BIological tools to Optimize Treatment Technologies to remOve micro Pollutants and Endocrine disrupters (BIOTTOPE)

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➢ I. Introduction

Industrial and urban development occurred in the 20th century allowed the emergence of thousands of anthropogenic chemicals in our environment. Once micro-pollutants are released into sewage, only an “end-of-pipe” solution remains to limit their presence in the natural aquatic environment. Removal rates in wastewater treatment plant of individual micro-pollutants suggest a reduction of eventual specific effects like endocrine disruption on aquatic animals. Considering this, it appears that treatment processes could be efficiently monitored using biological tools based on small aquatic organisms.

➢ II. Objectives

The aim of the BIOTTOPE project is to develop an automated tool, based on small biologic aquatic models, to evaluate endocrine disruption potential in wastewater and to adapt water treatments (here Actiflo® Carb) in order to improve WWTP effluent quality. Our first objective was to improve our laboratory pre-prototype (figure 2) to build an industrial prototype (figure 3).

➢ III. Methodology

A) Micro-pollutants removal

New prototype water treatment technology (Actiflo® Carb, figure 1) will be use to remove organic micro-pollutants. Treatment efficiency will be monitored using in parallel untargeted chemical analysis approach and *in vivo* endocrine disruption assessment.

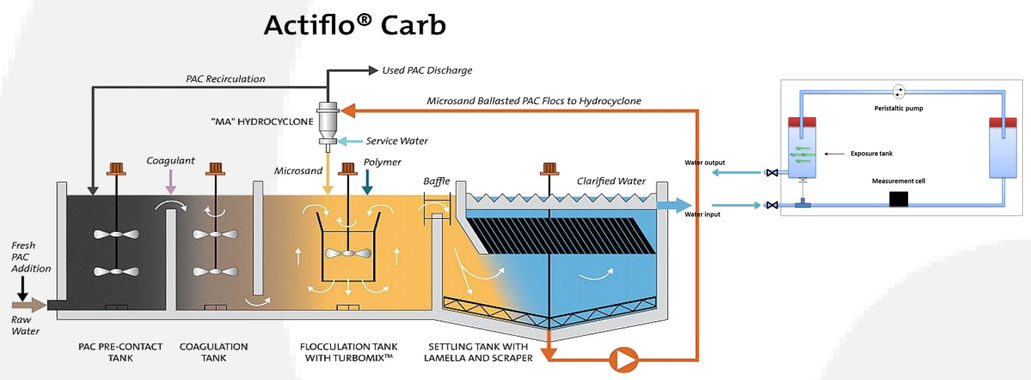

Figure 1. Scheme of the Actiflo® Carb technology to remove micropollutants.

B) Endocrine disruption assessment

Small models organisms derived from amphibian (*Xenopus laevis* tadpoles) and fish (*Oryzias latipes* fry) were modified to respond to the presence of endocrine disruptors in water. When an endocrine disrupter is present it activates the synthesis of fluorescent proteins in the transparent larvae through specific genetic response element. The models used in this study could detect thyroid or oestrogenic disruptors.

C) Flow-through Fluorescence Monitoring

In this tool, fluorescent organisms flowing through a transparent measurement flow cell, are illuminated by a blue LED with an excitation filter for eGFP. Images are taken using a digital camera and an emission filter for eGFP at a rate of 60 frames/s. After image processing steps, data are expressed in relative units of fluorescence (figure 2).

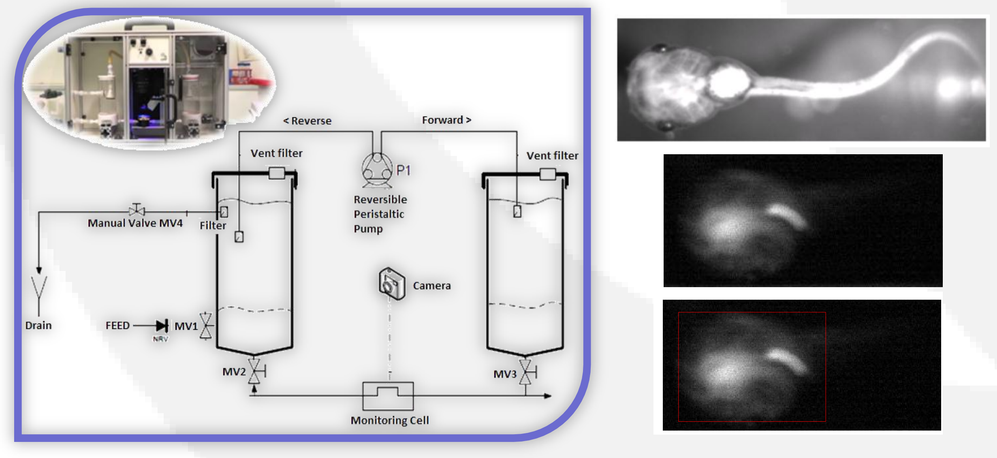

Figure 2. Scheme of the on-line readout system (left) to the endocrine disruption assessment. (Top right) Photo of tadpole in white light, offline. (Middle right) Image of the fluorescent part in blue light, acquired with the on-line readout system of figure 3. (Bottom right) Detection of the fluorescent part with image processing algorithms.

➢ IV. Results & Perspectives

Figure 3. Stylized industrial prototype of the on-line readout system developed in the BIOTTOPE project.

A multi-functional test rig was constructed in order to optimise the process and certain items of equipment. The measuring principle is based on determining and quantifying the fluorescent part of each larvae that passes under the camera by using image processing algorithms. The rig gave us the possibility to evaluate three types of lens, two types of camera, plus a research grade camera. Three different intensity LEDs and two x LED filters. Also, a new flow cell designed to be viewed from beneath, with either a direct flow path, or labyrinth design in order to maximise the exposure time. The first industrial prototype is underway (figure 3) and will be used on the Brussels-North WWTP in 2013 to evaluate performance of Actiflo® Carb pilot already on site.

Key words → **Wastewater treatment, Actiflo® Carb, Endocrine disruption assessment, Flow-through fluorescence**

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