Sulfide Effects on the Anaerobic Kinetics of Phosphorus-Accumulating Organisms

S. A. Saad*, L. Welles**, C. M. Lopez-Vazquez **, M.C.M. van Loosdrecht ***, D. Brdjanovic**,***

*Department of Civil Engineering, Faculty of Engineering, Ain Shams University, 1 El Sarayat St., Abbassia, Post code 11517, Cairo, Egypt. (E-mail: s.saad@unesco-ihe.org)
**Department of Environmental Engineering and Water Technology, UNESCO-IHE Institute for Water Education, Westvest 7, 2611 AX Delft, The Netherlands. (E-mail: l.welles@unesco-ihe.org; c.lopezvazquez@unesco-ihe.org; d.brdjanovic@unesco-ihe.org)
***Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands. (E-mail: m.c.m.vanloosdrecht@tudelft.nl; d.brdjanovic@unesco-ihe.org)

Abstract
Saline wastewaters can be produced from industrial activities, the use of seawater and brackish water in urban environments, or due to saline water infiltration into the sewer system. This can lead to sulfate-rich wastewater that under anaerobic conditions and the presence of electron donors can result in sulfide production. This study focuses on evaluating the sulfide effects on the biological phosphorus removal process which is a key process to prevent eutrophication on surface-water bodies. In this regard, anaerobic short-term sulfide inhibition tests were executed on an enriched culture of phosphorus-accumulating organisms (PAO) at different pH, and sulfide concentrations. Sulfide had a negative effect on PAO activity, and the effect seemed to be related to un-dissociated H\textsubscript{2}S concentration. 50% inhibition of the maximum acetate uptake rate of PAO was observed at around 60 mg H\textsubscript{2}S/l regardless the pH. With increasing H\textsubscript{2}S concentrations, higher ratios of phosphate release to HAc uptake rate were observed likely due to extra need for energy for cell detoxification. P-release for detoxification energy requirements (P\textsubscript{det}) were estimated relative to the total P-release rate at zero H\textsubscript{2}S, assuming a fixed ratio of P-release required for HAc uptake. Increasing H\textsubscript{2}S increased P\textsubscript{det} until a maximum of 50%. A further increase in H\textsubscript{2}S caused a decrease in P\textsubscript{det} possibly compensated by higher glycogen utilization. Mathematical expressions to describe the sulfide effect on acetate consumption and P-release have been proposed. These can be used to describe the anaerobic kinetics of PAO under the presence of sulfide when treating saline wastewater in general activated sludge models.

Keywords
Sulfide; phosphorus accumulating organisms; anaerobic; saline wastewater treatment.

INTRODUCTION
Saline wastewater rich in sulfate can be generated in food processing industries and leather tanneries (Lens, et al., 1998), which can also contain significant amounts of phosphorus, ranging from 14 to 100 mgP/l on average. (Gonzalez J.F., 1983; Orhon D., 1999). Seawater infiltration, intrusion into the sewerage and the use of hard water for domestic purpose can lead to up to 500 mg/L of sulfate in wastewater (Lens, et al., 1998). Sulfide, generated from the sulfate reduction under anaerobic conditions using organic carbon as electron donor, is at certain concentration considered toxic to microorganisms, especially its un-dissociated form (H\textsubscript{2}S) (Speece, 1983). Though the biological removal of carbon and nitrogen processes can deal with relatively high dissolved sulfide concentrations, up to 90 mg S/l (Lau, et al., 2006), the effect of sulfide on the enhanced biological phosphorus removal (EBPR) process is however unknown. EBPR, carried out by phosphorus-accumulating organisms (PAO), is a key process to avoid eutrophication by reducing the discharge of phosphorus into surface water bodies (Mino, et al., 1998; Oehmen, et al., 2007). On the other hand, the direct use of seawater for sanitation purposes is effective to reduce freshwater demand, contributing to water scarcity solutions (Li, et al., 2005). The later approach has been successfully supported by the development of the SANI saline wastewater treatment system for carbon and nitrogen removal where sulfide plays a key role (Wang, et al., 2009), however it does not incorporate the removal of phosphorus yet. Thus, it is interesting to evaluate the
effects of sulfide on PAO and to contribute to develop strategies to secure the satisfactory performance of the EBPR process under the eventual presence of sulfide as well as to support the incorporation of EBPR into the SANI process.

MATERIALS AND METHODS

Enrichment of PAO
A PAO culture was enriched in a double-jacketed laboratory sequencing batch reactor (SBR) operated in cycles of 6 hours following similar operating conditions used in previous studies (Brdjanovic, et al., 1997; Lopez-Vazquez, et al., 2009) with the exception of: (i) pH (maintained at 7.6 ± 0.05), (ii) dissolved oxygen concentration which was controlled at 20% of the saturation concentration (around 1.8 mg/l), and (iii) the influent carbon source comprised of 300 mgCOD/L as acetate (HAc) and 100 mgCOD/L as propionate (HPr).

Short term anaerobic batch experiments
Eight sets of experiments at three different pH values were performed at a controlled temperature of 20±0.5 °C using an automatic pH control (±0.1). Anaerobic batch test experiments were carried out in two double-jacketed laboratory reactors with a maximum operating volume of 0.5 L and a volatile fatty acid solution that contained an HAc-to-HPr ratio of 3:1. A defined volume of a sulfide stock solution was added to each reactor to reach the target sulfide concentrations of study.

The batch experiments were conducted with 150 ml of sludge transferred from the parent SBR at the end of aerobic phase to each batch reactor. Samples were taken throughout the experiment that lasted for 2.5 hrs. The initial substrate concentration was the same in all the experiments, but the sulfide concentrations were adjusted in accordance to the inhibitory effects observed during the execution of the tests.

RESULTS AND DISCUSSION

Sulfide effect on maximum acetate uptake rate of PAO
At the different tested pH values, a decrease in the maximum specific acetate uptake rate was observed relative to the control (with no sulfide present). 50% inhibition on the acetate utilization was found at around 60 mg H₂S/l regardless the pH value. The strong correlation between the hydrogen sulfide concentration in its uncharged form (H₂S) and the relative acetate uptake activity allowed its description using a non-competitive inhibition model (Figure 1). On the contrary, a clear trend for the activity with either total sulfide or bisulfide ion concentrations was not found (data not shown). The inhibition of the PAO acetate uptake process could have been caused by the dissociation of H₂S inside the cells which can induce anion and proton accumulation within the cells (Roe, et al., 1998). Also, the formed protons inside the cells have to be expelled out to maintain the proton gradient, on the expense of energy, leading to a reduction in cell growth rate and yield (Axe and Bailey, 1995; Russell, 1992).

Sulfide effects on PAO stoichiometry and phosphate release
Phosphate is released anaerobically to the bulk liquid by PAO for energy production that is mostly used for acetate uptake and maintenance. Consequently, the measured total P-release involves different processes. A decrease in the relative P-release rate relative to that at no sulfide, was observed at pH 6.5 and 7.0, while an increase followed by a decrease occurred at pH 7.8 (data not shown). On the other hand, the phosphate release to acetate uptake ratio at pH 6.5 and 7.0 values started with an increase up to 200 mgS/L and remained relatively constant for higher concentrations (Figure 2). At pH 7.8, it increased up to 80 mgS/L but suddenly dropped above this concentration (Figure 2). These trends suggested that, at pH 6.5 and 7.0, though the P-release and acetate utilization decreased, the still increasing P-release-to-acetate uptake ratio was caused by an increase
in maintenance requirements or detoxification processes rather than due to substrate uptake.

**PAO detoxification under the presence of sulfide**

Assuming a constant P-release needed for acetate utilization, and that the total P-release occurs as a consequence of the energy requirements for acetate uptake, maintenance and detoxification, the relative P-release for detoxification can be estimated by the difference between the total P-release at different pH values and the P-release needed for acetate uptake and maintenance (Figure 3). Thus, the detoxification P-release increased linearly up to around 55% when compared to the P-release observed under zero sulfide conditions. Above these values, another process, possibly an increase in glycolysis rather than poly-P hydrolysis, likely occurred to continue to provide the required energy, suggesting a potential shift in metabolic pathways. (Ye, et al., 2013) observed a similar detoxification effect on PAO metabolism (leading to a higher glycogen utilization) due to the presence of free nitrous acid (FNA). Remarkably, a detoxification model was able to provide a satisfactory description of the P-release profiles based on the energy provided by the poly-P hydrolysis for detoxification (Saad SA, et al., 2013).

**Figure 1.** Simulated (line) and measured (points) relative HAc uptake activity at different H₂S concentrations

**Figure 2.** Relative P/HAc ratio at different pH

**Figure 3.** Relative P-release for detoxification (points) and model simulation (lines) at different pH values
CONCLUSIONS
Sulfide in un-dissociated form appeared to have been the more inhibiting compound of PAO anaerobic kinetics in comparison to bisulfide or total sulfide. At around 60 mg H$_2$S/l, 50% inhibition of the anaerobic acetate uptake rate of PAO was observed. Nevertheless, H$_2$S will not likely hinder the integration of EBPR into the SANI process due to the high operating pH value in the SANI, which leads to lower H$_2$S in the water phase. An increase in maintenance phosphate release activity was observed coupled to an increase in hydrogen sulfide concentrations which is assumed be related to detoxification activity. Interestingly, the anaerobic P-release decreased at higher sulfide concentrations, suggesting a potential increase in glycogen utilization and therefore a shift in metabolic pathways. Remarkably, both the sulfide effects on acetate consumption and P-release were satisfactorily described by applying a non-competitive inhibition model and a detoxification model, respectively. These models can contribute to describe the anaerobic kinetics of PAO under the presence of sulfide when treating saline wastewater.

ACKNOWLEDGEMENTS
The author wishes to acknowledge the Egyptian Government which provided her with financial support through a government-sponsored scholarship. The authors like to thank the UNESCO-IHE laboratory staff for all their support.

REFERENCES
Lau GN, Sharma KR, Chen GH, Van Loosdrecht MCM. 2006 Integration of sulphate reduction, autotrophic denitrification and nitrification to achieve low-cost excess sludge minimisation for Hong Kong sewage. Water Science and Technology 53: 227-235
Speece RE. 1983 Anaerobic biotechnology for industrial wastewater treatment. Environmental science and technology - 179