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Culture of *Spirulina platensis* in Olive Mill Wastes and Used Engine Oils

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Graphical Abstract

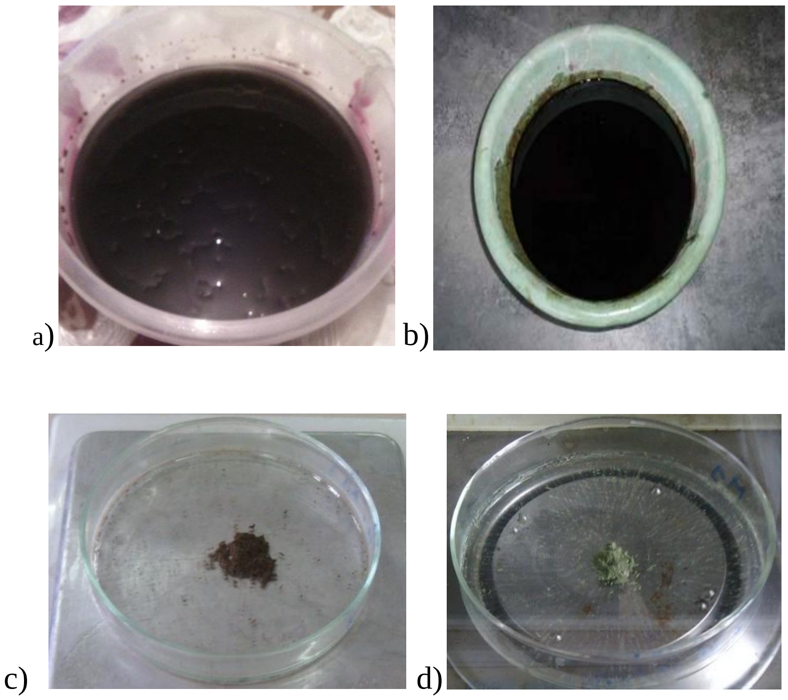

Fig.1. Appearance of Olive mill wastes (a), Used engine oils (b), Spirulina obtained from Olive mill wastes (c), and Spirulina obtained from Used engine oils (d).

Abstract

The objective of the present work is to cultivate the micro-alga "*Spirulina platensis*" from Burkina Faso in two types of wastes waters: olive mill wastes and used engines oils from the area of Tizi-Ouzou, with a view to reduce pollution. In order to minimize the pollution caused by these wastes, we chose to enrich them with a solution of fig wood ash as a source of alkalinity. The culture was carried out at 30 and 37° C. To be able to clean up these waste waters as much as possible, the recycling effect was studied. Some culture parameters (pH, phycocyanin level (%), Spirulina yields (%)), the analysis of functional groups and the antimicrobial activity of the obtained spirulina powders were determined.
The results obtained show that recycling has a positive effect on the culture of *S.platensis* in both natural media. A more or less significant production of phycocyanin was observed, in particular in the two recycled media at 30°C. These results show that temperature does not affect the phycocyanin production.

Spirulina produced from the used oils of engines is dark brown in color and is characterized by a high level of pigments and impurities. On the other hand, that from olive mill wastes is blue-green in color, which reveals the presence of phycocyanin, the latter being the source of this color.
The two Spirulina powders from both media contain hydroxyl and carbonyl groups. Consequently, they are highly recommended as a source of carbon to use in agriculture.
Key words: culture, olive mill wastes, used engine oils, *Spirulina platensis*, phycocyanin

1.Introduction

Olive mill wastes and used engine oils are releases known for their polluting effects. These discharges are viscous black liquids with a substantial acid pH. To minimize the pollution caused by these discharges, we opted for *Spirulina platensis* culture; and since this microalga requires an alkaline medium (pH between 8 and 10), both types of wastes were enriched with ash solution from fig wood. Indeed, in addition to its mineral salts content (Na, Ca, Mg), the fig wood ash is a source of alkalinity necessary for *S.platensis* (pH≥ 10 [1]. The olive mill wastes come from the rejection of an olive oil mill located in the Ouaguenoune region in Tizi-Ouzou (Northern Algeria). The used oils come from a dumping station located in the Bouzeguene region in the same province. This work is part of sustainable development and green chemistry with a view to producing spirulina while recovering waste and by-products.

2. Material and methods

2.1. Biological material

The spirulina (*Spirulina platensis*) was provided by the Research Laboratory INRA Morocco. This strain comes from Burkina-Faso.

2.2. Methods

2.2.1. Preparation of culture media

Two media (M1 and M2) were prepared using oils and olive mill wastewater respectively. These media were enriched with a fig wood ash solution (pH 12). The final pHs of the prepared media (M1 and M2) were 8.89 and 8.38 respectively. The pH values of these media was the lower limit required for the *S.platensis* culture [2, 3].

These media were sterilized by tyndallization, and then stored at 4°C until the start of the culture.

2.2.2. Culture trials

Two effects were investigated during this experiment:
1/Effect of temperature
2/ Effect of recycling media

The experiment was conducted at 37°C and 30°C. After biomass recovery, both media were reused for a new culture under the same culture conditions. The objective of recycling was to minimize the pollution generated by both industrial wastes.

Samples were taken to determine some parameters of the culture (pH, phycocyanin level (%), spirulina yields (%), and functional groups of the obtained spirulina powders.

The phycocyanin content was determined using colorimetry by measuring the OD absorbance at 615 nm and OD at 652 nm of the samples previously centrifuged at 6000 rpm [4]. The phycocyanin level was calculated according to the following formula :

$$\text{Phycocyanin level} = 1{,}873 \times (\text{OD615} - 0{,}474 \times \text{OD652}) \times \text{D/C}$$

C : Spirulina concentration g/ml
D : Dilution factor

At the end of the culture, the biomass was separated from the culture media (M1 and M2) -by centrifugation of Sigma type (6000rpm). Then washed with sterile physiological water and distilled water several times to remove impurities [5]. The obtained pellets were dried in an oven at 45°C.

3. Results and Discussion

The results found reveal an intense growth of *S.platensis* in both media (M1 and M2) at 30°C and 37°C followed by a decrease in pH (Fig. 1). This growth lasted 4 days, and then a growth disturbance was observed, indicating a possible inhibition. The decrease in pH was due to the release of CO_2 by *S. platensis* in aerobic conditions. The culture can be influenced by various factors; in our case, growth inhibition observed in M1 medium was due to lower pH [4], but in medium M2, it was related to increased pH. Temperature variation can also inhibit the growth of microalga [6]. In our case, the growth rate (Table 1) was affected by the medium composition but not by temperature. The same findings were reported by -Adel et al. [7], who cultivated spirulina in a medium based on date palm at a temperature of around 18°C.

These results can be explained by the phycocyanin production, which is not affected by temperature but by the medium composition. Indeed, both media have a complex composition (fatty acids, phenolic compounds, metals…etc.). For instance, olive mill wastes are rich in metal [8].

Table 1. pH and phycocyanin level of spirulina cultured in M1 and M2 media at 30°C and 37°C

	M1 Medium	M2 Medium	M1 recycled medium	M2 recycled medium
Temperature of growth		**30°C**		
pH at 20°C	7.64 ± 0.28	7.78 ± 0.35	8.42 ± 0.34	7.74 ± 0.41
Phycocyanin level (%)	30 ± 0.30	21 ± 0.30	24 ± 0.53	20 ± 0.49
Temperature of growth		**37°C**		
pH at 20°C	8.33 ± 0.08	8.13 ±0.01	7.32 ± 0.08	7.34 ± 0.09
Phycocyanin level (%)	25 ± 1.03	29 ± 0.91	31 ± 0.26	24 ± 0.80

M1-: medium prepared with used oil and fig ash solution
M2-: medium prepared with olive mill wastes and fig ash solution

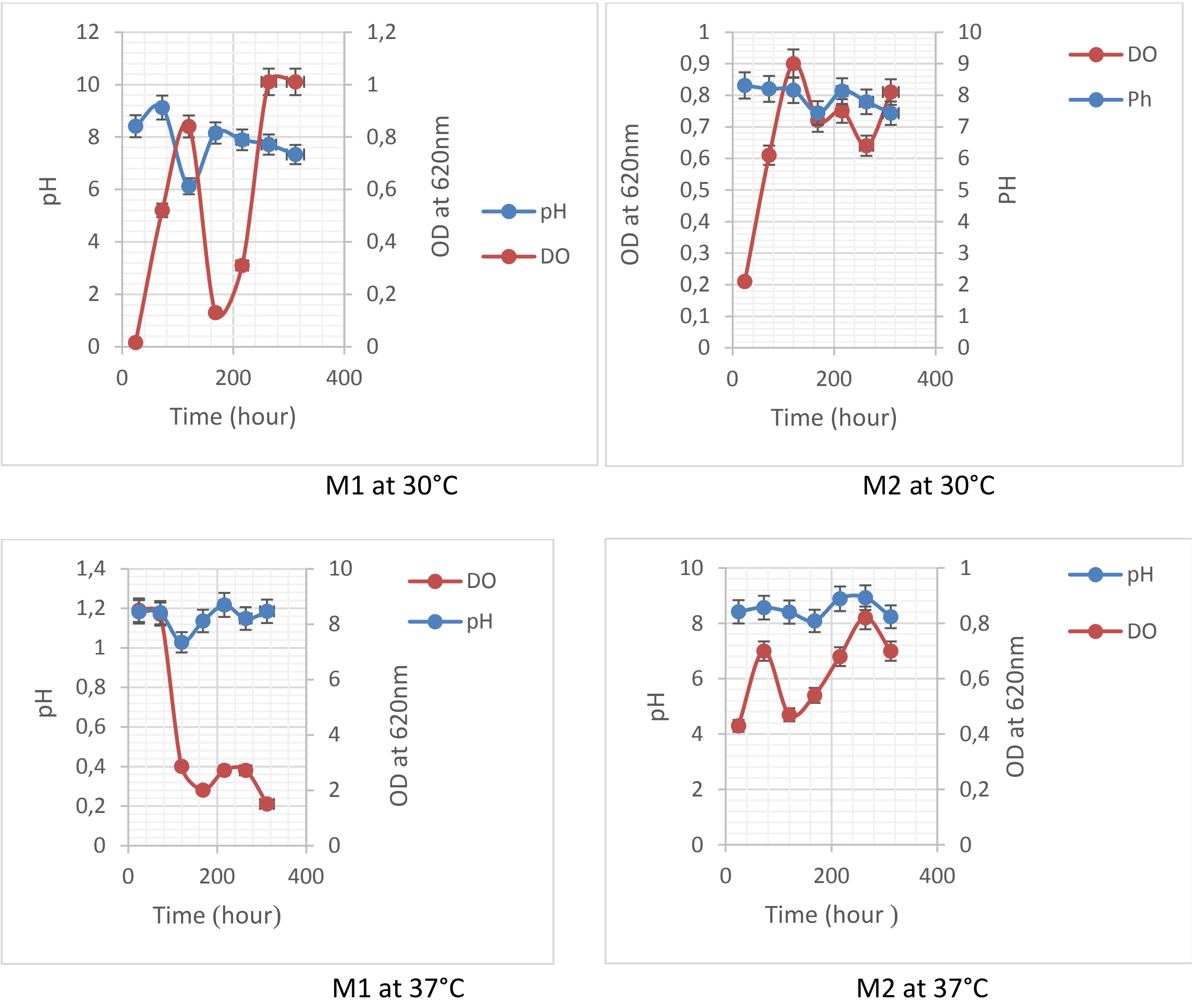

Fig1. Evolution of pH and growth of *S.platensis* in M1and M2 media at 30°C and 37°C

Recycling has a significant effect on the growth of spirulina in both media. This demonstrates that recycling enhances the availability of nutrients necessary for spirulina growth -such as bicarbonate.
Spirulina collected from the M2 medium has a dark brown color whereas the one cultured in the M1 medium has a blue-green color, which confirms the presence of phycocyanin. The obtained spirulina powders contain hydroxyl and carbonyl groups whereas the Burkinabe spirulina (reference strain)contains amino, carboxylic, hydroxyl and phosphate groups [1]. As a result, it is highly recommended to use the obtained spirulina as a source of carbon in agriculture.

4. Conclusion

One can conclude that spirulina can grow in olive mill wastes and used oils. It would therefore be useful to investigate the effect of other parameters such as aeration, agitation and thus to identify morphologically and genetically the protein profile of the obtained spirulina.

4. References

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