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## Critical analysis of biotechnologies on using resource potential of hydrobionts

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### Abstract

Hydrobionts are considered as highly potential source for bioproduction (including energy carriers and fertilizers) and many biotechnological processes that include hydrobionts, particularly their biomass as a substrate are used in different fields of energy, cosmetology, medicine, pharmaceuticals, aquaculture, agriculture, forestry etc. Latest developments prove efficiency in applying anaerobic digestion for purifying wastewaters from organic pollutants with the help of macrophytes and microphytes in conducting biomethanogenesis. Many studies have established that it is possible to reach high level of lipid extraction from algae (to 95%) with the help of organic solvents (methanol, acetone, hexane, diethyl ether etc). Blue – green algae biomass has been scientifically proved to be a good source for methane, methanol, ethanol, propanol, isopropanol, biodiesel and other biofuel types production. Macroalgae and microalgae contain  $\beta$ - carotene, biotin, folic acid, fucoidans, lectins, phenolics, sulphated polysaccharides and other derived biologically active compounds that can be used in producing vitamins, have anti-ulcer, antioxidant, antibiotic, antifouling, immune modulatory and other properties. *Cyanidioschyzon merolae*, *Ostreococcus lucimarinus*, *O. tauri*, *Micromonas pusilla* have shown high potential for hydrogen production while *Rhizoclonium sp.* has been experimentally used as a bounding material in briquetting miscanthus granules, resulting in 20 % higher dynamic strength. The article is a literature review and the purpose of this work is to classify and systemize hydrobionts, reveal regularity of their growth, conduct critical analysis on existing biotechnologies on using separate representatives of aquatic biomes as a raw material and also to review ways of intensification for these biotechnologies.

**Key words:** *bio-agents, biomass, biotechnologies, hydrobionts, target products*

### INTRODUCTION

In the scientific environment under circumstances of environmental challenges and problems, more and more attention is being paid to cycle approaches in the system of management and use of bioresources. Studies are being carried in direction to both discovering new biome species and studying their potential resource properties for balanced natural resource management.

A lot of scientific works have been dedicated to problems on using aquatic organisms for different economic purposes [FOMENKO 2007; KRAVCHENKO 2003; LIBANOVA, KHVESYK 2014; NYKYFOROV 2003; SINELNIKOV, KOZLOVA 2007; VDOVENKO 2017; ZAHIRNYAK *et al.* 2017]. But application of different forms of hydrobionts as a source for biofuel production of II–III generations, particularly as a substrate for methane digestion biotechnology is a new idea [BUZOVSKYI *et al.* 2008; KRUCHKOV *et al.* 2007; NYKYFOROV *et al.* 2008; 2011; VASSER *et al.* 1989].

Hydrobionts are organisms that inhabit hydroecosystems and are adapted to living in water medium. They inhabit water biotopes with different mineralization level, from freshwater to seas and salty lakes. By calculations there are around 150 000 animal species and 10 000 plant species inhabiting World Ocean, what is 7 and 8% of general quantity of species around Earth accordingly.

In particular, over the last 5 years in the Far East basin scientists discovered around 7000 hydrobiont species. This number exceeds all known species that cover European countries. The Sea of Japan is uniquely rich on new hydrobiont species. Several species (201 from 621) that have been registered in its water area are new to science. Highly valuable for medicine is the fact that many hydrobionts turned out to have oncostatic and bactericidal properties [MAYORAVA 2018]. Necessity to study biotechnologies on using resource potential of hydrobionts lays in determining perspective of using certain aquatic organisms' species in production of bioproducts. Many researches carry solely pilot character and under economic, political and financial circumstances do not reach mass production level of offered technologies.

## STUDY MATERIAL AND METHODS

Hydrobionts are marine and freshwater organisms that constantly (obligatory) or temporary (optionally) live in water medium [CHENG *et al.* 2011]. Over million years of evolution many of them have adapted to living under different conditions. Depending on way of transportation and residence in proper layers of water medium, among hydrobionts the following ecological groups are distinguished: nekton, plankton and benthos. Nekton (*nekton* – floating) – large animals that actively move and are capable to overcome large distances and strong currents: fishes, squids, pinnipeds, whales. In freshwater to nekton are referred amphibians and a lot of insects [BROCK 1994; HAYNRIKH, HERHT 2001; ROUND 1981].

Plankton (*plankton* – errant) is a complex of plants (phytoplankton: diatoms, green and blue – green algae etc.) and tiny animals (zooplankton: tiny crustacea, pteropoda molluscs, jellyfish, ctenophora, some worms) that live at different depth levels but are incapable of active transportations and of confrontation to currents. To plankton's content refer also larvae of animals creating specific group – neuston. This is «temporary» habitat of water's upper layer that passively floats, represented by different animals (decapoda, barnacle and copepod crustaceans, echinoderm, polychaete, fish mollusks etc.) at larva stage.

Organisms that live above surface layer refer to epineuston, below – to hyponeuston. Larvae, having grown up transfer to lower layers of pelagic zone. Above neuston is located pleuston – organisms, upper body part of which grows above the water and lower – in the water (duckweed – *Lemna*, siphonophorae etc.). Plankton plays very important role in trophic bounds of the biosphere, being nutrition for many water inhabitants, including main fodder for baleen whales (*Myatcoceti*) [MUSIENKO *et al.* 2004; ODUM 1986].

Benthos (Greek βενθος – depth) represents hydrobionts of the bottom. To composition of benthos refer mainly animals that live attached or slowly float to great distances (zoobenthos: foraminifera, some fish, sponges, coelenterata, worms, brachiopod mollusks, ascidiacea etc.), more numerous at shoalness where to composition of benthos refer plants (phytobenthos: diatoms, green, brown, red algae, bacteria). At the bottom where there is no light phytobenthos is absent. Near the shore occur flower plants: eelgrass (*zostera*), *ruppia*. The richest on phytobenthos are stony areas of the bottom. In lakes zoobenthos is less rich and various than in the sea. It is shaped by protozoa, crustacean, worms, mollusks, insect larvae etc. Phytobenthos of lakes is created by diatoms that float freely; green and blue – green algae; brown and red algae are absent [BOBROVSKIY 2005; MUSIENKO 2006; RAVEN *et al.* 2013].

Rooted coastal plants in lakes create clearly defined layers, species composition and look of which is coordinated with medium conditions in border zone «land–water». In water near the very shore grow hydrophytes – half submerged in water plants (*Sagittaria*, *Calla*, *Phragmites*, *Typha*, *Carex*). They change by hydatophytes – plants that are submerged into water but with leaves that float (lotus, duckweed, nuphar, water caltrop) and – further – fully submerged (pondweed, elodea, chara). To hydatophytes refer also plants that float on the surface (duckweed).

To massive forms of hydrobionts refer species, increasing number of which under certain conditions becomes explosive in waters, their biomass starts consistently prevail over biomass of species – competitors. Original classification of hydrobionts is illustrated on the scheme (Fig. 1).

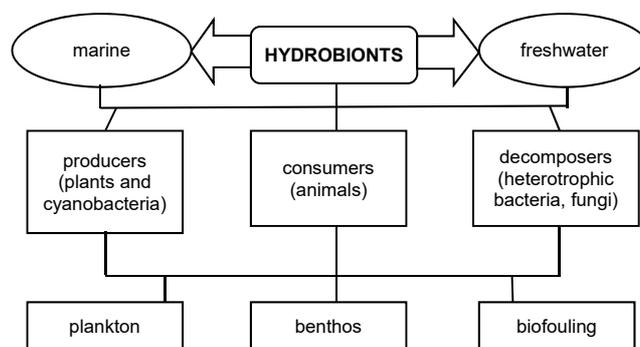


Fig. 1. Scheme of ecological classification of hydrobionts; source: own elaboration

According to modern system of organic world hydrobionts refer to different taxa and taxon groups; at kingdom level of live nature they are representatives of *Virabiota*, *Bacteriobiota*, *Phytobiota*, *Mycobiota*, *Zoobiota*.

Bacteria (*Bacteriobiota*) is a kingdom of microscopic, mostly unicellular, prokaryote organisms for whom is typical presence of cellular wall, cytoplasm with organelles, different specific insertions, absence of mitochondria and chloroplasts, shaped nucleus. Size of bacteria cells usually does not exceed several micrometers, seldom reaches 20

$\mu\text{m}$  (in average 0.1–1.0  $\mu\text{m}$ ). By nutrition physiology among bacteria are distinguished heterotrophs and autotrophs (photo- and chemotrophs), by aeration type – aerobic and anaerobic. Many species of pathogenic (moribific) bacteria cause diseases of humans, animals and plants. Some bacteria can exchange genetic information between each other, which is so called process of horizontal genes transfer [BUCKLEY *et al.* 2015].

Certain bacteria species can live both in marine and freshwater. Example of bacterium living in marine, salty (transitional) and freshwaters is *Nitrospira moscoviensis*. Some bacteria exist only in freshwater hydroecosystems (*Chroococcales*, *Chroococcophyceae*).

Studies have revealed dissemination of such bacteria groups in freshwater as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -proteobacteria [NDUKA 2011]. It was also discovered high content of  $\delta$ -proteobacteria in sediments of freshwater and marine waters [WANG *et al.* 2012]. Over the last five years many studies have been dedicated to bacteria resistance to antibiotics [TAE 2015].

Plants (*Phytobiota*) are considered to be one of the main groups of organic world on Earth; they refer to kingdom of eukaryotic autotrophic (photosynthetic) uni-, or multicellular organisms. By morphologic – anatomic structure and complexity of functioning plants are divided into lower with undifferentiated body – thallus (algae, some bryophyte, lichen) and higher, body of which is differentiated into root, stem, leaves. Higher plants are particularly divided into spore (bryophyte, equisetopsida, lycopodiopsida and polypodiidae) and seed (gymnosperms and angiosperms). Plants play very important role in nature: they create flora and plant cover of Earth and create favourable conditions for existence of representatives of animal world and humans, since they are primary producers of organic matter. There are around 500 thous. of modern plant species.

Algae (*Rhodobionta et Phycobionta*) is a group of lower divisions (thallophyta) of unicellular, multicellular and colonial autotrophic, chlorophyll plants from two plant subkingdoms. It is known over 35 thous. of species, majority of which are obligatory hydrobionts.

Upper water plants (macrophytes) – group of orders of mainly monocotyledons class (*Liliopsida*) of subkingdom *Embriobionta* – the most organized bryopsida organisms, ontogenesis of which takes place in water medium. Interaction of water plants with bacteria plays a very important role for many ecological processes that take place in freshwater biohydrocenoses and marine ecosystems, particularly water self-purification, nitrogen fixation, denitrification, oxidation of ammonia etc. [SRIVASTAVA *et al.* 2017]. Typical hydrophyte of freshwater biohydrocenoses is *Lemna minor* (*Alismatales*, *Liliopsida*).

Fungi (*Mycobiota*) refer to kingdom of heterotrophic eukaryotic organisms (around 100 thousands of species around the whole Earth territory). They consume organic compounds osmotically (adsorbition consumption) taking them from inanimate substrate – saprobionts, or from living organisms – parasites. Species of certain fungi remain in symbiosis with algae (lichens) and woody plants, creating mycorrhiza. Vegetative body consists of mycelium

(mashroom spawn) that is a system of thin branched hyphae. Cells of fungi are uni-, be- or multinuclear. Cellular wall of hyphae is solid, consists 80–90% from polysaccharides, the main of which in majority of fungi is chitin, and in oomycetes – cellulose. Important characteristic of upper fungi (ascomycota and basidiomycota) is ability to create sporocarps that develop from vegetative mycelium. Reproduction is asexual (vegetative) and sexual. In some fungi it is known even budding (yeast). Modern mycologists take such division of fungi: true fungi (*Eumycota*), water fungi (*Oomycota*), slime molds (*Myxomycota*) and lichens (*Lichenes*). Among fungi prevail terrestrial microorganisms, but there are some species that are popular in water ecosystems. To such species refer phycomycetes (*Zygomycetes*, *Chytridiomycetes*, *Plasmodiophoromycetes*, *Hyphochytridiomycetes*, *Trichomycetes*, *Oomycetes* etc. [NDUKA 2011].

Animals (*Zoobiota*) refer to kingdom of heterotrophic uni- or multicellular organisms; for cells of all animals, except tunicates, is typical absence of cellulose cellular wall. They are capable of growing to certain age, except alligators and tortoises. During evolutionary process structure and functions of multicellular animals become more complicated: appeared tissues, organs and their systems, adaptation that supply stability of inner medium (homeostasis); developed special complex forms of behaviour etc. It is known around 1.5 mln of animal species that live in our time, among them over 1 mln insects. To primary and secondary hydrobionts refer many representatives of invertebrates (protozoa, coelenterata, mollusks, arthropods etc.) and vertebrates (chordate) animals.

Protozoans (*Protozoa*) are types of unicellular animals (sizes 2–200  $\mu\text{m}$ ), body of which consists from cytoplasm (is divided into ectoplasm and endoplasm), pseudopodia (temporary formations of ectoplasm) and located in endoplasm nucleus and organelles. Digestion takes place with the help of digestive vacuoles, secretion and gaseous exchange – through contractile vacuoles or body surface. They reproduce mainly by division. They are capable of creating cysts under unfavourable conditions. There are more than 30 thous. species (in Ukraine – around 1500), popular around the world. They are divided into five classes: sarcodina, rhizopoda, radiolaria, sunflowers and zoomastigina [NDUKA 2011].

## RESULTS

It is important to take into account that over the last centuries took place an intensification of industrial development processes and urbanization that are connected to intensive extraction and use of metals, what causes significant environmental pollution by metal containing production wastes. Hence metal compounds usually fall into hydrosphere components through soil and rock erosion, industrial and municipal wastewaters, wastes that are created during mining and fossil fuel enrichment. Serious problem connected to pollution of hydrosphere components and pollutants accumulation in hydrobionts cells is possibility of their biomagnification in ecosystems' trophic chains. It has been also studied on metals affecting hydrobionts. In the result of this research it was discovered hazardous flow

into the hydrosphere non-essential compounds (Hg, Cd, Pb) that are capable of accumulating in hydrobionts cells and causing cumulative genetic toxicity. Essential metals are also capable of bioaccumulation and excessive accumulation of such elements as Fe, Zn, Cu, Mn, what leads to metabolic disorders in cells of aquatic biomes [GROSS, GROSS 2016].

Aquatic biome of regulated rivers should be viewed from three main aspects: as a natural resource, as an indicator of the environmental state and as a factor of shaping water quality. According to data from Dnieper.org, phyto-benthos of Dnieper and its reservoirs is characterized by great diversity of both in number of species representatives and in their quantitative development factors. In content of algae that are growing at the water/bottom division level depending on reservoir and season appear different species and interspecies taxa: in Kyiv – 181, in Kremenchuk and Kamianske – 128, in Kakhovka – 88. Periphytone single-stock reserves are very considerate: in Kremenchuk Reservoir in summer they reach up to 5000 Mg in dry matter [KLYMENKO *et al.* 2006].

Having adapted to certain living conditions, every species very vulnerably reacts on change of environmental factors. Quantitative and qualitative deviation of physical and biochemical indicators in any direction can be differently perceived by water ecosystem habitants. Substantial factor that causes disbalance in biohydrocencosis is eutrophication – artificial overfertilisation of water as a result of pollution by agricultural and municipal discharges, what leads to rapid algal growth and reduction of dissolved oxygen content in bottom layers through decomposition of dead organic matter that is created with increasing sedimentation [HOREV *et al.* 1994].

Eutrophication of surface water can be in the result of both anthropological impacts and biohydrocencosis succession. Main chemical elements that cause eutrophication are phosphorus and nitrogen, but presence of other plays defining role in the processes that take place in waters. This is why it is reasonable to view hydrochemical and hydrobiological regimes, on balance between which depends quality of surface waters of Dnieper reservoirs.

Eutrophication problem was dramatic for 54% of freshwaters in Asia, 53% – in Europe, 48% – in North America; 41% – in South America, 28% – in Africa. For artificial waters – reservoirs that do not possess full natural self-purification mechanisms eutrophication problem is even more dramatic than in case of natural lakes [SIRENKO 2002].

Reservoirs that are created in the result of hydroplants constructions now occupy territory of around 600 000 km<sup>2</sup> in the world (which approximately is full territory of Ukraine). During last decade there were more than eight large hydroelectric power stations (with dam height over 15 m) that supplied 19% of world electricity production [KHIZHNYAK, EVTUSHENKO 2014]. Human need in energy that is constantly growing and the need to convert to using renewable energy sources cause establishment of new hydroelectric power plants and construction of new and new reservoirs. As a rule on shores of such reservoirs takes place intense agrarian activity (through the access to water

for irrigation), large industrial objects are built that require a lot of water for their functioning and/or electricity (such as nuclear power stations, chemical, metallurgical enterprises etc.), a lot of people live.

## DISCUSSION

Hydrobionts are actively used for conducting bioindication. Representatives of different trophic levels are used for testing toxicity level in water medium. For instance, some of latest researches were concentrated on studying environmental and biological effect from metals and binary nanoparticles of different chemical nature with application of model organisms of such species: *Escherichia coli*, *Chlorella vulgaris*, *Paramecium caudatum*, *Daphnia magna*, *Danio rerio*. In the result of these studies it was revealed that the most sensitive to pollution of the medium with nanoparticles was *Chlorella vulgaris* [MORGALEVA *et al.* 2017]. JARQUE *et al.* [2016] assessed *Vibrio fischeri* in the assessment of seasonal and spatial patterns in toxicity of contaminated river sediments. Their studies confirmed the good correlation between the *V. fischeri* kinetic assay performed directly in sediments (contact test) and the toxicity values obtained in elutriates. This study demonstrated higher toxicity in samples from sediment traps in comparison to samples from bottom sediment collected at the same locality in the same time. Hydrobionts were also used for studying toxicity level of caesium oxide nanoparticles [MORGALEV *et al.* 2017]. The biotesting defined toxicity level according to EU Directive 93/67/EEC, SGS certified system.

DA ROS *et al.* [2013] showed that some species of *Gammarus* (*Amphipoda*, *Crustacea*) survived under unfavourable conditions in the glacial period. One of defining factors that affects life cycle of hydrobionts directly is salinity. Salinization of water leads to change of equatorial organisms' population. In the result of the latest studies it was discovered that in lake Zhaksy-Klych near Aral Sea a salty layer caused death of all protozoa organisms and in cells of filamentous algae – plasmolysis [ISSAEVA *et al.* 2018]. Researches on hydrobionts foresee defining such priority directions as complex study on structure and functioning of freshwater and marine ecosystems with purpose to create scientific basis of rational use and protection of water, particularly hydrobiological resources, creating optimum monitoring and forecasting systems on their conditions, avoiding negative anthropological impact, constructing cultivation technologies for hydrobionts of different trophic levels under both natural and artificial conditions, creating new information technologies and systems of accumulation, processing and analysis of data and also spreading scientific knowledge in the field of biology and ecology of inland waters and seas.

For studying complex and interdependent processes of hydrobionts dynamics population, quantitative methods on studying natural hydrobionts grouping (particularly for defining number of individuals from certain species and their biomass) and also spectrometric methods on defining chlorophyll contents in phytoplankton, bacterioplankton studying methods etc. gained great popularity. Microbial

diversity is a very important part of general global biodiversity that exists on our planet today. Species that are known to modern science appeared in the result of evolutionary process that lasts over 4 billion years. It is known that water microorganisms can be used for production of enzymes (cellulase, lipase, amylase, protease, catalase, phosphatase) [MORGALEVA *et al.* 2017].

For biological research on water medium different special devices are used. Recently mathematical modelling methods and application of computing devices have been significantly developed. Achievements in the field of studying hydrobionts nature are widely used for developing measures directed at water protection, water quality supply, biodiversity reservation (genetic, species, ecosystemic), complex application and enrichment of waters' biological resources etc. In biotesting system different hydrobionts are used with the purpose to study impact on them from polymeric derivatives of guanidine that in many countries are used as antiseptics and disinfectants. Results of some studies have revealed bacteriostatic effect under  $1 \text{ mg}\cdot\text{dm}^{-3}$  concentration and bactericidal effect from  $10 \text{ mg}\cdot\text{dm}^{-3}$  concentration and higher. As test objects were used infusoria (*Paramecium caudatum*), flat worms (*Dendrocoelum lacteum*), crustaceans (*Daphnia magna*), snails (*Lymnaea palustris*) and osteichthyes (*Poecilia reticulatum* *et Cyprinus carpio*) [LYSYTSA *et al.* 2017].

There are many biotechnological processes that include hydrobionts, particularly their biomass as a substrate, and obtained target products are used in different fields of energy, cosmetology, medicine, pharmaceuticals, aquaculture, agriculture, forestry etc. Some scientists based on blue-green algae biomass have studied possibility of producing biofuel (methane, methanol, ethanol, propanol, isopropanol, biodiesel etc.), and also glucuronic and hyaluronic acid [NYKYFOROV *et al.* 2016]. Algae are very perspective for further processing and production of target product, since they contain biologically active compounds. Algae pigments are astaxanthin, lutein, zeaxanthin, canthaxanthin, chlorophyll, phycocyanin, fucoxanthin; they contain also antimicrobial compounds, amino acids, proteins. To vitamins that can be derived from algae are biotin, riboflavin, nicotinic acid, pantothenate, folic acid. Algae contain also such polyunsaturated fatty acids (PUFAs) as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (ARA), gamma-Linolenic acid (GLA) and such antioxidants as catalase, polyphenol, superoxide dismutase, tocopherols. Among carotenoids,  $\beta$ -carotene can be derived from algae as well [SHARMA, SHARMA 2017].

ALASSALI *et al.* [2016] have dedicated their studies to establishing possible spectrum of low-tonnage useful products that can be extracted from microalgae and potential possibilities of their application. Both microalgae and macroalgae have components that can be used in different fields. For example, *Spirulina*, *Chlorella*, *Dunaliella*, *salina*, *Dunaliella* *pluvialis* are rich on lutein, sterols, astaxanthin, PUFA's that can be used in treatment of degenerative diseases, prevention of cardiovascular diseases.

Process of anaerobic digestion is popular in treatment of great variety organic wastes, particularly during produc-

tion of biogas. For organization of this process it can be used both macrophytes and microphytes. Chemical composition of substrate and parameters of process' organization define number and composition of biogas that is generated during anaerobic digestion (biomethanogenesis). Theoretically output of biogas can be defined with the help of Buswell equation [BUSWELL, MUELLER 1952]. Latest developments prove efficiency in applying anaerobic digestion for purifying wastewaters from organic pollutants. Nevertheless, using this process for purifying effluents has also drawbacks, particularly high concentrations of organic matter and ammonia in discharges after anaerobic digestion. Apart from that, anaerobic digestion does not supply with required efficiency of removing phosphorus [BOHUTSKYI, BOUWE 2012]. One of the key factors in achieving positive balance during biomethanogenesis of algae biomass is high level of transformation of organic matter into biomethane, since its increase appeared to be substantial factor in improvement of energy balance process and reduction of greenhouse vapors [MAYFIELD 2015; PECHSIRI *et al.* 2001]. Studies have also established efficiency in using fermentation process of freshwater algae, particularly *Chlorella vulgaris*, for producing biogas. In the result of these studies it was proven that utilization of *Chlorella* biomass as monosubstrate is problematic because of high content of protein [ADAMIETZ *et al.* 2018]. Possibly stable fermentation led to considerable accumulation of unfermented organic matter, what caused creation of 54% protein in digestate. This is why the scientists were incapable of achieving stability in the process of obtaining biogas under increased concentrations of proteins in biomass. Separate studies have been dedicated to biorefining – balanced process when biomass is converted into target product and additional energy carriers. Such process is already widely used in many countries: Great Britain, USA, Canada, China, Japan, Spain etc. [GONZALEZ-DELGADO, KAFAROV 2011]. Microalgae are considered as a raw material which can be used with obtaining superprofit. The purpose of this method is in production of products with added value alongside with conversion of residual biomass into biofuel [GATAMANENI *et al.* 2018]. Modern bioeconomy (bio-based economy) foresees construction of biocluster chains, main structural unit of which are biorefineries – enterprises that conduct conversion of biomass into fuel, energy and chemical compounds in full cycle. In this case biogas station can be regarded as biorefinery that will be energy plastic nucleus of biocluster infrastructure (gas generator, biogas liquefaction plant, fuel boiler on biomass, cogeneration unit, phycocyanin production enterprises, amino acid hydrolyzate, processing degistate into biofuel, greenhouse complex etc.) [ZAHIRNYAK *et al.* 2017].

Many studies have established that it is possible to reach high level of lipid extraction from algae (to 95%) with the help of organic solvents (methanol, acetone, hexane, diethyl ether etc.) [MALOVANYI *et al.* 2015]. Saponification process is used as first stage of processing extracted lipids for effective biofuel production and using algae raw material for other purposes (pharmaceutical, perfumery, etc.). Scientists [TAO *et al.* 2016] in the process of laboratory studies reached 96% of chlorophyll removal in

biomass *Scenedesmus*. It was established that content of fatty acids in the algae oil had decisive effect on biodiesel quality.

Biodiesel is produced from lipids that are extracted from algae during chemical-technological process which is called transesterification. Necessary condition for implementing this process is using heat, mechanical mixing and catalyzers. Average duration of reaction lasts from 4 to 6 h [Hielscher undated]. From algae biomass with the help of special extractors two phases (upper and lower) of oil can be extracted. Lower phase gives excessive methanol, water and in upper phase algae oil is obtained. Transesterification reaction with alcohol ( $\text{CH}_3\text{OH}$ ) after upper phase allows to obtain glycerol in lower phase and crude oil biodiesel in upper phase. Thus purified biodiesel can be obtained from algae biomass with the help of transesterification.

Hydrogen is regarded as pure, environmentally save fuel – renewable energy resource, and also acceptable alternative for substitution fossil fuels. Hydrogen is the only fuel, combustion of which is not supported by creating carbon dioxide.

It has been researched that many algae species can be potential source for production of hydrogen under certain conditions [LI, FANG 2007]. To such species refer *Cyanidioschyzon merolae*, *Ostreococcus lucimarinus*, *O. tauri*, *Micromonas pusilla* [KARS *et al.* 2006]. Nonetheless, commercial production of hydrogen from algae biomass is still unpractical, since biomass concentration is low and the very process is quite expensive. For hydrogen production can be used different types of bioreactors. As an example, hydrogen production process can be held in multi-stage reactor. At first stage takes place photolysis of water, then at nutrition medium takes place light and dark fermentation phases. At final stage takes place electrolysis with obtaining hydrogen [SHARMA, ARYA 2017].

Processed after biomethanogenesis or lipid extraction algae biomass (digestate) can be used as bonding material for briquetting miscanthus (popular energy culture, biomass of which is used for creating pellets and granules). Scientists have used for their researches algae species *Rhizoclonium sp.*, biomass of which at first was dried, later it was added in the shape of powder to ground miscanthus [THAPA *et al.* 2015]. After that material was compressed into granules with the help of press. Obtained biomass granules were tested on gross calorific value, dynamic strength and hydrogen content. In the result of studies it was revealed that granules from miscanthus mixture and ground algae (to 30%) had same gross calorific value as miscanthus granules without adding algae. But in miscanthus granules, mixed with algae dynamic strength was 20% higher than in solely miscanthus granules.

Effective way to intensify biotechnologies with using hydrobionts as a raw material alongside with chemical methods (pretreatment of biomass with hydrogen peroxide) can be processes that lead to destruction of cellular walls of water organisms and as a results – complete release of intercellular organic compounds that are substrate for digestion, extraction etc.

In case of algae cellular wall destruction with the help of ultrasound processing (cavitation) extraction of target

product increases from 4.8% to 25.9% [CRAVOTTO *et al.* 2008]. With hence pretreated raw material biodiesel is obtained with the help of traditional technology – transesterification of plant oils. Lipid raw material consists of 90–98% (weight) triglycerides and small amount of mono- and diglycerides, contains fatty acids 1–5% and small amount of phospholipids, phosphatides, carotenes, tocopherols, sulfur compounds and water traces [BOZBAS 2008].

One of methods for intensification of lipid extraction from microalgae is hydrodynamic cavitation. SETYAWAN *et al.* [2018] for their researches used biomass *Nannochloropsis sp.* In the result of their studies it was established optimum conditions for running process: energy supplied  $91.3 \text{ kJ}\cdot\text{kg}^{-1}$ , temperature  $42^\circ\text{C}$ , cavitation index 0.126 and algae concentration  $0.105 \text{ g}\cdot\text{g}^{-1}$ . Optimum conditions for extraction were under supply of  $16.743 \text{ mJ}$  energy per 1 kg of extracted lipids. Other scientists (LEE *et al.* [2015]) used algae *Tetraselmis suecica* for biofuel production after hydrodynamic cavitation.

## CONCLUSIONS

All energy biotechnologies connected to utilization of any wastes (organic municipal solid waste and wastewaters: residual activated sludge and primary sediment of pollution control facilities; agro-biomass, mainly liquid and solid wastes of post-harvest activity and cattle breeding that pollute environment; spoiled food products etc.) and also excessive biomass of organic origin (massive forms of hydrobionts, falls of deciduous forests, particularly forest litter, park zones of human settlements etc.) are environmentally reasonable. However their economic efficiency grows thanks to using digestate as an additional source of other target products, for instance biofuel, biologically active compounds.

For this biorefining is used, which is a balanced process when biomass is transferred into target products and additional energy carriers. Modern biobased economy foresees constructing biocluster chains, main structural unit of which are biorefineries – enterprisers that conduct conversion of biomass into fuel, energy and chemical compounds in entire cycle.

In our time microalgae are considered as a resource material suitable for receiving superprofit. In this case biomass processing biogas station can be regarded as a biorefinery which will be energy plastic nucleus of biocluster infrastructure (gas generator, biogas liquefaction plant, fuel boiler on biomass, cogeneration plant, plants with production of phycocyanin, amino acid hydrolysate, digestate to fertilizer processing, greenhouse complex etc.).

Hence, application of biotechnologies on utilization of excessive biomass, particularly of hydrobionts is an important component for supplying balanced natural resource management system and environmental safety in regions. Over the last decades many scientists have dedicated their works to study different massive forms of aquatic biomass: their function properties have been studied, living habitat peculiarities and cultivation conditions.

Apart from that, many studies have been dedicated to technologies for their effective harvesting, pretreatment

with the purpose to use as a raw material for newer biotechnologies. Every technology offered by scientists overall has pilot character and due to political and other aspects is incapable of being launched in full large scale production.

High production cost of target products is usually one of the main obstacles of practical implementation and application in real economy sector of projected biotechnology. Further studies in this field will help to better assimilate gained knowledge and also to offer innovation biotechnologies with applying hydrobionts as a substrate for diversification of energy sources and other target products.

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