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

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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: CO_2 for photosynthesis, O_2 for breathing, H_2S for chemosynthesis, and N_2 for its fixation. Within the vegetation period (70 to 120 days), one cyanobacterium cell can produce up to 10^{20} daughter cells, i.e. one bacterium can generate 10^{20} 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 man-made 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 *Cyanophycea*e.

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].

Table 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
I	*Chamaesiphon, Cyanothece, Gloeobacter, Microcystis, Gloeocapsa, Gloeothece, Myxobaktron, Synechococcus, Synechocystis*
II	*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 free-living 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.

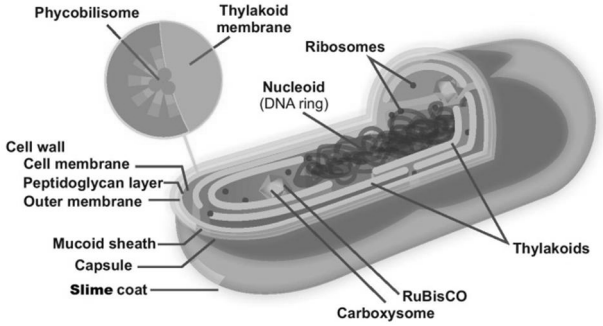

Fig. 1. Structural and functional organization of cyanobacteria cell (http://lakes.chebucto.org/)

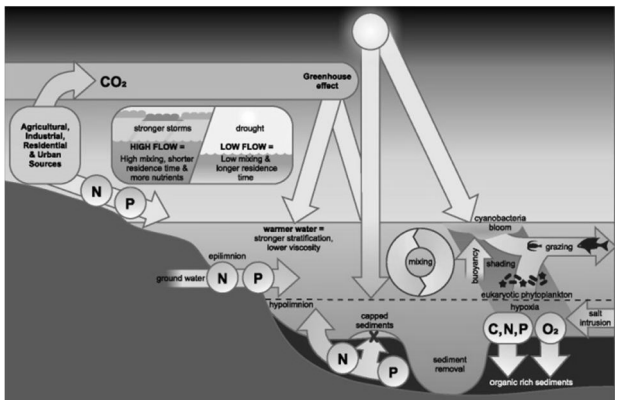

Fig. 2. Conceptual model of impact factors' influence on development of cyanobacteria (after Paerl et al. 2011)

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 and xanthophyll (lutein, zeaxanthin, oscilloxanthin, 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 – bacterio-starch. 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 conditions. Appearing under favourable 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 of phosphorus (Likens 1972, Schindler 1977, 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 non-stationary 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 CO_2 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 CO_2 (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
Flue gases	*Phormidium valderianum*	Coal combustion flue gases
	Atrhrospira platensis	
	Arthrospira sp.	Synthetic flue gas
	Synechocystis sp.	Flue gas from natural gas combustion
Biotechnological gases	*Arthrospira platensis*	CO_2-offgas from ethanol fermentation
		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).

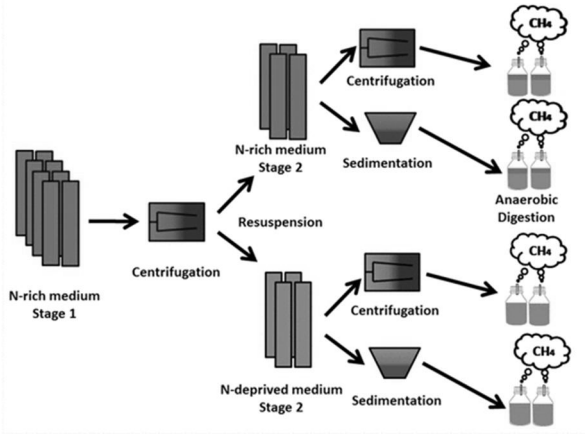

Fig. 3. Two-stage cultivation scheme by Gruber *et al*.

According to experimental construction of two-stage 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 of phototrophic microorganisms, especially 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 large-tonnage 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 Dnieper watercascades (species of *Microcystis, 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].

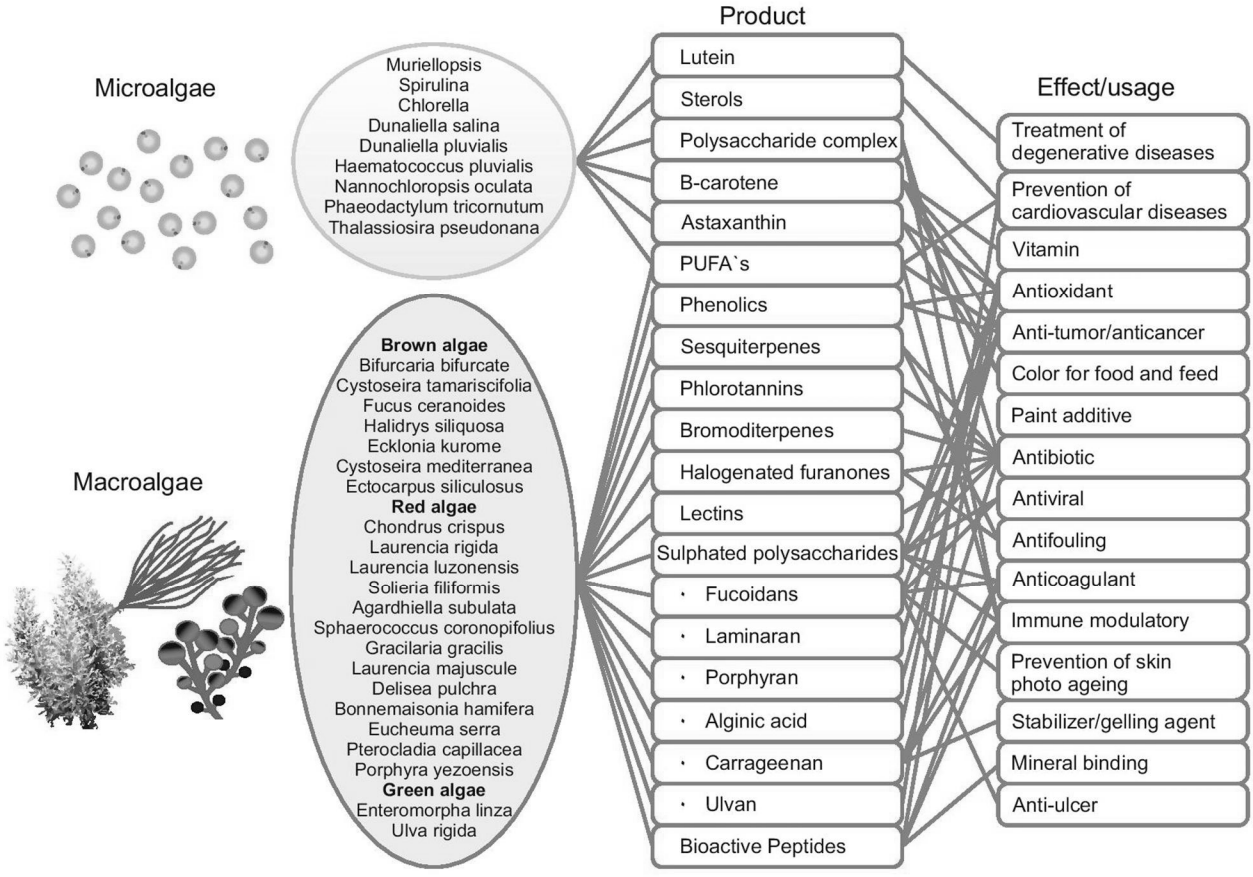

Fig. 4. Possible applications of target products from algae

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).

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