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Inhibitory effects of antibiotic combinations on syntrophic bacteria, homoacetogens and methanogens

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HIGHLIGHTS

- Acute impact of antibiotic combinations affected acetate, propionate and butyrate degradation pathways.
- Increasing antibiotics concentration will decrease the total methane production.
- Increasing antibiotics concentration will increase the accumulation of volatile fatty acid.
- Synergistic effect was observed in nearly all the antibiotic mixtures that included tetracycline as a component.

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ABSTRACT

Antibiotics have the potential to adversely affect the microbial community that is present in biological wastewater treatment processes. The antibiotics that exist in waste streams directly inhibit substrate degradation and also have an influence on the composition of the microbial community. The aim of this study was to evaluate the short-term inhibition impact that various antibiotic combinations had on the syntrophic bacteria, homoacetogenic and methanogenic activities of a microbial community that had been fed with propionate and butyrate as the sole carbon source and VFA mixture (acetate, propionate and butyrate). Acute tests were constructed using on a two way-factorial design, where one factor was the composition of antibiotic mixture and another was the concentration of antibiotics added. In addition, the inhibitory effect of antibiotics was evaluated by monitoring biogas production and the accumulation of individual volatile fatty acids. Specific methanogenic activity batch tests showed a significant ($p < 0.05$) decrease in the maximum methane production rate in the presence of 1 mg L^{-1} of antibiotics for the substrate in a VFA mixture and propionate; 1 mg L^{-1} of ETS, 25 mg L^{-1} of ET, 10 mg L^{-1} of ST and ES combination for substrates butyrate. The addition of antibiotics to the batch tests affected the utilization of acetate, propionate and butyrate. This study indicated that antibiotic mixtures have an effect on homoacetogenic bacteria and methanogens, which may exert inhibitory effects on propionate and butyrate-oxidizing syntrophic bacteria, resulting in unfavorable effects on methanogenesis.

1. Introduction

Within both high strength organic wastewater treatment processes and the generation of bioenergy resources, anaerobic wastewater treatment is highly desirable. However, the anaerobic process is complicated, and conversion of organic compounds to methane is carried out by several microbial groups in four steps including hydrolysis, acidogenesis, acetogenesis and methanogenesis (Town et al., 2014). In order to ensure that the process is successful and the systems perform in a stable manner, a sufficient methanogenic population needs to be maintained. This population is responsible for catalyzing the terminal stage of the process and are generally categorized in two main groups according to their substrate conversion capabilities (Demirel and Scherer, 2008). Acetoclastic methanogens are responsible for converting acetate to methane and CO_2 and, as such, they play an extremely important role in CH_4 production, as 70% of the methane that is produced as an output of the process is derived from acetate. Hydrogenotrophic methanogens are responsible for converting H_2/CO_2 to methane. To maintain anaerobic systems stability these species are so important because they maintain the very low partial pressures of H_2 that are necessary for the syntrophic communities of bacteria and archaea to function (Stams et al., 2012).

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Pharmaceutical wastewaters contain high concentrations of COD. As a result of this high organic content, anaerobic treatment represents a promising method of treating this waste. However, the effluents that are present within pharmaceutical wastewaters contain a range of recalcitrant compounds, such as antibiotics, and these have the potential to impact the treatment processes (Larsson et al., 2007; Santos et al., 2010; Ji et al., 2013a,b; Yu et al., 2014). Furthermore, there is a strong potential that these antibiotics could be released into the environment and cause the development of bacterial resistance (Auerbach et al., 2007; Kuemmerer, 2009; Munir et al., 2011).

Many existing studies have evaluated the effect that individual antibiotics have on treatment systems (Alexy et al., 2004; Amin et al., 2006; Christensen et al., 2006; Fountoulakis et al., 2008; Shimada et al., 2008; Alighardashi et al., 2009; Cetecioglu et al., 2012, 2013; Collado et al., 2013; Cetecioglu, 2014). Despite the fact that antibiotics are not present as single compound substances in environmental compartment (Backhaus et al., 2004), there is very little literature available on the inhibition effects of antibiotic mixtures (Cleuvers, 2004; Christensen et al., 2006; Pomati et al., 2008). This study focuses on the three types of antibiotics that are most commonly used within human and veterinary medicine: tetracycline, sulfamethoxazole and erythromycin. Erythromycin consists of macrocyclic lactone structures that work to prevent bacteria from growing by binding irreversibly to the 50S ribosomal subunits (Tenson et al., 2003). Tetracyline prevents bacterial protein synthesis by inhibiting the aminoacyl-tRNA from attaching to the ribosomal acceptor (A) site (Chopra and Roberts, 2001). Sulfamethoxazole is a sulfonamide that achieves an antibiotic effect through two main methods. It prevents nucleic acids and proteins from synthesizing or can inhibit the permeability of bacterial cell wall for glutamic acid, which is necessary element for folic acid synthesis to be successful (McDermott et al., 2003; Baran et al., 2011).

The purpose of this work was to study effect of these selected antibiotics on microbial groups involved in anaerobic process. The changes where in biological treatment of pharmaceutical wastewater on activity of methanogens has been evaluated with using specific methanogenic activity tests (SMA). SMA tests were conducted with various antibiotic combinations and evaluated the effects on syntrophic bacteria, homoacetogens and methanogens of acclimated biomass.

2. Material and Methods

2.1. Operation of anaerobic sequencing batch reactor (ASBR) systems

A 10-L jacketed bioreactor with an 8 L working volume was inoculated granular sludge from an anaerobic contact reactor treating raki and fresh grape alcohol wastewater and operated as an ASBR with 24 h cycles (10 min feeding, 22 h 45 min reaction, 1 h settling and 5 min decant) and intermittent mixing. The reactor was operated at 35 ± 2 °C with a hydraulic retention time (HRT) of 2.7 d, a solids retention time of 30 d. Amount of mixed liquor volatile suspended solid (MLVSS) is fixed as 12250 mg L^{-1} in reactors. Composition of synthetic wastewater was formed by starch, glucose, butyrate, propionate and acetate. Prior the steady-state condition, the influent COD was increased in a stepwise manner from 1250 mg L^{-1} COD to 6250 mg L^{-1} COD level. Stable operation was reached on day 30 of reactor operation.

The pH of the wastewater was adjusted seven through addition of NaOH. The pH in the reactor during the operation period varied between 6.8 and 7.2. Also, trace element solution (2 mg L^{-1} $FeCl_2 \cdot 4H_2O$, 2 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 0.32 mg L^{-1} $MnCl_2$, 0.024 mg L^{-1} $CuCl_2$, 0.05 mg L^{-1} $ZnCl_2$, 0.05 mg L^{-1} H_3BO_3, 0.09 mg L^{-1} $(NH_4)Mo_7O_{24} \cdot 4H_2O$, 0.068 mg L^{-1} Na_2SeO_3, 0.05 mg L^{-1} $NiCl_2 \cdot 6H_2O$, 1 mg L^{-1} EDTA, 0.5 mg L^{-1} resazurin, 0.001 mL HCl (36%)), vitamins (0.04 mg L^{-1} 4-aminobenzoic acid, 0.01 mg L^{-1} D(+)-biotin, 0.1 mg L^{-1} nicotinic acid, 0.05 mg L^{-1} calcium D(+)-pantothenate, 0.15 mg L^{-1} pyroxidine dihydrochloride, 0.1 mg L^{-1} thiamine in NaP buffer (10 mM, pH 7.1) and 0.05 mg L B_{12} solution) were added to the wastewater.

2.2. Acute tests

Acute tests were constructed as batch studies based on SMA test principles to evaluate syntrophic bacteria, homoacetogens and methanogens. Glass serum bottles with 120 mL total volume (100 mL liquid volume and 20 mL headspace volume) sealed with butyl rubber septa. Batch bottles were seeded by using acclimated biomass with 1000 mg L^{-1} VSS. The test medium and the trace element solution were prepared according to the OECD311 (2006) protocol under strict anaerobic conditions. The SMA tests were run for 15 d. The experiments were designed as three sets which were differ according to the carbon source (butyrate, propionate and VFA mixtures). Batch tests were conducted in duplicate bottles combining three antibiotics with four combinations (sulfamethoxazole-tetracycline (ST), erythromycin-sulfamethoxazole (ES), erythromycin-tetracycline (ET) and erythromycin-tetracycline-sulfamethoxazole (ETS)) and six concentrations of antibiotic mixture (0, 1, 10, 25, 50, 100 and 250 mg L^{-1}) as shown in Table 1. The concentrations of antibiotics used are based on inhibition levels of antibiotics reported by Gartiser et al. (2007) and Cetecioglu et al. (2013). According to the data were also observed by Cetecioglu et al. (2012), the maximum antibiotic concentration was 100 mg L^{-1} in ETS set.

2.3. Analytical Methods

Suspended solids (SS), volatile suspended solids and soluble COD measurements of ASBR were carried out according to American Public Health association APHA, 2005. Miligas Counter (Ritter Digital Counter, U.S.A.) was used for monitoring the biogas production in ASBR. Gas compositions and VFA concentrations were determined using gas chromatographs with a flame ionization detector (Perichrom, France and Agilent Technologies 6890N, USA, respectively). The column used was Elite FFAP(30 m × 0.32 mm). The set point of the oven and maximum temperature of inlet are 100 °C and 240 °C, respectively. Helium gas was used as a carrier gas at a rate of 0.8 ml min^{-1}. Headspace pressure was measured daily by hand-held pressure transducer (Lutron PM-9107, USA).

2.4. Statistical analysis

One way analysis of variance (ANOVA) and the Tukey test were used to determine whether significant differences existed for acute tests. In addition to calculating the global significance values (p values), the data were analyzed pairwise using the Tukey test to deliver detailed significant differences between total methane production and antibiotic concentrations. Significant difference were determined at the $p < 0.05$ level of significance.

3. Results and conclusions

3.1. Performance of ASBR

The ASBR that served as the stock reactor for biomass seeding was operated at an organic loading rate (OLR) of 6250 mg COD L^{-1} d. At steady-state, it exhibited a stable performance, with an average effluent soluble COD level of 475 ± 50 mg L^{-1}