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Application of real-time PCR to determination of combined effect of antibiotics on Bacteria, Methanogenic Archaea, Archaea in anaerobic sequencing batch reactors

Sevcan Aydin [a,*], Bahar Ince [b], Orhan Ince [a]

[a] Environmental Engineering Department, Istanbul Technical University, Maslak, Istanbul, Turkey
[b] Institutes of Environmental Sciences, Bogazici University, Bebek, Istanbul, Turkey

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ABSTRACT

This study evaluated the long-term effects of erythromycin-tetracycline-sulfamethoxazole (ETS) and sulfamethoxazole-tetracycline (ST) antibiotic combinations on the microbial community and examined the ways in which these antimicrobials impact the performance of anaerobic reactors. Quantitative real-time PCR was used to determine the effect that different antibiotic combinations had on the total and active Bacteria, Archae and Methanogenic Archae. Three primer sets that targeted metabolic genes encoding formylterahydrofolate synthetase, methyl-coenzyme M reductase and acetyl-coA synthetase were also used to determine the inhibition level on the mRNA expression of the homoacetogens, methanogens and specifically acetoclastic methanogens, respectively. These microorganisms play a vital role in the anaerobic degradation of organic waste and targeting these gene expressions offers operators or someone at a treatment plant the potential to control and the improve the anaerobic system.

The results of the investigation revealed that acetogens have a competitive advantage over Archaea in the presence of ETS and ST combinations. Although the efficiency with which methane production takes place and the quantification of microbial populations in both the ETS and ST reactors decreased as antibiotic concentrations increased, the ETS batch reactor performed better than the ST batch reactor. According to the expression of genes results, the syntrophic interaction of acetogens and methanogens is critical to the performance of the ETS and ST reactors. Failure to maintain the stability of these microorganisms resulted in a decrease in the performance and stability of the anaerobic reactors.

* *Corresponding author*. Istanbul Technical University, Civil Engineering Faculty, Environmental Engineering Department, 34469 Maslak, Istanbul, Turkey.
E-mail addresses: seaydin@itu.edu.tr, sevcan_aydn@hotmail.com (S. Aydin).
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1. Introduction

Antibiotics have a potential long-term adverse impact on microorganisms, put the natural environment at risk and have become an area of growing concern in the aquatic field (Kummerer, 2009). These substances are usually poorly degradable contaminants that cannot be removed entirely through wastewater treatment plants (WWTPs); as such they are regularly detected in wastewater effluents. Although the concentration of these antibiotics is relatively low in wastewater, they can be significantly higher in the effluents produced by hospital and pharmaceutical industry effluents, reaching up to 100–500 mg/L (Kummerer, 2001; Amin et al., 2006). This accumulation plays a role in the dissemination and development of antibiotic-resistant genes and bacteria, which may pose a risk to public health and can, cause serious changes in the native microbial population in the ecosystem (Selvam et al., 2012; Rodríguez-Mozaz et al., 2014). At high levels, antibiotics also have an effect on the performance of WWTPs and their ability to perform critical processes such as carbon removal (Cetecioglu et al., 2013; Aydin et al., 2014; Aydin et al., 2015). As a direct result of these issues, the quantitative detection of the microbial community inside bioreactors is extremely important for maintaining efficiency and stable reactors operation (Kindaichi et al., 2006; Wang et al., 2012).

Previous studies have indicated that anaereobic treatment processes can effectively treat some of the by-end products of pharmaceutical manufacturing wastewaters (Chelliapan et al., 2006; Oktem et al., 2008). However, anaerobic treatment is a unique process that involves several bacterial and archaeal groups and it includes a number of phases that must be followed in a sequential and parallel manner. There are four main steps involved in this process: hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis as seen in Fig. 1 (Narihiro and Sekiguchi, 2007). Acetogenic bacteria performs an important role through converting simple products, such as acetate, H_2, CO_2, and a series of other fermentation products, like propionate, butyrate and alcohols. These microorganisms are so important because complex substrates are not used by Archaea (Stams et al., 1994, 2012; Town et al., 2014). In order to ensure that the process is successful, and the systems perform in a stable manner, a special group of Archaea called Methanogens need to be maintained. This population, which is responsible for catalyzing the terminal and most sensitive step in the anaerobic process (methanogenesis), is generally categorized into two main groups according to their substrate conversion capabilities: acetotrophic and hydrogenotrophic methanogens. Acetotrophic methanogens play an extremely important role in the production of CH_4, as 70% of the methane that is produced as an output of the process is derived from acetate (Ince et al., 2011; Kim et al., 2013). The relationship between species in the microbial community and their distribution in the anaerobic process is not currently well understood in the context of the treatment of wastewater that contains antibiotic combinations. As such, comprehensive research into this subject is required, particularly in the form of studies that examine the based on 16S rRNA gene and several functional genes, as this will provide an understanding of the dynamics of the population and the way it impacts the performance of WWTPs (Lee et al., 2009).

Quantitative real-time PCR (qPCR), or real-time PCR, is highly important in the development of culture-independent approaches in microbial ecology research that aims to understand wastewater treatment systems because of its sensitivity, accuracy and specific quantification capacity (Kim et al., 2013; Cater et al., 2013). Moreover, qPCR is a more suitable approach to determine the composition of a functional group based on the analysis of the abundance gene and/or transcript numbers present in wastewater samples for the

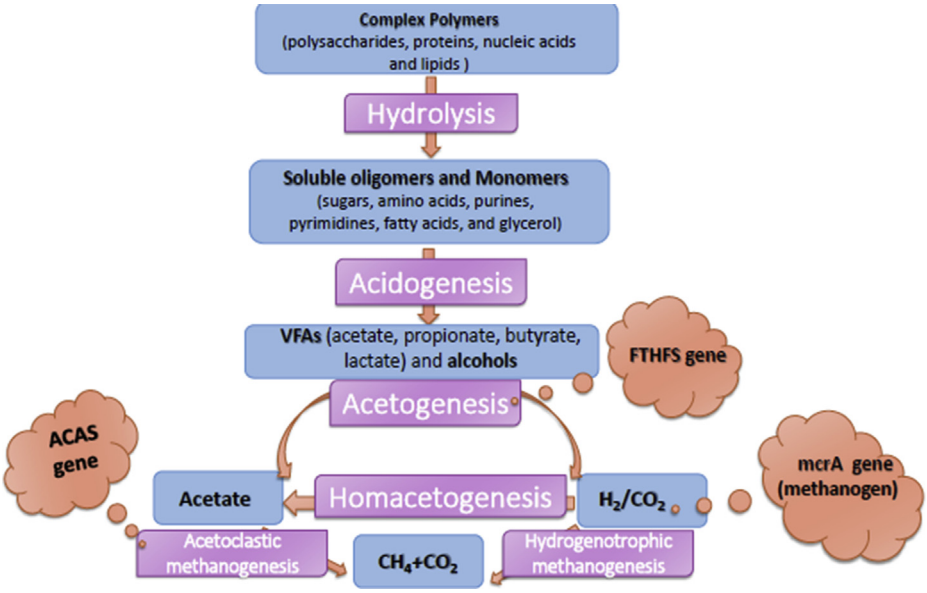

Fig. 1 – Anaerobic process and targeting metabolic genes encoding formylterahydrofolate synthetase (FTHFS), methyl-coenzyme M reductase (mcrA) and acetyl-coA synthetase (ACAS).