Short communication

Unreliability of cold-stored samples for assessment of chemical precipitates of phosphate in activated sludge

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Abstract

The effect on orthophosphate and polyphosphate levels was investigated for refrigerated or frozen activated sludge samples. A short fractionation procedure was used involvng removal of the supernatant, washing with physiological saline, and extraction at 0°C with perchloric acid. It was found that storage at 4°C or -20°C (followed by thawing to 15°C or room temperature) resulted in polyphosphate hydrolysis to orthosphosphate. The latter may have been erroneously interpreted in the past as 'chemical precipitate'.

Introduction

The mechanism of so-called enhanced biological phosphorus removal in activated sludge processes has received attention for over a decade (Fuhs and Chen, 1975; Nicholls and Osborn, 1979; Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Gerber *et al.*, 1987). Proposed biochemical models (Comeau *et al.*, 1986; Wentzel *et al.*, 1986) accept polyphosphate as the major form of phosphorus (P) accumulated in the sludge. Yet other workers (Menar and Jenkins, 1970; Arvin, 1985; Arvin and Kristensen, 1985; Kerdachi and Roberts, 1985) have argued that phosphates immobilised in the sludge as extracellular chemical precipitates incorporating calcium, magnesium, iron, aluminium or zinc cations should not be ignored. In fact, Arvin and Kristensen (1985) suggested on the basis of chemical analyses carried out on 25 sludge samples, that about 60% of the total sludge-P was chemical precipitate.

Whilst the hardness of the influent water may be a contributing factor in chemical phosphate precipitation (Arvin, 1985), the method of analysis for its occurence in sludge is equally important. Arvin and Kristensen (1985) applied the analytical method of Miya *et al.* (1984) but performed only the 5% perchloric acid (PCA) extraction at 4°C. In addition, the sludge samples were stored frozen (-20°C) over a period of 10 months prior to thawing and performing the above extraction. The effect of freezing the samples was not reported by Arvin and Kristensen (1985).

The purpose of this study was to investigate whether a freeze-thaw cycle produces artefacts in the analysis of activated sludge for chemically precipitated phosphate.

Materials and methods

Sludge samples were obtained from the final aerobic reactor of a three-stage Bardenpho[R] plant at Daspoort Sewage Works (Pretoria).

Chemical fractionation was performed as follows. An aliquot of sludge (200 mℓ) was centrifuged (1 500 x g) for 5 min. The supernatant was saved at 4°C. The pellet was resuspended at room temperature in 0,9% NaCl (25 mℓ) using a magnetic stirrer. After stirring for five minutes, the concentrated sludge (20 mℓ) was centrifuged as before. An aliquot (5 mℓ) of the remaining concentrated sludge was subjected to suspended solids determination (see above). The supernatant (designated NaCl wash) was saved at 4°C. The pellet was resuspended in 0,5 M PCA (15 mℓ) at 0°C and kept at that temperature for 25 min with intermittent shaking. Centrifugation was performed as before and the PCA extraction repeated five times in total. The PCA extracts were pooled and stored at 4°C. The residue was resuspended in distilled water (20 mℓ) and stored in the same manner. Total phosphate analyses were performed by persulphate digestion (*Standard Methods*, 1985, as modified by De Haas *et al.*, 1988) and orthophosphate analyses using the ascorbic acid method (*Standard Methods*, 1985, as modified by De Haas *et al.*, 1988). For polyphosphate, 7 min phosphate analyses were performed by hydrolysis according to Langen and Liss (1958) using filtration (Whatman no. 41) of the hydrolysate where necessary.

Results and discussion

The results of Experiments 1 and 2 are given in Tables 1 and 2 respectively.

Tables 1 and 2 indicate that the fractionation and analytical procedures proved satisfactory, with recoveries of phosphorus in the extracts exceeding 90%. A small margin of experimental error is nevertheless evident, for example, in the two negative 7 min phosphate values.

A freeze-thaw cycle has a significant effect on the fractionation pattern of phosphate compounds of activated sludge. The most marked effect is a breakdown of polyphosphate, the phosphorus appearing almost stoichiometrically as orthophosphate in the sludge supernatant, saline wash and PCA fractions, in that order of decreasing quantity (Table 1). It was not apparent from Experiment 1 (Table 1) whether the elevated levels of orthophosphate in the sample which had been frozen could be ascribed to the freezing process itself or to phosphorus release by the sludge bacteria under

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relatively anoxic - anaerobic conditons (Barnard, 1976; Gerber *et al.*, 1987). In this respect it is significant that the thawed sample in Experiment 1 may have remained at room temperature for about 60 min since it had been deliberately frozen as a thin layer to expedite thawing.

In Experiment 1, a single sludge sample was divided into two equal portions. The first was stored overnight (17 h) in a stoppered bottle at 4°C. The second was frozen overnight as a layer (ca. 8 mm thick) in a flat glass dish at -20°C. The following morning, the frozen sample was brought to room temperature over a period of about 120 min while the sample at 4°C was kept cold till fractionation commenced. The mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) concentrations were then determined for both samples *(Standard Methods,* 1985) and a portion of the remainder of each subjected to chemical fractionation (see below).

In Experiment 2, a single sludge sample was divided into three equal portions. The first was subjected to suspended solids determinations and chemical fractionation as soon a possible after sampling. The second was stored for three days in a stoppered bottle at 4°C. The third was frozen for three days in a 500 mℓ stoppered reagent bottle so as to form a cylinder of ice measuring approximately 80mm (diameter) by 80mm (height). The second sample was left at 4°C until fractionation commenced and the third was thawed from -20°C to 15°C over a period of about 15 h. Both these samples were then subjected to suspended solids determinations and chemical fractionation.

Hence in Experiment 2, the period of storage was increased, thawing occurred slowly, and no significant period was spent at room temperature by any sample (neglecting the delay in transportation from the sewage works.) Table 2 shows that immediately after sampling, orthophosphate concentrations in the supernatant, saline wash and PCA steps were markedly lower in relation to the samples which had been stored for 3 d in the refigerator or freezer. Furthermore, the difference between the sample stored at 4°C and the frozen sample was smaller than in Experiment 1. This is probably because the thawed sample did not attain room temperature for a significant period.

The results of Table 1 and 2 draw into question the results of Arvin and Kristensen (1985). These authors stored sludge samples frozen for months after decanting the supernatant. PCA was then used to extract so-called chemical precipitates of phosphate from the thawed samples. Tables 1 and 2 indicate that orthophosphate appearing in the supernatant and NaCl wash as a result of polyphosphate hydrolysis would be included in the PCA extract defined by Arvin and Kristensen (1985). In Table 2 this orthophosphate represents 25% of the sludge total phosphorus and could therefore be erroneously reported as chemical precipitate.

TABLE 1
EFFECT OF FREEZING ON PHOSPHATE ANALYSES OF ACTIVATED SLUDGE EXTRACTS - EXPERIMENT 1. FIGURES IN mg P/g VSS UNLESS OTHERWISE STATED

Sludge treatment	Extract	Ortho-P	7 min P*	Total
Not frozen (4°C, 17 h)	Sludge supernatant	2,220	(-0,066)	1,963
	NaCl wash	0,318	0,088	0,409
	PCA	3,748	33,504	42,291
	Residue	ND	≤8,143	11,311
	Whole sludge	ND	ND	59,300
	Recovery in extracts	ND	ND	94%
Frozen (-20°C, 17 h)	Sludge supernatant	9,620	0,683	12,317
	NaCl wash	1,517	0,099	1,751
Quick thaw	PCA	4,450	23,555	33,473
	Residue	ND	≤10,110	12,282
	Whole sludge	ND	ND	59,300
	Recovery in extracts	ND	ND	101%

*Definition: orthophosphate detected after hydrolysis (1M HCl, 95°C, 7 min) *less* orthophosphate detected before hydrolysis.
ND: Not determined.

TABLE 2
EFFECT OF FREEZING ON PHOSPHATE ANALYSES OF ACTIVATED SLUDGE EXPERIMENTS - EXPERIMENT 2. FIGURES IN mg P/g VSS UNLESS OTHERWISE STATED

Sludge treatment	Extract	Ortho-P	7 min P*	Total
None (immediately after sampling)	Sludge supernatant	0,002	0,116	1,347
	NaCl wash	0,092	0,104	0,261
	PCA	3,251	37,617	49,957
	Residue	0,005	6,683	13,636
	Whole sludge	ND	ND	70,480
	Recovery in extracts	ND	ND	91%
Not frozen (4°C, 3 d)	Sludge supernatant	11,416	(-0,632)	10,423
	NaCl wash	0,588	0,191	0,844
	PCA	2,899	34,188	41,386
	Residue	0,093	7,002	14,207
	Whole sludge	ND	ND	70,480
	Recovery in extracts	ND	ND	95%
Frozen (-20 °C, 3 d) Slow thaw	Sludge supernatant	13,472	1,861	16,743
	NaCl wash	1,616	0,038	1,774
	PCA	5,583	29,908	40,866
	Residue	0,097	5,273	12,148
	Whole sludge	ND	ND	70,480
	Recovery in extracts	ND	ND	102%

*Definition: orthophosphate detected after hydrolysis (1M HCl, 95°C, 7 min) *less* orthophosphate detected before hydrolysis.
ND: Not determined.

Moreover, if the sample is stored for periods of approximately 1 h or more before freezing or after thawing, this figure could be higher. It is apparent that significant polyphosphate breakdown (more than one-fifth of that present, representing 17% of the total sludge P in this case), even occurs at 4°C over 3 d (Table 2). Hence, polyphosphate hydrolysis during cold storage is probably due to the emergence of anaerobiosis.

It may be concluded that analysis of orthophosphate and polyphosphate in activated sludge are probably suspect unless conducted immediately after sampling. Marked overestimation of chemical precipitate is likely where 'dewatered' sludge samples have been stored in refrigerators or freezers for periods of days or months.

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