Significance of fractionation methods in assessing the chemical form of phosphate accumulated by activated sludge and an *Acinetobacter* pure culture

DW de Haas*
Division of Water Technology, CSIR, PO Box 395, Pretoria 0001, South Africa.

Abstract

Two chemical fractionation procedures aimed at the determination of phosphorus compounds were used to analyse the same activated sludge sample from a five-stage modified Bardenpho plant. In both procedures, taking due account of polyphosphate interference in the orthophosphate (orthoP) assay and polyphosphate (polyP) hydrolysis during acid extraction, at least 14% of the total phosphorus (totalP) was measured to be orthoP. Since intracellular orthoP levels were estimated to be negligible, it was concluded that the origin of the measured orthoP was chemical precipitates. Thus chemical precipitates of orthoP can form naturally in modified activated sludge systems not dosed with chemical precipitants, and, for the sludge tested, appeared to make a minor contribution to the overall P-removal. Comparing the two fractionation procedures, different chain lengths of polyphosphate (polyP) were measured in trichloroacetic and in perchloric acid extracts of the same sample. Furthermore, the chain lengths of polyP measured in subsequent (alkaline) steps were not greater than those found in the acid step. From these observations it was concluded that classification of acid-soluble polyP as "low molecular" and acid-insoluble polyP as "high molecular" is not justified. Also, quantification of acid-soluble and acid-insoluble polyP extracted from activated sludge was challenged since it was found that the distribution of polyP between the two fractions was influenced by the nature of the extractant and by hydrolysis of polyP to orthoP during extraction. Attention was also given to the occurrence of chemically precipitated orthoP in a stationary phase culture of *Acinetobacter* at pH ca. 9. Such precipitation occurred to a much lesser degree in an early log phase culture which had remained at pH ca. 7. Accordingly, it was recommended that metabolic studies of polyP formation by this organism should be conducted in a medium with pH control near neutrality.

Introduction

Modified Bardenpho and UCT activated sludge systems were designed for enhanced biological phosphorus removal as well as nitrogen and carbon removal from waste water (Barnard, 1976; Siebritz *et al*, 1980). Characterisation of the forms of accumulated phosphorus in the activated sludge of such systems has been the subject of numerous studies (Fuhs and Chen, 1975; Buchan, 1981, 1982; Kerdachi and Roberts, 1985; Lötter, 1985; Mino and Matsuo, 1985; Mino *et al*, 1985; Murphy and Lötter, 1986; Kerdachi and Healey, 1987; De Haas, 1989a). In most of these studies, polyphosphate (polyP) has been found to constitute the major storage form of phosphorus in the biomass with nucleic acids being quantitatively the next most important biochemical form of phosphorus. Mino *et al.* (1985), from chemical fractionation and radiotracer studies of activated sludge, concluded that two principal forms of polyP exist in the sludge microorganisms; "low molecular polyP (L-PP)" and "high molecular polyP (H-PP)". They derived the designation of these two "pools" from two fractions in their fractionation procedure: the L-PP was extracted with cold 0,5M perchloric acid (PCA) and determined as non PO_4-P, while H-PP was extracted with hot PCA and, after removal of nucleic acids by carbon adsorption, determined as labile P by degradation with IN sulphuric acid at 100°C.

Experiments performed by Mino *et al.* (1985) on ^{32}P-radioisotopes produced evidence of a turnover of phosphorus in H-PP under aerobic conditions but not (or to a lesser extent) in L-PP. Under anaerobic conditions, turnover in neither polyP form occurred. Mino *et al.* (1985) concluded that H-PP and L-PP fulfil different physiological functions. Because of the observed aerobic turnover observed in H-PP, it was considered to function as the phosphorus pool for microbial growth. (They noted, however, that since uptake and release of P to, or from, H-PP was found in some batch experiments, it was unclear whether H-PP served solely as a phosphorus pool). On the other hand, the L-PP was considered to function as the stored energy pool of the microbial cells. The concept of these two distinct polyP forms has not been incorporated in the biochemical models for enhanced P-removal (Comeau *et al.*, 1986; Wentzel *et al.*, 1986). The first aim of this paper is to investigate the validity of the conclusions drawn by Mino *et al.*, (1985) with the objective of assessing whether these two distinct forms of polyP should be included in future biochemical models.

The second aim of this paper is to investigate whether chemical fixation of orthoP in the activated sludge biomass plays a part in enhanced biological phosphorus removal from waste water. Numerous researchers (Fuhs and Chen, 1975; Arvin and Kristensen, 1985; Kerdachi and Roberts, 1985; Lötter, 1985; Mino and Matsuo, 1985; Mino *et al.*, 1985; Murphy and Lötter, 1986; Kerdachi and Healey, 1987; De Haas, 1989a) have found that variable amounts of orthoP are extractable from activated sludge with cold 0,5 M PCA, cold 0,061 M trichloroacetic acid (TCA) or 0,05 M EDTA. The origin of this orthoP has been ascribed to chemical precipitation (Arvin and Kristensen, 1985; Mino and Matsuo, 1985; Mino *et al.*, 1985; Kerdachi and Roberts, 1985; Kerdachi and Healey, 1987) and to "intracellular" orthoP (Lötter, 1985; Murphy and Lötter, 1986). Clearly, a re-examination of the magnitude and likely origin of orthoP in extracts obtained from activated sludge would be expedient. Any such re-examination should take due account of criticisms of previous experimental techniques: The storage of activated sludge samples by freezing (Arvin and Kristensen, 1985) has been criticised by De Haas and Dubery (1989); the possible interference of polyP in the orthoP assay (De Haas *et al.*, 1980a) and the possible hydrolysis of polyP to orthoP during acid extraction (Kerdachi and Roberts, 1985) have generally not been taken into account in previous studies.

In this paper the magnitude and likely origin of orthoP in extractions of activated sludge and pure culture samples are to be investigated, taking cognisance of criticisms of previous experimen-

•Present address: Umgeni Water, PO Box 9, Pietermaritzburg 3200, South Africa
Received 23 November 1989; accepted in revised form 16 July 1990.