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Research Article

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Interactions Effects between Thiamine and Heavy Metals on Biodiesel Production

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Abstract The date of this study, growth parameters (Dry weight, optical density and total photosynthetic pigments), total carbohydrates, total proteins and total lipid contents of stressed *Chlamydomonas reinhardtii* cultures were followed for 7 days. The growth parameters (Dry weight, optical density and total photosynthetic pigments), total carbohydrates and total proteins contents of *Chlamydomonas reinhardtii* cultures were significantly increased, when the algal cultures subjected to lower and moderate concentrations of $CoCl_2$. But, under relatively higher concentration of $CoCl_2$ all these parameters were significantly decreased. On the other hand the total lipid contents were significantly increased when the algal cultures subjected to lower concentrations of $CoCl_2$. Under moderate and higher concentration the total lipid contents were significantly decreased. When the stressed *Chlamydomonas reinhardtii* cultures treated with 200 ppm thiamine ($V.B_1$), Dry weight, optical density and total photosynthetic pigments), total carbohydrates, total proteins and total lipid contents of *Chlamydomonas reinhardtii* cultures were significantly increased, when the algal cultures subjected to lower and higher concentrations of $CoCl_2$.

Keywords thiamine, *Chlamydomonas reinhardtii*, pigments, total proteins, total lipid contents, algal cultures tance

1. Introduction

Heavy metals are prevalent in municipal and industrial effluents; they modify the structure and productivity of aquatic ecosystems [13]. As indicated by [6], the concentration 5 x 10^{-6} – 10^{-5} mol.L^{-1} Co^{2+} exerted maximal stimulatory effect on *Chlorella pyrenoidosa* cells at the exponential growth phase in terms of fresh weight (150-160 % increase), dry weight (50-60 % increase), chlorophylls a and b (45-65 % increase), total carotenoids (55-65 % increase), water-soluble proteins (19-20 % increase) and mono saccharides content (55-60 % increase), when compared to the control culture. [12] Studied the effect of Co^{2+} on *Chlamydomonas reinhardtii*. They observed reduction of growth at 10 ppm Co^{2+} and without change in the morphology of the cells or pH. At 20 ppm Co^{2+}, on the other hand, growth was considerably reduced compared to the control and the color of the organism became paler and the cells clumped. The lipid present in microalgae is mainly in the form of esters of glycerol and fatty acid, which are suitable for producing biodiesel [5] This investigation elucidates the stressing effects of cobalt and enhancing effects of thiamine upon the growth criteria and some metabolic activities of *Chlamydomonas reinhardtii* as a unicellular chlorophyta for enhancing the lipid contents as indicator on biodiesel production.

2. Materials and Methods

The microalgae species used in this study (*Clamydomonas reinhardtii*), were collected from culture collection of Algal and Plant Physiology Laboratory, Faculty of Science, Al-Azhar University, Assiut, Egypt. Culture medium BG11 nutritive culture was used as a medium for enrichment and growth of the tested algae, [17].

Treatments: *Clamydomonas reinhardtii* was subjected to 00 (control) and various concentrations (3, 6, 9 and 12ppm) of $CoCl_2$ and the same concentrations with 200 ppm of $V.B_1$ for 7 days were followed.

2.1. Analytical Methods

2.1.1. Determination of dry weight

A definite volume (100 mls.) of alga suspension was filtered through weighed glass fiber filter. The cells after being precipitated on the filter were washed twice with distilled water and dried overnight in an oven at 105 °C. The data were expressed as µg ml^{-1} algal suspension.

2.1.2. Determination of Optical Density (OD)

The cell concentration (Optical density) was determined by the method of measuring OD at 680 nm [15]. The data were calculated (g L^{-1} algal suspension).

2.1.3. Determination of total photosynthetic pigments

The pigment fractions (µg $m1^{-1}$ algal suspension) chlorophyll a, chlorophyll b and carotenoids extracted by 100 % acetone were calculated using the equations [11]: -

Chlorophyll a = 11.75 A_{662} - 2.350 A_{645}

Chlorophyll b = 18.61 A_{645} - 3.960 A_{662}

Carotenoids = 1000 A_{470} - 2.270 Chloro.a - 81.4 Chloro.b / 227

2.1.4. Determination of total carbohydrates

Using of anthrone-sulphoric acid reagent according to the method by [2] the data measured as µg mg^{-1} dry weight.

2.1.5. Determination of Proteins

Using Bradford reagent according the method adapted by [4] and [19] .The data were measured as µg mg^{-1} dry weight.

2.1.6. Determination of lipid contents

The lipid consents were determined according method by [3]. The data were measured as µg mg^{-1} dry weight.

2.1.7. Statistical Analysis

Four replicates were used in this study and the data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to [16].

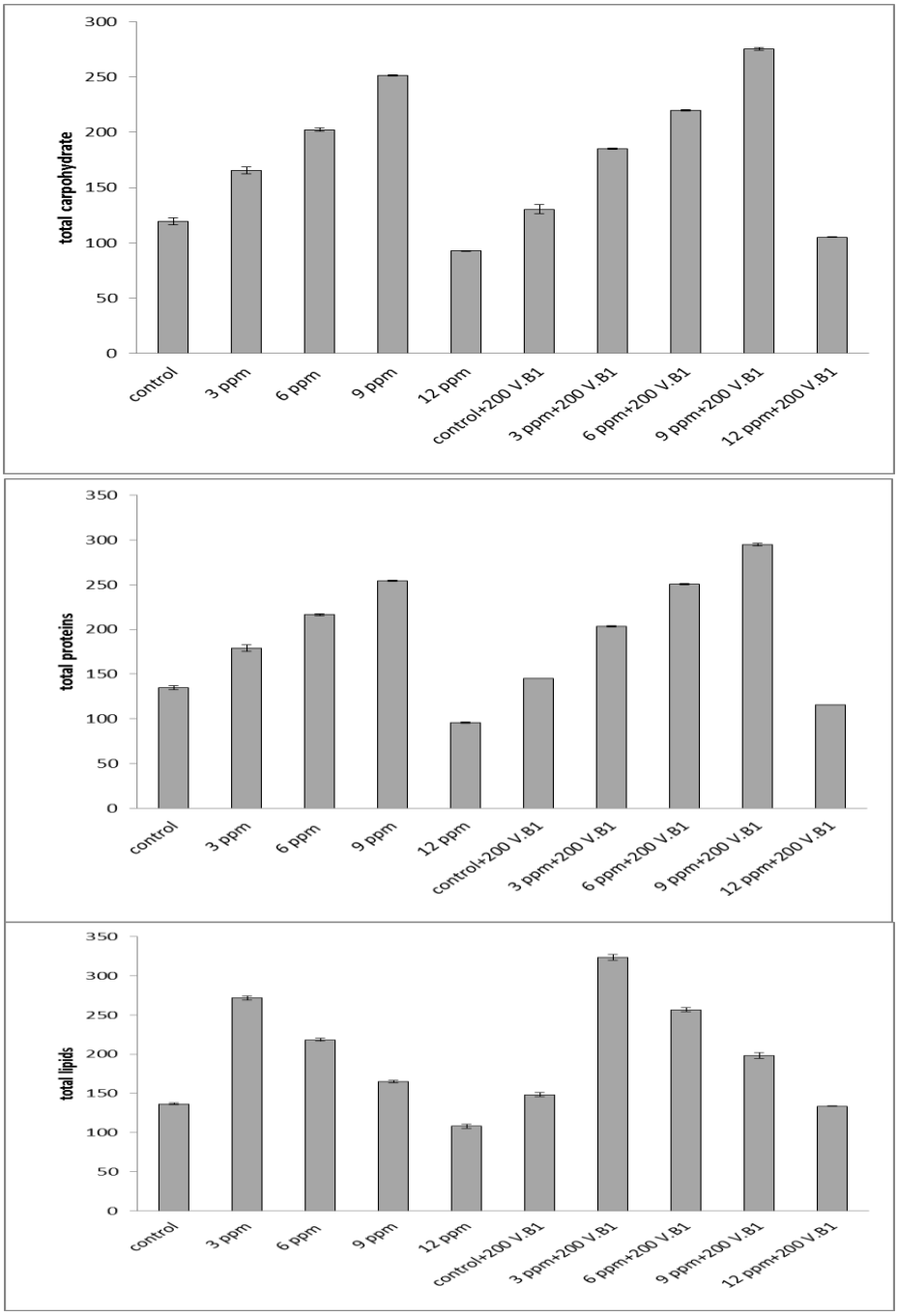
3. Results and Discussions

The date present in this investigation showed the interface effects of cobalt on growth parameters (dry weight, optical density, and total photosynthetic pigments), primary products (total carbohydrate, total protein, and total lipid contents) of *Chlamydomonas reinhardtii* cultures for 7 days. In this study, the growth criteria (dry weight, optical density and total photosynthetic pigments) of *Chlamydomonas reinhardtii* cultures were markedly increased up to level 9ppm of $CoCl_2$. However, under higher relatively concentration 12ppm of $CoCl_2$, all these parameters was markedly decreased, when compared with that of the control cultures. The maximum value of dry weight, of *Chlamydomonas reinhardtii* cultures was 136%, when the algal cultures subjected to 9 ppm $CoCl_2$ only, as compared with that the control cultures. When the stressed *Chlamydomonas reinhardtii* cultures treated with 200 (ppm) of thiamin the maximum value of dry weight reached to 143%, as compared with that the control cultures. The minimum value of dry weight, of *Chlamydomonas reinhardtii* cultures amounted to 88%, when the algal cultures subjected 12 ppm of $CoCl_2$ only, when compared with that the control cultures. But, the minimum value of dry weight, of stressed *Chlamydomonas reinhardtii* cultures was 95% of that the control cultures, when the algal cultures subjected 12 ppm of $CoCl_2$ and treated with 200 (ppm) of thiamin, as compared with the control cultures (Table1-a). the maximum value of optical density of *Chlamydomonas reinhardtii* was 118%, when the algal cultures subjected to the moderate concentration 9 ppm of $CoCl_2$ only, as compared with that the control cultures .When the stressed *Chlamydomonas reinhardtii* cultures treated with 200 (ppm) of thiamin the maximum value of optical density reached to 142%, when compared with that the control cultures. The minimum value of optical density, of *Chlamydomonas reinhardtii* cultures amounted to 62%, when the algal cultures subjected to higher concentration of 12 ppm of $CoCl_2$ only, when compared with that the control cultures. But, the minimum value of optical density of *Chlamydomonas reinhardtii* cultures was 69% of that the control cultures, when the algal cultures subjected to 12 ppm $CoCl_2$ and treated with 200 (ppm)

of thiamin as compare with the control cultures (Table1-b) The maximum value of the total pigments of *Chlamydomonas reinhardtii* reached to 135%, when the algal cultures subject to the moderate level of 9 ppm $CoCl_2$, only as compare with the control cultures. the maximum value of the total pigments of stressed *Chlamydomonas reinhardtii* reached to 198%, when the algal cultures subject to the moderate level of 9 ppm $CoCl_2$ and treated with 200 (ppm) of thiamin, as compare with the control cultures The minimum values of total pigments amounted to 66% of that the control cultures, when the algal cultures subject to 12 ppm $CoCl_2$ 0nly. Also, the minimum value of the total pigments of stressed *Chlamydomonas reinhardtii* was 79% of the that control cultures, when the algal cultures subject to the higher concentration of 12 ppm $CoCl_2$ and treated with 200 (ppm) of thiamin, as compare with the control cultures (Table1-c). The maximum values of total carbohydrate contents of *Chlamydomonas reinhardtii* amounted to 210%, of that the control cultures, when the algal cultures subjected to moderate concentration of 9 ppm $CoCl_2$ only. On other hand, maximum values of the total carbohydrate contents of *Chlamydomonas reinhardtii* treated with (200 ppm) thiamin were 230%, of that the control cultures, when the algal cultures subjected moderate concentration 9 ppm $CoCl_2$ and treated with 200 (ppm) of thiamin. The minimum values of total carbohydrate contents of *Chlamydomonas reinhardtii* were 78%, of that the control cultures, when the algal cultures subject to 12 ppm $CoCl_2$ only as compared with that the control cultures. Also, The minimum values of total carbohydrate contents of *Chlamydomonas reinhardtii* were 88% of that the control cultures, when cultures, when the algal cultures subject to 12 ppm $CoCl_2$, and treated with 200 (ppm) of thiamin (Fig. 1-a) the maximum values of the total lipid contents of *Chlamydomonas reinhardtii* reached to 199%, of that the control cultures, when the algal cultures subjected to lower concentration of 3 ppm $CoCl_2$ only. On the other hand, maximum values of the total lipid contents of stressed *Chlamydomonas reinhardtii* were 237%, of that the control cultures, when the algal cultures subjected lower concentration 3 ppm $CoCl_2$ and treated with 200 (ppm) thiamin (Fig. 1-b) the minimum values of total lipid contents of *Chlamydomonas reinhardtii* were 79%, of that the control cultures, when the algal cultures subject to 12 ppm $CoCl_2$ only as compared with that the control cultures. also, The minimum values of total lipids contents of stressed *Chlamydomonas reinhardtii* were 98% of that the control cultures, when the algal cultures subject to 12 ppm $CoCl_2$ and treated with 200 (ppm) 0f thiamin ($V.B_1$) (Fig. 1-b) the maximum values of the total protein contents of *Chlamydomonas reinhardtii* reached to 189 %, of that the control cultures, when the algal cultures subjected to moderate concentration of 9 ppm $CoCl_2$ only. on other hand, maximum values of the total protein contents of stressed *Chlamydomonas reinhardtii* were 219%, of that the control cultures, when the algal cultures subjected moderate concentration 9 ppm $CoCl_2$ and treated with 200 (ppm) of thiamin ($V.B_1$). The minimum values of total protein contents of *Chlamydomonas reinhardtii* were 71%, of that the control cultures, when the algal cultures subject to 12 ppm $CoCl_2$, only, as compared with that the control cultures. Also, The minimum values of total protein contents of stressed *Chlamydomonas reinhardtii* were 86% of that the control cultures, when cultures, when the algal cultures subject to 12 ppm $CoCl_2$, and treated with 200 (ppm) of thiamin ($V.B_1$) (Fig. 1-c)

Treatments	**Growth parameters of *Chlamydomonas reinhardtii***					
	Dry weight (µg ml^{-1})	**%**	**Optical density(g l^{-})**	**%**	**Total pigment (µgmg^{-1})**	**%**
Control	580 ± 3.33	100.0	0.783±0.001	100.0	8.042±0.029	100.0
3 ppm $CoCl_2$	696± 13.5	114.9	0.856±0.004	109.4	9.600±0.067	119.4
6 ppm $CoCl_2$	770± 3.33	132.7	0.886±0.004	113.2	10.45±0.026	130.0
9 ppm $CoCl_2$	793± 2.54	136.7	0.927±0.006	118.4	11.57± 0.048	134.9
12 ppm $CoCl_2$	513 ± 3.46	88.50	0.492±0.007	62.85	5.300±0.034	65.90
Control+ thiamin	596± 1.92	102.8	0.859± 0.029	109.7	10.69± 0.066	133.0
3 ppm $CoCl_2$ + thiamin	758± 2.54	130.7	1.008± 0.049	128.8	11.95± 0.064	148.6
6 ppm $CoCl_2$ + thiamin	800± 2.88	137.9	1.087± 0.004	138.7	14.24± 0.164	177.1
9 ppm $CoCl_2$ + thiamin	826± 3.46	142.5	1.113± 0.001	142.0	15.94± 0.035	198.2

12 ppm $CoCl_2$ + thiamin	553± 0.96	95.40	0.544± 0.001	69.40	6.323± 0.051	78.63
LSD=0.05	n.s		n.s		0.109	



Figuer 1: (a) Total carbohydrates (µg mg^{-1} dry weight), (b) Total lipids (µg mg^{-1} dry weight), (c) Total proteins (µg mg^{-1} dry weight), of Chlamydomonas reinhardtii cultures were subjected to various concentrations of $CoCl_2$ (ppm) and treated with (200) ppm of thiamin (vitamin B_1) for 7 days.

4. Conclusions

Adverse effect of heavy metals on green algae may lead to disturbances in plant metabolism which consequently lead to a reduction in the growth of these algae [1]. Many trials have been to help the plants (higher plants and

green algae) these disturbances using variable treatments in the laboratory aiming to be applied in the future in the field. The present results are in agreement with those obtained by [12] who reported that 10 and 20 ppm Co^{2+} resulted in partial inhibition of growth of *C. reinhardtii*, while concentrations of 30 ppm or higher completely prevented algal growth. In addition, [9] found that a lower Co^{2+} concentration (0.01 ppm) stimulated growth of *Nostoc muscorum*, while it showed a non-significant effect on *Calothrix fusca* growth. However, higher Co^{2+} concentrations were inhibitory for both organisms. On the other hand, growth promotion at low Co^{2+} concentrations may be due to Co^{2+} substitution for Zn^{2+} in some metalloenzymes *in vitro* and *in vivo* as reported by [14]. This study also detected the effect of $CoCl_2$ on growth criteria (optical density and total pigments), total carbohydrate, total protein and total lipid contents of *C. reinhardtii* and *C.reinhardtii* treated with thiamin (vitamin B_1) cultured for 7 days was as follwing and the present results are in agreement with those obtained by [12]. The growth parameters of *C. reinhardtii* and *C.reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to (3, 6 and 9 ppm $CoCl_2$). There above all these parameters were significantly decreased. the total carbohydrate contents of *C. reinhardtii* and *C. reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to lower and moderate concentrations (3, 6 and 9 ppm $HgCl_2$), but under relatively higher concentration (12 ppm $CoCl_2$) these parameters were significantly decreased. The total protein contents of *C.reinhardtii* and *C. reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to lower and moderate concentrations (3, 6 and 9 ppm $CoCl_2$), but under relatively higher concentration (12 ppm $CoCl_2$) these parameters were significantly decreased. The changes in the lipid contents of some plants types have vigorously been elevated with the rise of stress levels. Thus, the total lipid content of *C. reinhardtii* and *C. reinhardtii* treated with thiamin (vitamin B_1) were significantly increased, when the algal cultures subjected to lower concentration (3 ppm of $CoCl_2$), but under relatively highest concentrations (6, 9 and 12 ppm $CoCl_2$) these parameters were significantly decreased These results were in agreement with the results [7, 8, 10, 18] working with some plant types have suggested that the lipid contents were decreased with the rise of stress level.

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