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Valorization of Traditional Olive Mill Wastewaters as Culture Medium for Microalgae

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Abstract
The present work aims to valorize the treated olive mill wastewaters obtained after adsorption onto activated carbon by using them as a culture medium for the growth of microalgae. For this purpose the *Scenedesmus obliquus* (CCAP 276/3A) was cultivated in cylindrical photobioreactors. Common experimental conditions were continuous light, aeration rate (1v/v/min), stirring rate 350 rpm) and temperature at 25°C. Under these conditions, the microalgal biomass concentration reached 0.9 g/L after 120h of growth using OMW treated by activated carbon as culture medium. The modeling of the experimental results showed that the microalgae is following the Gompertz model and the specific growth rate (μ_m=0.075h^{-1}) which is much more important than the specific growth in the synthetic medium (μ_m=0.024h^{-1}).

Keywords: OMW, Valorization, Microalgae, Growth, Modeling

1. Introduction
The olive-oil industries based on discontinuous traditional process are installed on the entire Moroccan territory. They are using hydraulic presses or stone mills which press the olives and allow the production of three phases: the olive oil, the vegetation water and the pomace olive [1]. This arsenal process produces annually 50.000 t on average about 50% of the national production of olive oil [2]. The pollution generated by their wastewaters (OMW) is responsible of serious environmental problems (COD = 200 g COD/L [3]).

The physicochemical characteristics of OMW differ from country to another; they are generally related to the oil extraction technology used [4]. The OMW have a complex and heterogeneous composition. OMW organic fraction contains sugars, pectin's, tannins, polyphenols, proteins and organic acids. OMW mineral fraction contains potassium, phosphorus… [5].

A lot of researchers focused their studies on valorization of the OMW. This research line is limited by the presence of the phenolic compounds which have antibacterial effect [6]. Adsorption onto activated carbon is a technique which allows the reduction of the black color of the OMW by removing the phenolic compounds [7]. It is easily achievable and can use inexpensive and highly effective adsorbents which can be regenerated [8]. The Water treatment by microalgae is typically used as tertiary treatment in wastewater treatment plants for removing nitrogen and phosphorus and fixation of heavy metals such as iron, copper, chromium... [9].They are also used to provide dissolved oxygen for the bacteria to decontaminate water [10]. The biomass can be exploited to produce molecules with high added value, biodiesel, biogas, hydrogen etc. [11]. In this sense, culture in wastewater breweries allowed obtaining high levels of lipids up to 0.24 g/ L used in the biodiesel production [12].

Scenedesmus obliquus has shown great capacity of adaptation in the most difficult conditions. Several authors have studied its ability to grow in urban and industrial wastewater to different origins. It has proven effective for the removal of cyanide rejected by Golden industries at high concentrations ranging from 100-400 mg/L [13]. Also, the dry biomass of *S.obliquus* was used as biosorbant for the removal of chromium [14].

This study aims to evaluate the ability of the microalga *Scenedesmus obliquus* to grow on treated OMW by activated carbon and to study the feasibility of replacing the synthetic culture medium with the OMW.

2. **Experimental**

2.1. *Preparation of OMW*

The OMW was collected from a traditional olive oil extraction system situated in the province of Meknes (Morocco). The samples of OMW were diluted to 10% v/v with distilled water, then the pH value was adjusted to 2 and treated by activated bone char using a ratio 6 g per 50 mL during 20 min at T = 25°C. The mix was filtered by membrane with 0.45 µm pore size using a vacuum pump system. 0.1 M-NaOH was used to adjust the OMW pH to 7. Finally, the liquid phase was then sterilized through a membrane of 0.2 µm pore size using peristaltic pump. The OMW obtained was with clear color and was stored under sterile conditions until use.

2.2. *Microorganism, Photobioreactors, Procedure*

Microalgal strain was *Scenedesmus obliquus* (CCAP 276-3a) obtained from the algal collection at the University of Göttingen (Germany). The cells were incubated in mineral medium Rodriguez Lopez [15] for 7 days under continuous illumination. The reactor had 2L capacity (25cm in height and 7.5cm in diameter) equipped with thermostatically controlled water circulation, magnetic stirring at 350 rpm and continuously illuminated by *Osram L40w/10* fluorescent tube. The air was sterilized by filtration (0.2 µm pore diameter) and supplied to the microalgae cultures by air pumps (*Boyu air pump*).

All the laboratorial material and glassware were washed with water and detergent and autoclaved at 121ºC for 20 minutes and placed in closed room sterilized by UV light for 20 min before any cultivation. The inoculums were transferred to the photobioreactors and supplemented with treated OMW in two experiments and supplemented with synthetic medium RL in one experiment.

The initial biomass concentration in all experiments was 0.0357 ± 0.002g/L. The temperature was maintained at 25°C; the air was set to 1v/v/min and light supplied continuously. During the experiments the pH was maintained between 6.5-7 by using 0.1 M-NaOH and 0.1 M-HCl. At the end of the experiments the microalga biomass was harvest by centrifugation and stored in freezer.

2.3. *Analyses*

The samples of OMW were characterized by determining: electric conductivity, TS, COD, total phenols, nitrate, nitrite, ammonia and phosphate [15-18].

The biomass concentrations (g/L) were determined by spectrophotometric analysis. The samples were centrifuged at 3200 rpm for 5min and washed three times and re-suspended in distilled water. The absorbance was then measured at 685 nm by an UV-Visible spectrophotometer (Varian Cary® Type 50 UV-Vis). Biomass dry weight concentration was determined from OMW sample absorbance by application of a calibration line.

It is necessary to obtain a calibration curve of the microalgae in the OMW in order to follow the growth. In all experiments we diluted the samples with distilled water to maintain the absorbance under 0.4. All parameters were analyzed in triplicate.

The growth experiments were confirmed by calculation methods available in the basic version of MATLAB R2007a as well as in Curve fitting Toolbox (MathWorks).

3. **Results and discussion**

3.1. *Characterization of OMW*

The chemical characteristics showed that the OMW used have an acidic pH, the conductivity is very high compared to the OMW generated by the three phase- process (6.85 mS/cm). This is due to the excessive addition of salt during storage of olives [7].

The ratio COD/BOD_5 is less than 2, which explain that the OMW are readily biodegradable but their content of phenols inhibits the anaerobic process [19]. In this work the content of phenols is low (4.5g/L) compared to other studies (18 g/L) [6)], this difference is due to the degree of maturity, the retention period of the olives and the process of extraction [20].

The phenols responsible of the black color of OMW were removed by adsorption onto activated bone char. The capacity of adsorption was 3.5mg/g with a percentage of removal phenols by 91% after 30 min contact time. In fact, the acidification of the OMW with chlorhydric acid (pH=2) transformed their black color to red, the appearance of the red color confirms the presence of anthocyanins obtained after oxidative polymerization of tannins [21], when this group is adsorbed onto activated carbon the red color turns to pale yellow.

The charcoal used was prepared with bones (natural waste) the major constituents of this material were the calcium, phosphate and carbon [22]. The OMW obtained after adsorption was rich in nutrients, the presence of large amounts of calcium and phosphorus has been noticed, which is compatible with the presence in abundance of calcium, phosphate and carbonate in the charcoal. These results gave us the idea to use these OMW as a medium for the growth of microalgae.

The pH of the decolorized OMW was adjusted by NaOH (0.1N). The sodium hydroxide allows the neutralization to pH = 7 and the agglomeration of fats remaining in the OMW after adsorption and inhibits the growth of microalgae in the medium [23].

The sterilization was carried out by filtration because the autoclaving of OMW at 121°C causes blackening and precipitation of the OMW [24-25]. The characteristics of all the OMW used are summarized in Table 1.

Table 1: Physico-chemical properties of OMW from discontinuous traditional process

Parameters	pH	Conductivity (mS/cm)	Color	TS (g/L)	Fats (g/L)	BOD_5 (gO_2/L)	COD (gO_2/L)	Phenolic Compounds (g/L)	P-PO_4^{3-} (mg/L)	N-NO^{3-} (mg/L)	N-NO^{2-} (mg/L)	NH_4^+ (mg/L)
Raw OMW	4.5 - 5.5	34	Black	6.8	0.5	67	83	4.3	796	43.67	43.15	64.29
Diluted OMW	2 - 2.5	-	Red	-	0.2	-	45.16	0.43	75.9	4.36	4.31	6.42
Adsorbed OMW	4.5 - 5	-	Pale Yellow	-	-	-	6.64	0.009	146.79	4.64	4.58	10.14

3.2. *Kinetic of Growth*

The growth curve of the *S.obliquus* in the OMW was established after 7 days of cultivation and showed the existence of four phases (Figure 1a and 1b).

A very limited adaptation phase was recorded in the first 6 hours where the *S. obliquus* was trying to adapt to the conditions of the new medium. An exponential phase from 24-52h when the biomass concentration increased from 0.1946 g/L to 0.4013 g/L in which both the cell size and aggregate formation increased. This phase was followed by a stationary phase which may be due to a deficiency of a nutrient in the medium causes a slowdown in growth.

After 120h of growth a phase of decline was recorded. The microalga was in stress conditions due to a nutrient deficiency and the color of the OMW started to be brown, the element which may be responsible for the early arrest of microalgal growth is the depletion of nitrogen [26]. No lag phase was found probably due to the use of inoculums in exponential phase.

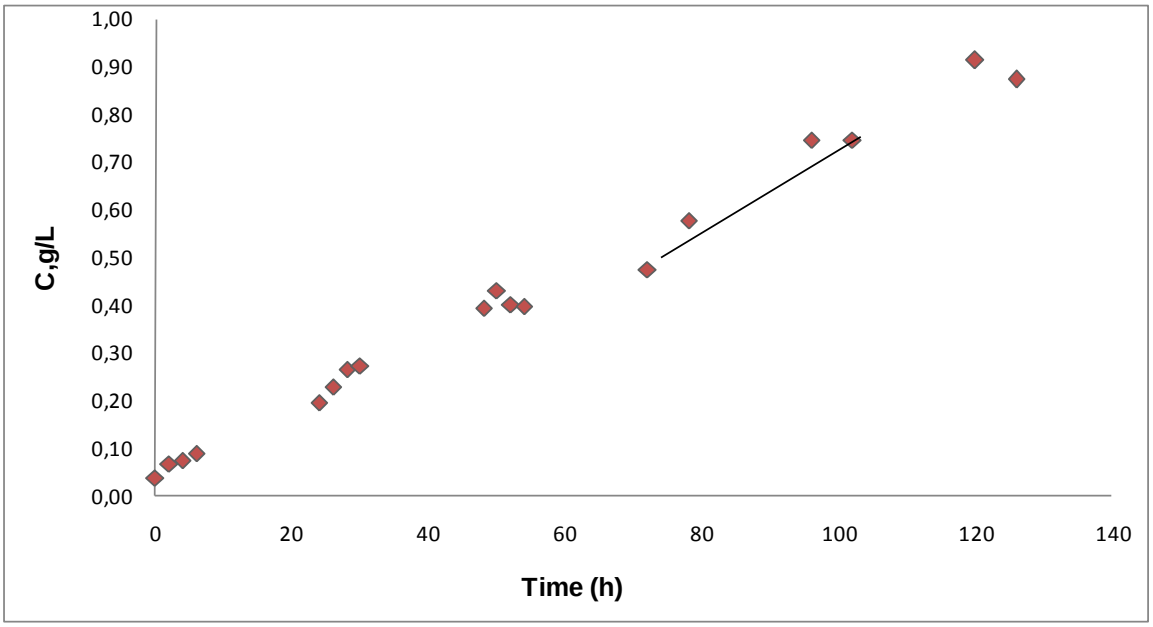

Figure 1a: Growth curve of *Scenedesmus obliquus* in OMW
Linear phase (The biomass is plotted as a function of time for the culture)

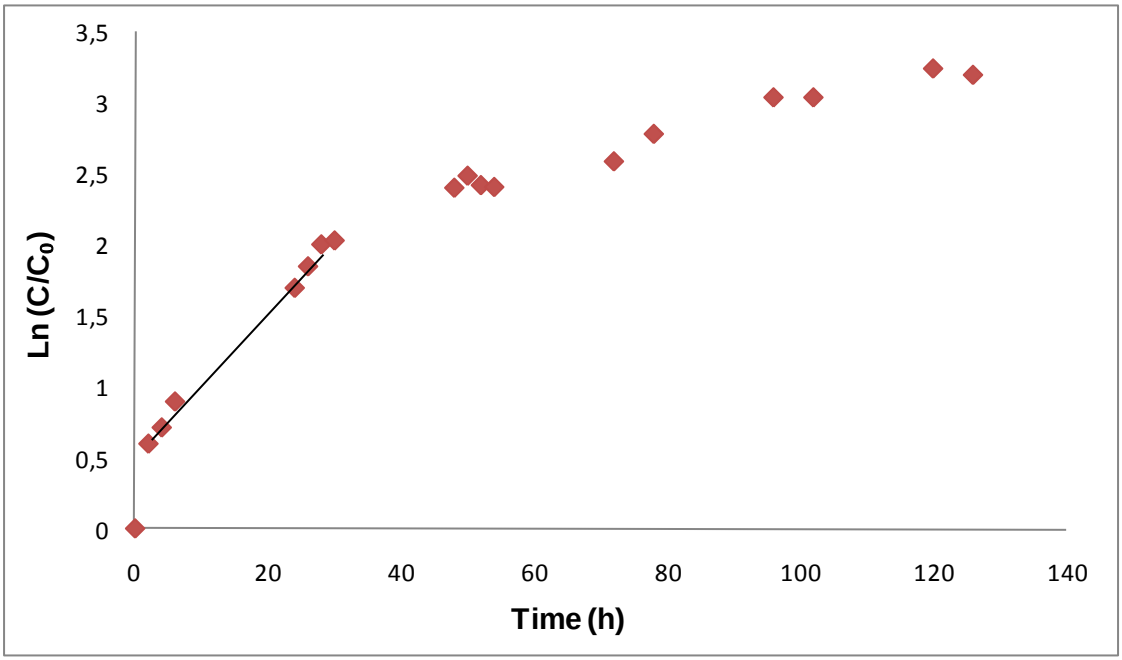

Figure 1b: Growth curves of the *Scenedesmus obliquus* in OMW
Exponential phase (The Ln (C/C0) is plotted as a function of time for the culture
Culture conditions: T: 25°C, pH=7, Treated OMW, aeration rate = 1 v/v/min, magnetic stirring=350 rpm

The biomass concentration was 0.9 g/L after 120h of growth. The cell concentration becomes important when the nutrients of the medium are completely consumed. The microalgae have grown in the OMW in the presence of light produced by fluorescent tubes without adding nutrients. The wastewater obtained was with low organic load attained COD = 3.25g O_2/L.

The specific growth rate during the exponential-growth phases can be calculated using the following equation1:

$$Ln(C/C_0) = \mu_m t + a \qquad Eq1$$

where 'C' represents the biomass concentration (g/L) at time t, 'x_0' is the initial biomass concentration after inoculation and 'a' is a constant value that includes the duration of the adaptation phase.

3.3. *Modeling Growth*

Modeling growth was performed by applying three different models. They are often used to model the growth of different species by the basic version of MATLAB 7.0 as well as in Curve fitting Toolbox.

These mathematical models give an idea about the behavior of the microalgae in the medium by the determination of the maximum concentration of biomass in the stationary phase, the maximum specific growth rate in the exponential phase and the time Lag latency which is the time required adapting the microalgae in the middle.

The models identified in this work are the model of Gompertz, Baranyi and Logistic [27], summarized in Table 2. They are used to determine the behavior of the *S. obliquus* in the OMW using experimental data without involving any parameter.

Table2: Models of growth of the *Scenedesmus obliquus*

Models	Equations
Gompertz	$\mu = B + A * e^{(-e\left(\left(\mu m * \frac{2,3}{A}\right) * (\mathrm{lag} - x) + 1\right))}$
Logistic	$\mu = \mathrm{B} + \left(\frac{\mathrm{A}}{(1+\mathrm{e}^{\left(\left(4 * \frac{\mu m}{\mathrm{A}}\right) * (\mathrm{lag} - \mathrm{x}) + 2\right)})}\right.$
Baranyi	$\mu = B + \mu m * \left(x + \left(\frac{1}{\mu m}\right) * \log\left(e^{(-\mu m * x)} + e^{(-\mu m * lag)} - e^{-\mu m * x - \mu m * lag}\right) - \log(1 + (e^{(\mu m * (x + 1\mu m * \log e^{-\mu m * x} + \mathrm{e}^{-\mu m * \mathrm{lag}} - \mathrm{e}^{-\mu m * \mathrm{x}} - \mu m * lag)) - 1) / (\mathrm{xm}x0))$

Where μ_m: Specific growth rate, A: maximum cell concentration in the stationary phase in mg/L, B: minimum cell concentration in the stationary phase in mg / L; Lag: Latency time.

Table 3 represents the parameters obtained after modeling the experimental data with growth models. From these results, we note that the correlation coefficients of the three models are close and exceed 0.9. According to the Gompertz model the specific growth rate achieved in the exponential phase μ_m = 0.075 h^{-1} with a very limited time of adaptation. Figure 2 illustrates the shape of the curves of the models used.

Table 3: Parameters of modeling with growth models

	Models		
Parameters	**Gompertz**	**Logistic**	**Baranyi**
μ_m	0.07487	0.06739	0.0677
A	2.865	2.922	-
B	-2.744	-2.883	-2.615
Lag	2.168e-008	2.17e-008	2.072e-011
$\mathbf{R^2}$	0.9358	0.9227	0.9452
$\mathbf{x_m}$	-	-	1.298
$\mathbf{x_0}$	-	-	0.08308
SSE	1.179	1.421	1.007
RMSE	0.2803	0.3077	0.2682

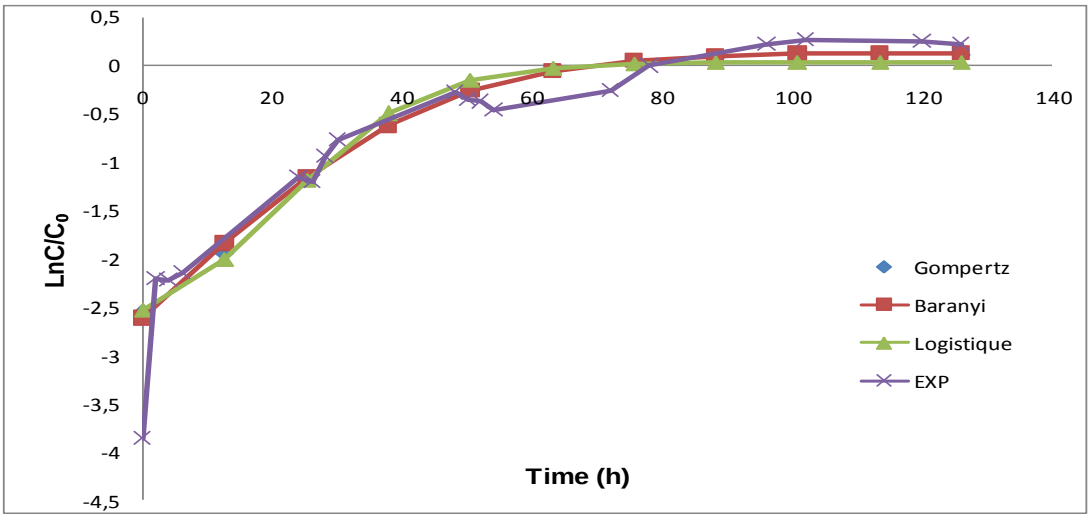

Figure 2: Models of growth of the *Scenedesmus obliquus* in the OMW

It is necessary to compare the growth of the *S. obliquus* in another medium to approve the effectiveness of the OMW. For this reason we achieved a growth monitoring of the microalgae in the synthetic medium Rodriguez Lopez, and we used the same models of growth which we used for the OMW.

Table 4 includes all the modeling parameters of growth by the three models in the two medium. From these results we note that the specific growth rates are close also in the medium RL, but they are very low compared to those in the OMW. The Gompertz model gives always the best growth rate in both culture mediums; the lag time is short but longer than in the OMW. Figure 3 shows the curves of the growth models in medium RL. We note that the growth follows the model of Baranyi whose correlation coefficient reached 0.95.

Table 4: Comparison of the modeling parameters of growth in the two mediums

	RL	OMW	RL	OMW	RL	OMW
Parameters	**Gompertz**		**Logistic**		**Baranyi**	
µm	0.02641	0.07487	0.02141	0.06739	0.02087	0.0677
A	456.3	2.865	3.013	2.922	-	-
B	-34.76	-2.744	-5.272	-2.883	-5.042	-2.615
Lag	0.002537	2.168e-008	2.34e-014	2.17e-008	2.391e-010	2.072e-011
R^2	0.8958	0.9358	0.9407	0.9227	0.9593	0.9452
x_m	-	-	-	-	1,829	1.298
x_0	-	-	-	-	0,09208	0.08308
SSE	2.412	1.179	1.374	1.421	0.9424	1.007
RMSE	0.3563	0.2803	0.2621	0.3077	0.2227	0.2682

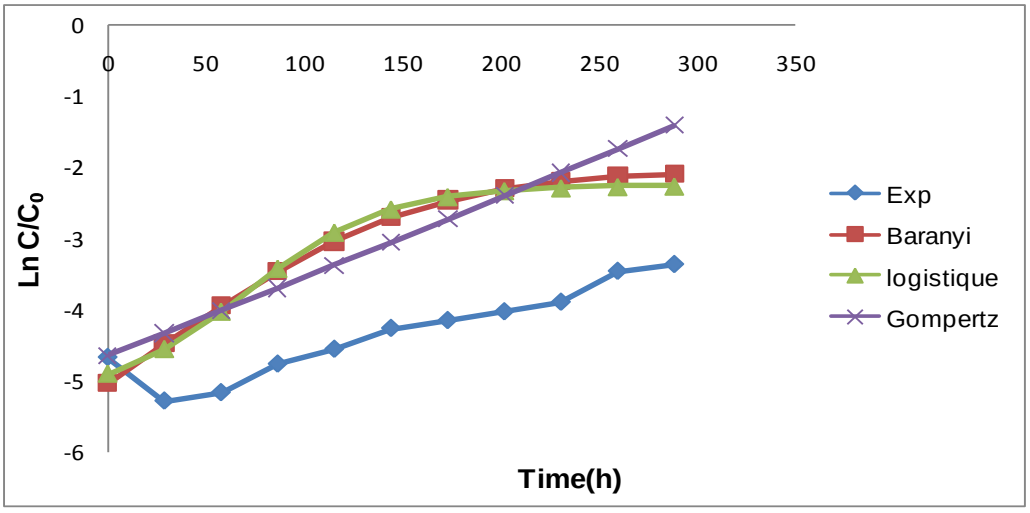

Figure 3: Models of growth of the *Scenedesmus obliquus* in the Rodriguez Lopez medium

We can therefore conclude that the presence of essential nutrients in the discolored OMW allowed the growth of the microalgae quickly without lag phase. The OMW can perfectly replace RL medium without adding nutrients but in limited period no more than 8 days. The deprivation of light and nutrients stops growth.

Conclusions

The valorization of the OMW by their use as a culture medium for *S.obliquus* can be an interesting environmental solution and can replace the synthetic medium based on chemical products. The use of these OMW without treatment inhibited the growth due to the presence of phenolic compounds in abundance. The adsorption onto activated carbon based on bones minimizes the toxicity of the OMW by eliminating their organic load and generated a discolored medium rich in calcium and phosphorus. The biomass concentration attained 0.9 g/L after

120h with a specific growth rate of $0.075h^{-1}$. The Monitoring the elimination of nutrients is needed to better understand the behavior of the microalgae in the OMW and on which element it depend for its growth.

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