Carbon-negative production process of second-generation bioethanol from *Typha* biomass: an innovative pilot-scale integrated solution for waste water treatment and 2G biorefineries

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This work aims to show the results of the development and up-scaling of an efficient and cost-competitive process to obtain second-generation bioethanol by using high-carbohydrate content bio-feedstock produced by aquatic green filter systems for waste water purification. The process was studies at three stages:

i) Development of an innovative technology based on Green Floating Filters (GFFs), where aquatic macrophyte plants (*Typha*) are utilized to treat industrial/farming waste water in an efficient and cost-competitive way.

ii) Development of an integrated biorefinery process - from the laboratory to the pilot plant scale- to convert lignocellulosic biomass with high starch/cellulose content (e.g. *typha*) into high purity bioethanol demonstrating a low/negative carbon footprint.

iii) Final testing of the produced 2G bioethanol for fuels blending.

Key performance parameters such as yield and productivity were optimized to demonstrate the scalability of the biomass-to-liquid process for future pilot/demo scale scenarios into integrated biorefinery and full circular bioeconomy concept.

First, *typha* biomass samples were produced and harvested from GFFs demos and further characterized to determine its composition as a biofeedstock (25.3 wt.% starch, 21.6 wt. % cellulose, 17.5 wt. % hemicellulose, 20.3 wt % lignin). Then, an experimental protocol and strategy for operation/process design were undertaken to maximize the production of 2G fermentable monosaccharides into bioethanol. The bioprocess comprised of the following stages: 1st) Biomass conditioning (drying, milling and sieving to 0.250 mm); 2nd) Biomass liquefaction/saccharification based on three-stage cascade process: enzymatic hydrolysis of starch, diluted acid hydrolysis and neutralization and enzymatic hydrolysis of cellulose; 3rd) Fermentation & downstream processing.

Optimal process parameters were determined for each stage (e.g. bbiomass solid concentration, enzyme / substrate ratio, temperature, pH, agitation speed, time, glucose concentration, etc.) to obtain a final glucose titer *ca.*40-45 g/L and a biomass-to-glucose yield of 65-70 wt.%. Also, *S. cerevisiae* Ethanol Red® yeast strain was selected to improve the fermentation to reach a final ethanol concentration up to 18% (v/v), followed-up with a final distillation to obtain 97-99 wt.%. pure bioethanol.

Finally, the process was scaled-up through the design and construction of a 200L multi-purpose bioreactor to perform a unique one-pot process which includes both the hydrolysis/saccharification and the fermentation processes.