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Stable isotope labelling to track the metabolic turnover of extracellular polymeric substances (EPS) of candidatus *Accumulibacter phosphatis*

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Ca. *Accumulibacter* is a phosphate-accumulating organism (PAO) responsible for enhanced biological phosphorus removal (EBPR) from wastewater. In granular sludge processes for nutrient removal, this bacterium grows embedded in granules formed by a matrix of extracellular polymeric substances (EPS). EPS comprise a complex mixture of biopolymers like polysaccharides or glycoproteins. Despite several previous EPS studies, little is known about the dynamics of EPS compounds in mixed cultures, and their production by the slow-growing Ca. *Accumulibacter*. EPS may act as substrate for other organisms. Studying EPS turnover can help elucidate their biosynthesis/biodegradation cycles and understand biofilm stability. In this study, we aimed to determine the turnover rates of proteins and polysaccharides in the EPS of an enrichment of Ca. *Accumulibacter* forming compact bioaggregates relative to the turnover of cell internal polymers. Hereto an anaerobic-aerobic sequencing batch reactor (SBR) simulating EBPR conditions was operated to enrich for Ca. Accumulibacter. After a stable culture was achieved, the carbon source was switched to uniformly labelled ^{13}C-acetate during one solids retention time (8 days, 32 SBR cycles). Samples were collected at the end of each aerobic phase. EPS were extracted by alkaline treatment. Labelling ratios of proteins and polysaccharides in extracted EPS were measured by mass spectrometry. Sequence-based subcellular location prediction tools were used to differentiate extracellular proteins. Incorporation of 13C in sugars was detected from the beginning of the experiment. However, incorporation in proteins presented a delay and label was only detected after 9 SBR cycles, probably caused by a ^{12}C-supply from intracellular storage polymers (poly-β-hydroxyalkanoates or glycogen). ^{13}C-labelling ratio after one SRT accounted for 81±6% and 76±8%, for proteins and polysaccharides respectively, higher than the theoretical 63%, based on the SRT. Stable isotope labelling helped tracking the EPS turnover, showing a faster recycling both for proteins and polysaccharides when compared to the SRT. Despite this higher turnover, there was not significant difference between extracellular and intracellular structures, indicating that they present a similar stability during the wastewater treatment process. It also indicates that often suggested cryptic growth on the extracellular polymers in natural biofilms in this case is very limited.