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Impacts of (NH4)2CO3, Ca(H2PO4)2, K2CO3 and CaCO3 additives on lipid accumulation in microalga Chlorella sorokiniana

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Abstract

At present, the major body of research is focused on weaning the world from fossil fuels. The problem is that the world is running out of fossil fuel. Therefore, an alternative source must be identified. The biofuels are promising alternatives. In the case of petrodiesel, a promising alternative is biodiesel production from algae. The ability of microalgae to generate large quantities of lipids with a fast growth rate made them superior biodiesel producers. An important factor of determining optimal microalgal activity is the bio response to changes in ions concentration and quantity. The effects of the addition of the following chemicals were investigated: ammonium carbonate (NH4)2CO3 with a concentration of 72 mg/L, monocalcium phosphate Ca(H2PO4)2 with a concentration of 14 mg/L, potassium carbonate (K2CO3) with a concentration of 4.5 mg/L, and calcium carbonate (CaCO3) with a concentration of 2 mg/L. Further treatment is a mixture of all additives with the same listed concentrations. According to the results of this study, it was found that nitrogen, phosphorous, potassium, and calcium concentration have great influence on the algal growth and lipid production. Furthermore, the mixture of all additives yielded the highest lipid (2.488 g/L) and the highest biodiesel production (114.39mg/L) among all treatments followed by the treatment of ammonium carbonate yielded 1.596 g/L lipids and 74.38 mg/L biodiesel.

Keywords: Biofuels; Biodiesel production; Lipid production; Chemical additives; Microalgae.

1. Introduction

Over recent years, the fast-increasing consumption and the expected depletion of fossil fuel reserves led to the classification of dependence of energy on fossil fuels as a kind of future challenge [1], and thus the increasing need for sustainable energy calls for the development of renewable and cost-effective alternative energy sources to reduce the use of fossil fuels. Phytoplankton cultivation in the best conditions and species are strongly improved lipids quantity and quality, through different factors to target the preferable designs to enhance the production from microalgae-based biodiesel [2].

They are recognized for CO_2 emission mitigation, fast growth rate and non-arable land usage for cultivation. These qualities present microalgae as beneficial over several or different other feedstocks [3]. There is a major reason, or the main advantage of microalgae makes it an interesting alternative to the most popular feedstock of food crops is that algae do not compete with food crops [3]. To circumvent the 'food vs fuel' problem which has strongly coupled with first generation biofuel [4]. The biological treatment of lignocellulosic non crop biomass comes as the base for the improvement of second-generation biofuel techniques [5].

Microalgae of the genus Chlorella are a likely source of biologically effective materials. Chlorella biomass contains a large amount of polyunsaturated fatty acids [6]. Moreover, these fatty acids can be transformed into biodiesel.

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Biofuels such as bioethanol, biohydrogen, and biodiesel are considered as alternative for petro-based fuels. Among the various biofuel options proposed, biodiesel came to be extremely promising fuel alternative [7, 8] . According to research findings, biodiesel was identified as a potential resource that can satisfy the world's energy needs whereas it can be used in diesel engines (blinded by 20%) without requiring any changes to the engine as their combustion properties are nearly like the petro-based diesel [9].

The acuteness of the greenhouse effect led researchers to look up alternatives for reducing greenhouse gas emissions to the atmosphere. Energy effectiveness plays the main essential role in the problem of climate change due to emission of greenhouse gas from power consumption [10].

In synthetic conditions, enhancement of lipid content can be done through several strategies, like response surface methodology [11].

At pH 9.2 and above, free ammonia become the dominant species. Although *C. sorokiniana* can tolerate ammonia concentration up to 96.3 $mg.L^{-1}$ NH_3 [12].

Algae strains require specific nutrients, which are: nitrogen (N), phosphorus (P) and potassium (K). Additionally, algae require some further nutrients like calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), boron (B), and zinc (Zn) which are required for good growth of the algae [13]. Thus, some of the above-mentioned nutrients will be prepared in form of metal carbonates and phosphate to treat the algal cells.

The research gap can be elucidated as follows: (1) the use of different chemical additives was not thoroughly investigated, and (2) more research is needed to cover the biodiesel production from algae to fulfil the world fuel demand. The major objective of this research was to increase lipid production from algal biomass using chemical additives. The general objectives can be further elaborated in terms of the following specific objectives: biostimulating algae using chemical additives for enhancing lipids accumulation of algae and, therefore, increasing oil production; and cultivating the algae in photobioreactors exposed to Light Emitting Diodes (LEDs), after being treated with metal carbonates and phosphate.

2. Experimental

2.1. Microalgae strain

The microalgal species employed in this research was Chlorella sorokiniana SAG 211-8k produced by the Marine Toxin laboratory at the Egyptian Agriculture Research Institute. This oleaginous strain was selected to be exposed to white LED light as a photobiostimulant, as described by [14 – 16], that could increase the lipids accumulation in the alga which have low oil contents (25 - 35%). Generally, Chlorella sp. is mainly preferred in biodiesel production by researchers because of its lipid concentration and composition.

2.2. Culture medium

The medium was Blue-Green (BG-11) media composed of: 240 mg/ l nitrogen ($NaNO_3$ 1.5 g/L), $K_2HPO_4.3H_2O$ 0.0314 g/L, $MgSO_4.7H_2O$ 0.036 g/L, $CaCl_2.2H_2O$ 0.0367 g/L, Na_2CO_3 0.02 g/L, citric acid 0.0056 g/L, Na_2Mg (EDTA) 0.001 g/L, ferric ammonium citrate 0.0071 g/L, Trace metal mix A5+Co 1 ml was sterilized at 121°C for 15 min with pH adjusted at 7.4 [17, 18].

2.3. Chemicals

Chemicals additives were provided from sigma Aldrich: Ammonium carbonate, ≥30.0% NH_3 basis, Monocalcium phosphate, ≥95%, Potassium carbonate, ≥99.0% and Calcium carbonate, ≥99.0%, powder.

2.4. Experimental setup

The experimental setup can be elaborated as follows: designing an array of photobioreactors, identifying the appropriate chemical additives, and selecting the microalgae strain. Generally, there are three stages to biodiesel production from algae as illustrated in Figure 1.

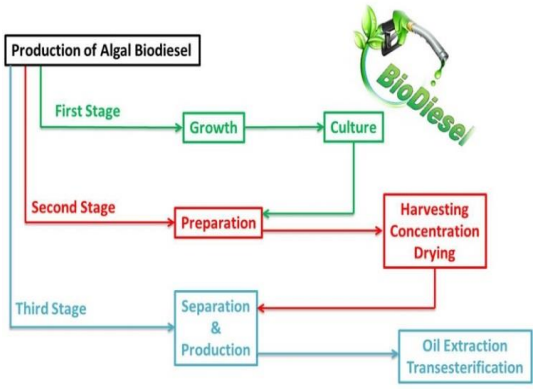

Fig. 1. Process flow chart for biodiesel production.

2.5. Culture condition

The implemented Lab-scale model is a closed photobioreactor (PBR) which consists of Erlenmeyer flask, an air pump (Shengzhe Bs-410, China), and sample purification filters (NY 0.45 µm, China). Microalga was grown in the laboratory [as shown in Figure 2] and was used as an experimental setup for *Chlorella sorokiniana* growth. Under sterilization conditions, using 2 L Erlenmeyer flask culture photobioreactor, 100 ml microalgal suspension (*Chlorella sorokiniana*) was inoculated into 900 ml of BG-11 media at 30 ± 5 °C with continuous stirring [5], pumping CO_2 and pH adjusted at 7.4. The experiments were carried out at the Department of Agricultural Engineering at the Faculty of Agriculture, Cairo University.

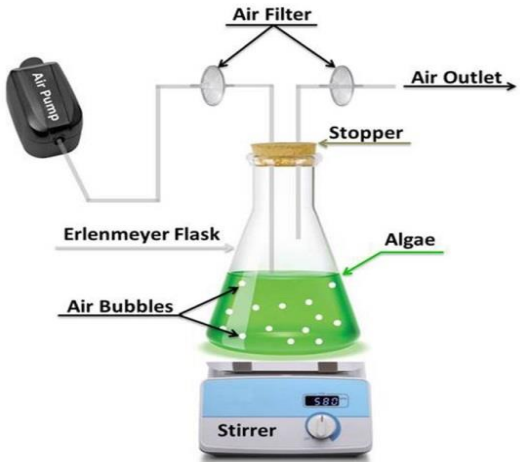

Fig. 2. Closed photobioreactor (PBR) system.

2.6. Biostimulation setup

An important factor of determining optimal microalgal activity is the bioresponse to changes in trace metal concentration and quantity [5]. The biostimulation was conducted at the Biofuel Laboratory at the Department of Agricultural Engineering, Faculty of Agriculture, Cairo University.

In this study, the effects of the addition of the following chemicals were investigated: ammonium carbonate $(NH_4)_2CO_3$ with a concentration of 72 mg/L as recommended by [19], Monocalcium phosphate $Ca(H_2PO_4)_2$ with a concentration of 14 mg/L as recommended by [20], potassium carbonate K_2CO_3 with a concentration of 4.5 mg/L as recommended by [13], and calcium carbonate $CaCO_3$ with a concentration of 2 mg/L as recommended by [13]. Further treatment is a mixture of all aforementioned additives with the same listed concentrations.

Algae were treated with the abovementioned chemicals then cultivated in the photobioreactors and exposed to Light Emitting Diodes (LEDs) source (Alobeidi, China) which irradiate the algae with a white light of complete spectrum (wavelength: 400-700 nm).

The hydraulic retention time (HRT) of the algae in photobioreactors was twenty-one days. All experiments were conducted in triplicate.

2.7. Experimental design

In order to investigate the effect of different chemical additives on lipid production, 100 ml algal biomass were inoculated into 2 L Erlenmeyer flask where the respective chemical additive was added with continuous stirring and were irradiated by white LEDs source (Fig. 3) compared with the control where no chemicals were added.

2.8. Oil extraction and analysis

Lipids were extracted from harvested microalgae biomass. The microalgae were harvested after twenty-one days of cultivation by centrifugation at 4500 rpm for 10 min. The algal biomasses were dried at 85 °C for 24 h before the extraction process. Total lipids were extracted using a Soxhlet Reflux Extractor with chloroform: methanol (2:1 v/v) from dried algae and was then gravimetrically quantified as described by [21].

The peroxide value was determined using the official method of the AOAC (1990). The acid value was determined using the official method of the AOAC (2000).

2.9. Transesterification

The transesterification of extracted oils and characterization of resulting biodiesel were conducted in this research according to [22]. The transesterification of the extracted oils was conducted using methanol (CH_3OH) and potassium hydroxide (KOH) and stirred for 3 h at 60 °C. The mixture was kept at room temperature for 18 h for separation of biodiesel and glycerol using a flask separator.

Fig. 3. Irradiation of algae using white LEDs source for twenty-one days.

2.10. Statistical analysis

The statistical analysis aimed at evaluating the effects of chemical additives on microalgae growth. Each experiment was conducted in three replicates. The statistical significance of difference was evaluated by one-way analysis of variance and KruskalWallis Test ($P \leq 0.05$) using SPSS Software (IBM, v. 20). The statistical analysis was conducted using the statistical package Origin (version 8, MA, USA). Data are represented as Standard Error (SE) of mean values. Analysis of Variance (ANOVA) and Fisher test as a post hoc one was performed to compare the DW of biomass, total lipid and biodiesel produced after variable microalgae treatments by visible light.

P-value < 0.05 was set as the significant level. For all results of statistical analysis, Sig equals to 1 indicates that the mean difference is significant at the 0.05 level, and Sig equals to 0 indicates that the mean difference is not significant at the 0.05 level.

3. Results and Discussion

3.1. Results

3.1.1. Effects of chemical additives on algal biomass

The effects of different chemical additives on the growth of microalgae were evaluated by using ammonium carbonate ($(NH_4)_2CO_3$) with a concentration of 72 mg/L, triple superphosphate ($Ca(H_2PO_4)$) with a concentration of 14 mg/L, potassium carbonate (K_2CO_3) with a concentration of 4.5 mg/L, and calcium carbonate ($CaCO_3$) with a concentration of 2 mg/L.

Further treatment is a mixture of all additives with the same listed concentrations. The control, where no additives were used, was operated in the same conditions for the microalgae conditions for the microalgal growth. As shown in Table 1 and Table 2, the mixture significantly produced the highest amounts of fresh and dry weights of microalgal biomass, followed in descending order by $(NH_4)_2CO_3$, $Ca(H_2PO_4)$, K_2CO_3, and $CaCO_3$. However, the lowest amount was delivered by the control.

TABLE 1
Fresh weights of algal biomass FW (g/L), after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	0.771	0.006	0.003
$(NH_4)_2CO_3$	3	1.895	0.033	0.019
$Ca(H_2PO_4)_2$	3	1.786	0.048	0.028
K_2CO_3	3	1.590	0.035	0.020
$CaCO_3$	3	1.450	0.052	0.030
Mixture	3	2.985	0.039	0.022

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	2.214	0.031	70.521	1	2.146	2.283
Mixture/$(NH_4)_2CO_3$	1.090	0.031	34.725	1	1.022	1.159
Mixture/ $Ca(H_2PO_4)_2$	1.199	0.031	38.185	1	1.131	1.267
Mixture/ K_2CO_3	1.395	0.031	44.438	1	1.327	1.464
Mixture/ $CaCO_3$	1.535	0.031	48.876	1	1.466	1.603

3.1.2. Effects of chemical additives on moisture content of algal biomass

The effects of different chemical additives on the moisture content of algal biomass were evaluated. Table 3 shows the moisture content of algal biomass after the addition of chemicals compared with the control, where both mixtures of all additives as well as the ammonium carbonate delivered the lowest moisture content of the algal biomass and, however, the control significantly delivered the highest moisture content.

TABLE 2.
Dry weight of biomass (g/L), after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	0.196	0.022	0.013
$(NH_4)_2CO_3$	3	1.110	0.013	0.007
$Ca(H_2PO_4)_2$	3	0.922	0.009	0.005
K_2CO_3	3	0.629	0.028	0.016
$CaCO_3$	3	0.637	0.008	0.005
Mixture	3	1.724	0.020	0.011

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	1.528	0.015	103.261	1	1.496	1.560
Mixture/$(NH_4)_2CO_3$	0.615	0.015	41.539	1	0.582	0.647
Mixture/ $Ca(H_2PO_4)_2$	0.802	0.015	54.199	1	0.770	0.834
Mixture/ K_2CO_3	1.095	0.015	74.000	1	1.063	1.127
Mixture/ $CaCO_3$	1.087	0.015	73.481	1	1.055	1.120

TABLE 3.
Moisture content of algal biomass after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	74.513	3.003	1.734
$(NH_4)_2CO_3$	3	41.427	0.490	0.283
$Ca(H_2PO_4)_2$	3	48.323	1.895	1.094
K_2CO_3	3	60.413	1.466	0.846
$CaCO_3$	3	56.053	1.046	0.604
Mixture	3	42.233	0.518	0.299

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
$(NH_4)_2CO_3$/Control	-33.087	1.348	-24.542	1	-36.024	-30.149
$Ca(H_2PO_4)_2$/Control	-26.190	1.348	-19.427	1	-29.127	-23.253
K_2CO_3/Control	-14.100	1.348	-10.459	1	-17.037	-11.163
$CaCO_3$/Control	-18.460	1.348	-13.693	1	-21.397	-15.523
Mixture/Control	-32.280	1.348	-23.944	1	-35.217	-29.343

3.1.3. Effects of chemical additives on total lipid

The effects of different chemical additives on the total accumulated lipids were evaluated. Table 4 shows the total lipid after the addition of chemicals compared with the control, where the mixture of all additives significantly delivered the highest total lipid and, however, the control delivered the lowest total lipid.

3.1.4. Effects of chemical additives on peroxide value

The effects of different chemical additives on the peroxide value were evaluated. Table 5 shows the peroxide value after the addition of chemicals compared with the control, where the mixture of all additives significantly delivered the highest peroxide value and, however, the control delivered the lowest peroxide value.

3.1.5. Effects of chemical additives on acid value

The effects of different chemical additives on the acid value were evaluated. Table 6 shows the acid value after the addition of chemicals compared with the control, where both mixture of all additives as well as the ammonium carbonate significantly delivered the highest acid value and, however, the control delivered the lowest acid value.

TABLE 4.
Total Lipid (g/100g) after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	0.352	0.037	0.021
$(NH_4)_2CO_3$	3	1.597	0.072	0.042
$Ca(H_2PO_4)_2$	3	0.899	0.018	0.010
K_2CO_3	3	0.431	0.030	0.017
$CaCO_3$	3	0.509	0.020	0.011
Mixture	3	2.488	0.049	0.028

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	2.136	0.034	62.520	1	2.062	2.210
Mixture/$(NH_4)_2CO_3$	0.891	0.034	26.089	1	0.817	0.966
Mixture/ $Ca(H_2PO_4)_2$	1.589	0.034	46.519	1	1.515	1.664
Mixture/ K_2CO_3	2.057	0.034	60.198	1	1.982	2.131
Mixture/ $CaCO_3$	1.979	0.034	57.934	1	1.905	2.054

TABLE 5.
Peroxide values after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	0.189	0.022	0.013
$(NH_4)_2CO_3$	3	0.802	0.030	0.017
$Ca(H_2PO_4)_2$	3	0.563	0.016	0.009
K_2CO_3	3	0.199	0.026	0.015
$CaCO_3$	3	0.310	0.033	0.019
Mixture	3	1.355	0.056	0.032

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	1.167	0.027	43.444	1	1.108	1.225
Mixture/$(NH_4)_2CO_3$	0.553	0.027	20.605	1	0.495	0.612
Mixture/ $Ca(H_2PO_4)_2$	0.792	0.027	29.505	1	0.734	0.851
Mixture/ K_2CO_3	1.157	0.027	43.072	1	1.098	1.215
Mixture/ $CaCO_3$	1.046	0.027	38.939	1	0.987	1.104

TABLE 6.
Acid values after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	0.599	0.053	0.031
$(NH_4)_2CO_3$	3	1.230	0.039	0.022
$Ca(H_2PO_4)_2$	3	1.107	0.094	0.054
K_2CO_3	3	0.919	0.028	0.016
$CaCO_3$	3	1.060	0.049	0.028
Mixture	3	1.342	0.025	0.014

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	0.743	0.043	17.124	1	0.648	0.838
Mixture/$(NH_4)_2CO_3$	0.112	0.043	2.574	1	0.017	0.206
Mixture/ $Ca(H_2PO_4)_2$	0.235	0.043	5.408	1	0.140	0.329
Mixture/ K_2CO_3	0.423	0.043	9.749	1	0.328	0.518
Mixture/ $CaCO_3$	0.282	0.043	6.499	1	0.187	0.377

3.1.6. Effects of chemical additives on biodiesel yield

The effects of different chemical additives on the biodiesel yield were evaluated. Table 7 shows the biodiesel yield after the addition of chemicals compared with the control, where both mixture of all additives as well as the ammonium carbonate significantly delivered the highest biodiesel yield and, however, the control delivered the lowest biodiesel yield.

3.1.7. Effects of chemical additives on algal cell count

The effects of different chemical additives on the algal cell count were evaluated. Table 8 shows the algal cell count after the addition of chemicals compared with the control, where the mixture of all additives significantly delivered the highest algal cell count and, however, the control delivered the lowest algal cell count.

TABLE 7.
Biodiesel yield after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	15.727	1.001	0.578
$(NH_4)_2CO_3$	3	74.383	1.668	0.963
$Ca(H_2PO_4)_2$	3	62.073	1.870	1.080
K_2CO_3	3	32.647	1.316	0.760
$CaCO_3$	3	38.277	1.691	0.976
Mixture	3	114.390	2.899	1.674

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	98.663	1.501	65.735	1	95.393	101.934
Mixture/$(NH_4)_2CO_3$	40.007	1.501	26.654	1	36.736	43.277
Mixture/ $Ca(H_2PO_4)_2$	52.317	1.501	34.856	1	49.046	55.587
Mixture/ K_2CO_3	81.743	1.501	54.462	1	78.473	85.014
Mixture/ $CaCO_3$	76.113	1.501	50.711	1	72.843	79.384

TABLE 8.
Algal cell count after the addition of chemicals.

	Sample Size	Mean	Standard Deviation	SE of Mean
Control	3	8.317	0.021	0.012
$(NH_4)_2CO_3$	3	13.840	0.104	0.060
$Ca(H_2PO_4)_2$	3	12.450	0.385	0.222
K_2CO_3	3	9.467	0.117	0.067
$CaCO_3$	3	11.523	0.356	0.205
Mixture	3	19.947	0.150	0.087

Fisher Test

	Mean Diff	SEM	t Value	Sig	LCL	UCL
Mixture/Control	11.630	0.189	61.463	1	11.218	12.042
Mixture/$(NH_4)_2CO_3$	6.107	0.189	32.273	1	5.694	6.519
Mixture/ $Ca(H_2PO_4)_2$	7.497	0.189	39.619	1	7.084	7.909
Mixture/ K_2CO_3	10.480	0.189	55.386	1	10.068	10.892
Mixture/ $CaCO_3$	8.423	0.189	44.516	1	8.011	8.836

3.2. Discussion

The results of this study show that the nitrogen concentration has a great influence on the algal growth and lipid production. This could be attributed to that the growth of all organisms depends on the availability of nitrogen which is required in large amounts as an essential component of peptides, proteins, enzymes, chlorophylls, energy-transfer molecules (ATP, ADP), genetic materials (RNA, DNA), and other cellular constituents [19]. However, excessively high, or low nitrogen concentration in the culture medium has inhibitory effect on the growth and lipid production of microalgae.

Nitrogen depletion causes a decrease in growth and protein content. though, mild nitrogen depletion (0.22 mM $NaNO_3$) causes maximum lipid yield [23, 24].

Also, the comparative content of starch increased obviously [25].

Nitrogen decreasing during growth led to increase the oil content. It is clear that under stress conditions, the entirety of carbon produced are changed to oil [26].

The results of this study show that phosphorus, like nitrogen, is a critical nutrient required for algae. The most common form of phosphorus used by biological organisms is phosphate (PO_4), which plays major roles in the formation of DNA, cellular energy, and cell membranes and cell wall [20]. However, excessive concentration of phosphorous can cause increased growth of algae, which can result in decreased levels of dissolved oxygen, a process called eutrophication. High levels of phosphorus can also lead to algae blooms that produce algal toxins.

The present study showed that the addition of potassium has an important positive effect on the growth of microalgae, which agrees with the statement of [13] who stated that potassium is essential for algal growth and mentioned that algae deficient in potassium could be stunted in their growth and lipid accumulation.

The outcome of this study indicates that calcium plays an important role in the growth of microalgae. This could be ascribed to that calcium starvation hinders cell division, thereby decreasing the cell concentration [13]. Thus, the addition of calcium had positive effect on algal growth.

The methodology of this study agrees with Khalifa et al. (2022) [27] who applied similar methodology but used different chemical additives.

The quality of biodiesel is related to carbon and nitrogen, an indicator of the quality of fuel in an engine (Stournas et al., 1995) [28]. The lower content of saturated fatty acids (SFAs) in Chlorella oil. Indeed, polyunsaturated fatty acids (PUFAs) which have at least four double bonds are led to oxidation, emit more nitrogen oxides, and have a thermal efficiency lower than biodiesel rich in SFAs, Such a biodiesel have low oxidative potential. So PUFAs are less acceptable for biodiesel production (Chisti, 2007) [29].

In addition, the ratio between SFAs and UFAs is remarkable when using microalgal oils for biodiesel production. Future research will focus on the biostimulation of microalgae using trace metals in form of nanomaterials as well as photoactivated nanomaterials using laser radiation, where the nanotechnology was implemented in biogas and biohydrogen production [30 - 37] but not yet in biodiesel production.

Another issue is the use of white LEDs in this study, where the amount of light produced from LEDs is the same amount of light produced from other energy sources, but LEDs use less energy. Further, heat generated during this process is almost null, which supports energy conservation [38]. Accordingly, in several different sectors, the LEDs topped instead of conventional light lamps owing to their low energy requirements, which makes it an environmentally friendly light source which agrees with [39], and the implementation of LEDs in microalgal cultivation affects the quantity and quality of the produced biomass. This happens primarily owing to the light's mono-chromaticity with effective control of photosynthetic photon flux density, a property not found in sunlight that agrees with [40].

Future research will focus on the addition of the trace metals, and chemical additives in form of nanomaterials which should be photoactivated using laser radiation to get better results. However, it is essential to conduct a life cycle assessment [37, 41]. An important future application is to develop an air purification system using algae to purify the exhaust air from industries, factories, and buildings [42 - 45] in order to replace current purification systems by an environmentally friendly algal purification system.

4. Conclusions

According to the results of this study, it can be concluded that:

1. Nitrogen plays a vital role in the growth of microalgae and lipid production.
2. Phosphorus concentration has a large influence on the algal growth and lipid production.
3. Potassium addition has an important positive effect on the growth of microalgae.
4. Calcium has an important positive effect on the growth of microalgae.
5. The mixture of all additives yielded the, the highest highest lipid and, therefore biodiesel production among all treatments.

In addition, the use of mixture additives in large-scale open cultivation of microalgae would

be effective way for biofuel production in desert areas in Egypt.

5. Conflicts of interest

Authors declare that there is no conflict of interest.

6. Formatting of funding sources

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