The effect of acetate and succinate on polyphosphate formation and degradation in activated sludge, with particular reference to *Acinetobacter calcoaceticus*

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Summary. The effects of acetate and succinate were compared to the effect of phosphate starvation on the formation and degradation of polyphosphate in an *Acinetobacter calcoaceticus* isolate from a five-stage Bardenpho activated sludge plant and in mixed liquor from the same plant. Both acetate treatment and phosphate starvation result in significant phosphate release from the cells. Succinate treatment showed little difference from the control. A reduction in polyphosphate was observed simultaneously with the phosphate release. On resuspension of the treated samples in a complete medium, uptake of phosphate was observed. In the acetate-treated samples, this was significantly higher than in the phosphate starved samples. Polyphosphate formation was also significantly enhanced after treatment.

Introduction

The phenomenon of enhanced phosphate removal by activated sludge systems has been well-documented (Srinath 1959; Nicholls and Osborn 1979). However, attempts to elucidate the exact mechanism have not yet been successful.

At the microbiological level, the identification of the dominant bacteria responsible for the phosphorus removal has been achieved (Buchan 1983; Kerdachi and Roberts 1983; Lötter 1985). These bacteria, *Acinetobacter calcoaceticus*, are capable of storing large amounts of phosphorus as polyphosphate (Buchan 1983). It has however, also been shown that the mere presence of these bacteria is not sufficient to guarantee good phosphorus removal (Lötter 1985).

Research on the molecular level has revealed the importance of substrate composition and in particular, the role of acetate in enhanced phosphorus removal (Fuhs and Chen 1975; Wentzel et al. 1985). It has become necessary to link these substrate effects to a particular state in *Acinetobacter calcoaceticus*, which stimulates its ability to accumulate polyphosphate. On addition of acetate to a mixed liquor sample from the aerobic zone of the plant, phosphate is released (Lötter 1985; Wentzel et al. 1985).

The amount of phosphorus released on addition of acetate is considerably higher than on the addition of succinate, which, unlike acetate, requires energy to enter the cell (Lötter 1985).

The phosphate released in this manner is utilised by the cell to reinstate the proton motive force, which has been dissipated by the absorption of acetic acid (Comeau et al. 1985).

Phosphate starvation stimulates rapid phosphate uptake on resuspension of *Escherichia coli* in a phosphate rich medium (Medveczky and Rosenberg 1971) and has been shown to induce polyphosphate accumulation in *Aerobacter aerogenes*. The release of phosphate on addition of acetate could stimulate a condition of phosphate starvation in the *Acinetobacter calcoaceticus*. In this study an attempt was made to compare the effect of acetate and succinate treatment with phosphate starvation on polyphosphate accumulation.

Materials and methods

Activated sludge

Mixed liquor samples were taken from the aerobic zone of a five-stage Bardenpho activated sludge plant. The volatile suspended solids content of the mixed liquor was determined by the ignition at 550°C of the residue retai-
charged on a glass fibre filter, dried at 105 °C (American Public Health Association 1981).

Five hundred millilitre aliquots of mixed liquor were used for each experiment. The sample treatments included the addition of acetic acid to a final concentration of 100 mg/l or re-suspension in acetate/sewage medium (Fuhs and Chen 1975) modified by the omission of phosphate and fermented raw sewage. The samples were stirred at room temperature for the duration of the experiment. Control samples were run simultaneously and all experiments were carried out in duplicate. Forty millilitre aliquots were taken from each sample at 40 minute intervals over the experimental period of 160 min. At the end of this period, each sample was centrifuged at 5000 g for 5 min and the residue resuspended in 250 ml acetate/sewage medium (Fuhs and Chen 1975), modified by the omission of fermented raw sewage.

The resuspended samples were aerated at room temperature and 40 ml aliquots taken at 40 minute intervals for 160 min.

The phosphorus fractions in the samples were determined as described below.

**Acinetobacter isolates**

Bacterial colonies were isolated from the aerobic zone of an activated sludge plant as described by Lötter and Murphy (1983) and identified by the analytical Profile Index API 20E system (Analytib Products 1977). *Acinetobacter calcoaceticus* isolates were also identified by staining with a fluorescein isothiocyanate antibody against *Acinetobacter calcoaceticus* with fluorescence microscopy (Lötter and Murphy 1985).

An isolate from a five-stage Bardenpho plant which had been maintained by weekly subculture onto GCY agar (Pike et al. 1972) and once monthly subculture onto acetate/sewage agar (Fuhs and Chen 1975), was used to inoculate acetate/sewage medium (Fuhs and Chen 1975). Samples were taken for the experiments during the stationary phase.

Five hundred millilitre aliquots were used for each treatment with sampling and resuspension in acetate/sewage medium as described for the mixed liquor. In addition to the treatments already described, succinate at 100 mg/l was added to one sample.

**Phosphorus fractionation**

The aliquots were centrifuged at 10000 g for 10 min, the residues washed with saline solution and the resulting suspension re-centrifuged. The orthophosphate in the combined supernatants was determined by the molybdenum blue method (Canelli and Mitchell 1975).

Fractionation of the phosphorus compounds in the sludge pellet was accomplished by Harold’s procedure (Harold 1960). The pellet was shaken with 40 ml cold 0.5 M perchloric acid, centrifuged and the extraction repeated. The extracts were pooled. The residue was then extracted with 40 ml ethanol for 30 min. After centrifugation, 40 ml ethanol:ethyl ether (3:1) was added to the residue. The mixture was boiled for one minute and then centrifuged. These two extracts were pooled. The residue was then extracted twice with 40 ml portions of hot 0.5 M perchloric acid at 70 °C. The extracts were pooled.

An aliquot of the cold perchloric acid extract was taken for an orthophosphate and total phosphorus determination. Approximately 500 mg phosphate-free charcoal was then added to the extract and the suspension shaken well. Total phosphorus and polyphosphate determinations were carried out on the filtrate.

The orthophosphate results provide the cellular orthophosphate level and the difference between the total phosphorus before and after charcoal treatment, the nucleotide phosphorus level. Total phosphorus was determined on the ethanol:ether extract to provide the lipid phosphorus level. The hot perchloric acid extract was subjected to total phosphorus determination, charcoal treatment and subsequent phosphorus determination as described above. The difference in total phosphorus before and after charcoal treatment provides the nucleic acid phosphorus level.

The polyphosphate in this fraction is defined as acid insoluble polyphosphate while that in the cold extract is known as acid soluble polyphosphate. Total phosphorus determinations were carried out by the digestion procedure of Jirka (1976), followed by the orthophosphate determination referred to above (Canelli and Mitchell 1975). Polyphosphate was determined by hydrolysis in 1 M hydrochloric acid at 70 °C for 15 min, followed by the orthophosphate determination.

The bacterial culture samples were treated in the same way, except that 10 ml of each reagent was used.

**Results and discussion**

**Activated sludge**

The results are expressed as mg P/g VSS (volatile suspended solids). Preliminary experiments showed that no significant differences in the nucleotide, nucleic acid and lipid phosphorus fractions were detectable during the experimental period. The determination of these fractions was therefore excluded from the subsequent experiments, which are described here.

Phosphate starvation and acetate treatment result in release of phosphate to the external medium. The effect of acetate is far more dramatic than phosphate starvation. Subsequent aeration of these samples resulted in uptake of phosphate in greater amounts than was released. A number of workers have reported a requirement for a pre-treatment to stimulate high phosphorus uptake (Nicholls and Osborn 1979; Marais and Ekama 1982) (see Table 1).

Increased phosphate uptake after a period of phosphate starvation has been reported for a

| Table 1. Phosphate released and absorbed by activated sludge during treatment |
|---------------------------------|---------|---------|
| Phosphate released mg P/g VSS   | Control | 3       |
| Phosphate absorbed mg P/g VSS   | Acetate treatment | 14      |
|                                 | Phosphate starvation | 5       |

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