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Acute impact of tetracycline on the utilization of acetate by activated sludge sustained under different growth conditions

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HIGHLIGHTS

- Acute exposure to tetracycline retarded growth and accelerated endogenous decay.
- Tetracycline induced a shift in acetate utilization toward (PHB) storage.
- TET significantly increased the PHB to acetate ratio under stress conditions.
- Adverse effect of TET was more pronounced under fast growing conditions.
- Inhibitory impact followed a physical adsorption mechanism of TET onto biomass.

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ABSTRACT

The study evaluated acute impact of tetracycline on the biodegradation of acetate by microbial cultures acclimated to different growth conditions. Two fill/draw reactors were operated to obtain acclimated cultures at sludge ages of 2 and 10 days. Acclimated biomass seeding was used in two series of batch experiments. The first run served as control and others were started with tetracycline doses of 100 mg/L and 400 mg/L. Parallel batch reactors were also operated for oxygen uptake rate (OUR) measurements. Acute impact was evaluated by model calibration of OUR, chemical oxygen demand (COD) and intracellular storage profiles. Exposure to tetracycline did not impair COD removal but induced a shift in acetate utilization toward polyhydroxybutyrate (PHB) storage. This shift was more pronounced for fast growing biomass; it identified itself both in related process kinetics and the modified stoichiometry between the magnitude of acetate directly used for microbial growth and converted to PHB.

1. Introduction

After their amazing discovery in the last century, antibiotics have been marketed and used at an escalating trend both as effective therapeutics for human and animal infections and as growth stimulatory additives for animal feedstuff. Increased exposure to antibiotics is now a major environmental concern: Due to their inherent properties and lethal action against microbial activities, they are naturally resistant to biodegradation, by-pass biological treatment when discharged and mixed with wastewaters and accumulate in the environment to alarmingly high levels (Daghrir and Drogui, 2013; Wu et al., 2010; Karci and Balcioglu, 2009). The tetracycline family, including *tetracycline*, *chlortetracycline*, and *oxytetracycline*, represent one of the most widely used antibiotic group in the world (Kim et al., 2007). *Tetracycline* (TET), is a broad-spectrum antibiotic and acts as inhibitor of bacterial protein synthesis by binding the 30S ribosomal subunit (Cetecioglu et al., 2013; Ozkok et al., 2011). Due to its persistent characteristics, TET residues have been detected in terrestrial and aquatic environment along with other antibiotics (Michael et al., 2013; Pena et al., 2010; Kolpin et al., 2002).

Previous studies were mainly focused on the fate of TET in wastewater treatment systems; they mostly reported that the principal removal mechanism for TET was sorption onto sludge, under favorable operating conditions (Prado et al., 2009; Kümmerer, 2009). Kim et al. (2005) observed that 75–95% of TET was adsorbed onto sludge within one hour during lab-scale biodegradability experiments; adsorption efficiency was reduced when tests were conducted with sludge acclimated to lower sludge retention times. Reported results on the biodegradation of TET remained highly inconclusive and controversial, varying in the wide range of 12–80%, depending on the specific conditions of the experiments (Michael et al., 2013; Spongberg and Witter,

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2008; Karthikeyan and Meyer, 2006). No significant research effort was devoted to the inhibitory/toxic impact of TET on the microbial community sustained in biological treatment systems.

On the other hand, traditional modeling approaches based on well-known enzyme analogy were recently modified with the recognition of dissolved oxygen as a significant model component in a multi-component model structure (Pala-Ozkok and Orhon, 2013; Cokgor et al., 2009). The rate of oxygen utilization, commonly defined as oxygen uptake rate (OUR) is now extensively used for identifying and understanding related process stoichiometry and kinetics based on different biodegradable chemical oxygen demand (COD) fractions under aerobic conditions. With the important instrumental support improved for this purpose, respirometry with activated sludge modeling also created a new dimension for the concept of inhibition and provided relevant information on the true extent of toxic effects of a wide range chemicals on substrate biodegradation applied to different industrial wastewaters (Cokgor et al., 2009; Insel et al., 2006). In the context of a comprehensive study, the research group at ITU adopted this novel approach for the evaluation of inhibitory/toxic effects of different antibiotics; Ozkok et al. (2011) studied the acute effects of TET, sulfamethoxazole (SMX), and erythromycin (ERY) on the biodegradation of complex substrate, where modeling of respirometric data exhibited inhibitory impact on all processes involved. Similarly, chronic impact of SMX on acetate utilization kinetics was assessed by Kor-Bicakci et al. (2014). Additionally, studies also compared acute and chronic impacts of ERY on the utilization of a complex substrate (Pala-Ozkok et al., 2014a; Pala-Ozkok and Orhon, 2013). Another study showed acute impact of ERY and TET on nitrification and organic carbon removal kinetics in slow growing microbial culture (Katipoglu-Yazan et al., 2013). Furthermore, chronic impact of TET under anaerobic conditions were explored by Cetecioglu et al. (2013). Reported findings have set the basis for comparison for the results obtained in this study with emphasis on the role of culture history and organic carbon source.

In this context, this study evaluated the acute (short-term) impact of TET on the biodegradation of acetate. The effect of TET was assessed using biomass acclimated to sludge ages of 2 and 10 days to better understand the response of fast and slow growing microbial cultures. Acute impact is quite significant as it reflects conditions likely to occur when treatment systems receive a pulse discharge of pollutants. Experiments were conducted with high TET concentration, mainly to simulate conditions in the effluents in pharmaceutical plants, hospitals, etc., where antibiotics must be controlled and removed, before being mixed with main sewage streams.

2. Methods

2.1. Experimental approach

The experimental approach essentially consisted of a two parallel laboratory-scale fill and draw reactor sustained at sludge ages (SRTs) of 2 and 10 days under aerobic conditions. The biomass was taken from a wastewater treatment facility operated in Istanbul; it was cultured and acclimated to pulse feeding using laboratory-scale bioreactors operated at steady-state and fed with the selected organic substrate with a net aeration volume of 4 and 10 L at SRTs of 2 and 10 days, respectively. After the initial start-up period, the steady state operation extended and continued at least for a period of three STRs; this duration was 10 days for the SRT 2 days reactor and 30 days for the SRT 10 days reactor.

Acetate representing readily biodegradable substrate was chosen as sole carbon source which also favors significant intracellular storage as PHB under intermittent/sequential operating conditions (Ciggin et al., 2012). Acetate feeding solution was prepared from sodium acetate anhydrous (CH_3COONa) (MERCK 127-09-3) and both reactors were fed with of 400 mg COD/L concentration (1.6 g COD/d) of acetate solution. In addition to carbon source, macro nutrients (NH_4Cl: 120 g/L, KH_2PO_4: 160 g/L, K_2HPO_4: 320 g/L) and micro nutrients ($MgSO_4{\cdot}7H_2O$: 15 g/L, $CaCl_2{\cdot}2H_2O$: 2.65 g/L, $FeSO_4{\cdot}7H_2O$: 0.5 g/L, $ZnSO_4{\cdot}7H_2O$: 0.5 g/L, $MnSO_4{\cdot}H_2O$: 0.41 g/L) were added to the reactor to support microbial activity. At steady state, biomass concentrations were stabilized approximately at 480 ± 25 mg VSS/L and 1800 ± 50 mg VSS/L, yielding an average food to microorganism (S_{AC0}/X_{T0} – initial acetate/total biomass) ratio of 0.85 mg COD/mg VSS d and 0.22 mg COD/mg VSS d, for SRTs of 2 and 10 days, respectively. pH was kept at neutral levels and the temperature was maintained at 20 ± 1 °C during reactor operation. Oxygen concentration was sustained above 2 mg/L to prevent any oxygen limitations.

Respirometric analyses were carried out in a series of batch experiments (i) to visualize and evaluate the short-term effect of TET on the utilization of acetate, and (ii) to observe the variation of this impact at different sludge ages. Consequently, they were started in two sets (Set 1 and Set 2), with an acclimated biomass seed taken from SRT 2 and 10 days reactors, respectively. Each set additionally contained a control batch experiment (Run 1.1 and 2.1) conducted with substrate and biomass only, to assess the biodegradation characteristic of acetate at two different SRTs, without TET addition. The other two runs included, aside from the organic substrate, an initial dose of 100 and 400 mg/L respectively of TET for each sludge age to examine its acute inhibitory impact. At first sight, the selected doses of TET appear to be objectionably high, in view of μg/L levels encountered in wastewaters (Michael et al., 2013). They are however carefully selected, in agreement with the new concept of wastewater management advocating control and removal of specific micro-pollutants like antibiotics at source, where they are most concentrated. Removal at source entails evaluation of unfavorable effect on biological treatment at high concentrations. In this context, the TET doses in the study are quite well-suited and characterizes waste streams and effluents generated by hospitals reported in the order of between 10 and 600 mg/L (Sponza and Çelebi, 2012). For example, tylosin concentration in the effluent of one pharmaceutical plant in the UK was measured between 20 and 200 mg/L (Chelliapan et al., 2006). Operating characteristics of batch experiments are given in Table 1. These respirometric experiments were run in duplicate. Parallel fully aerated batch reactors were also conducted with acclimated biomass seeding for monitoring OUR profiles together with COD and polyhydroxybutyrate (PHB) measurements used in the calibration of the selected model.

2.2. Respirometric analysis

Fully aerated batch reactors of 2.0 L volume each used for respirometric experiments were started with the biomass seeding alone to obtain the initial endogenous OUR level. Then substrate and antibiotic mixture was added to the biomass in the reactor at the desired S_{AC0}/X_{T0} ratios, and the OUR data was monitored during a period necessary for full depletion of external substrate and decrease of observed OUR down to endogenous respiration level. A continuous lab-scale respirometer (Applitek RA-Combo-1000, Nazareth, Belgium) was used for OUR measurements. Both initial acetate and biomass concentrations were diluted by approximately 50% to avoid oxygen limitation in respirometric tests. During each analysis, nitrification inhibitor (Formula 2533TM, Hach Company) was used to prevent any possible interference induced by nitrification.