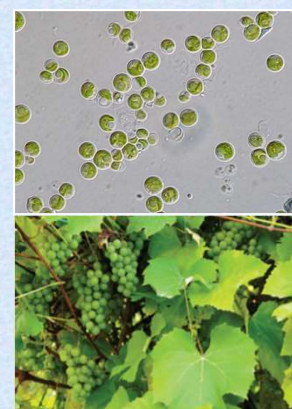


Cultivation of microalgae *Chlorella* using wine industry by-products

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Abstract

An approach of new and sustainable uses for by-products generated in the wine production industry, one of the agro-food sectors of importance, has been studied. Wine lees, a sediment obtained in different processes of decantation of wine, have been used to produce biomass of microalgae enriched in carotenoids as high added value biomolecules. Experiments to incorporate chemical components of wine lees into microalgae biomass to understand the effect of these residues on the growth and biosynthesis of carotenoids into commercial microalgae *Chlorella sorokiniana* have been done. Algae culture system has been optimized and preparation of culture media have been obtained by extracting in water the soluble nutrients contained in the lees at different concentrations between 5% and 50% p/v. Optimal growth was obtained using extraction of wine residues at 5% and 10% w/v. At 10% oxidative stress, measured as carotenoids production (specially lutein) and antioxidant activity (DPPH method), was more intense than the obtained using residues at 5%. Our results show that growth in culture media prepared with wine lees extracts stimulated the antioxidant activity and the production of carotenoids in *C. sorokiniana* cells. Preliminary information, not only to produce sustainable growth media for biomass of microalgae enriched in high value molecules, but also to reuse nutrients contained in wine industry by-products what is of particular interest in the context of a circular economy is provided.



Results and Discussion



Figure 2. HPLC analysis of carotenoids from *C. sorokiniana* extracts. Peak assignment is as follows: (1) Neoxanthin, (2) Violaxanthin, (3) Lutein, (4) Zeaxanthin, (5) Chlorophyll b, (6) Chlorophyll a, (7) β -carotene.

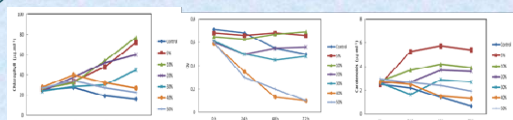


Figure 1. Evolution of chlorophyll, quantum yield and total carotenoid content in *C. sorokiniana* cultures prepared with MSN at different concentrations. Cell cultures of the microalgae were incubated with media obtained by extraction of wine residues (MSN) at 5%, 10%, 20%, 30%, 40% and 50% w/v. Both cultures were inoculated with 25 μg Chl/ml, with 100 μmol of photons/m²/s of white light, incubated at a temperature of 25 °C and were fluidized with air enriched in CO₂ (5% v/v). Chlorophyll content ($\mu\text{g ml}^{-1}$) (A); quantum yield (QY) (B) and total carotenoid content ($\mu\text{g ml}^{-1}$) (C) were calculated throughout the experiment.

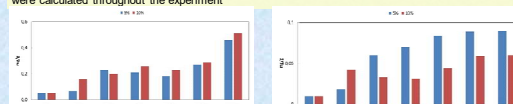


Figure 3. Carotenoid content of *C. sorokiniana* cultures prepared with MSN at different concentrations. Microalgae cultures were incubated with media obtained by extraction of wine residues (MSN) at 5% and 10%. Both cultures were inoculated with 25 μg Chl/ml, with 100 μmol of photons/m²/s of white light, incubated at a temperature of 25 °C and were fluidized with air enriched in CO₂ (5% v/v). Content of lutein (a) and β -carotene (b), expressed in mg/g, were calculated by HPLC throughout the experiment (192h).

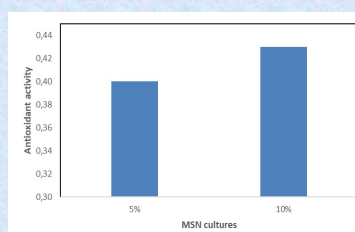


Figure 4. Antioxidant activity (DPPH) of *C. sorokiniana* cultures prepared with MSN at different concentrations. Microalgae cultures were incubated with media obtained by extraction of wine residues (MSN) at 5% and 10% w/v. Both cultures were inoculated with 25 μg Chl/ml, with 100 μmol of photons/m²/s of white light, incubated at a temperature of 25 °C and were fluidized with air enriched in CO₂ (5% v/v). Antioxidant activity expressed as ($\mu\text{mol DPPH min}^{-1}\text{ml}^{-1}\times 10^3$), was calculated at the end of the experiment (192h).



Conclusions

- 1- Culture media to grow microalgae *C. sorokiniana* using by-products from the wine industry has been optimized, extracting with distilled water at room temperature the soluble nutrients contained in wine lees using them at different concentrations w/v.
- 2- Stable cultures of the microalgae *C. sorokiniana* have been obtained using culture media prepared with soluble nutrients (MSN) extracted from lees as wine residues at concentrations from 5% to 30% w/v. Optimal growth was reached with MSN cultures prepared at both 5% and 10% w/v.
- 3- At 10% oxidative stress, measured as carotenoids production (specially lutein) and antioxidant activity (DPPH method), was more intense than the obtained using residues at 5%. Our results show that growth in culture media prepared with wine lees extracts stimulated the antioxidant activity and the production of carotenoids in *C. sorokiniana* cells.
- 4- A first approach in the search for new and sustainable uses of wine industry by-products in the context of a circular economy is presented as these residues could be used to obtain carotenoid-enriched microalgae biomass.

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