Comparison of several methods for BAP measurement

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Abstract It has been more important for management of water quality to estimate the amount of bioavailable phosphorus (BAP) in suspended solids (SS) entering lakes and estuaries. AGP test or extraction by 0.1 mol l^{-1} NaOH (C-BOD) is widely used. Recently, highly bioavailable phosphorus (HBAP) was introduced to indicate a more easily soluble and bioavailable fraction using successive extraction by 0.1 mol l^{-1} HCl and 0.1 mol l^{-1} NaOH. New biologically measured BAP (B-BAP) using bacterial respiration activity was introduced in this paper. B-BAP was estimated from oxygen uptake rate (OUR), which was measured by a respiratory meter for BOD measurement using a pressure sensor. B-BAP is useful for a rapid and direct measurement of phosphorus bioavailability. B-BAP, HBAP and C-BAP in river SS were measured and compared with each other. The percentages of HBAP and B-BAP to PP were large in the urban river, while the percentage of NaOH-P or C-BAP was large in the rivers flowing in agricultural areas. By comparison with phosphorus fractions in paddy soil and activated sludge it was suggested that SS in the rivers flowing in agricultural areas mainly consisted of clay, silt or sand, while the SS in the urban river consisted of a large percentage of organic particles as well. Phosphorus in SS was suggested to be more easily bioavailable in the urban river than the rivers in agricultural areas. The ratio of C-BAP/B-BAP was large in the rivers in agricultural areas and small in the urban river. As HBAP contents were almost similar to B-BAP contents in the river SS, HBAP can be a suitable index of phosphorus indicating easily and rapidly the bioavailable fraction in SS.

Keywords Bacterial respiration; bioavailable phosphorus; eutrophication; lake water management; oxygen uptake rate; phosphate analysis

Introduction

It has become more important to estimate bioavailable phosphorus (BAP) amount entering lakes or estuaries from non point sources in their basin for management of their water quality and environment. In order to measure BAP, chemical and biological methods have been applied (Okubo, 1996). In chemical methods 0.1 mol l^{-1} NaOH extraction is the most common to measure BAP in agricultural runoff (Dorich *et al.*, 1985; Butkus *et al.*, 1988; Parker, 1991; Sharpley *et al.*, 1991, 1992). Nakajima and Okubo (2003) improved the sensitivity of the BAP measurement by collecting SS using filtration procedures before 0.1 mol l^{-1} NaOH extraction. They also introduced 0.1 mol l^{-1} HCl extraction before 0.1 mol l^{-1} NaOH extraction in order to estimate the more easily soluble and bioavailable fraction (HBAP; highly bioavailable phosphorus) by applying the successive extraction method of soil phosphorus fractions (Chang and Jackson, 1957; Williams *et al.*, 1967; Sekiya, 1973). By measuring HBAP as well as BAP the seasonal changes of the phosphorus in SS and sediments were shown more clearly (Nakajima and Okubo, 2003).

In the biological method green algae such as *Selenastrum capricornutum* has been widely used as an algal growth potential (AGP) test to measure BAP (Cowen and Lee, 1976; Huettl *et al.*, 1979; Dorich *et al.*, 1980; Williams *et al.*, 1980; Ekholm and Krogerus, 1998). The chemical methods estimate the bioavailability of phosphorus by microorganisms indirectly, while the biological methods measure directly the algal

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growth potentials. However, the latter takes several weeks to get to the maximum growth of the algae. Bacteria have been also used for measurement of phosphorus in water using growth of *Pseudomonas fluorescens* (Lehtola *et al.*, 1999) and DO consumption by respiration (Nakamoto, 1977) as well as phosphorus in soil using CO_2 production by respiration (Nordgren, 1992; Demetz and Insam, 1999). The periods of the bioassay using bacteria are within several days and rather shorter than the case of using algae. Good correlations were shown as for the relationship between chemical and biological methods for BAP measurement (Williams *et al.*, 1980; Dorich *et al.*, 1980, 1985; Butkus *et al.*, 1988; Sharpley *et al.*, 1991).

Nakamoto (1977) introduced MBOD-P (modified biochemical oxygen demand) showing AGP by DO consumption in BOD assay using media added organics and nutrients without phosphorus. We applied MBOD-P to BAP measurement in this study because AGP without phosphorus addition seemed to be strongly related to BAP in the sample. Firstly the Winkler method using a DO bottle was examined in the measurement but the range of detection was small. Hence respiratory meters that were used in biodegradation tests (Larson and Perry, 1981; OECD, 1993; Reuschenbach *et al.*, 2003) were used and good results were obtained. The purpose of this study is to examine this new biological measurement method of BAP using bacterial respiration by applying it to river suspended solids (SS) and by comparing it with BAP measurement using the chemical method we proposed previously.

Materials and methods

Samples

Surface water samples were taken from five rivers in Shiga Prefecture entering Akanoi Bay in the southern part of Lake Biwa from January 2003 to May 2004 (96 samples), the Nakae River in Gifu Prefecture from April 2003 to June 2004 (41 samples) and seven points in the Yamato River in the southern part of Osaka from July to November 2003 (14 samples). The rivers entering Akanoi Bay and the Nakae River are located in paddy field and their water quality is influenced largely by agricultural cultivation, while Yamato River flows in an urban basin and the water quality was strongly influenced by sewerage and industrial wastewater as well as urban diffuse pollution. Samples were brought back to the laboratory and filtered through a glass fiber filter GS-25 (Advantec) to collect the suspended solids. In order to examine the characteristics of BAP in river SS, BAP in soil and sludge samples were also measured. Paddy soil samples were taken in the basin of the rivers entering Akanoi Bay (15 samples). Activated sludge samples were taken from experimental treatment plants in our laboratory (seven samples).

Measurement of B-BAP by bacterial respiration

Measurement of B-BAP (biologically measured BAP) fundamentally followed Nakamoto (1977) while the DO bottle was exchanged to a commercial respiratory meter for the BOD test in order to measure a precise DO change and to obtain its wide dynamic range. The filter paper collecting SS was put in a 125 mL bottle and 80 mL of modified BOD seeding media without phosphorus (Table 1) were added in the bottle. After putting a seal cup filled with 0.8 g of soda lime, in order to adsorb CO_2, the bottle was capped and connected to a pressure sensor part of BOD Track (HACH). The bottle was stored in a dark place for 36 hours at 25 °C with mixing by a magnetic stirrer. The DO concentration in the water layer in the bottle was estimated by measuring the pressure in the upper gas layer in the bottle and the data were stored in a memory. The DO consumption data were plotted according to the time and oxygen uptake rate (OUR: $mgO_2/L/hr$) was determined

Table 1 Media composition used for B-BAP measurement

Compound	Concentration (mg/L)
NH_4Cl	170
$MgSO_4 \cdot 7H_2O$	22.5
$CaCl_2$	27.5
$FeCl_3 \cdot 6H_2O$	0.25
Glucose	400
$NaHCO_3$	168
Seeding bacteria*	1 capsule/2 × 10^4 L

*Polyseed-US (Console)

by the change of the DO consumption. The B-BAP concentration of a sample was estimated by the calibration curve using the OUR of the standard PO_4-P solutions. B-BAP content (mgP/g) was then calculated using SS of the sample.

Measurement of HBAP and C-BAP by acid and alkali extraction

HBAP and C-BAP (chemically measured BAP) were extracted from the samples fundamentally according to Pearson (1940) as shown in Figure 1 (Nakajima and Okubo, 2003). The filter paper collecting SS (usually after drying and measuring its weight to estimate the SS value of the sample) was put in a 140 mL PTFE bottle. 50 mL of 0.1 mol l^{-1} HCl was added to the bottle and after shaking for 3 minutes the acid extract was obtained by filtration (Advantek No.5C). HBAP was determined as PO_4-P in the acid extract after neutralization. The residue of the filtration with the filter paper was put into the bottle again and 100 mL of 0.1 mol l^{-1} NaOH was added to the bottle. After shaking for 17 hrs the alkali extract was obtained by filtration. NaOH-P was determined as PO_4-P in the alkali extract after neutralization. C-BAP was obtained as the sum of HBAP and NaOH-P. HBAP and C-BAP contents (mgP/g) were then calculated using SS of the sample. Total phosphorus and dissolved phosphorus of the sample water were also measured and particulate phosphorus content (PP: total phosphorus in SS) was determined by their difference as shown in Figure 1.

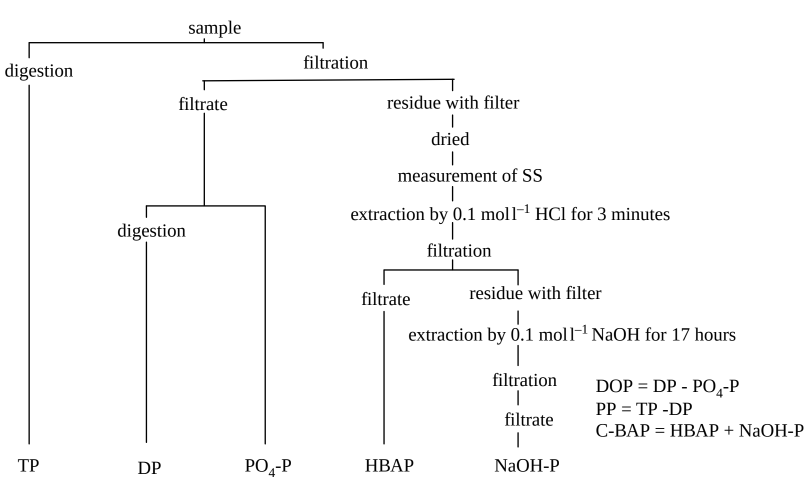

Figure 1 Measurement procedure of HBAP, C-BAP and PP

Results and discussion

Performance of B-BAP measurement method

The optimum experimental conditions such as incubation temperature, effect of equipment sterilization, concentration of nitrogen and glucose, kind of buffer solution and seeding as well as treatment method of filter paper were obtained by results of preliminary experiments. An example of the change in DO consumption using PO_4-P standard solution is shown in Figure 2. Usually a lag phase of 12 h was observed. DO consumption increased after that and then OUR was determined by its slope. An example of the calibration curve is shown in Figure 3. In this case, OUR was saturated at a concentration of 300 μgP/L. The range of determination was from 5 μgP/L to 250 μgP/L as the concentration of the solution in the bottle. The range was developed by increasing the concentrations of NH_4Cl and glucose in the media. The coefficient of variation of this method applied to river SS was 10% showing that the repeatability of the determination was good enough as a bioassay.

This method is carried out in less than 2 days, which is rather short compared to BAP bioassay using the growth of *Selenastrum capricornutum*. Moreover it seems reasonable that the phosphorus fraction that bacteria can utilize in a short period must also be easily utilized by algae directly or after bacterial utilization. Therefore B-BAP measured by bacterial respiration in this method will be useful as a rapid and direct measurement of phosphorus bioavailability.

BAP contents in river SS

The average values and the standard deviations of PP, HBAP, C-BAP and B-BAP contents of river SS in the three areas are shown in Table 2. PP was high in the Nakae River because of high C-BAP (or high NaOH-P) while HBAP and B-BAP were high in the Yamato River. The average percentages of the phosphorus fractions to PP are shown in Figures 4 and 5. The percentages of HBAP and B-BAP were small in the rivers entering Akanoi Bay and the Nakae River and large in the Yamato River. On the contrary the percentage of NaOH-P or C-BAP was large in the rivers entering Akanoi Bay and the Nakae River and small in the Yamato River.

River SS mainly consists of clay, silt or sand as well as organic particles such as microorganisms or organic detritus. The paddy soil was measured as a typical example of clay, silt or sand and the activated sludge was measured as a typical example of microorganisms or organic detritus. The average percentages of phosphorus fractions in paddy soil and activated sludge samples are also shown in Figures 4 and 5. The paddy soil had a large percentage in C-BAP and a small percentage in B-BAP, while the activated

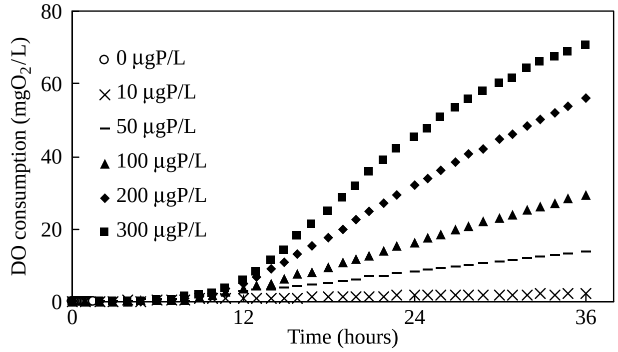

Figure 2 Change of DO consumption after blank correction

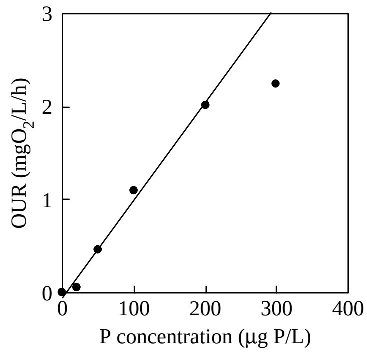

Figure 3 Calibration curve

Table 2 BAP contents in SS of three river groups

	n	Average ± standard deviation (mgP/g)			
		PP	HBAP	C-BAP	B-BAP
Rs. Akanoi	96	8.65 ± 3.29	1.37 ± 0.86	5.89 ± 2.75	1.29 ± 0.59
R. Nakae	41	20.8 ± 12.4	1.13 ± 0.79	18.7 ± 12.4	2.29 ± 1.33
R. Yamato	14	8.19 ± 1.71	2.14 ± 0.59	3.59 ± 0.80	2.78 ± 1.01

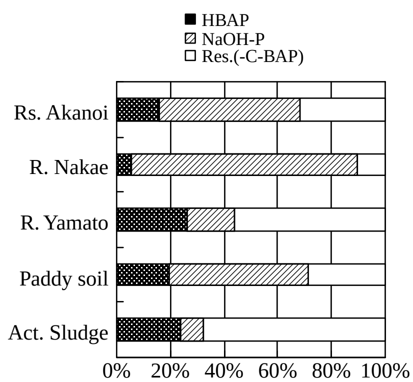

Figure 4 Percentages of HBAP and NaOH-P to PP

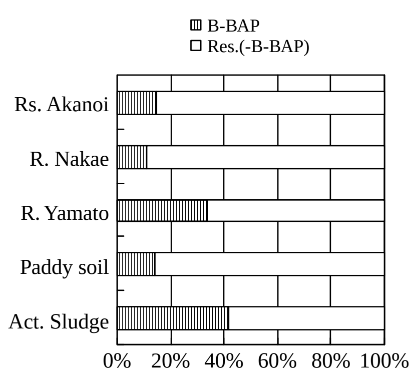

Figure 5 Percentages of B-BAP to PP

sludge had large percentages in B-BAP as well as HBAP and a small percentage in NaOH-P.

The percentages in the rivers entering Akanoi Bay and the Nakae River were similar to the percentages in the paddy soil, while the percentages in the Yamato River were similar to the percentages in the activated sludge. This suggests that the SS in the rivers entering Akanoi Bay and the Nakae River mainly consists of clay, silt or sand, while the SS in the Yamato River consists of a large percentage of organic particles as well. This seems reasonable because the former rivers flow in an agricultural area and the latter river flows in an urban area. Moreover the results suggest that phosphorus in SS is more easily bioavailable in urban rivers than rivers in agricultural areas.

Correlations between B-BAP and C-BAP

The relationships between B-BAP and C-BAP as well as HBAP are shown in Figure 6. HBAP and C-BAP increased with the increase of B-BAP in all cases, although their ratio was different in each river area. In the rivers entering Akanoi Bay and the Nakae River the difference between HBAP and C-BAP was large, while the difference was small in the Yamato River. The ratio of C-BAP/B-BAP was large in the rivers entering Akanoi Bay and the Nakae River and small in the Yamato River. The ratio of HBAP/B-BAP was around 1 in all cases. This means HBAP contents were almost similar to B-BAP contents in the river SS.

The relationships between B-BAP and C-BAP in the paddy soil and activated sludge samples are shown in Figure 7. The correlations were clear in the paddy soils between B-BAP and C-BAP as well as HBAP. The ratio of C-BAD/B-BAP was large like SS in the rivers entering Akanoi Bay and the Nakae River. On the contrary the ratio is small in the activated sludge like SS in the Yamato River. These results also suggest SS in the

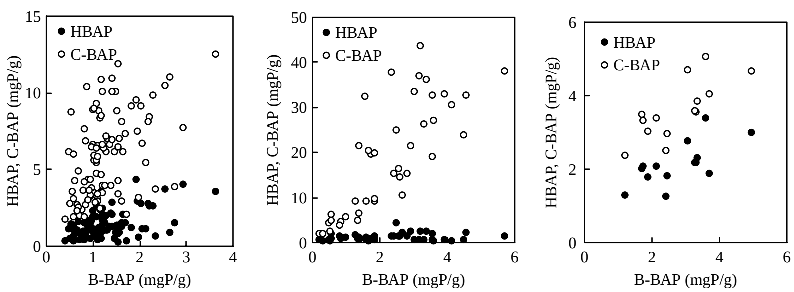

Figure 6 Relationships between B-BAP and C-BAP in the rivers entering Akanoi Bay (left), the Nakae River (center) and the Yamato River (right)

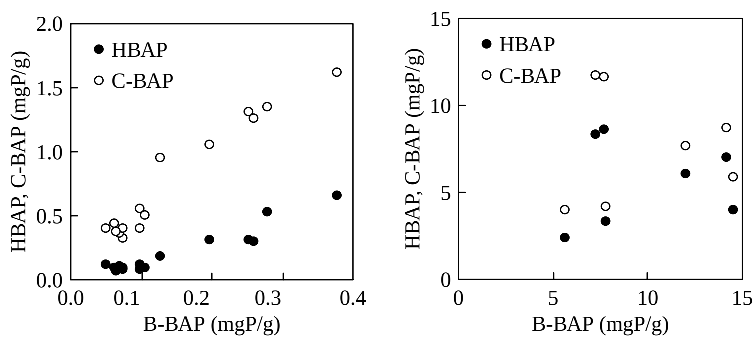

Figure 7 Relationships between B-BAP and C-BAP in paddy soil (left) and activated sludge (right)

agricultural area and urban area largely consists of soil and organic particulate, respectively.

The result that HBAP contents were similar to B-BAP contents supports the proposal by Nakajima and Okubo (2003) that HBAP is a suitable index of phosphorus indicating easily and rapidly bioavailable fraction. HBAP is able to be measured very simply and the sensitivity of the detection can be improved by filtration of a large volume of sample water. HBAP in river SS is also a part of reactive phosphorus (R-P) in water that can be dissolved from SS by acid reagent addition.

Conclusions

(1) A new biological BAP measurement method using bacteria respiration was introduced to rapid measurement of easily bioavailable phosphorus in river SS.
(2) The percentages of HBAP and B-BAP were large in the urban river, while the percentage of NaOH-P or C-BAP was large in the rivers flowing in the agricultural area.
(3) SS in the rivers flowing in agricultural areas mainly consists of clay, silt or sand, while the SS in the urban river consists of a large percentage of organic particles as well.
(4) Phosphorus in SS was suggested to be more easily bioavailable in the urban river than the rivers in the agricultural area.
(5) The ratio of C-BAP/B-BAP was large in the rivers in the agricultural areas and small in the urban river.
(6) HBAP contents were almost similar to B-BAP contents in the river SS. Therefore HBAP is a suitable index of phosphorus indicating easily and rapidly the bioavailable fraction in SS.

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