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Free nitrous acid inhibition on carbon storage microorganisms: Accumulated inhibitory effects and recoverability

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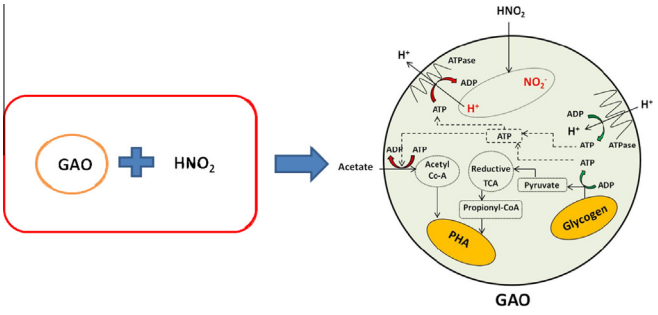
HIGHLIGHTS

- Application of GAO in the A-stage of the AB process for enhanced carbon entrapment.
- Nitrite leakage into the A-stage tank leads to FNA inhibition on GAO metabolisms.
- FNA inhibition on GAO continued to the following FNA-free phases.
- FNA-induced inhibition on carbon metabolisms of GAO was found to be reversible.
- Dynamics of cellular carbon storage could be linked to detoxification activity.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent research has shed light on utilization of carbon storage microorganisms in the A-stage of AB process for higher methane generation and resource recovery potential. Typically, organic matters are entrapped into biomass in the A-stage and subsequently channeled to the anaerobic digester for energy/resource recovery. In the following B-stage, nitrite shortcut strategy is often implemented to achieve low energy nitrogen removal. In this study, an enriched glycogen accumulating organism (GAO) culture was deployed as the A-stage carbon storage microorganisms to enhance the removal of soluble COD. This study aimed (1) to address the challenge arising from incidental nitrite leakage into the A-stage tank, leading to free nitrous acid (FNA) inhibition; and (2) to evaluate the continued (henceforth referred to as 'accumulated') inhibitory effects on GAOs' carbon metabolisms under the subsequent FNA-free condition. Upon FNA exposure, dynamics in carbon storage mechanisms were obtained and could be linked to higher cellular energy expenditure for detoxification activity. The inhibition on carbon transformation, however, was found to be reversible, suggesting the robustness of GAO towards FNA inhibition and its potential application in the nitrite-shortcut AB process.

1. Introduction

The AB process has received considerable attention lately due to concern over energy consumption in wastewater treatment. The AB process is a two-stage approach with an extremely high loaded carbon capture stage (A-stage), which is subsequently followed by a low loaded biological stage (B-stage) and so ensuring removal of

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dissolved organics and ammonia. In the recent years, there has been interest in application of the nitrite shortcut strategy for nitrogen removal in the B-stage due to its potential carbon and energy savings [1,2]. The entrapped organics (energy) during the A-stage is recovered from the solids train containing thickened sludge through biogas generation in the anaerobic digester located downstream.

It was proposed that entrapment of organics in the A-stage mainly involves three mechanisms: (i) fast microbial growth, (ii) bioflocculation and enmeshment (physico-chemical adsorption by cell surface typically known as biosorption or extracellular polymeric substances (EPS) entrapment), and (iii) bioaccumulation into the cell [3–6]. It is generally assumed that under non-substrate limiting condition, the A-stage microorganisms take up readily biodegradable COD in excess of and at a faster rate than its consumption for cell growth and subsequently store it as intracellular storage products, such as poly-hydroxyalkanoates (PHAs) or glycogen [2,7]. Upon depletion of the influent COD, the microorganisms would in turn utilize the stored substrate as carbon and energy sources. On the other hand, the particulate and colloidal fractions of the COD are removed via surface sorption and EPS entrapment [8]. Among the above mechanisms, microbial storage is considered important for the following reasons: (1) lower aeration energy requirement than mineralization; and (2) the ability to recover not only energy but also material resources. Indeed, a recent report had suggested that approximately 66.3% of the overall carbon removal capacity during A-stage was attributable to intracellular carbon storage as PHA [4]. In addition, higher methane generation was obtained from anaerobic digestion using PHA accumulating sludge as substrate as compared to the excess activated sludge [9,10]. Glycogen accumulating organisms (GAOs) are among the major groups of microorganisms highly capable for carbon removal via internal storage mechanism in the form of PHAs. GAOs phenotype anaerobically takes up soluble carbons for storage as PHA by utilizing intracellular glycogen as a source of energy. In the subsequent aerobic phase, PHA is used for active biomass production, glycogen replenishment and cellular maintenance. For the selection and acclimation of GAOs in the A-stage reactor, subdivision into anaerobic and aerobic zones is therefore required.

The suspended solids concentration of the mixed liquor in the A-stage reactor is maintained by returning sludge from the end of A- and B-stage [11]. A side stream sludge breeder tank may also be designed as an additional biomass source. In the effort to reduce energy expenditure and greenhouse gas emission from wastewater treatment, removal of nitrogen via partial nitrification has been increasingly applied in the following B-stage. In this configuration, an incidental nitrite leakage to A-stage with the recycle flow is likely to take place as a result of nitrite accumulation in B-stage. The accumulation of nitrite is often intensified during an upset process or a process during start-up period and by the high-strength ammonia stream of reject water originating from dewatering activity of digested sludge which is typically returned to the mainstream bioreactor as well as the infiltration of industrial wastewater [12]. Such leakage would cause inhibition on carbon storage microorganisms and lead to the deterioration of the A-stage performance.

Various researchers have reported the accumulation of nitrite or nitrous acid (FNA or HNO_2) inhibits various microorganisms existing in the wastewater treatment system, such as ammonia and nitrite oxidising bacteria (AOB and NOB) [13,14], denitrifiers [15], poly-phosphate accumulating organisms (PAOs) [16,17], and GAOs [18]. It has then been reported that FNA, instead of nitrite, is the key factor responsible for the inhibition [19]. While FNA inhibition on carbon uptake by GAO has been reported elsewhere [20], there are no reports on the recovery of GAO after FNA inhibition to date. It is indeed crucial to investigate the response of GAO after inhibition, so that long-term performance can be predicted. Furthermore, in order to address the incidental nitrite leakage as a possible challenge in the implementation of GAO as a supplemental factor responsible for enhanced sCOD removal in the A-stage of AB process and to study the post-inhibition recovery, a series of experiments have been carried out. The objectives of this study included: (1) to investigate the accumulated effect of FNA on carbon uptake and transformation by the GAO-acclimated sludge; and (2) to evaluate the recovery of GAO metabolisms in the subsequent FNA-free condition.

2. Materials and methods

2.1. Sludge acclimatization

GAOs were cultivated in a laboratory-scale sequencing batch reactor (SBR) under alternating anaerobic–aerobic conditions. Inoculum was taken from a conventional WWTP in Singapore. The SBR had a working volume of 4 L and was operated with a cyclic time of 4 h consisting of 70 min anaerobic, 160 min aerobic, and 10 min wasting, settling and decanting periods. To maintain anaerobic and aerobic conditions, nitrogen gas and air were sparged intermittently through the mixed liquor at a flow rate of 1.0 L/min during the respective periods. One liter of synthetic wastewater containing acetate as the sole carbon source, ammonium, and microelements was fed into the SBR in the first 10 min of the anaerobic period, resulting in a hydraulic retention time of 16 h. The initial COD and ammonium concentrations were 200 mg COD/L and 10 mg NH_4^+-N/L, respectively. The microelements solution was prepared in accordance with Smolders et al. [21]. 5 mg/L ATU (allylthiourea) was applied to inhibit nitrification. The solid retention time (SRT) was maintained at 8 d by wasting 83 mL of mixed liquor at the end of each cycle. The reactor was operated in a temperature-controlled room at 24 ± 1 °C. The pH was controlled during both the anaerobic and aerobic phases at a range of 7.0–7.3 by dosing 0.5 M HCl and 0.5 M NaOH as required. Weekly cycle study was performed to monitor the SBR performance. After the SBR had reached steady-state condition, as indicated by stable treatment performance and biomass concentration (4.4 ± 0.3 g MLVSS/L), sludge was withdrawn at the end of the cycle and used for batch experiments.

2.2. Batch experiments

A series of batch experiments were carried out with GAO sludge to investigate the accumulated effects of FNA on the metabolisms of GAO as well as the recoverability from the inhibition in the subsequent FNA-free cycles. Due to the absence of nitrification during acclimation period, the GAO culture used in this study was not adapted to nitrite/FNA. The sludge was withdrawn from the SBR at the cycle end and subsequently washed with phosphate buffer to remove any residual nutrient. The washed sludge was then distributed into 250 mL batch reactors where a 2-h anaerobic condition was first applied. N_2 gas was sparged through the headspace to ensure anaerobic conditions. Acetate and nitrite stock solutions were then injected, resulting in initial acetate concentration of 150 mg COD/L and the initial nitrite concentrations are as presented in Table 1. The FNA concentration was calculated using $S_{N\text{-}NO_2}/(K_a * 10^{pH})$, with the K_a value determined using $e^{-2300/(273+T)}$ for any given temperature T (°C) [13]. The control test was carried out in the absence of nitrite.

2 mL mixed liquor samples were taken at 15-min intervals using a syringe and immediately filtered through disposable Millipore filter units (0.45 μm pore size) for the analyses of acetate and nitrite. Solid samples (10 mL mixed liquor) for the analyses of PHA