	0.03698	0.9781
	TSP	0.03360	0.9876
MAP to K_2HPO_4 is 1 to 9	SRP	0.03742	0.9820
	TSP	0.02796	0.9784
	DOP	0.00131	0.9539
MAP to K_2HPO_4 is 1 to 7	SRP	0.03783	0.9834
	TSP	0.02745	0.9793
	DOP	0.00167	0.9908
MAP to K_2HPO_4 is 1 to 5	SRP	0.03514	0.9917
	TSP	0.02589	0.9864
	DOP	0.00106	0.9867
no K_2HPO_4	SRP	0.00165	0.9244
	TSP	0.00092	0.9834
	DOP	0.00196	0.9942
Phoxime to K_2HPO_4 is 1 to 264	SRP	0.03100	0.9812
	TSP	0.02764	0.9714
	DOP	0.00134	0.9843
Phoxime to K_2HPO_4 is 1 to 87	SRP	0.03743	0.9834
	TSP	0.02988	0.9844
	DOP	0.00008	0.9818
Phoxime to K_2HPO_4 is 1 to 60	SRP	0.03024	0.9745
	TSP	0.02431	0.9712
	DOP	0.00005	0.9878

or

$$\ln(\frac{a}{C_t}-1)=\ln\left(\frac{1-s}{s}\right)-k_s t \qquad (9)$$

Where $a = C_N / s$. The relationship between ln [(a / C_N) -1] and t was a linear correlation. Using the data C_t and t obtained from the kinetic curves of the concentration change of the phosphorus forms in each medium, suitable values of a were chosen in the calculations to give the best linearity of Eq. (9), and the rate constants of uptake of the phosphorus forms by the *M. aeruginosa* (k_s) were calculated. The results were listed in Table 2. It can be showed that the SRP could be assimilated by the algae at higher velocity
than that of other phosphorus forms, and few DOP were utilized by the algae. Compared with Table1, the faster of the algal grewth, the greater of the rate of the algal uptake SRP . All these results showed that the growth rate of the *M. aeruginosa* was dependent upon the concentration of phosphorus in the cell, and SRP was the preferential phosphorus form used by the algae. Many studied results (Perona et al.1991) considered that algal could use low concentration OPs as P source to accelerate the growth, but in this experiment, the DOP were utilized by the algal limitedly. In general, SRP was the preferential phosphorus form utilized by algae cells, but when the SRP existence at lower concentration in aquatic environment, the DOP could be utilized by algae cells through the action of enzyme (Zhou et al.1999). The Table 2 showed that when the MAP as unique P source for the algal

growth, alkaline phosphatase could hydrolyze phosphate ester to inorganic phosphate, which could be utilized by algal cells (Tian et al. 1997), and the activity of alkaline phosphatase would be inhibited when the concentration of SRP was higher. So the concentration of DOP changed very little in the medium. Therefore, the growth of the *M. aeruginosa* stimulated by MAP and phoxime at lower concentrations, and the MAP and phoxime cannot directly utilized by algal cells. The cause of the stimulation might be that the rate of the uptake SRP of the algal cells could be accelerated at lower concentration of the MAP and phoxime, and there was some coordination between inorganic P and organic P in stimulation (Mostafa et al. 1994). The mechanism of methamidophs and phoxime action on algal cells need further investigations.

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