Polyphosphate and glycogen accumulating organisms in activated sludge:
Biokinetics and competition for carbon

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Enhanced biological phosphorus removal (EBPR) is a popular modification of the activated sludge process that is critical for reducing phosphorus discharges to protect receiving waters from eutrophication. However, many fundamental aspects of EBPR are still not well understood. Competition between the bacteria responsible for EBPR, the polyphosphate accumulating organisms (PAOs), and glycogen accumulating organisms (GAOs) for volatile fatty acids (VFAs) is thought to be critical to successful EBPR, but little is known about the effects of substrate concentration on this competition. It is hypothesized that GAOs may benefit from higher acetate concentrations, while PAOs may be better at taking up acetate at lower concentrations, based on consideration of transport energetics. This research may be important because acetate concentrations are generally low in full scale, completely mixed flow reactors (CMFRs), while they tend to be high in laboratory scale sequencing batch reactors (SBRs), which suggests that GAO competition may be less important in full scale systems than previously thought. In our study, an acetate-fed (addition switched from fast to slow), anaerobic-aerobic sequencing batch reactor was operated to evaluate our hypothesis. The reactor was enriched in GAOs when the acetate addition was fast (produced high initial concentrations that decreased over time), while, the reactor was dominated by PAOs after switching the acetate addition to slow (resulted in overall low acetate concentrations), the experimental results (Figure 1) supported the hypothesis that PAOs have kinetic advantage at low acetate concentration by yielding higher ratios of anaerobic P release to acetate uptake. The PAOs and GAOs species were identified by Fluorescence In Situ Hybridization (FISH) (Figure 2, 3) and pyrosequence analysis (only tested for PAOs). The Rhodocycyclus species were the most abundant organism in the PAOs dominated phase, while the Defluviicoccus species were probably the dominated organism in the GAOs phase (FISH analyses are still underway). In addition, highly enriched PAOs and GAOs biomasses were used to investigate different membrane transport mechanisms for substrate uptake in the presence of different chemical inhibitors that target different membrane proteins in the anaerobic phase. Future investigations will include identification of GAOs using other probes and pyrosequence analysis.
Figure 1: Relationship between time and the ratio of anaerobic phosphorus release to acetate uptake.

Figure 2: High PAO concentration culture, with (a) FISH EUB probe (for all bacteria), and (b) PAOMIX probes (*Rhodocyclus* spp.).

Figure 3: High GAO concentration culture, with (a) FISH EUB probe (for all bacteria), and (b) TFO probe (*Deflaviicoccus* spp.).