



## Motivation

Antibiotics have made it possible to cure and prevent infections in humans and animals improving life standards enormously. Unfortunately, the overuse of antibiotics has caused a rapid increase of antibiotic resistance (AR) that is contained in bacteria's genome and plasmids and can be transferred to other bacteria (Fig.1a). Antibiotics and antibiotic resistant bacteria (ARB) from anthropogenic sources are not anymore restricted to clinical areas, but they happen to be released in the environment<sup>[1]</sup>. Wastewater coming from households and hospitals carries fecal residues including ARB from the human gut. Moreover it can also contain sub-inhibitory concentrations of antibiotics and other residual elements as heavy metals that contribute to increase antibiotic resistance by co-selection of the strains harbouring resistance to these compounds. Wastewater is treated in Wastewater treatment plants (WWTPs) which are primarily designed to remove nutrients rather than microorganisms. Despite of achieving significant reductions in bacterial concentrations, WWTP biological processes have been suggested to enhance gene transfer of antibiotic resistant genes (ARG), leading to an increase in the AR bacteria/ non AR bacteria ratio in the down-stream WWTP products<sup>[2]</sup> (Fig. 1b).

## Technological challenge

Wastewater from biological processes comprises a complex matrix of abiotic and biotic constituents including a wide uncultivable bacterial community. Monitoring horizontal gene transfer (HGT) requires a robust approach that begins with the selection of the adequate bacterial and plasmids models, followed by the design of robust molecular microbiology tools to deliver good quality plasmid DNA and the ability of selectively targeting the genes of interest on the background of a diverse community. Moreover, assessing all variables (process parameters, biotic and abiotic parameters) possibly influencing HGT represents a great challenge in understanding the WWTP dynamics.

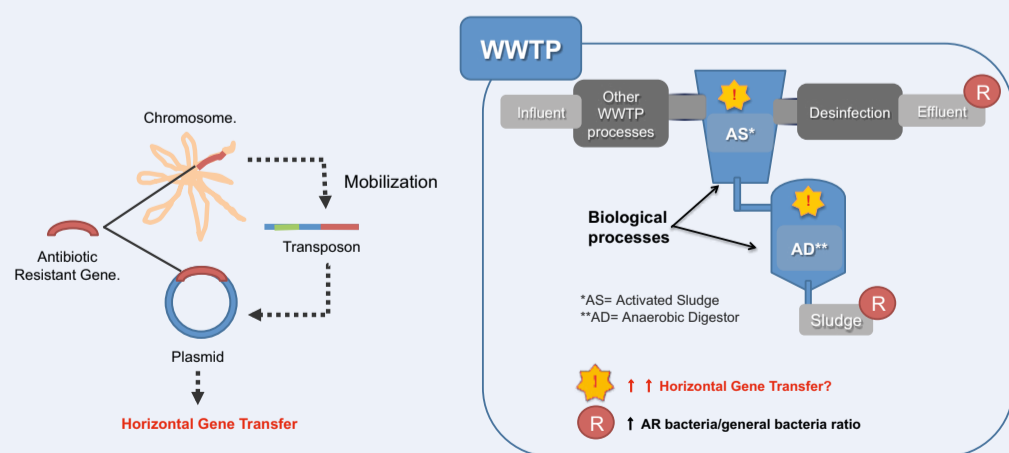
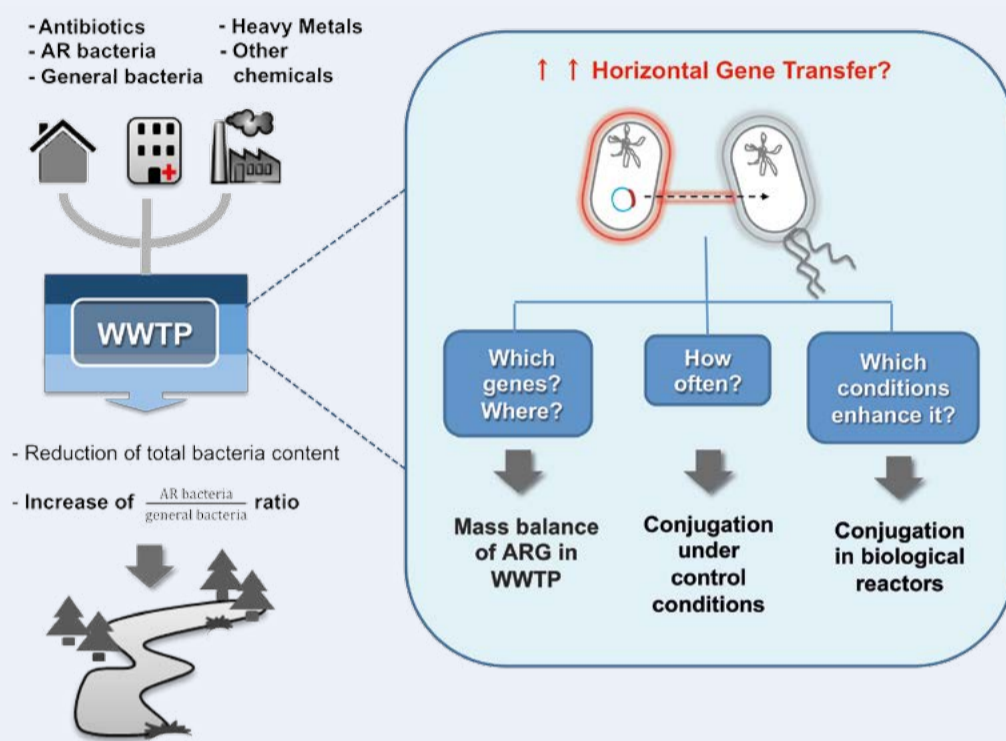


Fig 1a. Mechanims of mobilization of Antibiotic Resistant Genes.

Fig 1b. WWTP's Biological processes hipotesized as hotspots of Horizontal Gene Transference.



## Research goals

The goal of the present original research project is to conduct fundamental investigations of the mechanisms of ARG transfer and ARB proliferation in the complex microbial communities of biological wastewater treatment processes. Consequently, the research pursues the following goals:

1. Catalogue the knowledge on and status of AR in Dutch WWTPs and the real contribution of WWTPs in "breeding" the AR by means of a mass balance study of the ARG across the WWTP processes.
2. The development of a robust bio-analytical method to detect and quantify horizontal gene transfer in WWTPs.
3. The systematic exploration and examination of the process, operational, and environmental conditions that cause this unfavourable mechanism.
4. The engineering of bio-based operational strategies for the suppression and prevention of this detrimental microbial phenomenon.

[1] Baquero et al., (2008) Curr. Opin. Biotechnol. 19: 260–265.  
 [2] Czekalski, et al., (2012) Front. Microbiol. 3: 106-112.