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A critical look at the microalgae biodiesel

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ENHANCEMENT OF *TRIBULUS TERRESTRIS* L. YIELD BY SUPPLEMENT OF GREEN HOUSE SEEDLINGS

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ABSTRACT

The seeds of Tribulus terrestris L. show relatively low germination and a long period of dormancy which results in bare patches in its field cultures decreasing the yield. The yield could successfully be enhanced by supplementation of green house seedlings on the bare patches. A higher germination of the seeds was achieved in a green house by the help of photosynthetic microorganisms which stimulated both germination and young plant development. The composition of fatty acids and sterols of senescent plant biomass was analysed.

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Keywords: *Tribulus terrestris*, microalgae, green house, germination, fatty acids, sterols

Introduction

The plant puncture vine, *Tribulus terrestris* L., is a valuable source of pharmaceuticals which find a number of medical applications. Lately, being subject to versatile studies, many heeling properties of the plant have been found. Amid them are sexual impotence, oedemas, abdominal distention, cardiovascular diseases, dizziness, headache, hypertension, nettle rash treatment, cytotoxic and antibacterial activities, Alzheimer's Decease (1, 2, 3, 8, 9, 10, 11). The concentration of active substances, namely steroidal saponins, in Bulgarian plant material is one of the highest in the world (4, 5). Recently, the interest in the large scale cultivation of puncture vine has begun to increase quickly. The plant grows on dry and sandy areas in the wild. Its extremely low water requirement at large-scale field cultivation is a very useful quality (3, 6). On the other hand, the relatively low germination of the seeds and the long period of dormancy are real obstacles. Bare patches remain on the field, and the outcome as a whole is a scarce yield. In this respect research on the possibilities for increasing the crop density would be worth the effort from an economic point of view.

In a previous work we have described that a suspension of cyanobacteria and microalgae can successfully influence the seeds germination (13). The experiment with the puncture vine seeds and photosynthetic microorganisms, after the germination, was continued with the further development of the plants. Maintaining the increase of germination, the aim of this work was to find out how to grow seedlings to achieve quicker occupation of the bare patches on the field. In addition, a study on the biomass composition of senescent plants was also carried out.

Materials and Methods

Plant cultivation

Seeds of *Tribulus terrestris* L. were sown in transparent plastic leaky cups (10 cm in height, 7 cm in diameter), at a depth of 4-5 mm in sand, one seed in a cup. The transparent pots were placed on plates, where water had been added and, through the bottom holes, was capillary ascending to the surface. The experiment was carried out in a naturally lit green house, where a temperature in the range of 20-40°C was maintained. Soil photosynthetic microorganisms were once added to the water as previously described (13). The seedlings cultivated in the green house were planted in the field near Sofia.

Analytical procedures

Dry plant material was trice extracted with ethanol. Lipids were separated from the extract with chloroform. Saponification of lipids, methylation of fatty acids, their purification by thin layer chromatography (TLC), and identification by gas chromatography (GC) was previously described (7). The sterols were isolated by TLC, and identified by GC/MS (7).

Results and Discussion

Photosynthetic microorganisms, such as cyanobacteria and green microalgae, once added to the sand quickly begin to multiply, forming a substantial part of the soil microflora. So during its development from seed to a mature organism, puncture vine is involved in complex relationships, but mutual, or at least, positive for the higher plant. The conditions are made up for development of photosynthetic microorganisms. After several days they thrive even without adding of inoculum. As a matter of fact, the translucent side area of the pots, sand, and optimal temperature, are important factors that contribute to the development of microalgae.

As we have already described, the seeds begin to germinate 3 days after sowing (13). Seeds from the regions of

Do Cyanobacterial Lipids Contain Fatty Acids Longer Than 18 Carbon Atoms?

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Fatty acids of twelve species of cyanobacteria grown under different photoautotrophic conditions were studied and their composition was compared with literature data of many other species. We have come to the conclusion that the lipids of cyanobacteria do not contain fatty acids with a chain longer than 18 carbon atoms. In our opinion, omission of an analytical procedure, *i.e.* purification of fatty acid methyl esters before gas chromatography, leads to incorrect interpretation of the results. Absence or presence of fatty acids was suggested as a useful taxonomic marker and a proper diagnostic indicator in the commercial application of cyanobacterial biomass.

Key words: Chemotaxonomy, Cyanobacteria, Fatty Acids

Introduction

Historically, the cyanobacteria were the first photoautotrophic, oxygen-evolving prokaryotic organisms. Their membranes have a simple lipid composition compared to eukaryotic algal taxa. Indeed, their glycolipids and sulfolipids do not differ from those of eukaryotic algae. The difference is in the phospholipids, which are represented with a lower number of substances. Phosphatidyl glycerol is ubiquitous and predominant, and it is the only phospholipid of cyanobacteria (Nichols and Wood, 1968; Petkov and Furnadzieva, 1988; Domonkos *et al.*, 2004; Iliev *et al.*, 2006; Okazaki *et al.*, 2006). Lipids of cyanobacteria fulfil mostly a membrane function. Triacylglycerols, being storage substances, are normally present in small amounts and are not a part of the functional membranes. Also, small amounts of naturally occurring fatty acid methyl esters have been found (Petkov and Furnadzieva, 1993).

As a rule, the proportion of fatty acids in cyanobacteria follows temperature fluctuations (Wada and Murata, 1990; Varkonyi *et al.*, 2000). This relationship is very strongly expressed by some cyanobacteria, for example, the cold- and heat-resistant *Arthronema africanum* (Iliev *et al.*, 2006). This cyanobacterium maintains relatively constant fluidity of its membranes, adjusting the proportion of its fatty acids to the temperature. In other cases, the growth conditions have a smaller effect on fatty acid proportion (Piorreck *et al.*, 1984; Ronda and Lele, 2008).

Many previous studies on fatty acids of cyanobacteria used samples collected from a natural ecosystem and grown in the laboratory as a mixture, which was highly dominated by a single species. Consequently, the found fatty acid composition was not that of a single taxon. Similarly, when cyanobacteria are grown in open ponds, it is not a monoculture. Such studies do not allow chemotaxonomic conclusion and they are not mentioned here.

Chain length and number and position of double bonds of fatty acids are genetically determined. It is reasonable to suggest that a taxon has a specific maximal length of the fatty acid chain and maximal number of double bonds. Here obviously arises a question: which is the taxon in the hierarchy where all organisms have similar qualitative composition of fatty acids?

Based on the analysis of a large number of literature data on fatty acids of cyanobacteria and on our own experiments, we conclude that

Chapter X

Algae: Processes and Applications

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Abstract

Algae and cyanobacteria, as photosynthetic organisms, are cultivated in mineral nutrition media bubbled with carbon dioxide. In large scale they are grown in naturally illuminated open sloped areas, covered ponds or photobioreactors. In order to enhance the yield of algal biomass attention is paid to the processes hydrodynamics, heat transfer and mass transfer in the algal device. Normally, daily light intensity is sufficient for intensive algal growth, but longer time of exposition ensures higher algal yield. Centrifugation or filtration is the main method of microalgae harvesting.

As a whole, the algal biomass is a valuable nutritional additive for humans and animals. The biochemical composition of algae comprises all essential amino acids and minerals in the right natural ratio. Their qualities as food and forage are completed by almost all vitamins, valuable oils and phytosterols. The eggs of algae-fed hens have much higher carotene content, harder shells and the productivity as a whole is greater. Microalgae - zooplankton - fish is a nutritional chain, successfully applied in the industry. Dry algal biomass is a standardized product and can be kept in store for long.

Algae are important source of pharmaceuticals. Phycocyanin and phycoerythrin of cyanobacteria and red algae are used in the immunodiagnostic and radioactive protection. Algal biomass is rich of beta-carotene, tocopherol, vitamin K, coenzyme Q_{10} and other oil- and water soluble natural antioxidants. Highly unsaturated fatty acids with 4, 5, 6 double bonds, such as arachidonic, eicosapentaenoic, docosahexaenoic characterize the algal oils. Algal biomass, algal oils, hydrocarbons and hydrogen from microalgae are often pointed as proper renewable source of fuel.

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1. Introduction

When one speaks about algae most people have in mind the large green, red and brown fronds in the sea. They really are algae called macroalgae, but here we are going to discuss the microalgae, which are small, microscopic algae, and extremely wide spread in nature: water, soil, air, snow and ice, deserts, hot springs etc. and often called microalgae. The word microalgae is not strict taxonomic concepts but convenient for use in biotechnology. Cyanobacteria are prokaryotic organisms, without nucleus, and from taxonomic point of view are not algae. Both cyanobacteria and microalgae have similar environmental significance and, the methods of cultivation, separation of biomass, processing and commercial use are similar, too. Sometimes, the word

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THE ALGA *TRACHYDISCUS MINUTUS* (*PSEUDOSTAURASTRUM MINUTUM*): GROWTH AND COMPOSITION

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Summary. The growth and composition of xanthophycean *Trachydiscus minutus* (*Pseudostaurastrum minutum*) were studied. Algal density 3.5 g.dm^{-3} of dry weight was achieved in laboratory, and high lipid content (26 % of dry weight) due to the abundance of triacylglycerols was found. The lipids of alga were characterized with about 26 % myristic and 30 % eicosapentaenoic acids. The first lipid is used in cosmetics, the second as a nutrient additive. Sterols were quantified and GC-MS identified, too.

Composition of *T. minutus* was: proteins (43 %), carbohydrates (25 %), and chlorophyll *a* (0.6 %). The cultivation was successfully run in pilot plant scale (330 dm^3, 2 m^2, in greenhouse), for the first time ever. Growth optimum about 26°C, resistance to contamination, easy centrifugation and drying on foil in a greenhouse, by sunlight, are the technological advantages.

Key words: algae; fatty acids; pilot plant cultivation; PUFAs; sterols; *Trachydiscus minutus*.

Abbreviations: Chl – chlorophyll; EPA – eicosapentaenoic acid; GC – gas chromatography; GC-MS – gas chromatography-mass spectrometry; MGDG – monogalactosyldiacylglycerol; PAR – photosynthetically active radiation; PG – phosphatidylglycerol; PUFAs – polyunsaturated fatty acids; RT – retention time; SQDG – sulphoquinovosyldiacylglycerol; TAG – triacylglycerols; TLC – thin layer chromatography.

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COULD MICROALGAE ENHANCE THE GERMINATION OF *TRIBULUS TERRESTRIS* L. SEEDS?

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ABSTRACT

Seeds of Tribulus terrestris L. were sown in transparent plastic leaky boxes, at a depth of 4-5 mm in sand. The transparent pots were put on plates, where water has been added through the bottom holes and capillary ascending to the surface. The temperature of soil was 20-37 ^{0}C, temperature of air 20-40 ^{0}C. Either the soil microalgae, or preliminary grown microalgal suspension, single added with the water has been thriving at these conditions. At all experiments, the first seeds germinated exactly 3 days after they have been sown. Above 90 % of the seeds germinated to 7-9 day and single of them to 20 day. Inside, on the wall of the pots, green collour appeared due to microalgae and significantly later mosses appeared, too. Having worked with seeds from Pazardzhik a germination of 31 ± 12 % was achieved (74 seeds). Seeds gathered from nature, Black Sea coast, Zarevo, have germinated 7 of 10 in a single experiment. The manner of work is in consistency with a possible breeding of the plant as greenhouse seedlings.

Keywords: *Tribulus terrestris*, seeds, microalgae, germination

Introduction

Even a quick tentative run through the ethnopharmacological literature sources or study of people's ethnomedical customs says that the plant puncture vine, *Tribulus terrestris* L. had been used in days of yore. At the present time the healing properties of the plant have been used at a lot of illnesses and aberrations from the norm (1, 2, 3, 10, 13).

Breeding of the plant in large scale is bound with some drawbacks as law germination of the seeds and high period of dormancy (5, 7). The concentration of active substances is higher in the period of seed-immaturity coming in a heavy contradiction with production of seeds. In the wild, *T. terrestris* grows on bare places. Being tended as a field culture, *T. terrestris* does not occupy the whole planted ground but it keeps cropping up on patches. A good germination rate of 35 % was achieved after a series of heavy rains (5). Otherwise, the plant is definitely thermophilic and dwells southern, even desert regions.

On the other hand, the desert sandy soils are full of photosynthetic microorganisms. The cyanobacterium *Arthronema africanum* is an important resident of the deserts soils (9). It has been studied in laboratory and pointed as a highly tolerant to temperature and light variations (8). A cytokinin-like activity of *Arthronema* was described and isopentenyladenine, a cytokinin, was isolated (11, 15). One of the manners to increase the soil fertility is when add cyanobacteria or microalgae. Usually, there is natural abundance of them in the rich fertile soils. Many cyanobacteria, for example *Anabaena, Aulosira, Calotrix, Cylindrospermum, Nodularia, Nostoc, Oscillatoria, Plectonema, Scytonema, Tolypothrix* are described to stimulate the growth of higher plants (4, 14, 16, 17).

As a matter of fact, there is an interaction between higher plant and soil photosynthetic microorganisms. The aim of this preliminary study, save increasing the germination of *Tribulus terrestris* L. seeds, is to improve the whole technology, especially when green house seedlings are used for breeding of the plant on field.

Materials and Methods

Seeds of *Tribulus terrestris* L. were sown in transparent

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BIOLOGIE
Microbiologie

EXTRACELLULAR SUBSTANCES OF TOTAL BACTERIAL FLORA FROM CULTURES OF THE GREEN ALGA *SCENEDESMUS*

Georgi Petkov, Roumena Kambourova, Vassya Bankova

(*Submitted by Academician V. Golemansky on October 15, 2009*)

Abstract

The extracellular substances of concomitant bacteria of the green alga *Scenedesmus* cultures were studied, both in fresh and Black Sea water. Pentadecanoic and hydroxypentadecanoic acid dominate amid the lipophilic substances. Lactic and succinic acid and their esters are the main components of the water soluble material. Formation of esters in water medium is a proof of extracellular bacterial esterification activity. Fatty acids, sterols and unsaturated hydrocarbons excreted from algae are subjected to bacterial oxidation and assimilation. N- Dimethyl valine, an extracellular amino acid was found.

Key words: algae, bacteria, extracellular, N,N-dimethylvaline, *Scenedesmus*

Introduction. Growing of algae both in laboratory, in high volume photobioreactors, or in large scale ponds have been carried out at non-sterile conditions. Nevertheless, as a rule the whole weight of concomitant microorganisms compared to the algal biomass is 1:100 due to the photoautotrophic manner of cultivation [1]. The algal culture, being illuminated and oversaturated with oxygen, does not content other bacteria save aerobic. Not long ago we found that green alga *Scenedesmus* excretes in the medium small amount of organic matter [2,3]. That is why the weight of total concomitant bacteria is also small. It is known that they belong predominantly to the genera *Micrococcus*, *Pseudomonas*, *Bacillus*, *Flavobacterium* [4,5]. This relatively constant concomitant microflora

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SOLID AND LIQUID WASTE FULL TREATMENT AND APPLICATION IN CAR INDUSTRY

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ПЪЛНО ТРЕТИРАНЕ НА ТЕЧНИ И ТВЪРДИ ОТПАДЪЦИ И ПРИЛОЖЕНИЕ В АВТОМОБИЛНАТА ИНДУСТРИЯ

Филип Филипов*, Георги Петков, Анастас Иванов, Ненчо Ненов, Ангел Димитров, Валентин Чобанов, Демир Демирев, Ахмед Йосуф, Владимир Тодоров, Маия Куртева, Диана Банкова, Филип Михайлов

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Резюме

В разработката е представено ново технологично решение за пълно третиране на течни и твърди отпадъци. При проектиране на конструкциите за биогаз са отчетени термични, ветрови, динамични и сеизмични натоварвания. Получава се биомаса от инсталации за експериментална алгология. Тя се използва в модерна ферма за риба. В хранителната верига е включена и модерна ферма за гъби.

Abstract

New technology solution for solid and liquid waste full treatment is presented in the study. Thermal, wind, dynamic and seismic loadings are included in the biogas structural design. The proposed biomass is obtained from experimental algae. This biomass can be used in modern fishing farms. A modern cultivated mushroom farm is designed in the food chain.

Key words: Solid and Liquid Waste, Bio Gas, Bio Fuel, Methane Tanks, Finite Element Method.

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Which are fatty acids of the green alga *Chlorella*?

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Abstract

Fatty acid composition of three species of *Chlorella* were studied under conditions of photoautotrophic and heterotrophic cultivation, nitrogen starvation, and outdoor in a photobioreactor. The composition 14:0, 16:0, 16:1, 16:2, 16:3, 18:0, 18:1, 18:2, α-18:3 is confirmed for *Chlorella*. Fatty acids with 20 carbon atoms and four or five double bonds are considered not originating from *Chlorella*. Other exceptions of this composition are interpreted as mixed algal culture, bacterial contamination or impurities.

Keywords: Algae; Chemotaxonomy; *Chlorella*; Fatty acids

1. Introduction

Fatty acids are primary metabolites of acetyl CoA pathway which is genetically determined, evolutionary very old, and therefore conservative. Fatty acids in many species of *Chlorella* have been well studied for 50 years (Nichols, 1965; Pohl et al., 1971; Piorreck et al., 1984; Vladimirova et al., 2000). The correlations between cultivation conditions and percentage of fatty acids were subject of many studies (Table 1). These physiological studies described some quantitative changes of fatty acids, presenting one and the same qualitative composition. Nevertheless, there are studies reporting different qualitative fatty acid composition of *Chlorella* (Shinichi et al., 1983; Zhukova and Aizdaicher, 1995; Vanderploeg et al., 1996; Rosa et al., 2005). Studying marine *Chlorella*, Hirata et al. (1985) found abundant amount of arachidonic and eicosapentaenoic acids. These fatty acids appear more frequent when marine species of *Chlorella* are studied (Table 2). Obviously, some of the above mentioned publications have not been performed with *Chlorella* but with incorrect classified algae.

Marine *Chlorella* is widely used in artificial nutrition chain phytoplankton–zooplankton–fish (Elert and Woffrom, 2001; Wacker et al., 2002). Fatty acid composition of phytoplankton lipids is important to the success of aquaculture because the next members of this chain do not biosynthesize some fatty acids and also sterols (Martin-Creuzburg and Elert, 2004). It is evident that the algal species and its fatty acid composition are essential in these nutrition chains.

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MEMBRANE METABOLITES OF *ARTHRONEMA AFRICANUM* STRAINS FROM EXTREME HABITATS

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Summary. Two strains of scarcely studied *Arthronema africanum* (Cyanoprokaryota), originating from two desert habitats in Kuwait and Kathmandu (Nepal) were investigated. Both algae possess good adaptability and grow in a wide range of temperatures and light intensities. The lethal temperature for the Kuwait strain was 50 ± 1 °C while the one of Nepal origin died at 47 ± 1 °C. The content of phycobiliproteins enhanced at low light intensity while the content of chlorophyll and carotenoids increased at higher light intensity.

Fatty acids of monogalactosyldiacylglycerol, sulphoquinovosyldiacylglycerol, and phosphatidylglycerol as well as total lipids were analyzed. Percentage of linolenic acid decreased from 33 % at 16 °C to 0.5 % at 46 °C. In comparison to other algae and higher plants, *Arthronema* behavior represented a clearly expressed physiological response to temperature changes. Constantly high percentage of palmitoleic acid contributed to the endurance of the alga at stress temperature variations.

Keywords: *Arthronema africanum*, fatty acids, lipids, phycobiliproteins, stress.

INTRODUCTION

Blue-green alga *Arthronema africanum* was found in desert soil and marshes near to the sea in Kuwait, and in high plateaus in Nepal (Komarek and Lukavsky, 1988).

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BIOLOGIE
Biochimie

GROWTH, LIPIDS AND FATTY ACIDS OF THE DESERT TOLERANT BLUE-GREEN ALGA *ARTHRONEMA AFRICANUM*

I. Iliev, G. Petkov

(*Submitted by Academician V. Golemansky on September 20, 2006*)

Abstract

Desert tolerant blue-green alga *Arthronema africanum* was cultivated in laboratory. Growth and composition of three single cultures originating from one parent culture have simultaneously been studied and standard deviation is shown. Algal density was evaluated gravimetricaly. Content of lipids, pigments and total fatty acids is presented. Relatively high content of pigments is found. The alga shows qualities as a well-yielding subject of photoautotrophic biotechnology.

Key words: *Arthronema*, blue-green algae, fatty acids, growth, lipids

Introduction. Blue-green alga *Arthronema africanum* was found in desert soil near to the sea in Kuwait, and in high plateaus in Nepal [1]. It was also recently found in desert regions of Spain [2]. The alga has to withstand to rather variable light and temperature, drought and high salinity, demonstrating high endurance. The composition of *Arthronema* and its physiology have not yet been studied. Only a cytokinin-like activity of the alga is described and isopentenyladenine was isolated [3,4]. Cultivation of *Arthronema* in diluted pig manure has also been object of experiments [5]. The aim of our work as an initial step in the study of *A. africanum* is to specify reliably the growth, the lipid content, and also to analyse the fatty acid composition at constant cultivation conditions.

Material and methods. Blue-green alga *Arthronema africanum* KOMAREK and LUKAVSKY [1], strain Lukavsky 1981/1, isolated from desert soil (salt marsh) in Kuwait was kindly supplied from CCALA, Czech Academy of Sciences. An intensively growing culture of *A. africanum* was exactly divided into three similar cultivation vessels, and nutrition medium was added to equal volume. Constant volume of 200 ml was maintained in the three vessels during the cultivation. The algal cultures were grown at temperature 31 ± 1 °C, 8 klx uninterrupted light intensity and bubbled with $3\,cm^3.s^{-1}$ 0.5% (v/v) CO_2. Proportion of the salts described elsewhere [6] were used in $g.dm^{-3}$ 2.52 $NaNO_3$, 0.583 $K_2HPO_4.3H_2O$, 0.246 $MgSO_4.7H_2O$, 0.243 NaCl, 0.069

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Extracellular polar organic substances in cultures of the green alga *Scenedesmus*

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With 2 tables in the text

Abstract: The organic excretion of non-sterile monoalgal cultures of *Scenedesmus incrassatulus* grown in fresh and Black Sea water were investigated using gas chromatography/mass spectrometry. Lactic acid predominated, oxalic, hydroxybutiric, succinic acids, and some organic phosphates were also found. Dehydroabietic, 2-hydroxy-3-(4-hydroxyphenyl)-propanoic, N,N-Dimethylglycine, N,N-dimethylisopropenylglycine were identified, they are new found substances in algae. Algal cultures at exponential growth phase, which was characterized with high density around 4 g.dm^{-3}, excrete relatively less organic matter than the phytoplankton in natural ponds or starving algal cultures in model experiments.

Key words: Algae, N,N-Dimethylglycine, Extracellular, Lactic acid, *Scenedesmus*

Introduction

Green microalgae, especially *Chlorella*, are a subject of large-scale commercial cultivation. In open ponds or large-scale photobioreactors they are cultivated in growth conditions as non-sterile cultures. Aerobic, non-pathogenic bacteria mainly belonging to genera *Pseudomonas, Bacillus* and *Micrococcus* are always present in the culture of *Scenedesmus* (TONCHEVA-PANOVA 1978, MOUGET et al. 1995). A big variety of other bacteria could also be present accidentally. The bacteria, associated with a certain algal culture, produce substances against other bacteria (ACCORINTI 1981). Bacterial excretion in the medium could not be neglected, although the biomass of the bacteria is evaluated 100 times less than algal one (ORON et al. 1979). So, the polar water-soluble organic matter is a feature of the whole algal-bacterial culture. The complex excreted matter remains in the exhausted media after the cultivation and biomass harvesting.

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BIOLOGIE
Physiologie des plantes

EXTRACELLULAR LIPOPHILIC SUBSTANCES OF GREEN ALGA *SCENEDESMUS*

R. Kambourova, V. Bankova*, G. Petkov

(*Submitted by Academician E. Karanov on June 23, 2004*)

Abstract

Extracellular hydrocarbons, alcohols, aldehydes, cholesterol and its oxidated derivatives, free and esterified fatty acids in fresh and Black Sea water cultures of *Scenedesmus* were identified and quantified. It was proved that these substances did not originate from disintegrated cells. Cultivation in salt water leads to a growth of the extracellular algal lipophilic exudates presumably because of the salt stress.
Key words: algae, extracellular, *Scenedesmus*

Green microalgae are known to release extracellular substances in the medium. It is well documented that *Botryococcus* excretes substantial quantities of specific hydrocarbons [1,2]. *Botryococcus* is one of the exceptions among green microalgae having large extracellular lipophilic production. The quality and quantity of extracellular substances vary considerably between genera. The green microalgae *Chlorella* and *Chlamydomonas* release small amounts of extracellular lipiophilic substances [3]. Nevertheless, these substances are of ecological significance. They could affect the development of concomitant bacteria. Sometimes the undesirable foaming of large-scale algal cultures is ascribed to extracellular substances. For instance, extracellular free fatty acids at pH of the medium are foaming substances. Knowledge about them could help to improve the cultivation conditions. The following questions arise in the course of analysis of exudates from microalgae:

- Could substances be present as part of cellular debris?
- Do their composition and quantity depend on the cultivation conditions?

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GRAVIMETRIC QUANTIFICATION OF TOTAL EXTRACELLULAR ORGANIC SUBSTANCES OF MICROALGAE

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(*Submitted by Academician E. Karanov on March 24, 2004*)

Abstract

Total extracellular organic substances in the medium of green alga *Scenedesmus* are quantified. They vary about 10 mg.l^{-1} in fresh water and twice more in Black Sea water when alga is cultivated at exponential phase. The extracellular organic matter increases more than 10-fold at phase plateau. The gravimetric method used here is more exactly compared to the methods based on extractions or chemical oxygen demand.

Key words: algae, extracellular, *Scenedesmus*

Contradictory standpoints exist concerning the quantity of extracellular organic matter of microalgae. The conceptions about the green alga *Scenedesmus* are between "a minimal level of excretion" and "almost all metabolites found in the cell could be found in the medium" [1-3]. The latter opinion raises the question if these substances could appear as a result of cell disintegration.

The lipophilic extracellular substances can be relatively easily quantified after extraction with a proper, lipophilic solvent. Water-soluble substances are usually extracted with butanol. The residual nutrition substances in the medium could be an obstacle if they are soluble in butanol.

Chemical Oxygen Demand is a method often used for estimation of organic matter in waste water samples. Here, this method cannot give precise information about

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Volatile Substances of the Green Alga *Scenedesmus incrassatulus*

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Volatile substances of the green microalga *Scenedesmus incrassatulus*, cultivated in fresh and salt water, were studied. Cultivation in fresh water diversifies volatile secondary metabolites. Hydrocarbons and derivatives of the acetate pathway predominate when algae are grown in salt water; isoprenoids and aromatics are more abundant after fresh water cultivation.

Key words: Microalgae, *Scenedesmus*, Volatiles

Introduction

Volatile substances, which are excreted from algae in the water and then in the atmosphere, influence the environment to some extent. There are sufficient data about volatile substances from marine macrophytes. In red and brown algae, halogenated derivatives of acetate and mevalonate biosynthetic pathways and dimethyl sulfide were found (Jongaramruong and Blackman, 2000; Milkova *et al.*, 1997; Careri *et al.*, 2001; Yamamoto *et al.*, 2001). In green marine macrophytes acetate derivatives predominated, while the amount of isoprenoids was found to be relatively low (Sakagami *et al.*, 1991). Freshwater green macrophytes showed a more diverse volatile composition (Kamenarska *et al.*, 2000). The main volatiles in blue-green algae are hydrocarbons (Dembitsky *et al.*, 1999; Tellez *et al.*, 2001). The volatiles of only a few green freshwater microalgae have been investigated (Zolotovitch *et al.*, 1973; Rzama *et al.*, 1995).

The purpose of the present study is to investigate the quantity and composition of volatile substances from the industrially important green microalga *Scenedesmus incrassatulus* (Furnadzieva *et al.*, 1987). Volatiles of this alga should be compared when grown as high density cultures in fresh and Black Sea water.

Materials and Methods

Algal material

Green unicellular algae Scenedesmus incrassatulus Bohlin (R-83, Algal Culture Collection of the Plovdiv University). The algae were grown in the laboratory as non-sterile monoalgal culture at 33 °C, 9 klx uninterrupted light intensity and bubbled with $100\,l \cdot h^{-1}$ air enriched with 0.5 vol.% CO_2. Mineral nutrition medium and the same medium with $17\,g \cdot l^{-1}$ NaCl, analogously to Black Sea salinity were used. Proportions of both media were previously described (Petkov, 1995). Algae were harvested during the late exponential phase by centrifugation at $3000 \times g$.

Isolation of volatiles

The fresh biomass (5 g) was subjected to hydrodistillation in a Likens-Nickerson apparatus for 4 h, and the volatiles were collected in diethyl ether/*n*-pentane 1:1 (v/v) (50 ml).

GC-MS analysis

GC-MS analysis of the volatiles was performed on a Hewlett-Packard gas chromatograph 6890 equipped with a Hewlett-Packard MS 5973 detector. A HP5-MS capillary column was used (30 m × 0.25 mm, 0.25 mm film thickness). The temperature was programmed from 40 °C to 280 °C at a rate of $6\,°C \cdot min^{-1}$. Helium was used as a carrier

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Fatty acids and sterols of *Griffonia* seeds oil

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RESUMEN

Ácidos grasos y esteroles de aceite de semillas de *Griffonia*.

Se han estudiado los lípidos, ácidos grasos y esteroles del aceite de semillas de *Griffonia simplicifolia*. La composición en ácidos grasos es 18:2 - 60%, 16:0, 18:0, 18:1 - 9-18%, y 20:0 - 3-4%. El principal esterol es el ß-sitosterol - 60%, el estigmasterol constituye el 29%, y el campesterol el 11%. El ácido linoleico puede enriquecerse hasta el 95% separando los otros ácidos grasos como aductos de urea.

PALABRAS-CLAVE: Ácidos grasos – Esteroles – Griffonia – Lípidos.

SUMMARY

Fatty acids and sterols of *Griffonia* seeds oil.

Lipids, fatty acids and sterols of *Griffonia simplicifolia* seeds oil were studied. Fatty acid composition is 18:2 - 60 %, 16:0, 18:0, 18:1 - 9-18 %, and 20:0 - 3-4 %. The main sterol is ß-sitosterol - 60%, stigmasterol is 29%, and campesterol is 11%. Linoleic acid can be relatively simply enriched to 95% separating the other fatty acids as urea adducts.

KEY-WORDS: Fatty acids – Griffonia – Lipids – Sterols.

1. INTRODUCTION

The West African legume plant *Griffonia simplicifolia* Baill. (Caesalpinaceae) has been studied intensively since 1960. Lectins and hydroxytriptophan were detected, and these substances constitute the main interest to the plant (1). There are no data about the seeds oil.

The aim of this paper is to study the lipid, fatty acid, and the sterol composition of G. *simplicifolia* seeds oil.

2. MATERIALS AND METHODS

2.1. Materials

The plants G. *simplicifolia* were cultivated in their native land Ghana. The seeds were commercial product of Pharmline Inc. and the oil samples of the seeds were kindly supplied by the farm. Three, light yellow oil samples from different seed batches extracted with liquid CO_2 were analysed.

2.2. Methods

2.2.1. *Triacylglycerols*

Triacylglycerols (TG) were separated from the oil by thin layer chromatography (TLC) on silica gel G with hexane - diethyl ether (3:1 v/v). The spots were identified using reference compounds and quantified densitometrically. Plates were sprayed with H_2SO_4 - ethanol (2:3 v/v) and heated at 110°C to visualise the spots.

2.2.2. *Fatty acids*

The oil samples were converted to fatty acid methyl esters by heating in methanol containing 6 % m/m anhydrous HCl at 60°C for 1.5 h. The fatty acid methyl esters were extracted with hexane and purified by TLC on silica gel with hexane - diethyl ether (10:1 v/v).

2.2.3. *Sterols*

Another part of the oil sample was saponified with 5 % m/v KOH in 96 % v/v ethanol for 2 h under reflux. Unsaponifiable matter was extracted with diethyl ether and gravimetrically quantified. Sterols were separated on TLC with hexane - diethyl ether (1:1 v/v).

2.2.4. *Purification of linolic acid*

A mixture of 1 part fatty acid methyl esters, 10 parts urea, and 30 parts ethanol was heated for several minutes until dissolution, and then slowly cooled to obtain the solid urea adducts. The crystals were isolated and the solution was extracted with hexane, evaporated and analysed by gas chromatography.

2.2.5. *Gas chromatography*

Gas chromatography of fatty acid methyl esters was carried out on 10 % DEGS and 2.5 % SE-52,

USE OF GEOTHERMAL FLUIDS AND ENERGY FOR MASS MICROALGAL CULTIVATION

The search for natural raw materials rich in biologically active and harmless substances for the pharmaceutical, cosmetic and food industries is extremely significant nowadays, when modern life is characterized by numerous ecological problems. Microalgae are an untraditional but promising means to attain the above purpose owing to the possibilities to control, optimize and run large-scale production of useful and healthy algal biomass.
The technology for large scale microalgal cultivation (Figure 1) is based on the use of sun as energy and thermal source, natural (or collateral) CO2, alkaline, spring or sea water, mineral elements and simple pond constructions, i.e. all factors that are prerequisites for photosynthesis.

Geothermal energy could be used at the following stages:
1. Use of geothermal water for nutrition algal media preparation;
2. Use of geothermal CO2 and energy for optimizing of the photosynthesis;
3. Use of geothermal energy for drying of the algal biomass.
Good results on the use of geothermal energy and fluids for mass cultivation of the green algae Chlorella and Scenedesmus and the cyanobacterium Spirulina were obtained in Bulgaria and Greece.
The experimental base for production and processing of biomass of green algae in Bulgaria is situated at 42o northern latitude in the geographic area "Rupite". It consists of a total cultivation area of 2,690 sq. m.
Some other installations for algal cultivation have been constructed in a geographic area situated 70 km. South of pite, near town of Nigrita (Greece). The installations have a total area of 1,950 sq.m. and are used for large scale production of Spirulina biomass. Each unit is of concrete. The cultivation units are situated in a green house covered with plastic foil.
Both experimental bases are situated close to mineral spring water evaporating free CO2.
According to the features of the geothermal source (debit, temperature, water composition, amount of CO2), some parameters in the technological scheme of algal cultivation were improved.
It was estabilished that geothermal water that does not contain heavy metals or toxic compounds could be used for preparation of nutrition media; the use of geothermal CO2 decreased the technological expenses by approximately 20%.
By optimizing the daily and sesonal regime of radiation and temperature using geothermal energy, the algal cultivation season was extended and daily algal yield increased by 20-30%.
The use of geothermal energy and fluids optimizes the photosynthesis (yield of biomass) and decreases technological expenses.

Fig. 1. Technological scheme for production and application of microalgal biomass

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Absorber Tower as a Photobioreactor for Microalgae*

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Abstract—Green microalgae were grown under natural light in a photobioreactor similar to a transparent plate absorber. A proper temperature was maintained through the control of evaporation and the minimization of convective heat waste. Carbon dioxide desorption was lower in comparison to its level during cultivation in open or covered ponds. A yield of 1 g/l per day and over 100 g/m^2 projection area was achieved.

Key words: algae - biomass - photobioreactor

INTRODUCTION

The main requirements for an intensive large-scale cultivation of microalgae include a nutritional medium, a carbon dioxide supply, light, and the proper temperature. Nutritional media are preliminarily balanced, and the elements are proportionally exhausted. On the other hand, the desorption of carbon dioxide from cultivation ponds can present a difficulty. The mathematical description of this process is useful for finding the proper length of the cultivation bed [1–3]. Nevertheless, the absorption of CO_2 in a horizontal pond or sloped layer cannot be rationally intensified by the application of a model. Carbon dioxide desorption is a natural process, and the models in these cases remain predominantly cognitive and descriptive. As for light, it is normally more than sufficient [4–8]. In the temperate zone, throughout most of the day, water temperature remains too low. This is due to the intensive evaporation of water in the relatively dry air. That is why evaporation is a factor which must be limited and controlled in order to maintain a near-optimum temperature. According to heat waste and maintenance of optimal temperature, the cultivation devices for microalgae could be subdivided as follows: (1) open ponds [9–11], (2) devices without contact between the algal suspension and the cover [12–15], and (3) devices with direct contact between the algal suspension and the wall of the photobioreactor [16–24]. In all these devices, light absorption, gas exchange, heat exchange, and motion of the liquid take place. Having made rational compromises to combine all these processes, we can achieve a physiological optimum. Here, we present our experience in the outdoor cultivation of microalgae in a transparent counter-current absorber, in which mass exchange, heat exchange, light absorption, and a proper hydrodynamic are combined to insure more acceptable conditions for algal growth.

* This article was submitted by the author in English.

RESULTS AND DISCUSSION

Mass Transfer

The algal suspension is moved vertically, and mineral substances are dissolved and reach every single cell. Carbon dioxide, which is supplied in the algal suspension, and the oxygen which is given off, are unevenly distributed along the cultivation bed. Therefore, the mass transfer process treats mainly the absorption of CO_2 in the algal suspension and desorption of O_2 from it. Carbon dioxide is a weakly soluble gas, and excessively low concentrations are maintained in the suspension. It has been quickly consumed, which leads to the limitation of photosynthesis. The desorption losses increase when CO_2 is supplied in larger amounts. This is why we have introduced counter-current absorption (figure). Perforated plates of transparent plastic material are situated one over the other at a distance of 20–25% of their width in a metal frame. The frame is made of constructive steel rods 8 mm in diameter. In this arrangement, the whole frame is inserted into a transparent plastic sleeve. Carbon dioxide is supplied in the middle of the column, and the algal suspension trickles down through the perforated plates. Desorption losses in such a mass-exchange column are minimized because of a counter current between the gas phase and the great surface of the liquid phase. No hydrostatic pressure is to be overcome, and there is no need for compressors and their maintenance and energy costs. The perforated transparent plates serve as cultivation areas and perform the function of mass transfer.

In the available sloped-layer installations, which are in use in algological practice, there are metal bars. They create hydrodynamic resistance along the course of the flow and enhance the thickness of the layer [9, 10]. In the present column, the side edges of the plates which are 10–12 mm in height perform a similar function. By comparing columns of different sizes, we found that a diameter or width of 0.7 m and height of 2 m are the

USE OF GEOTHERMAL FLUIDS AND ENERGY FOR MASS MICROALGAL CULTIVATION (RESULTS FROM BULGARIA AND GREECE)

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KEYWORDS: Chlorella, Scenedesmus, Spirulina, mass cultivation, geothermal fluids

ABSTRACT

Alternatives for use of geothermal energy and fluids (water and CO_2) in biotechnology for large scale production of microalgae biomass are discussed. The presented results were obtained in green microalgae experimental bases in Bulgaria (2,690 sq. m., 42° northern latitudes, "Roupite) and Northern Greece (1950 sq. m, town of Nigrita). It was established that geothermal water that does not contain heavy metals or toxic compounds, could be used for preparation of nutrition media; the use of geothermal CO_2 decreased the technological expenses by approximately 20%.

By optimizing the daily and seasonal regime of radiation and temperature using geothermal energy, the algal cultivation season was extended and daily algal yield increased by 20-30%.

INTRODUCTION

The use of geothermal energy dates since ancient times and is continuously expanding at present as well (Dickson, Farnelli, 1993). One of the modern technologies for nontraditional and effective use of geothermal fluids and energy is the wide scale cultivation of microalgal biomass (Fournadzieva, et al., 1993).

Microalgae are ancient photosynthesizing life forms that appeared more than 3.5 million years ago. The large variation of existing species provides a wide array of choice for microalgal cultivation.

Attempts for their cultivation started in the last 40-50 years. Relatively few algal species have been used for mass cultivation: Chlorella, Scenedesmus, Dunaliella, Porphyridum, Haema-tococus, and Spirulina, therefore enormous potential for cultivation of other algal species exists.

Microalgae could be used as a source for controlled production of large-scale algal biomass enriched in specific nutrients and natural compounds used in the pharmaceutical industry, cosmetics and food processing.

The technology for large scale microalgal cultivation (Figure 1) is based on the use of solar energy as energy and thermal source, natural (or collateral) CO_2, alkaline, mineral, spring or sea water, mineral elements and simple pond constructions, i.e. all factors that are prerequisites for photosynthesis.

The use of geothermal energy and fluids optimizes the photosynthesis and decreases technological expenses.

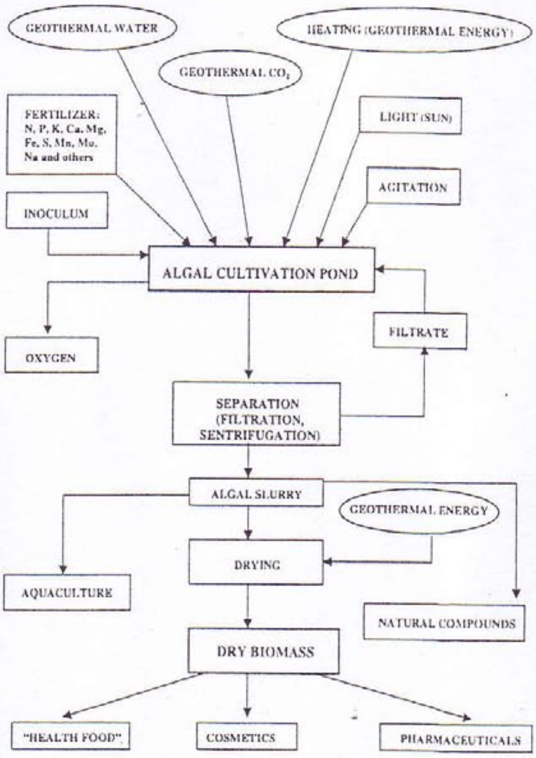

Figure 1. Technological scheme for production and application of microalgal biomass.

Geothermal energy could be used at the following stages:

1. Use of geothermal water for nutrition algal media preparation;
2. Use of geothermal CO_2 and energy for optimizing of the photosynthesis; and
3. Use of geothermal energy for drying of the algal biomass.

This report presents some results on the use of geothermal energy and fluids for mass cultivation of the green algae Chlorella and Scenedesmus and the cyanobacterium Spirulina obtained in Bulgaria and Greece.

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Sterols of the green alga *Coelastrum*

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With 2 tables in the text

Abstract: Sterols and hydrocarbons of the green microalga *Coelastrum cambricum* and *C. sphaericum* were studied. The main sterol in *C. cambricum* is chondrillasterol – 71% and the main sterol in *C. sphaericum* is ergosterol – 90% of the sterols.

Key words: *Coelastrum*, green algae, hydrocarbons, sterols.

Introduction

Green microalgae of the genus *Coelastrum* were studied as a subject for cultivation and production of biomass (RUNKEL et al. 1970, WITSCH & HEUSSLER 1970, DANG 1987, GROBBELLAR et al. 1990). The alga showed sufficient growth, resistance to parasites, technological adaptability, and they appear to be a proper biotechnological object.

A few studies of their lipid composition have been published. GELPI et al. (1970) showed presence of hydrocarbon with 17 carbon atoms and SCHNEIDER et al. (1970) investigated the fatty acid composition of *C. microporum*. In two papers we studied the fatty acid composition of total and unpolar acylolipids of *C. cambricum* and *C. sphaericum* (PETKOV et al. 1986, PETKOV & FURNADZIEVA 1993).

The aim of this paper is to study the composition of sterols and other lipophilic substances of *C. sphaericum* and *C. cambricum*.

Materials and methods

Coelastrum sphaericum NÄG. strain KOMÁREK 1964/84 and *C. cambricum* ARCHER were kindly supplied from Czech Academy of Sciences. They were cultivated in laboratory at 16 klx uninterrupted light intensity and bubbling with 100 $l.h^{-1}$ air enriched with 2–3% CO_2 in a nutrition medium described by ŠETLÍK et al. (1965, 1968) with 2 $g.l^{-1}$ $NaHCO_3$ as adopted here. Outdoor cultivation was carried out

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