

# From macro to lab-scale: Changes in bacterial community led to deterioration of EBPR in lab reactor

## Research Article

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**Abstract:** A laboratory scale sequencing batch reactor (SBR), fed with synthetic wastewater containing a mixture of organic compounds, was operated for nearly six months. Despite maintaining the same operational conditions, a deterioration of enhanced biological phosphorus removal (EBPR) occurred after 40 days of SBR operation. The  $P_{\text{rel}}/C_{\text{upt}}$  ratio decreased from 0.28 to 0.06 P-mol C-mol<sup>-1</sup>, and C requirements increased from 11 to 32 mg C h<sup>-1</sup> g<sup>-1</sup> of mixed liquor suspended solids. A FISH analysis showed that the percentage of *Accumulibacter* in an overall community of polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) dropped from 93% to 13%. An increase in abundance of *Gammaproteobacteria* (from 2.6% to 22%) and *Alphaproteobacteria* (from 1.8% to 10%) was observed. The number of *Competibacter* increased from 0.5% to nearly 9%. Clusters 1 and 2 of *Deffluicoccus*-related GAOs, not detected before deterioration, constituted 35% and 27% of *Alphaproteobacteria*, respectively. We concluded that lab-scale experiments should not be extended implicitly to full-scale EBPR systems because some bacterial groups are detected mainly in lab-scale reactors. Well-defined, lab-scale operational conditions reduce the number of ecological niches available to bacteria.

**Keywords:** Polyphosphate accumulating organism (PAO) • Glycogen accumulating organism (GAO) • Microbial ecology

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## 1. Introduction

Conventional activated sludge systems can be modified to substantially improve elimination of nutrients. Low effluent phosphorus (P) concentration is achieved by enhanced biological phosphorus removal (EBPR) without the need for chemical P precipitation. Activated sludge has to be exposed to alternating anaerobic and aerobic conditions in order to enrich polyphosphate accumulating organisms (PAOs), which in turn take up large amounts of P (in excess of metabolic demand for cell biosynthesis) and accumulate it as intracellular granules of polyphosphate (poly-P).

The sludge from EBPR systems exhibit characteristic transformations of poly-β-hydroxyalkanoates (PHAs), poly-P and glycogen during the wastewater treatment process. Under anaerobic conditions, where organic substances are available in bulk liquid (feast conditions), PAOs take up volatile fatty acids (VFAs) and store them

as PHAs. PAOs obtain energy for this purpose from the cleavage of the internally stored poly-P, while the reducing power for PHA synthesis is provided mostly by the hydrolysis of intracellular glycogen. It results in a release of inorganic phosphorus ( $P_i$ ) into bulk liquid, an increase in internally stored PHAs, and a decrease of the intracellular glycogen pool. Under subsequent aerobic conditions (famine conditions) PAOs use the stored PHAs as the intracellular carbon and source of energy for cell growth, for an uptake of  $P_i$  and synthesis of poly-P, as well as for replenishment of the pool of glycogen. Extracellular  $P_i$  concentrations fall, along with PHA content, while intracellular pools of glycogen and poly-P increase. PAOs accumulate  $P_i$  in excess of the anaerobic  $P_i$  release (luxury uptake over metabolic demand) and it results in efficient P removal through sludge wastage at the end of the aerobic period.

The alternating anaerobic-aerobic and feast-famine phases give PAOs a selective advantage in

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