Increasing biodiesel production from microalgae using chemical additives

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Abstract: The majority of current research is focused on weaning the world off of fossil fuels. 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 adding calcium chloride ($CaCl_2$) with a concentration of 2 mg L^{-1}, potassium chloride (KCl) with a concentration of 4.5 mg L^{-1}, and ferric chloride ($FeCl_3$) with a concentration of 1.2 mg L^{-1} were examined. Further treatment is a mixture of all additives with the same listed concentrations. According to the results of this study, it was found that calcium, potassium, and iron concentration have great influence on the algal growth and lipid production. Furthermore, among all treatments, the mixture of all additives produced the most lipid and, as a result, the maximum biodiesel yield.

Keywords: biofuels, biodiesel, microalgae, chemical additives, photobioreactor.

Citation: Faried, M., A. Khalifa, E. Abdelsalam, Y. Attia, M. A. Moselhy, R. S. Yousef, K. Abdelbary, and M. Samer. 2022. Increasing biodiesel production from microalgae using chemical additives. Agricultural Engineering International: CIGR Journal, 24(4): 101-110.

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 (Al-Ameri and Al-Zuhair, 2019), 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 and create a sustainable bioeconomy (Dickinson et al., 2017; Samer, 2022). So, microalgae have been widely investigated in recent years owing to their recognized benefits (Zhou et al., 2017). Algal biofuels are renewable fuels obtained from algae as feedstock through several bioprocesses of conversion. 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. Apart from its substantial oil fraction, its greatest advantage is its capacity to transform almost all the energy from the substrate into a variety of valuable products (Adeniyi

Received date: 2018-08-30 **Accepted date:** 2022-07-05

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et al., 2018). 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 (Akubude et al., 2019). There is a major reason, or the main advantage of microalgae that makes it an interesting alternative to the most popular feedstock of food crops is that algae do not compete with food crops (Akubude et al., 2019). To circumvent the ‘food vs fuel’ problem which has strongly coupled with first generation biofuel (Younis, 2020). The biological treatment of lignocellulosic non crop biomass comes as the base for the improvement of second-generation biofuel techniques (Faried et al., 2017). Especially that the lipid fraction of algal biomass comprises important fatty acids that play a vital role in anthropological nutrition (Díaz et al., 2017). Moreover, these fatty acids can be transformed into biodiesel (Zhou et al., 2017).

Biofuels such as bioethanol, biohydrogen, and biodiesel are being promoted as viable alternatives to petroleum-based fuels. Biodiesel, among the several biofuel solutions presented, has proven to be a very viable fuel alternative (Lee et al., 2017; Sivaramakrishnan and Incharoensakdi, 2018; Shomal et al., 2019). Biodiesel has been discovered as a viable resource that can meet the world's energy demands, according to research findings, and it can be utilized in diesel engines (blinded by 20%) without requiring any engine modifications because its combustion qualities are essentially identical to petro-based diesel (Mohd-Noor et al., 2018).

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 the emission of greenhouse gas from power consumption (Sun et al., 2017).

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 (Lam and Lee, 2014). 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 usage of various chemical additives has not been completely examined, and (2) additional study is needed to cover biodiesel synthesis from algae in order to meet global 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 Materials 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 Faried et al. (2021, 2022a, 2022b), 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^{-1}, $K_2HPO_4.3H_2O$ 0.0314 g L^{-1}, $MgSO_4.7H_2O$ 0.036 g L^{-1}, $CaCl_2.2H_2O$ 0.0367 g L^{-1}, Na_2CO_3 0.02 g L^{-1}, citric acid 0.0056 g L^{-1}, Na_2Mg (EDTA) 0.001 g L^{-1}, ferric ammonium citrate 0.0071 g L^{-1}, Trace metal mix A5+Co 1 mL was sterilized at 121 ℃ for 15 min with pH adjusted at 7.4 (Huesemann et al., 2013; Olasehinde et al., 2019).

2.3 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.

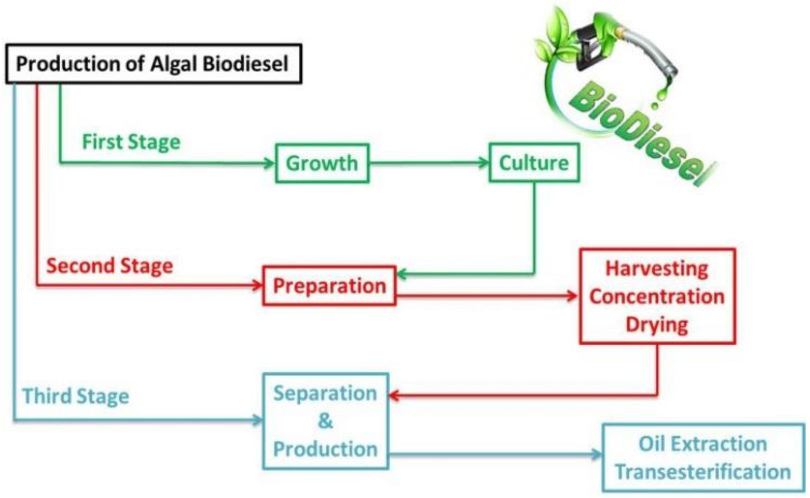

Figure 1 Process flow chart for biodiesel production

2.4 Culture condition

The implemented Lab-scale model is a closed photobioreactor (PBR) which consists of an 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 a 2 L Erlenmeyer flask culture photobioreactor, 100 mL microalgal suspension (*Chlorella sorokiniana*) was inoculated into 900 mL of BG-11 media at 30℃ ± 5℃ with continuous stirring (Faried et al., 2017; Khalifa et al., 2022), pumping CO_2 and pH adjusted at 7.4. The experiments were carried out at the Biofuels Laboratory, Department of Agricultural Engineering at the Faculty of Agriculture, Cairo University.

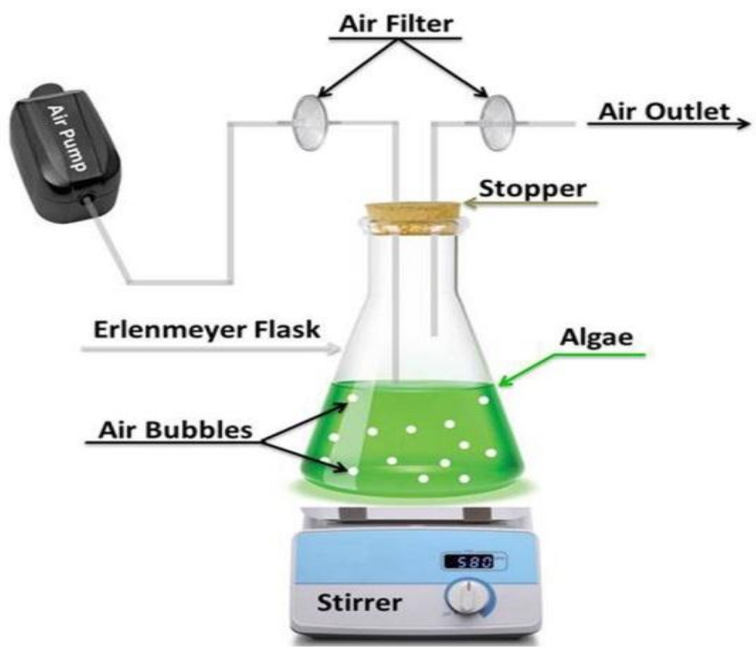

Figure 2 Closed photobioreactor (PBR) system

2.5 Biostimulation setup

An important factor in determining optimal microalgal activity is the bioresponse to changes in trace metal concentration and quantity (Faried et al., 2017). 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: calcium chloride ($CaCl_2$) with a concentration of 2 mg L^{-1} as recommended by Lam and Lee (2014), potassium chloride (KCl) with a concentration of 4.5 mg L^{-1} as recommended by Lam and Lee (2014), and ferric chloride ($FeCl_3$) with a concentration of 1.2 mg L^{-1} as recommended by Ren et al. (2014). 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 irradiates 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 was inoculated into a 2 L Erlenmeyer flask where the respective chemical additive was added with continuous stirring and irradiated by a white LEDs source (Figure 3) compared with the control where no chemicals were added.

2.7 Oil extraction

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 ℃ 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 (Kiran et al., 2014).

2.8 Peroxide and acid values determination

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 Oil transesterification

The transesterification of extracted oils and characterization of resulting biodiesel were conducted in this research according to Onay et al. (2014). The transesterification of the extracted oils was conducted using methanol (CH_3OH) and potassium hydroxide (KOH) and stirred for 3 h at 60 ℃. The mixture was kept at room temperature for 18 h for the separation of biodiesel and glycerol using a flask separator.

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 calcium chloride ($CaCl_2$) with a concentration of 2 mg L^{-1}, potassium chloride (KCl) with a concentration of 4.5 mg L^{-1}, and ferric chloride ($FeCl_3$) with a concentration of 1.2 mg L^{-1}. 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 microalgal biomass, ranging from 1.75 to 1.79 g L^{-1}, followed in descending order by $FeCl_3$

(1.11-1.15 g L^{-1}), KCl (1.02-1.08 g L^{-1}), $CaCl_2$ (1.35-1.41 g L^{-1}), and the control (0.69-0.70 g L^{-1}).

Table 1 Weights of algal biomass after the addition of chemicals

Treatments	Replicates	Fresh weight of biomass FW (g L^{-1})	Dry weight of biomass DW (g L^{-1})
	1	0.693	0.293
Control	2	0.696	0.158
	3	0.703	0.173
	1	1.366	0.539
$CaCl_2$	2	1.346	0.494
	3	1.406	0.527
	1	1.023	0.534
KCl	2	1.059	0.524
	3	1.079	0.525
	1	1.109	0.636
$FeCl_3$	2	1.120	0.634
	3	1.147	0.647
	1	1.748	0.994
Mixture	2	1.776	0.991
	3	1.794	1.012

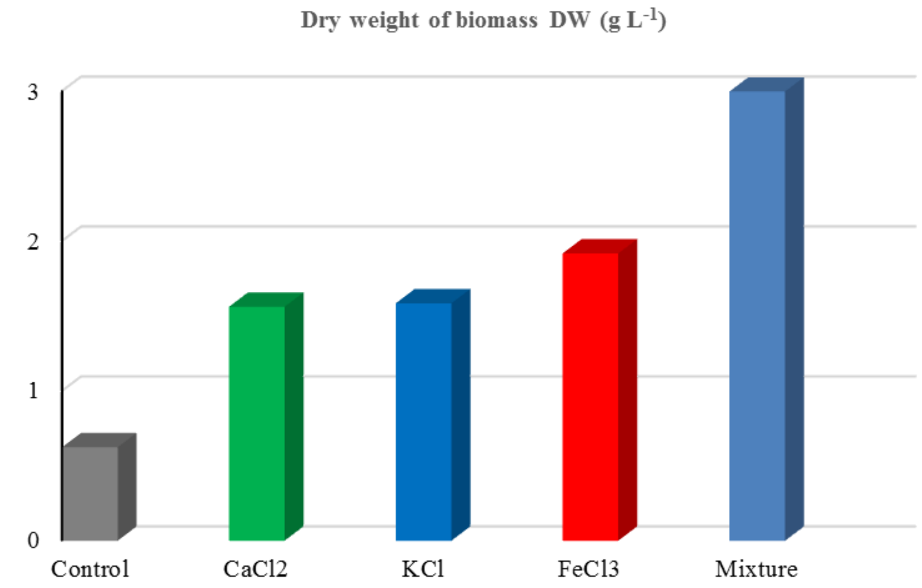

Figure 4 Weights of algal biomass after the addition of chemicals

3.2 Effects of chemical additives on the moisture content of algal biomass

Table 2 The moisture content of algal biomass after the addition of chemicals

Treatments	Replicates	Moisture (%)
	1	57.72
Control	2	77.30
	3	75.39
	1	60.54
$CaCl_2$	2	63.30
	3	62.52
	1	47.80
KCl	2	50.52
	3	51.34
	1	42.65
$FeCl_3$	2	43.39
	3	43.59
	1	43.14
Mixture	2	44.20
	3	43.59

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 mixtures of all additives as well as the ferric chloride delivered the lowest moisture content of the algal biomass and, however, the control delivered the highest moisture content.

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 (g/100g fresh weight) 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.

Table 3 Total lipid after the addition of chemicals

Treatments	Replicates	Total Lipid (g/100g)
Control	1	0.277
	2	0.331
	3	0.338
$CaCl_2$	1	0.464
	2	0.250
	3	0.274
KCl	1	0.523
	2	0.412
	3	0.516
$FeCl_3$	1	0.953
	2	0.873
	3	0.932
Mixture	1	1.429
	2	1.473
	3	1.421

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.

Table 4 Peroxide values after the addition of chemicals

Treatments	Replicates	Peroxide value (Millequivilent kg^{-1})
Control	1	0.280
	2	0.151
	3	0.165
$CaCl_2$	1	0.178
	2	0.140
	3	0.175
KCl	1	0.156
	2	0.186
	3	0.190
$FeCl_3$	1	0.217
	2	0.199
	3	0.212
Mixture	1	0.776
	2	0.758
	3	0.821

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 mixtures of all additives as well as the ferric chloride delivered the highest acid value and, however, the control delivered the lowest acid value.

Table 5 Acid values after the addition of chemicals

Treatments	Replicates	Acid Value (mg g^{-1})
Control	1	0.042
	2	0.534
	3	0.585
$CaCl_2$	1	0.618
	2	0.738
	3	0.754
KCl	1	0.727
	2	0.572
	3	0.716
$FeCl_3$	1	0.731
	2	0.704
	3	0.703
Mixture	1	0.721
	2	0.687
	3	0.721

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 mixtures of all additives as well as the ferric chloride delivered the highest biodiesel yield and, however, the control delivered the lowest biodiesel yield.

Table 6 Biodiesel yield after the addition of chemicals

Treatments	Replicates	Biodiesel (mg L^{-1})
Control	1	23.89
	2	13.15
	3	14.20
$CaCl_2$	1	34.55
	2	27.16
	3	25.80
KCl	1	36.37
	2	28.60
	3	35.88
$FeCl_3$	1	43.76
	2	40.11
	3	42.89
Mixture	1	67.82
	2	62.17
	3	66.48

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^{-1})	Final algal count (log10 cell mL^{-1})
	1	5.15	7.45
Control	2	5.15	7.44
	3	5.15	7.48
	1	5.15	7.84
$CaCl_2$	2	5.15	7.71
	3	5.15	7.90
	1	5.15	7.70
KCl	2	5.15	7.91
	3	5.15	8.05
	1	5.15	7.93
$FeCl_3$	2	5.15	8.04
	3	5.15	7.93
	1	5.15	11.50
Mixture	2	5.15	11.66
	3	5.15	11.50

4 Discussion

The findings reveal that iron content has a significant impact on algal growth and lipid production. This could be due to the fact that iron in the culture medium can alter the metabolic pathways involved in lipid production in microalgae (Ren et al., 2014). However, microalgae growth and lipid production are inhibited by extremely high or low iron concentrations in the culture medium.

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 Lam and Lee (2014) 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.

Calcium appears to play a crucial function in the growth of microalgae, according to the findings of this study. This could be explained by the fact that calcium deficiency prevents cell division, lowering cell concentration (Lam and Lee, 2014). As a result, calcium supplementation had a favorable impact on algal development.

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 (Abdelsalam et al., 2018; Abdelsalam and Samer, 2019; Abdelsalam et al., 2019a, 2019b; Abdelsalam et al., 2021; Attia et al., 2021; Hijazi et al., 2020a, 2020b; Samer et al., 2021, 2022b) but not yet in biodiesel production.

Another concern is the study's use of white LEDs, which generate the same amount of light as other energy sources but use less energy. Furthermore, the amount of heat generated during this operation is negligible, promoting energy conservation (Abdelsalam et al., 2018). As a result, LEDs have surpassed conventional light lamps in a variety of industries due to their low energy requirements, making them an environmentally friendly light source, according to Duarte and Costa (2018), and the use of LEDs in microalgal cultivation affects the quantity and quality of the biomass produced. This is due to the light's monochromaticity, which allows for effective regulation of photosynthetic photon flux density, a quality not found in sunshine, as Schulze et al. (2014) claim.

Future research will focus on the addition of 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 (Samer et al., 2021, 2022b; Ioannou-Ttofa et al., 2021; Samer et al., 2022a). An important future application is to develop an air purification system using algae to purify the exhaust air from industries, factories, and buildings (Samer et al., 2011; Samer, 2013; Samer and Abuarab, 2014; Samer et al., 2014) in order to replace current purification systems by an environmentally friendly algal purification system.

5 Conclusions

The effects of adding calcium chloride ($CaCl_2$) with a concentration of 2 mg L^{-1}, potassium chloride (KCl) with a concentration of 4.5 mg L^{-1}, and ferric chloride ($FeCl_3$) with a concentration of 1.2 mg L^{-1} were examined. Further treatment is a mixture of all additives with the same listed concentrations.

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

1. Iron concentration has a great influence on algal growth and lipid production.

2. Potassium addition has an important positive effect on the growth of microalgae.

3. Calcium plays an important role in the growth of microalgae.

4. The mixture of all additives yielded the highest lipid and, therefore, the highest biodiesel production among all treatments.

Future research will focus on the addition of 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. An important future application is to develop an air purification system using algae to purify the exhaust air from industries, factories, and buildings to replace current purification systems by an environmentally friendly algal purification system.

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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