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Simulating the fate of indigenous antibiotic resistant bacteria in a mild slope wastewater polluted stream

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ABSTRACT

The fate of indigenous surface-water and wastewater antibiotic resistant bacteria in a mild slope stream simulated through a hydraulic channel was investigated in outdoor experiments. The effect of (i) natural (dark) decay, (ii) sunlight, (iii) cloudy cover, (iv) adsorption to the sediment, (v) hydraulic conditions, (vi) discharge of urban wastewater treatment plant (UWTP) effluent and (vii) bacterial species (presumptive *Escherichia coli* and enterococci) was evaluated. Half-life time ($T_{1/2}$) of *E. coli* under sunlight was in the range 6.48–27.7 min (initial bacterial concentration of 10^5 CFU/mL) depending on hydraulic and sunlight conditions. *E. coli* inactivation was quite similar in sunny and cloudy day experiments in the early 2 hr, despite of the light intensity gradient was in the range of 15–59 W/m^2; but subsequently the inactivation rate decreased in the cloudy day experiment ($T_{1/2}$ = 23.0 min) compared to sunny day ($T_{1/2}$ = 17.4 min). The adsorption of bacterial cells to the sediment (biofilm) increased in the first hour and then was quite stable for the remaining experimental time. Finally, when the discharge of an UWTP effluent in the stream was simulated, the proportion of indigenous antibiotic resistant *E. coli* and enterococci was found to increase as the exposure time increased, thus showing a higher resistance to solar inactivation compared to the respective total populations.

Introduction

Antibiotic resistance is a public health issue of great concern worldwide (WHO, 2014) which calls for a multidisciplinary approach to minimize health and economic impact. In a recently published report, it was estimated that world population may decrease by 11 million (most optimistic scenario evaluated) and 444 million (more critical scenario for a world with no effective antimicrobial drugs) by 2050 (Taylor et al., 2014). Antibiotic resistance development is not only confined to hospitals but other facilities, such as urban wastewater treatment plants (UWTPs), are also suspected to contribute to antibiotic resistance spread (Rizzo et al., 2013a). UWTPs are not designed to control antibiotic resistant (AR) spread and antibiotic resistant bacteria (ARB) are continuously released into the environment. Several mechanisms are developed by bacteria to transfer/acquire resistance to antibiotics (Dodd, 2012). In particular, vertical gene transfer takes place when antibiotic resistant genes (ARGs) are directly transferred from an AR cell to all the progeny during deoxyribonucleic acid (DNA) replication. Horizontal gene transfer (HGT), beyond spontaneous mutation, is another mechanism whereby mobile genetic material can be transferred to individual bacteria of the same species or even

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different species. HGT includes three processes: transduction, transformation and conjugation. Transduction occurs when bacteriophages (bacteria-specific viruses) transfer DNA between two closely related bacterial cells. In transformation mechanism, free DNA (*e.g.*, released in water from death or damaged bacterial cells) is taken up by bacterial cells. Finally, and possibly the main mechanism of HGT, conjugation occurs when two bacterial cells are in strict contact and small pieces of DNA called plasmids transfer through pilus from one bacterial cell to the other. In spite of several authors detected the presence of ARB in aquatic ecosystem (Tao et al., 2010; Araújo et al., 2010; Korzeniewska et al., 2013) and investigated the behavior of fecal bacteria in rivers (Schultz-Fademrecht et al., 2008; Walters et al., 2014), no information is available about the fate of ARB in river under different conditions (*e.g.*, weather conditions, hydraulic regime) and mechanisms (*e.g.*, solar inactivation, sediment adsorption). In particular, antibiotics mode of action involves the inhibition of DNA, protein and bacterial cell wall synthesis as well as the disruption of membrane structure; therefore ARB may show a different behavior to environmental stressors in aquatic systems compared to the corresponding families of both antibiotic sensitive or no-resistant bacteria.

In current modeling efforts, it is usually assumed that fecal bacteria are free floating individuals that can also attach to sediment particles (Bai and Lung, 2005). In this context, bacteria detachment may be induced by a physical concurrent mechanism consisting of the application of the shear stress transferred by the flowing water. Sources, fate, and transport of fecal indicator bacteria in water bodies have been investigated and the effect of meteorological conditions has been remarked (Marsalek and Rochfort, 2004; Kim et al., 2007). In particular, the fate of microorganisms in aquatic systems has been significantly associated with the solar intensity (Brookes et al., 2004) and a different behavior under sunlight between (total) *Escherichia coli* (Agulló-Barcelo et al., 2013) and AR *E. coli* (Ferro et al., 2015) can be expected. The fate of microorganisms in water bodies has been investigated (1) under real conditions (von Sperling and de Lemos Chernicharo, 2005; Chapra and Pelletier, 2003; Kashefipour et al., 2006) and (2) in artificial systems simulating water bodies (Schultz-Fademrecht et al., 2008; Walters et al., 2014). The investigation of the fate of bacteria in artificial systems reproducing real water systems gives the opportunity to evaluate the effect of different variables under controlled conditions. Unfortunately only a few works are available in scientific literature, and weather and hydraulic conditions were not exhaustively investigated. Moreover, bacterial sunlight inactivation experiments are typically conducted with laboratory-cultured bacteria (Mostafa et al., 2016), but indigenous surface-water/wastewater bacteria may be more resistant than their laboratory-cultured counterparts. According to previous studies, indigenous wastewater *E. coli* and enterococci were characterized by slower sunlight inactivation rates than their laboratory-cultured counterparts (Fisher et al., 2012). These findings show that sunlight inactivation experiments utilizing laboratory-cultured bacteria may overestimate the inactivation rates of bacteria in real aqueous matrices.

The aim of the present work was to study the fate of indigenous surface-water and wastewater ARB in a mild slope stream simulated through a hydraulic channel, in outdoor experiments. Performed under both sunny and cloudy days to evaluate the effect of weather conditions on ARB inactivation.

1. Material and methods

1.1. *Experimental design and setup*

The fate of indigenous surface-water and wastewater ARB was investigated in outdoor experiments through a hydraulic channel which simulated a mild slope stream. The experimental setup includes a “U” shaped hydraulic channel with a 1% slope, purposely built to reduce the number of curvatures and to simulate a stream (Fig. S1, in Supplementary material). The hydraulic channel, made of aluminum, has trapezoidal section ($b = 30$ cm, $B = 50$ cm, $h = 18$ cm), in order not to hamper the path of natural solar radiation in the water flow. The system was equipped with a removable covering, to perform tests in dark conditions too. The experiments were carried outdoor at the Laboratory of Sanitary and Environmental Engineering, Department of Civil Engineering, University of Salerno, Italy (Lat. 40°46′33.7″N, Long. 14°47′13.1″E). The volumetric capacity of the hydraulic channel can be varied between 120 and 180 L by a Cipolletti weir set downstream. The water flows through the channel from the upstream to the downstream section where it is collected in a tank and recirculated back by means of a pump. The water retention time in the tank varied in a range of few seconds depending on the experimental condition (approximately 4 sec with Cipolletti weir set at 3 cm to 15 sec at 12 cm), thus making this time negligible compared to the total time of each experiment (4 hr). Although a full characterization of the contribution of sediment biofilm to the fate of bacteria in the stream was out of the scope of this work, it was believed useful to evaluate the effect of ARB adsorption to the sediment for the duration of the inactivation experiments. Accordingly, the channel bottom was covered with sand (about 0.8 cm deep, average diameter of sediment particles 0.2 mm), taken from Sele river (located in Salerno province, Southern Italy). Before each experiment, the sand was autoclaved at 121°C for 15 min to make the contribution of bacteria adsorption to the sediment comparable among the different experiments. In a typical test, the water was contaminated with an indigenous ARB strain selected from the Sele river, according to the procedure explained in the subsequent Section 2.2.

Tap water was used for the experiments. The water temperature was measured during the experiments, ranging between 10.0 and 12.0°C. Conductivity (410 μS/cm) and pH (7.5) were constant during the experiments. The water was spiked with the selected ARB strain to obtain an initial bacterial concentration of 10^5 CFU/mL.

Depending on the figure, the inactivation rate was plotted as a function of either (1) the experimental time (t) and (2) the cumulative energy per unit of volume (Q_{UV}) received in the channel, and calculated by Eq. (1):

$$Q_{UV,n} = Q_{UV,n-1} + \Delta t_n UV_{G,n} A_r / V_t, \Delta t_n = t_n - t_{n-1} \quad (1)$$

where, $Q_{UV,n}$ (kJ/L) and $Q_{UV,n\text{-}1}$ (kJ/L) is the utraviolet (UV) energy accumulated per liter at times n and $n-1$, $UV_{G,n}$ is the average

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