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**PhD Proposal:** Microalgae utilization for removal of organic compounds from wastewater: Circular economy concept

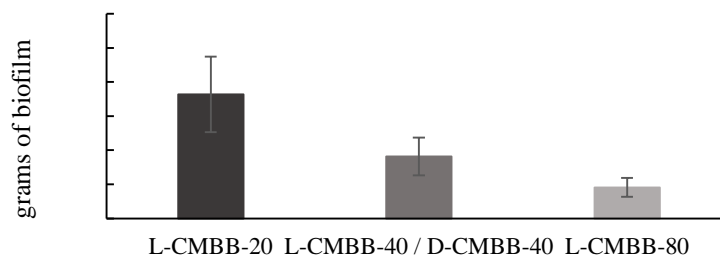
**Progress Report** jan/2021

The article Iohexol removal and degradation-product formation via biodegradation by the microalga *Chlorella vulgaris* was accepted and published in August 2020.

The joint study with Thapar Institute (TIET) continues, but we already wrote the first article, containing the first results obtained. The abstract of the manuscript is: “Treated wastewater can be an alternative source for depleting water sources for crop irrigation, however conventional wastewater treatment plants are energy intensive and costly to construct and operate, especially for low-middle income countries. The present study focused on improving the quality of wastewater by incorporating coupled microalgae-bacteria biofilm (CMBB) treatment to the wastewater ponds. Standard polyether sponges were dipped in the raw wastewater samples to enhance biofilm development on the sponges. The enriched sponges were used to treat wastewater, with or without external energy for aeration. Wastewater parameters were analyzed during the enrichment and treatment processes. The CMBB technology improved effluent quality at similar levels to aeration, however saving the energy costs, reducing 36% of chemical oxygen demand (COD<sub>t</sub>) within 24 hours and 71% within 4 days. The values of biochemical oxygen demand (BOD), ammonium and phosphates reduced by 80%, 64% and 95% within 7 days, respectively. The values for COD and BOD obtained were below the maximum allowed for reuse and discharge.”

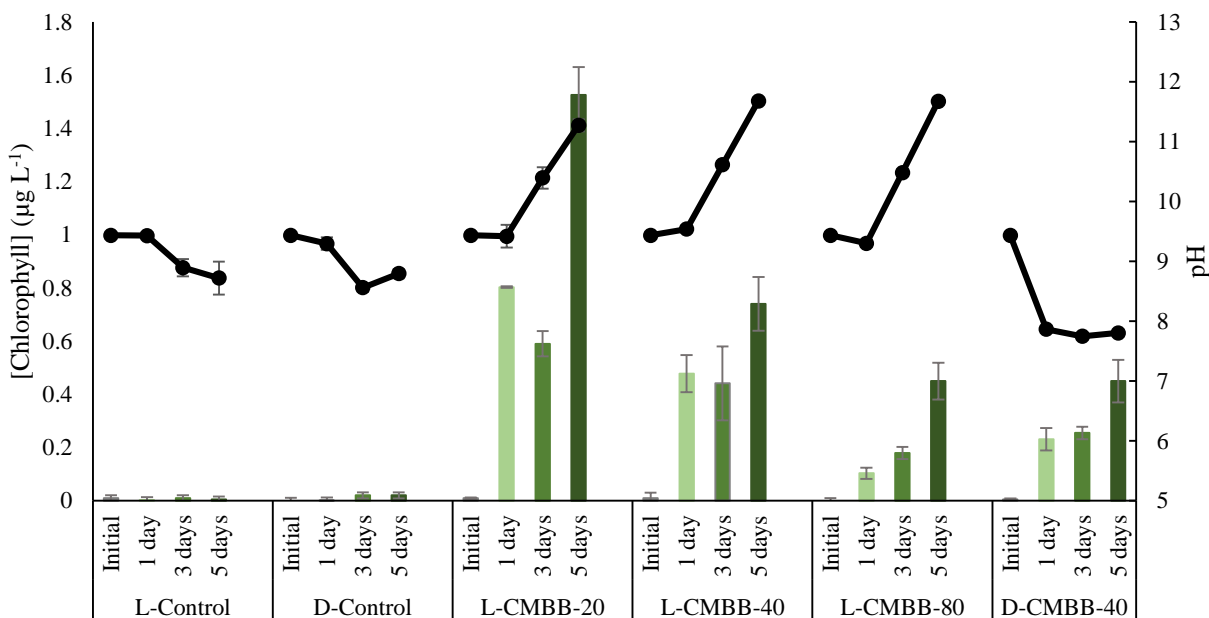
Following the improvement that was obtained using CMBB to improve wastewater parameters, we have decided to test the capability of the CMBB to remove recalcitrant pharmaceuticals from wastewater. We choose four pharmaceuticals (sulfamethoxazole, venlafaxine and carbamazepine and iohexol) and tested the ability of the consortia to remove this compounds. Also, we decided to characterize the biofilm, identifying what types of bacteria and microalgae are present at the biofilm.

And to understand their role at the removal. Three concentrations of CMBB were chosen (20, 40 and 80 milliliters of wastewater per sponge) (Fig.1), and light and dark conditions were tested (L and D).



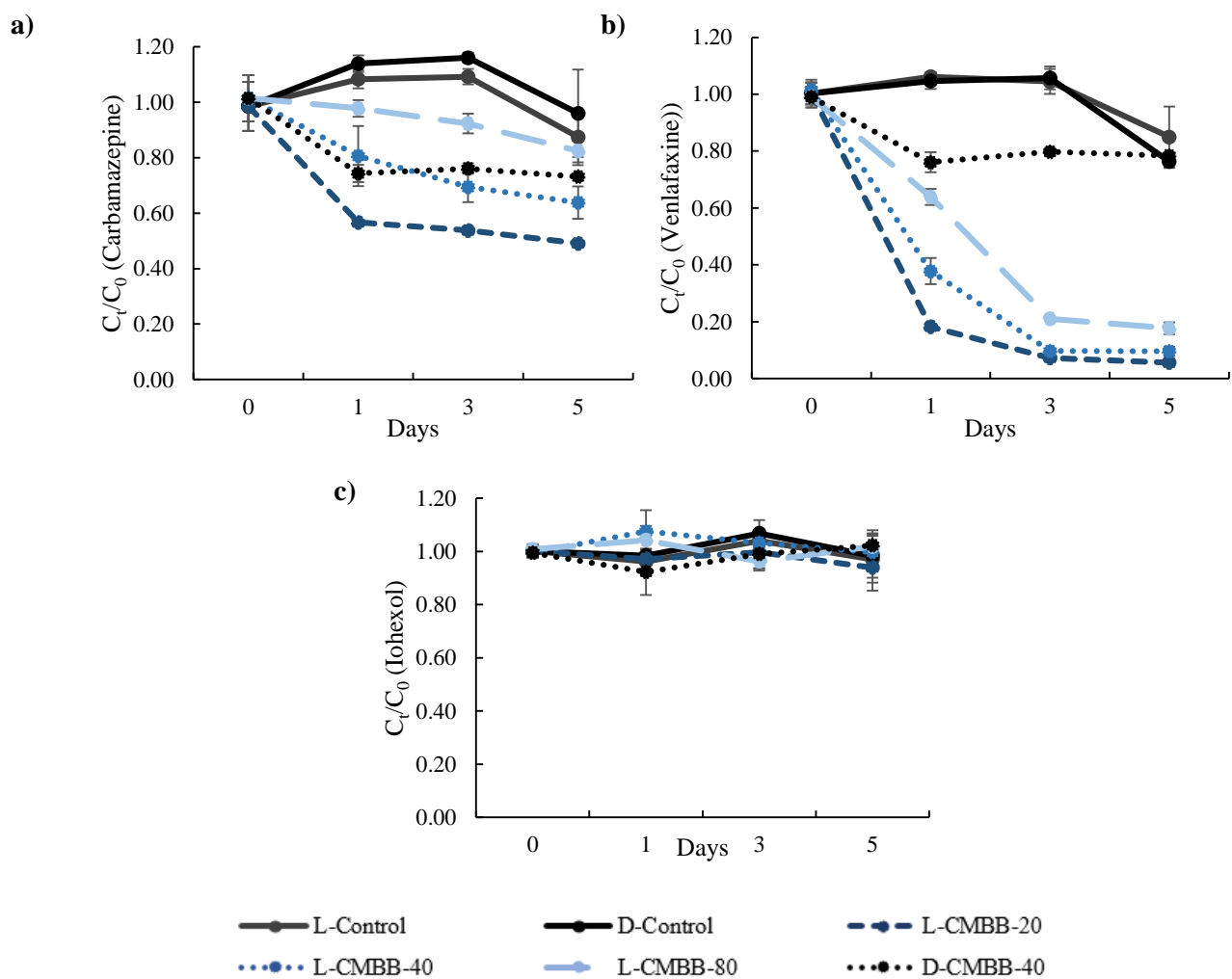
**Fig. 1.** Biofilm mass in grams in each experiment. Error bars represent the standard deviation of three independent experiments.

The pH were affected by the presence of microalgae and also by the light, L-CMBB-20,40 and 80, showed increase at pH with time and after 5 days pH was higher than 11, pH at D-CMBB-40 decreased from 9.4 to 7.8 and controls decreased from 9.4 to 8.7 (Fig. 2). The higher pH values are caused by photosynthesis, during this process microalgae uptake dissolved CO<sub>2</sub> in the water, then the enzyme carbonic anhydrase converts bicarbonate into CO<sub>2</sub> releasing hydroxyl ions, consequently increasing pH Fig 1. shows the concentration of chlorophyll during the experiment, together with pH.



**Fig. 2.** Chlorophyll concentration (green bars) and pH (black lines) correlation. Error bars represent the standard deviation of three independent experiments for CMBBs and two independent experiments for controls.

No removal of iohexol were seen after 5 days, on dark and light conditions. For venlafaxine, during the first day was possible to see 82% removal at L-CMBB-20, 62% removal for L-CMBB-40 and 36% removal for L-CMBB-80, after 5 days the removal reached 90% for L-CMBB 40 and 20. In the dark, 24% of venlafaxine were removed and kept steady until day 5. Carbamazepine removal reached 43 to 50% between 1-5 days for L-CMBB-20, and oscilated between 2 -36% between L-CMBB-40 and 80 during these 5 days. The results obtained for sulfamethoxazole were not satisfactory. Problems at the sulfamethoxazole recovery this during the solid phase extraction lead to inconclusive results, and for this reason we do not have the removal % of this pharmaceutical.



**Fig. 3.** Removal of carbamazepine (a), venlafaxine (b) and iohexol (c) from wastewater. Errors bars represent the standard deviation of three independent experiments.