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# Bacterial community evolutions driven by organic matter and powder activated carbon in simultaneous anammox and denitrification (SAD) process

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ABSTRACT

A distinct shift of bacterial community driven by organic matter (OM) and powder activated carbon (PAC) was discovered in the simultaneous anammox and denitrification (SAD) process which was operated in an anti-fouling submerged anaerobic membrane bio-reactor. Based on anammox performance, optimal OM dose (50 mg/L) was advised to start up SAD process successfully. The results of qPCR and high throughput sequencing analysis indicated that OM played a key role in microbial community evolutions, impelling denitrifiers to challenge anammox's dominance. The addition of PAC not only mitigated the membrane fouling, but also stimulated the enrichment of denitrifiers, accounting for the predominant phylum changing from *Planctomycetes* to *Proteobacteria* in SAD process. Functional genes forecasts based on KEGG database and COG database showed that the expressions of full denitrification functional genes were highly promoted in $R_C$, which demonstrated the enhanced full denitrification pathway driven by OM and PAC under low COD/N value (0.11).

## 1. Introduction

Recently, nitrogen-rich wastewater has received much attention due to its potential threat to aquatic ecosystem. Anaerobic ammonium oxidation (anammox) was regarded as a high efficient and cost saving process to remove nitrogen from wastewater. In this process, anammox bacteria directly convert ammonium to nitrogen gas using nitrite as electron acceptor under anaerobic condition, as shown in Eq. (1) (Strous et al., 1998).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Although the processes based on anammox were high efficient, anammox plants were still a little around the world (Ni and Zhang, 2013). The greatest challenge for implementation was the slow growth rate of anammox bacteria, i.e. the double time of 11 days (Strous et al., 1998). However, a shortened double time of 3 days for anammox was found in a membrane bioreactor (MBR) (Van der Star et al., 2008) and the availability of a fast anammox process opened real perspectives for practical application from laboratory scale. Moreover, MBR was proved to exhibit an excellent performance for the start-up of anammox process (Wang et al., 2012). MBR seemed to be a good solution for the development of anammox related process. Although MBR had remarkable advantages, the control method and mitigating strategy of membrane fouling were still challenging, which might depress the applications of MBR in anammox process (Wang et al., 2012). It was shown that the membrane fouling was caused mainly by the attachment of anammox to the membrane (Jiang et al., 2013). Based on that, Zhang et al. (2016a,b) tried to adopt the microbiological immobilization technique to mitigate the membrane fouling in a MBR successfully by introducing carriers. While, except those information, for anammox related processes, the strategies for controlling membrane fouling of MBR is still lacking. It was proved that in traditional process, powder activated carbon (PAC) could mitigate the membrane fouling by reducing the attachment of organic and inorganic matters on the membrane surface (Villamil et al., 2016). In this study, PAC was applied in a MBR, which was proved to be an effective method to mitigate membrane fouling of simultaneous anammox and denitrification (SAD) process.

It is worthy to note that in anammox process, nitrate (about 11%) is

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formed and accumulates gradually in effluent (Eq. (1)). Thus, the total nitrogen (TN) in effluent may exceed the standards for nitrogen discharge to the environment. Industrial wastewaters usually contain both nitrogen and organic matter (OM) (Boley and Müller, 2005; Sun et al., 2010). The OM in wastewater can be electron donors for the reduction of produced nitrate during anammox process. It was imagined that the effluent from aerobic tank was combined with the digester effluent and then treated in a granular-sludge anammox reactor to ensure the remaining nitrate be below the required effluent standards (Kartal et al., 2010). But what counted was that a combination of simultaneous anammox and denitrification was set up to realize simultaneous removal of nitrogen and OM (Li et al., 2016). In SAD process, anammox converts ammonium and nitrite to dinitrogen gas and nitrate, then the nitrate is transformed to nitrite via partial denitrification or dinitrogen gas via full denitrification. Subsequently, the produced nitrite and residual nitrite are consumed using OM as an electron donor.

Although OM was necessary for denitrification, several studies revealed its varying effects on anammox bacteria. Viet et al. (2008) found that acetate (below 640 mg COD/L) did not affect the anammox and there was no decrease in activity, while more acetate (up 1280 mg COD/L) could cause obvious inhibition on anammox. Excess acetate stimulated the denitrifiers to grow faster than the autotrophic anammox bacteria and this out-competition reduced the anammox nitrogen removal capability (Jin et al., 2012). Other research showed that anammox could bear glucose (below 180 mg COD/L) and work well, while be severely inhibited by methanol (24 mg COD/L) (Zhong and jia, 2013), which might due to the direct inhibition of methanol on anammox bacteria (Jin et al., 2012). Moreover, it was reported that anammox could even utilize propionate and acetate and showed a more versatile metabolism (Güven et al., 2005). Although denitrification process needs sufficient OM, for anammox, limited OM concentration in wastewater is preferred in most cases, which will avoid the inhibition of OM on anammox and high cost for external carbon. It is significant to find a way to enhance the denitrification pathway under limited OM concentration in SAD process.

Moreover, the addition of OM not only influence the activity of anammox, but also change the microbial community. It was reported that the microbial community shifted from a mixed anammox community to *Candidatus Brocadia fulgida* as the dominant anammox type under an elevated COD/N ratio (0.8), with acetate as OM source (Jenni et al., 2014). Other study revealed that a predominant class shift from *Alphaproteobacteria* and *Gammaproteobacteria* to *Betaproteobacteria* occurred in granular and flocculent sludge when the COD/N ratio was decreased from 4 to 2 and 1 (Luo et al., 2014). Until now, the effect of PAC on microbial community in SAD process is still unclear. The evaluation of bacterial community is necessary to investigate the effect of OM and PAC on community evolutions in SAD process.

The present investigation aimed at directly revealing the enhanced denitrification pathway driven by OM and PAC in SAD process. The primary goals were to: (1) start up and evaluate the SAD process performance in an anti-membrane fouling submerged anaerobic membrane bio-reactors (SAMBRs) under optimal OM dose with/without the addition of PAC, (2) reveal the microbial community variations in SAMBRs driven by OM and PAC, and (3) explore the effect of PAC on controlling membrane fouling.

## 2. Materials and methods

### 2.1. SAMBR setup and operation

Three parallel submerged anaerobic MBRs were constructed in this experimentation, and their schematic diagram was shown as Fig. 1. The reactors were made-up of plexiglas material, with an effective volume of 6 L, an internal diameter of 14 cm, and a height of 50 cm. Reactor temperature was controlled at 34 ± 1 °C via water bath. Some sliver papers on the surface of the reactor were covered to avoid the underlying growth of phototrophic organisms which had potential competition for substrates with anammox bacteria and denitrifiers.

The feed pump was kept at a rotation rate of 0.7 rpm, and the effluent pump was controlled by a level sensor in the reactor to maintain a HRT of 48 h. The membrane module was consisted of 100 microporous hollow fibers (length 30 cm; diameter 1 mm; micro-hole diameter 1 μm) with a total effective membrane surface of 0.094 $m^2$. The membrane modules and reactors were all sealed with vaseline to maintain an isolated environment. Aerating apparatus was set in the reactor and the influent tank, and nitrogen gas was supplied through the apparatus twice a day to remove the DO in the influent water and reactors. The pH sensor was installed to keep the pH ranging from 7.3 to 7.7 using the sodium hydrate and hydrochloric acid. Pure nitrogen gas was pumped into the influent tank and reactors twice a day to drive out any oxygen. Both influent tank and reactors were sealed carefully to keep strict anaerobic conditions. A magnetic mixture device was used to mix the biomass and liquid in the reactors. Besides, a vacuum gauge was connected with the membrane module to test the trans-membrane pressure (TMP).

Prior to the start-up of SAD process, experiment about the effect of OM on anammox was progressed in two SAMBR (Reactor A and B). Glucose and sodium acetate were added into reactor B, meanwhile, as a control, no OM was added into reactor A. Acetate and glucose were chosen as OM source in this study, instead of other organic substrates, for two reasons: (1) It was reported that *Ca. Brocadia fulgida* had the ability to oxidize acetate (Jenni et al., 2014); (2) Glucose had little effect on anammox bacteria (Kartal et al., 2007; Zhong and jia, 2013). The aim of this preliminary experiment was to attain a proper dose of OM for subsequent start up of SAD process without fatal inhibitory effects.

After this experiment, three SAMBRs were set up for next experiment, and reactor A ($R_A$) was operated to proceed anammox process as the control reactor, the reactor B and reactor C ($R_B$ and $R_C$) were set for SAD process (influent OM concentration was 50 mg COD/L). The influent ammonium and nitrite in three reactors were 100 mg/L and 130 mg/L, respectively. To investigate the effect of PAC on membrane fouling, $R_C$ was applied with PAC (1 g/L).

### 2.2. Synthetic medium and inoculation

The synthetic wastewater was consisted of mineral medium and trace elements. Ammonium and nitrite were prepared using $NH_4Cl$ and $NaNO_2$, and the carbon source was glucose and sodium acetate at a mole ratio of 1:1 in required amount. The composition of the mineral medium was as follows (mg/L): $KHCO_3$ 500, $CaCl_2{\cdot}2H_2O$ 180, $MgSO_4{\cdot}7H_2O$ 120, $KH_2PO_4$ 27 and 1 mL/L of trace elements solution. The trace elements were consisted of (mg/L): EDTA 15,000, $ZnSO_4{\cdot}7H_2O$ 430, $CoCl_2{\cdot}6H_2O$ 240, $MnCl_2{\cdot}4H_2O$ 990, $CuSO_4{\cdot}5H_2O$ 250, $NaMoO_4{\cdot}2H_2O$ 220, $NiCl_2{\cdot}2H_2O$ 190, $NaSeO_4{\cdot}10H_2O$ 210, and $H_3BO_4$ 14.

The SAMBR was seeded with mixed sludge (MLSS 4000 ± 50 mg/L) which was consist of one fourth anammox sludge and three fourths anaerobic granular sludge. The anammox sludge was taken from an up-flow anaerobic sludge blanket (UASB) reactor in our laboratory, which was operated at 31 ± 1 °C and continuously fed with ammonium (490 mg N/L) and nitrite (588 mg N/L). The anaerobic granular sludge was also taken from an UASB in our laboratory.

### 2.3. Analytical methods

The concentrations of ammonium ($NH_4^+$-N), nitrite ($NO_2^-$-N) and nitrate ($NO_3^-$-N) in the effluent were monitored by the Standard Methods (APHA, 1995). The COD was determined by potassium dichromate method. The DO was monitored by a digital DO meter (Hash HQ30d53LDO™), and pH was measured by a pH meter (pHS-3C acidometer). The extracellular polymeric substances (EPS) was extracted