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30 YEARS OF SCIENTIFIC AND PEDAGOGICAL ACTIVITY OF PROFESSOR VOLODYMYR V. NYKYFOROV

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Abstract. For the first time, full data on the scientific and pedagogical work of Professor Volodymyr Nykyforov is published. The main stages of his work for 30 years are considered. The basic results of fundamental research and applied developments of the scientist are discussed.

Key words: science, higher education, research and developments, biology, ecology, biotechnology.

Nykyforov, Volodymyr Valentynovych is a Ukrainian biologist, ecologist and biotechnologist. In 1987 he graduated from the Biology Department of Kharkiv State University (Diploma ПВ No. 659683). In 1987−1989, in accordance with the state distribution, he worked as a Biology and Chemistry teacher at secondary school № 20 in Kremenchuk, Poltava region.

From 1989 to 1992 he took a postgraduate course at the Department of Spore Plants of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (NASU), where he defended his candidate dissertation in the subject of “Golden Algae (*Chrysophyta*) of the Mountainous Crimea & the Ukrainian Carpathians”.

During the period of 1993−1995, he was an assistant of the Physics & Chemistry Department of the Kremenchuk branch of Kharkiv State Polytechnic University. In 1994 he was conferred the scientific degree of Candidate of Biological Sciences (Diploma КН No. 006250), in 2000 − the Academic title of Associate Professor of the Ecology Department (Certificate ДЦ No. 000744), in 2010 − Doctor of Biological Sciences (Diploma ДД No. 009128), and in 2012 − Academic title of Professor of the Ecology Department of Kremenchuk Mykhailo Ostrohradskyi National University (KrNU) of the Ministry of Education and Science of Ukraine (MESU) (Certificate 12пр No. 007673).

From 1995 to 1998 he worked as a senior teacher of the Applied Ecology Department of Kremenchuk State Polytechnic Institute. In 1998−2001, he was a doctoral candidate at the Department of Soil Science, Geobotany and Ecology of the Department of Biology, Medicine and Ecology of Dnipro Oles Honchar National University, where he defended his doctoral thesis in the subject of “Structural Organization of Biogeocenoses and Biohydrocenoses of the Middle Dnieper: Restoration, Protection and Rational Use”.

In 2001−2012, Volodymyr Nykyforov headed the administration of the regional landscape park “Kremenchuk Plavni” − an object of the Natural Reserve Fund of Ukraine. He is the initiator of the creation of the National Natural Park “Middle Dnieper Plavni”. From 2003 to 2013 he was the head of the Science & Research Department of KrNU. During 2012−2015 he headed the Department of Ecological Biotechnology and Bioenergy of KrNU. Since 2013 to the present time he has been the First vice-rector of the KrNU (responsible for the research and international activities).

The range of his scientific interests includes the next areas of research

− the inventory and protection of species and ecosystem biodiversity [1–5, 9, 10, 12, 17, 18, 20];

− the structural and functional organization of natural and anthropogenic aquatic ecosystems [7, 19];

− ecological safety, environment pollution and human health [8, 15, 16,];

− biotesting, bioindication and biomonitoring [8, 11, 13, 14];

− the development of ecological and bioenergy technologies [6, 17–20].

Professor Nykyforov is the author of educational and methodical complexes for 12 disciplines, at present he delivers the following courses: “General Biotechnology”, “Biochemistry”, “Genetics”, “Fundamentals of Pharmacognosy” and “Ecological Aquaculture”, and also the author of 37 manuals for practical and laboratory exercises, term papers and a supervisor of bachelor, master and PhD theses for the specialties: “Ecology and Environmental Protection”, “Biotechnology” and “Ecological Biotechnology and Bioenergy”. He is responsible for the PhD program in Ecology, within the framework of which he delivers the lecture courses in “Autecology, Synecology and Nature Protection”, “Structural and Functional Organization of Superorganismic Systems” and “Genetic, Species and Ecosystem Biodiversity”.

Volodymyr Nykyforov is the winner of the contest in 1993−1994 on biodiversity and the scholarship of the George Soros International Science Foundation, a participant of the state program of the Ministry of Education and Science of Slovakia “National Scholarships Program for the Slovak Republic for Foreign University Teachers and Researchers” (2010 and 2013), as well as an international project of the European Union “Project − Developing the Knowledge and Skills of Teaching Staff and Scientific Researchers and PhD Students at Matej Bel University in Banska Bystrica” (ITMS code: 26110230019, module 9 − Specialized Education in Natural Sciences, 2012). He is a participant of the bilateral Austrian-Ukrainian scientific project “Methods of Processing of Cyanobacteria (algae) Biomass Causing Water Reservoir Blooming” (No. SR 0117U003299, 2017−2018) and he has completed the training programme on teaching and learning in higher education (the Erasmus+ KA107) at Abertay University (Dundee Scotland, United Kingdom)in 2019.

He is Chairman of the Scientific and Technical Council, Deputy Chairman of the Academic Council of the KrNU, member of the Academic Council of the Department of Natural Sciences, member of the Specialized (dissertational) Scientific Councils D 08.051.04, specialty 03.00.16 − Ecology and K 45.052.05, specialty 21.06.01 − Environmental Safety. He is a Deputy Editor-in-Chief of the scientific journal Vestnik KrNU and member of the editorial boards of scientific journals “Ecology and Noospherology” (Dnipro, Ukraine), “Biology and Ecology” (Poltava, Ukraine), “Biological Bulletin of Melitopol State Pedagogical University” (Melitopol, Ukraine), “Environmental problems” (Lviv, Ukraine) and “Acta Facultatis Ecologiae” (Zvolen, Slovakia).

Professor Nykyforov V. V. is a co-executor of the fundamental research “Physical & Chemical Biology of Methanogenesis of Hydrobionts by the Example of Blue-green Algae” (No. SR 0108U002170, 2008−2010) and a scientific supervisor of the applied research on “Ecological Biotechnology of Methane Production from Blue-green Algae” (No. SR 0115U002528, 2015−2016). He has been Chairman of the Organizing Committees of International Scientific Conferences “Biosphere-Noospheric Ideas of V.I. Vernadsky and Modern Problems of Sustainable Development of Education and Science” since 2005, “Physical Processes and Fields of Technical and Biological Objects” and “Problems of Environmental Safety” since 2007. Since 2015 he has been a Member of the Presidium of Vice-Rectors for Scientific Work of the MESU, Member of the Middle Dnieper Basin Council of the Ministry of Ecologyand Natural Resources of Ukraine since 2018 and Expert of the Scientific Council of the Ministry of Education and Science of Ukraine (Section “Biology, Biotechnology and Actual Problems of Medical Sciences”) since 2019.

He is the holder of the Honor Certificate of the MESU (2008), the Honor Certificate of the NASU (2013), the Honorary Letters of the Poltava Regional State Administration, the Executive Committee of the Kremenchuk Municipal Council, international exhibitions, the administration of the KrNU. He was awarded with badges "Excellence in Education" (2011) and "For Achievements in Science and Education" (2015).He is the author of 147 scientific publications, including 75 papers in 25 specialized research journals, those indexed in WoS, Scopus and other international science-metric databases, as well as five copyrighted documents [52–55] and five monographs [11, 13, 15, 16].

Among his fundamental researches Volodymyr Nykyforov pays special attention to the comparative analysis of the structural and functional organization of natural and anthropogenic terrestrial and aquatic biocenosis. He has founded that algophytes which usually dominate higher plants by bioproduction and other indicators impose a significant impact on the structural organization of the phytocenosis in aquatic ecosystems. Nevertheless, under hydrocenosis conditions, as well as geocenosis conditions, the limiting abiotic factor is luminous flux intensity, which forms the vertical structure, although the layering is poorly expressed even in higher hydrophytes in the littorals. At the same time, a certain correlation between algae ecological groups and the type of morphological structure of their thallus (biomorph) was revealed (Table 1). The biogeocenoses has a specific relationship between the biomorphic and ecological embryobionts groups (higher plants) (Table 2). However, there is nothing in common between these relations in terrestrial and aquatic conditions.

The most important abiotic factors determining the hydroecosystems structure and absent in terrestrial conditions are temperature stratification, water flow, its vertical and horizontal turbulence, chemism (especially the gas mode, pH and salinity), transparency, etc. Taking into account everything mentioned above, the structures of the terrestrial and freshwater biogeocenoses are presented in the most general terms below (Fig. 1).

Table 1

Relationship between thallus type (biomorph) and algophytes ecological groups

ecological group \ biomorph	palmelloid	monadoid	trichoid	coccoid	amoeboid	lamelloid	siphonoid
phytoplankton	+	+	+	+	+	–	–
periphyton	–	–	+	+	+	+	+
phytobenthos	–	–	–	+	+	+	+

Table 2

Relationship between the life form (biomorph) and embryobionts ecological groups

ecological group \ biomorph	terophytes	cryptophytes	hemicryptophytes	hamephytes	phanerophytes
hygrophytes	–	+	–	–	+
mesophytes	–	+	+	+	+
xerophytes	+	+	+	+	+

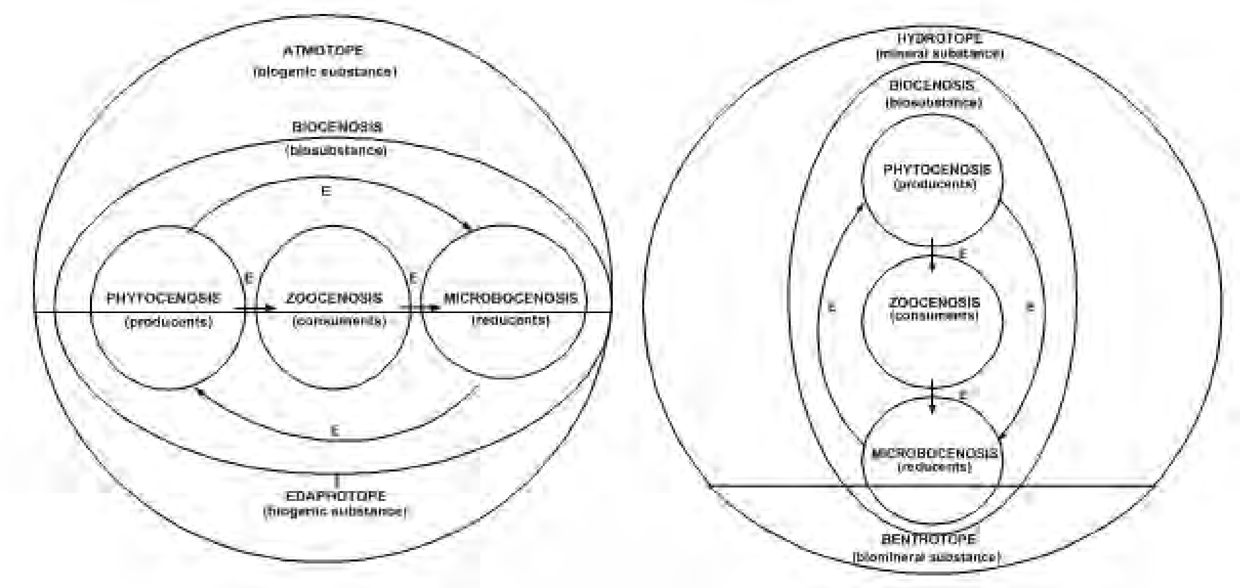

Fig. 1. Structural and functional organization of terrestrial and aquatic ecosystems

The principal difference in the functioning mechanisms of terrestrial and aquatic ecosystems is in the peculiarities of their structural organization, due to different abiotic factors modes. If in the biogeocenoses structure formation the hydrothermal pair is a prior, then in the biohydrocenoses the photothermal mode plays the leading role. The difference between the modes of the leading abiotic factors, in turn, is determined by the environment-forming ecotope: in terrestrial ecosystems these are atmotope and edaphotope, in aquatic ecosystems – hydrotope and benthotope, primarily by their physical and chemical specificity.

As a result of this, phanerophyte ecosystems are characterized by a high level of structural and functional organization due to the vertical and horizontal differentiation of the autotrophic block, in which the main energy reserves are accumulated. Aquatic ecosystems are characterized by a low level of organization as a result of primitive structuring and high differentiation of the heterotrophic block, as a result of which the energy is not concentrated in them, but due to the aquatic environment it is dispersed and moved over long distances.

Differences in the processes of energy-plastic transformation in water and on land Nykyforov V. V. explains as the significant difference between the consortia determinants in aquatic and terrestrial biocenoses. In the more ancient by origin biohydrocenosis (primary – marine and secondary – freshwater), the determinant is many species (sometimes up to 100 or more species) and the polyphylogenetic complex of lower photosynthesizing organisms, mainly planktonic organisms – algae, whose phytomass does not exceed 3 % (hydroconsument consort zoomass heterotrophy is up to 90 %). The opposite picture is observed in sylvacenoses, where the determinant is, as a rule, one species – phanerophytes (in monodominant phytocenoses) or several species in herbaceoses with different organization levels of autotrophic block, the main energy reserves of which are concentrated in the edaphotope. In any case, zoomass biogeocenoses do not exceed 1–2 % of their total biomass.

It is known that about 90 % of the biosphere biomass is terrestrial plants phytomass, the rest of it is aquatic vegetation and heterotrophic organisms. For marine ecosystems and large inland water reservoirs, a small plant biomass is characteristic, represented in the pelagial mainly by plankton. The biomass of plankton and benthic animals is several times higher. At great depths their biomass is insignificant. The total biomass of the World Ocean animals is 6·109 tons, which is 20 times more than all hydrophytes biomass. Soil microorganisms are characterized by the highest biomass among heterotrophic terrestrial organisms. The invertebrate soil animals biomass, mainly earthworms, can reach 4 t/ha. The average total warm-blooded vertebrate biomass is much less and is up to 0.015 t/ha.

In this regard, the fundamental difference in the structural and functional organization of terrestrial and aquatic ecosystems seems to be due to another reason, the primacy (antiquity) of the latter, since the aquatic consortia formation began at least 2.5 billion years earlier than the terrestrial one (the absolute age of the first cyanobacteria is about 3 billion years while land colonization by rhinophytes belongs to the end of the Silurian period – 400±10 million years ago).

Due to the autotrophic nature of magnoliophytes and evolutionary domination in modern biogeocenoses associated with it, the main consorts functions at different trophic levels are coupled with the autotrophs reproduction (lower heterotrophs, mainly insects) or the diasporas spread (higher heterotrophs). Algae do not need such “services”. Therefore, lower (invertebrates) and higher (vertebrates) heterotrophs – hydroconsorts perform one specific but very important function – uniform, diffuse dispersal of energy reserves in the aquatic environment.

There are also differences in the formation of structural and functional mechanisms stability of aquatic and terrestrial ecosystems. Sylvacenoses are characterized by high stability and opposition to external factors. Herbacenoses are less stable because their development is determined by the action of leading edaphic factor. The stability mechanisms of biohydrocenoses are determined solely by the processes of water self-purification due to the algobacterial complexes functioning. In the course of detailed analysis of the fallout sequence of the main trophic representatives’ levels in terrestrial and aquatic biogenesis as a result of their anthropogenic digression (Fig. 2), the following patterns were revealed:

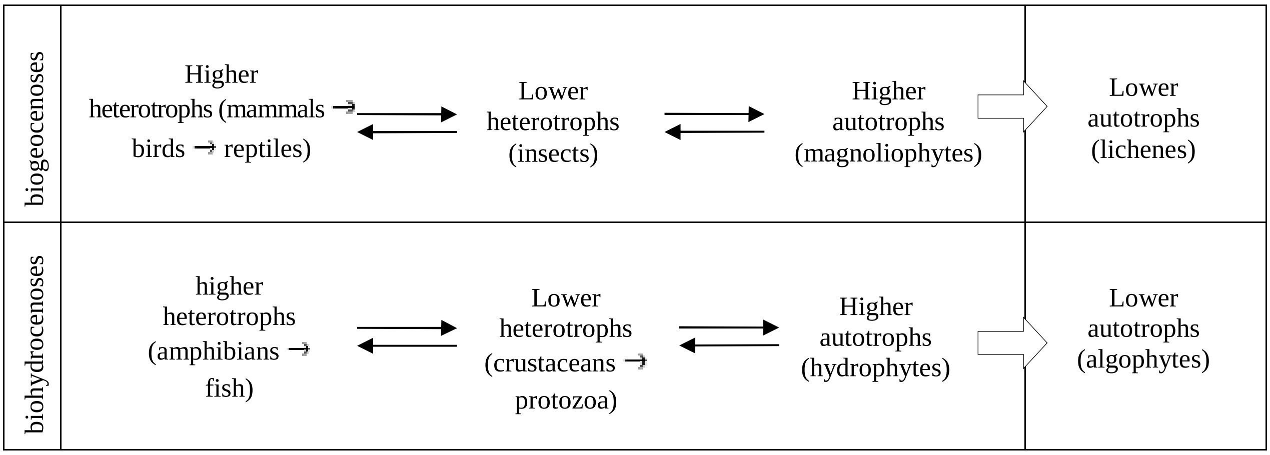

Fig. 2. Scheme of the main trends of digression (→) – demutation (←) processes in biogeo- and hydrocenoses

– trends of biogeo- and hydrocenoses digression with different types of anthropogenic impact on them (man-made, agrogenic, recreational) are almost identical;

– the decrease in the number of populations – the main biogenesis components in terrestrial and aquatic ecosystems – occurs in one direction: from higher heterotrophy to higher autographs;

– the most stable in both types of biocenoses are higher plants, and the most vulnerable are vertebrates;

– in case of autotrophic main structural elements preservation the digression process is reversible;

– demutation processes in disturbed biocenoses occur in the opposite order of digression.

Consequently, the lower organization level of living organisms is, the higher is their resistance to negative environmental factors. The last in the course of demutation processes in disturbed ecosystems higher heterotrophs are renewed. In this connection, analogy can be traced between the main trends in the organisms’ recovery – representatives of different trophic blocks of disturbed biocenoses and the phylogenetic sequence of their appearance in the evolution process.

Thus, all the differences and similarities in the structural and functional organization of terrestrial and aquatic ecosystems discussed above, according to Nykyforov V.V., must be taken into account when developing and implementing environmental protection measures aimed at rational nature management, optimization of all the living environments, destructive ecosystems restoration, as well as consortium biodiversity inventory.

Among the most important applied developments conducted under the supervision of Professor Nykyforov V. V. is “The Biotechnological Ways of Blue-green Algae Complex Processing”. The results of microscopy have allowed to determine that 95–99 % of the blue-green algae (BGA) biomass, characteristic of the Kremenchuk water reservoir of the Dnieper cascade (Ukraine) is Microcystis aeruginosa Kützing (Fig. 3) w weighing $4.14 \cdot 10^7$ tons for the vegetative period (70–120 days), which is about $2.51 \cdot 10^5$ tons of dry organic matter. Thanks to biomethanogenesis, it is possible to obtain up to 18.84 million m^3 of methane or extract about 11 thousand tons of lipids for the production of biodiesel, and also to obtain up to 25 million tons of liquid biological fertilizers.

This development set the urgent scientific and practical task − to get the maximum possible amount of energy and useful substances from the BGA biomass (and/or other aquatic organisms). Solutions to this problem, on the whole, are presented in the flow diagram of complex processing of algal biomass (Fig. 2). Each of the proposed stages is a process or method, relating to environmental and energy sectors of biotechnology, microbiological or chemical industry, forestry and agriculture. Currently, the method of production of the second (biomethane) and third (bioethanol, or biodiesel) generation biofuels from mass forms of BGA, as well as bio-fertilizers (specially prepared after the substrate biomethanogenesis) is known.

Since 2007, the first and major stages of the BGA biomass deep processing have been tested in the field and laboratory conditions: a) extraction of lipids after cavitation; b) allocation of the biogas resulting from methanogenesis; c) biotesting of the output mineral-organic fertilizer (Fig. 4). It should be emphasized that BGA are of interest in medicine, pharmacology, cosmetology and perfume industry, because they are a potential source of chromoproteids (complex colored proteins that change their color depending on the absorption spectrum), including phycobiliproteins (red and blue pigments). Hemoproteins, flavoproteins and phycobilins can also be isolated from BGA. The pigments, added to cosmetic compositions improve skin tissue respiration and thus contribute to rejuvenation.

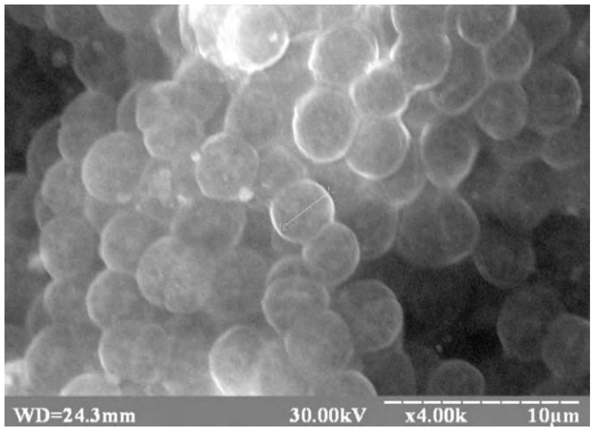

Fig. 3. The scanned image of the *Microcystis aeruginosa* colony surface (SEM−106 I, 4000^{x})

Hyaluronic and glucuronic acids are of particular interest. Their synthesis is very complex from the chemical point of view, therefore, BGA act as a valuable source of these compounds. They are readily removed from residual dry biomass through hydroalcoholic extraction followed by recrystallization at temperatures above 40–50 °C. At higher temperatures, the molecules of these polybasic organic acids lose biological activity, since become racemates or are decarboxylated.

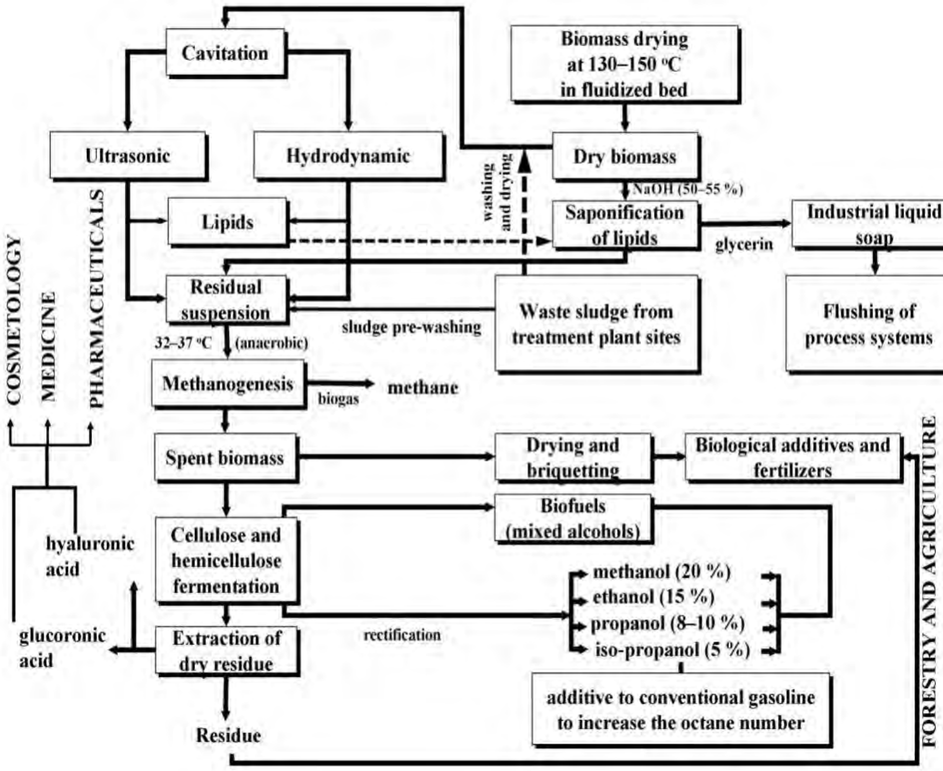

Fig. 4. Biotechnological ways of blue-green algae complex processing and the industries of application of its products application

During desktop studies, four lines of samples of the water suspension of the concentrated BGA biomass (cavitated and without cavitation) were subjected to extraction with carbon tetrachloride before and after methanogenesis. The experiments revealed that fresh suspension (without cavitation and before methanogenesis) after extraction for 60 min is divided into extracted and aqueous phases when using both CCl4 and CHCl3 as extractants, which may be due to the small density of fresh biomass (2.5 times lower than after methanogenesis). The remaining aqueous layer is a suspension, which after drying in the fluidized bed can be used for separating cellulose and hemicellulose.

As a result of the biochemical reactions occurring on the organic substrate under the influence of a number of symbiotic microorganisms, the gas mixture was obtained, in which methane accounted for from 65 to 80 %, and the rest were carbon and nitrogen oxides, hydrogen sulfide and other gases.

From the chemical point of view, biomethanogenesis includes three stages: dissolution and hydrolysis of organic compounds, acidogenesis and methanogenesis. Accordingly, at each stage there are three groups of bacteria: some (acidogenic) of them convert complex organic substrate compounds to butyric, propionic and lactic acids; others (acetogenic) turn these organic acids into acetic acid, hydrogen and carbon dioxide, and finally, the methanogenic bacteria recover CO_2 to methane with absorption of hydrogen, which might otherwise inhibit the acetic acid bacteria.

At In the next stage of the research, the calorific value of the biogas produced to be 33 MJ/m^3 was determined experimentally. The biogas density was in the range of 0.914–0.922 kg/m^3 and calorific value Q=5100–5200 kJ/m^3. Thus, the physical and thermodynamic parameters of the biogas produced are close to those of the natural gas – propane-butane mixture. The latter can be used for heating industrial and residential buildings.

The research resulted in the design, installation and testing of the laboratory setup, simulating the biomethanogenesis process using different types of substrates in natural temperature conditions and in the isothermal model systems. This approach is expedient when using the cyanogen concentrated organic matter as a substrate for biogas plants on an industrial scale. To collect a substrate on an industrial scale, special concentration columns are placed in the areas of maximum natural concentration of the blue-green algae biomass (Fig. 5).

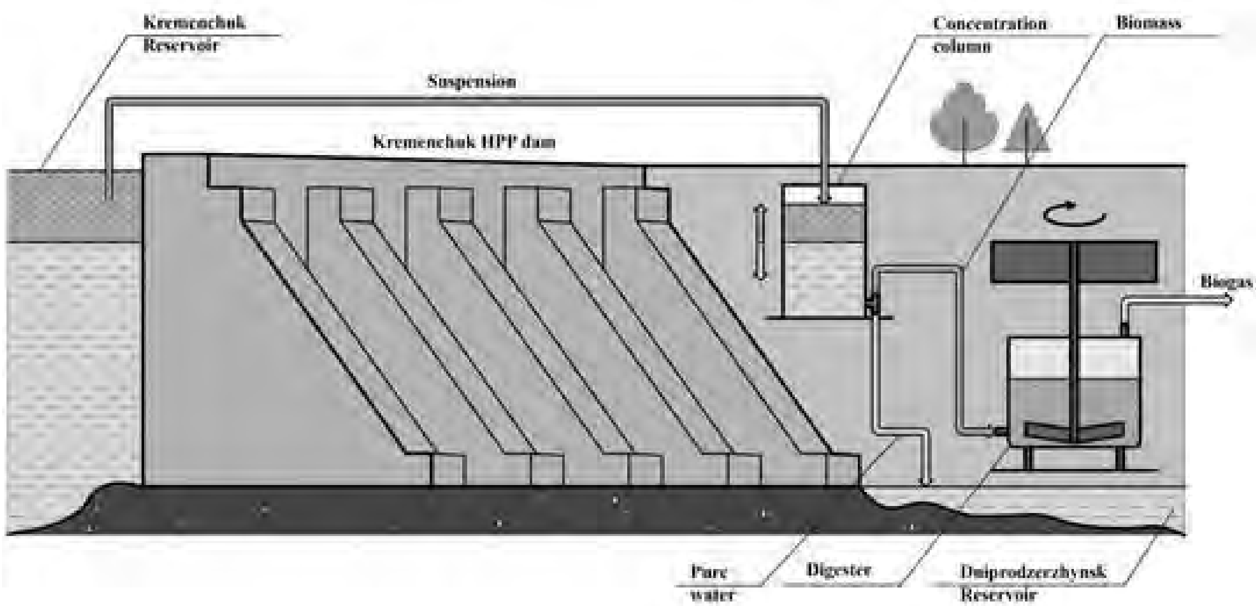

Fig. 5. The layout of process units of the methane and fertilizer biorefineer

The proposed process flow diagram of the biogas production is based on an intermittent feed of a substrate in a concentration column. In this case, the algal biomass is fed by gravity. Biomass floats to the surface due to the presence of gas bubbles therein, and clean water remains on the column bottom. As soon as the lower level of the biomass reaches a distribution valve, it is switched to feed the biomass in a digester to carry out methanogenesis. The remaining substrate is removed from the anaerobic chamber and sent for drying or briquetting to produce a biofertilizer. A part of the spent substrate from a digester contains methanogenic microbiocenosis, therefore is further used as seed stock for inoculation of feeding in the next sestonic biomass fermentation cycle.

The next stage of research was based on the need for further processing of the spent substrate remaining after methanogenesis. The conventional technology of biogas production in economically feasible quantities involves the use of waste of agriculture, primarily livestock as an organic substrate. In this case, the biogas technology allows producing natural biofertilizers, containing biologically active substances and microelements by means of anaerobic fermentation. Their main advantage over traditional fertilizers is the form, availability and balance of all nutrients, high levels of organic matter humification. Therefore, the introduction of such biofertilizers in the soil activates nitrogen-fixing and other microbiological processes.

Thus, the use of the substrate spent in methanogenesis (organic matter) as a fertilizer in agriculture and f orestry can be regarded as a promising waste-free biotechnological direction. In this respect, the study of the dynamics of quantitative germination of model organisms (test objects), typically agricultural crops, is a priority in determining the efficiency of the spent substrate impact on plants. To determine the seed germination rate as a criterion for assessing the possibility of using blue-green algae after methanogenesis, two species of crops: soft wheat − *Triticum durum* L. (Monocotyledones) and pea – *Pisum sativum* L. (Dicotyledones) were used as fertilizers.

Seed germination of the test objects was carried out in petri dishes using the spent substrate at different dilutions (1:10, 1:50, 1: 100, 1: 200, 1: 500 and 1: 1000) at T=25 °C and pH = 6.5−7.50. Germinating ability was determined by the number of germinated seeds out of 100 % as compared with the control (double-distilled water irrigation) in triplicate.The analysis of the results of studies on the pea and wheat germination on substrates with different concentrations revealed that the highest germination rate of wheat is at 1:100 and 1:200 dilutions of the substrate, and pea – at 1:50 and 1:100. Thus, 1: 100 dilution of the spent substrate is optimum for using the algae residues as bio-fertilizers in agriculture and forestry.

Proceeding from the fact that biotesting is an integral part of quality assessment and control of aqueous solutions of various industrial purposes, a number of diluted cyanogen substrates spent in the biogas production with different concentrations were subjected to biotesting in accordance with the National Water Quality Standard. The basic technique of the experiment provides for the use of crustaceans – cladocerans *Daphnia magna* Straus as a test object.

A thorough analysis of the results of studies of survival rate of water fleas in the substrate aqueous solutions with different concentrations allowed us to determine their levels of toxicity:

− the survival rate of test objects in the control is absolute;

− the survival rate of water fleas at 1:10 and 1:50 dilutions of the spent substrate decreased by 90 and 76 %, respectively;

− the survival rate of water fleas at 1:10 and 1:50 dilutions of fresh (toxic) substrate decreased by 93 and 83 %, respectively;

− the survival rate of test objects at 1:100, 1:200, 1:500, 1:1000 dilutions of fresh (toxic) spent substrates ranged from 90 to 100 %.

Thus, dilutions starting with 1:100 are optimum for the use of substrates as bio-fertilizers, which is fully consistent with the biotesting results using model organisms of peas and wheat. The feasibility of using the cyanogen spent biomass was also confirmed by the results of measurements of the mass fraction (%) of the basic chemical elements using the EXPERT 3L XRF analyzer (Tab. 3), the values of which correlate with the elemental qualitative and quantitative composition of living plant cells.

Table 3

Mass fraction of chemical elements of the cyanobacteria biomass after methanogenesis with the content of > 1 %

Chemical element	Mass fraction, %		
	sample 1	sample 2	average
$_{14}Si$	3.75±0.18	4.43±0.09	4.09±0.14
$_{15}P$	7.47±0.30	7.16±0.13	7.32±0.22
$_{16}S$	10.10±0.22	11.71±0.10	10.91±0.16
$_{17}Cl$	9.45±0.19	8.46±0.08	8.96±0.14
$_{19}K$	21.41±0.14	20.20±0.06	20.81±0.10
$_{20}Ca$	44.54±0.25	45.13±0.11	44.84±0.18
$_{25}Mn$	1.46±0.03	1.14±0.02	1.30±0.03
$_{26}Fe$	1.69±0.03	1.49±0.02	1.59±0.03

The results indicate that the elemental composition of the cyanobacteria biomass after methanogenesis is similar to the elemental composition of the green mass of plants. Therefore, the BGA spent substrate can be used as a biofertilizer, because it is similar in nutritional value.

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