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Review article

Antibiotic resistance in wastewater treatment plants: Tackling the black box

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ABSTRACT

Wastewater is among the most important reservoirs of antibiotic resistance in urban environments. The abundance of carbon sources and other nutrients, a variety of possible electron acceptors such as oxygen or nitrate, the presence of particles onto which bacteria can adsorb, or a fairly stable pH and temperature are examples of conditions favouring the remarkable diversity of microorganisms in this peculiar habitat. The wastewater microbiome brings together bacteria of environmental, human and animal origins, many harbouring antibiotic resistance genes (ARGs). Although numerous factors contribute, mostly in a complex interplay, for shaping this microbiome, the effect of specific potential selective pressures such as antimicrobial residues or metals, is supposedly determinant to dictate the fate of antibiotic resistant bacteria (ARB) and ARGs during wastewater treatment. This paper aims to enrich the discussion on the ecology of ARB&ARGs in urban wastewater treatment plants (UWTPs), intending to serve as a guide for wastewater engineers or other professionals, who may be interested in studying or optimizing the wastewater treatment for the removal of ARB&ARGs. Fitting this aim, the paper overviews and discusses: i) aspects of the complexity of the wastewater system and/or treatment that may affect the fate of ARB&ARGs; ii) methods that can be used to explore the resistome, meaning the whole ARB &ARGs, in wastewater habitats; and iii) some frequently asked questions for which are proposed addressing modes. The paper aims at contributing to explore how ARB&ARGs behave in UWTPs having in mind that each plant is a unique system that will probably need a specific procedure to maximize ARB&ARGs removal.

1. Introduction

Urban wastewater treatment plants (UWTPs) have a pivotal role in the protection of the environment, in particular, the natural water bodies. The removal of organic matter, chemical pollutants and undesirable microorganisms from sewage, using combinations of physicochemical and biological treatments, was a major technological achievement of the last century, allowing the return to the environment

Abbreviations: ARB, Antibiotic Resistant Bacteria; ARGs, Antibiotic Resistance Genes; BOD, Biological Oxygen Demand; COD, Chemical Oxygen Demand; epicPCR, Emulsion, paired isolation and concatenation Polymerase Chain Reaction; FISH, Fluorescence in situ hybridization; HGT, Horizontal gene transfer; PCR, Polymerase Chain Reaction; qPCR, Quantitative PCR; SWOT, Strengths, Weaknesses, Opportunities and Threats; UWTPs, Urban wastewater treatment plants

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of water with good quality. However, the final UWTPs effluents are far from being sterile and, hence, release to the environment high amounts of bacteria, many of which are of animal (e.g. pets or small husbandry or animal farms) or human origin (Berendonk et al., 2015; Manaia, 2017; Rizzo et al., 2013). Many of these bacteria harbour acquired antibiotic resistance genes (ARGs) and are potential carriers for the dissemination of these genes in the environmental microbiome (Berendonk et al., 2015; Manaia, 2017; Pruden, 2014). As such, these bacteria are considered a potential threat to humans and/or animals health since they may lead to more cases of difficult-to-treat infections. Moreover, although only part of the ARB released from UWTP will be able to cause disease in humans or animals, the risk of enriching the environmental resistome either through selection or horizontal gene transfer (HGT), and therefore contribute to the emergence of resistance in pathogenic bacteria cannot be neglected (Manaia, 2017). UWTPs bring together antibiotic resistant bacteria (ARB), antibiotic residues and other potential selectors that favour the selection towards these bacteria and, simultaneously, offer a rich supply of nutrients and close cell-to-cell interaction, capable of facilitating the horizontal transfer of ARGs. These arguments make the UWTPs environment one of the most exciting niches to unveil the fate of ARB&ARGs. This paper is the result of a think tank of Early Stage Researchers summer school organized by the Marie Skłodowska-Curie Innovative Training Networks, project ANSWER (http://www.answer-itn.eu/), and discusses the tools and the environmental conditions that may rule the fate of ARB&ARGs throughout the wastewater treatment.

The paper is divided into four major sections: 1) one dissecting the UWTPs compartments where analyses of ARB&ARGs may be relevant given the potential constraints that are imposed to the microbiota, as well as 2) the bio-physico-chemical conditions that may shape the dynamics of populations and genes within the bacterial communities; 3) another revising the pros and cons of the most commonly used methods to analyse antibiotic resistance in environmental samples; and 4) a final section where the previous three are combined to give an integrated overview of the major information on ARB&ARGs ecology, exemplified through the answers to some frequently asked questions. Above all, this work intends to serve as a guide for wastewater professionals who aim at optimizing wastewater treatment for the removal of ARB&ARGs.

2. Urban wastewater treatment plant, the big black box

UWTPs were first developed to assure the removal of debris, high organic loads and pathogens from sewage before discharging into environmental receptors (water streams/rivers, lakes, sea). Benefits of their worldwide implementation include avoidance of eutrophication and the spread of potentially harmful microorganisms (Henze et al., 2008). However, socio-economic evolution and increasing human population density created new challenges for an efficient wastewater treatment, with the consensual recognition that improvements are required in order to produce final effluents that effectively will protect the environment and humans.

Nowadays, a wide variety of UWTPs designs are available. Nonetheless, all of them assemble at least 3 sequential steps: the preliminary (pre)-, the primary-, and the secondary-treatment (Grady et al., 2011; Henze et al., 2008). Pre-treatment aims at removing from the raw wastewater all the materials that can damage the downstream equipment, including bulky solids and sand which are mechanically removed or settled. In addition, in some UWTPs this step includes an equalization tank, not only to avoid flow peaks but also to homogenise the raw wastewater composition, avoiding the sporadic income of high loads of chemicals, which could inhibit the following secondary treatment. The removal of the floating fat and grease is also undertaken in some large UWTPs. The remaining sedimentable solids are removed in the primary settling tanks, and channelled into the sludge treatment facilities, whereas the effluent of this primary treatment enters the secondary treatment. A wide variety of processes are nowadays available for secondary treatment, but all of them aim at removing the biodegradable compounds from wastewater (Henze et al., 2008). Suspended and/or dissolved compounds are mainly those resultant from human excreta, food waste, and detergents, but a wide variety of inorganic (e.g. heavy metals) and organic compounds (e.g. pharmaceutical residues, pesticides) is also present (Henze and Comeau, 2008; Köck-Schulmeyer et al., 2013; Rizzo et al., 2013; Tchobanoglous et al., 2003). Hence, wastewater does not only contain microorganisms and readily biodegradable compounds but also recalcitrant substances, some of which may be potentially toxic to at least a fraction of the cells entering and/or inhabiting the reactor(s), i.e., substances capable of generating selective pressure. Nevertheless, the high organic load of the wastewater supports the growth of the microbiota able to cope with the prevailing conditions, which consequently can reach high densities. The resultant excess of biomass must be removed, although its release to the environmental receptors should be avoided. This is possible thanks to the prevailing conditions in the secondary treatment that favour the floc/biofilm forming organisms. The extracellular polymer substances (EPS) produced by these cells act as adsorbents not only of microorganisms unable to produce EPS but also of organic and/or inorganic chemical compounds, the so-called activated sludge, which is settled in the secondary sedimentation tanks. Hence, the microbial load of the secondary effluent is 1 to 2 log-units lower than raw wastewater (EPA, 1986), and the spent biomass is channelled to the sludge treatment line. Indeed, microbes that enter, survive or even proliferate during the wastewater treatment can be pollutants themselves if released in the environment, in the sense that they will occur in an environment to which they do not belong, and where they can cause directly or indirectly any kind of damage.

At least in some countries, conventional wastewater treatment relies mostly upon activated sludge tanks to reduce the organic load of the primary effluent to values compatible with its discharge in the environment (EEA, 2017; Grady et al., 2011). However, upgraded UWTPs include re-circulation of the mixed liquor between aerobic, anoxic, or even anaerobic tanks to ameliorate the removal of inorganic N and P, respectively, from the secondary effluents (EEA, 2017; Grady et al., 2011). The tertiary treatment has been increasingly regarded as a measure to obtain a final treated wastewater of high quality, i.e., not only without readily organic metabolizable compounds but also free of nutrients (N/P) and recalcitrant chemical micropollutants as well as with very low microbiological loads (Henze et al., 2008). Given the high costs involved in the removal of the chemical micro-pollutants, most of the currently operating UWTPs if include any additional step, this is the disinfection of the secondary effluent, before discharge in the environment (EEA, 2017). Chlorination, UV radiation, and ozonation are the most common disinfection technologies currently applied in WWTPs (e.g., EPA-Victoria, 2002; EPA, 1986). Fig. 1 summarizes the main steps of the majority of the UWTPs operating nowadays worldwide.

2.1. Wastewater treatment events affecting ARB and ARGs

Looking into the process from a microbiological point of view, sharp variations occur in each wastewater treatment step. Sewage microbiota is mainly composed of human commensal bacteria, which is mixed with bacteria from distinct origins that may be entering and colonizing the sewage system (e.g., Cai et al., 2014; Shanks et al., 2013; Shchegolkova et al., 2016; Wang et al., 2014). In this environment, the fraction of ARB may reach more than 50% at least within a given group (e.g., enterobacteria or enterococci) (e.g., Manaia et al., 2016; Rizzo et al., 2013). A high fraction of the organisms thriving in sewage adheres to organic and/or inorganic particles, which in first instance can be removed from wastewater if retained in the primary sedimentation tank. Nonetheless, those in suspension or forming less dense flocs end up in the biological treatment tank(s). The secondary treatment is thus where the fraction of ARB&ARGs not removed in the primary treatment gets in