DART: Distribution of Antibiotic Resistant microorganisms in municipal wastewater treatment plants (WWTP) equipped with a step to remove micropollutants

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The emergence of antibiotic-resistant microorganisms represents a threat for human health as well as for environmental welfare. Micropollutants (e.g., antibiotics, pharmaceuticals) discharged in the waste streams pool in Waste Water Treatment Plants (WWTPs). Here, under the pressure of sub-inhibitory concentrations of micropollutants, microorganisms can mix, mutate, and exchange resistance mechanisms. Efforts are increasing to develop effective measures to prevent the release of micropollutants and resistant microorganisms into the environment. In Switzerland, WWTPs will be asked progressively up to 2025 to implement the steps designed to remove contaminants present in ppb.

Different processes and technologies have been evaluated as potential options to eliminate micropollutants. However, at present, the effects of such processes on the release of antibiotic-resistant microorganisms in municipal WWTP outputs are not yet completely understood.

The DART project focuses on a high effective method for post treatment of WWTP effluent: the combination of adsorption on powdered activated carbon (PAC), with a solid/water separation with dissolved air flotation (DAF). The DART project is funded by the Swiss Expert Committee for Biosafety (http://www.efbs.admin.ch).

The aims of the project are: 1) to evaluate the fate and distribution of pathogenic and antibiotic/antimycotic-resistant microorganisms in a wastewater treatment plant (WWTP) equipped with a PAC/ DAF treatment for micropollutants removal, and 2) to understand if the PAC treatment could positively select for antibiotic resistance as micropollutants will saturate the PAC particles on which microorganisms are adsorbed. To investigate the distribution of antibiotic-resistant microorganisms, a laboratory scale wastewater treatment plant, consisting in a sequencing batch reactor (SBR) composed by a biological nutrient removal step, a dissolved air flotation and an air dissolving tube, has been set-up. As a starter for the SBR, the aerated activated sludge from the wastewater treatment plant in Bioggio (190’000 PE) was used.

The PAC/DAF unit was inoculated with fluorescent-tagged antibiotic/antimycotic sensitive or resistant *E. coli* (bacteria) and *C. albicans* (yeast), which fate was followed along the entire treatment process, with epifluorescence microscopy, flow cytometry and Scanning Electron Microscopy.

The results of several tests demonstrated that the number of fluorescent-tagged cells retained in the floated PAC after treatment was significantly higher than the number of free cells found in the final effluent. The PAC/DAF process allowed removal of 96-100% of fluorescent-tagged microorganisms from the biological treatment effluent. Therefore, it was possible to conclude that the PAC/DAF treatment was more efficient in retaining microorganisms, included resistant ones, than the conventional treatment. It is highly conceivable that the wastewater treatment plants that will implement powdered activated carbon as advanced treatment step for the removal of micropollutants, will reuse part of the PAC until its saturation, in order of decrease their costs. In this case, used PAC would be re-injected in the system at the biological treatment phase, namely the activated sludge. Antibiotics that have been trapped into the PAC may have an influence on bacteria that are adsorbed on it. The reuse of PAC may lead to a selection and possibly to an increase of antibiotic resistant bacteria in the activated sludge and in the depurated effluent waters. This question is currently addressed and risks concerning recycling of PAC are evaluated.

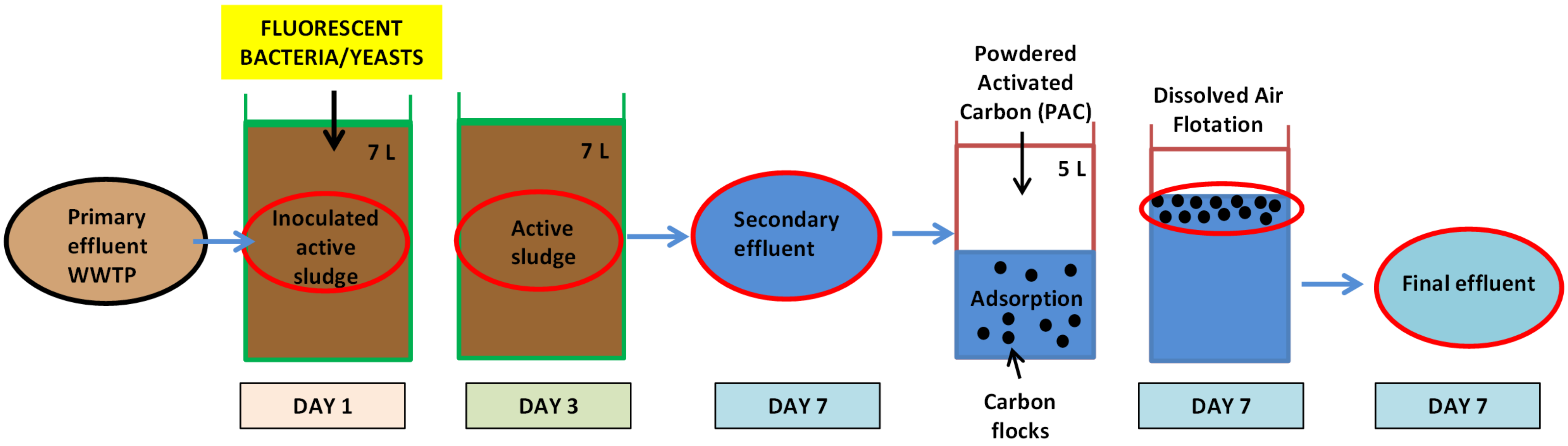

Figure 1: Scheme of the process simulated at lab scale

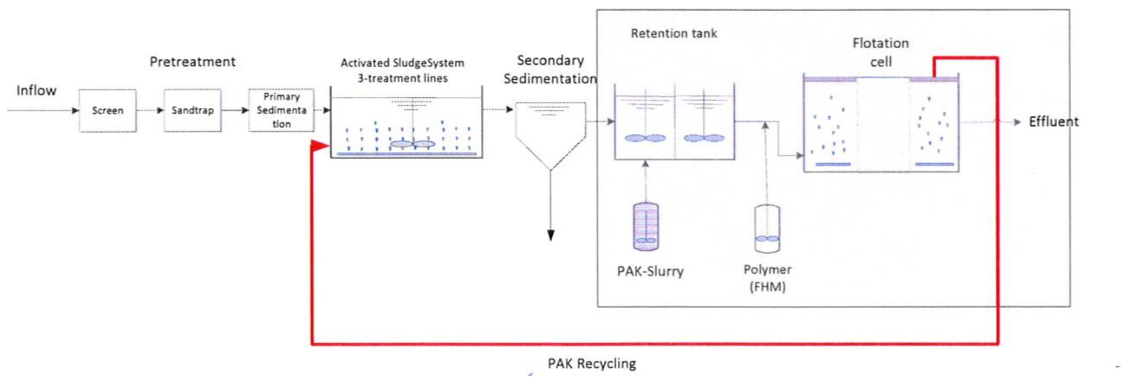

Figure 2: Scheme of the process of WWTP in which PAC is recycled after separation

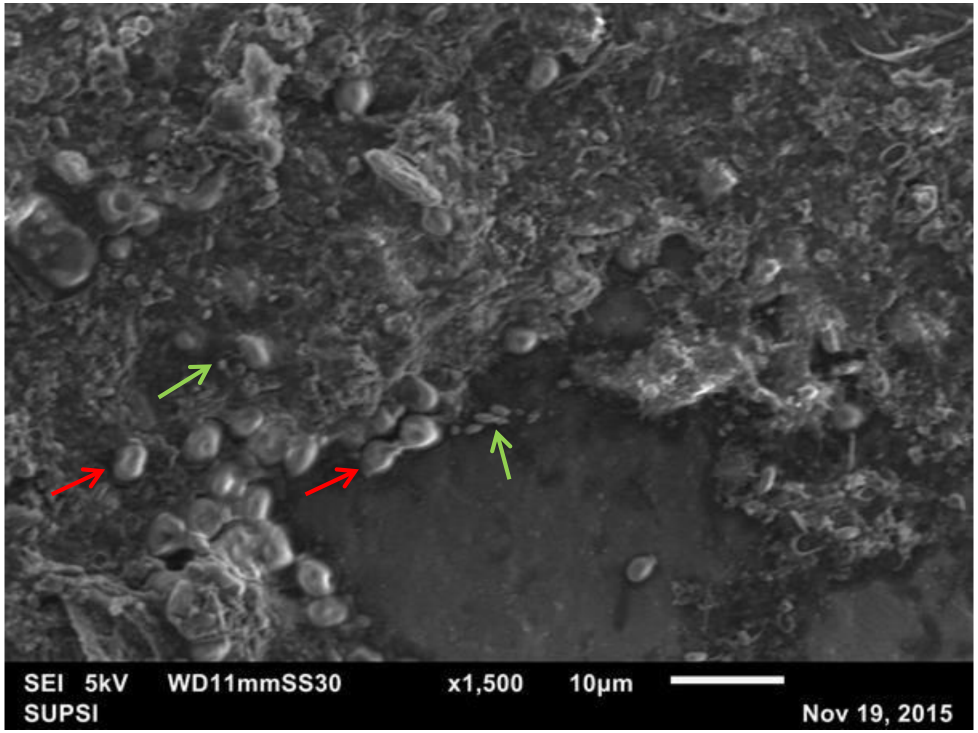

Figure 3: SEM observation of activated sludge sample collected during the lab-scale tests. Fungal and bacterial cells - putatively *Candida albicans* (red arrows) and *Escherichia coli* (green arrows) used in the inoculum - are clearly visible in the floc