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# OPENING THE BLACK BOX: FLUORESCENCE-BASED IMAGING OF SPATIAL PATTERNS OF MICROBIAL ENZYMATIC ACTIVITY IN A VEGETATED WALL FOR DOMESTIC GRAYWATER TREATMENT

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Green walls are vertical vegetated structures located on the unused surfaces of the buildings. They have positive effects on life quality and have also important environmental benefits (e.g. CO<sub>2</sub> absorption, heat island mitigation, biodiversity preservation). Unfortunately, their maintenance requires a considerable amount of water for irrigation. Water consumption is becoming a central issue nowadays, even in countries that do not traditionally suffer water crisis. Domestic wastewater can be used to irrigate green walls, thus reducing their water footprint and obtaining treated wastewater that can be reused. Light greywater (i.e. household wastewater from hand basins, showers, bathtubs and laundry machines) is the best target for these nature-based solutions in urban areas, due to its low pollutant content. Modular green walls work as small vertical flow constructed wetlands and their efficiency is usually evaluated comparing inflow and outflow properties. The microorganisms living in the plant rhizosphere play an important role during the treatment, releasing enzymes that contribute to the pollutants degradation, but few information are available on their activity.

The aim of this study is to employ a non-invasive method to better understand microbial reactions in a porous medium by visualizing enzymatic activity responsible for pollutant removal. This methodology uses fluorescein diacetate (FDA), which becomes fluorescent when enzymes break part of its chemical bond during its degradation. Since we need to measure fluorescent light emission, a dark room has been built to contain a Canon EOS 800D camera, transparent pots (10x10x15 cm) filled with monochromatic porous media and two blue LED lights. Unvegetated pots have been used with fluorescein to calibrate the optical system and correlate light intensity with solution concentration. Different materials (both for pots and porous medium), light configurations and camera options have been tested during the calibration step. A vegetated pot (the smallest component of the green wall) is daily fed by synthetic greywater to facilitate biofilm growing into the porous medium. To measure the enzymatic activity, the pot will be filled to saturation with a FDA solution on a periodical basis. Several pictures will be taken at short time intervals while the microbial enzymes break down FDA to its bright fluorescein compound.

The fluorescent concentration is measured by analysing the green band in each image, without disturbing the pot system. Calibration curves have been built, revealing linear correlation between green light intensity and fluorescent concentration. During the next months, spatial resolution will be refined as much as possible to control optical effects. Comparing the pictures of the vegetated pot rhizosphere at different instants after the injection of the FDA solution, an enzymatic activity reaction rate map will be drawn, providing important insight on microbial process into the porous medium. Knowledge of these processes will contribute to a better design process for the pots, increasing green walls treatment efficiency.

**BIO:** Elisa Costamagna is a first year PhD student. She is working on enzymatic activity analysis using image processing and fluorescent tracer. She is involved in SUPERGREEN project, building and testing a real scale green wall for synthetic greywater treatment.

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