Some considerations in polyphosphate determinations of 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 polyphosphate determinations when applied to extracts of activated sludge or artificial solutions simulating such extracts.

It was found that powdered activated carbon (PAC) in stirred batch systems may be used selectively to remove nucleic acids from mixtures with polyphosphates, but simultaneous polyphosphate adsorption occurs in weakly ionic environments at acidic, neutral or basic pH. The addition of 1% (m/v) trichloroacetic acid (TCA) and 0,5 M perchloric acid (PCA), or the sodium salts of these acids, served to abolish the adsorption of polyphosphate (Graham's salt, n = 16) at acidic, neutral or basic pH. Moreover, the adsorption of nucleic acids to PAC is enhanced by 1% TCA at neutral to basic pH. A convenient method for washing PAC free of phosphate is supplied. The significance of the above findings is discussed in relation to published results for chemical fractionation of activated sludge phosphorus compounds.

Introduction

Biochemical models recently have been proposed for enhanced biological phosphorus removal in modified activated sludge systems (Comeau *et al.*, 1986, 1987; Wentzel *et al.*, 1986). Polyphosphate is a key component of the mechanism suggested by these models, implying that it can be accumulated by activated sludge bacteria to concentrations as high as 7 to 13% phosphorus (P) on a dry weight basis of mixed liquor suspended solids (MLSS) in systems recieving domestic sewage as influent (Arvin, 1985; De Haas, 1988). In the past, however, attention has been given to the formation (or potential formation) of chemical phosphorus precipitates in activated sludge (Menar and Jenkins, 1970; Fuhs and Chen, 1975; Arvin and Kristensen, 1985; Kerdachi and Roberts, 1985; Kerdachi and Healey, 1987). In an attempt to distinguish between different forms of accumulated phosphorus, modified forms of the STS procedure (Schmidt and Thannhauser, 1945; Schneider, 1945) and Harold's procedure (Harold, 1963) have been applied to activated sludges (*inter alia* Mino *et al.*, 1985; Kerdachi and Roberts, 1985; Lötter, 1985; Murphy and Lötter, 1986; Kerdachi and Healey, 1987). However, uncertainty surrounds these results, particularly because the analytical accuracy of methods used to determine polyphosphate in the extracts was not reported.

During standard procedures for water and waste-water analysis, organic phosphate compounds and polyphosphates are generally grouped together as 'acid-hydrolysable phosphorus'. For example, after exclusion of suspended material (by 0,45 μm membrane filtration), dissolved reactive phosphorus (determined by direct colorimetry for orthophosphate) is distinguished from dissolved acid-hydrolysable phosphorus (after mild acid hydrolysis) and total dissolved phosphorus (acid digestion in the presence of strong acid and/or oxidising agent) (*Standard Methods*, 1985). Clearly in such procedures the acid concentration, time and temperature during mild acid hydrolysis are critical factors. In the above-mentioned example (*Standard Methods*, 1985), the total acid concentration is about 0,003 M and the sample is heated at 90 to 137 kPa for 30 min. Under these conditions polyphosphates and certain organic phosphates, that occur in some natural waters and which do not generally respond to reactive phosphorus tests, are hydrolysed to orthophoshate (*Standard Methods*, 1985). A standard method for distinguishing polyphosphates from organic phosphates such as nucleic acids has not been reported.

In the literature it is common to find polyphosphate concentration in cell extracts determined as the difference between orthophosphate concentration before and after hydrolysis in 1 M hydrochloric or 1 M sulphuric acid. Various times and temperatures of hydrolysis have been used, *inter alia* : 7 min at 100°C (Langen and Liss, 1958; Mino *et al.*, 1985), 15 min at 95 to 100°C (Harold, 1963; Rao *et al.*, 1985) and 15 min at 70°C (Lötter, 1985). These methods are often encountered in association with either activated carbon treatment of cell extracts, ostensibly to specifically remove nucleic acids (Langen and Liss, 1958; Liss and Langen, 1960; Harold, 1963; Lötter, 1985; Mino *et al.*, 1985), or barium precipitation of polyphosphates followed by carbon treatment to remove any coprecipitated nucleic acid (Rao *et al.*, 1985). Harold (1963) used Norit A powdered activated carbon, but in other cases (Langen and Liss, 1958; Liss and Langen, 1960; Lötter, 1985; Mino *et al.*, 1985; Rao *et al.*, 1985) the carbon type was not specified. Similarly, although the carbon was usually prewashed in acid, the exact conditions were not reported. Crane (1958) reported that in 0,05 M HCl (pH ~ 1,3), 8,4% of inorganic orthophosphate in a mixture with sugar and nucleotide phosphates was adsorbed to Norit A carbon (0,06) g/m(). Under these conditions, 100% of the phosphate as adenosine-5'-triphosphate (ATP) or as ribose-5'-triphosphate was adsorbed, and more than 90% of various hexose phosphates (Crane, 1958). In the procedures of Harold (1963), Lötter (1985) and Mino *et al.* (1985), activated carbon was also used to remove nucleic acids and nucleotides from strongly acidic solutions. Liss and Langen (1960) used activated carbon to remove nucleic acids and protein from a mixture containing polyphosphate at near-neutral pH; only 2% of the polyphosphate was adsorbed. Rao *et al.* (1985) used activated carbon to remove nucleotides from a barium precipitate of polyphosphate at pH 4,5; they did not report on the adsorption of polyphosphate.

The purpose of this paper is to evaluate the above-mentioned techniques for polyphosphate determination with a view to maximum accuracy in analysis of activated sludge extracts.

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

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Materials and methods

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

Polyphosphate determinations

Hydrolysis of polyphosphate (Graham's salt) (Merck) or ribonucleic acid (RNA Sigma Type IV from calf liver) was performed using standard solutions of these compounds in the presence of 1 M HCl at 95,5°C for 7 min (Langen and Liss, 1958; Mino *et al.*, 1985), 1 M HCl at 70°C for 15 min (Lötter, 1985) or ~ 0,0054 M acid (sulphuric mixed with nitric) in an autoclave at 100 kPa for 30 min (Standard Methods, 1985). Dilutions of at least ten-fold were performed following hydrolysis and the orthophosphate content of the hydrolysate determined using the ascorbic acid method (*Standard Methods,* 1985). Total phosphate determinations were performed by the persulphate digestion procedure given below using 1 M H_2SO_4 and 10 g/ℓ $(NH_4)_2S_2O_8$ (final concentrations) as well as by the magnesium nitrate fusion method described by Harwood *et al.* (1969).

Activated carbon studies

Two types of powdered activated carbon were studied: Norit A (medicinal type no longer in commercial use, obtained from CSIR, Pretoria) and Norit SA 4 (modern commercial equivalent, donated by Holpro Chemical Corporation, Johannesburg). These carbons were subjected to acid washing by one of various methods (see **Results and Discussion**) prior to testing their adsorption of polyphosphate (Graham's salt used without further purification), RNA or DNA.

These adsorption tests were performed in conical flasks or beakers with a carbon concentration in the range 1 to 4 g per 80 mℓ solution (see **Results and Discussion).** The resulting suspensions were stirred at room temperature for 60 min, followed by centrifugation and filtration through 0,45 μm membrane filters (Millipore). Such filtration was usually necessary to completely clarify the supernatant, owing to the fineness of the carbon particles. Highly polymeric samples (especially those of nucleic acids) were resistant to 0,45 μm filtration, in which case filtration through glass-fibre (Whatman GF/C) proved adequate. Adsorption of nucleic acids was measured by difference in absorbance at 260 nm, with distilled water dilution where necessary.

Standard absorbance curves were constructed using commercial preparation of RNA (Sigma Type IV from calf liver) and DNA (Sigma Type III from salmon testes). The total phosphate content of these preparations was determined by magnesium nitrate fusion (Harwood *et al.,* 1969). The protein content, by the method of Hartree (1972), was found to be 2,8% (m/v) and 4,6% (m/v) for the above-mentioned RNA and DNA preparations respectively.

Results and discussion

Polyphosphate determinations

7-min or 15-min phosphate

Table 1 gives the results of 7 and 15-min determinations by two methods for standard RNA and polyphosphate solutions. Table 1A indicates that although the recovery of polyphosphate by this method exceed 90%, close to 15% of the RNA phosphate is also hydrolysed with 1 M HCl at 95°C for 7 min (Langen and Liss, 1958). This interference by the RNA phosphate will be greater if a boiling temperature closer to 100°C is attained, or if a 15-min period of hydrolysis at 100°C is employed (Harold, 1963; Rao *et al.*, 1985). Using 1 M HCl at 70°C for 15 min (Lötter, 1985), RNA hydrolysis amounted to less than 2%, but only slightly more than 50% of the polyphosphate was hydrolysed (Table 1b). Although the exact level of interference will vary with the sample and with the RNA and polyphosphate species, these results indicate that the above-mentioned polyphosphate determinations are sub-

TABLE 1
COMPARISON OF ORTHOPHOSPHATE YIELDED BY (A) 7-MIN HYDROLYSIS (1 M HCl, 95,5°C); AND (B) 15-MIN HYDROLYSIS (1 M HCl, 70°C) WITH THE TOTAL PHOSPHATE CONTENT OF STANDARD RNA AND POLYPHOSPHATE (GRAHAM'S SALT) SOLUTIONS. AVERAGE OF DUPLICATES.

A	7-min phosphate (mg P/ℓ)	Total phosphate (mg P/ℓ)	% recovery of total P as 15-min-P
RNA	8,124	55,900*	14,5
Polyphosphate	182,498	196,105**	93,1
B	**15-min phosphate (mg P/ℓ)**	**Total phosphate (mg P/ℓ)**	**% recovery of total P as 15-min P**
RNA	1,175	83,200*	1,4
Polyphosphate	101,050	193,013**	52,3

* Determined by magnesium nitrate fusion (see **Materials and Methods)**
** Determined by persulphate digestion (1 M H_2SO_4, 1% $(NH_4)_2S_2O_8$) (see **Materials and Methods)**

TABLE 2
COMPARISON OF ORTHOPHOSPHATE YIELDED AS 'ACID-HYDROLYSABLE PHOSPHATE' WITH THE TOTAL PHOSPHATE CONTENT OF STANDARD RNA AND POLYPHOSPHATE (GRAHAM'S SALT) SOLUTIONS. AVERAGE OF DUPLICATES.

	Acid-hydrolysable phosphate (mg P/ℓ)	Total phosphate (mg P/ℓ)	% recovery of acid-hydrolysable P as total P
RNA	12, 575	58,640*	21,4
Polyphosphate	224, 152	237,233**	94,5

See footnote of Table 1

TABLE 3
EFFECT OF pH ON ORTHOPHOSPHATE ADSORPTION TO NORIT A ACTIVATED CARBON AT A CARBON CONCENTRATION OF 0,3 g/10 mℓ.

pH	Orthophosphate concentration before carbon treatment (mg P/ℓ)	Orthophosphate concentration after carbon treatment (mg P/ℓ)	% Δ *
1,5	3,6	6,1	169
4,0	3,6	0,9	25
7,0	3,6	0,9	25
10,0	3,5	1,3	37

$$* \% \Delta = \frac{100}{1} \times \frac{\text{mg P/ℓ after}}{\text{mg P/ℓ before}}$$

ject to significant errors. It may be advisable to determine the polyphosphate content of activated sludge or its extracts by the difference between total, phospholipid and nucleic acid phosphate levels. The latter may be based on sugar colorimetric reactions (Ceriotti, 1955).

Acid-hydrolysable phosphate

Table 2 presents the relevant results for using the procedure given by *Standard Methods* (1985) to determine acid-hydrolysable phosphate. It indicates that, using this procedure, polyphosphate cannot be accurately assayed in the presence of nucleic acid.

Activated carbon treatment for nucleic acid removal

Crane (1958) tested nucleotide adsorption to Norit A activated carbon at a carbon concentration of 0,3 g/5 mℓ. This produces a very dense slurry. Accordingly a lower carbon concentration was used. Table 3 presents the results of tests for orthophosphate adsorption to Norit A at a carbon concentration of 0,3 g/10 mℓ. It is clear that while phosphate was desorbed from the carbon at strongly acidic pH (1,5), it was adsorbed at higher pH.

The Norit A carbon used in the experiments for Table 3 was not free of phosphate. It had been washed at room temperature twice by stirring in ca. 1 M HCl, followed by stirring twice in distilled water. Low-speed centrifugation was used between successive steps and final drying performed at 100°C. One of the problems encountered in this washing procedure was caused by the small particle size of Norit A. This caused it to form a paste-like pellet upon centrifugation which was difficult to resuspend in the next washing step so that a large number of washing steps would be tedious to perform. It was found that at least 10 washing steps at room temperature, each of at least 10-min duration, using 0,2 M HCl, were necessary to reduce the orthophosphate of the supernatant to approximately 40 μg P/ℓ. At each step, 10 g Norit A was stirred in 500 mℓ acid at each step. Subsequently it was found that both Norit A and Norit SA 4 powdered carbons could be filtered using Whatman GF/A of GF/C filters and that hot acid-persulphate treatment (see *Total Phosphate Determinations* in *Standard Methods,* 1985) considerably speeded up the process of washing carbon free of phosphate (Table 4). The carbon may be washed on the filter until the filtrate is neutral to pH indicator paper.

Table 4 shows that after three hot treatments in the presence of acid and persulphate (the second and third steps rely on the residual levels from the first step), the filtrate still contains low but measurable concentrations of orthophosphate, similar to those after ten or more washings with dilute acid at room temperature. Furthermore, the phosphate content of the filtrate after step one for Norit A is about one fifth of that for Norit SA 4. However, the particle size of Norit SA 4 was qualitatively noted to be slightly larger than that of Norit A, giving a reduced tendency to form

TABLE 4
RESULTS OF PROCEDURE FOR REMOVING PHOSPHATE FROM NORIT POWDERED ACTIVATED CARBONS. CARBON CONCENTRATION = 10 g/500 mℓ.

Carbon	Step no.*	mg P/ℓ in filtrate	mg P released per g carbon
Norit SA 4	1	11,922	0,596
	2	0,097	0,005
	3	0,044	0,002
Norit A	1	2,408	0,120

* Step 1: 1 M H_2SO_4 + 10 g/ℓ $(NH_4)_2S_2O_8$. Autoclave (100 kPa) for 30 min.
Steps 2 and 3: Distilled water. Autoclave as before.

paste-like pellets upons centrifugation or filtration. The particle distribution of Norit SA 4 is given by the manufacturer as 10 to 44 μm; that of Norit A was unavailable. According to the manufacturer, Norit SA 4 is used commercially for purifying sodium tripolyphosphate to meet technical specifications for industrial and domestic softeners. It was therefore considered the carbon of choice for purifying extracts of activated sludge containing polyphosphates. However, to minimise analytical error, adsorption of polyphosphate to the carbon should be minimised, while that of nucleic acids should be maximised. On the basis of charge, polyphosphate adsorption to carbon in response to pH in the range 1 to 10 might be expected to be similar to orthophosphate (Table 3) although the strong (K_1) and weak (K_2) acid functions weaken with increasing polyphosphate chain length (i.e. K_1 and K_2 increase) (Van Wazer and Holst, 1950).

However, Norit SA 4 subjected to hot acid washing (Table 4) shows very different adsorption characteristics toward orthophosphate and polyphosphate in response to pH; the adsorption toward orthophosphate also differs from that of Norit A (Table 3 and Fig. 1d). Strong adsorption of polyphosphate to both Norit SA 4 and Norit A occurred at pH 1 and 1,5 with distilled water and 0,05 M NaOH as starting solutions (Figs. 1d and 1e). It is noteworthy that since HCl was used for adjusting the pH of the latter; at pH 1 and 1,5 the starting solution is really 0,05 M NaCl. This concentration of NaCl is nevertheless effective at neutral pH in preventing polyphosphate adsorption, as is unneutralised 0,05 M NaOH (pH 13). Reduced concentrations of

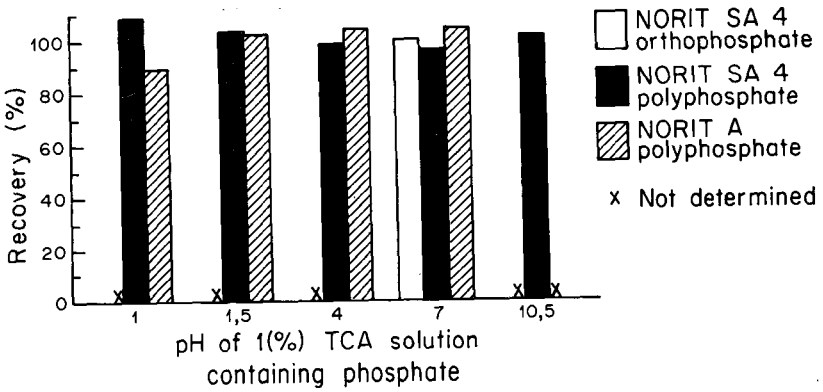

Figure 1a

Figure 1

Effect of pH and ionic composition of starting solution on adsorption of polyphosphate (ca. 100 mg P/ℓ as Graham's salt) and orthophosphate (ca. 100 mg P/ℓ) to powdered activated carbons Norit A and Norit SA 4. pH values are to the nearest 0,5 unit. X implies 'Not determined'.

$$\text{Recovery (\%)} = \frac{\text{TP after}}{\text{TP before}} \times \frac{100}{1}$$

where TP is the total phosphate content of the supernatant before and after carbon treatment. Carbon concentration = 1 g/80 mℓ throughout. See ***Results and Discussion*** *for further explanation.*

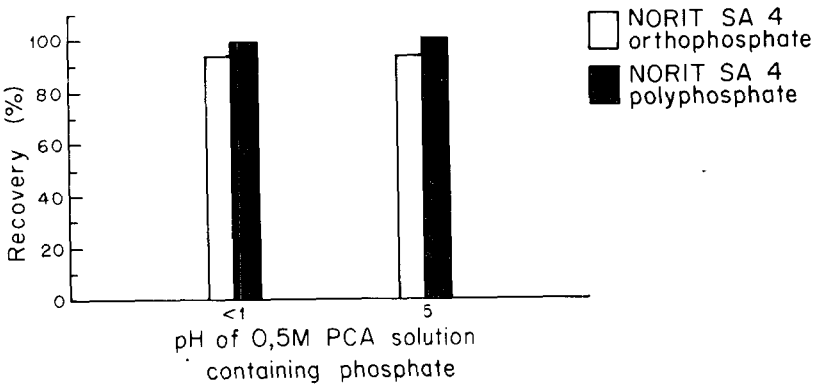

Figure 1b

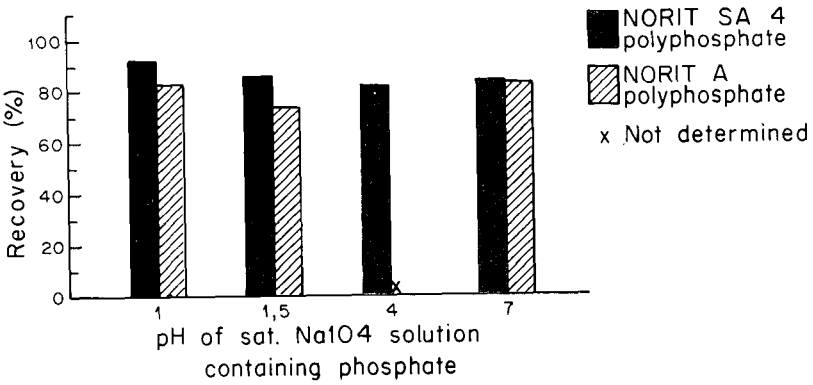

Figure 1c

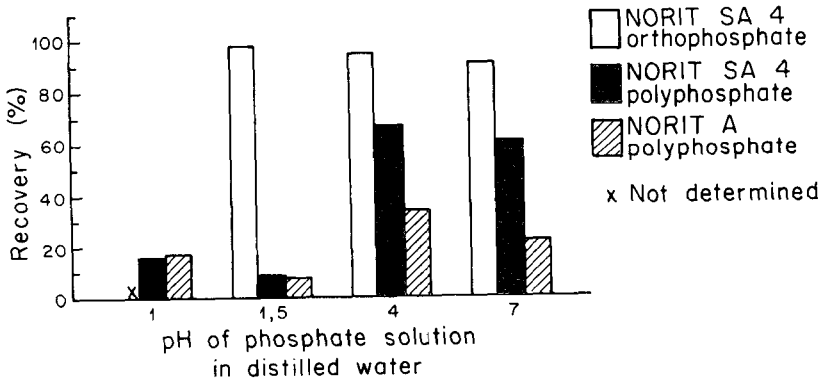

Figure 1d

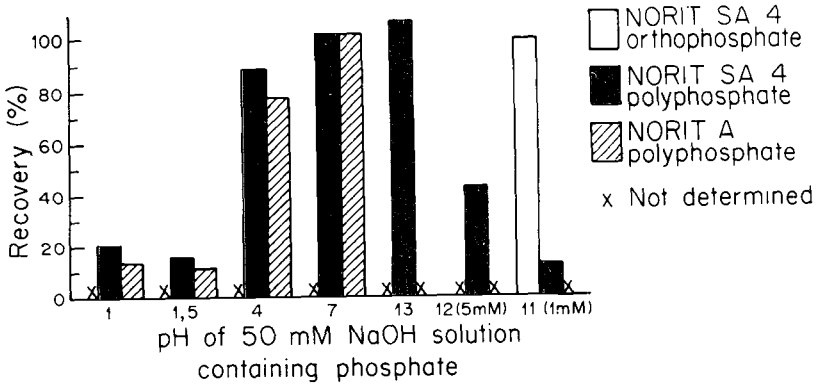

Figure 1e

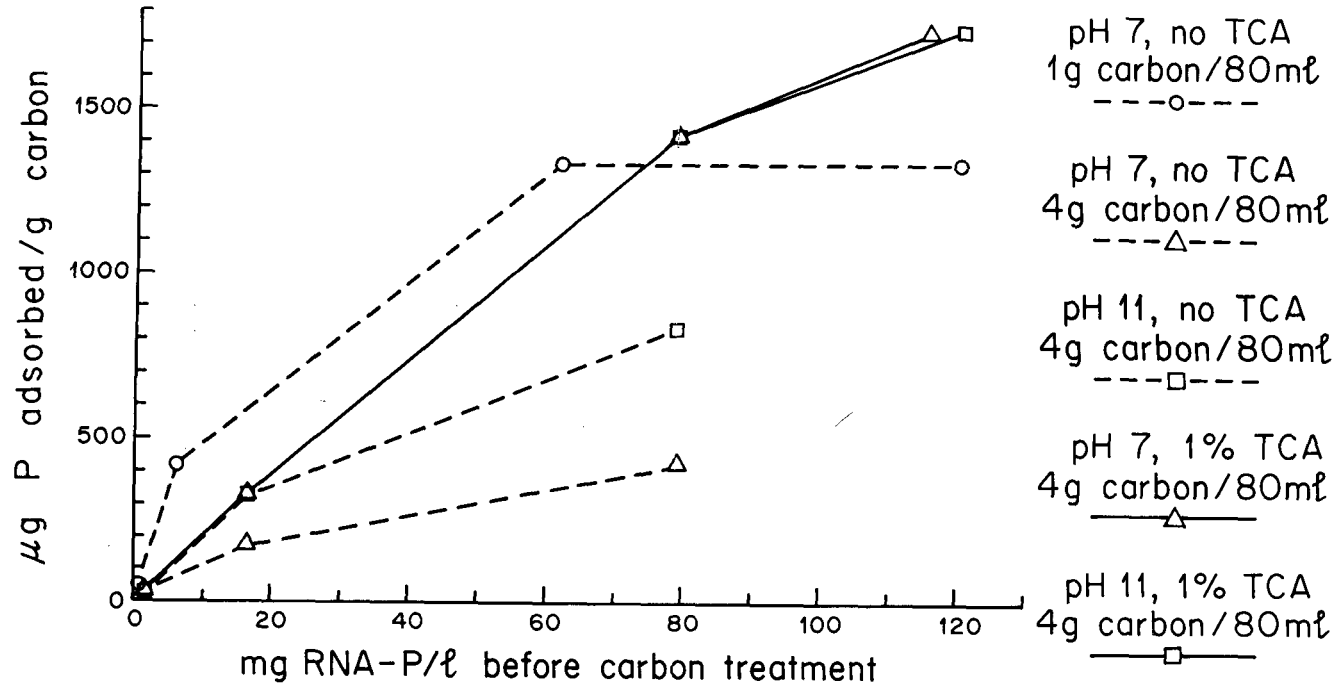

Figure 2a

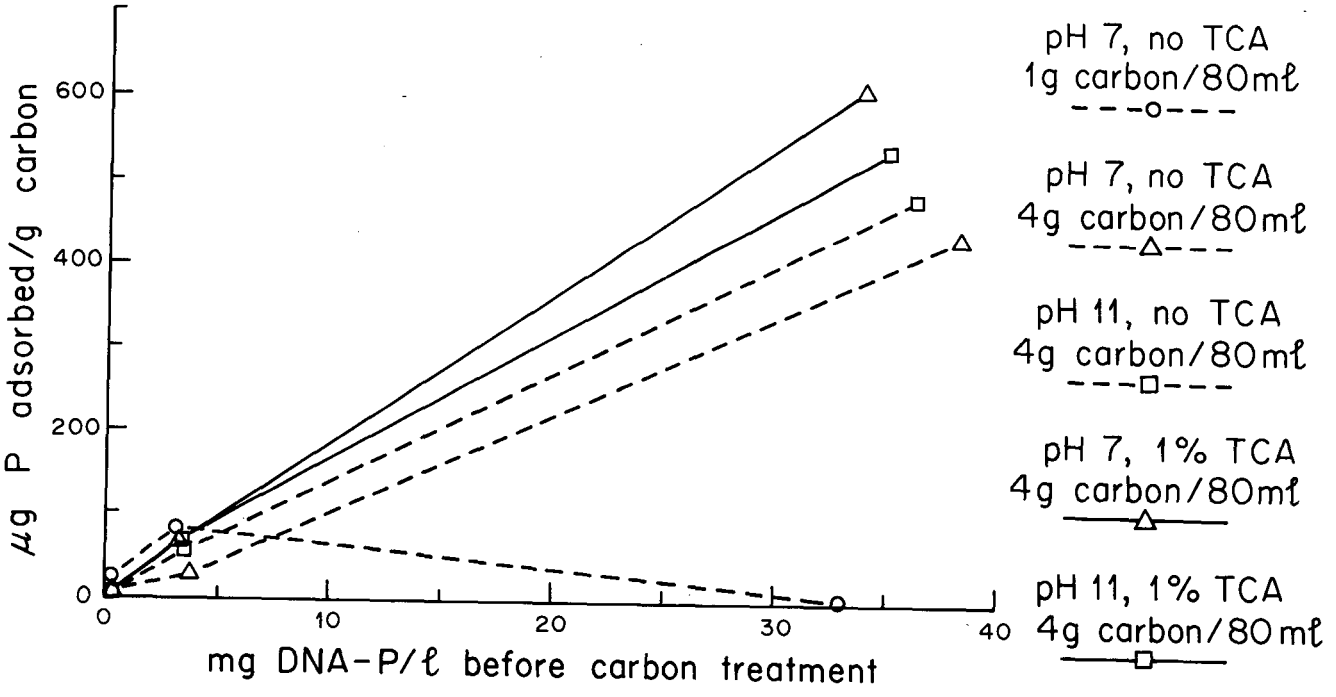

Figure 2b

Figure 2
Effect of TCA (1%) and pH on adsorptive behaviour of Norit SA 4 toward nucleic acids : RNA (Fig. 2a) and DNA (Fig. 2b).

NaOH, viz. 0,005 M (pH 12) and 0,001 M (pH 11) give reduced effects (Fig. 1e). The addition of 1% trichloroacetic acid (TCA) virtually abolishes the adsorption of polyphosphate across the pH range 1 to 10,5 (Fig. 1a). The same is true of 0,5 M perchloric acid (pH < 1) and 0,5 M sodium perchlorate (pH 5) (Fig. 1b). Saturated sodium perchlorate is slightly less effective (Fig. 1c).

Although the mechanism of polyphosphate adsorption is apparently complex, extracts of activated sludge containing 1% TCA or 0,5 M PCA at any pH in the acidic-neutral range will not loose significant amounts of polyphosphate by adsorption to Norit SA 4 at room temperature. The same will apply to alkaline extracts containing at least 0,05 M NaOH. Caution should be exercised with extracts containing more dilute concentrations of alkali, but addition of sodium trichloroacetate will prevent marked loss of polyphosphate.

Figs. 2a and 2b pertain to the results of tests for nucleic acid adsorption to Norit SA 4 at room temperature in the presence and absence of 1% TCA. The nucleic acid concentration ranges tested agree with the approximate concentrations which may be expected in activated sludge extracts (Lötter, 1985; Mino *et al.*, 1985). In the absence of TCA, a maximum of about 1,3 mg RNA-P/g carbon was removed at a carbon concentration of 1 g/80 mℓ and pH 7. The specific adsorption of RNA was lower at an increased carbon concentration (4 g/80 mℓ) in the absence of TCA; the reason for this lower adsorption is not clear. What is clear is that in the presence of 1% TCA the specific adsorption of RNA is practically identical at pH 7 and pH 11; a higher maximum (> 1,75 mg P/g carbon) is also attained than in the absence of TCA for both carbon loadings. Efficient RNA adsorption may therefore be expected in the presence of 1% TCA at neutral to alkaline pH. The same is true of DNA (Fig. 2b). In the case of DNA with no TCA addition, a carbon loading of 1 g/80 mℓ was ineffectual at initial concentrations exceeding about 5 mg DNA-P/ℓ. Finally, it should be noted that aqueous 1% TCA has an absorbance at 260 nm of approximately 0,16. This should be taken into account when converting absorbances at this wavelength to nucleic acid concentrations before and after carbon treatment. Limited adsorption of TCA by Norit SA 4 was noted in controls (data not shown).

Conclusions

Direct methods for the determination of polyphosphate involve mild acid hydrolysis. The various time-acid combinations proposed in the literature for these methods, are subject to significant errors in the form of incomplete polyphosphate hydrolysis and/or interference from nucleic acid hydrolysis. Of the experimental systems tested here the '7-min phosphate' method (1 M HCl, 100°C, 7 min) (Langen and Liss, 1958) was found to give the lowest error. However, the exact error is likely to be sample-dependent. Hence, it may be advisable to determine the polyphosphate content of activated sludge and its extracts by other methods for comparison. For example, the difference may be determined between the total phosphate content and the sum of phospholipid and nucleic acid phosphate, where the latter are based on colorimetric reactions.

The adsorption of orthophosphate and polyphosphate to powdered activated carbon is complex and cannot be explained solely on the basis of charge. Strong adsorption of polyphosphate occurs in weakly ionic environments at acidic, neutral or basic pH. Trichloroacetic acid (1%) and perchloric acid (0,5 M), or the sodium salts of these acids, served to abolish polyphosphate adsorption at acidic, neutral and basic pH. These tests were conducted using Graham's salt of mean chain length 16 phosphate units; extrapolation to other polyphosphate species was not attempted.

The adsorption of nucleic acids to powdered activated carbon at both neutral and basic pH is enhanced by the presence of 1% sodium trichloroacetate (TCA). It is possible to adsorb at least 1,5 mg RNA-P/g carbon and 0,5 mg DNA-P/g carbon (Norit SA 4, acid-washed) under these conditions at room temperature in 60 min. If absorbance measurements are made at 260 nm, the absorption by TCA at this wavelength should be taken into account.

Mino *et al.* (1985) and Lötter (1985) apparently did not examine the efficacy of activated carbon in adsorbing nucleic acids and apparently assumed that polyphosphate is not adsorbed. Furthermore, Mino *et al.* (1985) did not report the carbon concentration used. Results of this study imply that these considerations are important. They may explain why Lötter (1985) reported over 40% of the sludge total phosphate in the form of nucleotides in the cold 0,5 M perchloric acid extract. It should be stressed that the inhibition of Graham's salt adsorption to PAC in the presence of 0,5 M perchloric acid, as reported here, may not apply to other polyphosphate species. It seems advisable to check nucleic acid removal using colorimetric methods based on sugar determinations, and hence to estimate polyphosphate adsorption by difference with total phosphate results.

A suitable method for washing powdered activated carbon free of phosphate involves hot acid-persulphate treatment, followed by hot dilute acid and hot distilled water treatment. Filtration using glass-fibre filters is suitable and the carbon may be washed in the filter with distilled water until the filtrate is neutral to pH indicator paper.

References

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

ARVIN, E and KRISTENSEN, GH (1985) Exchange of organics, phosphate and cations between sludge and water in biological phosphorus and nitrogen removal processes. *Wat. Sci. Tech.* **17** 147-162.

CERIOTTI, G (1955) Determination of nucleic acid in animal tissues. *F. Biol. Chem.* **214** 59-70.

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

COMEAU, Y, OLDHAM, WK and HALL, KJ (1987) Dynamics of carbon reserves in biological dephosphatation of wastewater. In: *Advances in Water Pollution Control: Biological Phosphate Removal from Wastewater.* (Editor: Ramadori, R) Pergamon, Oxford. 39-56,

CRANE, RK (1958) Use of charcoal to separate mixtures of inorganic ester and nucleotide phosphates. *Science* **127** 185-286

DE HAAS, DW (1988) Fractionation of bioaccumulated phosphorus compounds in activated sludge. Poster paper presented at 14th IAWPRC Biennial International Conference, Brighton. 17-22 July.

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.

HAROLD, FM (1963) Accumulation of inorganic polyphosphate in *Aerobacter aerogenes.* I. Relationship to growth and nucleic acid synthesis. *J. Bact.* **86** 216-221.

HARTREE, EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* **48** 422-427.

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

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 Wastewater,* 28-30 Sept., Rome, Italy. 339-342.

KERDACHI, DA and ROBERTS, MF (1985) Further investigations into the modified STS procedure as used specifically to quantitatively assess "metal phosphates" in activated sludge. *Proc. Int. Conf. on Management Strategies for Phosphorus in the Environment,* Lisbon, Spain. 66-71.

LANGEN, P and LISS, E (1958) Über Bildung und Umsatz der Polyphosphate der Hefe. *Biochemische A.* **330** 455-466.

LISS, E and LANGEN, P (1960) Über ein hochmolekulares Polyphosphate der Hefe. *Biochemische Z.* **333** 193-201.

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

MENAR, AB and JENKINS, D (1970) Fate of phosphorus in waste treatment processes: Enhanced removal of phosphate by activated sludge. *Env. Sci. Technol.* **4** 1115-1121.

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, 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. Sppl. Microbiol. Biotechnol.* **24** 512-517.

RAO, NN, ROBERTS, MF and TORRIANI, A (1985) Amount and chain length of polyphosphates in *Escherichia coli* depend on cell growth conditions. *J. Bact.* 162 242-247.

SCHMIDT, G and THANNHAUSER, SJ (1945) A method for the determination of deoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. *F. Biol. Chem.* **161** 83-89.

SCHNEIDER, WC (1945) Phosphorus compounds in animal tissues. *F. Biol. Chem.* **161** 293-303.

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

VAN WAZER, JR and HOLST, KA (1950) Structure and properties of the condensed phosphates. *F. 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.