Journal of Chemical Health Risks

Journal of Chemical Health Risks (2017) 7(1), 61–69

ORIGINAL ARTICLE

The Effect of Aluminium on Antibacterial Properties and the Content of Some Fatty Acids in Microalgae, *Chlorella vulgaris* Beijernick, under Heterotrophic and Autotrophic Conditions

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(Received: 21 June 2016 Accepted: 20 August 2016)

KEYWORDS

Antibacterial; Autotrophic; Fatty acids; Green algae *Chlorella vulgaris* Beijernick; Heterotrophic

ABSTRACT: Microalgae are a group of organisms, which have a significant potential for industrial applications. These algae contain large amounts of lipids compounds that are beneficial to health, have antibacterial properties, and their extracted oil can be used for biofuel. In this study, microalgae *Chlorella vulgaris* Beijernick was grown in the culture medium BG-11 containing aluminium ($AlCl_3$) under autotrophic and heterotrophic conditions. In each case, survival and growth, dry weight, internal aluminium content of the sample, antibacterial properties, the content of fatty acids accumulated in the algae and secreted into the culture medium in the logarithmic growth phase were studied. Aluminium significantly increased ($P < .05$) growth and dry weight in autotrophic treatment compared to the heterotrophic one. Most antibacterial properties were observed in methanol extracts of heterotrophic treatments containing 0.05% glucose. Aluminium also decreased fatty acids accumulation in the algae and increased fatty acids excretion into the culture medium in heterotrophic treatment compared to the autotrophic treatment. Survival of the sample was maintained in heterotrophic conditions and showed growth without lag phase, which is indicative of rapid acclimation of organisms in heterotrophic conditions. It seems that the mentioned characteristics make the single-celled green algae *Chlorella vulgaris* more efficient in different ways.

INTRODUCTION

Microalgae are a group of organisms, used as a source of protein and fat for hundreds of years [1]. Microalgae, which are at the lowest level of the nutritive cycle in the aquatic ecosystems, are considered as the first determinant of the quality of nutrition. Since they are the main natural resources, are essential for the synthesis of fatty acids. Due to the use of fatty acids in health and nutrition, the importance of analyzing fatty acids has been doubled. Many research studies have been conducted on the

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nutritive and medical values of *Chlorella vulgaris*.Unsaturated fatty acids, especially linoleic acid and α- linolenic acid, omega-6 and omega-3, which is a combination of creative, human cells, have a key role in many metabolic processes, since there is no mechanism in the body for the synthesis of these unsaturated fatty acids [2, 3]. Lipids in algae produce higher returns than other products of the land. Algal cells use sunlight for photosynthesis. In *C. vulgaris* cells, the photosynthetic rate is about 10 to 100 times faster than the respiration rate. The protein content varies depending on the species of algae cultivation and harvesting methods, and is about 15% to 88% and an average of 50% of the dry weight [4].

The correlation between culture conditions and the amount of carotenoids, proteins and fatty acids has been the focus of many studies [5]. In autotrophic culture, the amount of fatty acids produced is small. To increase fatty acid production by algae, heterotrophic growth development is more appropriate [6]. The fatty acid composition of *C. vulgaris* is simple in comparison with other species and producing natural nutrients or natural pharmaceutical products using autotrophic cells, compared to heterotrophic cells, is under consideration [7]. *C. vulgaris* have a capability of producing a wide range of active substances with antibacterial activity [8].

In 1944, the first anti-bacterial material was isolated from *C. vulgaris*. A mixture of fatty acids called chlorellin, which had inhibitory activity against gram-positive and gram-negative organisms, were also isolated [9]. Cultures of *Chlorella* in media containing mineral nutrients, causes the production and accumulation of a substance, which inhibits the proliferation of cells. The extracted material from these cultures showed antibacterial properties on gram positive and gram-negative organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Bacterium coli* and *Pseudomonas pyocyanea*. Recently, due to the limited fossil resources and concerns regarding environmental pollution, the production of biofuels such as biodiesel from vegetable oils, animal fats and algae has gained remarkable attention [10]. Lipid production in algae has been shown to have more efficiency than that of other biological products. Biodiesel is produced through chemical reactions of oils to alcohols in the presence of catalysts [11-13]. The end products are methyl esters (actually biodiesel) and glycerol (a byproduct) and this reaction is called trans-esterification. These reactions are performed at high pH and catalysis by alkaline. The used alcohols in this reaction were mostly ethanol and methanol, to a lesser extent. This chemical reaction is sensitive to water. The presence of water leads to the saponification reaction, which affects the production and quality of biodiesel [11].

This study considered the effect of aluminium on fatty acids synthesis and antibacterial properties in green algae *C. vulgaris* under autotrophic and heterotrophic conditions.

MATERIALS AND METHODS

A) Algae cultivation

C. vulgaris Beijernick were obtained from pure microalgae collection of Applied Sciences Institute, Shahid Beheshti University, and Tehran, Iran of and cultured in BG-11 medium.

Treatments included in this study were as follows:

1. Heterotrophic Treatment, Al 300Mm and Glu
2. Heterotrophic Treatment, Al 300μM and Suc
3. Autotrophic treatment, Al 300μM.

Inoculated in 250 mL Erlenmeyer flask (in triplicate) was performed.

Autotrophic treatments, light conditions and temperatures between 27-25 °C and 1500 lux light for 20 h of light and 4 h of darkness and heterotrophic treatments were incubated at 28°C for 21 d were grown respectively.

B) Measurement of growth

Survival and growth was measured based on opacity survey by spectrophotometers (spectronic LABOMED, INC, USA).

C) Measurement of dry weight

Algal cells in test tubes were dried in the oven at 105 ºC dry weight of algae based on the (algae suspension 10 mg/ml) were obtained from the following equation:

Dry weight = (Weight of Tube + Algae) – Tube Weight

D) Measurement of aluminium by acid extraction method with atomic spectrophotometer (Atomic Absorption Spectroscopy) model VARIAN AA240

The algae with nitric acid and chloridric acid were mixed and centrifuged; the supernatant solution absorption was measured by atomic absorption.

E) Evaluate the antibacterial properties

Methanol extracts were prepared of Super Natant and disks impregnated with extracts from three different treatments in Muller Hinton agar media containing *E. coli*, *Pseudomonas pyogenes* and *Staphylococcus aureus* were incubated for 8h at 37°C.

F) Lipid extraction

Sonication steps of algae, removing the chloroform phase by decanter (Separatory funnel) of methanol phase and fatty acid hydrolysis by soxhlet. Fatty acid methylation method for sample preparation was raised for injection into the GC [10].

STATISTICAL ANALYSIS

Data were analyzed through a completely randomized design and comparison between treatment and control groups at 5% level was performed according to Duncan test and T2Tamhane by SPSS Ver. 16 (Chicago. IL, USA). Results are presented based on $\overline{X} \pm SD$.

RESULTS

Effect of Autotrophic and Heterotrophic treatments upon growth

According to Figure 1, significant differences in growth were observed between treatment and control samples (P<.05).

Effect of Autotrophic and Heterotrophic treatments on dry weight

According to Figure 2, significant difference was seen in terms of dry weight in all treatments than controls (P<.05).

Effect of Autotrophic and Heterotrophic treatments on internal Al content

According to Figure 3, the aluminium content in autotrophic treatment than other treatments significantly decreased (P<.05).

Effect of Autotrophic and heterotrophic conditions on antibacterial properties

According to Figure 4, the methanol extract of heterotrophic treatment, was effective only against gram-negative bacteria *E. coli* and *P. aeruginosa*, and thus gram-positive bacterium *S. aureus* resistant to the antibiotic. Finally, according to Figure 5, methanol extracts of autotrophic plants has affected only the Gram-negative bacterium *E. coli.*

Effect of Autotrophic and Heterotrophic conditions on fatty acids content

Accumulated fatty acids in the presence of aluminium and light in autotrophic treatments increased compared to heterotrophic treatments (Figure 6).

The presence of aluminium and light in autotrophic treatment than to heterotrophic reduced fatty acids secreted into the culture medium (Figure 7).

The models for Chlorella vulgaris Beijerinck behavior for fatty acids synthesis in the presence of Aluminium in the heterotrophic and autotrophic culture medium

According to Figures 8, 9 in aluminium presence, accumulation of saturated and unsaturated fatty acids in autotrophic treatments have increased than heterotrophic ones, secretion of saturated and unsaturated fatty acids into the culture medium have declined in autotrophic treatments than heterotrophic ones.

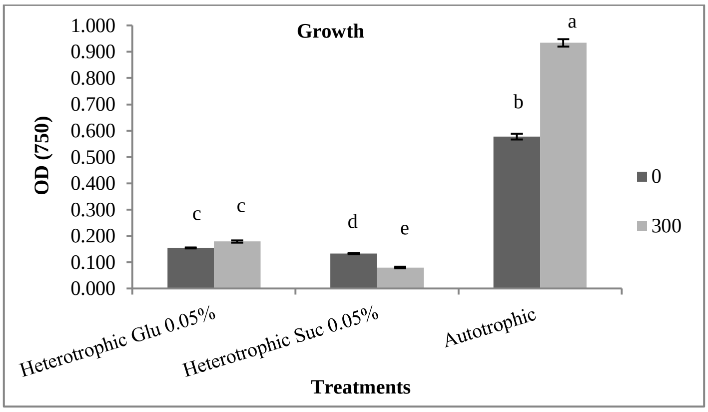

Figure 1. Comparison between treatments (Al300) and control (Al0) in *Chlorella vulgaris*

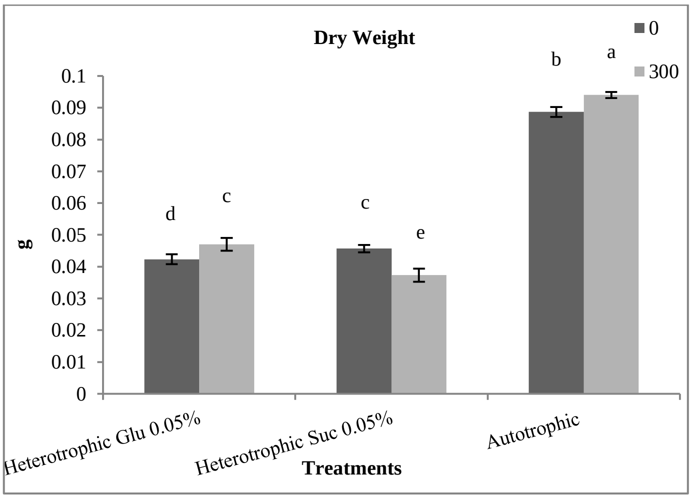

Figure 2. Comparison of dry weight between treatments (Al300) and control (Al0) in *Chlorella vulgaris*

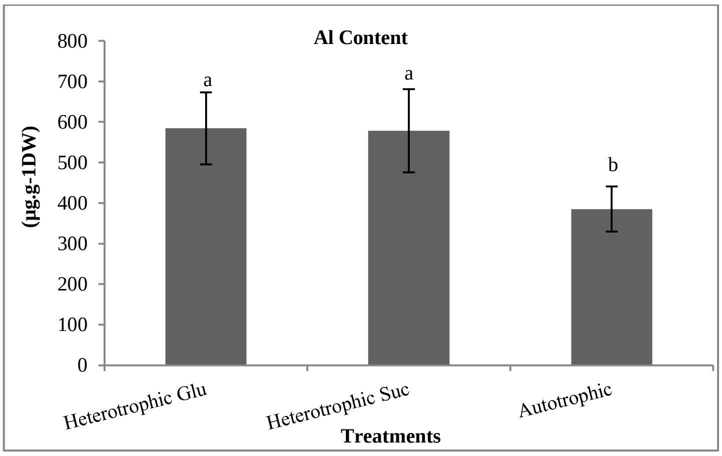

Figure 3. Comparing internal aluminium content in different treatments of *Chlorella vulgaris* in aluminium 300 mM**.**

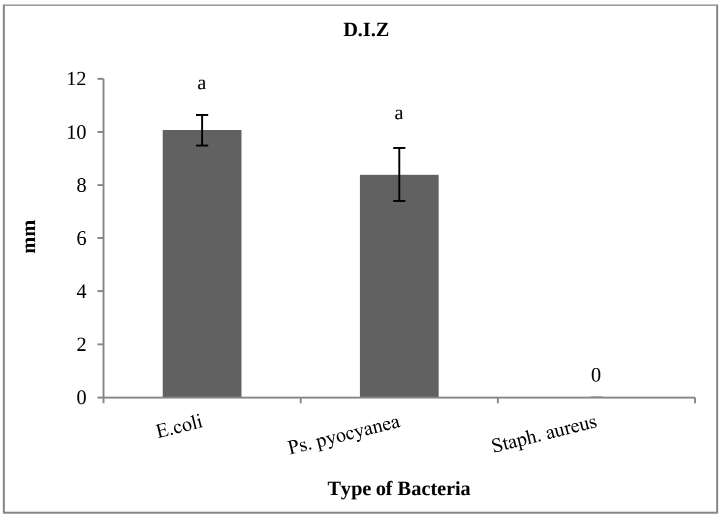

Figure 4. Bacterial Inhibitory Zone the presence of methanol extract in heterotrophic treatment**,** DIZ: Diameter of Inhibitory Zone

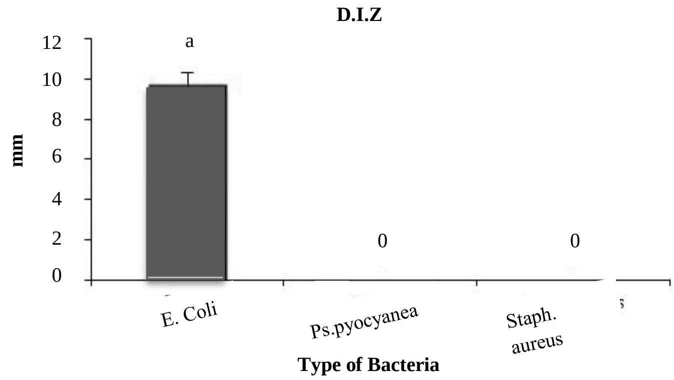

Figure 5. Bacterial Inhibitory Zone the presence of methanol extract in autotrophic treatmentDIZ: Diameter of Inhibitory Zone

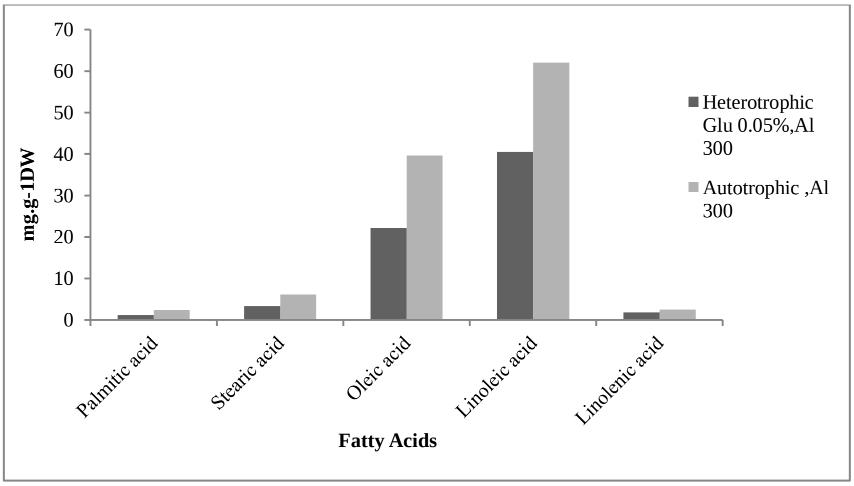

Figure 6. The content of fatty acids accumulated in two treatments autotrophic (Al 300) and heterotrophic glucose (Al 300)

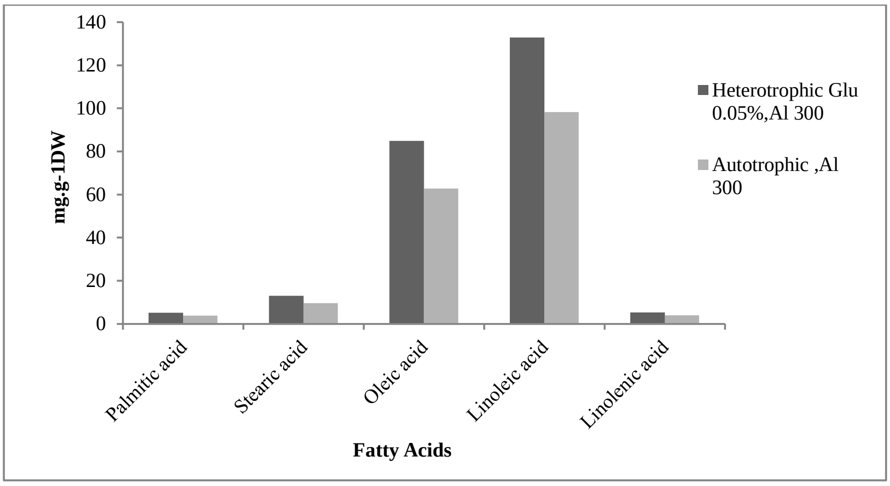

Figure 7. The content of fatty acids secreted into the culture medium in the treatment of autotrophic (Al 300) and heterotrophic glucose (Al 300)

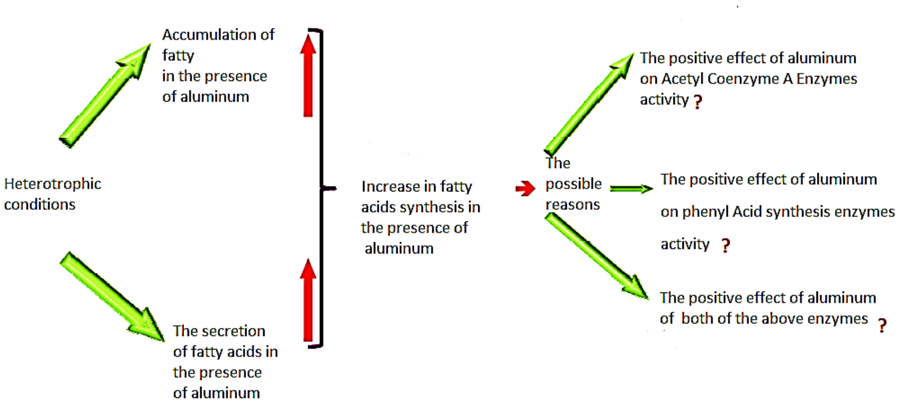

Figure 8. The behavior of *Chlorella vulgaris* Beijerinck. on fatty acids synthesis in the presence of aluminium in heterotrophic culture medium

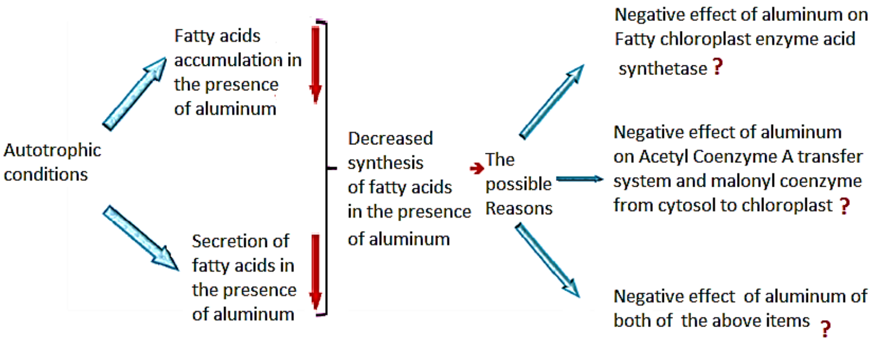

Figure 9. *Chlorella vulgaris* Beijerinck. behavior for fatty acids' synthesis in the presence of Aluminium in the heterotrophic culture medium.

DISCUSSION

"It is well known that algal cells exposed to heavy metals may suffer serious morphological and biochemical alterations "[14]. Although the effects of heavy metals depend on their concentration and kind of organisms, rarely uptake of heavy metal from culture medium depends to the amount of element in the water [15].

Our results showed that Al increased algae growth, and this is similar to the results of some researches about Cucumis melo showed good or even increased growth in low Al concentrations, and pH 4.0, in culture medium, [16-18].

Aluminium increased accumulation of saturated and unsaturated fatty acids in autotrophic treatments compared to the heterotrophic ones. In the presence of Al, saturated and unsaturated fatty acids secreted into the culture medium and declined in autotrophic treatments in comparison to the heterotrophic ones. Similar to El-Sheekh and Fathy results [7], the amount of oleic acid, linoleic acid and linolenic acid in these treatments, compared to heterotrophic treatments, reduced considerably.

In normal conditions, antibiotics are less effective on gram-positive bacteria because their multilayer cell wall structure is more complex, and therefore, more difficult to penetration of such materials to the bacterial cell [19]. A variety of solvents with different polarity for the isolation of bioactive compounds of microalgae was used [20-23]. The antimicrobial activity was not observed in the extraction with hexane, which was probably due to the polar nature of these compounds. This shows that the chances of finding the antimicrobial activity of methanol extracts and supernatants culture were higher.

El-Sheekh and Fathy [7] proposed that the biological mechanisms of glucose consumption by Chlorella is not clear but in normal conditions in which the autotrophic growth of Chlorella shifts to heterotrophic growth, may also make the consumption of exogenous carbon source possible.They said: "In the presence of exogenous carbon source glucose, growth and production of fatty acids has increased". Compared with other species, *C. vulgaris* has a quite simple composition of fatty acid. The qualitative composition of fatty acids is not only different in species, but also varies in different phenotypes of a species [13, 24]. Dickson [25], quantitatively and qualitatively analyzed the important fatty acids of *C. fusca* in autotrophic and heterotrophic cultures.

Increases in ambient CO_2 concentration (from 1 to 30%) resulted in a 40 and 50 percent increase of total lipids and fatty acids in heterotrophic cultures, respectively. Milner and Spoehr [26] reported that the assimilation of carbon as an organic carbon or CO_2, possibly after the end of cell division by environmental factors (such as nitrogen limited) would be continued. CO_2 has a direct inhibitory effect on cell division in *Chlorella* [27]. New protoplast production and growth may be very sensitive to environmental stresses, whereas carbon assimilation continues and can be stored as product storage such as triglycerides.

CONCLUSION

Autotrophic growth conditions caused a significant increase in dry weight ratio compared to the heterotrophic growth conditions. Most anti-bacterial feature was revealed in methanol extract of heterotrophic bacteria against *E. coli* and *P. pyocyanea*. In heterotrophic conditions, the sample survived and tended more to glucose. Besides, in glucose heterotrophic conditions, the sample grew without delay phase and this showed that the organism was quickly adapted to heterotrophic conditions.

ACKNOWLEDGMENTS

The authors would like to thank all the individuals who cooperated in this study. The authors declare that there is no conflict of interests. The authors did not receive any financial aids.

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