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

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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 (> 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; Letter, 1985; Murphy and Letter, 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

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