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Response of nitrogen removal performance, functional genes abundances and *N*-acyl-homoserine lactones release to carminic acid of anammox biomass

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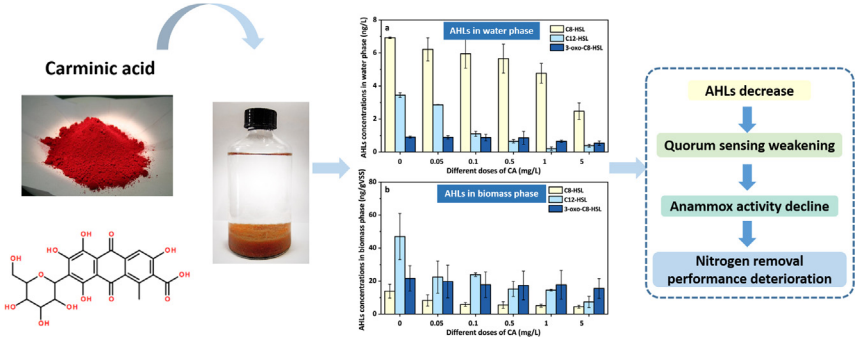
GRAPHICAL ABSTRACT



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ABSTRACT

Carminic acid (CA) can serve as a redox mediator and influence the electron transfer process. CA dosages of 0–5 mg/L were added to anaerobic ammonia oxidation (anammox) biomass. The results illustrated that CA not only reduced the inorganic nitrogen removal efficiency, but also decreased the nitrogen removal rate. The deterioration of nitrogen removal performance was due to the excess production of nitrate-nitrogen. The concentration of extracellular polymeric substances showed a decrease together with a decline in *N*-acyl-homoserine lactones release. CA addition decreased the activity of anammox bacteria while increasing the nitrifying potential. Quantitative reverse transcription polymerase chain reaction showed a decrease in anammox functional genes (*nirS*, *hzo*, and *hzsB*) and promotion of the expression of the *nxrB* gene, which corresponded with a decrease in anammox bacteria activity and the improvement of nitrifying potential. As a result, CA should not be added to anammox biomass.

1. Introduction

The anaerobic ammonia oxidation (anammox) process is a biological nitrogen removal process by which ammonium-nitrogen (NH_4^+- N) is directly transferred to nitrogen gas using nitrite-nitrogen (NO_2^-- N) as the electron acceptor (Jetten et al., 2001). Compared with traditional biological nitrogen removal technologies (Klein et al., 2017a; Klein et al., 2017b), anammox is recognized as a sustainable and environmentally friendly process for inorganic nitrogen removal in wastewater treatment because the process does not require oxygen or

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organic carbon and reduces the carbon dioxide and nitrous oxide emissions (Lackner et al., 2014; Rikmann et al., 2018; Zekker et al., 2019). This process can reduce the cost of wastewater treatment (Rikmann et al., 2018; Zekker et al., 2019).

A redox mediator is a molecule that can function as an electron shuttle between electron acceptors and bacteria (Rabaey et al., 2005). Electron shuttles have the ability of transferring electrons between an extensive variety of compounds in redox reactions, including inorganic and organic compounds (Van der Zee and Cervantes, 2009). Various redox mediators have been applied in wastewater treatment, especially the microbial electron transfer process (Van der Zee and Cervantes, 2009). Qiao et al. (2014) investigated the short-term effect of three redox mediators (anthraquinone-2,6-disulfonate, 2-hydroxy-1,4-naphthoquinone, and anthraquinone-2-carboxylic acid) on the anammox process, and the results showed that the increase in the dosage of redox mediators had a more negative impact on nitrogen removal. Rikmann et al. (2014) also reported that anthraquinone-2,6-disulfonate improved the production of hydrazine (N_2H_4) and that the total nitrogen removal rate was further increased by 20%. Graphene oxide had been reported as an electron shuttle to increase the redox conversion process (Colunga et al., 2015). In the study conducted by Wang et al. (2013), a maximum increase of 10.26% in anammox activity together with greater extracellular polymeric substances (EPS) production was obtained with 0.1 g/L of graphene oxide. Furthermore, reduced graphene oxide was reported to have a greater ability of electron transfer than graphene oxide by approximately three orders of magnitude, with the total nitrogen removal rate and enzyme activity increasing by 10.2% and 1.5–2 fold, respectively (Wang et al., 2013).

Quorum sensing (QS) refers to the cell-cell communication process of the ability of bacteria to sense their density by exchanging communication molecules called autoinducers (Jemielita et al., 2018). Recently, the metabolic pathways and communication mechanism of *N*-acyl-homoserine lactones (AHLs)-mediated QS regulation have been mentioned in the anammox consortia (Tang et al., 2018a; Tang et al., 2018b). In particular, *N*-(3-oxohexanoyl)-$_{DL}$-homoserine lactone (3-oxo-C6-HSL), *N*-hexanoyl-$_{DL}$-homoserine lactone (C6-HSL), and *N*-octanoyl-$_{DL}$-homoserine lactone (C8-HSL) regulate the electron transport carriers that influence bacterial activity (Tang et al., 2018b). Adding *N*-dodecanoyl-$_{DL}$-homoserine lactone (C12-HSL) into the reactors can reduce the anammox process start-up period to 66 d compared with the control group start-up period of 80 d, thereby indicating that C12-HSL has the ability to promote the specific anammox activity (Zhao et al., 2018).

Carminic acid (CA) is a deep red anthraquinone that is extracted from scale insects, and it is widely used as a pigment in different fields, e.g., crimson ink, paints, cosmetics, and food coloring (Dapson, 2007). CA has the redox group of anthraquinone and the oxidation group of hydroquinone, and it can act as a redox mediator (Li et al., 2013). Wolf et al. (2009) explored the effects of quinones containing various redox functional groups on microbial ferrihydrite reduction kinetics by *Geobacter metallireducens* at low concentrations, and found that CA concentrations of 0.1–10.0 μm had no apparent effect on the iron reduction kinetics. Li et al. (2013) also investigated the effects of CA on goethite reduction and current production by *Klebsiella pneumonia* L17, and the result showed that there was no clear effect on Fe(III) reduction.

To date, study on CA addition to anammox biomass was lacking. In this study, different concentrations of exogenous CA were added to anammox biomass, and the following aspects were determined: (1) the response of nitrogen removal performance, (2) the change in QS signal molecule concentrations and EPS concentrations, and (3) the variation of anammox bacteria activity, nitrifying potential, and the change in functional genes expression activities according to quantitative reverse transcription polymerase chain reaction (qRT-PCR) after CA addition.

2. Materials and methods

2.1. Original anammox biomass and CA

The anammox biomass was obtained from a 4 L biofilm reactor that had been operating in a laboratory for 1.5 years with a stable total inorganic nitrogen (TIN) removal efficiency of more than 80%, with 100 mg/L of NH_4^+- N and 132 mg/L of NO_2^-- N in the influent under 35 ± 1 ℃. The hydraulic retention time was maintained at 24 h.

CA (CAS No. 1260-17-9) was purchased from Dr. Ehrenstorfer GmbH (Germany).

2.2. Set up of batch experiments

Serum flasks with an effective volume of 250 mL were used for batch exposure assays, and the volatile suspended solids (VSS) concentration was 2200 mg/L in each flask. Before feeding into the flask, anammox biomass was washed in phosphate-buffered saline (PBS) (0.14 g/L of KH_2PO_4 and 0.75 g/L of K_2HPO_4) three times in order to eliminate the residual nitrogen in the biomass. The synthetic wastewater contained 50 mg N/L of $(NH_4)_2SO_4$ and 50 mg N/L of Na_2NO_2 as the ammonia and nitrite sources, respectively, 500 mg/L of $NaHCO_3$ for alkalinity addition, and 100 mg/L of $MgSO_4{\cdot}7H_2O$, 180 mg/L of $CaCl_2{\cdot}2H_2O$, and 27 mg/L of KH_2PO_4. In addition, 1 mL/L of trace element solutions I and II were added to the influent (Sliekers et al., 2002). The liquid was purged with highly purified nitrogen gas for at least 10 min to remove dissolved oxygen (DO). Six sets of batch experiments were conducted with CA concentrations of 0, 0.05, 0.1, 0.5, 1, and 5 mg/L, and each set of experiments was conducted in triplicate. The control group was set as 0 mg/L of CA addition. The original pH was fixed at 7.8 by 1 M HCl and 1 M NaOH. The operating temperature of the shaker was maintained at 37 ± 0.2 ℃ and the shaker speed was 170 rpm to maintain full contact. Liquid samples were gathered by a syringe with a long needle and periodically purged through 0.45 μm membranes for measurement of nitrogen species.

2.3. Analytical methods

The concentration of nitrate-nitrogen (NO_3^-- N) was determined by ion-exchange chromatography (IC-1100, Thermo Fisher, USA). NH_4^+- N, NO_2^-- N, mixed liquor suspended solids, and VSS concentrations were detected based on the APHA Standard Methods (APHA, 2005). The concentration of TIN was calculated as the sum of NH_4^+- N, NO_2^-- N, and NO_3^-- N. DO and pH were determined by a portable DO meter (HQ 30d, HACH, USA) and a digital pH meter (JENCO Model 6010, China), respectively.

2.4. Determination of anammox bacteria activity and nitrifying potential

Anammox biomass was gathered at the end of 48 h to test the anammox bacteria activity and nitrifying potential. The anammox activity test was described by Dapena-Mora et al. (2010). Briefly, biomass samples were washed with PBS solution three times, and substrates of 20 mg/L of NH_4^+- N and 20 mg/L of NO_2^-- N were added. Each serum bottle contained approximately 400 mg VSS/L of biomass and was stripped with nitrogen gas for 10 min. Supernatants were obtained at intervals of 30 min to measure the NH_4^+- N and NO_2^-- N concentrations in order to calculate the anammox bacteria activity. This test was conducted in duplicate and the average value and standard deviation were calculated for analysis.

For the nitrifying potential test, 20 mg/L of NH_4^+- N or NO_2^-- N was fed separately with adequate aeration. The liquid sampling interval was 30 min (Rich et al., 2008; Sun et al., 2019) and the concentrations of NH_4^+- N, NO_2^-- N, and NO_3^-- N were detected to test the nitrifying potential (Almstrand et al., 2011).