An evaluation of the methods used for the determination of orthophosphate and total phosphate in activated sludge extracts

DW de Haas[1]*, LH Lötter[2] and IA Dubery[3]
[1]*Division of Water Technology, CSIR, PO Box 395, Pretoria 0001, South Africa*
[2]*Cydna Laboratory, City Health Department, PO Box 1477, Johannesburg 2000, South Africa*
[3]*Department of Biochemistry, Rand Afrikaans University, PO Box 524, Johannesburg 2000, South Africa*

Abstract

A need exists for routine analysis of the phosphorus fractions stored in activated sludge from waste-water treatment plants designed to accomplish biological phosphate removal. An investigation was conducted into the suitability of published methods for orthophosphate and total phosphate determinations when applied to extracts of activated sludge or artificial solutions simulating such extracts. It was found that the standard orthophosphate method for water could be used, but attempts to increase its range are likely to introduce errors due to turbidity formation, especially with samples at high ionic strength. The standard persulphate digestion procedure for total phosphate (TP) was found to give slightly inferior recoveries from activated sludge samples relative to a procedure in which the acid and persulphate concentrations were raised, and in which samples of high TP ($\geq$ 100 mg P/ℓ) were prediluted. Irrespective of the latter, a post-digestion dilution is necessary to avoid interferences in the subsequent orthophosphate determination due to the higher ionic strength and acidity of the digest. Acid-hydrolysable phosphates at concentrations ca. 1 to 75 mg P/ℓ (as TP) interfere positively in the standard molybdate-ascorbic acid orthophosphate determination, and to approximately the same extent in related methods which were designed to limit such interference. Negative interference (to as far as complete inhibition) is caused by acid-hydrolysable phosphates at high concentrations (> ca. 75 mg P/ℓ) in the standard molybdate-ascorbic acid orthophosphate determination. Determination of orthophosphate in the presence of relatively large concentrations of polyphosphates may therefore be subject to significant errors. It is advisable to use a modified method for orthophosphate determination which reduces these errors and to check the result by gel chromatography.

Introduction

Nutrient removal from waste water has received international attention over the past two decades as a result of eutrophication of inland and coastal water supplies. However, implementation of biological nutrient removal processes has advanced faster than an understanding of the mechanisms involved, particularly in the case of phosphorus removal (Arvin, 1985). Recently, biochemical models have been proposed for enhanced biological phosphorus removal in modified activated sludge systems (Comeau *et al.*, 1986; Wentzel *et al.*, 1986). Testing the validity of these models is partially dependent on the availability of reliable analytical methods to resolve the different chemical forms of phosphorus accumulated in activated sludge.

Activated sludge, particularly that from nutrient removal plants, contains upward of 1% phosphorus (P) on a dry weight basis of mixed liquor suspended solids, with maximum concentrations of approximately 7% P observed for systems receiving mainly domestic sewage (Riding *et al.*, 1979; Sutton *et al.*, 1980; Arvin, 1985). Polyphosphate is reputedly an important component of this stored phosphate but occurs to varying degrees along with nucleic acids, orthophosphate and phospholipids in chemical extracts of activated sludge (Fuhs and Chen, 1975; Mino *et al.*, 1985; Lötter, 1985; Murphy and Lötter, 1986). The phospholipid fraction is quantitatively the smallest and can be readily isolated and determined (Mino *et al.*, 1985). With regard to the determination of the other phosphorus fractions, it is potentially difficult to distinguish orthophosphate from polyphosphate and other acid-labile phosphate species: colorimetric orthophosphate determinations commonly take place in the presence of strong acid (Murphy and Riley, 1962; Edwards *et al.*, 1965; Harwood *et al.*, 1969a; *Standard Methods*, 1985). In the presence of such strong acid both the polyphosphate and the nucleic acid phosphate undergo hydrolysis to orthophosphate, the rate of hydrolysis being not only pH-dependent, but also temperature and species-dependent (Leloir and Cardini, 1957; Clesceri and Lee, 1965). The implication is that, in the colorimetric orthophosphate determination, some polyphosphates and nucleic acid phosphates might be converted to orthophosphate. In an attempt to solve this problem Saheki *et al.* (1985) devised an orthophosphate determination which is performed at pH 5 using zinc acetate as catalyst. They claimed minimal positive interference from the acid-labile compound glucose-1-phosphate and no negative interference from metal-chelating agents (EDTA and citrate) and thiol compounds. In these respects Saheki *et al.* (1985) found their method to be superior to that of Lowry and Lopez (1946) which is performed at pH 4 in the absence of zinc ions. Similarly Chifflet *et al.* (1988) made use of citrate to avoid organic phosphate hydrolysis during colour development. Citrate serves to complex excess molybdate in this method. Chifflet *et al.* (1988) also introduced sodium dodecyl sulphate to their assay, making it suitable for samples containing high amounts of protein.

Apart from interference by labile phosphates, colorimetric orthophosphate determinations have limited ranges. That of *Standard Methods* (1985) gives a linear function between approximately 0,15 and 1 mg P/ℓ (for a 1 cm light path), the absorbance being around 0,640 at the upper limit. However, modern spectrophotometers can measure absorbance accurately as high as 2,5, implying that the test may be suitably modified to allow measurement over a wider range of phosphate concentrations.

Finally, total phosphorus (TP) determinations of activated sludge, its extracts or similar starting material have been performed by various methods. Mino *et al.* (1985) reported simply that 'potassium persulphate degradation' of the sample was used. Heinke and Norman (1969) found their persulphate digestion method superior to perchloric acid and sulphuric-nitric acid

*To whom all correspondence should be addressed. Present address: Umgeni Water, PO Box 9, Pietermaritzburg 3200.
Received 19 October 1988

methods. Similarly Harwood *et al.* (1969b) compared the persulphate oxidation method of Gales *et al.* (1966) with other methods for samples of algae, water plant, tryptone and peptone and found that although a long tedious method involving magnesium nitrate fusion was statistically slightly superior, the persulphate method was simple, reliable and quick.

Using the latter method Harwood *et al.* (1969b) found that although undigested particles remained in some samples, phosphate analyses did not show any loss of phosphorus. The method of Gales *et al.* (1966) is very similar to that given by *Standard Methods* (1985) for persulphate digestion. The final sulphuric acid concentration after sample addition is close to 0,1 M, and that of potassium persulphate is 1% (m/v). This mixture is autoclaved at 98 to 137 kPa for 30 min. The accuracy of this method when applied to activated sludge has not been reported. For activated sludge and its extracts, Lötter (1985) used a digestion procedure (Jirka *et al.*, 1976) suitable for both TP and total Kjeldahl nitrogen (TKN) analyses involving HgO (ca. 1,5 mM), K_2SO_4 (ca. 0,13 M) and H_2SO_4 (ca. 0,6 M). For soil and sediment samples Nelson (1987) used a modified persulphate digestion procedure with final concentrations of 2,75 M H_2SO_4 and 20% potassium persulphate, the time of autoclaving (at 130°C) being increased to 1 h.

Clearly many small differences exist in published methods for assaying the amount and type of phosphorus-containing compounds present in water-related samples. However, little attention has been given to the implications of these differences. The purpose of this paper is to evaluate the above-mentioned techniques for orthophosphate, polyphosphate and total phosphorus determinations with a view to maximum accuracy in analyses of activated sludge extracts.

Materials and methods

Analytical grade reagents were used throughout and all glassware was prewashed in dilute hydrochloric acid, followed by distilled water.

Orthophosphate determinations

The ascorbic acid methods described by *Standard Methods* (1971) and *Standard Methods* (1985) were used with the following exceptions. For the convenience of using test tubes in place of Erlenmeyer flasks, 8 mℓ sample (pH between approximately 2 and 8) was mixed with 1,25 mℓ mixed reagent. No ethanol was added to the sample (*Standard Methods,* 1971). The colour was developed at 25 to 30°C for 30 min and read against a distilled water blank at 880 nm using a Milton-Roy Spectronic 601 spectrophotometer with a 1 cm path length. The mixed reagent was prepared by one of three methods: Method A according to *Standard Methods* (1971); Method B according to *Standard Methods* (1985); and method C identical to A and B except that the potassium antimonyl tartrate reagent was prepared at a concentration of 13,3445 g/ℓ (5 g Sb/ℓ). In each of these three methods, $K(SbO)C_4H_4O$ was used instead of $K(SbO)C_4H_4O.{}^1/_2\ H_2O$ since the former was more readily dissolved in distilled water during preparation of the antimonyl tartrate reagent. Correction was made for the molecular mass difference. In all cases, sodium dihydrogen orthophosphate ($NaH_2PO_4.H_2O$) was used as standard.

The effect of ionic strength on orthophosphate determinations by Methods A, B and C was assessed by the inclusion of 0,50 M or 1,00 M sodium chloride in the orthophosphate standard solutions. The effect of 0,01 M to 1,0 M sulphuric acid and 0,05% to 5,0% (m/v) ammonium persulphate was examined in a similar manner. In the case of ammonium persulphate the concentrated solution was freshly prepared and autoclaved (100 kPa) for 30 min beforehand in the absence of oxidisable material, in order to simulate the effect of heat on residual persulphate concentrations during digestion for total phosphate assays.

The effect of acid-hydrolysable ('condensed') phosphates on the orthophosphate assay was determined by replacing the orthophosphate standard solutions in Methods A and B (Table 1) with standard solutions of sodium pyrophosphate, sodium tripolyphosphate, sodium polyphosphate (Graham's salt) or disodium adenosine triphosphate (ATP). Furthermore, to ensure the absence of ortho, pyro, and tripolyphosphate (Ueno and Ohashi, 1976), the sodium polyphosphate was subjected to gel chromatography (Sephadex G-25) at room temperature using a column (1,6 x 90 cm) and 0,1 M KCl (pH ~ 6) as eluent at a flow rate of 18 mℓ/h. Graham's salt (25 mg), which corresponds to about 5 mg P, was loaded onto the column in approximately 1 mℓ distilled water using a sample applicator. One hundred fractions (3 mℓ each) were collected. A sample (0,05 mℓ) was withdrawn from each fraction, diluted by the addition of 1 M HCl (3 mℓ), heated at 100 kPa in a pressure cooker for 30 min, cooled, diluted tenfold using distilled water and subjected to the orthophosphate determination (Method B). It was found that two major phosphate peaks were eluted in fractions 24 and 31 inclusive. The first corresponded to the void volume (Vo) of the column determined previously using blue dextran. Fractions 24 and 31 were pooled and labelled as the 'high molecular weight' fraction of the sodium polyphosphate sample. The high molecular weight fraction had a mean chain length close to 16 phosphate units, as determined by end-group titration (Van Wazer and Holst, 1950) using 0,01 M standard sodium hydroxide as titrant in place of tetramethylammonium hydroxide.

For comparative purposes, the effect of acid-hydrolysable phosphates was also tested as outlined above using the orthophosphate determinations of Saheki *et al.* (1985) and Chifflet *et al.* (1988).

The effect of condensed phosphates on the orthophosphate determination (Method A) when the former is in admixture with the latter was investigated using an orthophosphate standard solution containing varying amounts of sodium pyrophosphate.

Total phosphate determinations

The persulphate digestion procedure given by *Standard Methods* (1985) was followed except for the following modifications: Digestion was carried out in ordinary Pyrex test tubes (16 x 150 mm) fitted with aluminium caps. It was found that negligible loss of volume occurred during autoclaving. To a 5 mℓ aliquot of sample (prediluted by tenfold in certain instances - see **Results and discussion),** 1 mℓ acid and 1 mℓ *freshly dissolved* ammonium persulphate solution was added. The acid solution was in the form of either 0,7 M or 7 M H_2SO_4 and the ammonium persulphate solution at a concentration of either 7% or 35% (m/v), giving final concentrations as follows: 0,1 M or 1 M for the acid, and 1% or 5% for the $(NH_4)_2S_2O_8$. The procedure given by *Standard Methods* (1985) has final concentrations (upon autoclaving) of approximately 0,1 M H_2SO_4 and 0,8% $(NH_4)_2S_2O_8$. The test tubes were autoclaved at 100 kPa for 30 min, cooled quickly in a basin of tap water and the tubes mixed to remove condensate from the inner walls. The digest was then filtered (Whatman No. 41) where necessary to remove residual particles and diluted at least twenty-fivefold prior to measuring the orthophosphate concentration (see above).

The magnesium nitrate fusion method (Harwood *et al.*, 1969b)

was used for comparative purposes on activated sludge and nucleic acid samples.

Results and discussion

Orthophosphate determination

Effect of potassium antimonyl tartrate concentration

A comparison of the final concentrations of reagents in various modifications of the molybdate blue-ascorbic acid orthophosphate determination is given in Table 1.

From Table 1 it is clear that an increased range for orthophosphate determination is achieved by using higher concentrations of antimonyl tartrate reagent: Although Harwood *et al.* (1969a) also used a higher ascorbic acid concentration than that of *Standard Methods* (1971, 1985), it can be seen from Fig. 1 that a higher antimonyl tartrate concentration alone produces an increased linear range. However, Fig. 1 also indicates that using 8 g Sb/ℓ in the mixed reagent makes the method unreliable since two independent attempts produced different standard curves with turbidity being qualitatively noted in the blank as well as at 0,5 and 1,0 mg P/ℓ during attempt 2. Method C did not exhibit turbidity in a limited number of tests, but in view of ionic strength constraints (see below), a large number of independent trials were not performed to verify this. Harwood *et al.* (1969a) reported turbidity when using the method of Edwards *et al.* (1965) upon which that of *Standard Methods* (1971) is based. Edwards *et al.* (1965) added ethanol (final concentration approximately 5%) to the orthophosphate assay with the purpose of eliminating turbidity arising from elevated potassium antimonyl tartrate concentrations; they postulated that an antimony-phosphorus-molybdenum complex is responsible for such turbidity. Harwood *et al.* (1969a),

TABLE 1
COMPARISON OF REAGENT CONCENTRATIONS AND RATIOS IN MODIFIED MOLYBDATE-ASCORBIC ACID METHODS FOR ORTHOPHOSPHATE DETERMINATIONS.

Method and source	Reagent*	Concentration upon preparation	Volumetric ratio in mixed reagent	Final concentration after addition to sample	Maximum recommended orthophosphate concentration in the sample**
Harwood	I	4 N	5	0,4 N	3,0 mg P/ℓ
et al.	II	1 g Sb/ℓ	2	40 mg Sb/ℓ	
(1969a)	III	9,6 g/ℓ	5	0,96 g/ℓ	
	IV	100 g/ℓ	2	4 g/ℓ	
Standard	I	5 N	10	0,35 N	≥2,0 mg P/ℓ
Methods (1971)	II	8 g Sb/ℓ	1	55,2 mg Sb/ℓ	
and Edwards	III	40 g/ℓ	3	0,83 g/ℓ	
et al. (1965)	IV	17,6 g/ℓ	6	0,73 g/ℓ	
Standard	I	5 N	10	0,35 N	1,3 mg P/ℓ
Methods (1985)	II	1 g Sb/ℓ	1	6,9 mg Sb/ℓ	
	III	40 g/ℓ	3	0,83 g/ℓ	
	IV	17,6 g/ℓ	6	0,73 g/ℓ	
This paper	I	5 N	10	0,39 N	3,0 mg P/ℓ
Method A	II	1 g Sb/ℓ	1	54,1 mg Sb/ℓ	
cf	III	40 g/ℓ	3	0,81 g/ℓ	
SM (1971)	IV	17,6 g/ℓ	6	0,71 g/ℓ	
This paper	I	5 N	10	0,39 N	1,0 mg P/ℓ
Method B	II	1 g Sb/ℓ	1	54,1 mg Sb/ℓ	
cf	III	40 g/ℓ	3	0,81 g/ℓ	
SM (1985)	IV	17,6 g/ℓ	6	0,71 g/ℓ	
This paper	I	5 N	10	0,39 N	1,0 mg P/ℓ
Method C	II	5 g Sb/ℓ	1	33,8 mg Sb/ℓ	
	III	40 g/ℓ	3	0,81 g/ℓ	
	IV	17,6 g/ℓ	6	0,71 g/ℓ	

*I = Sulphuric acid; II = Potassium antimonyl tartrate; III = Ammonium molybdate; IV = Ascorbic acid

** = Assuming 1 cm path length.

SM = *Standard Methods*

using the method of Edwards *et al.* (1965), found, however, that turbidity arose in both samples and blanks and persisted in spite of numerous reagent and ethanol concentrations being tested.

Effect of ionic strength

Results for orthophosphate determinations by Methods A, B and C (refer Table 1) at elevated sample ionic strengths are given in Figs. 2a, 2b and 2c. Method C was identical to Methods A and B with the exception that potassium antimonyl tartrate was prepared at 5 g Sb/ℓ, giving a final concentration after addition to the sample of 34 mg Sb/ℓ.

It is evident from Fig. 2 that at a sample ionic strength of 0,5 M NaCl, only Method B gives no interference. At a sample ionic strength of 1,0 M Method B is also free of interference up to 1 mg P/ℓ. At higher phosphate concentrations this interference was qualitatively observed as turbidity in samples coupled with reduced colour formation. With extended periods of colour development, the particles producing turbidity enlarged and ultimately settled out, leaving little colour in solution.

The importance of ionic strength arises from the need to digest sludge or sludge extract samples for total phosphate analysis. Using the persulphate method described by *Standard Methods* (1985), the ionic strength of the digest following autoclaving

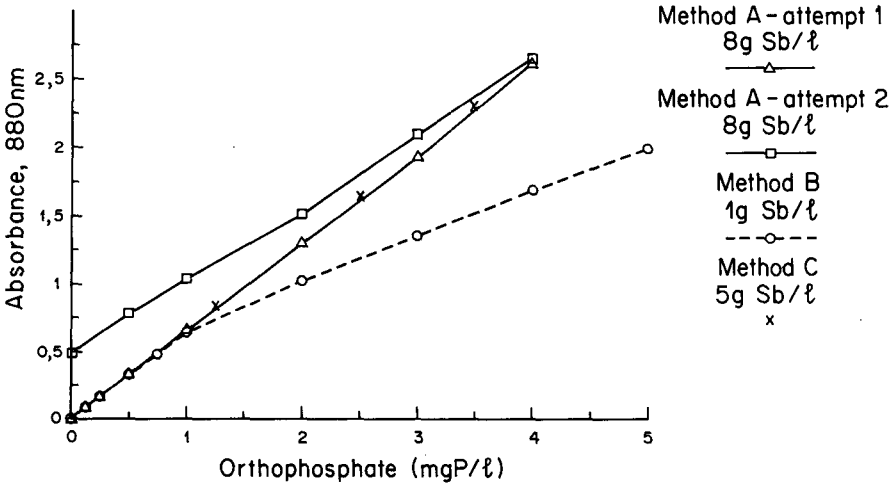

Figure 1

Relationship between absorbance and orthophosphate concentration using different concentrations of potassium antimonyl tartrate reagent in the molybdate blue - ascorbic acid method. Concentrations of the reagent are those upon preparation (Table 1).

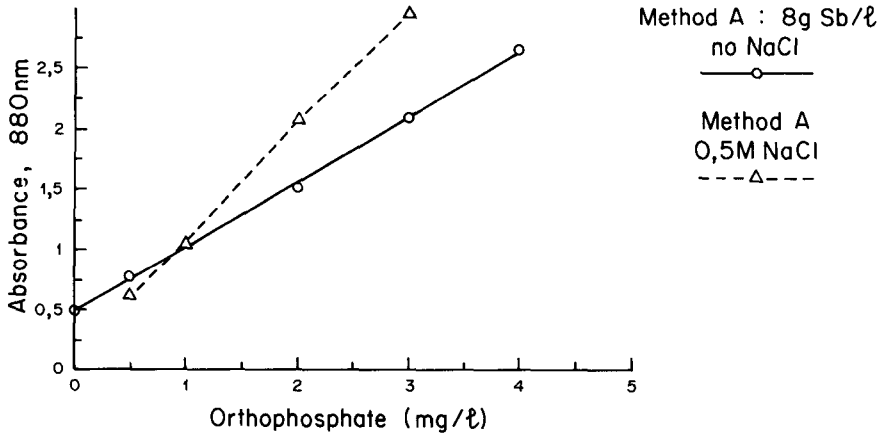

Figure 2a

Effect of sample ionic strength on orthophosphate determination using the molybdate blue - ascorbic acid method with a 8 g Sb/ℓ concentration of potassium antimonyl reagent

(neglecting any contribution from the sample) is approximately 0,4 M. If the sulphuric acid concentration is increased to 1 M (after admixture with sample) and ammonium persulphate to 1% (m/v), then the ionic strength of the digest is at least 3,1 M. Finally, 1 M H_2SO_4 and 5% $(NH_4)_2S_2O_8$ gives a digest ionic strength of over 3,6 M. It follows that if the sample (such as a sludge extract) contains 0 to 1 M NaOH (or 1 M NaCl after neutralisation) the total phosphate digest could have an ionic strength in the range 1,4 to 4,6 M. Hence on the basis of ionic strength alone, at least a threefold dilution of the digest to give ≦ 0,3 M H_2SO_4 and ≦ 0,3% $(NH_4)_2S_2O_8$ is necessary to circumvent ionic interference in subsequent orthophosphate determinations using Method B. This is in agreement with the recommendation of *Standard Methods* (1985) for a twofold dilution of the persulphate digest following autoclaving, but the results suggest that greater dilutions may be necessary if higher antimonyl tartrate concentrations are used (*Standard Methods*, 1971).

Effect of acid concentration

Standard Methods (1985) recommended neutralisation of the persulphate digest following autoclaving prior to the orthophosphate assay. Table 2 shows the effect of sulphuric acid concentration on the orthophosphate assay using Method B. A strong inhibitory ef-

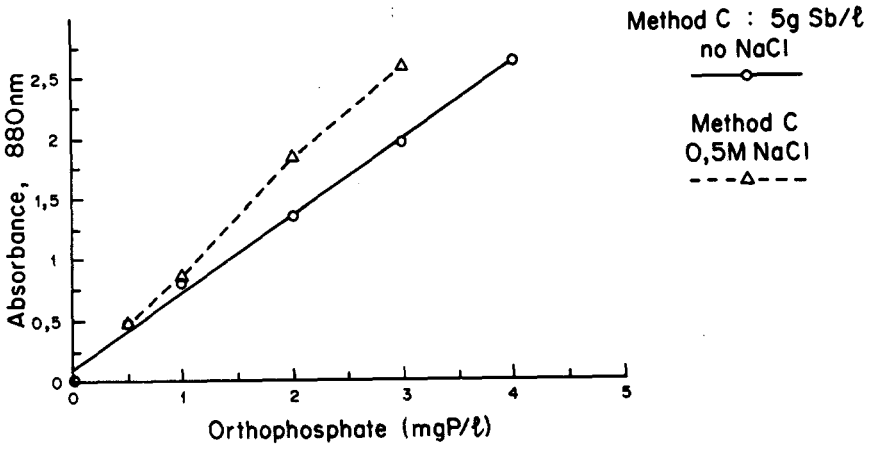

Figure 2b
Effect of sample ionic strength on orthophosphate determination using the molybdate blue - ascorbic acid method with a 5 g Sb/ℓ concentration of potassium antimonyl reagent

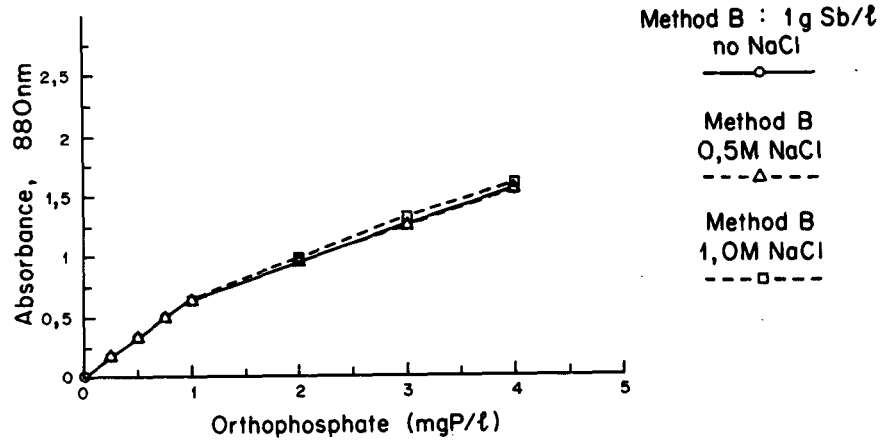

Figure 2c
Effect of sample ionic strength on orthophosphate determination using the molybdate blue - ascorbic acid method with a 1 g Sb/ℓ concentration of potassium antimonyl reagent

fect is exerted on colour formation above 0,2 M H_2SO_4. At or below 0,05 M H_2SO_4, the inhibitory effect is completely absent (allowing for 3% experimental error). If the persulphate digest has a final concentration close to 0,1 M H_2SO_4, then the results indicate agreement with the minimum twofold dilution given by *Standard Methods* (1985). However, if 1 M sulphuric acid is used in the digest, or if the sample (such as a sludge extract) contains strong acid, post-digestion dilutions of at least twentyfold will be necessary to avoid interference on the basis of pH alone.

Effect of persulphate

Allowing for an experimental error of 2%, Table 3 shows that after autoclaving (see **Materials and methods),** ammonium persulphate at an original concentration of ≤ 1% (m/v) has a negligible effect on the orthophosphate determination by Method B (Table 1). Furthermore, the residual persulphate concentrations present in the samples used after autoclaving (Table 3) are maximal in view of the absence of oxidisable material; for samples such as activated sludge extracts, the residual concentrations will be lower.

Effect of acid-hydrolysable phosphates

The effects of ATP and various polyphosphate species on orthophosphate determination by a method employing strong acid (Method A, Table 1), a method employing a mildly acidic mixed reagent (Saheki *et al.,* 1985), and one using citrate to complex excess molybdate (Chifflet *et al.,* 1988) are compared in Figs. 3a, 3b, 3c and 3d. A comparison of the relative interferences in the orthophosphate determination is given in Table 4 for the data in Figs. 3a, 3c and 3d. Similar results were reported by Edwards *et al.* (1965) for pyro and tripolyphosphate using a method similar to Method A (see Table 1). From Figs. 3c and 3d and from Table 4, it is clear, firstly, that contrary to the findings of Saheki *et al.* (1985) and Chifflet *et al.* (1988) the methods employing mildly acidic conditions (pH 5) are only slightly superior to the conventional strongly acidic molybdate blue-ascorbic acid method for the acid-hydrolysable phosphate species tested here; moreover from Figs. 3a and c and Table 4 the method of Saheki *et al.* (1985) appears to be inferior in the case of ATP. Secondly, determination of orthophosphate in the presence of large unknown amounts of polyphosphates and nucleic acids may be subject to large errors. The results for determination (Method A, Table 1) of 1 mg P/ℓ as orthophosphate in the presence of 50 to 200 mg P/ℓ as pyrophosphate are given in Fig. 4. Inhibition of the colorimetric reaction is thus the most likely explanation of the curves in Figs. 3a and 3b obtained at concentrations of acid-hydrolysable phosphates exceeding ca. 75 mg P/ℓ. Although such large amounts of pyrophosphate, tripolyphosphate or ATP may not be present in extracts of activated sludge, longer-chain polyphosphate concentrations in the range 50 to 200 mg P/ℓ could conceivably exist in extracts of activated sludge containing large amounts of stored phosphorus. This is especially true because it is often convenient to concentrate the sludge during an extraction procedure in order to measure the minor phosphate constituents (such as nucleic acid phosphate) by colorimetric methods. Furthermore, it is impossible to measure the polyphosphate concentration independently of the orthophosphate concentration; difference between the orthophosphate levels before and after acid hydrolysis is the standard method for measuring polyphosphate concentration. Because it is not possible to know the exact polyphosphate species extracted, appropriate correction curves also cannot be constructed. For example, a major difference exists in the curves obtained for Graham's salt before and after gel chromatography (Fig. 3a).

Mino *et al.* (1985), Lötter (1985) and Kerdachi and Healey (1987) reported that 0,5 M perchloric acid (PCA) can be used to extract 'low molecular weight' polyphosphates as well as orthophosphate originating from extracellular chemical precipitates or intracellular sources (minimal). The orthophosphate concentrations are determined directly in this extract. In view of the above findings, the orthophosphate concentration determined by strongly acidic methods may be subject to either large positive or negative errors. The methods of Saheki *et al.* (1985) and Chifflet *et al.* (1988) are superior in that negative interference from acid-hydrolysable phosphate compounds was not noted. Since positive interference is not completely avoided in these methods either, it seems advisable to check the result of orthophosphate determinations of activated sludge extracts by means of gel chromatography which separates orthophosphate from polyphosphate (Ueno *et al.,* 1970).

TABLE 2
EFFECT OF SULPHURIC ACID CONCENTRATION IN SAMPLE ON ORTHOPHOSPHATE COLORIMETRIC REACTION (METHOD B). ALL SAMPLES CONTAINED 1,000 mg P/ℓ ORTHOPHOSPHATE.

H_2SO_4 concentration in sample (M)	Orthophosphate determined (mg P/ℓ)
0 (Control)	1,000
0,01	0,996
0,05	0,996
0,1	0,979
0,2	0,968
1,0	0,000

TABLE 3
EFFECT OF SAMPLE AMMONIUM PERSULPHATE CONCENTRATION (FOLLOWING AUTOCLAVING AT 100 kPa FOR 30 MIN) ON ORTHOPHOSPHATE COLORIMETRIC REACTION (METHOD B). ALL SAMPLES CONTAINED 0,510 mg P/ℓ ORTHOPHOSPHATE.

$(NH_4)_2S_2O_8$ concentration in sample prior to autoclaving (% m/v)	Orthophosphate determined (mg P/ℓ) Average of replicates
0 (Control)	0,512
0,05	0,525
0,1	0,521
0,2	0,511
0,5	0,515
1,0	0,525
5,0	0,197

Total phosphate determination

Table 5 summarises the relative total phosphate recoveries using different persulphate digestion methods relative to a magnesium nitrate fusion method. It is evident that although differences in recovery between the methods do not exceed 8%, as the total phosphate content of the activated sludge rises, so the persulphate digestion procedure involving sample predilution and 1 M H_2SO_4 and 5% $(NH_4)_2S_2O_8$ becomes more reliable. The

TABLE 4
PER CENT INTERFERENCE OF ACID-HYDROLYSABLE PHOSPHATES IN ORTHOPHOSPHATE DETERMINATIONS BASED ON STEEPEST POSITIVE GRADIENTS RECORDED IN FIGS. 3a, 3b, 3c and 3d.

Method	Curve	% interference in orthophosphate determination*
Strongly acidic (Method A)	pyrophosphate	3,7
	tripolyphosphate	1,6
	polyphosphate (Graham's salt)	3,9
	polyphosphate (purified Graham's salt)	1,3
	ATP	1,2
Strongly acidic (Method B)	pyrophosphate	4,5
	polyphosphate (Graham's salt)	4,6
Mildly acidic (Saheki *et al.*, 1985)	pyrophosphate	1,5
	polyphosphate (Graham's salt)	2,2
	ATP	10,6
Citrate complexing (Chifflet *et al.*, 1988)	pyrophosphate	1,5
	polyphosphate (Graham's salt)	3,9

$$*\text{Interference} = \frac{100}{I} \times \frac{\text{steepest positive gradient of acid-hydrolysable phosphate curve}}{\text{gradient of orthophosphate curve}}$$

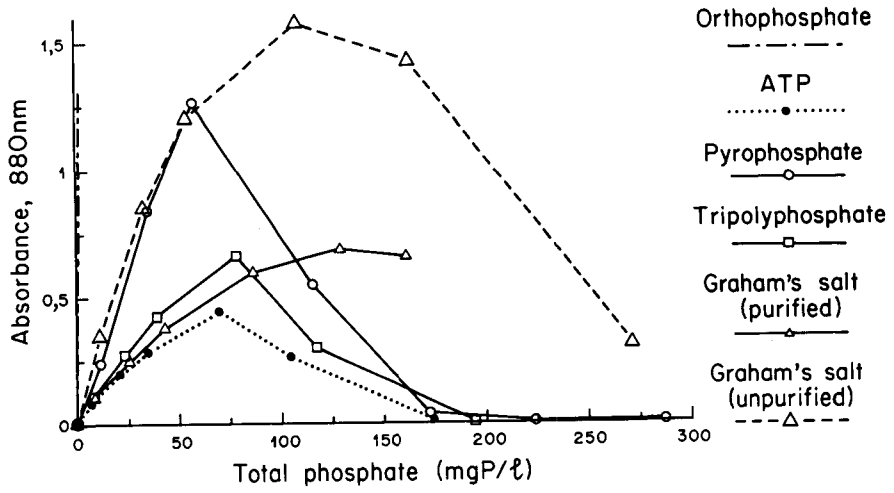

Figure 3a
Effect of acid-hydrolysable phosphates on orthophosphate determinations by Method A - strongly acidic

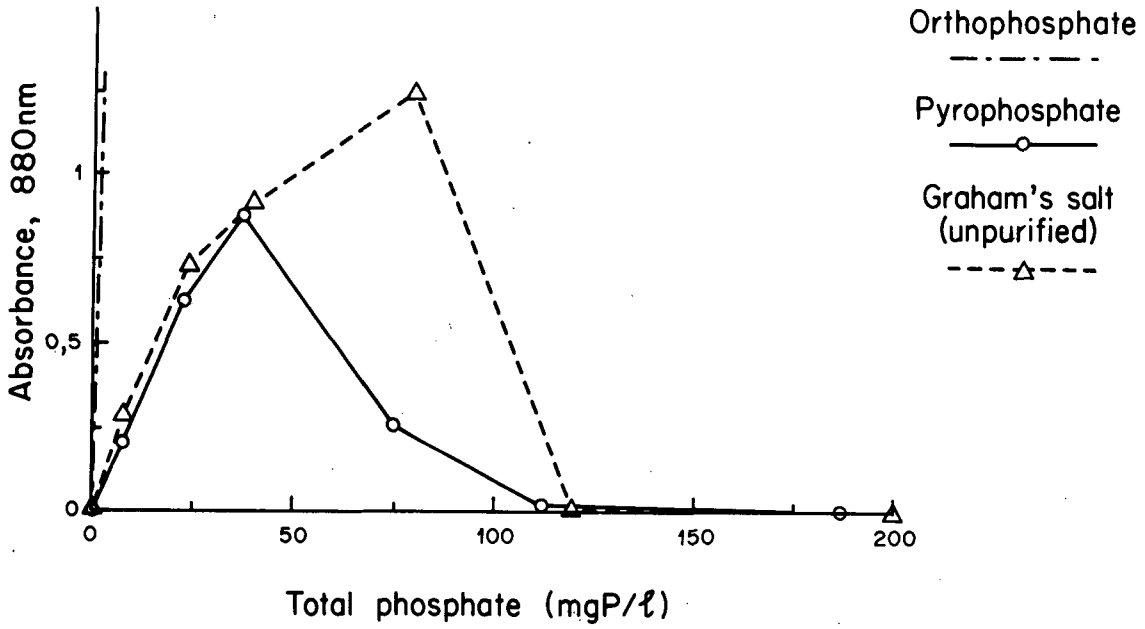

Figure 3b
Effect of acid-hydrolysable phosphates on orthophosphate determinations by Method B - strongly acidic

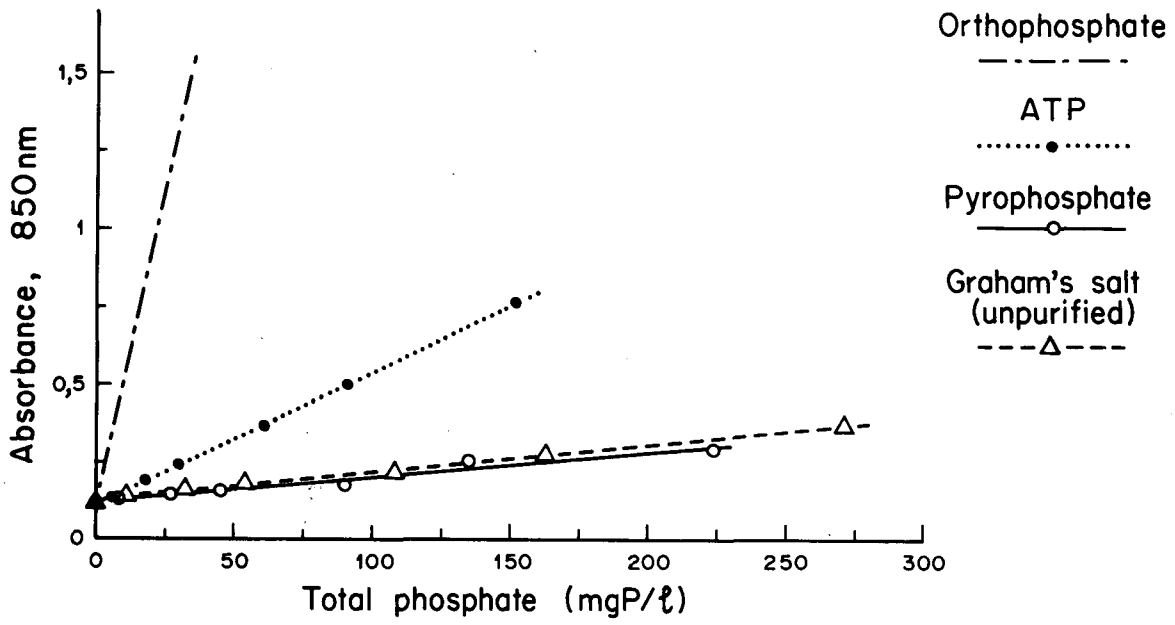

Figure 3c
Effect of acid-hydrolysable phosphates on orthophosphate deteminations by method of Saheki et al. *(1985) - mildly acidic*

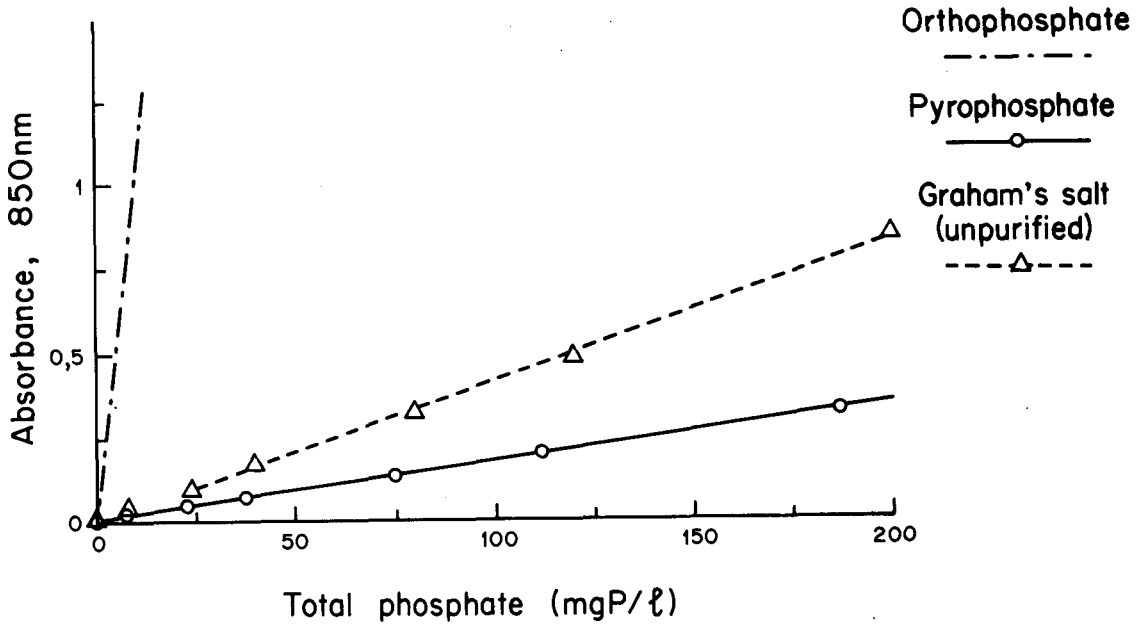

Figure 3d

Effect of acid-hydrolysable phosphates on orthophosphate determinations by method of Chifflet et al. *(1988) - citrate complexing*

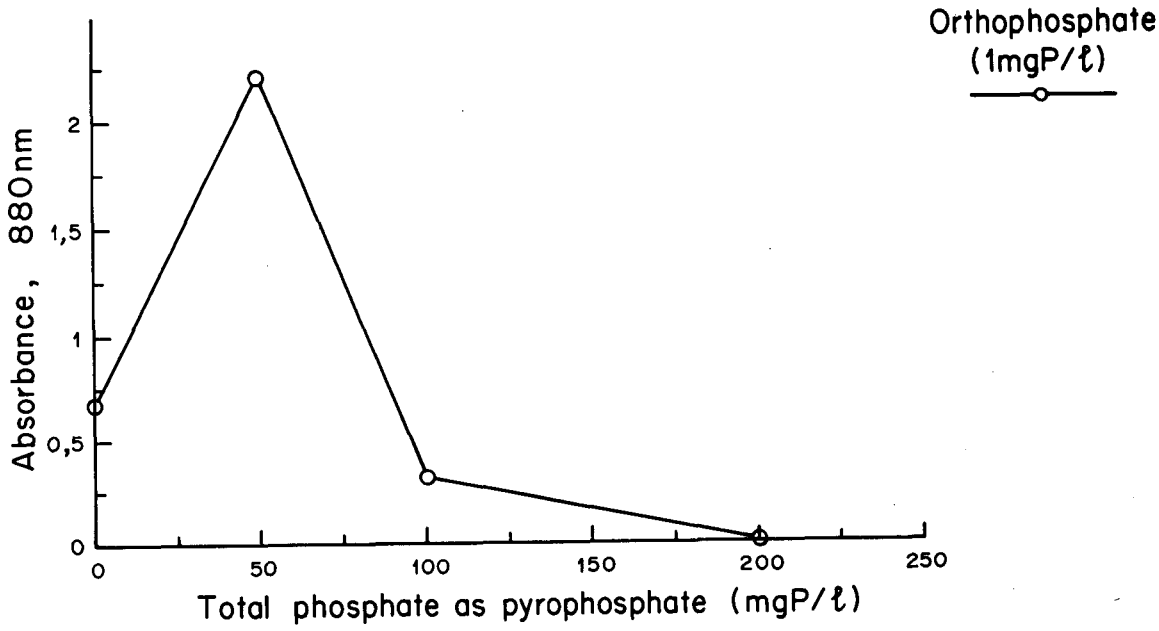

Figure 4

Variation in colour formation by samples containing 1 mg P/ℓ orthophosphate and increasing concentrations of polyphosphate using the strongly acidic molybdate blue - ascorbic acid method (Method A). Absorbance of blank (0 mg P/ℓ) = 0,008

tediousness of the magnesium nitrate method precludes it from routine use. It does not offer significantly higher recoveries (lower in certain cases) than the 1 M H_2SO_4 –5% $(NH_4)_2S_2O_8$ method for activated sludge and RNA samples. A similar result was obtained by Harwood *et al.* (1969b) for other biological materials including algae and samples from blood, sewage and brewery digesters. However, Harwood *et al.* (1969b) used a persulphate method closely resembling Method IV (Table 5) which here was found to be slightly inferior.

Conclusions

- Although it is possible to achieve an increased range for orthophosphate determination with elevated antimonyl tartrate concentrations in the molybdate blue-ascorbic acid method, the method becomes unreliable with this modification since turbidity may arise in blanks and samples, especially at high ionic strengths (≥ 0,5 M). The method given by *Standard Methods* (1985) is satisfactory up to 1 mg P/ℓ and 1 M ionic strength. This method may be modified to allow determinations to be performed in test tubes rather than Erlenmeyer flasks.
- For activated sludge and nucleic acid samples, the persulphate digestion procedure given by *Standard Methods* (1985) for total phosphate determinations was found to give slightly inferior recoveries relative to other methods. Of the other methods, a convenient one involves final concentrations of 1 M sulphuric

TABLE 5
COMPARISON OF RECOVERIES OF TOTAL PHOSPHATE BY DIGESTION OF ACTIVATED SLUDGE AND NUCLEIC ACID SAMPLES USING MODIFIED PERSULPHATE METHODS AND A MAGNESIUM NITRATE FUSION METHOD.

Sample*	Sample predilution	Digestion method**	Total phosphate determined (mg P/ℓ)	Recovery relative to Method 1 (%)
Activated sludge No. 1	-	I	184,5	-
	-	II	177,7	96,3
	-	III	171,1	92,7
	-	IV	167,8	91,0
Activated sludge No. 2	-	I	322,0	-
	-	II	325,7	101,2
	10x	II	343,3	106,6
	-	III	308,2	95,7
	10x	III	310,4	96,4
Activated sludge No. 3	-	I	467,5	-
	-	II	466,1	99,7
	10x	II	500,1	107,0
	-	III	463,9	99,2
	10x	III	486,9	104,2
Activated sludge No. 4	-	I	528,0	-
	-	II	558,2	105,7
	10x	II	570,3	108,0
	-	III	550,5	104,3
	10x	III	561,5	106,3
Ribonucleic (RNA)	-	I	16,5	-
standard (251 mg/ℓ)		II	17,4	105,4
(Sigma Type IV)		III	16,3	98,5
Orthophosphate		I	100,1	-
standard (101,7 mg P/ℓ)		II	101,0	-

*Activated sludge No. 1 = Municipal full-scale Bardenpho plant
Activated sludges Nos. 2-4 = Laboratory-scale Bardenpho units receiving synthetic feed

** I = Magnesium nitrate fusion
II = Persulphate digestion : 1 M H_2SO_4, 5% $(NH_4)_2S_2O_8$
III = Persulphate digestion : 1 M H_2SO_4, 1% $(NH_4)_2S_2O_8$
IV = Persulphate digestion : 0,1 M H_2SO_4, 1% $(NH_4)_2S_2O_8$

acid and 5% ammonium persulphate (after mixing with sample). In the case of samples having high total phosphate contents ($\geq$ 100 mg P/ℓ), a tenfold predilution of sample may be advisable. Irrespective of sample predilutions, a post-digestion dilution of at least twentyfold with distilled water is needed to avoid interference in the method for orthophosphate determination due to the effects of high ionic strength and acidity.

- Contrary to the methods of Mino *et al.* (1985) and Lötter (1985), it is inadvisable to determine orthophosphate (especially at relatively low concentrations) in the presence of acid-hydrolysable (condensed) phosphates such as polyphosphates and nucleotides. Cross-checking for interference may be possible if ortho and polyphosphates are separated by gel chromatography (Ueno *et al.*, 1970). At concentrations up to approximately 75 mg P/ℓ (total P), these substances give positive interference of up to 4% in the strongly acidic molybdate blue-ascorbic orthophosphate determination *(Standard Methods,* 1985). At higher concentrations, they exert negative interference (inhibition). In an orthophosphate method which functions at pH 5 (Saheki *et al.*, 1985) and a related method (Chifflet *et al.*, 1988), inhibition was not noted although similar levels of positive interference occurred, and the latter method was markedly less sensitive towards orthophosphate.

References

ARVIN, E (1985) Biological removal of phosphorus from wastewater. *CRC Critical Reviews in Environmental Control* **15** 25-64.

CHIFFLET, S, TORRIGALIA, A, CHIESA, R and TOLOSA, S (1988) A method for the determination of inorganic phosphate in the presence of labile organic phosphate and high concentrations of protein : Application to lens ATPase. *Anal. Biochem.* **168** 1-4.

CLESCERI, NL and LEE, FG (1965) Hydrolysis of condensed phosphates - I. Non-sterile environment. *Int. J. Air Wat. Poll.* **9** 723-742.

COMEAU, Y, HALL, KJ, HANCOCK, REW and OLDHAM, WK (1986) Biochemical model for enhanced biological phosphorus removal. *Wat. Res.* **20** 1511-1521.

EDWARDS, GP, MOLOF, AH and SCHNEEMAN, RW (1965) Determination of orthophosphate in fresh and saline waters. *J. Am. Water Works Ass.* **57** 917-925.

FUHS, GW and CHEN, M (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microbial Ecology* **2** 119-138.

GALES, ME, JULIAN, ER and KRONER, RC (1966) Method for quantitative determination of total phosphorus in water. *J. Am. Water Works Ass.* **58** 1363-1368.

HARWOOD, JE, VAN STEENDEREN, RA and KÜHN, AL (1969a) A rapid method for orthophosphate analysis at high concentrations in water. *Wat. Res.* **3** 417-423.

HARWOOD, JE, VAN STEENDEREN, RA and KÜHN, AL (1969b) A comparison of some methods for total phosphate analyses. *Wat. Res.* **3** 425-432.

HEINKE, GW and NORMAN, JC (1969) Hydrolysis of condensed phosphates in wastewater. *Proceedings of 24th Industrial Waste Treatment Conference,* May, Purdue University, Lafayette, Indiana. 644-654.

JIRKA, AM, CONKER, MJ, MAY, D and FULLER, FD (1976) Ultra-micro semi-automated method for simultaneous determination of total phosphorus and total Kjeldahl nitrogen in wastewaters. *Env. Sci. Techno.* **10** 1038-1044.

KERDACHI, DA and HEALEY, KJ (1987) The reliability of cold perchloric acid extraction to assess metal-bound phosphates. *Proceedings of IAWPRC Specialized Conference on Biological Phosphate Removal from Wastewaters,* 28-30 Sept., Rome, Italy.

LELOIR, LF and CARDINI, CE (1957) Characterisation of phosphorus compounds by acid lability. *Methods Enzymol.* (Eds. Colowick, SP and Kaplan, NO) **III** 840-850.

LÖTTER, LH (1985) The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. *Wat. Sci. Tech.* **17** 127-138.

LOWRY, OH and LOPEZ, JA (1946) The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.* **163** 421-428.

MINO, T, KAWAKAMI, T and MATSUO, T (1985) Location of phosphorus in activated sludge and function of intracellular polyphosphates in biological phosphorus removal process. *Wat. Sci. Tech.* **17** 93-106.

MURPHY, J and RILEY, JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta.* **27** 31-36.

MURPHY, M and LÖTTER, LH (1986) The effect of acetate and succinate on polyphosphate formation and degradation in activated sludge, with particular reference to *Acinetobacter calcoaceticus. Appl. Microbiol. Biotechnol.* **24** 512-517.

NELSON, NS (1987) An acid-persulphate digestion procedure for determination of phosphorus in sediments. *Comm. in Soil Sci. Plant Anal.* **18** 359-370.

RIDING, JT, ELLIOT, WR and SHERRARD, JH (1979) Activated sludge phosphorus mechanisms. *J. Wat. Pollut. Control Fed.* **38** 990-995.

SAHEKI, S, TAKEDA, A and SHIMAZU, T (1985) Assay of inorganic phosphate in the mild pH range, suitable for measurement of glycogen phosphorylase activity. *Anal. Biochem.* **148** 277-281.

Standard Methods for the Examination of Water and Wastewater (13th edn.) (1971) American Public Health Association, Washington DC.

Standard Methods for the Examination of Water and Wastewater (16th edn.) (1985) American Public Health Association, Washington DC.

SUTTON, PM, JANK, BE and VACHON, D (1980) Nutrient removal in suspended growth systems without chemical addition. *J. Water Pollut. Control Fed.* **52** 98-109.

UENO, Y, YOZA, N and OHASHI, S (1970) Gel chromatographic behaviour of linear phosphates. *J. Chromatog.* **52** 481-485.

VAN WAZER, JR and HOLST, KA (1950) Structure and properties of the condensed phosphates. *J. Am. Chem. Soc.* **72** 639-663.

WENTZEL, MC, LÖTTER, LH, LOEWENTHAL, RE and MARAIS, GvR (1986) Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal - A biochemical model. *Water SA* **12** 209-224.