Growth kinetics and biomass production of *Chlorella sorokiniana* grown only on industrial wastewaters for a sustainable process development

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Keywords: microalgae, biodiesel, wastewater, mixotrophy

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The management of wastewaters (WW) is essential to face the climate change effects and reduce the impact of many industrial sectors. In fact, WW treatment is necessary to lower pollution concentrations below prescribed limits, resulting in energy- and money-related expenses. An alternative to the industrial treatments currently applied is the utilization of WW as cultivation substrate for microorganisms that produce several chemicals. Thus, while many pollutants are removed through biological treatments, the utilization of fresh water and disposal costs are drastically reduced. In this context, industries can satisfy financial needs and tackle society's impact on the environmental issues.

Lately the production of third generation biofuels, derived from microorganism biomasses, has attracted the interest of the scientific community. The photosynthetic microalgae are good candidates for this purpose because of their adaptability to different cultivation conditions (e.g. pH, temperature, salinity etc.) and the ability to efficiently uptake several pollutants present in wastewaters such as nitrogen, phosphorous, organic carbon, metal elements or salts (Daneshvar et al., 2019). Additionally, some strains of microalgae are able to couple photosynthesis to organic carbon catabolism, in a cultivation mode known as mixotrophy or grow in complete absence of light using only organic carbon as C source, in a mode known as heterotrophy. This biotechnological approach allows to simultaneously bioremediate industrial WW and produce valuable algal biomass rich in lipid, which can be subsequently turned into biodiesel, in accordance with the circular economy principle.

The present study aims at optimizing microalgal growth on WW from dairy industry (dairy wastewater, DWW) and gas fermentation. Different WW have been considered for microalgal growth discriminating for their availability, composition complexity and for their life cycle. In fact, the WW we selected are generally sent to WW treatment plants, to reduce the content of pollutants without a precise valorization. Specifically, exhausted sludge from DWW, scotta (derived from the production of ricotta cheese), and gas fermentation effluents (derived from fermentation process of *Clostridium carboxidivorans* developed in our facilities) have been selected to be furtherly studied as growth substrates for microalgae cultivation.

Firstly, we assessed three different growth conditions with *Chlorella sorokiniana* CCAP 221/8k in a Multi-cultivator photobioreactor: photoautotrophy, heterotrophy and mixotrophy. Heterotrophy and mixotrophy were obtained through the addition of acetate, as carbon source, to the synthetic medium, maintaining the former in the darkness and the latter with constant illumination, as well as for photoautotrophy, where the CO_2 was the unique carbon source. Our results showed a significant difference in growth kinetics, highlighting that the combination of photosynthetic and respiratory metabolisms (i.e. mixotrophic condition) lead to a higher growth rate of *C. sorokiniana*. Consequently, the aim was to replicate a mixotrophic cultivation condition using real WW.

So far the two WW under investigation have been separately assayed for the growth of *C. sorokiniana*. Exhausted sludge derived by the treatment of dairy wastewater (DWW) was tested in different percentages diluted with synthetic standard Bold Basal Medium (BBM) with 3-fold Nitrogen and Vitamins (3N BBM +V). Here, the highest growth rate was registered with 50% of exhausted sludge, followed by 70% and 80% (Fig. 1). Unfortunately, ammonium can be toxic for microalgae, depending on concentration and strain tolerance (De Lourdes et al., 2017). To solve this limitation, it would be enough to properly dilute exhausted sludge with other WW, reducing ammonium content while increasing the carbon sources to boost the biomass production.

Meanwhile, gas fermentation effluent (GFE) was tested in different ratios diluted with water and pH level was adjusted to be around 7. Here *C. sorokiniana* grew better when 50% of GFE was used, compared to the standard medium (Fig.1). Once observed that both DWW and GFE need to be diluted to allow the microalgal growth, the mixture of them in a 1:1 ration was performed, and very promising results were achieved. The highest biomass production (0.166 ± 0.007 g L^{-1} d^{-1}) and growth rate (0.289 ± 0.005 d^{-1}) were recorded using 50% of DWW and 50% of GFE. Interestingly, the mixture of DWW and our GFE appears to be an ideal substrate to produce microalgal biomass, employable for subsequent biomass production.

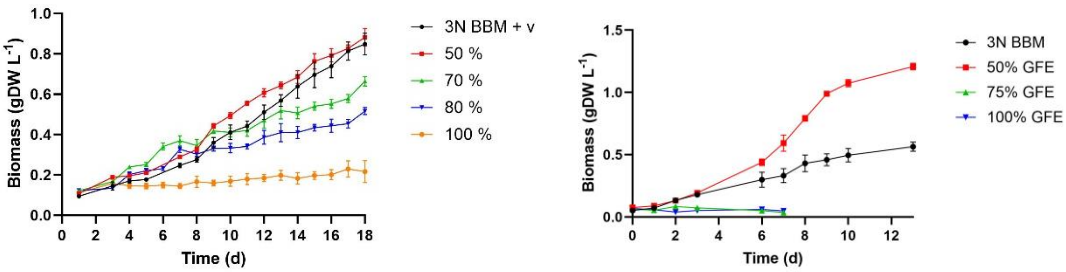

Figure 1. on the left growth curves of *C. sorokiniana* cultivated on different percentages of DWW, diluted with 3N BBM + V, represented as gDW L^{-1} of biomass measured per day. On the right the growth curves are obtained by cultivation of *C. sorokiniana* on different percentages (50%, 75% and 100%) of GFE diluted in water

In conclusion, both DWW and GFE are promising substrates for *C. sorokiniana* cultivation. We present here the development of a smart growth process based on the integration and balancing of different types of WW to fully sustain algal biomass and lipid production. In the future, to refine the process and confirm its feasibility and sustainability, a laboratory scale-up will be assessed. The final goal is to reduce the energy-sector environmental impact by decreasing fossil fuels utilization and creating an alternative and innovative method for WW treatment.

References:

Daneshvar, E., Zarrinmehr, M. J., Koutra, E., Kornaros, M., Farhadian, O., & Bhatnagar, A. (2019). Sequential cultivation of microalgae in raw and recycled dairy wastewater: Microalgal growth, wastewater treatment and biochemical composition. *Bioresource Technology*, *273*, 556–564. https://doi.org/10.1016/j.biortech.2018.11.059

De Lourdes, F. M. M., Dolores Josefina, R. R. M., Ulises, M. M. C., & De Jesús, M. R. A. (2017). Tolerance and nutrients consumption of Chlorella vulgaris growing in mineral medium and real wastewater under laboratory conditions. *Open Agriculture*, *2*(1), 394–400. https://doi.org/10.1515/opag-2017-0042