CONDITIONS FOR DEVELOPMENT AND CULTIVATION OF CYANOBACTERIA FOR MULTI-PURPOSE APPLICATION (LITERATURE REVIEW)

¹Myroslav Malovanyy, ¹Khrystyna Soloviy, ²Volodymyr Nykyforov, ¹Jaromir Wojtowicz

¹Lviv Polytechnic National University, 79013, 12, S. Bandery Str., Lviv, Ukraine ²Kremenchuk Mykhailo Ostrohradskyi National University 39600, 20, Pershotravneva Str., Krenemchuk, Ukraine christina.gf@gmail.com

Received: 19.11.2017

© Malovanyy M., Soloviy Kh., Nykyforov V., 2018

Abstract. This article contains current data on biology, ecology, and cyanobacteria taxonomi. The results of analysis of bibliographic sources on conditions for nutrition, development and reproduction of cyanobacteria, pecularities of their life cycle, requirements for creating suitable conditions for their cultivation and methods for increasing their biomass production are discussed. An impact factor model on cyanobacteria development is considered and analyzed. Special attention is paid to analysis of information concerning optimal conditions for cyanobacteria cultivation, biomass separation and ways of biomass application for obtaining the target products.

Key words: cyanobacteria: morphology, systematics, biomass, nutrition, target product.

Introduction

The object of our studies is cyanobacteria – the most ancient group of living organisms, remnants of which were discovered in pre-cambrian stromitolites aged 2.7–3.2 billion years. Their cosmopolitism is caused by their almost unlimited adaptive capacities – wide range of environmental tolerance, high reactivity and resistance etc. Among them there are cryophiles (discovered in Antarctic ices at a temperature of –83 °C) and thermophiles (live in hot springs at a temperature of + 90 °C). The reason for such a universal tolerance is the polytropicity of cyanobacteria – the only organisms on the planet that are able to assimilate the following four types of gas: CO₂ for photosynthesis, O₂ for breathing, H₂S for chemosynthesis, and N₂ for its fixation. Within the vegetation period (70 to 120 days), one cyanobacterium cell can produce up to 10²⁰ daughter cells, i.e. one bacterium can generate 10²⁰ such bacteria in this time interval. Such a high rate of reproduction causes their massive development - "bloom" of water. Cyanobacteria are the first primary producers of oxygen on Earth, that under influence of ultraviolet rays transforms into ozon; the letter allowed in due time living organism to get out of water on land. They are also the most efficient accumulative converters of solar energy, because the value of efficiency (η) of their photosynthesis energy conversion reaches 20%; that 200 times exceeds the average value of photosynthesis efficiency (η) of terrestrial plants.

Because cyanobacteria are photosynthetic and aquatic (mainly plankton) microorganisms, they are also often called "blue-green algae" (BGA). Cyanobacteria can produce great amount of biologically active compounds with bactericidal, oncostatic, ultraviolet- and radioprotective and other qualities. For rapid increase of biomass of phototrophic organisms, the most prospective way is the using of CO_2 – product of manmade activity - in order to minimize its negative influence on the environment in general and on atmospheric and climatic processes in particular. It is reasonable to use selected biomass during «blooming» of artificial water bodies for production of target products, in particular, as feedstock for production of energy carriers (biogas, bioethanol, biodiesel), organic mineral fertilizers, etc.

The purpose of our work is to provide critical analysis of literature sources of information on conditions for development and cultivation of cyanobacteria for using their biomass as feedstock for obtaining target products for different application (energy, fuel, chemical substances, etc).

1. Systematics of cyanobacteria

First attempts create a system of BGA were made in XIX century. (Agardh - 1824, Khtzing - 1843, 1849, Thuret - 1875). Further construction of system was continued by Kirchner (1900), and since 1914 considerable alteration of system begun, and whole range of new systems Cyanophyta was published (Elenkin - 1916, 1923, 1936, Bortsi - 1914, 1916, 1917, Heitler - 1925, 1932). Among them the most successful in that period of time was considered a system of A. A. Elenkin, published in 1936. New phase of development started since the 70s of 20th century, when wide range of alhologist admitted that the cell of BGA has no nucleus, and according to system of organic world by Takhtajan (1972), they refered it to subkingdom Oxyphotobacteriobionta of kingdom Bacteriobiota superkingdom Procaryota. In the suggested by Parker (1982) system of algae, blue-green algae are refered to kingdom Procaryota, division Cyanophycota, class Cyanophyceae.

In modern classification of microorganisms, the following hierarchy of taxonomic categories is adopted: domain, phylum, class, order, family, genus, species. The category of domain was suggested as superior to kingdom in order to emphasize significance of division of living organisms into three groups - Archaea, Bacteria, and Eukarya. According to such hierarchy, cyanobacteria are refered to domain Bacteria, phylum B10 Cyanobacteria, which is devided into five subgroups (according to taxonomic scheme of Bergey's Manual of Determinative Bacteriology). I and II subgroups include unicell ular (coccoid) forms or non-trichal colonies (palmelloid) cells, united by layers of gel-like capside. Bacteria of each of the two subgroups differ in ways of their reproduction. III, IV and V subgroups include filamentous (trichal) organisms. Bacteria of each of these subgroups differ from each other in ways of their cellular division and, as they differ in forms of their trichomes (branched or unbranched, uniseriate or multiseriate). Hence, there are 42 genera distinguished among cyanobacteria, including the division into the subgroups: I - 9, II - 6, III - 9, IV – 7, V – 11 [1].

Modern taxonomy of cyanobacteria (Madigan, Michael T., Martinko, John M. (2006) Brock Biology of Microorganisms. 11 ed. Pearson Prentice Hall. New Jersey, USA. p. 396)

subgroup	genera
Ι	Chamaesiphon, Cyanothece, Gloeobacter,
	Microcystis, Gloeocapsa, Gloeothece,
	Myxobaktron, Synechococcus, Synechocystis
П	Chroococcidiopsis, Dermocarpa,
	Dermocarpella, Myxosarcina, Pleurocapsa,
	Xenococcus
III	Arthrospira, Crinalium, Lyngbya, Microcoleus,
	Oscillatoria, Pseudanabaena, Spirulina, Starria,
	Trichodesmium
IV	Anabaena, Aphanizomenon, Cylindrospermum,
	Nodularia, Nostoc, Scytonema, Calothrix
v	Chlorogloeopsis, Fisherella, Geitleria,
	Stigonema, Cyanobotrys, Loriella, Nostochopsis,
	Mastigocladopsis, Mastigocoleus, Westiella,
	Hapalosiphon

2. Biology and ecology of cyanobacteria

Cyanobacteria are oxygenic phototropic prokaryotes that include chlorophylls and phycobilins. Some species of these organisms can fixate nitrogen under their freeliving condition or in symbiosis with aquatic plants, for instance Azolla (Becking, 1978). Cyanobacteria are resistant to extreme conditions of different ecotopes. Cyanobacteria form a large, morphologic heterogenous group of hydrophilic bacteria. Several morphologic levels of cyanobacteria organization are distinguished coccoid, palmelloid, trichal, heterotrichal (uniseriate or multiseriate). Cyanobacteria play significant role for balanced development of hydro-ecosystems, since they are the main, and sometimes the only, producers of primary organic substance in them. Cyanobacteria have adapted to all types of ecotopes of salt water, freshwater, soil, air, etc. During period of time between 2 and 4 billion years ago, cyanobacteria gained ability to photosynthesis, owing to which they secret oxygen till now as product of their vital activity. Thanks to wide and massive spread of cyanobacteria, ancient atmosphere, that at that time had been saturated with carbon dioxide, started to change significantly by oxygen saturation. It is estimated today that from 20 to 30 % of oxygen, obtained from photosynthesis in our planet, is owed to cyanobacteria. This is exactly why they played central role in alteration of air content and atmosphere structure. Cyanobacteria perform also important functions in edaphotops, providing fertility of soils via nitrogen fixation. Some plants evolved thanks to mutualism between colonies of cyanobacteria, that develop in rhizosphere of plants. Other species of cyanobacteria create mycorhiza with fungi hyphae. Cyanobacteria fix oxygen not only in soils, but in coral reefs as well as in other marine ecotopes, making nitrogen available to other organisms under conditions of different ecosystems [2].

3. Cytology, morphology, and biochemistry of cyanobacteria

Cyanobacteria cells are bigger in sizes and more complex as to their structure than other bacteria. Structural and functional organization of cyanobacterial cell (Fig. 1) has typical of procaryots organelles: mucous capside of murein, that wraps cellular wall from the outside from four layers of peptidoglycan, bare ring DNA –nucleoid, submerged in sol-gel hyaloplasm, tiny compared to eukaryotic ribosomes without endoplasmic reticulum, plasmalemma, that separates cellular wall and cytoplasm, mesosomes – mono-membrane formations, that perform functions of mitochondria in prokaryotes and also simplified in structure golgiosomes. Ectoplasm contains photosynthetic thylakoids, that carry title of chromatophores, that migrate in parietal litine space alongside with movement of cytoplasm.

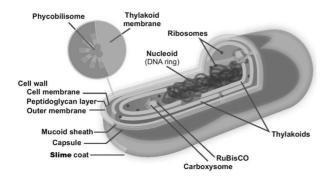


Fig. 1. Structural and functional organization of cyanobacteria cell (http://lakes.chebucto.org/)

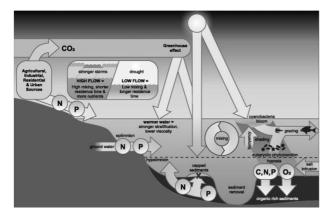


Fig. 2. Conceptual model of impact factors' influence on development of cyanobacteria (after Paerl et al. 2011)

Blue-green algae are coloured mainly in blue-green colour, under extreme conditions they have often colour with different shades of red. Green shade of cells is given by bacteriochlorophyll "a". Red or blue colour is caused by presence of phycobilin pigments - phycocyanin and allophycocyanin (blue) and phycoerythrin (red). Carotenoids are represented only by β -carotenes xanthophyll (lutein, zeaxanthin, oscilloxanthin, and myxoxanthin, aphanin, and aphanizophyll, etc). Differentiated functioning of phycobilins provides photosynthetic apparatus of cyanobacteria with ability to absorb maximum quantity of sunlight - assimilation of energy of almost full spectrum of photosynthetic active radiation, this caurses maximum value of η . [3].

Almost in all cyanobacteria the main reserve product of assimilation is glycogen-like polysaccharide - bacteriostarch. Besides carbohydrates, they also accumulate cyanophicin and volutin. Size of cyanobacteria cell can vary from 0.5 to 40 µm in diameter. Structure of wall is similar to gram-negative bacteria (Gerba, Maier and Pepper, 2000). Some cyanobacteria form complex or multilayer photosynthetis membrane system, that consists of slime coats that combine cells in the trichomes or colonies. Some filamentous cyanobacteria form heterocysts - round, empty looking cells, that specialize in nitrogen fixation, that are spread usually along or at the end of trichomes [4]. Some species are toxic (the most studied toxin is microcystin, that is produced by species Microcystis aeruginosa particularly) or conditionally pathogenic (species of genus Anabaena). Main agents of water "blooming" causing massive fish exhaustion and toxication of animals and humans, appear for instance during "blooming" of reservoirs of Ukraine.

4. Physiology and ecology of cyanobacteria

The life cycle of cyanobacteria consists of alternation of the following stages: bacterial mitosis (division), vegetative growth, and dormant state (spores). Before cellular division, the amount of DNA is doubled and then divided in two. Mitosis in cyanobacteria appears in such a way that on the side wall of cell there appears a ring plica, created by cytoplasmic membrane and inner layers of cellular wall. Growing in centripetal direction, this plica locks like diaphragm of microscope, forming cross web - septum that cuts thylakoids and protoplast in two daughter cells that during vegetation phase are increasing due to assimilation of substances and creation of new organelles, hence preparing to the next stage - mitosis or creation of spores. Under unfavorable conditions, creation of spores takes place. As in case of heterocysts, vegetation cells that transform into spores lose their gas vacuoles. This process is stimulated by different

environmental factors, but mainly by phosphorus. Contents of DNA in spores noticeably increases, sometimes in 20-30 times comparing to vegetation stage. Spores can bear desiccation and other extreme Appearing under favourable conditions, conditions. they can start growing immediately after their emergence, without any dormant state. Under absence of such favourable conditions, spores keep vital capacity for lasting period of time (several decades). During growing of spores, one sprout is created that is released through wrack of coat. Most unicellular and colonial cyanobacteria reproduce with the help of division of cells in two, in some cases it is with help of tiny cells gonidia, that are created inside maternal cell or ribbed from the top of maternal cell. Majority of filamentous forms reproduce with the help of hormogonia - spaces where filaments are disintegrated.

Cyanobacteria do not need vitamins for existence and development. They can use nitrates or ammonia as source of nitrogen and also phosphorus compounds and microadditives of such elements as ferrum, sulphur, zinc, copper, manganum, cobaltum, molybdenum, etc. Most of their species are phototrophs, but some filamentous types can grow in darkness, using some carbohydrates (glucose or saccharose) as source of energy. Optimum conditions for cyanobacteria growth lay in complex of interrelated, mostly abiotic factors. Problem of cyanobacteria growth influence factors of Anabaena, Aphanizomenon, Cylindrospermopsin, Nodularia, Lyngbya, Oscillatoria, Microcystis, Planktothrix was researched by variety of scientists (Chorus and Bartram 1999, Carmichael 2008, Paerl and Huisman 2008, Hudnell 2010, O'Neill et al. 2012, Paerl and Paul 2012). Among other factors that form conditions of cyanobacteria growth, they concentrated their studies on salinity, temperature, pH, trophism, radiation, hydrodynamics of the environment. In Fig. 2, a conceptual model of factors influencing a life cycle of cyanobacteria created by Paerl et al. in 2011 is shown [5]. This model involves temperature, laminar conditions in growth environment, exposition of residence of cyanobacteria in the environment, supply of carbon (C), nitrogen (N) and phosphorus (P) from sources of man-made and arable contamination.

4.1. Salinity (mineralization)

Marine cyanobacteria *Prochlorococcus*, *Synechococcus* and *Trichodesmium* have shown high salinity tolerance as a result of laboratory experiments. This is why they are universally tolerant species. For instance, stenohaline tolerant species of genus *Cylindrospermopsis* vegetate in water with mineralization up to 2.5 ppt, and universally tolerant species of genera *Anabaenopsis* and *Nodularia* vegetate under salinity of 5–20 ppt (Moisander *et al.* 2002). Species *Microcystis aeruginosa* tolerate the level of salinity up to 10 ppt at which no change in growth can be found in comparison to freshwater (Tonk *et al.* 2007). Based on results of these experiments, one may claim that under optimum growth conditions these species can vegetate in regions where the water is more salty. Over the last decades, there is a tendency of extension of area of these cyanobacteria species in littoral ecosystems under conditions of middle salinity (5–15 ppt) (Paerl and Paul 2012). For instance, «blooming» caused by *Microcystis aeruginosa* appears in Baltic Sea (Maestrini *et al.*1999) and the San Francisco Estuary (Lehman *et al.* 2013). These scientists prove that special extension of microcystis is affected not by salinity, but by other environmental factors.

4.2. Level of concentration of biogenic elements

Like in other phytoplanktons, under optimum conditions of temperature and irradiance (so called phototermal pair of ecofactors), production of biomass in cyanobacteria proceeds in direct proportion to the quantity of biogenic elements, mainly nitrogen and phosphorus, that are available in the environment. Results of many scientists' research have shown that the growth of cyanobacteria in freshwater natural (rivers and lakes) and artificial (channels, reservoirs, ponds) ecosystems is mainly caused by excessive concentration (Likens 1972, Schindler 1977, of phosphorus Edmondson and Lehman 1981, Elmgren and Larsson 2001, Paerl 2008, Schindler et al. 2008). Unlike freshwater biohydrocenosis, estuarial and marine ecosystems are more sensitive to concentration of nitrogen, and eutrophication caused by cyanobacteria growth also is often related to excessive concentrations of nitrogen. (Ryther and Dunstan 1971, Nixon 1986, Suikkanen et al. 2007, Paerl 2008, Conley et al. 2009, Ahn et al. 2011).

The supply of nutrients from stationary and nonstationary sources (man-made and arable unpurified wastewaters) causes simultaneous increase of phosphorus and nitrogen concentrations (Paerl and Paul 2012, Paerl et al. 2014b). Results of research done in summer have shown that under conditions of achievement of maximum biomass of cyanobacteria under minimum concentration of nutrients, nitrogen and phosphorus both equally affect biomass accumulation in ecosystem (Paerl et al. 2014a). Overall, domination of both nitrogen fixating and non-fixating species Aphenizomenon flos aquae, Nodularia spumigena, Microcystis aeruginosa and Cylindrospermopsin raciborskii in phytoplankton's structure is caused by increase of both nitrogen and phosphorus concentrations (Chapman and Schelske 1997, Jacoby et al. 2000, Gobler et al. 2007, Burford et al. 2006, Burford and O'Donahue 2006, Hong *et al.* 2006, Suikkanen *et al.* 2007, O'Neill *et al.* 2012). For the decrease of phytoplankton bioproductivity, concentrations of biogenic elements must be several times lower than those of phytomass. Herewith, the need in nitrogen and phosphorus compounds in cyanobacteria is higher than in eukaryotic phytoplanktons because of high demand in proteins.

4.3. Irradiance and water clarity

Due to carotenoid pigments, cyanobacteria cells dissipate excessive light energy, which, in its turn, provides access for irradiance to their photosynthetic apparatus without photoinhibition (Paerl *et al.* 1983, 1985). Due to buoyancy, such cyanobacteria as *Microcystis* vegetate very close to water surface, having tolerance to levels of irradiance that are limitation for other representatives of phytoplankton. As a result, cyanobacteria can increase density of their cells, while under typical conditions they would limit the access to light with their shadow. Due to growing almost in neuston zone, cyanobacteria are insensitive to irradiance limitation, even if there exists a high concentration of suspended pollutants in water.

4.4. Temperature and pH level

One may talk about temperature as of one of the most limiting abiotic factors that affect growth of cyanobacteria (Robarts and Zohary 1987, Butterwick et al. 2005, Reynolds 2006, Paerl and Huisman 2008). The range of thermotolerance for majority of cyanobacteria species in all climate types with exception of polar zones, is from 25 to 35 °C (Reynolds 2006, Lürling et al. 2013). During researches on eight species of cyanobacteria, it was discovered that optimal temperature for vegetation of Microcystis aeruginosa is 30-32.5 °C, for Aphanizomenon gracile optimal temperature is 32.5 °C, for species Cynlindrospermopsis raciborskii and Planktothrix agardhii 27.5 °C, and optimal temperature for «bloom» of species Anabaena sp. is 25 °C (Lurling et al. 2013). Overall, vegetation can take place under conditions from cryophilic (+4 °C) to thermophilic (+75 °C), for example in the case of Synechococcus lividus [6, 7]. Photosynthetic activity without visible changes takes place even at a temperature of +30 °C [6, 8]. Miyake [9] and Nishioka [10] reported on synthesis of polyhydroxyalkanoates in Synechococcus MA19 at a temperature of +50 °C, when almost all researchers conducted experiments in the temperature range from +20 to +30 °C. Thermophilic conditions are favourable for photosynthesis because of speeding up of biochemical processes of metabolism and due to considerably lower risk of autointoxication. Nevertheless,

cyanobacteria can produce polyhydroxyalkanoates very seldom and thermophilic synthesis in large bioreactor causes high costs of thermal isolation [6].

According to Brock, cyanobacteria are unable to grow under a level of pH lower than 4–5. For representatives of alkaliphiles, optimal pH range for growth is between 7.5 and 10. Though pH level with alkalinity and temperature affect modification of dissolved organic carbon, influence of pH on cyanobacteria growth is independent. Of course, it is impossible to generalize requirements for optimal level of pH for normal vegetation of cyanobacteria, since it depends on strain (culture) or wild race of particular species and on the pH of its natural environment [7].

5. Factors for optimum cultivation of cyanobacteria

5.1. Application of man-made carbon dioxide

In order to provide balanced environmental management and minimize negative impact from anthropogenic activity, it is reasonable, in our opinion, to have such an approach that is based on using carbon dioxide as second photosynthesis reagent for increase of bioproductivity of cyanobacteria, hence as the only source of carbon for all biomolecules of the cell. For intensification of mitosis, that provides increase of biomass in natural or artificial, opened and closed systems, the use of industrial gas that contains CO₂ is widespead [11]. For modern cultivation systems which allow us to use obtained cyanobacteria biomass for production of high quality products (food products and nutritional supplements), the price for CO_2 is not critical. If cultivation aimed at production of polyhydroxyalkanoates, that have lower economic value then serious question emerges of the search for cheap sources of carbon dioxide. There is great number of researches who suggest using different formations of CO₂ (flue, production and other industrial gases) (Tabl. 2) [11].

Table 2

Application of carbon dioxide of different genesis for cyanobacteria cultivation

Gas type	Cyanobacteria species	Source of CO ₂
	Phormidium valderianum	Coal combustion flue
Flue gases	Atrhrospira platensis	gases
	Arthrospira sp.	Synthetic flue gas
	Synechocystis	Flue gas from natural
	sp.	gas combustion
Biotechnological	Arthrospira platensis	CO ₂ -offgas from
e		ethanol fermentation
gases		Biogas

5.2. Alternative nutrition environment for cyanobacteria

Among modern researches of dynamics of cyanobacteria biomass development under cultural conditions, high attention is being payed to application of synthetic sources of nutrition [11]. It is proved that by using alternative sources of biogenic macroelements (agricultural and industrial wastewater, degistate after biomethanogenesis, exhausted activated sludge etc) it is possible to ensures stable increase of cyanobacteria biomass [11]. The biomass weight gained in opened and closed cultivation systems reaches 0.5-1 and 2-9 g/l, respectively [12]. The necessary condition is a need in large amount of water. Purification of industrial effluents is the next important condition for providing optimum growth of microalgae, that supplies both production of valuable biomass and microalgae purification [13]. On the other hand, new requirements appear concerning contamination of cultural liquid with microbes, heavy metals, antibiotics and other growth inhibitors that are contained in wastewater. Seasonal fluctuation of concentrations is typical of all these components [14].

This is why modern researches are aimed at cultivation of cianobacteria with using for their nutrition exhausted biomass of anaerobic digestion, so called degistate and also wastes and effluents from agricultural complex [4, 11, 15–18], aiming also to purify the latter. After primary purification of wastewater, the water can be transferred to the next level of purification or used as reclaimed, and lipids obtained through extraction of received cyanobacteria biomass can be used for production of biodiesel. [12].

5.3. Two-stage cultivation

In the case of cultivation of cyanobacteria biomass for production of polyhydroxyalkanoates, two stages of technology were studied [6]. At first, cyanobacteria grew in medium, saturated with nutrients then the obtained at first stage biomass was carried into a medium with impoverished concentrations of biogenic elements for initiation of polyhydroxyalkanoates and other products synthesis. However, under conditions of large-scale production there appear difficulties with separation of large amount of biomass from primary medium and with transportation to another stage. Moreover, it leads to emergence of stressed state in cells, and with application of transversal forces in the process of transportation of biomass and oxygen deficiency causes lagging in biomass accumulation [6]. The method of two-stage cultivation was used in researches by Gruber et al., 2016 [19]. Researches were conducted with using species of green microalgae

Acutodesmus obliquus for optaining a biomass in nutrient environment with excess and deficiency of nitrogen compounds. In this case the method of dehydration was used before carrying to anaerobic degistation (Fig. 3).

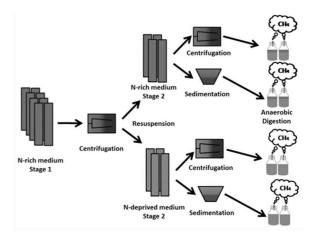


Fig. 3. Two-stage cultivation scheme by Gruber et al.

According to experimental construction of twostage cultivation, microalgae were cultivated and harvested in stationary stage. After that, biomass was separated from nutrition medium by centrifugation and resuspended (inserted) at first in a nitrogen enriched medium and then in nitrogen impoverished medium. After that, biomass of microalgae was subjected to dehydration: centrifugation and sedimentation. During bioproduction processes on dynamics of methane creation under conditions of anaerobic degistation was studied [19].

5.4. Peculiarities of biomass separation

After accumulation of enough amount of biomass resulted from cyanobacteria cultivation, its excessive fraction is periodically harvested. Technical water that contains residiol nutrients after biomass harvesting can be transfered to the next purification stage. Biomass can be processed in anaerobic way, or after dissolving in hydrothermal way it can be mineralized. Purification of technical water can contain cleaning from sediments of dissolved organic compounds [11–13]. It is necessary to note that cyanobacteria cultivation is accompanied by processes of autoselection. Autoselection - combination of cultivation conditions, favourable for application of strain, and non-favourable for contaminants substence are important goals for any biotechnological process. For cyanobacteria, the selection process can be achieved by conditions of maintenance of some parameters simultaneously: the need in dissolved organic compounds of carbon, limitation of nitrogen and phosphorus compounds, walue of pH not lower than 8.5 etc. Researches [6] have shown secondary growth of

green algae *Chlorella sp.* When the culture reaches its stationary phase, cells die and release in the environment secondary metabolites that can be nutrition for growth of new cells. When conducting cyanobacteria cultivation, it is important to remember that under non-sterile conditions, microbic, in particularly fungi, contamination is inevitable. Hence, purification turns out to be one of necessary requirements for final stages of cyanobacteria biomass separation for further processing [11].

5.5. Ways of utilization of cyanobacteria biomass

Cyanobacteria biomass contains many target products that are valuable for different fields of modern bioeconomics: food, pharmaceutical and perfumery industry. Under natural conditions, these bacteria massively grow for centuries as primary source of organic compounds [20]. In our time, much effort has been put in field of genetic engineering for modification phototrophic microorganisms, especially of cyanobacteria, - producers of new useful compounds (target products) that are not synthesized in natural way [21, 22]. Topical direction of modern studies is also environmental biotechnology and bioenergetics that anticipate direct application of cyanobacteria largetonnage biomass and other massive forms of hydrobionts as raw material for biofuel production (biomethane, bioethanol, and biodiesel) and mineralorganic fertilizer [23, 24].

Task concerning management of processes of cyanobacteria biomass production depends on the choice of target product, that in its turn defines methods of its production cultivation. For bioethanol production, a method of yeast fermentation is used. In this case, the target for biotechnology is to create strains of cyanobacteria that synthesize large amount of bacteria starch or bacteria glycogen that are substrate for alcohol fermentation. In this strategic case of biomass utilization, cyanobacteria have some benefits over eukaryotes, since their cellular walls contain peptidoglycan layers [25], it can perform lysis, unlike majority of algae, walls of which consist of polysaccharides and proteoglycan [26]. Moreover, the way of residence of carbohydrates is very important, if biomass was used as substrate for fungi, in particular, for alcohol fermentation.

Different ways [20] (fermentative, chemical, physical, including desiccation, heating, acid and alkani hydrolysis) of preliminary procession of biomass *Synechococcus* aiming to release monomeric hexose were also studied. It is also familiar that species of *Synechococcus* accumulate reserve carbohydrates glycohen and cyanophicin and do not synthesize

polyhydroxybutyrate, as is seen in other cyanobacteria [27-29]. Accumulation of nitrogen compounds in biomass during process of growth of Synechococcus causes coordinative complex physiologic adaptations, that allow photosynthesis and growth to continue to certain limit [30-33]. It manifests itself in increase of productivity of process of polysaccharides assimilation (mainly glycogen) and dissimilation of nitrogen compounds, including light-absorbing pigments phycobilisome proteids [27, 33]. Biomass of Synechococcus can be significantly degraded under impact of fermentation processes and serve simultaneously both as substrate and as source of nutrition elements for fungi fermentation. This in its turn manifests itself in greater productivity with respect to ethanol than in previous studies, where cyanobacteria cellular biomass was used [24]. During the studies it was also established that fermentative hydrolysis of biomass of cyanobacteria can be also used as a source of nutrients for increase of alcohol fermentation completeness [34-37].

Production of another fuel type - biogas (biomethane) - using method of anaerobic fermentation of cyanobacteria (biomethanogenesis) that uncontrollably grew during summer period in reservoir water area of watercascades (species of Microcystis, Dnieper Phormidium, Merismopedia, Aphanizomenon, Anabeana and Oscillatoria) was studied by other scientists [38-54]. It was shown that an effective method of reducing of environmental risk level from uncontrolled development of cyanobacteria growth in artificial reservoirs of Dnieper watercascades can be the method of harvesting of cyanobacteria and using them as raw material for standarded biogas. There was also established an effectiveness of preliminary cyanobacteria biomass processing in field of hydrodynamic cavitation for the increase of completeness of biomass decay (amount of extracted lipids increases in 3.2 times, amount of synthesized biogas in 1.4 times) [45-54].

In many publications, advantages of using of microalgae for biodiesel fuel production in comparison to another accessible feedstock [55-64] is described. All these researchers focused their studies on artificial cultivation of microalgae. The advantage consists in the ability to use for aquatic algae plantation, which are unconsumable for the human, in life support of the microalgae by solar energy, the latter during photosynthesis converts into chemical energy of primary organic compounds, mainly carbohydrates; and another advantage is that one generation of cyanobacteria life cycle lasts several days [57]. Biodiesel production technology from microalgae includs biomass growth stage, extraction of lipids from biomass, and production of biodiesel from them with application of existing processes and technologies that were used for other types of feedstock. For effective utilization of biomass, important is an operation of harvesting and concentration of microalgae. The price for this operation is of 20–30 % of the general price of biodiesel production [63]. Technology of microalgae concentration can include several processes (physical, chemical or biological), with the help of which a necessary level of solid and liquid phases seperation is achieved.

Experiments have shown that although no universal method for harvesting and concentration of microalgae exists (it is still productive field for studies), for each particular algae species optimal economical ways and methods can be made [65, 66]. After concentration, in majority of cases, biomass dehydration is applied, this leads to the increase in its maximum term of storage. For microalgae, The following ways of dehydration are used: drum, pulverizer, sublimation or solar desiccation [67]. Extraction of lipids and pendent fatty acids from biomass is conducted directly from lyophilized biomass. For extraction, there can be used such solvents as

hexane, ethanol, or mixture of both hexane, and ethanol which allows us to extract up to 98 % of purified lipids, and fatty acids [68]. Studies [69] have shown that in case of damage of cellular wall of algae with help of ultrasound procession, extraction of target product increases from 4.8 % to 25.9 %. From the obtained feedstock, biodiesel can be produced using traditional technology– repeated esterification of plant oils. Lipid feedstock consists of 90–98 % (weight) of triglyceride and of small amount of mono- and diglyceride, it contains free fatty acids (1–5 %) and small amounts of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and remnants of water [70].

Alassali *et al.* (2016) dedicated their studied to establishment of possible spectrum of small-tonnage target products, that can be obtained by means of extracting them from microalgae and to potential abilities of their application. In Fig. 4, alternatives of application of secondary metabolite from micro and macroalgae is shown [71].

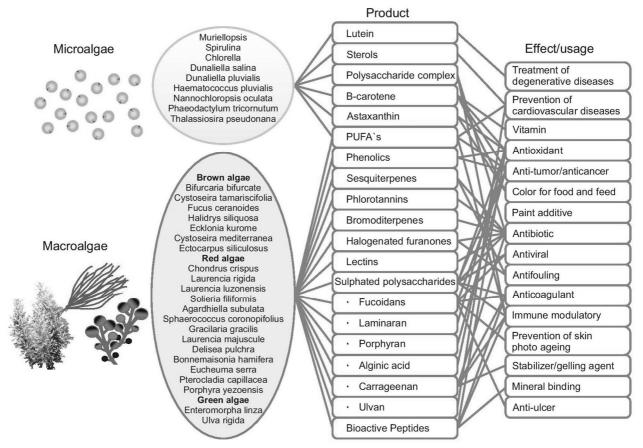


Fig. 4. Possible applications of target products from algae

Conclusions and recommendations

Cyanobacteria, that caused "oxygen revolution" at the beginning of emergence of live on the Earth, even till now play significant role in enrichment of atmosphere with oxygen (about 30 % of all planetary photosynthetic oxygen received yearly), and in nitrogen fixation. Conditions for cyanobacteria development include a complex of interrelated environmental factors: salinity, temperature, pH, nutrients concentration, irradiance, hydrodynamic situation, optimal parameters of which for different cyanobacteria species are in broad ranges, this determins broad extension of them in ecosystems and their ability to adapt under conditions of change of these factors. Cyanobacteria cultivation strategy, as actual direction of modern biotechnology and bioenergetics, involves the using of industrial wastes, products of fuel combustion and exhausted gases, man-made and arable wastewaters as sources of biogenic elements of nutrition (carbon-, nitrogen- and phosphorus elements). Cyanobacteria biomass released from cultural medium can be used for production of small-tonnage valuable products with unique properties. Large tonnage biomass of natural origin can be used for fuel production (biomethane, bioethanol, biodiesel).

References

- [1] Efimova M. V., Efimov A. A. Sinezelenie vodosorti ili zianobakterii? Voprosy sistematiki // Sovremennye problem nauki I obrazovaniya, 2007. – № 6–1; URL: https://www.science-education.ru/ru/article/view?Id=710 (date of last access 30.11.2017)
- [2] Scott Johnson (2017) Roles of Cyanobacteria in the Ecosystem. https://sciencing.com/.Last accessed 30.09.2017
- [3] Manisha M. Cyanobacteria: Occurrence, Morphology and Cell Structure. 2017.http://www.biologydiscussion. com. Last accessed 30.09.2017
- [4] McMurdo Dry Valleys Long-Term Ecological Research Site. Cyanobacteria. http://huey.colorado.edu.2017. Last accessed: 30.09.2017
- [5] Berg M. and Sutula M. 2015. Factors affecting the growth of cyanobacteria with special emphasis on the Sacramento-San Joaquin Delta. Southern California Coastal Water Research Project Technical Report 869 August 2015
- [6] Drosg B. et al., Photo-autotrophic Production of Poly(hydroxyalkanoates) in..., Chem. Biochem. Eng. Q., 29(2) 145–156 (2015)
- [7] Castenholz, R. W., A Handbook on Habitats, Isolation, and Identification of Bacteria, Section B, Springer, Berlin Heidelberg 1981, pp. 236–246.
- [8] De Vera, J.-P. P., Möhlmann, D., Leya, T., Photosynthesis activity of frozen cyanobacteria, snow alga and lichens as pre-tests for further on studies with simulation of Mars equatorial latitude temperatures, EPSC Abstracts 4(2009) 355.
- [9] Miyake, M., Erata, M., Asada, Y., J Ferment Bioeng 82 (1996) 516–518. doi: http://dx.doi.org/10.1016/S0922-338X(97)86995-4
- [10] Nishioka, M., Nakai, K., Miyake, M., Asada, Y., Taya, M., Biotechn Letters 23(2001) 1095–1099. doi: http://dx.doi.org/10.1023/A:1010551614648
- [11] Clemens Troschl, Katharina Meixner and Bernhard Drosg. Cyanobacterial PHA Production—Review of Recent Advances and a Summary of Three Years' Working Experience Running a Pilot Plant,

Bioengineering 2017, 4, 26; doi:10.3390/bioengineering 4020026

- [12] Kumar, K.; Mishra, S. K.; Shrivastav, A.; Park, M. S.; Yang, J.W. Recent trends in the mass cultivation of algae in raceway ponds. Renew. Sustain. Energy Rev. 2015, 51, 875–885. [CrossRef]
- [13] Markou, G.; Vandamme, D.; Muylaert, K. Microalgal and cyanobacterial cultivation: The supply of nutrients. Water Res. 2014, 65, 186–202. [CrossRef] [PubMed]
- [14] Markou, G.; Georgakakis, D. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. Appl. Energy 2011, 88, 3389–3401. [CrossRef]
- [15] Chaiklahan, R.; Chirasuwan, N.; Siangdung, W.; Paithoonrangsarid, K.; Bunnag, B. Cultivation of spirulina platensis using pig wastewater in a semicontinuous process. J. Microbiol. Biotechnol. 2010, 20, 609–614. [CrossRef] [PubMed]
- [16] Cicci, A.; Bravi, M. Production of the freshwater microalgae scenedesmus dimorphus and arthrospira platensis by using cattle digestate. Chem. Eng. Trans. 2014, 38, 85–90. [CrossRef]
- [17] Fouilland, E.; Vasseur, C.; Leboulanger, C.; Le Floc'h, E.; Carré, C.; Marty, B.; Steyer, J.-P.P.; Sialve, B. Coupling algal biomass production and anaerobic digestion: Production assessment of some native temperate and tropical microalgae. Biomass Bioenergy 2014, 70, 564–569. [CrossRef]
- [18] Olguín, E.J.; Galicia, S.; Mercado, G.; Pérez, T. Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. J. Appl. Phycol. 2003, 15, 249–257. [CrossRef]
- [19] M. Gruber-Brunhumer et al. Elsevier Ltd. 2016. http://dx.doi.org/10.1016/j.algal.2016.04.0162211-9264/
- [20] K Benedikt Möllers et al., Biotechnology for Biofuels.2014, vol 7, no. 1. DOI: 10.1186/1754-6834-7-64
- [21] Sauer J., Schreiber U., Schmid R., Völker U., Forchhammer K: Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Plant Physiol.
- [22] Van Baalen C: Studies on marine blue-green algae. Bot Mar. 1962, 4: 129–139.
- [23] John R. P., Anisha G., Nampoothiri K. M., Pandey A.: Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol. 2011, 102: 186–193. 10.1016/j.biortech.2010.06.139.
- [24] Aikawa S., Joseph A., Yamada R., Izumi Y., Yamagishi T., Matsuda F., Kawai H., Chang J.-S., Hasunuma T,. Kondo A.: Direct conversion of Spirulina to ethanol without pretreatment or enzymatic hydrolysis processes. Energy Environ Sci. 2013, 6: 1844–1849. 10.1039/c3ee40305j.
- [25] Hoiczyk E., Hansel A.: Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol. 2000, 182: 1191-1199. 10.1128/JB.182.5.1191-1199.2000.
- [26] Domozych D. S.: Algal cell walls. eLS. 2011, Chichester: John Wiley and Sons
- [27] Stevens S., Balkwill D., Paone D.: The effects of nitrogen limitation on the ultrastructure of the

cyanobacterium Agmenellum quadruplicatum. Arch Microbiol. 1981, 130: 204-212. 10.1007/BF00459520.

[28] Stevens S. E., Paone D. A., Balkwill D. L.: Accumulation of cyanophycin granules as a result of phosphate limitation in Agmenellum quadruplicatum. Plant Physiol. 1981, 67: 716–719. 10.1104/pp.67.4.716.

10

- [29] Beck C., Knoop H., Axmann I. M., Steuer R.: The diversity of cyanobacterial metabolism: genome analysis of multiple phototrophic microorganisms. BMC Genomics. 2012, 13: 56-10.1186/1471-2164-13-56.
- [30] Luque I., Forchhammer K.: Nitrogen assimilation and C/N balance sensing. The cyanobacteria: molecular biology, genomics and evolution. Edited by: Herrero A., Flores E. 2008, Norfolk: Caister Academic Press, 335– 382.
- [31] Schwarz R., Forchhammer K.: Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. Microbiology. 2005, 151: 2503-2514. 10.1099/mic.0.27883-0.
- [32] Sauer J., Schreiber U., Schmid R., Völker U., Forchhammer K.: Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Plant Physiol.
- [33] Paone D. A., Stevens S. E.: Nitrogen starvation and the regulation of glutamine synthetase in Agmenellum quadruplicatum. Plant Physiol. 1981, 67: 1097–1100. 10.1104/pp.67.6.1097.
- [34] Choi S. P., Nguyen M. T., Sim S. J.: Enzymatic pretreatment of Chlamydomonas reinhardtii biomass for ethanol production. Bioresour Technol. 2010, 101: 5330–5336. 10.1016/j.biortech.2010.02.026.
- [35] Harun R., Danquah M. K., Forde G. M.: Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol. 2010, 85: 199–203.
- [36] Harun R., Danquah M. K.: Influence of acid pretreatment on microalgal biomass for bioethanol production. Process Biochem. 2011, 46: 304–309. 10.1016/j. procbio.2010.08.027.
- [37] Harun R., Jason W., Cherrington T., Danquah M. K.: Exploring alkaline pre-treatment of microalgal biomass for bioethanol production. Appl Energy. 2011, 88: 3464– 3467. 10.1016/j.apenergy.2010.10.048.
- [38] Nikiforov V. V. O metodakh podavleniya massovoho razvitiya sinezelenykh vodorostey / Nikiforov V. V. // Visnyk problem biolohii I medytsyny. – 2002. – Vyp. 4. – S. 27–31.
- [39] Nikiforov V. V. Khimiko-toksykolohichni problemy pidhotovky pytnoi vody pry dii ekstremalnikh pryrodnykh chynnykiv / V. V. Nikiforov, T. F. Kozlovska // Visnyk KDPU. – 2002. – Vyp. 5 (16). – S. 106–108.
- [40] Elizarov O. I. Pro mozhlyvist vykorystanna hidrobiontiv dlya otrymanna biohazy / O. I. Elizarov, A. V. Luhovoy, V.V. Nikiforov //Visnyk KPDU. – 2006. – Vyp. 6(41). – S. 43–44.
- [41] Nikiforov V. V. O metodakh podavleniya massovoho razvitiya sinezelenykh vodorostey / V. V. Nikiforov // Visnyk problem biolohii i medytsyny. – 2002. – Vyp. 4. – S. 27–31.

- [42] Nikiforov V. V. Osobennosti khozyaystvennoho znacheniya sinezelenykh vodorostey v usloviyakh Kremenchuhskoho I Dneprodzerzhinskoho vodokhranilishch / V. V. Nikiforov, T. F. Kozlovskaya / Visnyk KDPU. – 2002. – Vyp. 5(16). – S. 109–108.
- [43] Nikiforov V. V. Khimiko-biolohicheskie prichiny ukhudsheniya kachestva prirodnoy vodi / V. V. Nikiforov, T. F. Kozlovskaya // Visnyk KDPU. – 2002. – Vyp. 6(17). – S. 82–85.
- [44] Nikiforov V. V. Rezultati biotestirovaniya pitevoy vodi na raznikh stadiyakh ee podhotovki k potrebleniyu / V. V. Nikiforov, T. F. Kozlovskaya // Ekolohiya ta noosferolohiya. Naukoviy zhurnal Dnipropetrovskoho natsionalnoho universitetu. – 2001. – T. 10, No. 1–2. – S. 99–105.
- [45] Malovanyy V. S. Optimalni umovi otrymanna enerhii iz tsianobakteriy / M. S. Malovanyy, O. D. Sinelnikov, O. V. Kharlamova, A. M. Malovanyy // Khimichna promislovist Ukrainy. – 2014. – No. 5. – S. 39–43.
- [46] Malovanyy M. S. Otsinuvanna ekolohichnoi nebezpeky v akvatoriyakh Dniprovskikh vodoskhovyshch vnaslidok nekontrolovanoho rozvitku tsianobakteriy / M. S. Malovanyy, V. V. Nikiforov, O. V. Kharlamova, O. D. Sinelnikov // Naukoviy visnyk NLTU Ukrainy. – 2015. – Vyp. 25.6. – S. 159–164.
- [47] Malovanyy M. S. Vpliv hidrodynamichnoi kavitatsii na biolohichni objekty / M. S. Malovanyy, V. V. Nikiforov, O. D. Sinelnikov ta in. // Tekhnolohichnyy audit ta rezervy vyrobnytstva- 2015. – No. 5/4(25). – S. 41–45.
- [48] Malovanyy M. Priropokhrannye i enerheticheskie aspekty biotekhnologii utilizatsii tsianobakteriy kak ekoloho-ekonomicheskiy imperativ ustoychivoho razvitiya / M. Malovanyy, V. Nikiforov, E. Kharlamova, A. Sinelnikov // Teoriya ustoychivoho razvitiya, Varna. – 2015. – №1(22). – S. 4–9.
- [49] Malovanyy M. S. Vykorystanna zianobakteriy dlya otrymanna enerhonosiiv – shlyakh do unyknenna ekolohichnoi nebezpeky vid ikh nekontrolovanoho rosvytku v vodoskhovyshchakh Dniprovskoho kaskadu / M. S. Malovanyy, V. V. Nikiforov, O. V. Kharlamova, O. D. Sinelnikov // Stalyy rozvytok – XXI stolitta: upravlinna, tekhnolohii, modeli: Dyskusii 2015. – Cherkasy, 2015. – S. 352–361.
- [50] Malovanyy M. Prospects of combining in complex usage of different types of renewable energy and creation of renewable energy sources / M. Malovanyi, Y. Mahera, O. Zakhariv, O. Kharlamova O. Synelnikov // Naukovyy visnyk Natsionalnoho universitetu bioresursiv I pryrodokorystuvanna Ukrainy, Seriya "Biolohiya, biotekhnolohiya, ekolohiya". – 2015. – No. 214. – S. 155–163.
- [51] Malovanyy M. Mathematical model of the process of synthesis of biogas from blue-green algae / M. Malovanyy, V. Nykyforov, O. Kharlamova, O. Synelnikov // Ekolohichna bezpeka. – 2015. – № 1/2015(19). – S. 58–63.
- [52] Malovanyy M. S. Ratsionalna tekhnologiya utylizatsii synyo-zelenykh vodorostey / M. S. Malovanyy, V. V. Nikiforov, O. V. Kharlamova, O. D. Sinelnikov //

Naukovyy visnyk NLTU Ukrainy. – 2015. – Vyp. 25.10. – S. 140–149.

- [53] Malovanyy Myroslav Reduction of the environmental threat from uncontrolled development of cyanobacteria in waters of Dnipro reservoirs / Myroslav Malovanyy, Volodymyr Nykyforov, Olena Kharlamova, Olexander Synelnikov, Khrystyna Dereyko // Environmental Problems. – 2016. – No. 1. – P. 61–64/
- [54] Malovanyy Myroslav Production of renewable energy resources via complex treatment of cyanobacteria biomass / Myroslav Malovanyy, Vladimir Nikiforov, Elena Kharlamova and Alexander Synelnikov // Chemistry & Chemical Technology. – Vol. 10, No. 2, 2016. – P. 252–254.
- [55] Li Y. Effects of nitrogen sources on cell growth and lipid production of Neochloris oleoabundans / Y. Li, B. Wang, N. Wu, C. Q. Lan // Applied Microbiology and Biotechnology. – 2008. – No. 81(4). – P. 629–636.
- [56] Li Y. Biofuels from microalgae / Y. Li, M. Horsman, N. Wu, C. Q. Lan, N. Dubois-Calero // Biotechnology Progress. – 2008. – No. 24(4). – P. 815–820.
- [57] Sheehan J. A look back at the U. S. Department of Energy's aquatic species program: biodiesel from algae / J. Sheehan, T. Dunahay, J. Benemann, P. Roessler // NREL/TP-580-24190, National Renewable Energy Laboratory, USA. – 1998. – 256 P.
- [58] Chisti Y. Biodiesel from microalgae / Y. Chisti // Biotechnology Advances. – 2007. – No. 25(3). – P. 294– 306.
- [59] Hossain A. Biodiesel fuel production from algae as renewable energy / A. Hossain, A. Salleh, A. N. Boyce, P. Chowdhury, M. Naqiuddin // American Journal of Biochemistry and Biotechnology. – 2008. - No. 4(3). – P. 250–254.
- [60] Hu Q. Sommerfeld Microalgal triacylglycerols as feedstocks for biofuels production: perspectives and advances / Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Seibert M., et al. // The Plant Journal. – 2008. – No. 54. – P. 621–639.
- [61] Rodolfi L. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a lowcost photobioreactor / Rodolfi L., Zittelli G. C., Bassi N., Padovani G., Biondi N., Bonini G., et al. // Biotechnology and Bioengineering. – 2009. – No. 102(1). – P. 100–112.
- [62] Rosenberg J. N. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution / J. N. Rosenberg, G. A. Oyler, L. Wilkinson, M. J. Betenbaugh // Current Opinion in Biotechnology. – 2008. – No. 19(5). – P. 430–436.
- [63] Schenk P. M. Second generation biofuels: high-efficiency microalgae for biodiesel production / Schenk P. M., Hall SR. T., Stephens E., Marx U. C., Mussgnug J. H., Posten C., et al. // Bioenergy Research. – 2008. – No. 1. – P. 20–43.
- [64] Tsukahara K. Liquid fuel production using microalgae / K. Tsukahara, S. Sawayama // Journal of the Japan Petroleum Institut. – 2005. – No. 48(5). – P. 251–259.

- [65] Grima M. E. Recovery of microalgal biomass and metabolites: process options and economics / M. E. Grima, E. H. Belarbi, F. G. A. Fernandez, A. R. Medina, Y. Chisti // Biotechnology Advance. – 2003. – No. 20 (7-8). – P. 491–515.
- [66] Weissman J. C. Design and analysis of microalgal open pond systems for the purpose of producing fuels: a subcontract report / J. C. Weissman, R. P. Goebel. – US DOESERI, 1987. – 456 p.
- [67] Richmond A. Handbook of microalgal culture: biotechnology and applied phycology / A. Richmond. – Blackwell Science Ltd, 2004. – 322 p.
- [68] Mata Teresa M. Microalgae for biodiesel production and other applications: A review / Teresa M. Mata, Antonio A. Martins, Nidia. S. Caetano // Renewable and Sustainable Energy Reviews, Vol. 14(1), 2010. – P. 217–232.
- [69] Cravotto G. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. / G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro, P. Cintas // Ultrasonics Sonochemistry. – 2008. – No. 15(5). – P. 898–902.
- [70] Bozbas K. Biodiesel as an alternative motor fuel: production and policies in the European Union / K. Bozbas // Renewable and Sustainable Energy Reviews. – 2008. – No. 12. – P. 542–552.
- [71] Alassali et al., Adv Tech Biol Med 2016, 4:1. http://dx.doi.org/10.4172/2379-1764.1000163
- [72] Abed R., Dobretsov S., Sudesh K.: Applications of cyanobacteria in biotechnology. J Appl Microbiol. 2009, 106: 1-12. 10.1111/j.1365-2672.2008.03918.x.
- [73] Canadian Council of Ministers of the Environment. Updated 1992. Canadian Water Quality Guidelines. Environment Canada.
- [74] Carmichael, W. W., and L. D. Schwartz. 1984. Preventing Livestock Deaths from Blue-Green Algae Poisoning.
 U. S. Dept. of Ag., Farmer's Bull. No. 2275. 11 pp.
- [75] Carmichael, W. W. 1991. Blue-Green Algae: An Overlooked Health Threat. In Health & Environment Digest, Freshwater Foundation. July, 1991. 5(6):1-4.
- [76] Carmichael, W. W. 1992. A Review, Cyanobacteria secondary metabolites- the cyanotoxins. In J. Applied Bacteriology. 72:445-459.
- [77] Carmichael, W.W. 1992. A Status Report on Planktonic Cyanobacteria (Blue-Green Algae) and Their Toxins. USEPA #EPA/600/R-92/079. 141 pp. (includes 867 references)
- [78] Chen, H. W.; Yang, T. S.; Chen, M. J.; Chang, Y. C.; Lin, C. Y.; Wang, E. I. C.; Ho, C. L.; Huang, K. M.; Yu, C.C.; Yang, F. L.; et al. Application of power plant flue gas in a photobioreactor to grow Spirulina algae, and a bioactivity analysis of the algal water-soluble polysaccharides. Bioresour. Technol. 2012, 120, 256– 263. [CrossRef] [PubMed]
- [79] Dineshbabu, G.; Uma, V. S.; Mathimani, T.; Deviram, G.; Arul Ananth, D.; Prabaharan, D.; Uma, L. On-site concurrent carbon dioxide sequestration from flue gas and calcite formation in ossein effluent by a marine

cyanobacterium Phormidium valderianum BDU 20041. Energy Convers. Manag. 2016, in press. [CrossRef]

- [80] Echlin, P. 1966. The Blue-Green Algae. Scientific American, 214(6):74–81.
- [81] Fay, P. 1983. The Blue-greens (Cyanophyta- Cyanobacteria). Edward Arnold (Pubs.), Baltimore, MD. 88 pp.
- [82] Ferreira, L. S.; Rodrigues, M. S.; Converti, A.; Sato, S.; Carvalho, J. C. M. Arthrospira (spirulina) platensis cultivation in tubular photobioreactor: Use of no-cost CO2 from ethanol fermentation. Appl. Energy 2012, 92, 379–385. [CrossRef]
- [83] Haynes, R. C. 1988. Deptt of Env. Qual. Engg., Commonwealth of Massachusetts. An Introduction to the Blue-Green Algae (Cyanobacteria) with an Emphasis on Nuisance Species. North Am. Lake Manage. Soc. 19 pp.
- [84] He, L.; Subramanian, V. R.; Tang, Y. J. Experimental analysis and model-based optimization of microalgae growth in photo-bioreactors using flue gas. Biomass Bioenergy 2012, 41, 131–138. [CrossRef]
- [85] Kumari, A.; Kumar, A.; Pathak, A. K.; Guria, C. Carbon dioxide assisted Spirulina platensis cultivation using NPK-10:26:26 complex fertilizer in sintered disk chromatographic glass bubble column. J. CO2 Util. 2014, 8, 49–59. [CrossRef]
- [86] Lambou, V. W., F. A. Morris, R. W. Thomas, M. K. Morris, L. R. Williams, W. D., Taylor, F. A. Hiatt, S. C. Hern, and J. W. Hilgert. 1977. Distribution of phytoplankton in West Virginia lakes. Working Paper No. 693, National Eutrophication Survey, USEPA. 23 pp.
- [87] Madigan, Michael T., Martinko, John M. (2006) Brock Biology of Microorganisms. 11 ed. Pearson Prentice Hall. New Jersey, USA. p. 396
- [88] Markou, G.; Chatzipavlidis, I.; Georgakakis, D. Cultivation of Arthrospira (Spirulina) platensis in oliveoil mill wastewater treated with sodium hypochlorite. Bioresour. Technol. 2012, 112, 234–241. [CrossRef] [PubMed]

- [89] Needham, J. G., and P. R. Needham. 1964. A guide to the study of Fresh-Water Biology. 5th Ed. Holden-Day, Inc., San Francisco, CA. 108 pp.
- [90] Nishiwaki-Matsushima, R., T. Ohta, S. Nishiwaki, M. Suganuma, K. Kohyama, T. Ishikawa, W. W. Carmichael, and H. Fujiki. 1992. Liver tumor promotion by the cyano- bacterial cyclic peptide toxin microcystin-LR. In J. Cancer Res. Clin. Oncol, Springer-Verlag. 118:420-424.
- [91] Prajapati, S. K.; Kumar, P.; Malik, A.; Vijay, V. K. Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation process. Bioresour. Technol. 2014, 158, 174–180. [CrossRef] [PubMed]
- [92] Rosgaard L., de Porcellinis A. J., Jacobsen J. H., Frigaard N.-U., Sakuragi Y: Bioengineering of carbon fixation, biofuels, and biochemicals in cyanobacteria and plants. J Biotechnol. 2012, 162: 134–147. 10.1016/ j.jbiotec.2012.05.006.
- [93] Soil & Water Conservation Society of Metro Halifax. 1992. Phytoplankton assemblages in six Halifax County lakes. 56 p.
- [94] Soil & Water Conservation Society of Metro Halifax.
 1993. Phytoplankton Assemblages in 21 Halifax Metro Lakes (Phase-B3 Limnology project), November 1993.
 130 p.
- [95] Soil & Water Conservation Society of Metro Halifax. 1993. Synopsis titled "Phytoplankton" (of fresh waters). 11p.
- [96] Sumardiono, S.; Syaichurrozi, I.; Budi Sasongko, S. Utilization of Biogas as Carbon Dioxide Provider for Spirulina platensis Culture. Curr. Res. J. Biol. Sci. 2014, 6, 53–59.
- [97] Wang B., Wang J., Zhang W., Meldrum D. R.: Application of synthetic biology in cyanobacteria and algae. Front Microbiol. 2012, 3: 344
- [98] Wedepohl, R. E., Knauer D. R, Wolbert G. B., Olem H., Garrison P. J., and Kepford K.. 1990. Monitoring Lake and Reservoir Restoration. EPA 440/4-90-007. Prep. by N. Am. Lake Manage. Soc. for U.S.E.P.A. 142 pp.

12