An examination of the factors involved in determining phosphatase activities in estuarine water. 1: Analytical procedures

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Keywords: phosphatase, analytical procedures, pH, magnesium, sample preservation, estuarine

Abstract

The effects of assay pH, magnesium addition, temperature, sodium azide, and of sample storage and filtration on the apparent phosphatase activities in natural water bodies, and in particular estuaries, have been examined. Phosphatase activity was assessed by monitoring the cleavage of p-nitrophenol from p-nitrophenyl phosphate (pNPP).

Magnesium concentrations in natural waters were found to be generally in excess of the requirement of the assay procedure. Several pH maxima occurred for phosphatase activities in the estuarine waters examined. These optima were not constant with respect to sampling stations or sampling time. This was considered to be, in part, the result of differences in the biomass composition. Sample storage times and temperatures employed in the present study did not influence the phosphatase activities of these low biomass waters. Phosphatase activities increased with increased incubation temperature. The rate of orthophosphate release from pNPP during incubation was linear over time to 120 h. Sodium azide was found to be an adequate preservative when long incubation times were required.

The effects of assay conditions are discussed in relation to the assay procedures and data interpretation.

Introduction

When in the organic form, phosphorus is not directly utilizable by most organisms. Phosphatases hydrolyze the ester linkages of organic phosphorus molecules, making orthophosphate available for uptake and metabolism. Many organisms will synthesize phosphatases in response to phosphorus deficient conditions. Conversely, in many organisms, synthesis of phosphatase may be repressed by excess available orthophosphate (cf. Aaronson et al., 1976). Therefore, it has been suggested that the phosphatase activity in a body of water can be used as an index of the phosphorus nutritional status of the system (cf. Berman, 1970; Jones, 1972; Perry, 1972). In order to be meaningful as an index, adequately defined assay procedures must be followed.

The most commonly used procedures for assaying phosphatase activity are based on monitoring cleavage of artificial substrates, either p-nitrophenyl phosphate (Reichardt et al., 1967) or 3-o-methyl fluorescein phosphate (Perry, 1972). Use of the latter substrate is more sensitive.

Problems with assessing phosphatase activities in natural waters can be grouped into two categories. The first is related to the details of the assay method and sample treatment, while the second deals with adequate sampling procedures. Meaningful comparisons within and between studies can be made only when both are adequately defined. In this paper, the variations in apparent phosphatase activities due to assay conditions (pH, cation addition, incubation time and temperature, preservative addition, sample filtration, and sample storage time and temperature) are examined.

Materials and methods

1. Routine phosphatase assay

Phosphatase activities were measured using essentially the method of Reichardt et al. (1967).

The reaction mixture was 2:2:1 (v/v/v) of water sample, buffer, and substrate. The buffer systems used were 0.1 M Tris-HCl, pH 7.5 or 8.6, for neutral or alkaline phosphatases, or 1 M citric acid-sodium citrate buffer, pH 5.6, for acid phosphatases. The substrate was 0.7 mM p-nitrophenyl phosphate (pNPP, Sigma 104) in a 10⁻² M MgSO₄ solution. Incubation was at 25 °C in the dark for 24 or 48 h. Sodium azide (5 mM) was added as a preservative to inhibit microbial growth and to preclude phosphatase synthesis. Substrate and sample controls were included in all experiments. The enzyme activity was measured on a Varian Techtron (Model 635) spectrophotometer at 410 nm. For alkaline and neutral phosphatases this was done directly on the samples, but for acid phosphatases the colour of the reaction mixture was developed by adding an equal volume of 0.1 M NaOH. This solution was made up just prior to the use with high purity NaOH, since carbonates which formed in the NaOH solution caused precipitates in the reaction mixture. Measurements were corrected for sample and substrate controls. Results are generally reported in terms of absorbance units at 410 nm in a 1 cm quartz cuvette.

Error bars have not been included in Figs. 8, 9 & 10, but the variances in the assay method are ± 11.4% after 24 h sample incubation, and ± 5.7% after 48 h incubation. These are based on the March 1978 grid study data (see accompanying paper, Huber & Kidby). Variances were calculated using:

\[ S^2 = \frac{1}{2n} \sum_{i=1}^{n} (\log x_{1i} - \log x_{12})^2 \]

where \(i\) = the sampling site (\(n = 36\)) and \(x_{1i}\) and \(x_{12}\) are replicate assays of a sample. Sample variation within one sampling site is discussed in the accompanying paper (Huber & Kidby).

Details of variations of the basic methods are given in Results and Discussion.

2. Standard phosphatase assays

Sigma alkaline phosphatase (calf intestinal mucosa, 1.2 activity units mg⁻¹; 1 unit converts 1 μmol pNPP min⁻¹ at 37 °C, pH 9-10) was used. The standard solution was 8.28⋅10⁻³ activity units per 5 ml total volume. The time response was examined over a 3 h period.

3. Sampling stations

All samples were taken from the Peel-Harvey Estuary located in the south-west of Western Australia. The locations of the sampling sites are shown in Fig. 1.

Results and discussion

1. Standard phosphatase assay

The results of a standard phosphatase assay (bo-