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The impact of aeration on the competition between polyphosphate accumulating organisms and glycogen accumulating organisms

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ABSTRACT

In wastewater treatment plants (WWTPs), aeration is the major energetic cost, thus its minimisation will improve the cost-effectiveness of the process. This study shows that both the dissolved oxygen (DO) concentration and aerobic hydraulic retention time (HRT) affect the competition between polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). At low DO levels, *Accumulibacter* PAOs were shown to have an advantage over *Competibacter* GAOs, as PAOs had a higher oxygen affinity and thus largely maintained their aerobic activity at low DO levels, while GAO activity decreased. Bioreactor operation at low DO levels was found to increase the PAO fraction of the sludge. Furthermore, an increase in aerobic HRT (at a DO level of 2 mg O_2/L), promoted the proliferation of GAOs over PAOs, decreasing the EBPR efficiency. Overall, this study shows that low aeration can be beneficial for EBPR performance through selecting for PAOs over GAOs, which should be incorporated into WWTP models in order to minimise energetic costs and improve WWTP sustainability.

1. Introduction

In wastewater treatment plants (WWTPs), the economic, environmental and social factors should be considered in order to improve process sustainability (Molinos-Senante et al., 2012; Muga and Mihelcic, 2008). Improving the energetic efficiency of WWTPs is central to this theme, as minimising energetic inputs both lowers operational costs and reduces indirect greenhouse gas emissions. Several factors contribute towards WWTP energy consumption, where the aeration requirements are typically the most significant. Indeed, it has been estimated that the aeration requirements correspond to 45–75% of WWTP energetic costs (Rosso et al., 2008), so it is necessary to decrease the oxygen requirements in order to improve the cost-effectiveness of the WWTP.

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Enhanced biological phosphorus removal (EBPR) is an economic and sustainable process used to remove phosphorus (P) from wastewater. In this process, it is necessary to control the competition between polyphosphate accumulating organisms (PAOs) and another group of microorganisms known as glycogen accumulating organisms (GAOs) (Oehmen et al., 2007). Minimising the growth of GAOs improves the efficiency of organic carbon and oxygen utilisation for P removal, thus minimising P effluent concentrations.

The competition between PAOs and GAOs, and consequently the efficiency of EBPR, can be affected by several factors, such as pH (Filipe et al., 2001; Lopez-Vazquez et al., 2009; Weissbrodt et al., 2013; Zhang et al., 2007), temperature (Lopez-Vazquez et al., 2009; Weissbrodt et al., 2013; Whang and Park, 2006), volatile fatty acids (VFAs) composition of the wastewater (Lopez-Vazquez et al., 2009; Oehmen et al., 2006), sludge age (Whang and Park, 2006) and the ratio of P to organic carbon in the influent (Liu et al., 1997). However, the effect of aeration on the PAO–GAO competition has never been systematically studied.

Previous studies have shown that PAOs can be selected over GAOs at various dissolved oxygen (DO) concentrations, including low DO levels of about 0.5 mg/L (Lemaire et al., 2006), and high DO concentrations > 3 mg/L (Oehmen et al., 2005; Pijuan et al., 2004), achieving successful EBPR operation. Griffiths et al. (2002) performed morphological observations of WWTP sludges and hypothesised that the DO concentration could affect the quantity of tetrad forming organisms thought to be GAOs. Numerous studies (Li and Chen, 2011; Li et al., 2008; Zheng et al., 2009), have operated EBPR systems at very low DO concentrations (0.15–0.45 mg/L), with variable P removal efficiency achieved (61–99%). However, none of these studies have investigated the effect of DO concentration on the microbial community and their metabolism, thus it is still unknown how the DO level impacts the PAO–GAO competition and EBPR efficiency.

Furthermore, it is not only the DO concentration that can affect the success of EBPR, but also the length of the aerobic period. Brdjanovic et al. (1998) showed through a series of short-term tests that extended aerobic periods can promote the deterioration of the EBPR process through excessive consumption of their internal storage polymers. Since both PAOs and GAOs depend on the consumption of storage polymers such as polyhydroxyalkanoates (PHA) aerobically, it is unclear if increased PHA consumption would be more beneficial for PAO or GAO. Thus it is important to study the impact of aerobic hydraulic retention time (HRT) on the competition between PAOs and GAOs, since its effect on the microbial selection of PAOs vs GAOs is not yet known.

In this study, the effect of aeration on the competition between PAOs and GAOs was investigated for the first time. For this purpose, batch tests of enriched PAO and GAO cultures were performed at different DO levels, in order to determine how the aerobic kinetics of each population varies as a function of the DO concentration. Moreover, long-term tests were performed to assess the performance and microbial population of each system operated at different DO levels. Finally, the impact of the aerobic HRT on the microbial population and EBPR performance was also studied. This knowledge will contribute towards the understanding of the competition between PAOs and GAOs as a function of both the DO concentration and the aerobic HRT. This could lead to the development of strategies to minimise WWTP energetic costs, improving the EBPR efficiency, and enabling greater sustainability of these processes.

2. Materials and methods

2.1. *SBR operation*

Two sequencing batch reactors (SBR) with 2 L of working volume were operated to obtain enriched cultures of PAOs and GAOs. The reactors were seeded with sludge from a wastewater treatment plant in Lisbon, Portugal. Each cycle consisted of 6 h, with a 2 h anaerobic period, 3 h aerobic period and 1 h settle/decant period. Both reactors were fed, during the first 5 min of the anaerobic phase, with 1 L of synthetic medium and were operated with a hydraulic retention time (HRT) of 12 h and a solids retention time (SRT) of 8 days. The initial chemical oxygen demand (COD) concentration in each reactor was 200 mg/L, where the PAO reactor contained a mixture of acetate (HAc) and propionate (HPr) as carbon source (75–25% HAc–HPr), while the GAO reactor was fed with only HAc. The difference in carbon source fed to each system was based on a previous study (Lopez-Vazquez et al., 2009) that predicted that PAOs would proliferate with a 75–25% HAc–HPr ratio and GAOs would proliferate with only HAc. The 1 L synthetic medium was composed of 250 mL of solution A and 750 mL of solution B. Solution A contained per liter, in both reactors: 0.59 g NH_4Cl, 0.95 g $MgSO_4.7H_2O$, 0.44 g $CaCl_2.2H_2O$, 11.7 mg allyl-N thiourea (ATU), 31.7 mg ethylenediaminetetraacetic (EDTA), 3.17 mL of a micronutrients, 2.55 g $C_2H_3O_2Na.3H_2O$ and 270 μL $C_3H_6O_2$ in the PAO reactor or 3.40 g $C_2H_3O_2Na.3H_2O$ in the GAO reactor. The micronutrient solution (based on Smolders et al. (1994)) contained per litre: 1.5 g $FeCl_3.6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4.5H_2O$, 0.18 g KI, 0.12 g $MnCl_2.4H_2O$, 0.06 g $Na_2MoO.2H_2O$, 0.12 g $ZnSO_4.7H_2O$, 0.15 g $CoCl_2.6H_2O$. Solution B contained per liter: 124.1 mg K_2HPO_4 and 96.8 mg KH_2PO_4 in the PAO reactor and 7.50 mg K_2HPO_4 and 5.86 mg KH_2PO_4 in the GAO reactor. In both reactors, the temperature was controlled at $20 \pm 1\,°C$. pH was controlled, by automatic addition of 0.1 M HCl, at 7.5 and 7.0 for PAO and GAO reactors, respectively, which is also consistent with previous predictions for the selection of PAOs and GAOs, respectively (Lopez-Vazquez et al., 2009). To ensure anaerobic conditions in the reactors, argon was bubbled at a flow rate of approximately 15 mL/min. In the aerobic phase, the dissolved oxygen was maintained at 8 ± 0.2 mg O_2/L (with continuous aeration) or controlled at 2 ± 0.2 mg O_2/L using an on/off control valve. Both reactors were operated for >3 SRT at the aforementioned conditions with continuous aeration before commencing DO control at 2 mg O_2/L. Operation with DO control continued for >3 SRT prior to performing the batch tests detailed below. The objective of this decrease in DO concentration to 2 mg O_2/L was to mimic the DO level often experienced in full-scale EBPR plants.

The SBRs were monitored through routine sampling of volatile fatty acids (VFAs), P, PHAs and glycogen along the anaerobic/aerobic cycles. Total suspended solids (TSS) and