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Sulfide effects on the anaerobic metabolism of polyphosphate-accumulating organisms

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HIGHLIGHTS

- Sulfide inhibits HAc-uptake rate of '*Candidatus* Accumulibacter phosphatis' type I.
- Sulfide affects the anaerobic stoichiometry of PAO I.
- Exposure to sulfide induces P-release presumably for detoxification.
- Sulfide effects on P-rel & HAc-uptake rates well described by mathematical model.

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ABSTRACT

Sulfate rich wastewaters can be generated from industry, use of seawater in urban environments, or by saline water infiltration into the sewerage. Under anaerobic conditions sulfate can be converted to sulfide, which may affect micro-organisms performing biological nutrient removal. The objective of this study was to evaluate the effect of sulfide on the activity of polyphosphate-accumulating organisms (PAO) in the anaerobic stage of the enhanced biological phosphorus removal process (EBPR). In this regard, a highly enriched culture of PAO was exposed in short-term activity tests to a range of sulfide concentrations at different operational pH values. The PAO activity was mainly affected by un-dissociated H_2S. The specific acetate uptake rate was inhibited by 50% at around 60 mg $H_2S.L^{-1}$. With increasing H_2S concentrations, higher phosphate release rate to acetate uptake rate ratios were observed, possibly due to increased energy requirements for cell detoxification. Mathematical expressions were developed, which satisfactorily described the sulfide effects on the acetate uptake rate and phosphate release rate. The results show that, dependent on the pH, EBPR might be negatively affected by total sulfide concentrations exceeding 275 mg $SO_4.L^{-1}$ at pH 6.5 or 1200 mg $SO_4.L^{-1}$ at pH 7.8 mg $SO_4.L^{-1}$ or when freshwater is partially replaced by seawater more than 45% (pH 7.8) or 10% (pH 6.5) used as secondary quality water. The findings of this study imply that sulfide, which is commonly found in different type of wastewaters, affects the anaerobic metabolism of PAO and may play an important role in the process performance of treatment plants treating wastewaters with high sulfide content.

1. Introduction

Different types of activities or events can lead to the generation of wastewaters containing elevated sulfate and phosphorus concentrations. For instance, saline wastewater rich in sulfate can be generated in food processing industries and leather tanneries [1]. These industrial wastewaters often contain significant amounts of phosphorus, ranging from 14 to 100 mgP.L^{-1} [2,3]. Due to seawater infiltration into the sewerage during high tides, intrusion of brackish groundwater in coastal areas as well as the use of hard water for domestic purpose [1], sulfate concentrations as high as 500 mg.L^{-1} can be found in wastewaters. In addition, direct use of seawater or brackish ground water for sanitation purposes or in the urban water cycle, a promising option to mitigate fresh

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water shortage in urban areas, introduces salinity and sulfate in domestic wastewaters [4]. In different stages of the wastewater collection and treatment, sulfate can be converted to sulfide under anaerobic conditions in the sewerage [5], in anaerobic tanks with long hydraulic retention times in conventional nutrient removal systems [6,7], or in the sludge treatment facilities from where supernatant is recycled back to the beginning of the main stream of the waste water treatment plant (WWTP). Sulfide formation in conventional systems is generally considered as an unwanted process due to the risks for human health, the potential harmful effects of sulfide on the treatment facilities and the biological nutrient removal processes for carbon, nitrogen and phosphorus.

Sulfide is toxic to microorganisms, especially its un-dissociated membrane permeable form (H_2S) [8]. The micro-organisms responsible for biological removal of carbon and nitrogen processes can still tolerate relatively high dissolved sulfide concentrations, up to 90 mg $S.L^{-1}$ [9]. Some studies showed that sulfide indirectly affected the EBPR systems, by the development of filamentous bulking, leading to wash out of sludge from the reactor and subsequent deterioration of EBPR [10,11]. However, the direct effect of sulfide on the enhanced biological phosphorus removal (EBPR) process remains unclear. Therefore there is a need to study the sulfide effects on the EBPR process.

The enhanced biological phosphorus removal process is a widely implemented process for removal of excessive phosphate concentrations through the storage of intracellular polyphosphate by polyphosphate-accumulating organisms (PAO) and subsequent removal of PAO biomass through wastage of activated sludge. The PAO are able to anaerobically take up volatile fatty acids and store them in the form of PHAs, and acquire energy for this process from the degradation of intracellularly stored polyphosphate, releasing ortho-phosphate to the bulk liquid. In the subsequent aerobic/anoxic phase PAO grow and take up ortho-phosphate to recover their poly-P pools, leading to phosphorus removal from the bulk liquid [12,13].

The objective of this study was to evaluate the direct effect of sulfide on the different anaerobic metabolic processes of "*Candidatus* Accumulibacter phosphatis" clade IC (a well-known PAO) and to develop an inhibition model that is capable of describing the sulfide effects on the anaerobic metabolism. The study provides fundamental insight in the toxicity effects on the metabolism of PAO and contributes to the development of measures for securing satisfactory EBPR performance in conventional biological nutrient removal processes exposed to sulfide. In addition the study helps to explore the possibilities of incorporating EBPR into the SANI® (Sulfate reduction, Autotrophic denitrification, Nitrification Integrated) process for saline wastewater treatment [14].

2. Materials and methods

2.1. Enrichment of PAO, and operation of parent SBR

For the enrichment of PAO, activated sludge was used as inoculum from wastewater treatment plant (WWTP) Harnaschpolder, a municipal wastewater treatment plant in The Netherlands performing simultaneous biological nitrogen and phosphorus removal. A PAO culture was enriched in a double-jacketed laboratory sequencing batch reactor (SBR) of 2.5 L working volume. The SBR was automatically controlled and online operating data (e.g. pH and DO) was stored. It was operated in cycles of 6 h (135 min anaerobic, 135 min aerobic and 90 min settling and decanting phase), pH was maintained at 7.6 ± 0.05, and temperature was controlled at 20 ± 1 °C. In the aerobic phase, dissolved oxygen was controlled at 20 ± 2% of dissolved oxygen saturation level (around 1.8 $mg.L^{-1}$). Exchange volume in the reactor was 1.25 L, sludge retention time (SRT) and hydraulic retention time (HRT) were controlled at 8 days, and 12 h respectively. Since this study is a continuation of a previous study with the same sludge, a detailed description of the PAO enrichment is reported elsewhere [15,16].

The influent of the reactor contained per liter: 637.5 mg $NaAc{\cdot}3H_2O$ (9.5 C-$mmol.L^{-1}$, 300 mg $COD.L^{-1}$), $6.675x10^{-2}$ ml $C_3H_6O_2$ (2.72 C-$mmol.L^{-1}$, 100 mg $COD.L^{-1}$), 107 mg NH_4Cl (2 N-$mmol.L^{-1}$), 111.3 mg $NaH_2PO_4{\cdot}H_2O$ (0.81 P-$mmol.L^{-1}$, 25 mg PO_4^{3-}-$P.L^{-1}$), 90 mg $MgSO_4{\cdot}7H_2O$, 14 mg $CaCl_2{\cdot}2H_2O$, 36 mg KCl, 1 mg of yeast extract, and in addition, 2 mg of allyl-N-thiourea (ATU) was added to inhibit nitrification. The trace element solution was prepared as described by [17].

The performance of the SBR was regularly monitored by measuring orthophosphate (PO_4^{3-}-P), acetate (Ac-C), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Pseudo steady-state condition in the reactor was confirmed based on the daily measurements of the aforementioned parameters and the observation of pH and DO profiles

When the sludge performance in the parent SBR reached steady-state conditions, cycle measurements and microbial analyses were carried out to determine the biomass composition and activity. Subsequently, the sludge was transferred to small batch reactors and exposed to sulfide to assess the effect on the metabolism of the PAO.

2.2. Short term effect of sulfide Stress on PAO anaerobic metabolism experiments

In nine sets of experiments, a total number of 30 tests were conducted over a period of 24 weeks at three different pH values (pH 6.5, 7.0, 7.8). To assess the relative sulfide effects on the metabolism and to avoid potential interferences of periodic changes in the biomass performance, a control test (without sulfide) was conducted at each pH and in each set of experiments. The longest time span of a set of experiments with a control test was 2 weeks (less than 2 SRTs). The experiments were performed at a controlled temperature of 20 ± 0.5 °C and pH 6.5, 7.0 or 7.8 (±0.1). Anaerobic batch experiments were conducted in two double-jacketed laboratory reactors in parallel with a working volume of 0.3 L (maximum operating volume of 0.5 L). A concentrated mineral stock solution was prepared, which provided after dilution the same concentration of minerals as the influent of the parent SBR. A concentrated volatile fatty acid (VFA) stock solution was prepared with an HAc:HPr ratio of 3:1 that provided after dilution the same VFA load per biomass (F/M ratio) as applied in the parent reactor. From each stock solution, 75 ml were introduced to each reactor and the mixture was sparged with nitrogen gas for 15 min to obtain anaerobic conditions in both the liquid and headspace. Subsequently the reactors were made air tight to avoid intrusion of oxygen or loss of sulfide. To avoid changes in pressure due to addition of solutions or sampling, while keeping an air-tight system, the vents of the reactors were connected to a closed empty Teddlar bag. When the nutrient solution and sludge were introduced to the reactors, the bags became partially filled by nitrogen gas. A sulfide stock solution of 24000 $mgS.L^{-1}$ was prepared freshly for every new experiment by dissolving $Na_2S{\cdot}9H_2O$ in nitrogen sparged 0.1 M NaOH. A specific amount of sulfide stock solution was added to each reactor to reach the target sulfide concentrations and concentrated 2 M HCl was dosed to adjust the pH to the target pH according to the experimental plan. During the rest of the test, automatic pH control was maintained by adding 0.1 M NaOH and 0.1 M HCl. At the end of aerobic phase in the parent SBR, 150 ml of sludge was transferred to each batch reactor to reach a final working volume of 300 mL (including the 75 mL added from each stock solution) and the transfer was considered as the start of the test. The sludge concentration in the batch reactors was 50% of that in the parent