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ORIGINAL ARTICLE

Effect of Mn^{2+}, Co^{2+} and H_2O_2 on biomass and lipids of the green microalga *Chlorella vulgaris* as a potential candidate for biodiesel production

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Abstract The present study examined the effect of heavy metals (Mn^{2+} and Co^{2+}) and hydrogen peroxide (H_2O_2) on biomass, lipid content and lipid productivity of *Chlorella vulgaris*. Manganese chloride at 2 μM, 10 μM and 12 μM increased the lipid content significantly by 14 %, 16 % and 15 %, respectively, above the corresponding controls after 8 days of incubation, resulting in a significant (18 %) increase in lipid productivity with respect to the control at 12 μM manganese chloride. All applied concentrations of cobalt nitrate increased the lipid content up to 25 % more than the corresponding controls. The optimum concentration of cobalt nitrate for high lipid productivity was 2.5 μM, which resulted in a 22 % increase in lipid productivity over the control. Furthermore, lipid productivity was increased significantly by 29 % over the control when 4 mM hydrogen peroxide was included in the culture medium. Additionally, the proportion of total saturated fatty acids extracted from *Chlorella vulgaris* treated with 12 μM manganese chloride, 2.5 μM cobalt nitrate and 4 mM hydrogen peroxide ranged between 40 % and 45 % of total fatty acids. The present study concluded that heavy metals and oxidative stress efficiently increased the lipid productivity of the promising biodiesel feedstock chlorophyte *Chlorella vulgaris*. In addition, the type and proportion of individual fatty acids meet the biodiesel standards.

Keywords Biodiesel · *Chlorella vulgaris* · Cobalt · Fatty acids · Hydrogen peroxide · Manganese

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Introduction

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure (Mata et al. 2010). Algal biomass can play an important role in solving the ethical problems in using human food for renewable energy production (Chaumont 1993; Nigam and Singh 2011). Algae, like plants, convert solar energy into chemical energy by photosynthesis and store this energy in the form of high energetic compounds such as carbohydrates, proteins and oils (Ozkurt 2009). Microalgae, due to their rapid biomass production and ability to store large amounts of lipids, are ideal sources for biodiesel production (Ozkurt 2009). Biodiesel, which is eco-friendly and renewable, is an alternative liquid fuel produced by a chemical reaction between plant oils or animal fats in the presence of a catalyst (Meher et al. 2006; Marchetti et al. 2007). Using of edible vegetable oils for fuel production competes with human food, and this would result in undesirable increase in food and biodiesel costs (Demirbas 2008). Many microalgal species have oil contents in the range of 20–50 % of their cellular dry weight (CDW), and may reach up to 90 % of dry weight under stress conditions (Spolaore et al. 2006; Chisti 2007). Chisti (2007) reported that the oil content of *Chlorella* sp. ranges between 28 % and 32 % of CDW. Abomohra et al. (2013) recorded biomass productivity

for *Chlorella vulgaris* of 0.16 g CDW L^{-1} day^{-1} and lipid productivity of 34 mg L^{-1} day^{-1}. Because of the relative high biomass and lipid productivities of *C. vulgaris*, it was selected for the present study. Lipid content, type of fatty acids and other biochemical compositions in algae can be controlled by altering the environmental growth conditions (Fábregas et al. 1995; El-Sheekh et al. 2013). One of these environmental growth conditions is nutrient limitation, which efficiently increases the lipid content of microalgae (Rodolfi et al. 2009).

Heavy metals are essential micronutrients for all biota (Lustigman et al. 1995) because they act as precursors of vitamins and as essential cofactors in metal enzymes (Munda and Hudnik 1988). Algae are able to tolerate certain concentrations of heavy metals through intracellular detoxification by binding with metal-binding peptides and proteins (Gekeler et al. 1988), binding and precipitation within the cytoplasm and/or vacuole (Reed and Gadd 1990; Chekroun and Baghour 2013) or by sequestration with electron-dense polyphosphate granules (Jensen et al. 1982; Jin-fen et al. 2000). In spite of this, high concentrations of heavy metals have an inhibitory effect on algal growth (Rachlin and Grosso 1993) and chlorophyll synthesis (El-Naggar et al. 1999; Küpper et al. 2002), as well as inducing changes in photosynthetic activity (Tiwari and Mohanty 1996; Küpper et al. 2002) and lipid peroxidation, which leads to disruption of membrane functions and harmful effects on plant cells (DeLong and Steffen 1997; Abo-Shady et al. 2008). Hydrogen peroxide (H_2O_2) is formed normally within the cell during photorespiration (Grant and Loake 2000). It is a strong oxidant that can initiate localized oxidative damage, leading to the disruption of metabolic functions and loss of cellular integrity at sites where it accumulates (Mallick and Mohn 2000). The present work was intended to study the effect of heavy metals, Co^{+2} and Mn^{+2}, and H_2O_2 on biomass and lipid productivity of *C. vulgaris* as a promising microalga for biodiesel production.

Materials and methods

Alga strain and growth conditions

The unicellular green microalga *C. vulgaris* was obtained from the Laboratory of Phycology, Faculty of Science, Zagazig University, Egypt. *Chlorella vulgaris* was grown axenically on bold basal medium (BBM) according to Nichols (1973). Under aseptic conditions, 100 mL BBM in 250-mL Erlenmeyer flasks was inoculated with 5 mL precultured *C. vulgaris*, then incubated under a photoperiod of 16:8 h light:dark regime at 25±2 °C with a light intensity of 5,000 lux. Cultures were illuminated by tubular fluorescent lamps (PHILIPS Master TL-D 85 W / 840) for 12 days.

BBM medium, containing 7 μM manganese chloride, 1.7 μM cobalt nitrate and devoid of hydrogen peroxide, was used as a control. The effect of different concentrations of manganese chloride (2 μM, 4 μM, 10 μM and 12 μM), cobalt nitrate (1 μM, 2 μM and 2.5 μM) and hydrogen peroxide (2 mM, 4 mM and 6 mM) on growth and lipid content were studied. Since the change in cobalt nitrate concentration also affects the nitrogen content of the medium, the final nitrate concentration of 2.2 mM in each culture was achieved using sodium nitrate. A certain volume of exponentially growing *C. vulgaris* cells was inoculated into 500 mL BBM medium in 1 L Erlenmeyer flasks at an initial OD_{450} of 0.25. Sterile filtered air enriched with 3 % (v/v) CO_2 was applied continuously into the cultures.

Biomass assay

Biomass and lipid content were measured every other day. Algal growth was monitored spectrophotometerically using the optical density at 450 nm (OD_{450}) according to Hsieh and Wu (2009) and by determination of algal cellular dry weight (CDW, g L^{-1}). Biomass productivity was calculated according to Abomohra et al. (2013),

Biomass productivity (g CDW $L^{-1}day^{-1}$)=$(CDW_L - CDW_E)/(t_L - t_E)$ with CDW_E and CDW_L representing the CDW (g L^{-1}) at days of early exponential phase (t_E) and late exponential phase (t_L), respectively.

Lipid extraction and determination

The lipid content of *C. vulgaris* was determined according to Sadasivam and Manickam (1996) by Soxhlet apparatus using *n*-hexane as an extraction solvent for 6 h under reflux. Lipid extracts were dried under a stream of argon. The pre-weighed glass vials containing lipid extracts were dried at 80 °C for 30 min, cooled in a desiccator and weighed; lipid content was determined as mg g^{-1} CDW. The total lipid productivity was calculated according to Abomohra et al. (2013)

Lipid productivity (mg L^{-1} day^{-1})=$(L_L - L_E)/(t_L - t_E)$ with L_E and L_L representing the total lipid content (mg L^{-1}) at days of early exponential phase (t_E) and late exponential phase (t_L), respectively.

Fatty acid analysis

The extracted lipids were saponified overnight with ethanolic KOH (20 %, w/v) at room temperature. Fatty acids were librated from their potassium salts by acidification with 5 *N* hydrochloric acid followed by extraction using petroleum ether at 40–60 °C. The ether extract containing fatty acid methyl esters was washed three times with distilled water and dried over anhydrous sodium sulfate (Vogel 1975). To determine the fatty acid profiles, 1 μL fatty acid methyl esters was injected into a column of internal diameter 6 feet×1/8 inch (182.88 cm × 0.3175 cm) packed with 20 % diethylene

glycol succinate (DEGS) on chromsorb 60–80 mesh using a Hewlett-Packard GC-MS device.

Statistical analysis

Results are presented as mean±standard deviation (SD) from three replicates. The statistical analyses were carried out using SAS (v 6.12, SAS, Cary, NC). Data obtained were analyzed statistically to determine the degree of significance using one way analysis of variance (ANOVA, $P \leq 0.05$).

Results

Figure 1 shows the effect of different concentrations [control (7 μM), 2 μM, 4 μM, 10 μM and 12 μM] of manganese chloride on the growth of *C. vulgaris* during 12 days of incubation. During the late exponential phase (after 8 days of incubation), the OD_{450} at 10 μM and 12 μM manganese chloride was increased by 2 % and 3 %, respectively, higher than the control. However, cultures treated with 2 μM and 4 μM manganese chloride showed a reduction in the OD_{450} by 15 % and 6 %, respectively. A concentration of 10 μM manganese chloride led to insignificant changes in biomass productivity of *C. vulgaris*, while 12 μM increased biomass productivity significantly to 3 % higher than the control (Table 1). On the other hand, the results in Table 1 reveal that there was no significant effect on lipid content at 4 μM manganese chloride. However, 2 μM, 10 μM and 12 μM caused increases of 14 %, 16 % and 15 %, respectively, in lipid content of *C. vulgaris* compared to the control after 8 days of incubation. The increase in lipid content at 10 μM and 12 μM manganese chloride led to a significant increase in lipid productivity, which rose to 18 % with respect to the control after 8 days of incubation (Table 1).

Table 1 Effect of different concentrations of manganese chloride on lipid content, biomass productivity and lipid productivity of *Chlorella vulgaris* after 8 days of incubation. Each value is the mean of three readings± standard deviation (SD). *CDW* Cell dry weight

Manganese chloride (μM)	Lipid content (mg g^{-1} CDW)	Biomass productivity (mg CDW L^{-1} day^{-1})	Lipid productivity (mg L^{-1} day^{-1})
Control (7 μM)	202.47±1.50	25.42±0.38	5.15±0.08
2	230.67±7.64*	21.62±0.25*	4.99±0.22 (ns)
4	202.17±2.57 (ns)	23.94±0.51*	4.84±0.09*
10	234.73±2.25*	25.87±0.21 (ns)	6.07±0.03*
12	231.97±0.06*	26.16±0.11*	6.07±0.03*

* Significant at $P \leq 0.05$ using one way analysis of variance (ANOVA), *ns* non significant at $P \leq 0.05$ using one way analysis of variance (ANOVA)

Treating *C. vulgaris* with 2 μM cobalt nitrate resulted in stimulation of growth to 4 % higher than the corresponding control after 8 days of incubation (Fig. 2), while treatment with 1 μM and 3 μM resulted in 10 % and 18 % decreases in growth, respectively, compared to the corresponding control. The data in Table 2 show that biosynthesis and accumulation of lipids increased significantly at different concentrations of cobalt nitrate. After 8 days of incubation with 1 μM, 2 μM, 2.5 μM and 3 μM cobalt nitrate, the lipid content increased by 7 %, 13 %, 21 % and 25 %, respectively, compared with the corresponding control. Consequently, lipid productivity also increased by 18 % and 22 % higher than the control at 2 μM and 2.5 μM cobalt nitrate, respectively (Table 2).

Regarding the effect of hydrogen peroxide, Fig. 3 shows that growth of *C. vulgaris* decreased upon treatment with different concentrations of hydrogen peroxide up to 4 mM, while treatment of *C. vulgaris* with 6 mM hydrogen peroxide caused a lethal effect and considerable suppression of algal growth. After 8 days of treating *C. vulgaris* with 2 mM and

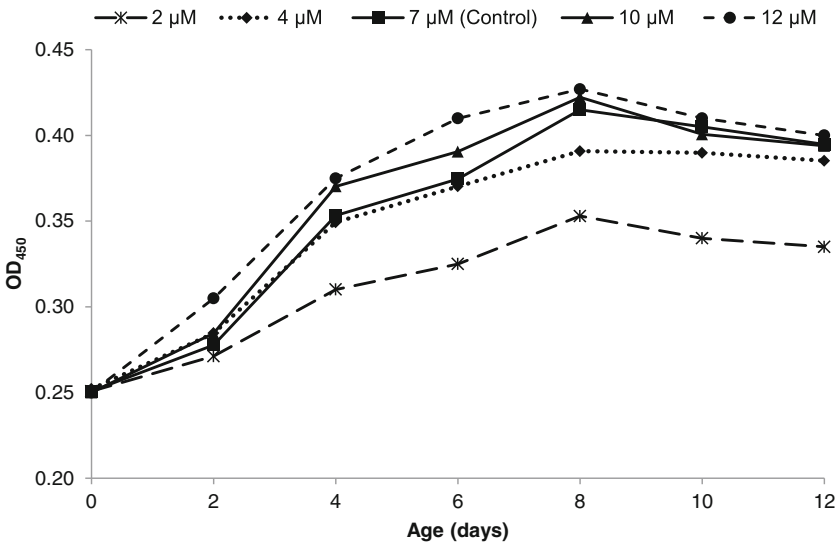

Fig. 1 Effect of different concentrations of manganese chloride on growth of *Chlorella vulgaris* cultivated for 12 days

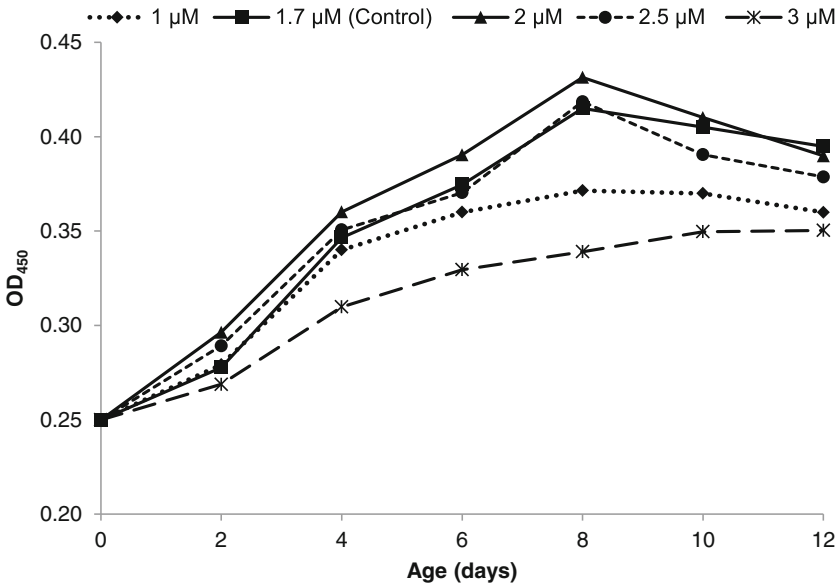

Fig. 2 Effect of different concentrations of cobalt nitrate on growth of *C. vulgaris* cultivated for 12 days

4 mM hydrogen peroxide, growth was inhibited by 15 % and 31 %, respectively, as compared with the corresponding control (contains no hydrogen peroxide). Interestingly, analysis of the impact of different concentrations of hydrogen peroxide on the lipid content of *C. vulgaris* revealed that the biosynthesis and accumulation of lipids was increased with increasing concentrations of hydrogen peroxide up to 4 mM (Table 3). After 8 days of treating *C. vulgaris* with 2 mM and 4 mM hydrogen peroxide, the lipid content increased by 20 % and 87 %, respectively, compared to the control. Consequently, lipid productivity increased significantly by 29 % at 4 mM of hydrogen peroxide after 8 days of incubation (Table 3). The obtained data manifested that among the used treatments, 12 μM of manganese chloride, 2.5 μM of cobalt nitrate and 4 mM of hydrogen peroxide considered as feasible conditions for the over-production of lipids of *C. vulgaris*, where it maximally recorded 12 %, 22 % and 29 %, respectively, increase over the corresponding controls.

Table 2 Effect of different concentrations of cobalt nitrate on lipid content, biomass productivity and lipid productivity of *C. vulgaris* after 8 days of incubation. Each value is the mean of three readings±SD

Cobalt nitrate (μM)	Lipid content (mg g^{-1} CDW)	Biomass productivity (mg CDW L^{-1} day^{-1})	Lipid productivity (mg L^{-1} day^{-1})
Control (1.7 μM)	202.47±2.50	25.42±0.38	5.15±0.12
1	216.37±3.99*	22.75±0.33*	4.92±0.02 (ns)
2	229.37±1.52*	26.43±0.10*	6.06±0.05*
2.5	244.33±3.79*	25.64±0.50 (ns)	6.26±0.06*
3	253.03±3.59*	20.77±0.56*	5.26±0.20 (ns)

* Significant at $P \leq 0.05$ using one way analysis of variance (ANOVA), *ns* non significant at $P \leq 0.05$ using one way ANOVA

The relative proportions of fatty acids in *C. vulgaris* treated with 12 μM manganese chloride, 2.5 μM cobalt nitrate and 4 mM hydrogen peroxide are presented in Table 4. The data revealed that high heavy metal stress (Mn^{2+} and Co^{2+}) and the oxidative stress of hydrogen peroxide led to a great variation in fatty acid composition of *C. vulgaris*. In general, 13 fatty acids ranging from capric acid (C10:0) to lignoceric acid (C24:0) were identified. The dominant fatty acids recorded in the control culture of *C. vulgaris* were myristic (C14:0), palmitic (C16:0), oleic (C18:1) and linoleic (C18:2). Treating of *C. vulgaris* with 12 μM manganese chloride and 2.5 μM cobalt nitrate resulted in a significant decrease in total saturated fatty acids (SFAs) to 15 % and 19 %, respectively, lower than their corresponding control with a noticeable increase in unsaturated fatty acids (UFAs). In contrast, treating with 4 mM hydrogen peroxide showed insignificant (one way ANOVA, $P \leq 0.05$) changes in SFAs and UFAs with respect to the control. The reduction in SFAs was due mainly to the inhibition of lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) anabolic pathways, whereas the increase in UFAs was due mainly to the stimulation of oleic (C18:1) and linoleic (C18:2) production. Also, it was found that all treatments applied in the present work led to the formation of lignoceric (C24:0), which was not recorded in the lipid profile of the corresponding controls.

Discussion

In general, the results obtained showed that changes in the concentrations of heavy metals and addition of hydrogen peroxide to the medium resulted in consequent changes in the growth and lipid content of *C. vulgaris*. The results obtained showed that 12 μM manganese chloride enhanced the

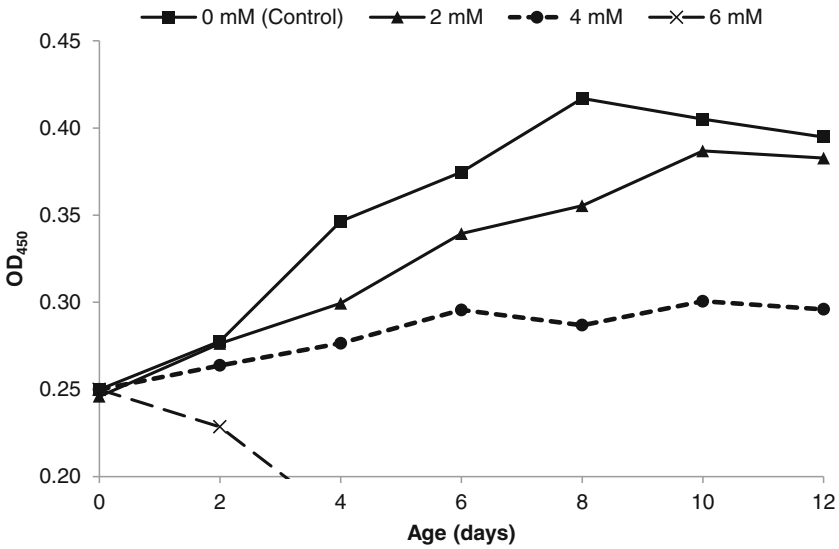

Fig. 3 Effect of different concentrations of hydrogen peroxide on growth of *C. vulgaris* cultivated for 12 days

growth of *C. vulgaris*, while Mn^{2+} deficiency resulted in growth inhibition. This finding is in agreement with those of Reisner and Thompson (1956) who found that in the absence of Mn^{2+} there was no measurable growth of *C. vulgaris*, and Huntsman and Sunda (1980) who reported that the growth rate of marine phytoplankton was inhibited under Mn^{2+} deficiency. In addition, Pirson and Bergmann (1955) recorded a 50 % reduction of growth in Mn^{2+}-deprived cultures. Growth enhancement at high Mn^{2+} supplemented media might be explained by the importance of manganese as an essential constituent of a number of metalloenzymes, proteins and vitamins that perform key roles in algal metabolism (Coates et al. 1972; Clarkson and Hanson 1980; Burnell 1988), while manganese deprivation in algae causes chlorosis and interferes with the maintenance of the chloroplast membrane structure and with electron transport of photosystem II (Rains 1976). Regarding the increase in lipid content at high concentrations of manganese chloride, our results are in agreement with those of Pirson et al. (1952) who reported that Mn^{2+} salts has an inhibitory effect on photosynthesis of algae and hence stimulates the storage of lipids in favor of carbohydrates. The recorded increase in growth and lipid content at high manganese chloride concentrations (12 μM) increased lipid productivity by 18 % over the corresponding control after 8 days of incubation.

Table 3 Effect of different concentrations of hydrogen peroxide on lipid content, biomass productivity and lipid productivity of *C. vulgaris* after 8 days of incubation. Each value is the mean of three readings±SD

Hydrogen peroxide (mM)	Lipid content (mg g^{-1} CDW)	Biomass productivity (mg CDW L^{-1} day^{-1})	Lipid productivity (mg L^{-1} day^{-1})
Control (0 mM)	202.47±3.50	25.54±0.56	5.17±0.10
2	243.77±3.75*	21.77±0.49*	5.31±0.18 (ns)
4	379.00±1.00*	17.57±0.58*	6.66±0.24*
6	0*	0*	0*

* Significant at $P \leq 0.05$ using one way analysis of variance (ANOVA), *ns* non significant at $P \leq 0.05$ using one way ANOVA

As indicated from the present results, different concentrations of cobalt nitrate affected both growth and lipid content of *C. vulgaris*. An increase in cobalt nitrate concentration to 2 μM resulted in enhancement of growth to 4 % higher than the control. Furthermore, an increase in cobalt nitrate concentration to 3 μM caused an increase in lipid content of *C. vulgaris* by 27 % over the control. These results are in agreement with those obtained by Rachlin and Grosso (1993) who found that *C. vulgaris* has a great resistance to Co^{2+}, and that toxic effects of Co^{2+} were recorded at very high concentrations. Price and Morel (1990) concluded that the growth promotion at low Co^{2+} concentrations may be due to Co^{2+} substitution for Zn^{2+} in some metalloenzymes. On the other hand, the toxic effect at high Co^{2+} concentrations was attributed to retardation of plasma membrane function by interacting with sulfhydryl groups on proteinaceous membranes to produce S–metal–S bridges that can alter membrane permeability (Rachlin and Grosso 1993; Afkar et al. 2010). In agreement with our results, Chia et al. (2013) recorded an increase in total lipid concentration with a decrease in growth rate of *C. vulgaris* under cadmium stress. The increase in lipid content of *C. vulgaris* at high concentrations of Co^{2+} might be attributed to the disruption of algal metabolism by inactivation of the photosynthetic machinery, which results in the formation of lipids as storage compounds in favor of carbohydrates (Mallick and Rai 1992; Rai et al. 1998; Bellou and Aggelis 2012). Shifting between pools of sugars and lipids has been studied recently; Li et al. (2011b) suggested that the oleaginous green microalga *Pseudochlorococcum* sp. uses starch as a primary carbon and energy storage product but when

Table 4 Fatty acid profiles of *C. vulgaris* treated with the optimum concentrations of manganese chloride, cobalt nitrate and hydrogen peroxide for maximum lipid productivity. Fatty acids were measured after 8 days of incubation. Values are given as percent (%) of total fatty acids. Each value is the mean of three replicates±standard deviation. *SFA* Saturated fatty acid, *UFA* unsaturated fatty acid

Fatty acids	Control	Manganese chloride (12 μM)	Cobalt nitrate (2.5 μM)	Hydrogen peroxide (4 mM)
Capric (C10:0)	1.38±0.09	0.64±0.05	nd	2.58±0.18
Lauric (C12:0)	4.67±0.61	nd[a]	nd	nd
Myristic (C14:0)	14.57±0.91	2.86±0.06	0.99±0.03	4.51±0.29
Palmitic (C16:0)	19.83±1.15	14.89±0.75	8.09±0.96	10.5±0.99
Palmitoleic (C16:1)	7.57±0.49	0.18±0.05	nd	1.59±0.43
Hexadecadienoic (C16:2)	2.79±0.28	2.89±0.12	1.04±0.05	1.36±0.09
Stearic (C18:0)	5.54±0.22	1.43±0.51	3.33±0.23	4.13±0.28
Oleic (C18:1)	10.69±0.64	23.26±1.02	17.44±0.65	16.25±0.76
Linoleic (C18:2)	17.66±0.77	27.09±0.52	19.95±0.78	19.31±0.66
α-Linolenic (C18:3)	12.24±0.66	4.7±0.32	22.07±0.53	16.15±0.69
Arachidic (C20:0)	1.57±0.14	9.05±0.23	0.39±0.10	0.48±0.36
Behenic (C22:0)	1.49±0.12	2.67±0.12	0.66±0.21	nd
Lignoceric (C24:0)	nd	10.34±0.35	26.04±0.92	23.14±0.44
SFAs	49.05±3.24	41.88±2.07*	39.50±2.45*	45.34±2.54 ns
UFAs	50.95±2.84	58.12±2.03*	60.50±2.01*	54.66±2.63 ns

[a] Not detected

* Significant difference with respect to the corresponding control, $P \leq 0.05$; *ns* non significant at $P \leq 0.05$ using one way ANOVA

nitrogen was depleted, cells shift into neutral lipid formation as a secondary storage product. The increase in lipid content and the insignificant effect on growth at 2.5 μM cobalt nitrate resulted in maximum lipid productivity (6.26 mg L^{-1} day^{-1}).

The present study showed that hydrogen peroxide significantly affects growth and lipid content of *C. vulgaris*. Using 2 mM and 4 mM hydrogen peroxide showed inhibition of growth with synchronous stimulation of lipid production, while 6 mM hydrogen peroxide had a lethal effect on *C. vulgaris* cells. These results are in agreement with those of Drábková et al. (2007) and Barrington and Ghadouani (2008), who reported that hydrogen peroxide is a strong oxidizing agent that is considered an effective inhibitor of microalgal growth. The toxicity of hydrogen peroxide for algal growth arises from its conversion into extremely reactive hydroxyl radicals (˙OH), which cause severe damage to membranes, proteins, and DNA (Halliwell and Gutteridge 1984; Abo-Shady et al. 2008). Stimulation of lipid production in *C. vulgaris* under hydrogen peroxide stress might be explained by the findings of Hu et al. (2008) and Li et al. (2011a), who demonstrated that microalgae can change their biosynthetic pathways towards the formation and accumulation of lipids under stress conditions that then serve as energy storage rather than for the formation of structural compounds. Sakthivel et al. (2011) mentioned that many microalgae have the ability to produce substantial amounts of triacylglycerols as a storage lipid under oxidative stress. Skerratt et al. (1998) stated that exposure of *Phaeocystis antarctica* to high UV-B irradiation results in oxidative stress and increases the concentration of total lipids, triacylglycerols and free fatty acids. Although growth was inhibited in the presence of 4 mM hydrogen peroxide, lipid productivity was significantly increased by 29 % over the control due to the accumulation of lipids.

The properties of biodiesel are determined mainly by its fatty acid methyl esters (Knothe 2005; Yoo et al. 2010). Damiani et al. (2010) reported that not all oils extracted from algae are suitable or compatible for use as biodiesel. UFAs with four or more double bonds are susceptible to oxidation during storage, thus reduce the acceptability of microalgal oil for biodiesel production (EN 14214 2003; Chisti 2007). Biodiesel quality is related directly to cetane number, which determines ignition quality in engines (Gerpen 2009). Our results revealed that the SFA content at 12 μM manganese chloride, 2.5 μM cobalt nitrate and 4 mM hydrogen peroxide was recorded as 41.88 %, 39.50 % and 45.34 % of total fatty acids, respectively. Moreover, lipids extracted from *C. vulgaris* presented linoleic acid (C18:2) contents within European standard specifications (≤ 12 %, EN 14214 2003), thus highlighting the oxidative stability of *C. vulgaris* biodiesel.

Conclusion

The results of the present study suggested that Mn^{2+}, Co^{2+} and H_2O_2 stresses are an effective approach to enhance lipid production by *C. vulgaris*. Despite inhibition of growth by most treatments, the increase in lipid content led to an increase in lipid productivity up to 29 % higher than the control. Although heavy metals and oxidative stress resulted in a decrease of SFAs proportion by 8–19 % compared to the control, the proportion of SFAs in the treated cultures (40–45 % of the total fatty acids) was still in the range specified for biodiesel standards. This study nominates *C. vulgaris* as an

eligible microalga for further research as a feedstock for biodiesel production. Measurements of other parameters, including density, viscosity, flash point, cold filter plugging point and saponification value will be important to determine the suitability of *C. vulgaris* lipids as a feedstock for biodiesel.

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