Identification and Quantification of Fatty Acids in Cyanobacteria Cells

Original Article

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Abstract

Cyanobacteria biomass lipid extraction was performed by the method of Bligh and Dyer [5] in chloroform/methanol mixture and methanol/water mixture. The mixture separation in three specific phases made possible the lipid extraction in chloroform (lower phase). Quantitative determination of fatty acids was performed by gas-liquid chromatography of the fatty acid methyl esters using an Agilent 7890 N gas chromatograph. Analyses were performed in the Environmental Analysis Laboratory of the Research Institute for Analytical Instrumentation, ICIA Cluj by the means of specialist Mrs. Miclean M. Data processing was performed using the OriginPro 8 program.

Keywords: cyanobacteria, lipid extraction, chloroform, methanol, chromatography, fatty acids.

1. Introduction

Fatty acids are used in membranes formation maintaining cell viability. Knowing the mechanisms of fatty acids synthesis provides a major economic effect on the achievement of the biotechnological processes in order to obtain the desired products. The importance as biotechnological objects and their use in biodiesel production was determined by the cyanobacteria specific fatty acids and their ability to modify lipid profile depending on the cultivation conditions. The biodiesel properties depends on the chemical structure of fatty acid methyl esters. Saturated fatty acids gives a more stable biodiesel than unsaturated fatty acids. Changing the cultivation conditions leads to fatty acids biosynthesis [4].

2. Materials and methods

Biological material which has been subject of this study are *Synechocystis* sp. PCC 6803, *Anabaena variabilis* sp. ATCC 29413, *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 51142 strains.

For the study of fatty acid, lipids were extracted from cyanobacterial cells by the method of Bligh and Dyer [5] (fig. 1) Esterified fatty acids were detected by gas chromatographic analysis using a commercial standard consisting of fatty acid methyl esters of known concentration as reference.

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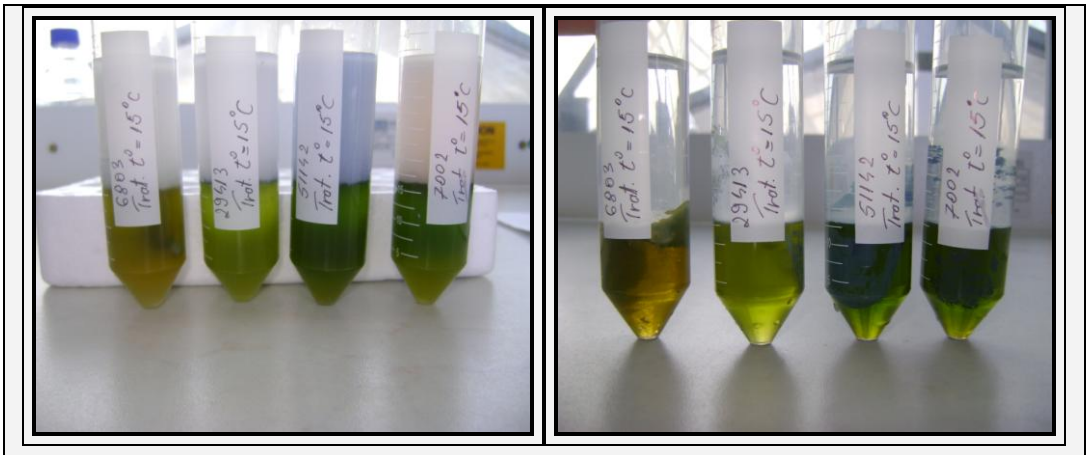


Fig. 1. Lipid extraction (original)

3. Results and Discussion

3.1. Identification and quantification of fatty acids in *Synechocystis* sp. PCC 6803 strain

In figure 2 is shown treatment effect on the fatty acid composition of extracted lipids from *Synechocystis* sp. PCC 6803 strain. The most obvious changes in thylakoid membrane was in acids: C8:0 (caprylic), C10:0 (capric), C12:0 (lauric), C16:0 (palmitic), C16:1 (palmitoleic) and C18:0 (stearic). High light intensity treatment, low temperature (15^0C) and high temperature treatments (35^0C) has increased the amount of capric, lauric, palmitic, stearic acids. Treatment with 5% carbon dioxide stimulated the synthesis of capric, lauric, palmitoleic, alpha-linolenic acids. All performed treatments had determined the inhibition of palmitic acid synthesis. 5% CO_2 treatment, low temperature and high temperature treatments had a negative effect on the concentration of caprylic acid. Compared with the control sample treatments had no effect on the heptadecanoic, oleic, elaidic, linoleic, linolelaidic acids.

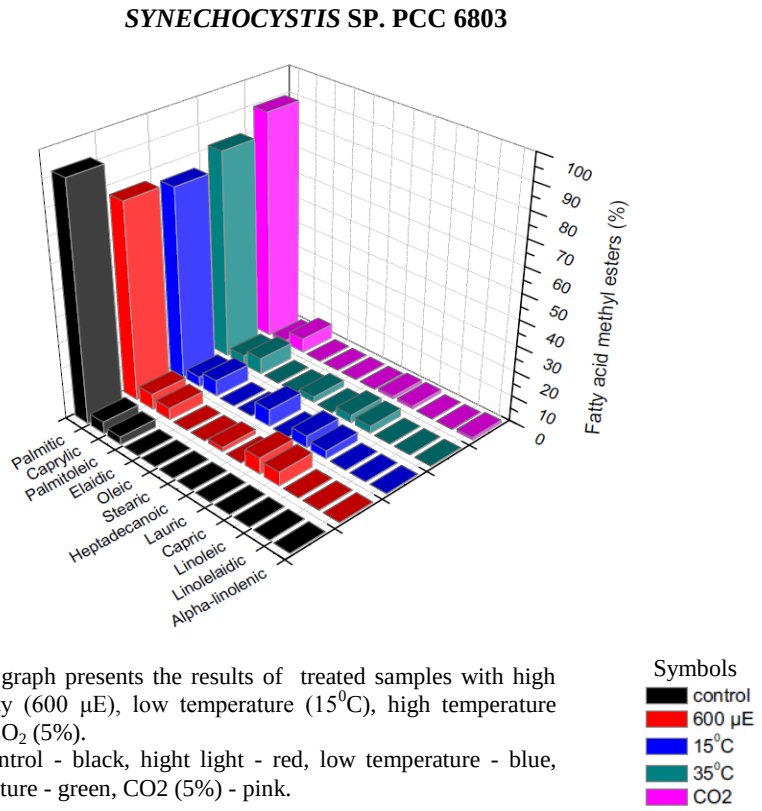


Fig. 2. This graph presents the results of treated samples with high light intensity (600 µE), low temperature (15^0C), high temperature (35^0C) and CO_2 (5%).
Symbols: control - black, hight light - red, low temperature - blue, high temperature - green, CO2 (5%) - pink.

3.2. Identification and quantification of fatty acids in *Synechococcus sp.* PCC 7002 strain

The fatty acid composition of extracted lipids from the cells of *Synechococcus* sp. PCC 7002 strain is shown in fig 3.

The obtained data shows that performed treatments on *Synechococcus* sp. PCC 7002 strain have a stimulatory effect over different types of fatty acids.

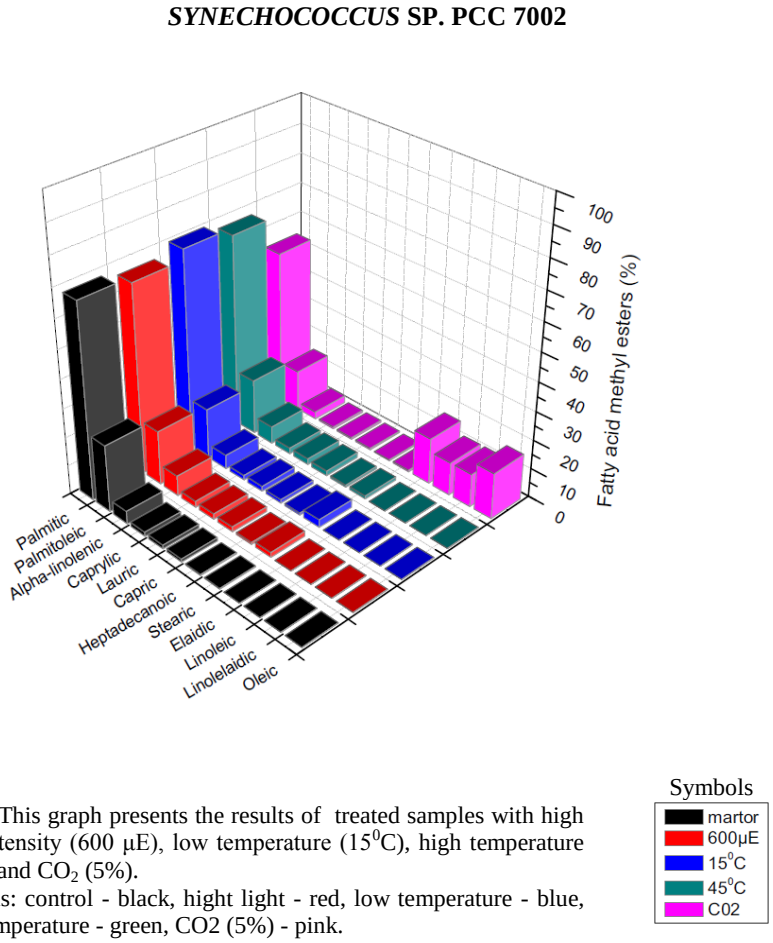
Significant changes in response to performed treatments were recorded in oleic, elaidic, linoleic and linolelaidic acids. These types of fatty acids were present only in extracted lipids from CO_2 treated cells.

Based on theoretical assumption that fatty acids are used in membranes formation it is likely that lipid membrane fluidity affects the genes transcription by the fatty acid desaturase that introduce a double bonds in fatty acid molecules [6].

High light intensity treatment (600 µE), low temperature (15^0C) and high temperature treatments (45^0C) has a positive influence on the amounts of caprylic acid, capric, lauric, heptadecanoic, stearic and alpha-linolenic acids compared to control sample. In addition low temperature treatment (15^0C) had also a stimulatory effect on the palmitic acid concentration.

CO_2 treatment improved concentration of stearic, oleic, elaidic, linoleic and linolelaidic acids. Carbon dioxide was the only one that had a stimulating effect on these types of fatty acids. However on the other types of fatty acids 5% concentration of CO_2 had a negative effect as compared with the control sample.

High light intensity treatment and high temperature treatment had a negative effect on the palmitoleic and palmitic acid. Low-temperature treatment produced a negative effect on the palmitoleic acid.



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3.3. Identification and quantification of fatty acids in *Anabaena variabilis* sp. ATCC 29413 strain

The fatty acid composition of total lipids isolated from cells of *Anabaena variabilis* sp. ATCC 29413 is shown in fig 4.

The predominant fatty acids were caprylic, capric, lauric, palmitic, palmitoleic, stearic and alpha-linolenic acid.

Lipid analysis performed on this strain reflect a slight stimulation of some fatty acids concentration. High light intensity treatment (600µE) showed a positive effect by stimulating the synthesis of caprylic, capric, lauric, stearic acids and negative action by inhibiting the synthesis of palmitic, palmitoleic and alpha-linolenic acids.

Low temperature treatment (15^0C) stimulated the concentration of caprylic acid, capric, lauric, stearic and alpha-linolenic acids. The treatment had also a negative effect on palmitic and palmitoleic acids, these acids values were lower than control values. Treatment with high temperature (35^0C) had a positive influence on caprylic, capric, lauric, palmitoleic, stearic, alpha-linolenic acids and a negative action on the palmitic acid.

Some of the performed treatments have led to changes in the amount of unsaturated fatty acids in the cell. On the palmitoleic acid high temperature and 5% carbon dioxide treatments had a positive effect; on the alpha-linolenic acid the positive effect had been achieved by the influence of low temperature and high temperature treatment.

By increasing the proportion of unsaturated fatty acids in thylakoid membranes photosystem II recovery is accelerated. The recovery necessity appeared after the heat stress or other stress type [1].

Influence of CO_2 treatment on the concentration of caprylic, capric, lauric, palmitic and palmitoleic acids has been positive. The concentration of stearic and alpha-linolenic acids decreased after treatment. Compared with the control sample the performed treatments had no significant influence on the concentration of heptadecanoic, oleic, elaidic, linoleic and linolelaidic acids.

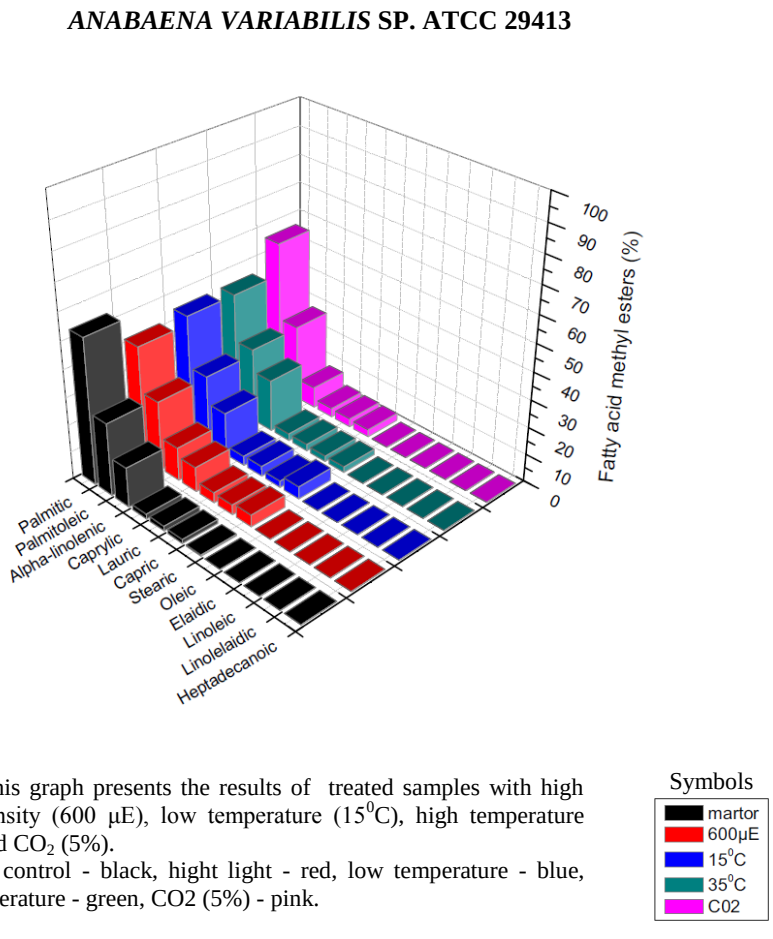

Fig. 4. This graph presents the results of treated samples with high light intensity (600 µE), low temperature (15^0C), high temperature (35^0C) and CO_2 (5%).
Symbols: control - black, hight light - red, low temperature - blue, high temperature - green, CO2 (5%) - pink.

3.4. Identification and quantification of fatty acids in *Cyanothece* sp. ATCC 51142 strain

Fatty acid composition of total lipids extracted from the biomass of *Cyanothece* sp. ATCC 51142 is shown in fig. 5.

Performed treatments on *Cyanothece* strain sp. ATCC 51142 were associated with increased concentration of some fatty acid types.

In total lipid extract palmitic acid was predominant; the proportion of this acid was more than 70% after 5% carbon dioxide treatment.

High light intensity treatment (600 μE) stimulated concentration of capric, lauric, palmitoleic, heptadecanoic and stearic acids; values of alpha-linolenic, caprylic and palmitic acids decreased compared with control sample.

Concentration of lauric acid, stearic acid and alpha-linolenic acid was stimulated by low temperature (15^0C) treatment, but concentration of caprylic, capric, palmitic and palmitoleic acids decreased after treatment.

Thylakoid membrane fluidity is affected by low temperature; the proportion of unsaturated fatty acids in this increase in other words membrane cell fluidity decreases. Increasing the proportion of unsaturated fatty acids compensates the cold action so that membrane functions such as hydrogen ions transport are not affected [2, 3].

High temperature treatment (35^0C) stimulated concentration of palmitoleic, stearic, alpha-linolenic acids and decreased the concentration of caprylic acid, capric, lauric and palmitic acids.

CO_2 treatment stimulated the palmitic acid synthesis, heptadecanoic, stearic, linoleic and linolelaidic acids and inhibited the synthesis of caprylic, capric, lauric, palmitoleic and alpha-linolenic acids.

On the concentration of oleic and elaidic acids performed treatments had no significant influence compared with the control.

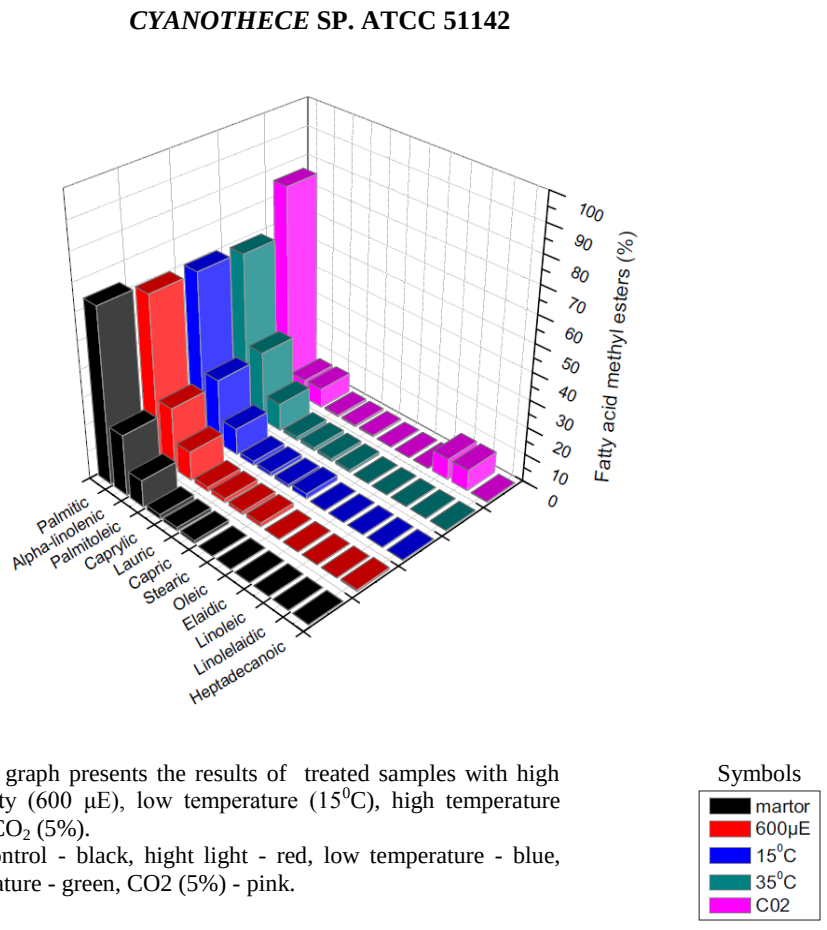

Fig. 5. This graph presents the results of treated samples with high light intensity (600 μE), low temperature (15^0C), high temperature (35^0C) and CO_2 (5%).
Symbols: control - black, hight light - red, low temperature - blue, high temperature - green, CO2 (5%) - pink.

Conclusions

1. High light intensity, low temperature, high temperature and 5% carbon dioxide treatments are associated with increased biosynthesis of certain types of fatty acids in photosynthetic membranes components. This treatments offer the possibility to increase the desired product quantity.
2. For analyzed fatty acids treatments may also manifest as stress factors which are expressed mainly by decreasing the amount of palmitic acid in all four studied cyanobacteria strains.
3. The majority fraction of total fatty acids has been shown by the palmitic acid (C16: 0) in all four studied strains while the C18: 1n9c (oleic) and C: 181n9t (elaidic) acids were synthesized only by *Synechococcus* sp. PCC 7002 strain.
4. In lipid extracts were found six types of saturated and six types of unsaturated fatty acids. Except heptadecanoic, oleic, elaidic, linoleic and linolelaidic acids most of the fatty acids were present in all lipid extracts.
5. The proportion of oleic, elaidic, linoleic and linolelaidic fatty acids increased only after CO_2 treatment and only in *Synechococcus* sp. PCC 7002 and *Cyanothece* sp. ATCC 51142 strains.
6. Identified fatty acids were better represented in *Synechococcus* sp. PCC 7002 strain fact which shows the ability of this strain to synthesize various types of fatty acids compared with the other studied strains.

„Acknowledgments

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