Agriculture fertilizer-based media for cultivation of marine microalgae destined for biodiesel production

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In recent years, biodiesel from microalgae has received large interest around the world, as sustainable energy for biofuel production. Mineral fertilizers can be a promising source for the development of low-cost culture media. We investigate the influence of fertilizer-based media: MAP (Mono-ammonium phosphate), TSP (triple superphosphate), Phosphoric acid, and Ammonitrate on cell viability, nutrients uptake, biomass, lipids production, and lipids profile of 3 microalgae strains. The best biomass production was 2.105 $g.L^{-1}$, 1.95 $g.L^{-1}$, and 1.75 $g.L^{-1}$ for *D. tertiolecta*, *Isochrysis sp.*, and *Tetraselmis sp.* cultured in TSP, MAP and H3PO4 (54%) based-media respectively, compared to control medium (1.85, 1.76 and 1.71 g/L respectively). The lipid content of all strains in fertilizer-based media was similar to control. The lipid profile showed that FAMEs (Fatty Acids Methyl Esters) of all microalgae underwent a significant reduction in PUFAs (Polyunsaturated Fatty Acids) for fertilizers based-media, which improves the quality of biodiesel. Mineral fertilizers are a promising source that can be a low-cost microalgae production base at the industrial level.

keywords: *Microalgae, Fertilizers, Culture, Medium, Biodiesel.*

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1. INTRODUCTION

Microalgae are eukaryotic or prokaryotic photosynthetic microorganism as a new source of biomass, microalgae have only really begun to attract the attention of scientists and economic communities until the middle of the last century. Nowadays, there are numerous commercial applications of microalgae such as bioenergy, cosmetic, medical, agriculture, environment, food, and feed [1]. The possibility of industrial production has been under evolution for many years,' since the use of microalgae for producing bioactives or proteins appeared to be of potential commercial interest [2]. The complexity of current technologies permits only small production with a high cost compared to the requirements of markets such as energy or food and feed. For example, the biofuel industry does not produce more than 20 kt/year at a production cost of $20,000/t [3]. The estimated price for 10,000 tons of microalgae biomass with 30% lipid is about $2.80/liter, including conversion, tax, and marketing cost [4]. A significant low-cost biomass production capacity increase is necessary to compete in large-scale markets such as energy and commodities. One of the alternatives to reduce microalgae biomass production costs is the use of low-cost culture media based on industrial grade inorganic salts such as agriculture fertilizers [5]. For most of these applications, the use of agriculture fertilizers as culture media promotes better economic and environmental feasibility.

Biodiesel production has recently received a lot of attention around the world, not only for their economic interest but also for the environment as well as after the global warming crisis.

Algal biomass can become one of the most important bioresource s in the biological industry if the cost of microalgae production is stable and economical [6]. Microalgae with high photosynthetic efficiency compared to conventional crops are a promising bioresource for biodiesel production [7]. Biodiesel from microalgal lipid is more sustainable and eco-friendly than petroleum-derived diesel fuels. The microalgae accumulate large quantities of lipids, grow at high rates, fix CO2 from the atmosphere , adapt to a wide area including extreme environment and utilize nutrient s from wastewater [8, 9]. Because using pure chemical media is very expensive and not cost-effective in large scale microalgae culture, the pure chemical media is being

replaced with a cheaper alternative that is commercial fertilizer. Agriculture fertilizers can be used and are being used widely in current fish production practices. In addition, these fertilizers contain important nutrients such as N, P, and K and a few trace elements (Ca, Cu, Zn, Fe, and Mg). All of these nutrients and elements happen to be essential and utilized by most microalgae for growth.

Nevertheless, microalgae biodiesel is not economically viable. Different strategies have been proposed to reduce the production cost of biomass and lipids conversion to biodiesel. According to biodiesel production using microalgae, one of the main problems related to the large-scale production is the high cost of the culture medium, which supports an optimal biomass and lipids productivities [2]. Generally, seawater enriched by analytical quality macronutrients and micronutrients or conventional culture media such as f/2 and Walne are used for the cultivation of marine microalgae. These media are costly to formulate, and therefore are somewhat limiting factor s in the development of industrial production of microalgae [10]. In that sense, if we develop low-cost media based on agriculture fertilizers, the cost of algal biomass production can be reduced significantly. Agriculture fertilizers are mainly composed of macro-elements as nitrogenous and phosphorus and some of other elements such as iron, sodium, and potassium. However, they do not contain, in general, all micro-nutrients and vitamins necessary for the growth of microalgae [11]. Several studies have investigated the performance of low-cost culture media based on fertilizers for the cultivation of marine microalgae [12–14].

Different key parameters affecting the economic feasibility of algal for biodiesel production, such as biomass and lipids productivity [15].

This study is conducted to know the potential of commercial fertilizer as cheaper culture media for microalgae growth. The preparation of pure chemical media for mass algae culture is very expensive and labor-intensive . Therefore, with the finding of other cheaper alternative culture media for microalgae culture, this could contribute to the further development of large scale microalgae culture for different fields. In this particular study, commercial fertilizer will be used because it is readily available and easy to find in Morocco.

The aim of this work is to develop three culture media based on agriculture fertilizers (Monoammonium phosphate (MAP), Triple superphosphate (TSP), Phosphoric acid (H3PO4 (54%)), and ammonium nitrate) and study their effect on biomass and lipid of marine microalgae *Dunaliella tertiolecta*, *Tetraselmis sp.*, and *Isochrysis sp.* destined to biodiesel production. As well as a study to improve biomass and lipids productivity by adding phytohormones.

2. MATERIAL AND METHODS

A. Microalgae strains and culture conditions

Three marine microalgae *Dunaliella tertiolecta MS029*, *Tetraselmis sp MS052*, and *Isochrysis sp MS053* isolated from Moroccan Atlantic Ocean and maintained in MAScIR's Collection of Moroccan Microalgae [16]. These microalgae strains were cultivated in three different culture media prepared from filtered and sterilized seawater enriched by mineral fertilizers: MAP (N:P:K 11:54:0), TSP (N:P:K 0:46:0), phosphoric acid (H3PO4 (54%)) (N:P:K 0:54:0) and ammonium nitrate (N:P:K 35:0:0).

These low-cost products have been used as sources of nitrogen and phosphorus. However, agriculture grade products were used to complete the enrichment culture media by the remainder of the macronutrients (iron, boron, and manganese), micronutrients (zinc, copper, cobalt, and molybdenum), and vitamins. Walne medium was used as a control for all microalgae treatments. The composition of all culture media that were tested is given in Table 1.

Microalgae cultures for each formulation were initiated with an optical density (OD) at 680nm of 0.1 in a volume of 250ml, at a temperature of 25°C and under continuous illumination150 $\mu mols^{-1}m^{-2}$. Agitation of cultures was 130 rpm in an orbital shaker. All experiments were carried out in triplicate.

B. Monitoring and modeling of microalgae growth

The monitoring of microalgae growth was performed by measuring the optical density OD at 680nm after every two days. The maximum specific growth rate (μ_m), lag phase (t_{lag}) and the potential maximum biomass production concentration (A) were determined after the modeling of the growth curves by modified Gompertz model given by Eq. (1) [17]:

$$\ln(OD/OD_0) = A \quad \exp\{-\exp[(2.72 \quad \mu_m/A)\cdot(t_{lag}-t)+1]\} \tag{1}$$

where
$A = \ln(OD/OD_0)$: Potential maximum biomass production concentration.
OD and OD_0: Optical density at 680nm at any time t and at t=0.
OD_m: Maximum optical density at 680nm.
μ_m: Maximum specific growth rate (d^{-1}).
t_{lag}: Delayed time (d).

C. Microalgae cell viability analysis by Flow cytometer

In order to evaluate the effect of the mineral fertilizers basedmedia on the microalgae strains viability, cells were stained with Propidium Iodide (PI) (81845 FLUKA), and analyzed by flow cytometer (FCM) using the FACS-Calibur (Becton Dickinson, BD) cytometer. PI is an intercalating agent when associated with DNA of damaged cells , it emit s fluorescence and cannot cross with the cytoplasmic membranes of living cells. When excited at 488 nm, PI emit a fluorescence measured at 585 nm (FL2 channel of the cytometer).

Samples from each culture taken at day 14 were diluted using filtered seawater ($0.2\mu m$). To cancel the natural fluorescence of strains pigments (phycoerythrine and chlorophyll), samples without staining were analyzed as a negative control. Cells were stained with 10 $\mu g/ml$ of PI and incubated at room temperature for 10 min (modified protocol of [18]). The number of events (cells) analyzed, was fixed at 3000 for each sample, results are expressed as a percentage of viable cells (percentage of displaced cells in FL2 channel).

D. Nutrients analysis in culture media

Continuous Flow Analyzer (SKALAR, San^{++}) determined nitrogen sources and phosphorus. The analysis was done according to the Skalar methods for Ammonia, Nitrate, and phosphorus, respectively . Calibration curves were prepared using NH_4Cl, $NaNO_3$, and $NaHPO_4$ as standards of nitrogen and phosphorus, respectively.

E. Lipids extraction

Total lipid extraction is performed according to a modified protocol of [19]. The lyophilized biomass is rehydrated with water supplemented with 2% (Butylated hydroxytoluene) BHT and then sonicated (Branson Sonifier 450) at 40 KHz for 15 min at

Table 1. Composition of culture media in g/l

		Walne's medium	MAP-based medium	TSP-based medium	AP54-based medium
Macronutrients	$NaH_2PO_4, 2H_2O$	$2.00.10^{-2}$	-	-	-
	$NaNO_3$	$1.00.10^{-1}$	-	-	-
	MAP	-	$1.7.10^{-2}$	-	-
	TSP	-	-	$2.00.10^{-2}$	-
	PA54	-	-	-	$1.70.10^{-2}$
	NH_4NO_3	-	$4.5.10^{-2}$	$5.10.10^{-2}$	$5.10.10^{-2}$
	$FeCl_3, 6H_2O$	$1.30.10^{-3}$	$1.30.10^{-3}$	$1.30.10^{-3}$	$1.30.10^{-3}$
	H_3BO_3	$3.36.10^{-2}$	$3.36.10^{-2}$	$3.36.10^{-2}$	$3.36.10^{-2}$
	$MnCl_2, 4H_2O$	$3.60.10^{-4}$	$3.60.10^{-4}$	$3.60.10^{-4}$	$3.60.10^{-4}$
Micronutrients	$ZnCl_2$	$2.10.10^{-5}$	$2.10.10^{-5}$	$2.10.10^{-5}$	$2.10.10^{-5}$
	$CuSO_4, 5H_2O$	$2.00.10^{-5}$	$2.00.10^{-5}$	$2.00.10^{-5}$	$2.00.10^{-5}$
	$CoCl_2, 6H_2O$	$2.00.10^{-5}$	$2.00.10^{-5}$	$2.00.10^{-5}$	$2.00.10^{-5}$
	EDTA	$4.50.10^{-2}$	$4.50.10^{-2}$	$4.50.10^{-2}$	$4.50.10^{-2}$
Vitamins	VitaminB12	$1.00.10^{-6}$	$1.00.10^{-6}$	$1.00.10^{-6}$	$1.00.10^{-6}$
	VitaminB1	$1.00.10^{-6}$	$1.00.10^{-6}$	$1.00.10^{-6}$	$1.00.10^{-6}$
	Vitamin H	$2.00.10^{-8}$	$2.00.10^{-8}$	$2.00.10^{-8}$	$2.00.10^{-8}$

room temperature. The lipids are extracted using a mixture of water/chloroform/methanol (0.8:2:1) and the extract was centrifuged at 5000 rpm for 5 min. The lower phase was recovered and the upper one was subjected to the same extraction procedure to extract the remainder of the lipids. A physiological saline solution (0.9% NaCl) is mixed with the organic phase (5:1 v/v) then a separating funnel carries out the separation. The chloroform is evaporated to constant weight by nitrogen gas and the lipids are finally weighed.

F. Plant hormones treatment of microalgae

Microalgae were cultivated in filtered and sterilized seawater enriched with TSP-based media. The synthetic plant hormones were incorporated to culture medium from the beginning of culture at different concentrations: auxin 2,4-D (Sigma AldrichD7299): 0.5, 1, and 1.5 mg/L, Myo-inositol (Sigma Aldrich 15125): 50, 100, and 150 mg/L, Abscisic acid (ABA)(Sigma Aldrich A1049): 1, 5 et 10 mg/L.

G. Fatty acid methyl esters profile

The basic transesterification was done according to the protocol described by [20] to determine fatty acid methyl esters (FAME) profile. 2% NaOH (w/w) in methanol 1:20 was added as a reaction catalyst, the reaction was done at 80°C and atmospheric pressure during 6 hours. The gas chromatography (GC) (Agilent 7890A Series GC) coupled to mass spectrometry (MS) equipped with the multimode injector and 5-ms column with dimension of 30m x 250μm x 0,25μm, and electron impact ionization used in this study for FAME characterization.

Two μl of FAME solubilized in chloroform was injected into the column by splitless mode using helium as carrier gas at 1.5 ml/min. The ion source and quadruple temperatures were 230°C and 150°C, respectively . The oven temperature program was started at 70°C and maintained 1 min, increased at 20°C/min until 120°C, then, held one minute before to be increased until 200°C by 30°C/min and held one minute then, increased at 250°C at 10°C/min and held one minute, then increased until 270°C at 5°C/min and finally kept constant for 5 min. FAME composition was calculated as a percentage of the total FAMEs presents in the sample, determined from the peak areas. Detection was done using full scan mode between 35 to 600 m/z and with gain factor 5 and the identification was performed using NIST 2011 MS Library and confirmed by known standards Supelco ®37 Component FAME Mix (47885-USigma Aldrich) [20, 21].

3. RESULTS AND DISCUSSION

A. Microalgae growth

The parameters A, μm, and t_{lag} obtained by fitting Gompertz model to experimental data using nonlinear regression are collected in Table 2.

The regression coefficient (r^2), the sum of squared residuals (SSR) and the root-mean square error (RMSE) are also included to evaluate the goodness of fits.

Gompertz model exhibited a good fit sowing to the low SSR and RMSE values and high (r^2) values. In addition, the low values of (t_{lag}) indicate that the lag-phase in all treatments was negligible.

D.tertiolecta (Fig. 1a) cultivated in MAP and TSP-based media showed the highest significant effect on growth compared to the Walne medium (control) and the biomass production was improved to 1.94 $g.L^{-1}$ and 2.1 $g.L^{-1}$, respectively compared to control (1.850 $g.L^{-1}$).

These results were consistent with those found by [10] who compared the growth of *D. tertiolecta* in a medium based on commercial fertilizer, namely Maxicrop, with f/2 medium as a control. Likewise, it has been demonstrated that a medium based on a fertilizer N: P: K 10:26:26 has improved, significantly, the growth of *D. tertiolecta* its compared to f/2 medium [22]. In our study, the specific growth rate was also increased to 0.2363 d^{-1} and 0.3328 d^{-1}, in MAP and TSP-based media, respectively compared to control (0.2099 d^{-1}) (Table 2).

While, H_3PO_4 (54%)-based media, showed a similar biomass production to control was (1.820 vs. 1.850 $g.L^{-1}$),and the specific growth rate was found higher (0.3199 d^{-1} vs. 0.2099 d^{-1}). Ref. [10] was reported that no considerable influence of a medium based on commercial fertilizer, namely Miracle-gro,

Table 2. Growth parameters estimated using Gompertz model of three microalgae cultured in Walne's medium (control) and different low-cost media: MAP-based medium, TSP-based medium, and AP54-based medium

		A	$\mu_(d^{-1})$	$t_{lag}(d)$	r^2	SSE	RMSE
D. tertiolecta	Walne	1.85	0.2099	$2.22.10^{-14}$	0.9239	0.2069	0.1857
	MAP-based medium	1.94	0.2363	$9.90.10^{-9}$	0.9263	0.2081	0.204
	TSP-based medium	2.105	0.3328	$1.00.10^{-10}$	0.991	0.032	0.078
	AP54-based medium	1.82	0.3199	$4.16.10^{-9}$	0.9732	0.0712	0.1194
Tetraselmis sp.	Walne	1.709	0.2662	$4.68.10^{-9}$	0.9673	0.0761	0.1234
	MAP-based medium	1.747	0.2206	$9.50.10^{-3}$	0.9717	0.0682	0.1306
	TSP-based medium	1.551	0.3472	$1.00.10^{-10}$	0.9671	0.0648	0.1138
	AP54-based medium	1.208	0.1872	$2.23.10^{-14}$	0.9127	0.1021	0.1429
Isochrysis sp.	Walne	1.762	0.4281	$2.22.10^{-14}$	0.9464	0.149	0.1576
	MAP-based medium	1.913	0.3136	$3.69.10^{-10}$	0.9609	0.1143	0.1512
	TSP-based medium	1.886	0.4404	$1.10.10^{-10}$	0.9487	0.1524	0.1746
	AP54-based medium	1.946	0.3323	$2.24.10^{-14}$	0.94	0.1832	0.1914

compared with f/2 medium , was found on biomass production of *D.tertiolecta*. Similar results were also obtained by [12] who used Brazilian fertilizer, namely Super Our Verde as culture medium and Conway medium as control.

For Tetraselmis sp. (Fig. 1b), no considerable effect was found on the potential of biomass production in fertilizers-based media, MAP (1.75 $g.L^{-1}$), and TSP (1.75 $g.L^{-1}$) compared to control (1.709 $g.L^{-1}$).The specific growth rate slightly decreased in fertilizer based-media MAP medium (0.2206 d^{-1} vs. 0.2662 d^{-1}) and improved to 0.3472 d^{-1} in TSP medium. However, both the potential of biomass production and specific growth rate were decreased in H_3PO_4 (54%)medium (1.208 and 0.1872 d^{-1}, respectively) (Table 2).It was reported by [12] that among 8 commercial fertilizers from the Brazilian market tested to cultivate *Tetraselmis sp.*, only one, namely Ultra Fertil, has given a good growth.

For Isochrysis sp. (Fig. 1c), in all fertilizer-based media , the potential of biomass production was, slightly, higher than control, it's were 1.913, 1.886, and 1.946 in MAP, TSP and H3PO4 (54%) media, respectively (in control was 1.762). A specific growth rate in TSP medium (0.4404) was, practically, similar to control (0.4281) and, slightly, decreased in MAP (0.3136) and AP54 (0.3323) media (Table 2). Similar results were obtained with *Isochrysis aff. Galbana*, where the use of agricultural fertilizer-based media showed no significant effect on biomass production and growth rate when compared to f/2 medium [13]. Also, [12] was reported that a good growth was obtained using médium based on a fertilizer, namely S.O.V, compared to Walne medium.

B. Mineral fertilizers based-media effect on cell microalgae viability

Flow cytometer coupled with PI was used in this study, to monitor the stress caused by the application of chemical fertilizers on the different strains, in comparison with the reference medium.

Dot plots are given in Fig. 2, as an example for flow cytometer analysis of the *D. tertiolecta*.

Results of cell viability obtained in all media tested, calculated as a percentage , are presented in Table 3.

The percentage of viable cells, for all the media tested (including the reference medium), was between 94.36% and 99.71%. The MAP, TSP, H_3PO_4 (54%)fertilizers based-media showed a comparable effect of conventional medium on cell viability.

PI coupled with FCM was used to monitor microalgae cell viability in many studies [23]. Since only the viable cells are involved in the production, this fast and easy method is suitable to study the effect of culture conditions on the microalgae living cells, such as nutrients starvation.

Table 3. Percentages of viable cells at day 14 of culture in the three mineral fertilizers based-media in comparison with the reference medium

Strains	Walne	FW MAP	FW TSP	FW AcP54
D. tertiolecta	99,09	99,21	99,56	99,35
Tetraselmis sp	99,63	99,31	99,54	99,71
Isochrysis sp	94,36	98,86	99,31	98,32

C. Fertilizers based-media effect on nutrients uptake of microalgae

The monitoring of the consumption of the major elements N and P provides information on the ability of microalgae to assimilate the proposed new form. It thus focuses on the optimal concentration of each element necessary for each stage of growth. In this study, the consumption of N and P was studied during different growth stages.

The result presented in Fig. 3 shows that the majority of nitrogen and phosphate was consumed during the growth cycle. At the end of the exponential phase of *D. tertiolecta growth*, P is assimilated almost entirely (Fig. 3a).

For *Isochrysis sp.*, the nitrogen consumption during the culture cycle of this strain is not very important, indicating an excess of N in both the reference and the fertilizers based-media (Fig. 3c). At the end of the exponential phase, no more than 50% of N has been consumed, which gives the possibility of reducing the N/P ratio in this culture medium. While for *Tetraselmis sp.* the majority of N and P were consumed during the first 7 days of culture in all media included control medium (Fig. 3e,f). The study by [24] showed that the growth is proportional with the assimilation of nitrogen by *Chlorella vulgaris*, this study confirms the result that we obtained in the different fertilizers based-media. Another study showed that nitrogen assimilation in Chlorella is strongly related to the concentration and source of phosphate, as well as the N/P ratio that can influence lipid and protein metabolism in microalgae this strain [25]. The majority of conventional culture media have nitrate as their sole source

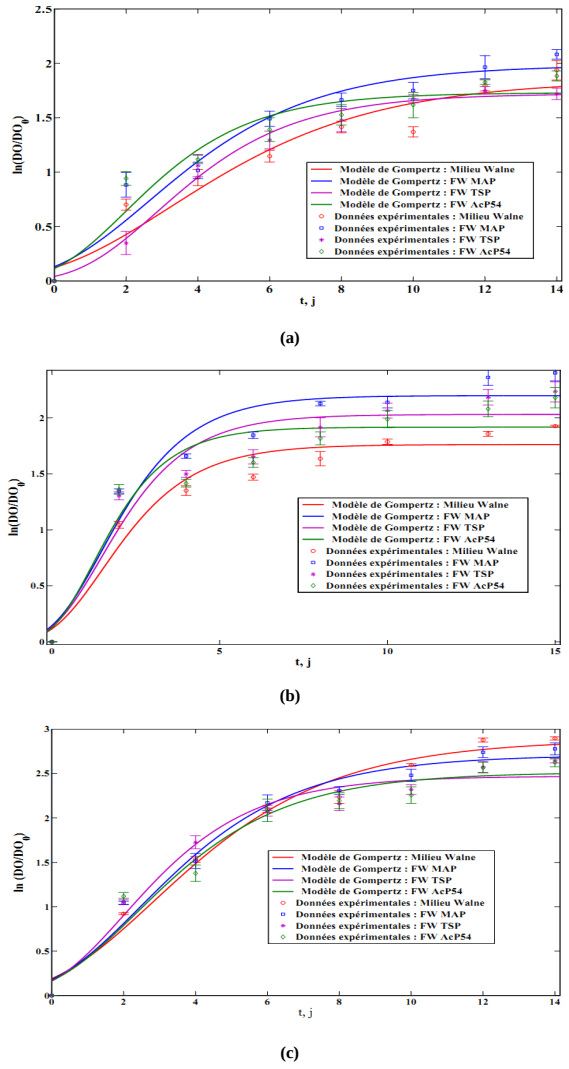

Fig. 1. Modeling of growth and growth rate of three microalgae (a) *D. tertiolecta*, (b) *Tetraselmis sp*, (c) *Isochrysis sp* in Walne's medium (control) and different low-cost media: experimental data for control (♦), MAP-based medium (■), TSP-based medium (×) AP54-based medium (•), Gompertz model for control (___), and fertilizer-based media (− − −−).

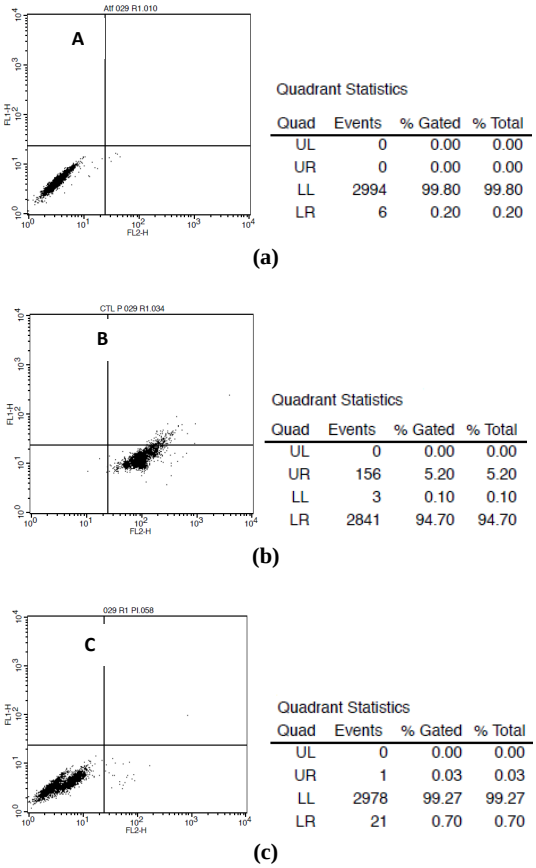

Fig. 2. (a) *Dunaniella tertiolecta* cells auto-fluorescence canceled (population in the double negative of the plot) of one of the triplicate sample s in the reference medium, (b) heat-killed cells (in a water bath at 100°C for 10 min) stained with PI, population moved in the FL2 channel corresponding to cells with permeabilised cytoplasmic membrane (0,1% viable cells), (c) sample is grown in the reference media stained with PI, no displacement of the population in the channel FL2 (99,27% viable cells). UL : UpperLeft, UR : Upper Right, LL : LowerLeft (double negative), LR: Lower Right.A.

of nitrogen. While fertilizers based media that we propose is composed of nitrate and ammonium, which could explain the improvement of the assimilation of all these nutrients by these microalgae studied.

D. Lipids content

One of the key criteria for biodiesel production from microalgae is lipid content. Based on total gravimetric lipids, the lipids yield was evaluated in the stationary phase. The challenge of biodiesel production is to minimize the cost by low-cost inputs, especially for the formulation of culture media that give a good growth without affecting the quantity and quality of lipids.

Fig. 4 shows that the mineral fertilizers based-media has no significant effect on lipid content for the microalgae strains studied. All agriculture fertilizer media are comparable to Walne medium. The best lipids content of three strains microalgae studied was 21% in TSP based-medium, 41% in TSP based medium and 45% in MAP based media for *D. tertiolecta*, *Tetraselmis sp.*, and *Isochrysis sp.*, respectively compared to control medium (20%, 39% and 42%).

Low-cost medium is a key factor in the industrial cultivation of microalgae to minimize the cost of lipids production. However, using local resources can be potential sources of microalgae culture media formulation in large-scale production. The agriculture fertilizers as low-cost chemicals should be more practical and at least one-step closer for industrial development [2, 26–29]. With the abundance of cheap agriculture fertilizers in the world, it provides a great opportunity to develop large-scale cultivation of microalgae for various applications such as feed-in aquaculture, food, medical uses, and even biofuel.

The total lipid content found in Moroccan strains is generally similar to those reported in the bibliography [30–32]. Ref. [33] has studied the effect of the nitrogen sources on the lipid content of microalgae. The results of this study showed that nitrate was the suitable nitrogen source for both cell growth and lipid accumulation of *Monoraphidium sp.* SB2 under the investi-

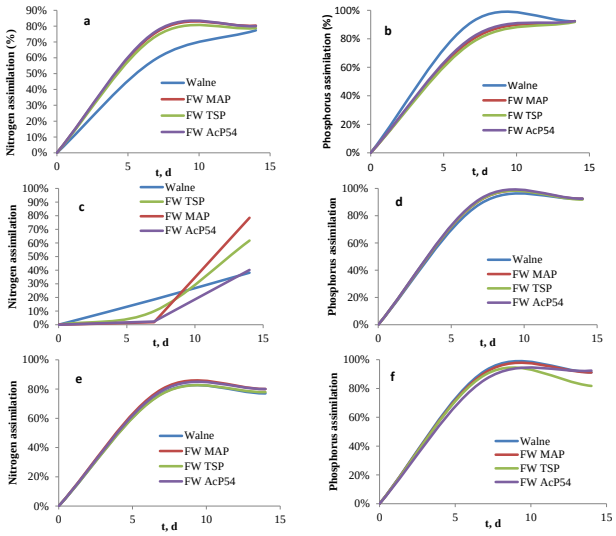

Fig. 3. Nitrogen and phosphorus assimilation by microalgae strains cultured in different fertilizers based-media: *Dunaliella tertiolecta* ((a): nitrogen and (b): phosphorus), *Isochrysis sp.* ((c): nitrogen and (d): phosphorus), *Tetraselmis sp* ((e): nitrogen and (f): phosphorus). All microalgae were cultured at 25°C with continuous illumination of 150-$\mu molm^{-2}s^{-1}$ during 15 days.

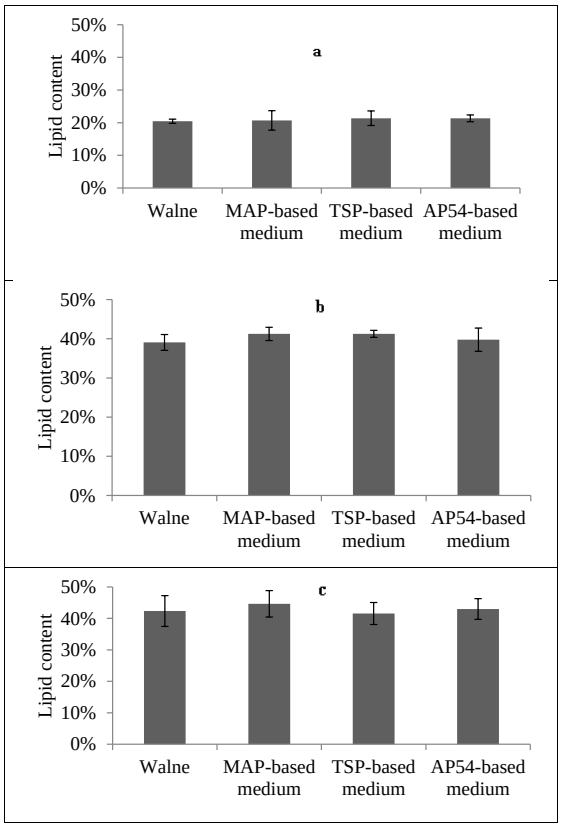

Fig. 4. Lipid content of three microalgae (a) *D. tertiolecta*, (b) *Tetraselmis sp.*, (c) *Isochrysis sp.*) cultured in fertilizer-based media. All microalgae strains were cultured at 25°C, in 7.8 pH and Luminosity of 150 $\mu mols^{-1}m^{-2}$.

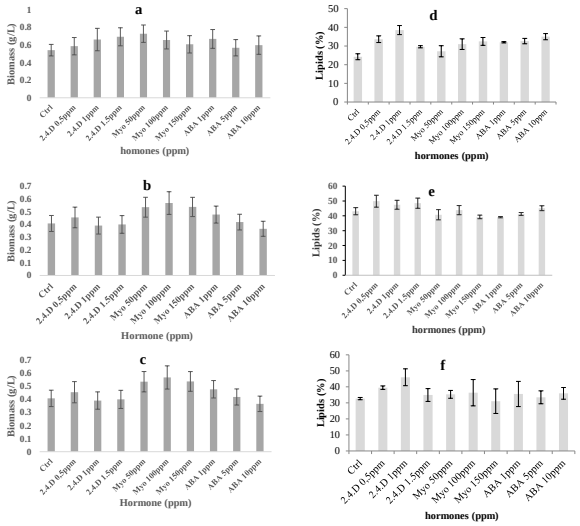

Fig. 5. Biomass production and lipids percentage of three microalgae cultivated in TSP based-media and treated by plant hormone (2.4.D, Myo-inositol, and ABA). (a),(b), (c) biomass production and (d), (e), (f) for lipids content of *D. tertiolecta*, *Isochrysis sp.*, and *Tetraselmis sp.*, respectively.

gated conditions compared to another's nitrogen sources. Other study show s that utilization of formulation based in fertilizer medium improved biomass, lipid, and eicosapentaenoic acid productivity in *Nannochloropsis sp.* [34]. The culture medium composition affected the chemical composition of microalgal cells, such as proteins and lipids. In our case, nitrate is the nitrogen source of the control medium, whereas the nitrogen sources of the fertilizer media are ammonium and nitrate. The simulation results of [35] showed that the combination of nitrate and ammonium in the culture of the microalgae strains *Mychonastesafer* HSO-3-1 slightly improved the lipid content in this strain. Contrariwise, the greatest TAG content (58.56%) of total lipids was obtained when $NaNO_3$ was used as the nitrogen source [35]. Therefore, using 100% $NH4^+$ as a nitrogen source was not a suitable nitrogen source for the cultivation of this strain.

E. Effect of phytohormone combined to fertilizer based-media on biomass and lipids content of microalgae

Microalgal biodiesel represents a promising alternative to fossil fuels as an eco-friendly energy source. However, mastery of culture conditions is necessary to maximize productivity (biomass and lipid) for commercial biodiesel production. In order to improve the performance of the low-cost culture medium, we added phytohormones in TSP-based media to boost biomass and lipid production. The result presented in Fig. 5 shows that the plant hormones improves the biomass and lipids content of three microalgae cultured in mineral fertilizers based-media (TSP and Ammonitrate) compared to control. The biomass was improved by 35,2%, 40%, and 35% in 50 mg/L Myo-inositol treatment for *D. tertiolecta*, *Isochrysis sp.*, and *Tetraselmis sp.*, respectively compared to control (Fig. 5a,b,c). While the auxin 2.4.D of 1 mg/L enhances the lipids content by 58.4, 10.5, and 40.6% *D. tertiolecta*, *Isochrysis sp.*, and *Tetraselmis sp.*, respectively compared to control (Fig. 5d,e,f).

These results of microalgae biomass production are similar to other studies when the growth stimulating effect of auxins has

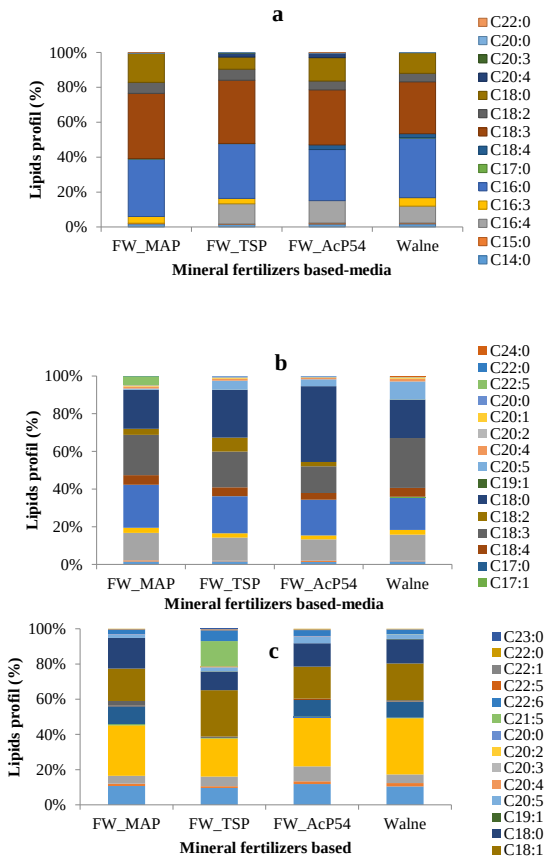

Fig. 6. FAMEs profile of microalgae cultured in Walne's medium (control) and different low-cost media: MAP-based medium, TSP-based medium, and AP54-based medium. (a) *D. tertiolecta*, (b) *Tetraselmis sp.*, (c) *Isochrysis sp.* All microalgae strains were cultured at 25°C, in 7.8 pH and Luminosity of 150 $\mu mols^{-1}m^{-2}$.

been demonstrated in *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Haematococcus pluvialis*, *D. tertiolecta*, and *Spirulina platensis* [28]. The lipids content improved by 58.4% after the treatment of *D. tertiolecta* with 1 mg/L of auxin 2.4.D [20].

F. Fatty acid methyl esters profile

The lipid profile of *D. tertiolecta* (Fig. 6a) has remained stable for tested fertilizer media. Nevertheless, the MAP-based medium caused the loss of 4,7,10,13-hexadecatetraenoic acid (C16:4), which was present at 9,8-12.8% for the control and other tested media. This leaded to the extinction of PUFA (≥ 4). This change on the profile may improve the quality of biodiesel obtained with this formula. Usually, FAME profile of *D. tertiolecta* grown under our experimental conditions is similar to that reported in the literature [28, 36].

Tetraselmis sp. lipid profile in fertilizer based-media was changed (Fig. 6b); the main fatty acids are similar to the control but, stearic acid (C18:0) increased in TSP and H_3PO_4 (54%)media compared to control (25, 40% vs 20% respectively), which is interesting to improve the quality of the resulting biodiesel. Linolenic acid (C18:3) decreased 21%, 19%, and 14% vs 26% (control) in MAP, TSP, and AP54 media, respectively, which also indicating a better quality of biodiesel.

The profile is clearly different from that reported by [37] for the majority of the acids except for C20:0, C20:4, and C24:0. It is also different compared to that reported by [36].

FAME profile of *Isochrysis* was slightly changed using TSP formula. While the profiles obtained using MAP and H3PO4 (54%)based-media are similar to the control medium. PUFAs (≥ 4) are similar to the control in MAP and H3PO4 (54%)based-media (14-16%) but increased by using TSP to 23% (Fig. 6c).

The simulation results of [38] showed that PUFAs increase significantly after stress using a basic ammonium culture medium (100%) relative to the medium based on nitrate as a nitrogen source [38]. Mostly, FAME profile found in this study is similar to those reported in the literature [36, 37].

4. CONCLUSION

To conclude, commercial fertilizer used in this experiment is able to be used as a cheaper alternative culture medium for the growth of microalgae. The three microalgae strains cultured in mineral fertilizers based-media showed better growth than the conventional medium (control). Better nitrogen assimilation in *D. tertiolecta* and *Tetraselmis sp.* cultured in the three mineral fertilizers based-media compared to control medium. The lipid content of three strains cultured in fertilizers media was similar to control. However, lipid profiles of microalgae in all fertilizers media showed a decrease of polyunsaturated fatty acids, which can improve the quality of biodiesel. The addition of phytohormones to increase productivity in biomass and lipids by improving the performance of mineral fertilizers based-media. For the density, growth rate, biomass and lipids, the cells cultured in commercial fertilizer based culture medium showed compared results to control media. In general, commercial fertilizer based culture media can replace conventional media for the production of microalgae biomass in order to minimize the production, cost of algal biodiesel.

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CONFLICT OF INTEREST STATEMENT

All authors declare that there is no conflict of interest between them.

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