

Polyphosphate accumulation by bacteria isolated from activated sludge

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Abstract

Biological phosphorus removal is an acknowledged phenomenon applied to reduce phosphorus concentrations in wastewaters. Under aerobic conditions some heterotrophic organisms store polyphosphate intracellularly which serves as a phosphorus and energy source during periods of phosphorus starvation. The objective of this study was to investigate polyphosphate accumulation by all bacteria isolated from the aerobic zone of a nutrient removal plant. Polyphosphate organisms were isolated on casitone glycerol yeast agar. Procedures for phosphate uptake studies included exposure of each monoculture to anaerobic environments for 2 h followed by aeration for 5 h. Extracellular orthophosphate concentrations were determined after aeration using a Merck SQ118 spectrophotometer. Identification was conducted using various biochemical tests, API 20E and 20NE. *Acinetobacter calcoaceticus* var. *lwoffii*, *Aeromonas hydrophila*, gram-positives and *Pseudomonas* spp. were among the predominant phosphate removers. Neisser staining confirmed that the organisms had stored polyphosphate intracellularly. Findings confirm that *Acinetobacter calcoaceticus* var. *lwoffii* is the predominant micro-organisms involved in enhanced phosphorus uptake.

Introduction

Biological phosphorus removal from wastewater is based on the enrichment of activated sludge with phosphate-accumulating organisms (PAOs). To achieve a phosphorus-removing bacterial population in an activated sludge system, exposure of sludge to alternating anaerobic and aerobic (or anoxic) conditions is necessary (Bdrjanovic et al., 1997). Under anaerobic conditions, P-removing bacteria convert volatile fatty acids (VFAs) synthesised in the zone by fermenters to polyhydroxybutyrate (PHB) which is stored intracellularly. Under aerobic conditions, stored PHB is used to generate cell growth, poly-P synthesis and glycogen formation and maintenance, resulting in the uptake of phosphate (Bdrjanovic et al., 1997).

The dominant bacteria in the activated sludge system are aerobic heterotrophs that degrade and eventually mineralise organic compounds present in wastewater to carbon dioxide and water. It is the small size of bacteria and their resultant large surface area to volume ratio which makes them efficient in terms of nutrient and catabolic exchange (Gray, 1989). Heterotrophic bacterial populations remain relatively stable throughout the plant with various environments in the three zones allowing different bacteria to dominate in terms of metabolic activity (Lötter and Murphy, 1985).

Several early studies have shown that the removal and release of phosphorus within a sludge are the results of the dominance of a single genus of bacteria known as *Acinetobacter* spp. and more specifically a single species, *Acinetobacter calcoaceticus*, was implicated (Buchan 1980, 1983; Horan, 1991; Starckenburg et al., 1993). *Acinetobacter* spp. are able to accumulate more phosphate than is required for cell synthesis; the so-called **luxury phosphate uptake**.

Acinetobacter spp. are normally present in activated sludge, but in the minority due to the low growth rate. *Acinetobacter* organisms prefer VFAs, especially acetate, as a growth substrate which are present or can be produced from wastewaters in an activated sludge system. This is achieved by incorporating an anaerobic zone, mostly at the beginning of the aeration tank, where the return sludge meets the incoming wastewater (Starckenburg et al., 1993).

Controversy surrounds the notion that *Acinetobacter* spp. is the predominant micro-organism involved in enhanced phosphorus uptake. Using respiratory quinone profiles some researchers found no correlation between the number of *Acinetobacter* spp. and the extent of phosphorus removal (Cloete and Steyn 1988). Other researchers reported that *Acinetobacter* spp. were predominant when enumerated using the analytical profile index method. For example, Hart and Melmed (1982) estimated *Acinetobacter* spp. at 56% to 66% of the total population, Buchan (1983) reported 48% to 66%, Lötter (1985) 56% to 66%, Lötter and Murphy (1985) ca. 60% to 70% and Kerdachi and Healey (1987) 73%. However, it was shown that *Acinetobacter* spp., as detected by the biomarker diaminopropane, was the dominant organism only in wastewater plants with low organic loading (Auling et al., 1991). Using oligonucleotide probes specific for *Acinetobacter* spp., it was found that the genus formed less than 10% of the total bacterial population (Wagner et al., 1994). Probing techniques, compared to plating techniques, do not overestimate *Acinetobacter* spp. populations (Wagner et al., 1994). Nutrient-rich medium favours growth of gamma-subclass proteobacteria (e.g. enterobacteria) and selects against beta-subclass proteobacteria (Wagner et al., 1993). This is mainly due to the selectivity of media and culture conditions (Wagner et al., 1994). In addition to *Acinetobacter* spp., the Gram-positives are also able to accumulate polyphosphate (Lötter and Murphy, 1985). Bacteria like *Pseudomonas* spp., *Aerobacter* spp., *Moraxella* spp., *Escherichia coli*, *Mycobacterium* spp., *Beggiatoa* spp. and *Klebsiella* spp. also have the ability to accumulate phosphorus at approximately 1 to 3% of the cell dry mass (Bitton,

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