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Combined effect of erythromycin, tetracycline and sulfamethoxazole on performance of anaerobic sequencing batch reactors

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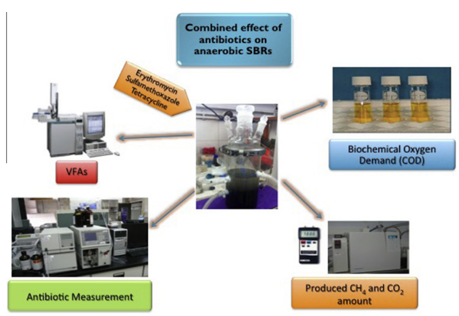
HIGHLIGHTS

- Combined effects of antibiotics on anaerobic SBRs was examined.
- Anaerobic pre-treatment of antibiotic combinations can be a suitable alternative.
- Erythromycin, tetracycline and sulfamethoxazole were partially biodegraded.
- Dual effects of antibiotics are more toxic than triple effects in the anaerobic SBRs.

GRAPHICAL ABSTRACT



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ABSTRACT

The combined effects of erythromycin–tetracycline–sulfamethoxazole (ETS) and sulfamethoxazole–tetracycline (ST) antibiotics on the performance of anaerobic sequencing batch reactors were studied. A control reactor was fed with wastewater that was free of antibiotics, while two additional reactors were fed with ETS and ST. The way in which the ETS and ST mixtures impact COD removal, VFA production, antibiotic degradation, biogas production and composition were investigated. The effects of the ETS mixtures were different from the ST mixtures, erythromycin can have an antagonistic effect on sulfamethoxazole and tetracycline. The anaerobic pre-treatment of these antibiotics can represent a suitable alternative to the use of chemical treatments for concentrations at 10 mg/L of S and 1 mg/L of T; 2 mg/L of E, 2 mg/L of T and 20 mg/L of S for the ST and ETS reactors respectively, which corresponds to min 70% COD removal efficiency.

1. Introduction

Antibiotics, which are one of the most commonly used pharmaceutical products, have a wide range of uses in both human and veterinary medicine. These active compounds cause damage to existing enzyme systems are discharged to receiving bodies without or with a little, mineralization from treatment plants and accumulated in soil and sediment day by day (Kümmerer, 2009). This accumulation may result in the proliferation of antibiotic-resistant-pathogens that threaten public health through including changes in the native microbial population of the ecosystem (Resende et al., 2014). Although concentrations of these antibiotics are relatively low in raw domestic wastewater (100 ng/L–6 μg/L) they can be significantly higher in hospital and pharmaceutical industry effluents, reaching the 100–500 mg/L level (Martín et al.,

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2012). Additionally, antibiotics typically occur within some forms of mixtures that have antagonistic or synergistic effects. The effects of mixtures of this nature are generally stronger than the impact of the individual components from which they are formed, even if all components are only present in low concentrations that do not provoke significant toxic effects (Aydin et al., 2015a; Ozbayram et al., 2014).

Biological treatment processes have been reported to effectively treat some of the products of pharmaceutical manufacturing wastewaters (Shi et al., 2014). Although activated sludge treatment processes have been used to treat these types of wastewaters, they can be unsuitable when the COD levels exceed 1500 mg/L. The high COD of the wastewaters makes them a favorable alternative for anaerobic biotechnology. Anaerobic digestion also uses less energy, has a lower sludge yield and lower nutrient requirements, is cheaper to implement, uses less space, and offers improved biogas recovery (Chelliapan et al., 2006; Oktem et al., 2008).

Various anaerobic treatment systems have been used to treat single antibiotic compounds (Sreekanth et al., 2009; Auerbach et al., 2007). Although research in this area is promising, the number of experimental studies that have investigated the combined effects of antibiotics on the anaerobic treatment of pharmaceutical wastewaters is scarce. For example, Massé et al. (2000) investigated the presence of individual and combined antibiotics on the anaerobic digestion of swine manure slurry in SBRs. Their results indicated that the presence of 55 mg Carbadox/kg, 110 mg Tylosin/kg and 16 mg Penicilin/kg combinations in manure slurries did not have a noticeable adverse effect on methane production. However, the presence of individual penicillin and tetracycline (550 mg/kg) in manure slurries reduced methane production by 35% and 25%, respectively. Furthermore, Álvarez et al. (2010) observed the significant inhibition of antibiotic mixtures, including oxytetracycline and chlortetracycline on the anaerobic digestion of pig manure. Similarly, Beneragama et al. (2013) demonstrated the individual oxytetracycline (OTC) and combined OTC and cefazolin (CFZ) at concentrations of 30, 60 and 90 mg/L resulted in a 70.3%, 68.6% and 82.7%, 70.3% reduction methane production respectively. Aydin et al. (2015a) also found that various tetracycline, sulfamethoxazole and erythromycin combinations resulted in a remarkable synergistic effect on anaerobic digestion. This study revealed that antibiotic mixtures does have an adverse effect on homoacetogenic bacteria and methanogens and that this may have inhibitory effects on propionate (e.g. *Syntrophobacter* species, *Pelotomaculum* species) and butyrate-oxidizing syntrophic bacteria (e.g. *Syntrophomonas spp.* and *Syntrophospora spp.*), resulting in unfavorable effects on methanogenesis.

This study focused on the tetracycline, sulfamethoxazole and erythromycin antibiotics that are most commonly used in human and veterinary medicine in Turkey. Erythromycin is among the main representative of the macrolide group of antibiotics for clinical use. An important difference between erythromycin and other macrolides, such as clarithromycin and roxithromycin, is the sensitivity of erythromycin to pH. Tetracycline are also the cheapest classes of antibiotics available today, making them attractive for use in developing countries that have limited health care budgets. Erythromycin and tetracycline prevent bacteria from growing by binding irreversibly to the 50S and 30S bacterial rRNA subunits respectively (Tenson et al., 2003). Sulfamethoxazole is one of the most frequently detected sulfonamide group of antibiotics in wastewater treatment systems. It achieves an inhibitory effect through two main methods: it inhibits the synthesis of nucleic acids and/or inhibits glutamic acid ability to permeate the bacterial wall, which prevents folic acid synthesis from being successful (McDermott et al., 2003). They are also active against a broad spectrum of Gram-positive and Gram-negative bacteria including species of the genus *Syntrophobacter*, *Pelotomaculum*, *Syntrophomonas spp.*, *Syntrophospora spp*, *Streptococcus*, *Staphylococcus*, *and Clostridium* (Le-Minh et al., 2010).

This research aimed to develop an understanding of the triple effects of sulfamethoxazole–erythromycin–tetracycline (ETS) and dual effects of sulfamethoxazole–tetracycline (ST) on the performance of anaerobic sequencing batch reactor systems (SBRs) throughout a year operation. The chronic joint effects of ETS and ST on the COD removal efficiency, VFA production, antibiotic removal, biogas production and composition in SBRs were investigated.

2. Methods

2.1. The experimental approach

The experimental set-up consisted of three anaerobic SBRs of identical dimensions and configurations. The anaerobic SBRs were run in a daily "fill and draw" mode using a synthetic substrate mixture that included volatile fatty acids, glucose and starch (Aydin et al., 2014; Aydin et al., 2015b). The mixture was formulated to resemble the wastewater produced by the pharmaceutical industry, as per the solution described in a study conducted by Amin et al. (2006). The operation of the anaerobic SBRs included a start-up period of approximately 90 days to allow acclimation and establishment of steady-state conditions. In the steady-state condition, the SBRs exhibited a stable performance, with an average effluent soluble COD of 92 ± 19 mg/L, which corresponded to a COD removal of around 95% and an average biogas production of 1247 ± 3 mL/day. The concentration of the influent antibiotics were gradually increased through successive phases, each lasting for 30 days, until the metabolic collapse of the anaerobic batch reactors, which was inferred when there was no COD removal or biogas production in the SBRs (Aydin et al., 2015b). The antibiotic concentrations used at each stage are provided in Table 1. During the operation, the ST and ETS reactors operated effectively until the 10th (360 days operation) and 12th stages (420 days operation) respectively. The concentrations of antibiotics used were based on the inhibition levels of antibiotics reported by Cetecioglu et al. (2013) and Aydin et al. (2015a). Daily antibiotics dosing was stopped after the total collapse of the ST and ETS reactors, which were then operated for a further 30 days in order to observe whether the reactors recovered. A third anaerobic SBR that was fed free of antibiotics was operated for the entire research period under identical conditions, and this served as the control reactor.

The evaluation of the performance of the anaerobic SBRs was predominantly based on measurements of soluble COD, and volatile fatty acid (VFA) concentrations determined both in the influent and effluent streams. These measurements were supplemented with parallel daily measurements of the biogas production and

Table 1
Tested antibiotic concentrations.

	Sulfamethoxazole (mg/L)	Erythromycin (mg/L)	Tetracycline (mg/L)
Stage 1	0.5	0.1	0.1
Stage 2	5	0.2	0.2
Stage 3	5	0.5	0.5
Stage 4	10	0.5	0.5
Stage 5	10	1	1
Stage 6	15	1	1
Stage 7	15	1.5	1.5
Stage 8	20	1.5	1.5
Stage 9	20	2	2
Stage 10	25	2.5	2.5
Stage 11	40	2.5	2.5
Stage 12	40	3	3