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Inhibition of the anammox activity by aromatic compounds

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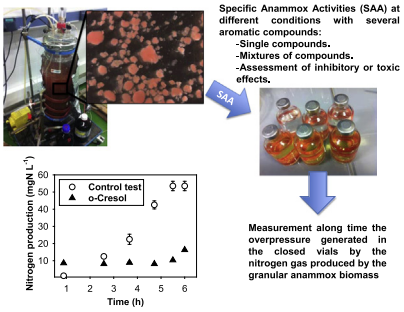
HIGHLIGHTS

- Short-term effect of several aromatics was evaluated over granular anammox sludge.
- The higher the concentrations of the aromatics, the higher the reduction of the SAA.
- Depending on the aromatic compound, toxic or inhibitory effect was measured.
- Synergistic effects were observed when mixtures of aromatic compounds were studied.

GRAPHICAL ABSTRACT



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ABSTRACT

The short-term effect of several aromatic compounds (*o*-cresol, *p*-nitrophenol, *o*-chlorophenol and quinoline) was evaluated over granular anammox sludge cultivated over 2 years in a Sequencing Batch Reactor (SBR). The anammox granular sludge had an average size of 1.0 ± 0.2 mm and was enriched in *Brocadia* sp. Specific Anammox Activity (SAA) batch tests with this granular biomass were carried out in the presence of *o*-cresol, *p*-nitrophenol, *o*-chlorophenol, quinoline and their mixtures. The anammox biomass was never exposed to the tested aromatic compounds, prior to the SAA tests. The concentration and the mixture of aromatic compounds had a strong effect over the loss of the anammox activity. The higher the concentrations of the aromatic compounds, the higher the reduction of the SAA. Quinoline and *p*-nitrophenol have a lower negative effect compared to *o*-cresol and *o*-chlorophenol. The Luong inhibition model seems to adjust better the inhibition of anammox biomass by the tested aromatic compounds. Depending on the aromatic compound, toxic or inhibitory effect was measured. *o*-Cresol and *o*-chlorophenol caused a toxic effect whereas *p*-nitrophenol and quinoline produced an inhibitory effect. In general, synergistic effects were observed when mixtures of aromatic compounds were studied.

1. Introduction

Complex industrial wastewaters composed by a mixture of high-strength ammonium and toxic/recalcitrant organic compounds are produced by industries like coking, petrochemical, waste management, steel manufacturing and coal gasification factories [1–8]. The main recalcitrant/toxic organic compounds present in these wastewaters are aromatic hydrocarbons, which include phenolic compounds, poly-aromatic hydrocarbons and mono- and poly-cyclic nitrogen-containing aromatics. Phenolic compounds, such as phenol, nitrophenols, methylphenols (cresols) and chlorophenols are commonly found. Other important aromatic compounds frequently found are heterocyclic compounds, such as quinoline. These industrial wastewaters are often treated by physic-chemical processes. However, these technologies have serious drawbacks such as: (i) high costs (due to the high temperature and pressure conditions needed and the chemicals required such as neutralising and oxidizing agents) [2,9–11]), (ii) do not allow complete degradation of the aromatic compounds and (iii) they

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can generate other hazardous by-products (secondary pollutants) [11,12]. On the contrary, biological treatments can satisfactorily overcome the disadvantages of physic-chemical treatments, but they are known to be sensitive to the aromatic compounds, mainly due to inhibitory effects [11]. Nevertheless, biological systems based on granular biomass can solve the problem associated with these inhibitory effects [13].

The application of a two-step granular process to treat high-strength ammonium and aromatic compounds wastewaters has been recently proposed [14–16]. In that configuration, the first aerobic granular reactor allows ammonium to be oxidized to nitrite by ammonia-oxidising bacteria and aromatic compounds to be totally biodegraded by specialized heterotrophic biomass [14–16]. In this sense, a suitable effluent for a subsequent anammox reactor can be achieved with: (i) a nitrite/ammonium ratio close to 1.0 and (ii) no presence of organic matter [14,15]. In this way, the anammox reactor would only receive aromatic compounds if the aerobic granular reactor is affected by shock-loads or sequentially alternating pollutants events [14,15].

The Specific Anammox Activity (SAA) has been demonstrated to be a very useful tool to assess the behaviour of anammox biomass at short-term under different conditions, including exposition to potentially endogenous or exogenous inhibitory compounds such as ammonia, nitrite, nitrate, inorganic salts and organic carbon sources [17–22]. Therefore, SAA tests seem to be appropriate to get some light about the behaviour of the anammox process in the presence of aromatic compounds. Nowadays, few references are reported regarding the effect of aromatic compounds over the anammox bacteria being phenol [20,23–27] and toluene [28] the only compounds that have been evaluated. Both aromatic compounds produce inhibition of the anammox biomass but the activity can be recovered (reversible inhibition) [20,23–28]. To the best of our knowledge, there are no references regarding the effect of other aromatic compounds over the anammox process. Therefore, this study aims to quantify, at short-term, the effect of several aromatic compounds: *o*-cresol, *p*-nitrophenol, *o*-chlorophenol and quinoline over a granular anammox biomass.

2. Materials and methods

2.1. Granular anammox biomass

A Sequencing Batch Reactor (SBR) of 10 L of effective volume was employed to enrich the anammox biomass during more than 2 years. The reactor has a diameter of 20 cm and a height of 61 cm. The temperature was controlled at 35 °C. pH of the SBR varied between 7.5 and 8.5 while the pH of the influent was between 6.5 and 7.0. Complete mixing was achieved using a mechanical stirrer with a rotating speed between 75 and 110 rpm. The reactor was operated in 6 h cycles distributed in four periods: mixed fill (300 min), mix (30 min), settle (15 min) and draw (15 min). The exchange volume was fixed at 25%, thus, the hydraulic retention time was 1 d. Low dissolved oxygen concentration was assured in the SBR by continuous addition of N_2 to the SBR headspace at a constant flow of 300 mL min^{-1}. Control of the pumps and different periods of the operational cycles was performed with a PLC system (Siemens LOGO! 230RC).

The SBR was fed with the following synthetic autotrophic medium [29] (in mg L^{-1}): 100 of $KHCO_3$, 50 of H_2PO_4, 100 of $CaCl_2 \times 2H_2O$, 200 of $MgSO_4 \times 7H_20$, 6.3 of EDTA and 1.25 mL L^{-1} of a trace elements solution. The ammonium to nitrite ratio in the feeding media was kept ca. 1.0 to operate in ammonium excess, avoiding nitrite accumulation in the reaction medium. The nitrogen loading rate (NLR) applied was 0.5 g N L^{-1} d^{-1} with a total nitrogen removal efficiency about 90%. The biomass concentration was 1.2 g VSS L^{-1}. The sludge volumetric index at 5 min (SVI_5) remained stable around 30 mL g^{-1} VSS, with a SVI ratio at 5 and 30 min (SVI_5/SVI_{30}) close to one during the whole operational period. The average particle size was also stable at 1.0 ± 0.2 mm.

2.2. Specific Anammox Activity (SAA) tests

(SAA) Batch experiments were employed to determine the SAA and to study the short-term effects of the chosen monosubstituted phenols (*o*-cresol, *p*-nitrophenol and *o*-chlorophenol) and a heterocyclic compound (quinoline). The applied methodology for the SAA determination was according to Dapena-Mora et al. [17], based on the measurement along time of the overpressure generated by the nitrogen gas produced by the anammox culture in closed bottles.

Completely closed bottles with a total volume of 60 mL (50 mL of reaction volume) were used to conduct the specific anammox activities. The bottles were inoculated with granular anammox biomass from the SBR described in Section 2.1. Previous to each test, the biomass was washed and re-suspended in phosphate buffer (0.14 g L^{-1} KH_2PO_4 and 0.75 g L^{-1} K_2HPO_4). The initial pH and biomass concentration in each vial were fixed at 7.8 and 1.0 g L^{-1}, respectively. Bottles were sealed tightly with butyl rubber caps. The headspace of the vial was gasified and purged with nitrogen gas to remove the oxygen. The bottles were placed in a thermostatic shaker, at 150 rpm and 30 °C until stable conditions were reached. Finally, the substrates ($(NH_4)_2SO_4$ and $NaNO_2$ at 35 mg N L^{-1} each one) and eventually, the aromatic compounds were added. Pressure was equalized to the atmospheric one prior to start the measurements. The batch tests were based on the measurement of nitrogen gas production and were tracked by measuring the overpressure in the headspace with a certain time frequency, by means of a portable transducer (range 0–5 psi; Centrepoint electronics, Ireland). The length of the test was established by the nitrogen production rate of the control test (without the presence of the aromatic compounds), which happened not later than 5–6 h after the start of each test (see Fig. 1). In each case, at least three bottles without any aromatic compound were used as control tests to assess the maximum specific activity of the biomass (SAA_{max}), while two other bottles were used for each tested concentration of aromatic compounds to measure its short-term effect over the anammox activity. The SAA presented in the results were denoted as relative SAA, referred to the SAA in the presence of the aromatic compounds over the SAA_{max} and the data are given in percentage (Eq. (1)):

$$\text{Relative SAA} = \frac{\text{SAA}_{\text{in the presence of aromatic compound(s)}}}{\text{SAA}_{(\text{max})}} \times 100 \quad (1)$$

In a first set of experiments, single aromatic compounds were added to the flasks. The range of the concentrations tested was 5–25 mg L^{-1} for *o*-cresol, *p*-nitrophenol and quinoline and 5–9 mg L^{-1} for *o*-chlorophenol. These concentrations ranges were tested taken into consideration two facts: (i) low concentrations can enter to an anammox reactor if a two-step granular process is applied (as is indicated in the introduction) and (ii) the effects obtained in the current study (see Section 3). Then, a second set of experiments was carried out to evaluate the effects of mixture of aromatic compounds (Table 1). The selection of the mixtures was done according to the behaviour obtained with the single aromatic compounds in the first set of experiments. Finally, a third set of experiments was performed with a slightly different methodology. In this case, after about 6 h of SAA test in presence of a single or a mixture of aromatic compounds, the bottles were opened and the biomass was carefully washed with the phosphate buffer. Subsequently, the bottles were closed again and flushed with nitrogen gas and a new SAA test was performed with the same