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Acute impact of erythromycin and tetracycline on the kinetics of nitrification and organic carbon removal in mixed microbial culture

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HIGHLIGHTS

- TET inhibited nitrification kinetics and stopped nitrite oxidation at higher dose.
- Both steps of nitrification were totally blocked by ERY at two tested doses.
- Both antibiotics induced partial inactivation of heterotrophic community.
- Both antibiotics increased substrate storage and accelerated endogenous respiration.
- Major inhibitory effect was binding and partial utilization of available substrate.

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ABSTRACT

The study evaluated acute impact of erythromycin and tetracycline on nitrification and organic carbon removal kinetics in mixed microbial culture. Acclimated biomass was obtained from a fill and draw reactor fed with peptone mixture selected as synthetic substrate and operated at a sludge age of 10 days. Acute inhibition was tested in batch reactors involving a control unit started solely with substrate and the others with additional doses of each antibiotic. Modeling indicated that both steps of nitrification were totally blocked by erythromycin. Tetracycline inhibited and retarded nitrification kinetics at 50 mg/L and stopped nitrite oxidation at 200 mg/L, leading to nitrite accumulation. Both antibiotics also affected organic carbon removal by inducing partial inactivation of the heterotrophic community in the culture, increased substrate storage and accelerated endogenous respiration, with a relatively slight impact on heterotrophic growth. Major inhibitory effect was on process stoichiometry, leading to partial utilization of organic substrate.

1. Introduction

Effective control and removal of nitrogen in wastewaters is usually secured in single-sludge biological treatment systems (Henze et al., 2000). Single-sludge activated sludge process is one of the most complex microbiological systems engineered for a specific purpose, which should control and sustain autotrophic microorganisms for nitrification, together with heterotrophs for organic carbon removal; it should also provide for the same heterotrophs, anoxic conditions suitable for the removal of oxidized nitrogen compounds – nitrite, nitrate – by means of anoxic respiration (Orhon et al., 1994, 2009). Nitrification is the essential preliminary part in nitrogen removal proceeding in two steps, where ammonia is first oxidized to nitrite – commonly called nitritation – by ammonia oxidizing bacteria (AOB) and nitrite is then converted to nitrate by nitrite oxidizing bacteria (NOB). The efficiency of nitrification is quite critical for nitrogen removal mainly because it determines the magnitude of oxidized nitrogen – mainly nitrate – available for denitrification and it controls the ammonia concentration in the process effluent (Sozen and Orhon, 1999; Henze et al., 2002). Nitrification is also considered as the rate limiting step in the design of single-sludge systems (Dold and Marais, 1986), since it proceeds at a much lower rate compared to heterotrophic activities and also, it is highly susceptible to adverse effects such as dissolved oxygen concentration, temperature, pH and inhibitory/toxic chemicals (Orhon et al., 2000; Sarioglu et al., 2009). Therefore, assessment of adverse effects is imperative for ensuring a sustainable nitrification.

In general, impact of various antibiotics on microbial cultures was evaluated mainly by means of inhibition tests yielding toxicity results on selected microorganisms (Halling-Sørensen, 2001). Observed adverse effects, although interesting, were mostly

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unsuitable for practical application as they were mostly related to specific environmental conditions – i.e. pH, temperature, time scale, etc. – associated with the selected testing procedure (Koller et al., 2000). Several studies also focused on the inhibitory impact of selected antibiotics and their mixtures on nitrifying bacteria in domestic sewage with conflicting results: Acute inhibition experiments as well as long-term inhibition tests indicated that antibiotics mainly reduced nitrification rate, with the exception of a few studies reporting no appreciable effect or even stimulatory effects (Campos et al., 2001; Christensen et al., 2006; Dokianakis et al., 2004; Ghosh et al., 2009; Halling-Sørensen, 2001; Louvet et al., 2010a).

Two different type of antibiotics, *tetracycline* and *erythromycin*, both affecting protein synthesis in bacteria, were selected for this study, as they were included in most inhibition studies. Previous work investigating the impact of these antibiotics on pure cultures and activated sludge also remained mostly qualitative, as they similarly reported inhibitory effect on the nitrification rate and toxic impact on nitrifying bacteria, without emphasizing process kinetics (Ghosh et al., 2009; Halling-Sørensen, 2001; Louvet et al., 2010a,b). Only nitrogenous compounds were monitored in most of these studies, except a few which also involved respirometric analyses (Dokianakis et al., 2004; Ghosh et al., 2009). Existing information in the literature does not cover simultaneous evaluation of nitrification and organic carbon removal under adverse effects of antibiotics in single sludge systems by available modeling techniques.

However, some recent studies involved respirometry and model evaluation, not for antibiotics, but for a range of inhibitory substances i.e. acetone, phenol and 2,6-DHBA (Contreras et al., 2008; Cokgor et al., 2011; Kaelin et al., 2009). The inhibitory impact was investigated on activated sludge by calibration of oxygen uptake rate (OUR) profiles for the assessment of process kinetics related to organic carbon removal, ammonia and nitrite oxidation. So far, only Pala-Ozkok et al. (2011) reported results on the impact of tetracycline and erythromycin on organic carbon utilization kinetics based on modeling of respirometric data. However, the impact on nitrification kinetics with a similar approach has not been fully investigated.

In practice, both the autotrophic and heterotrophic fractions of the microbial community sustained in single-sludge systems would be affected to a different extent by antibiotics in the influent stream. In this context, the objective of the study was to determine and evaluate the acute impact of tetracycline and erythromycin on nitrifying mixed microbial culture. It was conducted as part of a comprehensive research effort on the impact of antibiotics on mixed microbial systems, where the earlier work mainly focused on organic carbon removal under aerobic and anaerobic conditions (Cetecioglu et al., 2012; Pala-Ozkok et al., 2011). The acute impact was essentially assessed on process kinetics by model evaluation of respirometric data – oxygen uptake rate profiles - as well as experimental profiles of different nitrogen fractions. Heterotrophic activity was considered as an integral part of model evaluation on nitrification as a prerequisite for a reliable assessment of the fate of ammonia nitrogen in single-sludge systems. Accordingly, modeling covered heterotrophic processes and components for carbon removal, along with process kinetics and stoichiometry for nitrification.

2. Methods

2.1. Experimental rationale

The basis of the selected experimental system targeted the evaluation of the inhibitory impact of selected antibiotics on the nitrification process in a microbial system operated for simultaneous organic carbon removal and nitrification, i.e. sustaining a mixture of heterotrophic and autotrophic microbial fractions, as commonly observed in the biological treatment of domestic sewage (Cameau, 2008). For this purpose, a peptone/meat extract mixture – called *peptone mixture* for simplicity – was used as the synthetic substrate. This mixture is quite compatible with the objectives of the study, mainly because it is the recommended standard substrate for similar inhibition tests (ISO 8192, 1995) and it exhibits a COD fractionation and a corresponding biodegradation pattern, closely approximating sewage characteristics (Cokgor et al., 2011). First, an acclimated microbial community was sustained in a fill and draw reactor operated at steady-state at a sludge age of 10 days, enabling full nitrification. The COD concentration in the feed stream was adjusted to about 500 mg/L; at this level it also included a Total Kjeldahl Nitrogen (TKN) concentration of 50–55 mg N/L, corresponding to a COD/N ratio of 8–10 much like domestic sewage (Sozen and Orhon, 1999). This system also served in the earlier phases of the study for the evaluation of substrate biodegradation (Pala-Ozkok et al., 2012) and ammonification (Katipoglu-Yazan et al., 2012).

The essential part of the experiments involved testing a series of batch runs, started with the acclimated biomass seed taken from the fill and draw reactor, essentially for the assessment of ammonia oxidation kinetics. Each run included two parallel batch reactors, one for the respirometric analysis yielding the specific OUR profile for the selected operating conditions, and the other for monitoring concentration profiles of nitrogen fractions. Three different runs were conducted for this purpose: The first run was started only with around 500 mg COD/L of peptone mixture and served as the *control reactor*; the other two runs included, aside the organic substrate, an initial dose of 50 and 200 mg/L respectively of the antibiotic selected for its acute inhibitory impact. Batch experiments were conducted separately for erythromycin and tetracycline.

2.2. Batch experiments

The effects of selected antibiotics on nitrification and carbon removal were monitored by batch experiments. They were conducted in 2 L reactors with biomass taken from fill and draw reactor. Peptone mixture was supplied with 50 and 200 mg/L of TET or ERY solutions. Initial food to microorganisms ratio (S_0/X_0) was adjusted to around 0.49–0.55 mg COD/mg VSS. In order to prevent oxygen limitation in batch reactors, slightly higher S_0/X_0 ratios were selected compared to fill and draw reactor. Macro and micro nutrients were supplied with peptone mixture and antibiotics. The activity of biomass was monitored by means of nitrite and nitrate nitrogen generation, ammonia and COD removal efficiencies for 24 h. pH was also recorded in the course of experiments. Pertinent information related to batch experiments is outlined in Table 1.

2.3. Respirometric experiments

Oxygen uptake rate (OUR) measurements were conducted parallel to batch experiments. They were started with biomass seeding alone to obtain the initial endogenous OUR level; then, peptone mixture and antibiotic solutions were added for the desired S_0/X_0 ratios. Respirometric experiments which lasted for 15 h were performed with a Ra-Combo (Applitek Co., Nazareth, Belgium) continuous respirometer. The activity of nitrifying biomass and heterotrophs were monitored with oxidized nitrogen and COD compounds as well as pH and temperature. Dissolved oxygen level was kept between 6 and 9 mg/L. Corrections for O_2, pH and temperature parameters were considered in related rate expressions. Internal storage polymers polyhydroxyalkanoates