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The role of sulfate in aerobic granular sludge process for emerging sulfate-laden wastewater treatment

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A B S T R A C T

Sulfate-rich wastewaters pose a major threat to mainstream wastewater treatment due to the unpreventable production of sulfide and associated shift in functional bacteria. Aerobic granular sludge could mitigate these challenges in view of its high tolerance and resilience against changes in various environmental conditions. This study aims to confirm the feasibility of aerobic granular sludge in the treatment of sulfate containing wastewater, investigate the impact of sulfate on nutrient removal and granulation, and reveal metabolic relationships in the above processes. Experiments were conducted using five sequencing batch reactors with different sulfate concentrations operated under alternating anoxic/aerobic condition. Results showed that effect of sulfate on chemical oxygen demand (COD) removal is negligible, while phosphate removal was enhanced from 12% to 87% with an increase in sulfate from 0 to 200 mg/L. However, a long acclimatization of the biomass (more than 70 days) is needed at a sulfate concentration of 500 mg/L and a total deterioration of phosphate removal at 1000 mg/L. Batch tests revealed that sulfide promoted volatile fatty acids (VFAs) uptake, producing more energy for phosphate uptake when sulfate concentrations were beneath 200 mg/L. However, sulfide detoxification became energy dominating, leaving insufficient energy for Polyhydroxyalkanoate (PHA) synthesis and phosphate uptake when sulfate content was further increased. Granulation accelerated with increasing sulfate levels by enhanced production of *N*-Acyl homoserine lactones (AHLs), a kind of quorum sensing (QS) auto-inducer, using S-Adenosyl Methionine (SAM) as primer. The current study demonstrates interactions among sulfate metabolism, nutrients removal and granulation, and confirms the feasibility of using the aerobic granular sludge process for sulfate-laden wastewaters treatment with low to medium sulfate content.

1. Introduction

Successful water management is vital for the development of coastal cities as the number of people who live within 200 kms of the coast are estimated to account for 75% of the world's population by the end of 2050 (Gladwin, 2008). The use of seawater as an alternative water source for municipal non-potable water purposes (e.g., cooling, toilet flushing, etc.) is a preferable approach in view of energy efficiency and brine discharge (Grant et al., 2012). For instance, Hong Kong has implemented seawater toilet flushing for nearly 60 years, achieving a 22% reduction in freshwater demand (Lu et al., 2011). Extensive application of seawater as an alternative water resource in Hong Kong results in 1.7 million cubic meters/day saline wastewater containing chloride and sulfate concentrations of 5000 and 550 mg/L on average, respectively (Ding, 2010). Both salinity and sulfide generation caused by sulfate reduction pose noteworthy operational problems and challenges to conventional

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activated sludge processes when treating these brackish or saline wastewaters. In light of these considerations, aerobic granular sludge (AGS), a rapidly growing wastewater treatment technology, might provide a suitable solution for saline/brackish wastewater treatment in coastal regions in view of its excellent settling-ability, high biomass retention, reactor compactness, and high resilience against variations in pollutants and environmental conditions (de Bruin et al., 2004; Beun et al., 1999; de Kreuk and Van Loosdrecht, 2006; Pronk et al., 2015). Successful application of AGS in coastal areas can alleviate space and energy shortage as well as potentially tackle the threats posed by sulfate-laden wastewater. However, the influence of sulfate on biological nutrients removal (particularly phosphate) and sludge granulation is yet to be understood fully, which is necessary for facilitating optimization of AGS for sulfate-laden saline/brackish wastewater treatment.

For AGS systems, alternative anaerobic/aerobic operation is commonly applied in a Sequencing Batch Reactor (SBR) to achieve simultaneous removal of organics and nutrients (Beun et al., 1999). The anaerobic phase enables acidification, hydrolysis, denitrification and phosphate release, while organics removal, nitrification and phosphate uptake occur during the aerobic phase. However, anaerobic treatment of high sulfate-containing (>500 mg/L) wastewater inevitably leads to a shift in the functional microbial community and change in the metabolic pathways (Guo et al., 2016). Conventional biological nutrient removal processes are threatened by out- competition of sulfate-reducing bacteria (SRB), leading to inhibition of ordinary heterotrophic organisms (OHO), including phosphate-accumulating organisms (PAO), by sulfide generated from sulfate reduction (Muyzer and Stams, 2008; Karhadkar et al., 1987; Jin et al., 2013). Recently, sulfate-associated phosphate removal has been observed in a full-scale enhanced biological phosphorus removal (EBPR) process (Ginestet et al., 2015) and a lab-scale sulfate-driven AGS process named DS-EBPR, consisting of completely different microorganisms from that of conventional AGS (Wu et al., 2013). However, the exact mechanism for phosphate removal under high sulfate content as well as the necessary process conditions of the DS-EBPR are not well understood.

To evaluate the feasibility of applying aerobic granular sludge for sulfate-laden wastewater treatment, the overall role of sulfate in the AGS process requires a systematic investigation. This work thus focuses on studying the interrelations among sulfur metabolism, sludge granulation and nutrients removal, especially phosphate removal under alternating anoxic/aerobic conditions. Investigations of five lab-scale granular sludge systems with different sulfate concentrations was conducted by continuous examination of intermediates, internal storage compounds and internal electron shuttles generated during the operation. From the above study, key metabolic relationships among sulfate metabolism, nutrient removal and granule formation are elucidated.

2. Materials and methods

2.1. Reactor setup and operational condition

Five lab-scale air-lift SBRs, each having a working volume of 1.1 L (100 cm in height and 5 cm in diameter), were operated in parallel with different influent sulfate concentrations of 0, 50, 200, 500 and 1000 mg/L respectively. The operation cycle of these reactors were identical, consisting of 10 min feeding, 100 min anoxic phase with dissolved oxygen (DO) concentration of 0.1–0.3 mg/L, 100 min aeration with DO of 3–6 mg/L, 10 min settling, 10 min decant and 10 min idle. All reactors were inoculated with activated sludge taken from a local saline wastewater treatment plant in Hong Kong at an initial mixed liquid suspended solid (MLSS) concentration of 10 g/L. Sludge retention time (SRT) was not deliberately controlled through sludge wastage but was naturally stable at around 35 days due to natural washout of biomass in the effluent. All reactors were operated for 130 days, during which hydraulic retention time (HRT) was 8 h and temperature was controlled at 23 ± 1 °C.

2.2. Feeding

Synthetic wastewater was prepared from a stock solution containing organics, mainly comprising of acetate and glucose, ammonia and phosphate as well as micronutrients and alkalinity. Detailed components of the stock solution are shown in Table S1 and S2 of Supplementary Information (SI). The stock was diluted with tap water 100 times. Then, additional sodium acetate, NH_4Cl, K_2HPO_4 and KH_2PO_4 were added to set COD, ammonia-N and phosphate concentrations of the influent at 600, 100 and 40 mg/L respectively. The ratio of bicarbonate ($NaHCO_3$) to ammonium-nitrogen (N) in each reactor influent was set at 4 in order to maintain pH at moderate level. The designated sulfate concentration for each reactor was achieved by addition of sodium sulfate to the necessary level.

2.3. Supplementary experiments

Three supplementary experiments were conducted after 130 days of operation. The first experiment (A) aimed to distinguish the impacts of sulfate concentrations from changes in ionic strength on nutrient removal efficiency. In the second (B) and third (C) tests, VFA and phosphate uptake were monitored after using 2–4 dinitrophenol to eliminate proton motive force (PMF) (Morley et al., 1992) and adding sodium molybdate for sulfate reduction inhibition (de Jesus et al., 2015). In this way, the chain effects imposed by sulfate reduction on proton motive force (PMF), volatile fatty acid (VFA) intake, PHA storage, energy availability, and phosphate uptake were investigated. Detailed descriptions of these supplementary experiments are supplied in the Supplementary information (SI).

2.4. Analytical methods

Total organic carbon (TOC), ammonia-N and phosphate of the influent and effluent were measured regularly for reactor performance monitoring. Batch tests of five reactors were conducted at day 60, 90 and 120. In these tests, TOC, ammonia-N, phosphate, nitrate, nitrite, sulfate and sulfide concentrations were analyzed according to the Standard Methods (APHA, 2005). Detailed analytical procedures are provided in the SI.

Polyhydroxyalkanoate (PHA), including poly-*b*-hydroxybutyrate (PHB) and poly-*b*-hydroxyvalerate (PHV), was quantified by gas chromatography mass spectrometry (GC-MS) (Agilent 7890A-5975C) following Wu et al. (2013). Particle size distribution was determined using laser diffraction particle size analyzer (LSI3 320, Beckman Coulter). MLSS, Mixed Liquid Volatile Suspended Solid (MLVSS), and Sludge Volume Index at 5 and 30 min (SVI_5 & SVI_{30}) were measured periodically according to the Standard Methods (APHA, 2005). Extracellular Polymeric Substances (EPSs) were extracted and analyzed for polysaccharides and protein quantity according to Liu and Fang (2002). Polysaccharides components of EPSs were analyzed using Fourier transformation infrared spectroscopy (FTIR) (Vertex 70 Hyperion 1000, Bruker). Detailed extraction and analysis methods are provided in the SI.

Adenosine triphosphate (ATP) and zeta-potential of sludge were measured on day 85 and 110 at the beginning and end of the anoxic phase as well as the end of the entire cycle using an ATP Determination Kit (Thermo Fisher) and Zetasizer Nano Z (Malvern, UK),