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Acute effect of erythromycin on metabolic transformations of volatile fatty acid mixture under anaerobic conditions

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HIGHLIGHTS

- At lower doses, VFA mixture was completely removed but partially utilized for biogas.
- At higher doses, propionate utilization was impaired with reduced butyrate removal.
- Remaining VFAs were partly converted to new VFA isomers and polymers.
- High *erythromycin* doses induced total inactivation of microbial metabolism.

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ABSTRACT

The study explored the acute inhibitory impact of *erythromycin* on the methanogenic activity of acclimated biomass fed with a volatile fatty acid mixture and acetate alone. Parallel batch reactors were operated for six days, with increasing *erythromycin* dosing in the range of 1–1000 mg L^{-1}. Substrate removal was monitored by means of soluble COD and volatile fatty acid (VFA) measurements together with parallel observations on biogas and methane generation. The inhibitory impact was variable with the initial erythromycin dose: At lower doses, the VFA mixture was completely removed but partially utilized, leading to reduced biogas and methane generation, suggesting the analogy of uncompetitive inhibition. At higher doses, propionate utilization was totally impaired and butyrate removal was reduced, but acetate was still fully removed. Remaining VFAs were partly converted to new VFA compound through isomerization and polymerization reactions. High *erythromycin* doses induced total inactivation of microbial metabolism with negligible methane generation.

1. Introduction

One of the most important environmental concerns of last decades is to generate alternative energy from clean, environmental friendly and renewable sources. Waste is now regarded as a resource with a significant potential energy and bioenergy production from wastes/wastewaters by means of anaerobic processes is now the most widely implemented technology in the world (Weiland, 2010). Biogas formation under anaerobic conditions is extensively studied (Liu et al., 2009; El-Mashad and Zhang, 2010; Fermoso et al., 2010); however, the ever increasing number of chemicals introduced into the wastewaters makes it necessary to explore the inhibitory impact of these chemicals on the performance of anaerobic processes.

Anaerobic technology with biogas recovery is likely to be a promising approach for the pharmaceutical industry generating strong wastewaters with high levels of different organics (Chelliapan et al., 2006; Fountoulakis et al., 2008). Aside from high levels of different organics, these wastewaters also include a wide array of synthetic chemicals such as residual antibiotics, active compounds and solvents, mostly classified as xenobiotics and/or micropollutants (Kummerer, 2008). Studies indicated that antibiotics, like many xenobiotics are resistent to biodegradation by virtue of their nature and expected function and they mostly bypass treatment and accumulate in the environment (Kummerer, 2008). Despite serious environmental concern, approximately 100 000–200 000 tonnes of antibiotics are produced and consumed in the world every year (Wise, 2002). They are widely used as therapeutic agents for human and veterinary medicines and their fate and occurrence in the environment have been extensively studied drawn due to increasing antibiotic levels detected in natural and

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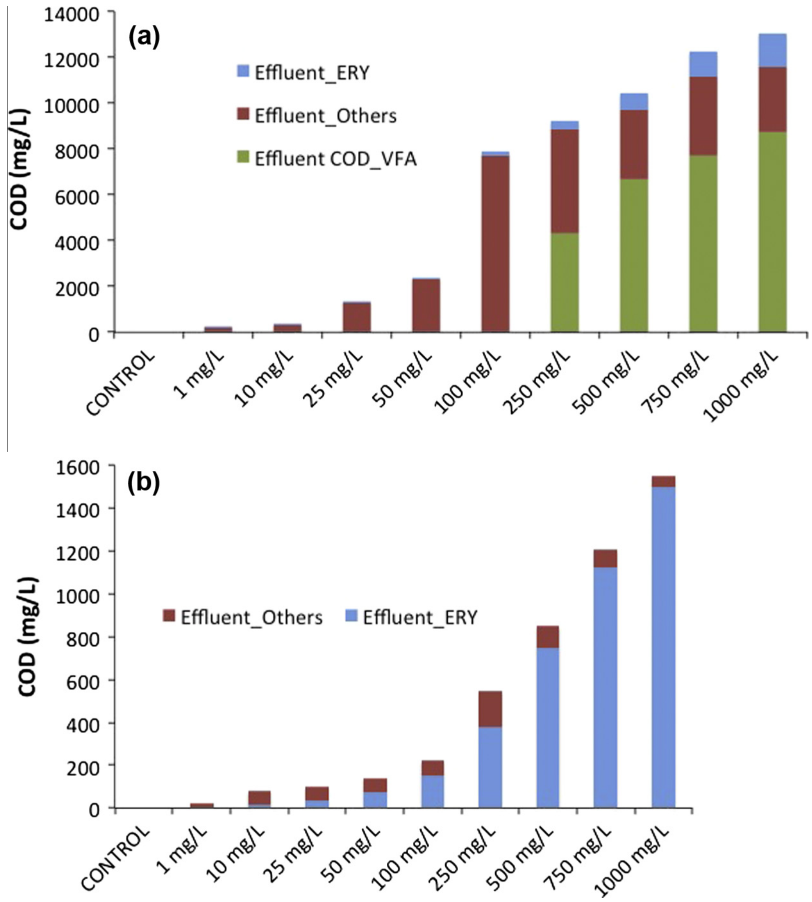

Fig. 1. Effluent COD profile with (a) VFA mixture and (b) acetate.

engineered ecosystems (Kümmerer, 2001; Xiao et al., 2008; Li and Zhang, 2010).

Erythromycin (ERY) is a one of the widely used macrolide antibiotic ($C_{37}H_{67}NO_{13}$) and produced by an actinomycete, *Saccharopolyspora erythraea* (Fig. 1). ERY consists of macrocyclic lactone structures and prevents bacterial growth by binding to the 23S ribosomal RNA and this way, it inhibits protein synthesis as other macrolide group antibiotics. The antibiotics belonging to this group have relatively broad-spectrum effects on Gram-positive and Gram-negative bacteria (Sweetman, 2009).

Wastewater generated from ERY production stream contains high concentration of this active compound and it may negatively affect the microbial community in the biological treatment units of wastewater treatment plants in terms of directly substrate utilization and/or shifting microbial community and indirectly metabolic pathways (Cetecioglu, 2011). This effect could be observed both under short-term and long-term operations. Acute and chronic inhibitory studies are two different approaches to reveal the effects of the recalcitrant compounds. In the first approach, non-acclimated biomass is tested with first exposure of the selected compound. In the other, biomass is being acclimated in a stepwise manner to the selected compound and its long-term inhibitory effect is assessed. Both two approaches should be used to provide a full knowledge about the behavior of undefined mixed biomass against to the selected compound (Kümmerer et al., 2004; Alighardashi et al., 2009). While acute and chronic impact of ERY on heterotrophic and autotrophic microbial communities have been studied under aerobic conditions (Alighardashi et al., 2009; Louvet et al., 2010; Furlong et al., 2013; Katipoglu-Yazan et al., 2013; Pala-Ozkok and Orhon, 2013; Cetecioglu, 2014), a similar research effort under anaerobic conditions was limited (Amin et al., 2006; Chelliapan et al., 2006; Shimada et al., 2008; Cetecioglu et al., 2012) and they did not provide any information on the acute and/or chronic inhibitory effect of ERY on syntrophic pathways of anaerobic degradation.

In this context, this study was undertaken to explore the short-term effect of ERY on the substrate utilization within the *homoacetogenic* and *methanogenic* phases of anaerobic degradation. It mainly focused on volatile fatty acid (VFA) utilization and methane production mechanisms to assess the acute inhibitory impact of ERY on *syntrophic* anaerobic degradation.

For this purpose, a VFA mixture including butyrate, propionate and acetate was selected as the organic substrate to sustain *butyrate* and *propionate degraders* together with acetoclastic and *hydrogenotrophic methanogens* in the microbial community. Specific methanogenic activity tests were set-up to evaluate the different biochemical steps of anaerobic degradation. Methane production, COD removal and also, volatile fatty acid profiles obtained in batch experiments were monitored in this context. The next step of this study will also cover the chronic inhibitory effect of ERY to provide a full extent of ERY inhibition on anaerobic processes.

2. Materials and methods

2.1. Experimental approach

The experimental set up involved running a series of batch reactors seeded with acclimated microbial culture and initially fed with VFA mixture (butyrate, propionate and acetate). Each reactor was