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Biodegradation of oxytetracycline and enrofloxacin by autochthonous microbial communities from estuarine sediments

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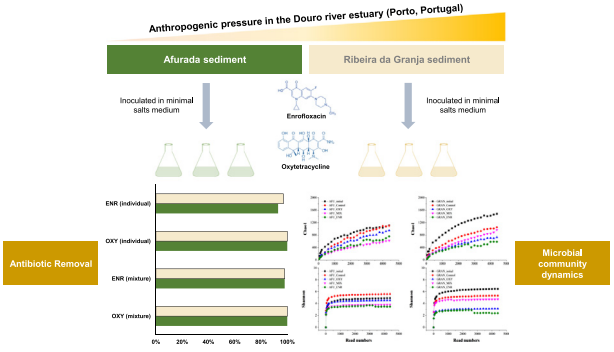
HIGHLIGHTS

- Microbial communities of estuarine sediments have potential to degrade ENR and OXY.
- Microbial cultures from both estuarine sediments removed over 95% of ENR and OXY.
- The mixture of the two antibiotics did not influence their individual removal.
- OXY removal was highly influenced by abiotic mechanisms.
- Exposure to antibiotics led to clear shifts on microbial structure and diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

This work investigated the potential of microbial communities native to an estuarine environment to biodegrade enrofloxacin (ENR) and oxytetracycline (OXY). Sediments collected from two sites in the Douro river estuary (Porto, Portugal) were used as inocula for the biodegradation experiments. Experiments were carried out for one month, during which ENR and OXY (1 mg L^{-1}) were supplemented individually or in mixture to the cultures at 10-day intervals. Acetate (400 mg L^{-1}) was added to the cultures every 3 days to support microbial growth. A series of experimental controls were established in parallel to determine the influence of abiotic breakdown and adsorption in the removal of the antibiotics. Removal of antibiotics was followed by measuring their concentration in the culture medium. Additionally, next-generation sequencing of the 16S rRNA gene amplicon was employed to understand how microbial communities responded to the presence of the antibiotics. At the end of the biodegradation experiments, microbial cultures derived from the two estuarine sediments were able to remove up to 98% of ENR and over 95% of OXY. The mixture of antibiotics did not affect their removal. ENR was removed mainly by biodegradation, while abiotic mechanisms were found to have a higher influence in the removal of OXY. Both antibiotics adsorbed at different extents to the estuarine sediments used as inocula but exhibited a higher affinity to the sediment with finer texture and higher organic matter content. The presence of ENR and OXY in the culture media influenced the dynamics of the microbial communities, resulting in a lower microbial diversity and richness and in the predominance of bacterial species belonging to the phylum

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Proteobacteria. Therefore, microbial communities native from estuarine environments have potential to respond to the contamination caused by antibiotics and may be considered for the recovering of impacted environments through bioremediation.

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1. Introduction

Antibiotics are an important group of pharmaceuticals widely consumed worldwide. Between 2000 and 2010 the worldwide use of antibiotics increased 36% (Van Boeckel et al., 2014) and in Europe alone, ca. 10,000 tons of these compounds are consumed every year (J. Yang et al., 2009, Q. Yang et al., 2009). Antibiotics have been employed both in human and veterinary medicine, with the veterinary sector being responsible for the use of a considerable fraction (ca. 50%) of these compounds (Teuber, 2001). Fluoroquinolones and tetracyclines are two of the most used classes of antibiotics with broad-spectrum activity. The antibacterial properties of fluoroquinolones rely on the inhibition of key enzymes involved in the replication of bacterial DNA (Suzuki and Hoa, 2012; Fàbrega et al., 2009), while tetracyclines exert antibacterial activity through the inhibition of bacterial protein synthesis (Chopra and Roberts, 2001). Enrofloxacin (ENR) and oxytetracycline (OXY) are two antibiotics representative of the fluoroquinolone and tetracycline classes, respectively, and are among the antibacterial compounds most frequently used in veterinary applications (FAO, 2010). As a result, OXY and ENR have been found in wastewaters (Karthikevan and Meyer, 2006; Seifrtová et al., 2008; Larsson et al., 2007; Ben et al., 2013; Qiting and Xiheng, 1988) and river waters (Li et al., 2008; Pena et al., 2007) in concentrations in the range of ng L^{-1}–μg L^{-1}, and in some cases at mg L^{-1} levels, as well as in agricultural soils up to the mg kg^{-1} range (Li et al., 2009; Li et al., 2011; Li et al., 2015; Andreu et al., 2007; Uslu et al., 2008; Martínez-Carballo et al., 2007).

Generally, antibiotics are introduced in the environment through wastewater treatment plants effluents, as a result of their incomplete removal from the influents (Corcoran et al., 2010), or through the application of livestock waste as natural fertilizers, because a significant fraction of the veterinary antibiotics consumed by livestock are excreted unchanged in urine and feces (Allen et al., 2010; Arikan et al., 2009a, 2009b). Estuaries are common sinks of environmental contaminants, including antibiotic compounds. Microbial communities native to these areas are in most cases the first-line responders to inherent contamination, but their potential to adapt and respond to the presence of contaminants, like antibiotics, is largely unknown. Knowledge on the environmental degradation of antibiotics is paramount, as their presence in the environment has negative impacts on the biota and can lead to the promotion and dissemination of antibiotic resistance phenomena (Ferri et al., 2017). For the particular case of OXY and ENR, resistance genes associated with these antibiotics have been recently detected in various environmental compartments, either as a result of their poor degradability or due to their adsorption to soil/sediment matrixes (Huang et al., 2013; Xiong et al., 2015; Xu et al., 2015; Guo et al., 2018). As such, this work had as objective to investigate the potential of microbial communities from an estuarine region subjected to anthropogenic impacts from different pollutants, which include antibiotics (e.g. sulfamethoxazole and trimethoprim) and other pharmaceuticals, pesticides and endocrine disruptors (Ribeiro et al., 2009; Mucha et al., 2004; Madureira et al. 2009 and 2010; Waszak et al., 2014), to biodegrade ENR and OXY, two widely used antibiotics in veterinary applications. For this, sediments from two locations of the Douro river estuary (Porto, Portugal), with different physicochemical properties and subjected to different levels of anthropogenic pressure were used as microbial inocula for the biodegradation study. Biodegradation of the selected antibiotics was investigated in batch mode, by supplementing the compounds individually and in mixture, in the presence of acetate as a co-metabolite. In addition, the effect of the target antibiotics on the microbial communities used as inocula was analyzed by next-generation sequencing of 16S rRNA gene amplicons.

2. Materials and methods

2.1. Reagents

All chemical reagents used were of the highest purity grade available. ENR (purity ≥98%) and OXY (purity >90%) were purchased from Sigma-Aldrich (Barcelona, Spain). Sodium acetate (purity 99%) was purchased from Alfa Aesar (Karlsruhe, Germany). Stock solutions of the antibiotics were prepared in methanol (Sigma-Aldrich) at the concentration of 1 g L^{-1} and kept at −20 °C.

2.2. Collection of sediment samples

Sediment samples from the estuary of Douro river (Porto, Portugal) were used as microbial inocula for the biodegradation experiments. Samples were collected at low tide, from two locations with different levels of anthropogenic pressure, situated on opposite sides of the margins of the estuary - Afurada and Ribeira da Granja - with the latter location having a higher level of pollution (Madureira et al., 2010; Ribeiro et al., 2009; Waszak et al., 2014). Approximately 100 g of each sediment were collected in sterile zip bags and kept in ice and protected from light until arrival at the laboratory.

2.3. Biodegradation experiments

Biodegradation experiments were conducted in batch mode, in closed 250 mL Schott flasks containing 50 mL of a sterile minimal salts medium (MM) (Alexandrino et al., 2017) inoculated with 5 g of sediment sample (Afurada or Ribeira da Granja sediment). The flasks were fed with 1 mg L^{-1} of the antibiotics ENR and OXY, supplemented individually and in mixture, and 400 mg L^{-1} of sodium acetate. The concentration of ENR and OXY tested was selected to couple a concentration that could be realistic in terms of environmental contamination and that could be at the same time accurately analyzed.

Flasks were incubated along a 10-day period at room temperature (ca. 25 °C), in static conditions and protected from light. Along the incubation period, cultures were fed with sodium acetate every 3 days at the same concentration supplemented initially. After the 10-day incubation period, 25 mL of each microbial culture (without sediment) were transferred to a new sterilized 250 mL flask containing equal volume of fresh MM and the resultant cultures were re-fed with 1 mg L^{-1} of the target antibiotics and 400 mg L^{-1} of sodium acetate and incubated for another 10-day period as above indicated. This procedure was repeated for an additional 10-day incubation period, resulting in a total experimental period of one month. Each experimental condition was tested in triplicate. Biodegradation of ENR and OXY was followed by measuring the concentration of these antibiotics in the culture medium at the beginning and at the end of each 10-day incubation period.

Experimental controls were also established in parallel to estimate abiotic degradation of the antibiotics and their adsorption potential. Abiotic controls were set up using sterile MM supplemented with the target antibiotics, both individually and in mixture, at the concentration of 1 mg L^{-1}. The potential of ENR and OXY to adsorb either to the sediments used as inocula or to the microbial biomass was assessed by

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