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Acute inhibitory impact of antimicrobials on acetoclastic methanogenic activity

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ABSTRACT

The study evaluated the short-term inhibition impact of three antimicrobials, sulfamethoxazole, erythromycin and tetracycline, on the methanogenic activity of acclimated biomass fed with acetate. Batch reactors were inoculated each with a different antimicrobial concentration in the range of 1–1000 mg/L and they were operated during 6 days. Organic substrate removal was monitored by both soluble COD and acetate measurements, together with daily measurements of biogas and methane generation. While acetate was almost fully removed in all experiments, methane generation exhibited a significant drop with increasing antimicrobial doses. Almost complete methane inhibition was observed for antimicrobial doses above 500 mg/L. Together with adverse impact on process kinetics in the early phases of the experiments, the final acute impact of antimicrobials was on process stoichiometry, preventing complete utilization of acetate removed in metabolic reactions. The observed effect was found compatible with uncompetitive inhibition, which similarly exerts a binding impact on substrate–enzyme complex.

1. Introduction

The increasing pace in the use of the wide array of different pharmaceuticals has become a major environmental concern. Major research effort is now devoted to better understand their fate in the environment and their toxic/inhibitory effect on natural and engineered ecosystems (Kummerer, 2009). Antimicrobials are among the most important pharmaceuticals to be considered, in view of their role and effect on bacterial pathogens. The yearly consumption of antimicrobials worldwide is estimated between 100,000 and 200,000 tons (Wise, 2002). Approximately 90% of the consumed antimicrobials are excreted via urinary or fecal pathways from the body after partial or no metabolism and they are transferred to the domestic sewage plants or directly to the environment. Conventional biological treatment of domestic sewage provides very low – if any – reduction for the antimicrobials, which usually by-pass treatment and accumulate in the receiving waters (Ternes, 1998).

The concentration of these materials in domestic wastewaters and surface waters are observed in a range between 0.3 μg/l and 150 μg/l (Alexy and Kümmerer, 2006). However, pharmaceutical plants, hospitals, concentrated animal feeding operations, and aquaculture generate effluents having much higher antimicrobial concentrations in the range of 100–500 mg/L (Kummerer, 2001; Amin et al., 2006). In this context, stream segregation and at-source treatment of the concentrated streams appear to be the most effective abatement strategy for these chemicals. Consequently, it is imperative to gather information on the fate and effect of antimicrobials at high concentrations for setting the basis for related practical treatment schemes.

As antimicrobials are designed and manufactured to inhibit microbial activities, their presence in waste streams will negatively affect biological wastewater treatment processes, either by direct inhibition of substrate degradation or by influencing the composition of the microbial community and potentially creating system instabilities (Al-Ahmad et al., 1999). Additionally, release of antimicrobials and their metabolites into the environment increases the risk of developing bacterial resistance. Resistance to these antimicrobials has been previously reported (García-Rey et al., 2002); moreover, the resistance genes themselves are considered as emerging contaminants.

Inhibitory action of a selected chemical may be experimentally evaluated in two different approaches: Short-term (acute) and long-term (chronic) tests: Acute experiments involve a microbial community selected and sustained by the selected organic substrate in the system and not previously exposed to the inhibitor. In this case, the test measures impairment of the substrate utilization pattern inflicted by the inhibition mechanism. Kummerer and his colleagues (2004) argue that short-term assays would not be sufficient to investigate the effect of antimicrobials on complex microbial systems because of different mechanisms associated with acute and chronic inhibition. In long-term experiments with continuous feeding of the inhibitor, the test may reflect, aside changes in substrate removal and utilization, adaptation and/or

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