BIOSTIMULANT PROPERTIES OF Euglena gracilis BIOMASS GROWN ON PRE-TREATED EXHAUSTED DAIRY BYPRODUCTS

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Introduction

Some byproducts of the dairy industry, such as the exhausted whey from Bovine Ricotta cheese production (scotta), do not have real ways of valorization as the bovine whole whey but, being still rich in nutrients, need to be disposed of for not impacting on the environment. Current conventional treatment technologiesare of hard management and feature high costs. Among the possible and more sustainable solutions, microalgae-based bioprocesses could be a valid alternative (Gramegna et al., 2020). *Euglena. gracilis* is a microalga able to grow under heterotrophic and mixotrophic conditions, accumulating higher amounts of paramylon than under photoautotrophic conditions (Lewis et al., 2020). Paramylon can be used in many fields of commercial interest. Some studies also indicated biostimulant properties of this polysaccharide (Barsanti et al., 2019)

In this work, the ability of *E. gracilis* to grow on diluted ricotta exhausted whey was verified. The obtained biomass was than characterized for protein, paramylon and lipid content and used for evaluation of its biostimulant effects.

Materials and methods

A sample of ricotta whey, coming from an Italian dairy, underwent a preliminary treatment consisting in thermocalcic precipitation (Bosco et al.,2018), in order to remove the lipid fraction and obtain a clarified phase. This phase had a significantly lower COD (from 55 g L^{-1} to 35 g L^{-1}). A part of the clarified phase was then, in turn, subjected to microfiltration, in order to reduce its bacterial count. Three growth media were tested with *E. gracilis* (1224-5/25): i) Standard Cramer-Myers medium (SM, Cramer & Myers, 1952) with glucose, used as control (SMG); ii) clarified whey, diluted 1:3 with water (TCW1); iii) microfiltered clarified whey diluted 1:3 with water (TCW2). Growth tests were performed in 100-L bubble-column photobioreactors; each medium was tested in duplicate in batch mode, at a temperature of 20-25° C and an irradiance of 80 µmol photons m^{-2} s^{-1}, with a dark/light cycle of 12/12 hours. At the end of the growth-phase, the biomass was harvested, analyzed for biochemical parameters and tested to assess seed germination on cress (Lepidium sativum L.) by a phytotoxicity test (Ronga et al., 2019).

Results

The best growth was found in TCW2, where the maximum cell density at day 10 was 0,88 ± 0,04 Mcell mL^{-1} and COD was removed by about 75%. The inorganic nitrogen was removed by 90-95% in both TCW1 and TCW2 and fully depleted in the control (SMG). The culture tests of *E.gracilis* on exhausted whey showed a content of proteins almost 2-fold higher than the control (SMG: 25.4 ± 0.6%; TCW1: 49.8 ± 8.4%; TCW2: 49.5 ± 3.4%). On the contrary, the content of paramylon (% of dry matter) was 2-time higher in the control compared with the tests (SMG: 21.9 ± 0.9%; TCW1: 9.7 ± 1.4%; TCW2: 12.0 ± 2.1%). The results of phytotoxicity test revealed an interesting biostimulant effect on cress seeds of the TCW2 extract, with an average germination index of 150.9 ± 17.6% (with respect of water, control thesis), while the extracts of the SMG (108.9 ± 9.2%) and TCW1 (96.8 ± 2.0%) revealed neither biostimulant nor phytotoxic effects.

Discussion and Conclusion

This study showed the ability of *E. gracilis* to grow on a pre-treated and water-diluted exhausted whey without additional nutrient supplementation.

The biochemical composition of the *E. gracilis* biomass grown on the dairy byproduct was found to be significantly different from the one grown on synthetic medium. In particular, the microalgae biomass grown on dairy byproduct, thus with lactose as source of carbohydrate and whey proteins and peptides as additional organic and nitrogen sources, showed a higher protein content, at the expense of paramylon and lipids. On the contrary, the *E. gracilis* biomass grown on synthetic medium, thus with glucose as the only organic source, showed a low content of protein and higher content in lipids and paramylon. The only microalgae biomass showing a biostimulants effects was that grown on microfiltered exhausted whey (TCW2).

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