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Metabolic modelling of full-scale enhanced biological phosphorus removal sludge

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ABSTRACT

This study investigates, for the first time, the application of metabolic models incorporating polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) towards describing the biochemical transformations of full-scale enhanced biological phosphorus removal (EBPR) activated sludge from wastewater treatment plants (WWTPs). For this purpose, it was required to modify previous metabolic models applied to lab-scale systems by incorporating the anaerobic utilisation of the TCA cycle and the aerobic maintenance processes based on sequential utilisation of polyhydroxyalkanoates, followed by glycogen and polyphosphate. The abundance of the PAO and GAO populations quantified by fluorescence *in situ* hybridisation served as the initial conditions of each biomass fraction, whereby the models were able to describe accurately the experimental data. The kinetic rates were found to change among the four different WWTPs studied or even in the same plant during different seasons, either suggesting the presence of additional PAO or GAO organisms, or varying microbial activities for the same organisms. Nevertheless, these variations in kinetic rates were largely found to be proportional to the difference in acetate uptake rate, suggesting a viable means of calibrating the metabolic model. The application of the metabolic model to full-scale sludge also revealed that different *Accumulibacter* clades likely possess different acetate uptake mechanisms, as a correlation was observed between the energetic requirement for acetate transport across the cell membrane with the diversity of *Accumulibacter* present. Using the model as a predictive tool, it was shown that lower acetate concentrations in the feed as well as longer aerobic retention times favour the dominance of the TCA metabolism over glycolysis, which could explain why the anaerobic TCA pathway seems to be more relevant in full-scale WWTPs than in lab-scale systems.

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1. Introduction

The biological removal of phosphate (also known as enhanced biological phosphorus removal or EBPR) has been incorporated into several wastewater treatment plant (WWTP) configurations and provides a more economical and sustainable alternative to chemical precipitation methods of P removal (Mino et al., 1998; Oehmen et al., 2007).

For more than 20 years, an effort has been made to develop and apply activated sludge models (ASM) to describe and predict the activated sludge processes, which are a useful tool for plant design and optimisation (Henze et al., 2000). While ASM models use a grey-box approach and focus on macroscopic processes, a different modelling approach, relying on metabolic and biochemical pathways, describes the energy, redox and mass balances of the cell processes (Smolders et al., 1994a). When comparing the two strategies, ASM models require a plant-tailored calibration procedure that can affect a higher number of variables, whereas metabolic models have been reported to require a simpler calibration procedure, since all of the equations for the microbial processes are interdependant (Seviour et al., 2010). Both approaches have been combined in the Technical University of Delft model (TUDP) and successfully applied for full-scale WWTPs, describing anaerobic, anoxic and aerobic processes of polyphosphate accumulating organisms (PAOs) (Van Veldhuizen et al., 1999; Brdjanovic et al., 2000; Meijer et al., 2001).

EBPR is carried out by PAOs using three different internal storage compounds that generate energy and/or reducing power, i.e., polyphosphate (poly-P), glycogen and polyhydroxyalkanoate (PHA). Anaerobically, volatile fatty acids (VFAs) like acetate and propionate are converted into PHA by consuming poly-P and glycogen, while PHA is then consumed for P uptake and glycogen production under anoxic and aerobic conditions. Additionally, PAOs have to withstand competition from glycogen accumulating organisms (GAOs), for which external parameters, such as temperature, pH, COD:P ratio and carbon source, play a significant role (Oehmen et al., 2007). Hence, the initial metabolic models developed for PAOs (Smolders et al., 1994a, 1995; Kuba et al., 1996; Murnleitner et al., 1997), were expanded to include the metabolic pathways of two main GAOs, i.e., *Competibacter* and *Defluviicoccus vanus*-related organisms, as well as the effects of temperature, carbon source and pH on their metabolism (Lopez-Vazquez et al., 2009). Moreover, the denitrification capacities of *Accumulibacter*, the main PAO known, and *Competibacter*- and *Defluviicoccus*-GAOs were further incorporated in the model by Oehmen et al. (2010b).

However, these new additions have only been validated in lab-scale systems containing enrichments of PAOs and GAOs and have not previously been tested on full-scale sludge. While simplified metabolic model calibration strategies have been proposed based on lab-scale results (Oehmen et al., 2010b), it is necessary to test these theories using full-scale sludge in order to evaluate their applicability to more complex situations. It is noteworthy to mention that when modelling the performance of full-scale systems, there is an added complexity, since not only could there be unknown PAOs and/or GAOs whose contribution to the phosphorus removal process is still unknown, but known PAOs such as *Tetrasphaera* could be active, whose metabolism related to EBPR is still largely unclear. Adding to this complexity is the fact that wastewater influents contain a wider diversity of organic carbon sources that are subject to much variability, and PAOs and GAOs make up a much smaller fraction of the total microbial community in full-scale sludge as compared to lab-scale systems.

Although PAOs have been typically modelled as using the glycolysis pathway as their sole source of anaerobic reducing power generation, it has been suggested that the role of the anaerobic TCA cycle in real WWTPs might be greater than expected as compared to lab-scale results (Pijuan et al., 2008; Lanham et al., 2013). In fact, Zhou et al. (2009) have shown that the TCA might have a particularly prominent role when PAOs face conditions of glycogen shortage. Since WWTPs deal with variable influent compositions and often with limited carbon substrate availability, this might be the reason for a greater reliance on the TCA cycle in WWTPs as opposed to lab-scale reactors (Lanham et al., 2013). Therefore, in order to improve the applicability of metabolic models, particularly with respect to full-scale situations, the relevance of incorporating the TCA cycle activity into the model should be assessed.

Furthermore, in previous metabolic models the aerobic maintenance processes predict cell decay at low PHA levels, which is not consistent with experimental findings. Experiments on the endogenous metabolism of PAOs (Lopez et al., 2006; Lu et al., 2007; Vargas et al., 2013) observed that the aerobic maintenance processes were dependant on glycogen and polyphosphate degradation following PHA depletion, with minimal cell decay. This is a particularly relevant factor to include when applying the model to full-scale systems, where the level of polymers stored by the sludge is much lower as compared to lab-scale systems.

In this study, a simplified version of the metabolic models previously developed by Lopez-Vazquez et al. (2009) and Oehmen et al. (2010b) was adapted in order to incorporate the anaerobic TCA utilisation of PAOs, in addition to the previously implemented glycolytic pathway. The resulting model was tested by describing the anaerobic/aerobic chemical transformations observed in activated sludge batch tests fed with acetate as carbon source. The tests were carried out with sludge from four different EBPR WWTPs with differing microbial compositions (including different fractions of *Accumulibacter*, *Competibacter* and *Defluviicoccus*) and metabolisms, as shown in Lanham et al. (2013). Special attention was paid to the required calibration procedure necessary in order to describe the activity of each biomass, and where possible, simplified calibration procedures that could be applicable to the modelling industry were evaluated. In addition, theoretical simulation studies were conducted between PAOs using solely the TCA cycle anaerobically (PAO_TCA) and PAOs using glycolysis (PAO_Glyc) in order to better understand the conditions which may lead to the use of one metabolic pathway over the other. Thus, this study is also relevant to improve our knowledge about factors that influence the microbial metabolism in EBPR systems, which is necessary in order to better understand and optimise the performance of the process.