Egypt. J. Chem. **Vol. 65,** No. 1, pp. 511 - 519 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effects of Fe_2O_3, MnO_2, MgO and ZnO additives on lipid and biodiesel production from microalgae

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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 bioresponse to changes in trace metal concentration and quantity. The effects of the addition of the following chemicals were investigated: ferric oxide (Fe_2O_3) with a concentration of 1.2 mg/L, manganese dioxide (MnO_2) with a concentration of 1 mg/L, magnesium oxide (MgO) with a concentration of 7.3 mg/L, and zinc oxide (ZnO) with a concentration of 5 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 iron, manganese, magnesium, and zinc concentration have great influence on the algal growth and lipid production. Furthermore, the mixture of all additives yielded the highest lipid and, therefore, the highest biodiesel production among all treatments.

Keywords: Biofuels, Biodiesel, Microalgae, Chemical additives, Photobioreactor.

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 [2]. So, microalgae have been widely investigated in recent years owing to their recognized benefits [3]. Algal biofuels are a renewable fuel derived from the algae as feedstock by different conversion bioprocesses. This is owing to the oil-rich structure of this substrate that can be coupled with its capability to alter metabolism under certain stress conditions. Its main advantage is the ability to convert almost all the energy from the substrate into several types of useful products apart from its large oil fraction [4]. 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 [5]. 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 [5]. To circumvent the 'food vs fuel' problem which has strongly coupled with first generation biofuel [6]. The biological treatment of lignocellulosic non crop biomass comes as the base for the improvement of second-generation biofuel techniques [7]. Especially that the lipid fraction of algal biomass comprises important fatty acids that play a vital role in anthropological nutrition [8]. Moreover, these fatty acids can be transformed into biodiesel [3].

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Receive Date: 23 June 2021, Revise Date: 06 July 2021, Accept Date: 25 July 2021
DOI: 10.21608/EJCHEM.2021.82155.4051

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 [9-11]. 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 [12].

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 [13].

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 [14]. Thus, some of the above-mentioned nutrients will be prepared in form of metal oxides 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 sunlight, after being treated with metal oxides.

2. Material and methods

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 with low oil contents was selected to be exposed to white LED light as a photobiostimulant, as described by [15], that could increase the lipids accumulation in the alga which have low oil contents (25 - 35%) and therefore increase biodiesel production from low-oil microalgae.

2.2 Culture medium

The medium was Blue-Green (BG-11) media composed of: $NaNO_3$ 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 [16, 17.

2.3 Experimental design

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.

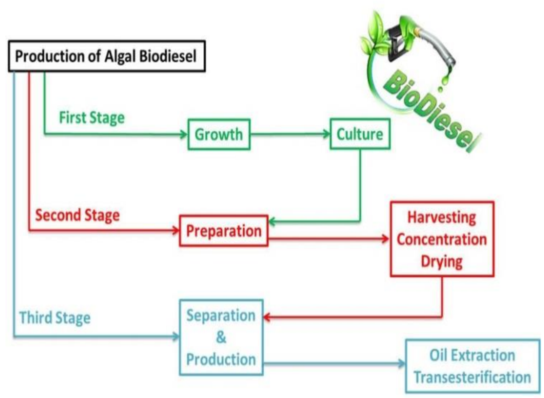

Figure 1: Process flow chart for biodiesel production.

2.4 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 [7], 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.

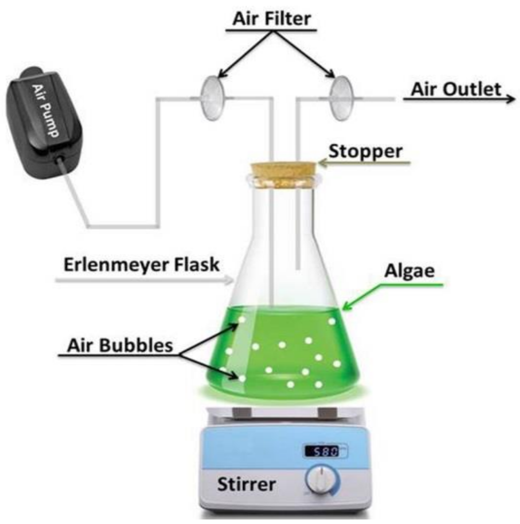

Figure 2: Closed photobioreactor (PBR) system.

2.5 Biostimulation setup

An important factor of determining optimal microalgal activity is the bioresponse to changes in trace metal concentration and quantity [7]. 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: ferric oxide (Fe_2O_3) with a concentration of 1.2 mg/L as recommended by Ren et al. [18], manganese dioxide (MnO_2) with a concentration of 1 mg/L as recommended by Lam and Lee [14], magnesium oxide (MgO) with a concentration of 7.3 mg/L as recommended by Ren et al. [18], and zinc oxide (ZnO) with a concentration of 5 mg/L as recommended by Dinesh et al. [19]. 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.

2.6 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.

2.7 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 Kiran et al. [20].

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).

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

3. Results

3.1 Effects of chemical additives on algal biomass

The effects of different chemical additives on the growth of microalgae were evaluated by using ferric oxide (Fe_2O_3) with a concentration of 1.2 mg/L, manganese dioxide (MnO_2) with a concentration of 1 mg/L, magnesium oxide (MgO) with a concentration of 7.3 mg/L, and zinc oxide (ZnO) with a concentration of 5 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 Figure 4, the mixture produced the highest of microalgal biomass, ranging from 1.84 to 1.89 g/L, followed in descending order by Fe_2O_3 (1.17–1.2 g/L), MgO (1.42–1.48 g/L), MnO_2 (1.08–1.14 g/L), ZnO (1–1.08 g/L), and the control (0.73–0.74 g/L).

Table 1: Weights of algal biomass after the addition of chemicals.

Treatments	Replicates	Fresh weight of biomass FW (g/L)	Dry weight of biomass DW (g/L)
Control	1	0.729	0.308
	2	0.733	0.166
	3	0.740	0.182
Fe_2O_3	1	1.167	0.669
	2	1.179	0.667
	3	1.207	0.681
MnO_2	1	1.077	0.562
	2	1.115	0.552
	3	1.136	0.553
MgO	1	1.438	0.567
	2	1.417	0.520
	3	1.480	0.555
ZnO	1	1.077	0.452
	2	1.005	0.442
	3	1.026	0.443
Mixture	1	1.840	1.046
	2	1.869	1.043
	3	1.888	1.065

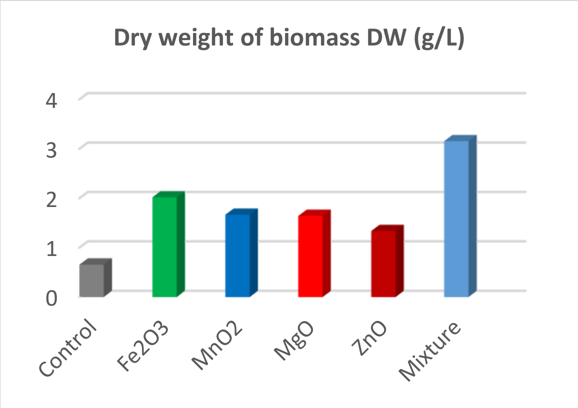

Figure 4: Weights of algal biomass after the addition of chemicals.

3.2 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 2 shows the moisture content of algal biomass after the addition of chemicals compared with the control, where both mixture of all additives as well as the ferric oxide delivered the lowest moisture content of the algal biomass and, however, the control delivered the highest moisture content.

Table 2: Moisture content of algal biomass after the addition of chemicals.

Treatments	Replicates	Moisture (%)	SD
Control	1	77.69	1.22
	2	77.35	
	3	75.42	
Fe_2O_3	1	42.67	0.48
	2	43.41	
	3	43.57	
MnO_2	1	47.85	1.81
	2	50.52	
	3	51.31	
MgO	1	60.52	1.44
	2	63.32	
	3	62.48	
ZnO	1	58.07	1.02
	2	56.05	
	3	56.81	
Mixture	1	43.14	0.52
	2	44.18	
	3	43.57	

3.3 Effects of chemical additives on total lipid

The effects of different chemical additives on the total accumulated lipids were evaluated. Table 3 shows the total lipid after the addition of chemicals compared with the control, where the mixture of all additives delivered the highest total lipid and, however, the control delivered the lowest total lipid.

3.4. Effects of chemical additives on peroxide value

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

3.5 Effects of chemical additives on acid value

The effects of different chemical additives on the acid value were evaluated. Table 5 shows the acid value after the addition of chemicals compared with the control, where both mixture of all additives as well as the ferric oxide delivered the highest acid value and, however, the control delivered the lowest acid value.

Table 3: Total lipid after the addition of chemicals.

Treatments	Replicates	Total Lipid (g/100g)	SD
Control	1	0.292	0.035
	2	0.348	
	3	0.356	
Fe_2O_3	1	1.003	0.044
	2	0.919	
	3	0.981	
MnO_2	1	0.551	0.065
	2	0.434	
	3	0.543	
MgO	1	0.488	0.123
	2	0.263	
	3	0.288	
ZnO	1	0.369	0.014
	2	0.356	
	3	0.342	
Mixture	1	1.504	0.030
	2	1.551	
	3	1.496	

Table 4: Peroxide values after the addition of chemicals.

Treatments	Replicates	Peroxide value (Millequivilent/Kg)	SD
Control	1	0.295	0.075
	2	0.159	
	3	0.174	
Fe_2O_3	1	0.228	0.010
	2	0.209	
	3	0.223	
MnO_2	1	0.164	0.020
	2	0.196	
	3	0.200	
MgO	1	0.187	0.022
	2	0.147	
	3	0.184	
ZnO	1	0.582	0.021
	2	0.561	
	3	0.540	
Mixture	1	0.817	0.034
	2	0.798	
	3	0.864	

3.6 Effects of chemical additives on biodiesel yield

The effects of different chemical additives on the biodiesel yield were evaluated. Table 6 shows the biodiesel yield after the addition of chemicals compared with the control, where both mixture of all additives as well as the ferric oxide delivered the highest biodiesel yield and, however, the control delivered the lowest biodiesel yield.

Table 5. Acid values after the addition of chemicals.

Treatments	Replicates	Acid Value (mg/g)	SD
Control	1	0.044	0.316
	2	0.562	
	3	0.616	
Fe_2O_3	1	0.769	0.016
	2	0.741	
	3	0.740	
MnO_2	1	0.765	0.091
	2	0.602	
	3	0.754	
MgO	1	0.651	0.078
	2	0.777	
	3	0.794	
ZnO	1	0.769	0.034
	2	0.703	
	3	0.751	
Mixture	1	0.759	0.021
	2	0.723	
	3	0.759	

Table 6: Biodiesel yield after the addition of chemicals.

Treatments	Replicates	Biodiesel (mg/L)	SD
Control	1	25.15	6.234
	2	13.84	
	3	14.95	
Fe_2O_3	1	46.06	2.007
	2	42.22	
	3	45.15	
MnO_2	1	38.28	4.583
	2	30.10	
	3	37.77	
MgO	1	36.37	4.956
	2	28.59	
	3	27.16	
ZnO	1	34.18	4.654
	2	26.88	
	3	25.53	
Mixture	1	71.39	3.109
	2	65.44	
	3	69.98	

3.7 Effects of chemical additives on algal cell count

The effects of different chemical additives on the algal cell count were evaluated. Table 7 shows the algal cell count after the addition of chemicals compared with the control, where the mixture of all additives delivered the highest algal cell count and, however, the control delivered the lowest algal cell count.

Table 7. Algal cell count after the addition of chemicals.

Treatments	Replicates	Initial algal load (log10 cell/ml)	Final algal count (log10 cell/ml)	SD
Control	1	5.15	7.84	0.021
	2	5.15	7.83	
	3	5.15	7.87	
Fe_2O_3	1	5.15	8.35	0.064
	2	5.15	8.46	
	3	5.15	8.35	
MnO_2	1	5.15	8.11	0.181
	2	5.15	8.33	
	3	5.15	8.47	
MgO	1	5.15	8.25	0.101
	2	5.15	8.12	
	3	5.15	8.32	
ZnO	1	5.15	8.02	0.056
	2	5.15	7.98	
	3	5.15	7.91	
Mixture	1	5.15	12.11	0.092
	2	5.15	12.27	
	3	5.15	12.11	

4. Discussion

The results of this study show that the iron concentration has a great influence on the algal growth and lipid production. This could be attributed to that the metabolic pathways related to lipid synthesis in microalgae could be modified by the addition of iron in culture medium [18]). However, excessively high, or low iron concentration in the culture medium has inhibitory effect on the growth and lipid production of microalgae.

The present study showed that the addition of manganese has an important positive effect on the growth of microalgae, which agrees with the statement of Lam and Lee [14] who stated that manganese is essential for algal growth and mentioned that algae deficient in manganese could be stunted in their growth and lipid accumulation.

The outcome of this study indicates that magnesium plays an important role in the growth of microalgae. This could be ascribed to that magnesium starvation hinders cell division, thereby decreasing the cell concentration [18]. Thus, the addition of magnesium had positive effect on algal growth.

The present study showed that the addition of zinc has an important positive effect on the growth of microalgae, which agrees with the statement of Dinesh et al. [19] that zinc has been established to be required for the growth of algae.

Future research will focus on the biostimulation of microalgae using trace metals in form of nanomaterials, where the nanotechnology was implemented in biogas and biohydrogen production [21-29] 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 [24]. 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 Duarte & Costa [30], 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 Schulze et al. [31].

In some cases, the trace metals, and chemical additives in form of nanomaterials should be photoactivated using laser radiation [32] to get better results. However, it is essential to conduct a life cycle assessment [33].

An important future application is to develop an air purification system using algae to purify the exhaust air from industries, factories, and buildings [34-37] in order to replace current purification systems by an environmentally friendly algal purification system.

5. Conclusion

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

1. Iron concentration has a great influence on the algal growth and lipid production.
2. Manganese addition has an important positive effect on the growth of microalgae.
3. Magnesium plays an important role in the growth of microalgae.
4. Zinc has an important positive effect on the growth of microalgae.
5. The mixture of all additives yielded the highest lipid and, therefore, the highest biodiesel production among all treatments.

6. Conflicts of interest

There are no conflicts to declare.

7. Funding sources

Science and Technology Development Fund (STDF) project number 26272.

8. Acknowledgments

The authors would like to acknowledge the Science and Technology Development Fund (STDF) of Egypt for funding this paper through research project number 26272.

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