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# Variation of Alkaline Phosphatase Activity in Sediments of Shrimp Culture Ponds and Its Relationship with the Contents of C, N and P

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**Abstract** Nine enclosures (5 m × 5 m) were built in a *Fenneropenaeus chinensis* culture pond of Rushan Gulf in April, 2001. The probiotics and BIO ENERGIZER solution were applied for disparate treatments. Variations of alkaline phosphatase activity (APA) and its relationship with the contents of C, N and P in sediments were studied. Results show that APA of sediments increases from 3.096 nmol $g^{-1}min^{-1}$ to 5.407 nmol $g^{-1}min^{-1}$ in culture period; the bacteria biomass is not the only factor to determine APA; the contents of total P and total organic carbon have a significant positive correlation with APA, while that of total nitrogen has a negative correlation. In addition, the contents of inorganic P and organic P are not regular with APA. By comparison, TOC shows a more significant coherence with APA, meaning that organic pollution in sediments affects APA remarkably.

**Key words** APA; N; C; P; sediments; *Fenneropenaeus chinensis*

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## 1 Introduction

Activities of extracellular enzymes can serve as the indicators of organic matter content. Enzyme assaying has been used in the analyses of dissolved organic materials in aquatic environments (Chróst, 1991), mass loss of plant litter in terrestrial systems (Sinsabaugh *et al.*, 1992; Sinsabaugh *et al.*, 1994), processing of particulate and dissolved organic carbon (POC, DOC) in streams (Sinsabaugh and Findlay, 1995; Findlay *et al.*, 1998), and processing of particulate organic matter (POM) in wetlands (Jackson *et al.*, 1995).

Alkaline phosphatase (EC3.1.3.1), one of extracellular enzymes, can catalyse the hydrolysis of phosphate monosters into alcohol and inorganic phosphate. It plays a central role in phosphorus (P) cycling in aquatic ecosystems. Both phyto- and bacterioplankton take up inorganic P. Therefore, they are competitors when P is scarce in the environment. During P depletion, these organisms can ensure their P intake by producing alkaline phosphatase (Jansson *et al.*, 1988). APA in the aquatic environment originates from either phyto- or bacterioplankton or both, depending on the nutritional status (Chróst and Overbeck, 1987; Vrba *et al.*, 1993; Thingstad *et al.*, 1993; Jamet *et al.*, 1995). APA is positively correlated with the physiological P limitation of the plankton (Thingstad *et al.*, 1993; Huang and Hong, 1999). It is a highly sensitive parameter of biomass and plankton. The regulation of APA by different nutritions in aquatic environments has been described in natural conditions (Boetius *et al.*, 2000; Koike and Nagata, 1998; Huang and Hong, 1999) or in artifical situations (Darrah and Harris, 1986; Chróst, 1991; Hoppe, 1993; Shand and Smith, 1997; Nausch, 2000). The studies of APA have been widely carried out in coastal or deep-sea ecosystems, and a few studies have been done in fresh water lakes of aquiculture (Zhou *et al.*, 2001; Alvarez, 2000). However, the study of APA in mariculture ecosystems has not been attempted. The present study is designed to determine the role of APA in sediments of shrimp culturing ponds.

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## 2 Materials and Methods

### 2.1 Experimental Design

The experiment was conducted from August 12 to September 23, 2001. Nine enclosures (5 m × 5 m) with homogeneous muddy condition were established in a shrimp pond of Rushan Gulf. The enclosures consisted of vertical polyethylene sheets, fixed to poles and buried in mud at the bottom. The water depth in the enclosures was about 1.5 m and the sediments were

argillo-arenaceous. *Fenneropenaeus chinensis* was fed at a density of 60 prawns $m^{-2}$ and every enclosure was equipped with an inflator of ACO-009.

Three enclosures, marked $V_1$, $V_2$ and $V_3$, were used as the control while those named $VI_1$, $VI_2$ and $VI_3$ used for probiotics treatment, consisted of three species of *Bacillus* SPM2, SPF3, 3041. The other three unites, named $VII_1$, $VII_2$ and $VII_3$, were treated probiotics and BIO ENERGIZER solution with a ratio of 1∶10000. This solution is a product of the company of BIO HUMA NETICS and can balance the natural microbial ecosystem.

### 2.2 Sampling

Sediment columns were obtained at 10 a.m. every 10 d, using a hand-driven perspex corer of 150 cm long with an internal diameter of 3.5 cm. The surface part (0.5 cm) was sliced for assay and divided into two parts, one for Total nitrogen (TN), Total phosphate (TP), Total inorganic phosphate (TIP), and Total organic carbon (TOC), and the other for APA determination. This part was stored in a −70 ℃ ultralow freezer before use.

### 2.3 Assay

APA was determined using the method described by Hong (1989) with modifications. Sediment samples were slurried by a Tris-HCL buffer (pH = 8.7). 1 mL of 3-o-meth-ylfluorescein phosphate (2 $\mu g\,cm^{-3}$) was added to 0.5 g sediment with 10 mL Tris-HCL buffer, and the samples were incubated at 37 ℃. After 1 h, 1 mL of NaOH (10 N) was added to stop the reaction, then the sample was centrifuged at 3000 r $min^{-1}$. Absorption was measured in a Hitachi-850 Fluorospectrometer with the excitation and emission wavelengths set at 400 and 520 nm, respectively. APA was converted to absolute units using a standard curve of 3-o-methylfluorescein and was expressed as nmol 3-o-methylfluorescein $g^{-1}$ $min^{-1}$.

TP of sediments was mensurated by phosphoric colorimetry adapted from Menzel and Corwin (1964). TIP was tested by phosphoric colorimetry after being distilled by HCL-$NH_4F$ (9:1). The result of subtracting TIP from TP is the content of Total organic phosphate (TOP). TN was determined based on the modified Kjeldahl method (Liu and Zhu, 1997). TOC was measured by the combustion-infrared method with TOC Analyzer-5000 A.

### 2.4 Data Analysis

The analysis of experimental data used the one-way ANOVA by Excel 2000 of Microsoft, with possible differences among data being tested by t-test. All statistical significance tests were at the $P < 0.05$ level.

## 3 Results

An increase of APA during the culture period was observed, except for the final period (Fig.1). APA fluctuated from 3.296 nmol $g^{-1}$ $min^{-1}$ to 4.605 nmol $g^{-1}$ $min^{-1}$ in control, and from 3.096 nmol $g^{-1}$ $min^{-1}$ to 5.407 nmol $g^{-1}$ $min^{-1}$ in experimental enclosures. Although the experimental enclosures were treated with probiotics and BIO ENERGIZER solution, there were no significant differences in APA of sediments among them ($P > 0.05$). This suggests that the bacteria biomass is not the only factor to determine APA in mariculture sediments.

In order to verify the role of APA in the P cycling of mariculture ecosystems, TP, TIP and TOP were monitored in the same sampling of shrimp pond sediments, with the results shown in Table 1. The correlation coefficient between TP and APA is 0.752 ($P < 0.05$), while that between APA and TOP or TIP is not significant.

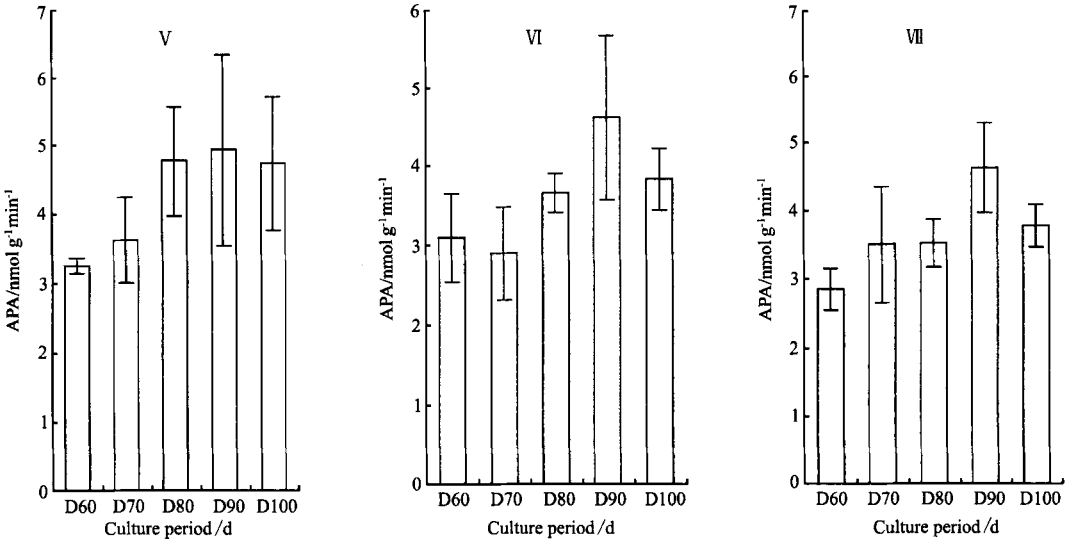


Fig.1 Variations of APA in sediments of *Fenneropenaeus chinensis* enclosures in culture period.

Table 1 The concentrations of APA, TP, TIP, TOP, TOC, TN and the correlation coefficients among them

| Unit | Culture period | Enclosures | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_1$ | $V_2$ | $V_3$ | $VI_1$ | $VI_2$ | $VI_3$ | $VII_1$ | $VII_2$ | $VII_3$ |
| APA/(nmol g$^{-1}$min$^{-1}$) | D60 | 3.33 | 3.13 | 3.10 | 2.74 | 2.79 | 3.73 | 2.94 | 2.52 | 3.10 |
| | D70 | 3.06 | 3.51 | 4.27 | 2.24 | 3.05 | 3.38 | 4.46 | 2.80 | 3.26 |
| | D80 | 4.05 | 5.62 | 4.61 | 3.89 | 3.39 | 3.66 | 3.80 | 3.13 | 3.64 |
| | D90 | 4.92 | 6.33 | 3.53 | 5.31 | 5.12 | 3.40 | 4.22 | 3.28 | 5.41 |
| | D100 | 4.27 | 5.84 | 4.05 | 3.81 | 3.44 | 4.22 | 3.60 | 3.58 | 4.14 |
| TP†/($10^2$ nmol g$^{-1}$) | D60 | 0.25 | 0.25 | 0.29 | 0.30 | 0.29 | 0.32 | 0.30 | 0.30 | 0.30 |
| | D70 | 0.30 | 0.33 | 0.29 | 0.31 | 0.27 | 0.27 | 0.26 | 0.27 | 0.26 |
| | D80 | 0.32 | 0.51 | 0.31 | 0.28 | 0.28 | 0.29 | 0.31 | 0.28 | 0.27 |
| | D90 | 0.33 | 0.36 | 0.30 | 0.32 | 0.30 | 0.26 | 0.27 | 0.33 | 0.33 |
| | D100 | 0.11 | 0.22 | 0.13 | 0.13 | 0.12 | 0.10 | 0.10 | 0.15 | 0.14 |
| TIP††/($\times 10^2$ nmol g$^{-1}$) | D60 | 0.24 | 0.24 | 0.24 | 0.25 | 0.26 | 0.32 | 0.30 | 0.30 | 0.30 |
| | D70 | 0.27 | 0.30 | 0.28 | 0.24 | 0.24 | 0.27 | 0.26 | 0.27 | 0.26 |
| | D80 | 0.23 | 0.14 | 0.22 | 0.22 | 0.21 | 0.23 | 0.25 | 0.22 | 0.26 |
| | D90 | 0.20 | 0.25 | 0.30 | 0.23 | 0.25 | 0.19 | 0.20 | 0.25 | 0.29 |
| | D100 | 0.08 | 0.12 | 0.07 | 0.07 | 0.08 | 0.08 | 0.05 | 0.09 | 0.06 |
| TOP†††/($\times 10$ nmol g$^{-1}$) | D60 | 0.16 | 0.01 | 0.46 | 0.50 | 0.29 | 0.01 | 0.01 | 0.00 | 0.00 |
| | D70 | 0.35 | 0.28 | 0.12 | 0.67 | 0.32 | 0.02 | 0.00 | 0.00 | 0.00 |
| | D80 | 0.86 | 3.75 | 0.87 | 0.63 | 0.66 | 0.59 | 0.60 | 0.62 | 0.13 |
| | D90 | 1.38 | 1.11 | 0.00 | 0.95 | 0.48 | 0.75 | 0.70 | 0.76 | 0.36 |
| | D100 | 0.37 | 0.97 | 0.61 | 0.58 | 0.43 | 0.24 | 0.44 | 0.62 | 0.74 |
| TOC††††/% | D60 | 0.25 | 0.57 | 0.52 | 0.19 | 0.20 | 0.84 | 0.70 | 0.40 | 0.45 |
| | D70 | 0.86 | 0.70 | 0.86 | 0.23 | 0.38 | 0.60 | 0.31 | 0.33 | 0.23 |
| | D80 | 0.56 | 1.38 | 0.65 | 0.66 | 0.65 | 0.60 | 0.41 | 0.36 | 0.64 |
| | D90 | 0.74 | 1.29 | 0.39 | 0.44 | 0.41 | 0.25 | 0.41 | 0.62 | 0.77 |
| | D100 | 0.68 | 1.22 | 0.82 | 0.35 | 0.40 | 0.59 | 0.28 | 0.35 | 0.42 |
| TN†††††/% | D60 | 0.13 | 0.06 | 0.06 | 0.10 | 0.09 | 0.07 | 0.06 | 0.08 | 0.07 |
| | D70 | 0.12 | 0.09 | 0.07 | 0.13 | 0.15 | 0.09 | 0.10 | 0.13 | 0.13 |
| | D80 | 0.23 | 0.12 | 0.19 | 0.18 | 0.23 | 0.20 | 0.17 | 0.20 | 0.20 |
| | D90 | 0.06 | 0.04 | 0.06 | 0.05 | 0.04 | 0.06 | 0.06 | 0.05 | 0.04 |
| | D100 | 0.12 | 0.06 | 0.07 | 0.08 | 0.07 | 0.07 | 0.08 | 0.08 | 0.07 |

Notes: †The correlation coefficient of APA with TP, $r=0.752$ ($P<0.05$).
††The correlation coefficient of APA with TIP, $r=0.168$.
†††The correlation coefficient of APA with TOP, $r=0.235$.
††††The correlation coefficient of APA with TOC, $r=0.801$ ($P<0.05$).
†††††The correlation coefficient of APA with TN, $r=-0.513$ ($P<0.05$).

The other two nutritional factors TOC and TN of sediments were also analyzed (Table 1). There is a positive correlation between TOC and APA ($r = 0.801$), but a negative one between TN and APA ($r = -0.513$). From the relationships between APA and C, N and P, we conclude that APA seems to be a sensitive indicator of ecological stability and an integrated parameter for organic pollution in sediments.

## 4 Discussion

Although probiotics and BIO ENERGIZER solution have been used in the treatment, APA shows no significant difference in the control and experimental enclosures, which suggests that the bacteria biomass is not the only factor to determine APA in sediments. APA in the aquatic environment originates from either phyto- or bacterioplankton or both, depending on the nutritional status. The various groups contribute to the total APA in different degrees, ranging from the predomination of algae (Chróst and Overbeck, 1987; Vrba *et al.*, 1993), to the main contribution of bacteria (Thingstad *et al.*, 1993; Jamet *et al.*, 1995). In addition, a study about lake-sediment with cage culture of *Oreochromis niloticus* has shown that the fish feces exhibit a remarkable APA (Zhou *et al.*, 2000). In general, the main sources of APA in mariculture sediments are deposited plankton, bacteria and feces of cultured-object, which determines the variation of APA together. Therefore an increase of APA is observed in sediment as the deposition of dead plankton, bacteria and feces of cultured-objects.

Since APA is a non-specific enzyme, it can catalyse the hydrolysis of phosphate monosters into alcohol and inorganic phosphate and plays a central role in the P cycling of aquatic ecosystems. In the research of circu-

lation pattern of P in sediments, Froelich (1988) offered the definition of the zero equilibrium phosphate concentration (EPC). It represents the concentration at which the sediments display the maximum buffering capacity. On the basis of EPC, P is not accumulated but in a releasing course in mariculture sediments (Yuan and Cun, 1999). Organic phosphate can hydrolyze to inorganic phosphate under the action of APA. If the inorganic phosphate coming from organic phosphate were utilized entirely by bacteria or phytoplankton, the correlation between APA and organic phosphate should be positive. If the inorganic phosphate were released to seawater completely, the correlation should be negative. However, both of the hypothesis were concomitant in P cycling, resulting in an insignificant correlation between organic phosphate and APA (Table 1). In addition, inorganic phosphate in sediments is not reserved entirely but set free to pore water. It also shows an unstable relationship with APA (Table 1). In contrast to organic phosphate and inorganic phosphate, the concentration of total phosphate is determined by the nutrition concentration of sediments and the biomass. With increasing of total phosphate, the APA must be rising, which is reasonable. Results show the highly correlation between total phosphate and APA (Table 1).

Kim *et al.* (1995) reported that animal wastes contain high carbon contents and can stimulate phosphate activity. In two shallow ponds, APA has shown significant correlation with POM, in which the main content is TOC (Alvarez and Guerrero, 2000). Since APA in mariculture sediments originates from deposit plankton, bacteria and feces of cultured-objects, the significant correlation between APA and TOC is obvious. Garcia *et al.* (1993) thought that kinetic parameters of phosphatase are correlative with the degree of evolution of the organic matter contained in the wastes. In our experiment, APA mainly correlates with TOC, which indirectly determines the degree of evolution of the organic matter. By contrast with phosphate, TOC shows a more significant coherence with APA, meaning that organic pollution in sediments affect APA remarkably. In contrast to TOC, TN is in a course of accumulation in mariculture sediments (Yuan and Cun, 1999), showing a negative correlation with APA. In the Baltic Sea, APA shows the same pattern: APA increases with dissolved inorganic nitrogen (DIN) decreasing in seawater (Nausch, 2000).

To summarize, APA plays a central part in the P cycling of mariculture ecosystems and has significant relationships with P, N and C, especially with the latter. It seems to be a sensitive indicator of ecological stability and an integrated parameter for organic pollution in sediments. Furthermore, researches on APA dynamics in benthonic ecosystems should be carried out to explain the process mechanism between APA and the contents of C, N and P in future work.

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## References

Alvarez, S., and M. Guerrero, 2000. Enzymatic activities associated with decomposition of particulate organic matter in two shallow ponds. *Soil Biology & Biochemistry*, **32**: 1941-1951.

Boetius, A., T. Ferdelman, and K. Lochte, 2000. Bacterial activity in sediments of the deep Arabian Sea in relation to vertical flux. *Deep-Sea Research*, **47**: 2835-2875.

Chróst, R., 1991. Environmental control of the synthesis and activity of aquatic microbial enzymes. In: *Microbial Enzymes in Aquatic Environments*. Chróst, R., ed., Springer, New York, 29-84.

Chróst, R., and J. Overbeck, 1987. Kinetics of alkaline phosphatase activity and P availability for phytoplankton and bacterioplankton in Lake Plusssee (North German Eutrophic Lake). *Microbial Ecol.*, **13**: 229-248.

Darrah, P. R., and P. J. Harris, 1986. A fluorometric method for measuring the activity of soil enzymes. *Plant and Soil.*, **92**: 81-88.

Findlay, S., R. Sinsabaugh, D. Fisher, and P. Franchini, 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems*, **1**: 227-239.

Froelich, P. N., 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: A primer on the phosphate buffer mechanism. *Limnol Oceanogr*, **33**: 649-668.

Garcia, C., T. Hernandez, F. Costa, B. Ceccanti, and G. Masciandaro, 1993. Kinetics of phosphatase activity inorganic wastes. *Soil Biol. Biochem*, **25**: 561-565.

Hong, H., 1989. Distribution of all kinds of phosphatase in Xiamen Gulf. *Marine Environmental Science*, **8**: 1-10.

Hoppe, H. G., 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. In: *Handbook of Methods in Aquatic Microbial Ecology*. Kemp, P. F., *et al.*, eds., Lewis Publishers, Boca Raton, 423-431.

Huang, B., and H. Hong, 1999. Alkaline phosphatase activity and utilization of dissolved organic P by algae in subtropical coastal waters. *Marine Pollution Bulletin*, **39**: 205-211.

Jamet, D., L. Aleya, and J. Devaux, 1995. Diel changes in the alkaline phosphatase activity of bacteria and phytoplankton in the hypereutrophic Villerest reservoir (Roanne, France). *Hydrobiology*, **300/301**: 49-56.

Jackson, C. R., C. M. Foreman, and R. L. Sinsabaugh, 1995. Microbial enzyme activities as indicators of organic matter processing rates in a lake Eirecoastal wetland. *Freshwater Biology*, **34**: 329-342.

Jansson, M., H. Olsson, and K. Pettersson, 1988. Phos-

phatases: origin, characteristics and function in lakes. *Hydrobiology*, **170**: 157-175.

Kim, K.Y., D. Jordan, and G.A. McDonald, 1998. Enterobacter agglomerans, phosphate solubilizing bacteria, and microbial activity in soil: effect of carbon sources. *Soil Biol. Biochem.*, **30**: 995-1003.

Koike, T., and T. Nagata, 1998. High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. *Deep-Sea Research* Ⅱ, **44**: 2283-2294.

Liu, Z., and F. Zhu, 1997. Improvement in kjadehl's method determinining protein content of flounder muscle. *Marine Sciences*, **22**: 24-26.

Nausch, M., 2000. Stimulation of peptidase activity in nutrient gradients in the Baltic Sea. *Soil Biology & Biochemistry*, **32**: 1973-1983.

Sinsabaugh, R.L., and S. Findlay, 1995. Microbial production, enzyme activityand carbon turnover in surface sediments of the Hudson River estuary. *Microbial Ecology*, **30**: 127-141.

Sinsabaugh, R.L., R.K. Antibus, A.E. Linkins, C.A. McClaugherty, L. Rayburn, *et al.*, 1992. Wood decomposition over a first-order watershed: mass loss as a function of lignocellulase activity. *Soil Biology & Biochemistry*, **24**: 743-749.

Sinsabaugh, R.L., D.L. Moorhead, and A.E. Linkins, 1994. The enzymatic basis of plant litter decomposition: emergence of an ecological process. *Applied Soil Ecology*, **1**: 97-111.

Shand, A.C., and S. Smith, 1997. Enzymatic release of phosphate from modelsubstrates and P compounds in soil solution from peaty podzol. *Biologyand Fertility of Soils*, **24**: 183-187.

Sundby, B., C. Gobeil, and N. Silverberg, 1992. The P cycle in coastal marine sediments. *Limnol Oceanogr*, **37** (6): 1129-1145.

Thingstad, T.F., E.F. Skjoldal, and R.A. Bohne, 1993. P cycling and algal-bacterial competition in Sandsfjord, western Norway. *Mar. Ecol. Prog. Ser.*, **99**: 239-259.

Vrba, J., J. Komarkova, and V. Vyhnalek, 1993. Enhanced activity of alkaline phosphatases phytoplankton responseto epilimnetic P depletion. *Wat. Sci. Tech.*, **28**: 15-24.

Yuan, Y., and Y. Cun, 1999. Vertical distributions of TOC TP TN and pH in sediments of shrimp pond. *Journal of Fisheries of China*, **23**: 363-368.

Zhou, Y., J. Li, Y. Fu, and M. Zhang, 2001. Kinetics of alkaline phosphatase in lake sediment associated with cage culture of *Oreochromis niloticus*. *Aquaculture*, **203**: 23-32.