MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL AVIATION UNIVERSITY
FACULTY OF ENVIRONMENTAL SAFETY,
ENGINEERING AND TECHNOLOGIES
DEPARTMENT OF ENVIRONMENTAL SCIENCE

APPROVED TO DEFENCE
Head of the Graduate Department
_____________ T. V. Dudar
«_____» ____________ 2022

BACHELOR THESIS

(EXPLANATORY NOTE)

SPECIALTY 101 “ECOLOGY”,
EDUCATIONAL AND PROFESSIONAL PROGRAM:
“ECOLOGY AND ENVIRONMENT PROTECTION”

Theme: « The role of microalgae in the removal of phosphates from freshwater ecosystems »

Done by: student of EK-411 group, Anastasiia A. Hetman
(student, group, surname, name, patronymic)

Supervisor: Ph. D. in Biol. Sc., Associate Professor of the Ecology Department, Tetiana I. Bilyk
(academic degree, academic rank, surname, name, patronymic)

Standards Inspector: _____________ Andrian A. Iavniuk
(signature) (S.N.P)

KYIV 2022

МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА ЕКОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової кафедри
_____________ Т. В. Дудар
«_____» ____________ 2022 р.

ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ВИПУСКНИКА ОСВІТНЬОГО СТУПЕНЯ БАКАЛАВРА

ЗА СПЕЦІАЛЬНІСТЮ 101 «ЕКОЛОГІЯ»
ОПП «ЕКОЛОГІЯ ТА ОХОРОНА НАВКОЛИШНЬОГО СЕРЕДОВИЩА»

Тема: «Роль мікроводоростей в очищенні прісноводних екосистем від фосфатів»

Виконавець: студентка групи ЕК-411, Гетьман Анастасія Олексіївна
(студент, група, прізвище, ім'я, по батькові)

Керівник: кандидат біологічних наук., доцент кафедри екології Білик Тетяна Іванівна
(науковий ступінь, вчене звання, прізвище, ім'я, по батькові)

Нормоконтролер: _______________ (підпис) Явнюк А. А. (П.І.Б.)

КИЇВ 2022

NATIONAL AVIATION UNIVERSITY

Faculty of Environmental Safety, Engineering and Technologies

Department of Environmental Science

Specialty, Educational and Professional Program: specialty 101 “Ecology”, Educational and Professional Program: “Ecology and Environment Protection”

(code, name)

APPROVED
Head of the Department
___________ Dudar T.V.
«____» _________ 2022

BACHELOR THESIS ASSIGNMENT

Anastasiia A. Hetman

1. Theme: « The role of microalgae in the removal of phosphates from freshwater ecosystems» approved by the Rector on April 18, 2022, № 388/ст.

2. Duration of work: 23.05.2022 to 19.06.2022.

3. Output work (project): scientific literature on pollution of aquatic ecosystems by phosphates, international and domestic data on the analysis and methods of determining surface water pollution, literature sources on the use of microalgae for freshwater treatment.

4. Content of explanatory note: (list of issues): Contents of the explanatory note: analytical review of literature sources on the subject of the diploma. Consideration of phosphates as a factor that pollutes surface waters. Introduction to methods of cleaning water bodies from phosphate pollution, and the role of microalgae in this process. Conclusion on the role of microalgae in the ecosystem and their cleaning ability.

5. The list of mandatory graphic (illustrated materials): tables, figures.

6. Schedule of thesis fulfillment

№	Task	Term	Advisor's signature
1	Receive themes task, search the literature and legislation	08.04.2022	
2	Preparing the main part (Chapter I)	23.05-29.05.2022	
3	Preparing the main part (Chapter II)	30.06-01.06.2022	
4	Preparing the main part (Chapter III)	02.06-04.06.2022	
5	Formulating conclusions and recommendations of the thesis	05.06.2022	
6	Making an explanatory note to the previous presentation of the department, consultation with the norms controller	05.06 - 07.06.2022	
7	Presentation of the work at the department	08.06.2022	
8	Taking into account the comments and recommendations and training to protect	09.06 - 13.06.2022	
9	Thesis defense at the department	14.06.2022	

7. Date of task issue: «08» _April__ 2022

Diploma (project) advisor: _________________ _Bilyk T.I._____
(advisor's signature) (S.N.P.)

Task is taken to perform: _________________ __Hetman A.A._______
(graduate's signature) (S.N.P.)

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

Факультет екологічної безпеки, інженерії та технологій

Кафедра екології

Спеціальність, освітньо-професійна програма: спеціальність 101 «Екологія», ОПП «Екологія та охорона навколишнього середовища»

(шифр, найменування)

ЗАТВЕРДЖУЮ
Завідувач кафедри
___________Дудар Т. В.
«____» _________ 2022 р.

ЗАВДАННЯ

на виконання дипломної роботи

Гетьман Анастасія Олексіївна

1. Тема роботи «Роль мікроводоростей в очищенні прісноводних екосистем від фосфатів» затверджена наказом ректора від «18» квітня 2022 р. № 388/ст.

2. Термін виконання роботи: з 23.05.2022 по 19.06.2022 р.

3. Вихідні дані роботи: наукова література про забруднення водних екосистем фосфатами , міжнародні і вітчизняні данні щодо аналізу та методики визначення забруднення поверхневих вод, літературні джерела про використання мікроводоростей для очистки прісноводних водойм.

4. Зміст пояснювальної записки: аналітичний огляд літературних джерел з тематики диплома. Розгляд фосфатів як фактора що забруднює поверхневі води. Ознайомлення з методами очищування водних об'єктів від фосфатного забруднення, та роль мікроводоростей у даному процесі. Висновок про роль мікроводоростей в екосистемі та їх очисна здатність.

5. Перелік обов’язкового графічного (ілюстративного) матеріалу: таблиці,рисунки.

6. Календарний план-графік

№ з/п	Завдання	Термін виконання	Підпис керівника
1	Отримання теми завдання, пошук літературних джерел та законодавчої бази	08.04.2022	
2	Підготовка основної частини (Розділ І)	23.05-29.05.2022	
3	Підготовка основної частини (Розділ ІІ)	30.06-01.06.2022	
4	Підготовка основної частини (Розділ ІІІ)	02.06-04.06.2022	
5	Формулювання висновків та рекомендацій дипломної роботи	05.06.2022	
6	Оформлення пояснювальної записки до попереднього представлення на кафедрі, консультація з нормоконтролером	05.06 - 07.06.2022	
7	Представлення роботи на кафедрі	08.06.2022	
8	Урахування зауважень, рекомендацій та підготовка до захисту	09.06 - 13.06.2022	
9	Захист дипломної роботи на кафедрі	14.06.2022	

7. Дата видачі завдання: «08» квітня 2022 р.

Керівник дипломної роботи (проекту): ________________ Білик Т. І.
(підпис керівника) (П.І.Б.)

Завдання прийняв до виконання: ______________ _Гетьман А.О______
(підпис випускника) (П.І.Б.)

ABSTRACT

Explanatory note to the thesis on the topic « The role of microalgae in the purification of freshwater ecosystems from phosphates »: 42 pages, 23 figures, 1 tables, 22 references.

Object of research the role of microalgae in the process of water treatment

Aim of work – to determine the role of microalgae in the purification of freshwater ecosystems from phosphates

Mehods of research: analytical, chemical, statistical methods of data processing, toxicological.

Practical importance: studies of the ability of freshwater ecosystems to purify water due to their high photosynthetic activity can be used to solve pollution problems with both phosphates and synthetic water detergents.

PHOSPHATES, WATER POLLUTION, MICROALGAE,EUTROPHICATION

CONTENT

INTRODUCTION

Relevance of the work. Today, the pollution of freshwater ecosystems with phosphorus is facilitated by household wastewater containing phosphates as synthetic detergents and water softeners. Another important factor is the leaching of phosphorus fertilizers and pesticides from agricultural lands, livestock industry, and industrial waste. Excessive supply of phosphorus in water sources leads to their eutrophication, and therefore to the accumulation of biotoxins, deterioration of water quality, water life, etc. Improving the ecological state of water resources is a goal for many developing countries, especially by reducing the content of phosphorus in wastewater. As part of wastewater treatment, in recent years are gaining interest in microalgae. This is directly related to their ability to use phosphorus in wastewater for their growth, with the desired result of a decrease in the concentration of these substances in water.

Aim and tasks of the diploma work

Aim of the work – to determine the role of microalgae in the purification of freshwater ecosystems from phosphates

Tasks of the work:

1.Analysis of phosphate pollution problems

2.Investigate the problems of eutrophication

3.Carry out a model experiment with phosphate and phosphate-free detergents as factors that pollute water bodies

4.Draw conclusions about the role of microalgae in ecosystems and their cleaning capacity

Object of research pollution of surface waters with phosphorus

Subject of research the role of microalgae in the process of water treatment

Methods of research **–** analytical, chemical, statistical methods of data processing, toxicological.

Personal contribution of the graduate: analysis of scientific literature on the topic of work, conducting research on the basis of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine.

Approbation of results.

1. Білик Т.І., Бондаренко А.О., Гетьман А.О., Тирінова А. І. Перспективи удосконалення систем очищення стічних вод від фосфатів. Комплексне забезпечення якості технологічних процесів та систем (КЗЯТПС – 2020): матеріали тез доповідей X Міжнародної науково-практичної конференції (м. Чернігів , 29–30 квітня 2020 р.): у 2-х т. Національний університет «Чернігівська політехніка». – Чернігів: ЧНТУ, 2020. – Т. 2. – С.145-147.

2. Bondarenko A.A., Hetman A.A. Current state of implementation of phosphate-free detergents in Ukraine and environmental awareness. (c. Kyiv November 25-26, 2020), c. Kyiv. 2020. P. 32-41.

3. Bondarenko AO, Hetman AO, Bilyk TI., All-Ukrainian competition of student scientific works in natural, technical and humanities in the specialty "Ecology"., Ecological safety of modern phosphate-free detergents for aquatic ecosystems, March 17-19, 2021, Poltava.-P.14-15.

4. Білик Т.І.1, Веренікін О.М., Бондаренко А.О., Гетьман А.О., Тремасова П. С.,Підвищення екологічної безпечності безфосфатних мийних засобів для водних екосистем зміною компонентного складу.Комплексне забезпечення якості технологічних процесів та систем (КЗЯТПС –2021): матеріали тез доповідей XI Міжнародної науково-практичної конференції (м. Чернігів, 26–27 травня 2021 р.): у 2 т. / Національний університет «Чернігівська політехніка». Чернігів : НУ «Чернігівська політехніка», 2021. – Т. 2. – С.150 – 151

CHAPTER 1
DANGER OF PHOSPHATE POLLUTION

Pollution of surface waters with phosphorus contributes to the inflow of domestic sewage containing phosphates as components of synthetic washing agents, photo-agents and water softening agents. Another important factor is the washing of phosphorous fertilizers and pesticides from agricultural lands, livestock and industrial enterprises.

It is worth noting the double role of phosphorus compounds. On the one hand, they play a major role in the process of photosynthesis and are a necessary material for the construction of phytoplankton cells, on the other - an excess of phosphorus compounds leads to the development of eutrophication of reservoirs [1].

Eutrophication is a complex process that occurs in freshwater or marine bodies, causes the rapid development of certain types of microalgae, and disturbs the balance of aquatic ecosystems, which leads to changes and deterioration of biological indicators of water. At the same time, the fauna of water objects changes: some organisms die, and the qualitative and quantitative composition of fish decreases. Due to the intense reproduction of parasitic organisms, the incidence of hydrobionts increases.

1.1. Phosphate content in surface waters and sources of their receipt

Phosphorus has the highest bioaccumulation ratio, so it is an important nutrient for all life forms. It determines the trophic state of freshwater ecosystems. It is because of this special attention devoted to studying the transformation and migration of phosphorus compounds in reservoirs.

If an excessive amount of phosphorus comes to the reservoir, this causes eutrophication. It is the eutrophication that leads to the accumulation of biotoxins, the death of hydrobionts, deterioration of water quality, etc.

Thus, it can be argued that the concentration of phosphorus is one of the most common causes of eutrophication of primeval lakes, reservoirs, and rivers, resulting in excess production of autotrophs, especially algae and cyanobacteria. This high productivity leads to the growth of bacterial populations and high respiratory rates, which causes hypoxia or anoxia in poorly mixed bottomed waters, as well as in surface waters at night in quiet, warm conditions. Low levels of dissolved oxygen cause the death of aquatic animals and the release of many substances, usually bound by bottom sediments, including various forms of phosphorus. This, in turn, enhances eutrophication. [2,3].

Sources from which phosphates fall into surface water can be divided into two categories: point sources (for example, industrial enterprises, wastewater treatment plants of housing and communal enterprises) and diffuse springs (for example, runoff from farmland).

The main sources from which phosphorus compounds will enter the reservoirs are: atmospheric precipitation, surface runoff from built-up areas, river runoff, soil erosion, bottom sediments, drainage water irrigation systems, tributary of phosphorus from deep sea waters. In addition, sources of phosphates are also considered continental weathering, fertilizers, animal waste and direct disposal of phosphates [1,4].

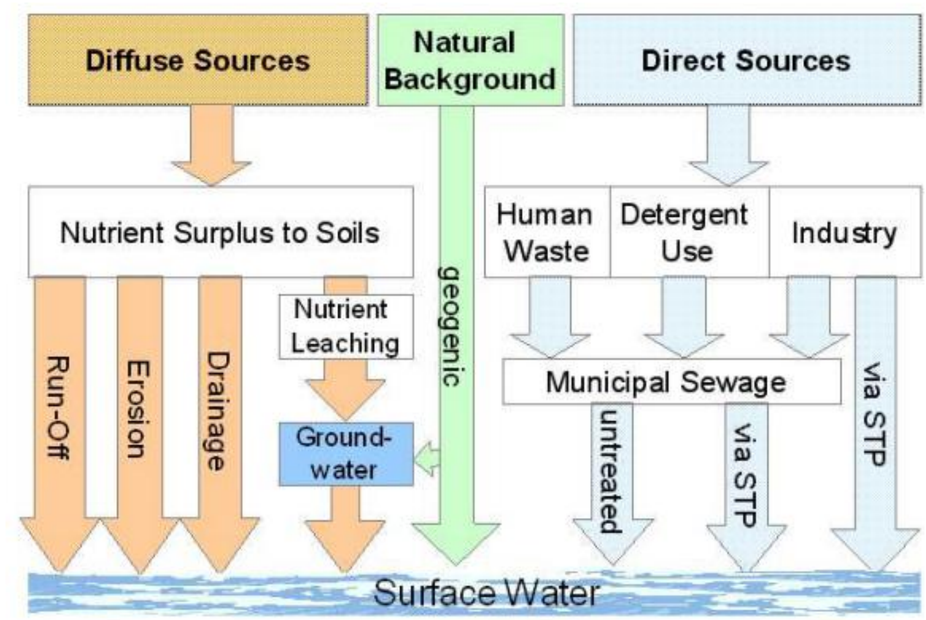

Fig. 1.1. Schematic image of phosphorus flow distribution in surface waters

The contribution of each of these sources to phosphorus-contaminated aquatic ecosystems is (average according to EU data) [5]:

- municipal sewage - 1.1%;
- detergents - 38.8%;
- agricultural activity (fertilizers, plant protection products) - 18.0%;
- soil erosion - 4.7%;
- seasonal regeneration from bottom mineralized organic deposits - 12.0%;
- industrial activity - 3.1%;
- other sources of income - 5.5%.

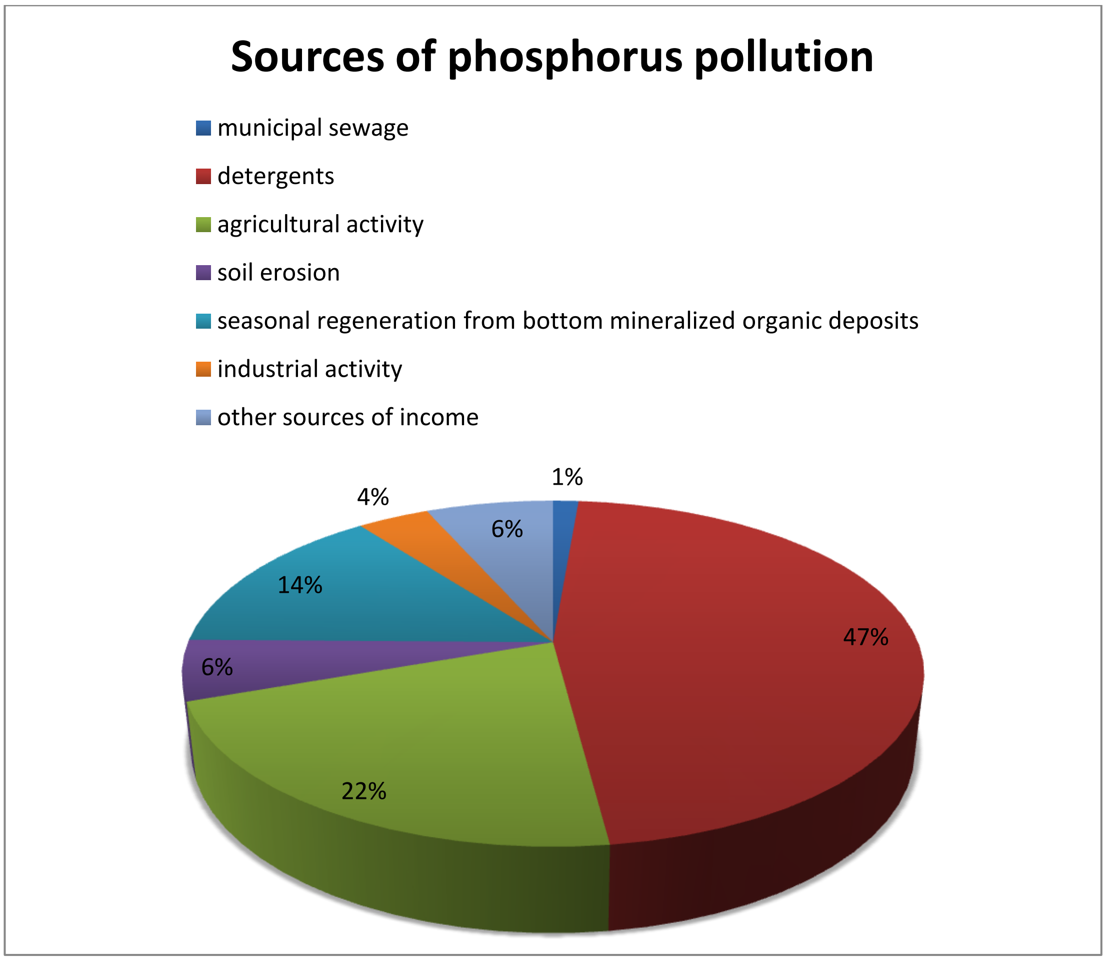

Fig. 1.2. Sources of phosphorus pollution of aquatic ecosystems (average according to the European Union)

The share of each of these sources of water pollution with phosphorus may differ from the Middle European, depending on specific weather conditions and the level of human impact.

In Ukraine, the largest water pollutant is industrial enterprises and car washes. Detergents based on polyphosphates have also contributed to this problem, increasing the biological load on aquatic ecosystems. Wastewater from the car wash flows directly into the sewer. Since the content of phosphates in such detergents is practically not normalized, one car wash that drains water without any purification can cause harm to more than two apartment buildings.

Draining domestic and industrial drains directly into the reservoir without any stages of cleaning. This is widely practiced in the highlands and on the coast of the Azov and Black Seas. If the rivers of Western Ukraine, due to the relatively small population density it does not lead to critical problems, the sea coast suffers greatly as a result of such actions.

The maximum allowable concentrations of phosphates in drinking water and water for household needs in Ukraine is 3.5 mg PO_4/dm^3, and for fisheries water, MDC of phosphates is also 3.5 mg PO_4/ dm^3, or 0.2 mg P/ dm^3.

According to the information prepared by the State Water Agency on discharges into surface water objects of phosphates as part of reverse wastewater based on state water use accounting data in 2019. So, in 2019, 5708 tons of phosphates were dropped on Ukrainian water bodies.

The largest pollutant of reservoirs is housing and communal services - 5354 tons. The second is the industry - 319.5 tons. Next: agriculture - 10.1; trade and catering - 8.3; transport - 5.1 and healthcare - 4.1 tons [6].

It is important to note that phosphorus is nutrients from limited and non-renewable sources, its operating rate at this time exceeds the percentage of phosphorus that returns to the natural cycle. It is assumed that the known available sources of phosphorus will soon be exhausted with serious and irreversible economic, social and environmental consequences.

Therefore, waste control is important for improving water quality, environmental protection, and public health [7].

Thus, the level of phosphorus in water indicates the quality of the aquatic environment, indicates the processes that occur in ecosystems, and also indicates the sources of pollution of water bodies with phosphates.

1.2. Microalgae as an important component of the purification capacity of the freshwater ecosystem

Microalgae are microscopic algae that are phytoplankton, commonly found in freshwater and marine systems that live in both water and sediment. They are single-celled eukaryotic organisms that have unique replication qualities.[8]

Microalgae contain chlorophyll and other auxiliary pigments capable of photosynthesis. Microalgae and bacteria form the basis of the food network and provide energy to all trophic levels above them. Microalgae biomass is often measured by chlorophyll concentration. Microalgae can grow in three ways: autotrophic, heterotrophic, and myxotrophic. During autotrophic growth, microalgae produce the necessary organic matter and energy, using carbon dioxide as a carbon source and sunlight. Autotrophic cultivation is usually carried out in open pond systems and closed photobioreactors. In heterotrophic growth, organic compounds are used as sources of energy and carbon.

Microalgae are used in cosmetics and pharmaceuticals as a source of essential amino acids, antioxidants, pigments, and vitamins . These substances contain growth stimulants, vitamins, amino acids, polypeptides, polymers, as well as antibacterial and antifungal substances. Microalgae are also a direct source of fertilizer, food and feed, fuel and even serve as indicators of pollution (Fig. 1.3.).

Fig. 1.3.Areas in which microalgae are used

Green algae are widespread in surface water. Chlorella (Chlorella vulgaris), and Chlamidomonas (Chlamidomonas) are most common in freshwater from unicellular forms. (Fig.1.4.).

Blue-green algae belong to the low-organized forms, they are the most adapted to life in bodies of water contaminated with organic matter. In their cells, unlike other types of algae, there are no vacuoles with cell sap and separated nuclei. Chlorophyll and other pigments (blue - phycocyanin, red - phycoerythrin, orange - carotene) are distributed in the form of grains in the outer layer of the cytoplasm. Among the blue-green algae are unicellular (Aphanizomenon flos aqna) and (Anabaena)(Fig.1.5.).

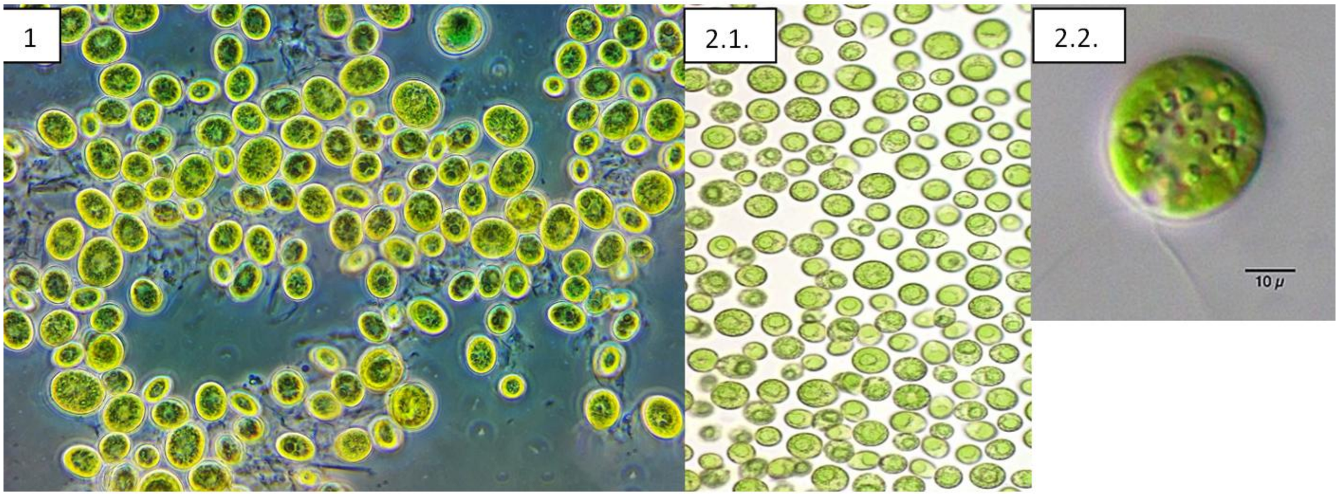

Fig.1.4. Unicellular green algae that are most common in freshwater (*1. Chlorella vulgaris; 2.1., 2.2. Chlamidomonas*)

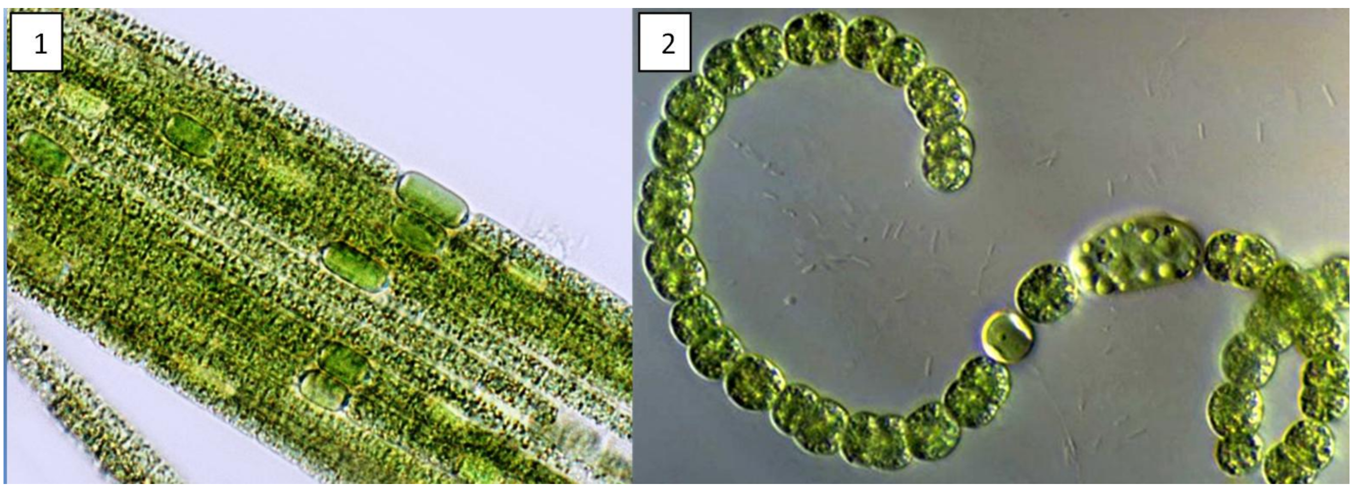

Fig.1.5. Blue and green algae (1. *Aphanizomenon flos aqna; 2. Anabaena*)

Diatoms are unicellular microorganisms that have a solid silicate shell. The pigments that make up their cells are brown or yellow. They reproduce by simple division or by spores. The structure of the silicon shell, consisting of two leaves, is a characteristic feature of the species. Of the diatoms in freshwater, the most common are Asterionella, Sinedra, and Melosira (Fig.1.6.)[9].

Fig.1.6. Diatom (*1. Asterionella; 2. Sinedra; 3. Melosira*)

An example of cleaning fresh water can take chlorella. Chlorella is a genus of microscopic single-celled green algae, with a microscopic fixed ball of 2 to 10 μm in diameter. The shell of many species contains a layer of sporopolenin, which gives it chemical resistance and strength. The cytoplasm contains one wall-shaped chloroplast with one pyrenoid in the thickened part of it. The pyrenoid is usually surrounded by a starch shell. The nucleus is one, but in a living cell, it is not visible without special processing. (Fig.1.7.) Spare substances - starch and colorless oil. Colonies and aggregates do not form. Chloroplasts in chlorelles contain chlorophyll a and chlorophyll b. It is unpretentious to the conditions of existence and due to a simple life cycle (Fig.1.8.) is capable of intense reproduction, so it is cosmopolitan: in fresh water, seas, soil. For photosynthesis, it needs only water, carbon dioxide, light.

Use of chlorella to clean water began in the mid - 20th century. At the present stage, an innovative approach that significantly reduces the level of water pollution and improves the organoleptic properties of water is the bioremediation of water bodies with a chlorella suspension, which is based on alcoholization of reservoirs with plankton strains of Chlorella Vulgaris green microalgae. This technology is based on the biological properties of living planktonic chlorella, which inhibits the action of blue-green algae (cyanobacteria).

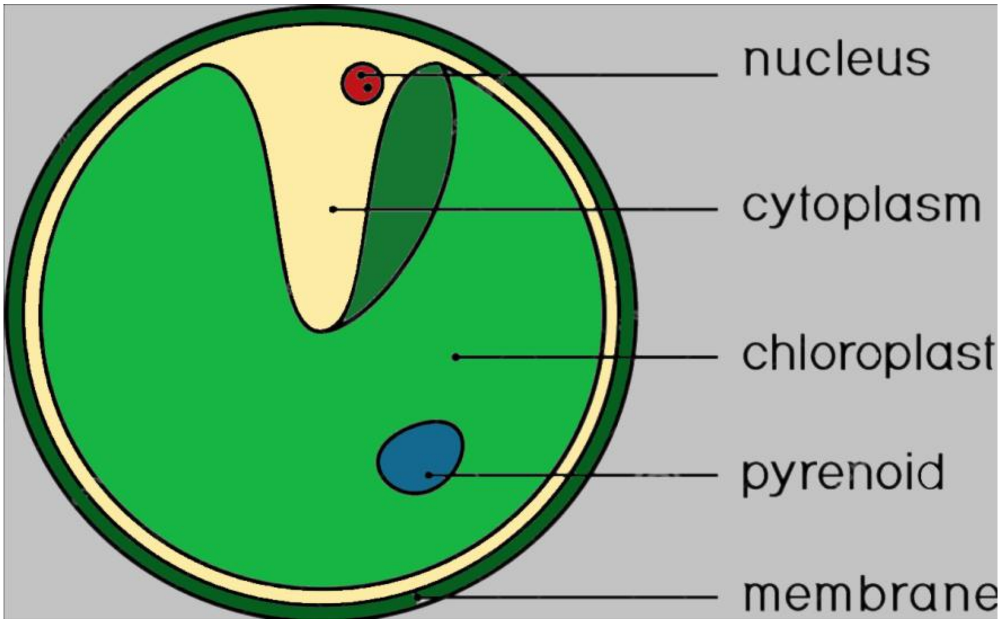

Fig.1.7. Chlorella’s structure

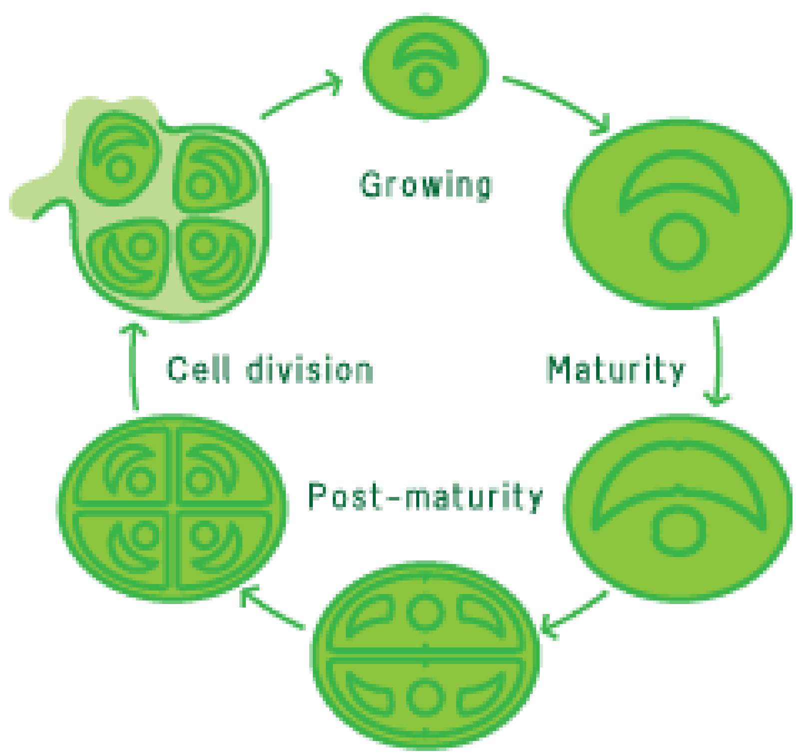

Fig.1.8. Chlorella’s life cycle

Chlorella shows natural competition and is able to influence the displacement of blue-green algae from water bodies, as well as eliminates the effects of "flowering": purifies water, saturates it with oxygen, restores the population of phytoplankton and zooplankton. Due to this, it provides fish with a natural feed base and, in general, increases the immunity of the fish herd. Algae and higher aquatic plants play an important role in the self-cleaning of the aquatic environment. Cleansing the aquatic environment from biogenic elements (N and P), water

1.3. Conclusion to chapter

Microalgae are unicellular eukaryotic organisms that have unique properties of replication, they can both purify reservoirs and cause the so-called "flowering water." It is the excess of phosphates in the aqueous environment that leads to eutrophication. In general, eutrophication, when moderate, has a positive effect on biological indicators in volumes including fish productivity. Contamination of natural water with surfactants and other compounds, especially phosphates, generally reduces the biological productivity of reservoirs. Trophic chains die, many living organisms lose a life, and entire ecosystems eventually die. Contamination of surface water with phosphorus is associated with the receipt of domestic wastewater, which contains phosphates and synthetic detergents, as well as the leaching of phosphorus fertilizers and pesticides from agricultural lands, leaching from livestock and industrial enterprises.

CHAPTER 2

MATERIALS AND METHODS OF PHOSPHATE REMOVAL INVESTIGATION OF DETERGENTS USING MICROALGAE

The work was performed on the basis of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine on the basis of a cooperation agreement.

2.1. Characteristics of freshwater microalgae and experimental methods

Washing powders have been used for research due to the fact that in recent years they are one of the main causes of water pollution with phosphorus. To compare their impact on microalgae, 4 types of washing powders were chosen. Of these, 3 do not have phosphates in their composition, and one - phosphates.

- D-1- phosphate-free, medium price category (Ukraine);
- D-2 - phosphate-free, medium price category (Ukraine);
- D-3- phosphate-free, medium price category (Germany);
- D-4 - phosphate, low price category (Ukraine).

Environmental assessment of the safety of detergents was performed by biotesting solutions in concentrations ranging from 0.01 to 100 mg / l in experimental ecological and toxicological studies using as test objects algae species Microcystis aeruginosa and Desmodesmus brasiliensis.

Microcystis aeruginosa is a species of freshwater cyanobacteria, or blue-green algae. Cyanobacteria produces neurotoxins and peptide hepatotoxins such as microcystine and cyanopeptoline. Microcytin is a large cyclic peptide that damages the liver, and especially sensitive to its toxic effects are pets and livestock.

Microcystis aeruginosa flowering has an ecological impact and is a danger to fish and wildlife and can also be harmful to humans during recreational water use due to contact with the body, unintentional ingestion or inhalation of water drops. For example, after contact with the body during rest in water can lead to slight skin irritation or allergic skin reactions, including eye irritation and the formation of blisters on the lips [10].

The flowering of cyanobacterial algae thrives due to the high content of phosphorus in agricultural effluents. In addition to phosphorus consumption, Microcystis aeruginosa thrives on glyphosate, although high concentrations may inhibit it.

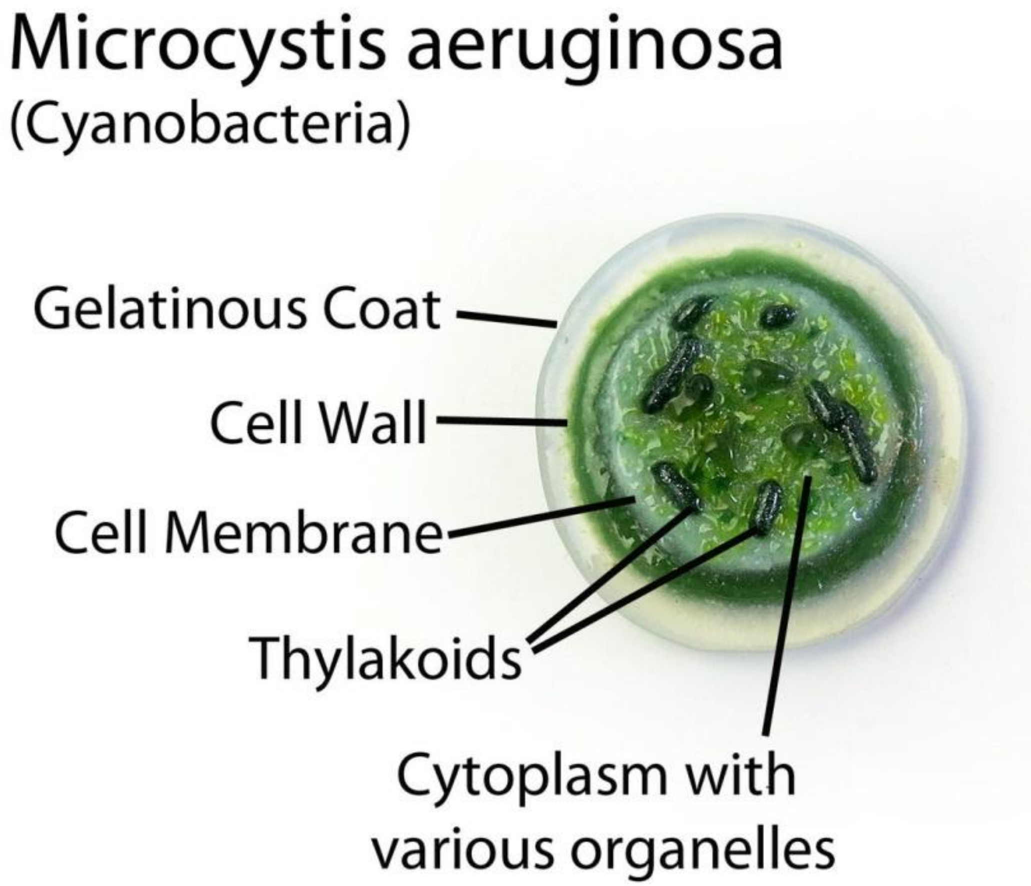

Fig.2.1. Structure of *Microcystis aeruginosa*

Desmodesmus brasiliensis is a representative of green algae. They are common in freshwater reservoirs of Ukraine. Desmodesmus brasiliensis also multiplies in large quantities during the eutrophication of water bodies due to pollution.

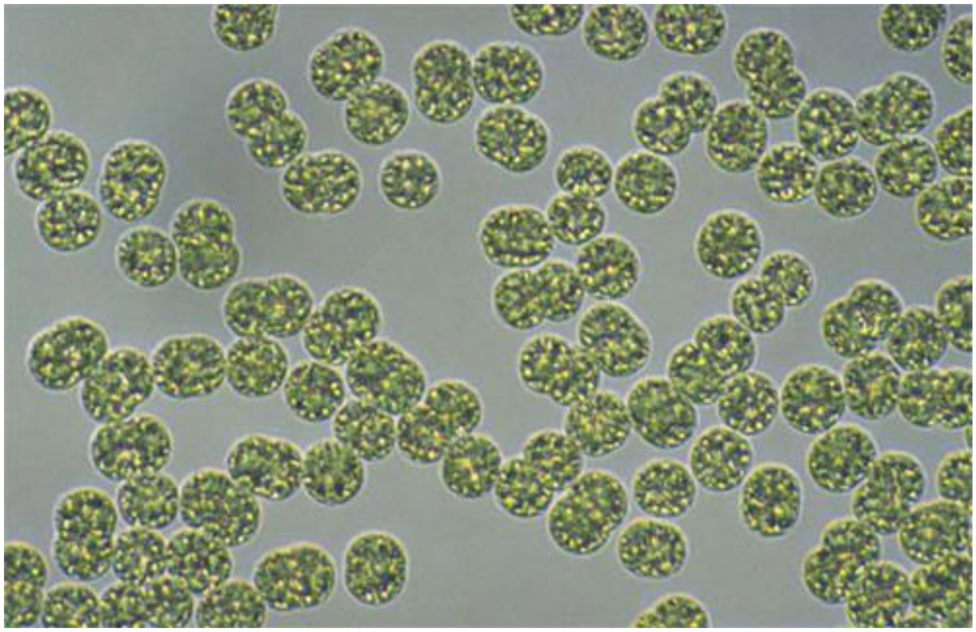

Fig.2.2. *Microcystis aeruginosa*

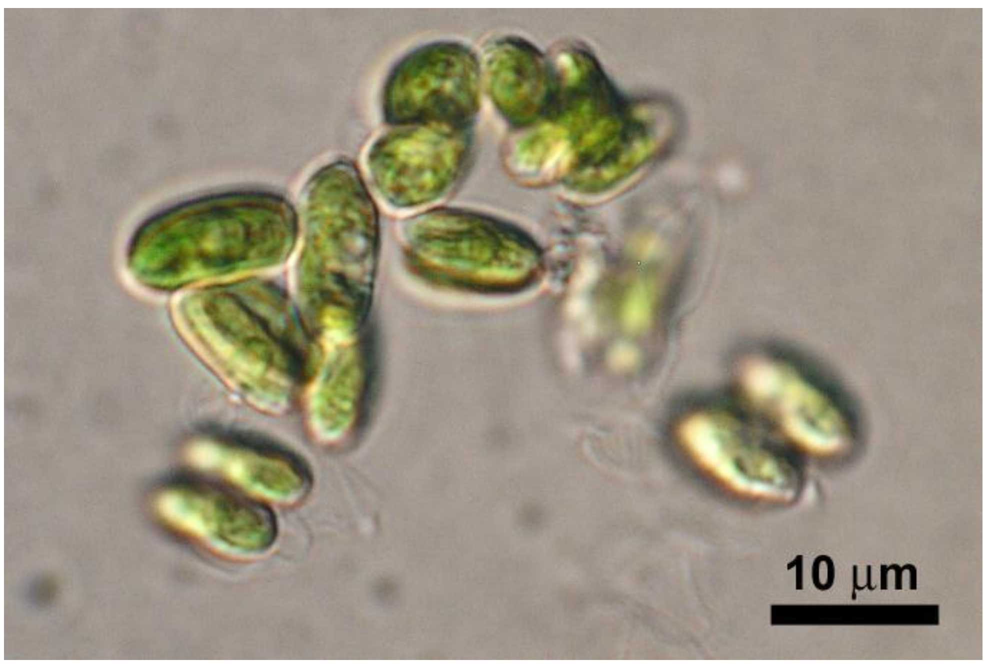

Fig.2.3. Desmodesmus brasiliensis

Studies of the effect of detergents on the algae Microcystis aeruginsa and Desmodesmus brasiliensis were performed on solutions of certain concentrations of 0.1, 1.0, 10 and 100 m/l, with oxygen saturation - 100%, at a temperature of 23ºC and lighting (Fig.

2.5., 2.6). The calculated concentrations of powders were obtained by adding appropriate amounts to the culture medium, the composition of which is given in table.2.1.

Algae test cultures were placed in glass cuvettes, and the number of cells was measured by density in five replicates for each detergent concentration. During biotesting, the exposure time was 1, 4 and 7 days.

Conditions for holding the test culture:

1. Temperature: 24±2ºC.

2. The medium for cultivation is prepared on distilled water (Fig.2.4). To avoid the formation of sediment in the solution, each of its components is prepared in advance in a concentrated form separately in 100 cm^3 of distilled water. The resulting solutions of concentrated salts are boiled for 10-15 minutes, cooled, after what they may be suitable for preparation of the solution.

3. Cleanliness of the test object. Because algae reproduce vegetatively, plant populations are clones (genetically homogeneous).

4. Control solution. Before testing, algae colonies are placed in the control solution for 2 days. The control solution is prepared as a combination of distilled water (1/2) and nutrient environment. The total volume is 200 ml [10].

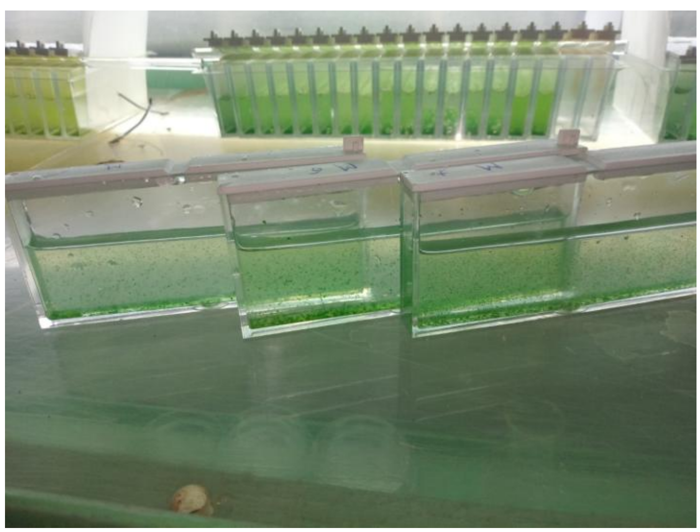

Fig.2.4. Preparation of the experiment of biotesting the toxicity of detergents on the test objects Microcystis aeruginsa and Desmodesmus brasiliensis

Table 2.1

The composition of the nutrient medium for the cultivation of algae

Microelement	Molar mass	mg / dm ^{3}
KNO_3	101,12	350,0
$Ca(NO_3)_2$ x $4H_2O$	236,15	295,0
KH_2PO_4	136,09	90,0
K_2HPO_4	174,18	12,6
$MgSO_4$ x $7H_2O$	246,37	100,0
H_3BO_3	61,83	120,0
$ZnSO_4$ x $7H_2O$	287,43	180,0
Na_2MoO_4 x $2H_2O$	241,92	44,0
$MnCl_2$ x $4H_2O$	197,84	180,0
$FeCl_3$ x $6H_2O$	270,21	760,0
Trilon B*	372,24	1500

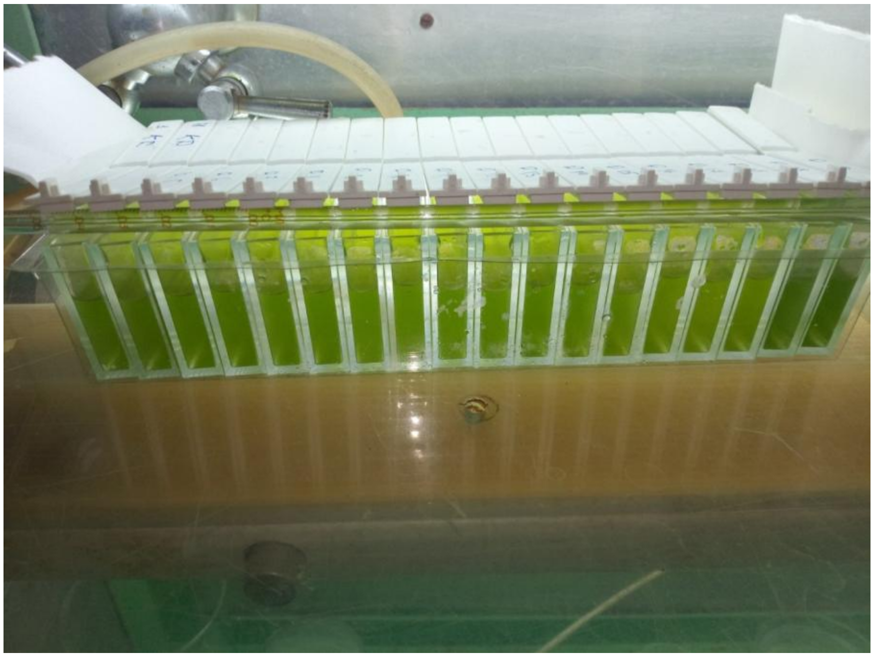

Fig.2.5. Growth of Desmodesmus brasiliensis culture in detergent solutions

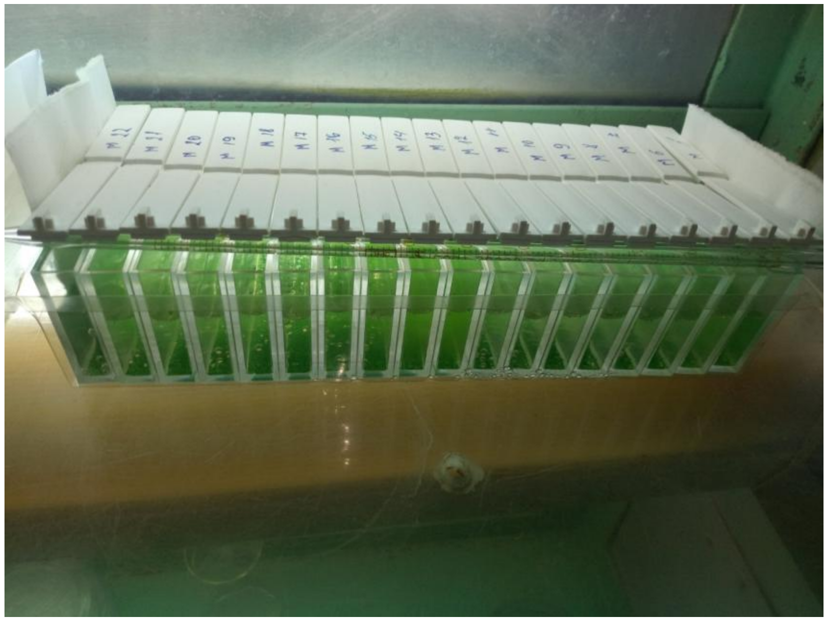

Fig.2.6. Growth of Microcystis aeruginsa culture in detergent solutions

2.2. Determination of phosphate content in water

The total content of phosphorus in detergent solutions, based on the content in the powder that needs to be dissolved. To determine the colorimetric method using molybdate and trumpet salts, the resulting complexes were restored with ascorbic acid. The reaction was carried out at room temperature. In this case, polyphosphates are not hydrolyzed, and organic phosphates do not decompose.For analysis, 50 ml of unfiltered, well-mixed sample was measured in a glass or other glass made of chemically resistant glass. 0.15 ml (3 drops) of hydrogen peroxide and exactly 1.0 ml of 37% sulfuric acid solution were added to this sample. At the same time, a blank experiment was performed with 50 ml of distilled water. The mixture was heated to 160 ° C and evaporated for 6 hours. The mineralized sample must be clean and colorless. After cooling, the sample was adjusted to 30 ml of distilled water, boiled for some time, cooled and quantitatively transferred to a flask, bringing the volume to 50 ml. After that, orthophosphate ions began to be determined. To do this, 2 ml of ascorbic acid solution was added to the sample. The mixture was stirred. Next, the optical density was measured over a period of 5 to 15 minutes. The total content was determined using a calibration graph.

The results of the analysis of washing powders to determine the mass fraction of phosphorus (P_{common},%) Showed its absence in D-1, D -2, D-3; in D -4 this figure was 9.2%.

2.3. Conclusion to chapter

An experiment with microalgae of Microcystisaeruginosa and Desmodesmus brasiliensis species was conducted. This work was carried out on the basis of the Institute of Hydrobiology of the NAS of Ukraine on the basis of the cooperation agreement. 4 types of washing powders, both phosphate and phosphate-free, were used for the work. The study of the effect of detergents on algae Microcystis aeruginsa and Desmodesmus brasiliensis was carried out on solutions of certain concentrations 0,1, 1,0, 10 and 100 m/l, with oxygen saturation - 100%, at 230C temperature and lighting.

CHAPTER 3

THE ROLE OF MICROALGAE FRESHWATER ECOSYSTEMS IN WATER TREATMENT FROM PHOSPHATES

Organic and inorganic substances that enter the environment as a result of domestic, agricultural, and industrial waters cause organic and inorganic pollution. Traditional processes of primary and secondary wastewater treatment are carried out in numerous places to remove easily settling material and oxidation of organic substances in wastewater. The end result is transparent, seemingly clean sewage that flows into natural ponds. However, these secondary runoffs are filled with inorganic nitrogen and phosphorus and cause eutrophication. Microalgae culture is an interesting step toward wastewater treatment, as it provides tertiary bio-cleaning combined with the production of potentially valuable biomass that can be used for several purposes. Microalgae offer excellent solutions for tertiary and heavy processing due to the ability of microalgae to use inorganic nitrogen and phosphorus for their growth. As for their ability to remove heavy metals and some toxic organic compounds, they do not cause secondary pollution. In this review, we will focus on the role of microalgae in wastewater treatment.

3.1. Assessment of detergents safety for aquatic ecosystems using algae

The study of the effect of selected detergents on the cultivation of algae in solutions in the range of concentrations from 0.1 to 100 mg / l compared to the control (nutrient medium only) showed the following results. At all studied concentrations of green algae Desmodesmus brasiliensis found on the fourth day of cell growth almost 100 times for solutions of all D, the same as the control (Fig. 3.1..-3.4.). After that, from 4 to 7 days there was a decrease in algae production by 15-20% in both D solutions and for control.

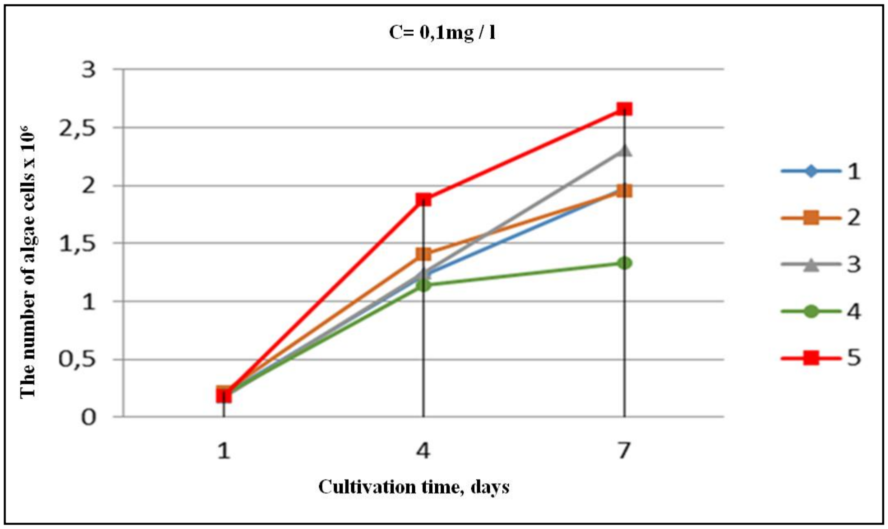

Fig.3.1. The effect of phosphate and phosphate-free detergents on the cultivation of algae Desmodesmus brasiliensis at a concentration of 0.1 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - M3-4, 5 - control.

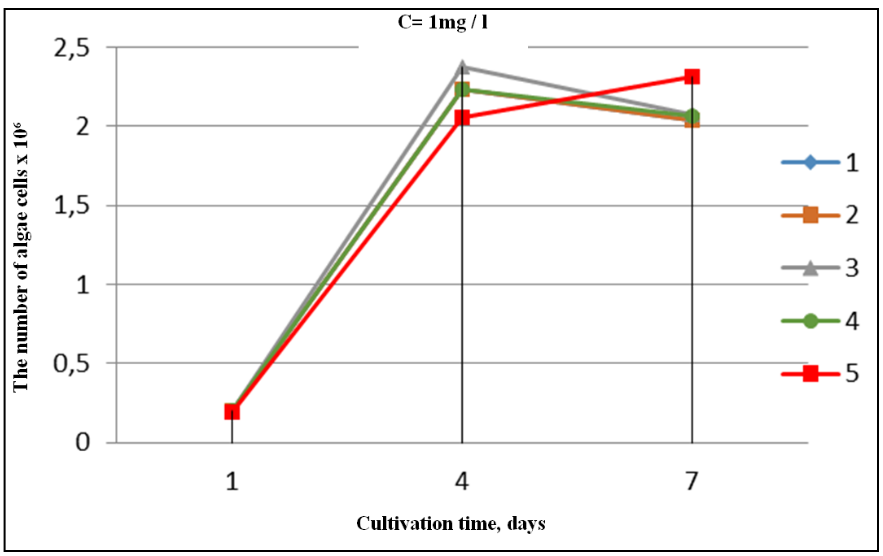

Fig.3.2. The effect of phosphate and phosphate-free detergents on the cultivation of algae Desmodesmus brasiliensis at a concentration of 1 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - M3-4, 5 - control.

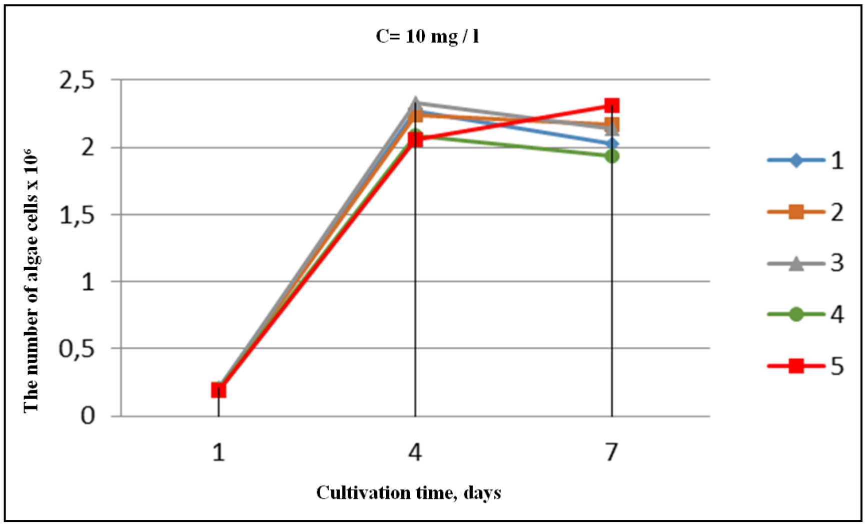

Fig.3.3. The effect of phosphate and phosphate-free detergents on the cultivation of algae Desmodesmus brasiliensis at a concentration of 10 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - M3-4, 5 - control.

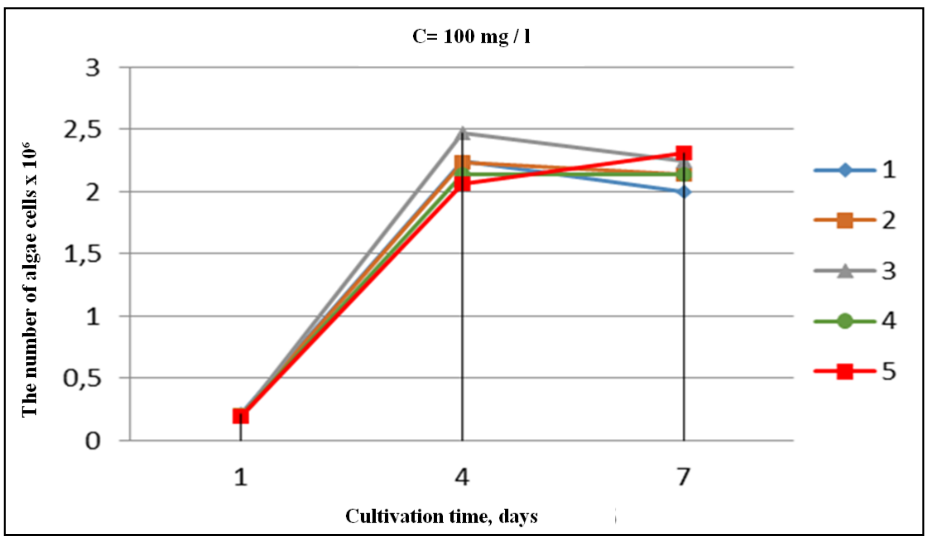

Fig.3.4. The effect of phosphate and phosphate-freedetergents on the cultivation of algae Desmodesmus brasiliensis at a concentration of 100 mg / l. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - M3-4, 5 - control.

A slightly different situation was observed for blue-green algae

Microcystis aeruginosa: growth of cultures was observed evenly throughout the cultivation period in solutions of all studied D, in parallel with the control, except with less intensity (Fig. 3.5.-3.8.). It is obvious that the nutrients in this case have not yet been exhausted and the culture continued to grow. Longer observations would lead to the same result as the case of green algae, which simply used up reserves faster.

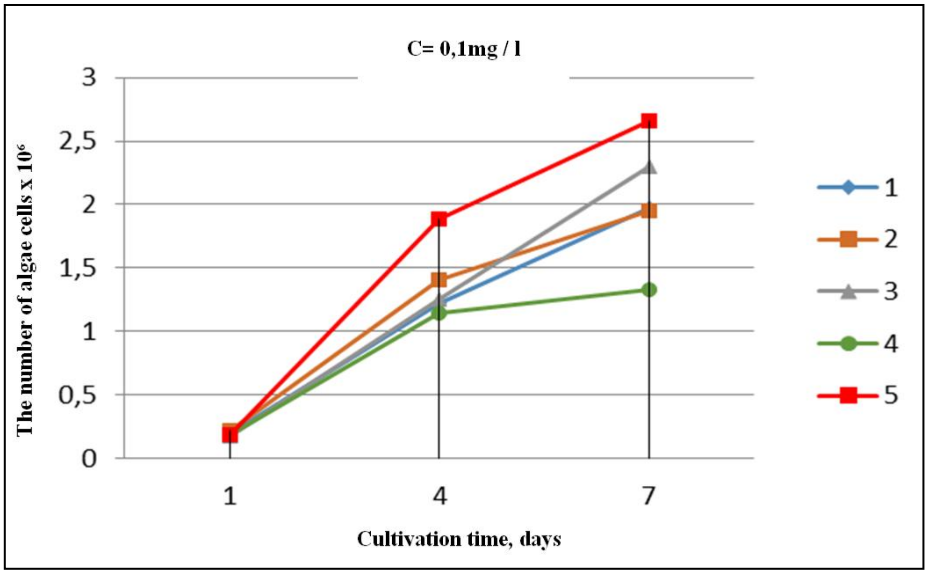

Fig.3.5. The effect of phosphate and phosphate-free detergents on the cultivation of algae Microcystis aeruginosa at a concentration of 0.1 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - D-4, 5 - control.

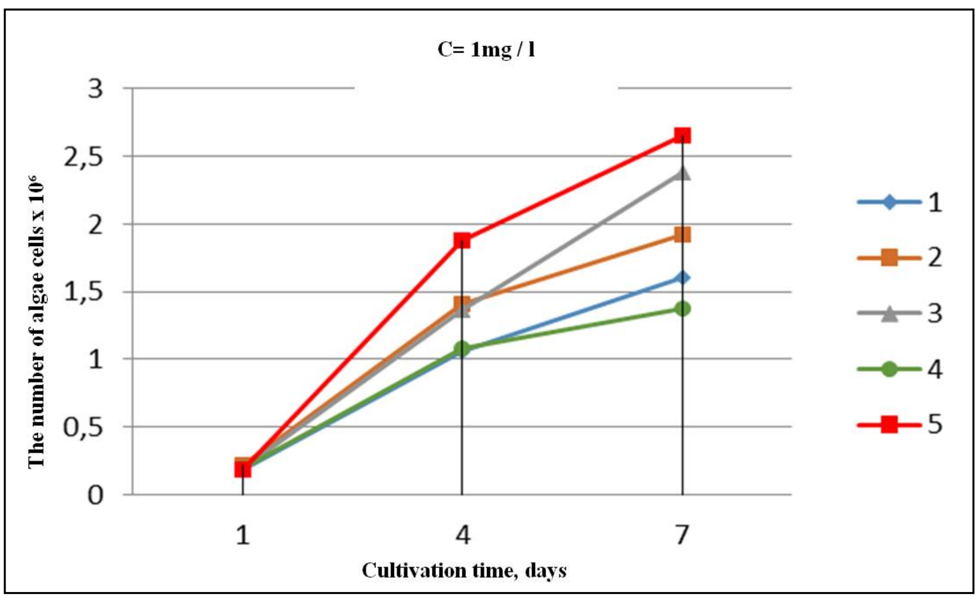

Fig.3.6. The effect of phosphate and phosphate-free detergents on the cultivation of algae Microcystis aeruginosa at a concentration of 1 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - D-4, 5 - control.

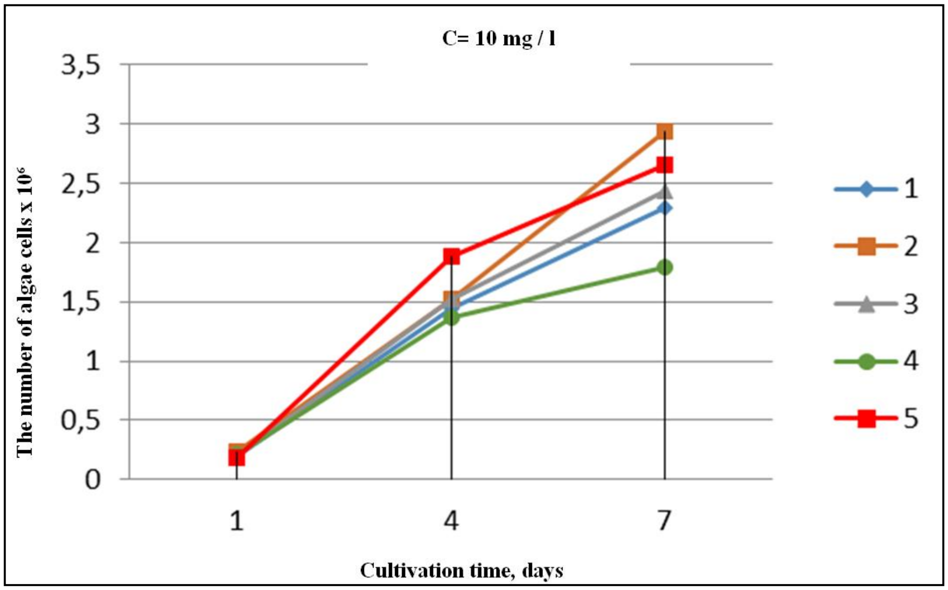

Fig.3.7. The effect of phosphate and phosphate-free detergents on the cultivation of algae Microcystis aeruginosa at a concentration of 10 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - D-4, 5 - control.

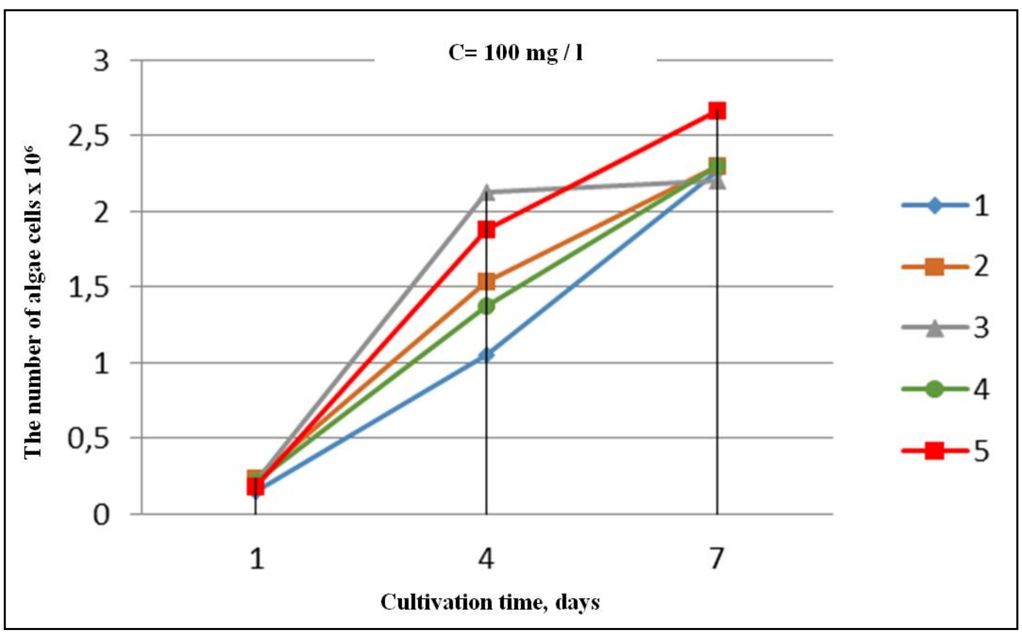

Fig.3.8. The effect of phosphate and phosphate-free detergents on the cultivation of algae Microcystis aeruginosa at a concentration of 100 mg / liter. Designation: 1- D-1; 2 - D-2; 3 - D-3; 4 - D-4, 5 - control.

3.2. Limiting the content of phosphates in washing powders as a promising measure to prevent water pollution

Phosphate synthetic detergents have become very widely and often used, which has led to the creation of a new stable chemical element in the human environment. As it was already mentioned earlier, it is sewage that contains detergent waste that are the main source of pollution.

In Ukraine, more than 90% of detergents contain sodium phosphate and potassium. They make detergents more effective and soften water. But after we have used them, they get into the water and become an excellent fertilizer for plants. Due to increased nutrition, blue-green algae grow and reduce the oxygen content in water during their biological growth. The water starts to bloom.

Back in the 1960s, on the basis of studying the impact of phosphates, civilized countries began to take measures to prevent contamination of reservoirs with phosphate-containing detergents, namely:

1.Legislative restrictions and prohibitions on the use of phosphate detergents [12].

2.Voluntary restrictions on the production of washing powders by manufacturers and the conclusion of relevant agreements with the government.

3.Introduce fines and taxes for phosphorus-containing disturbances in wastewater associated with the use of phosphorus detergents and their use in some countries.

4.Construction of additional systems for wastewater treatment containing phosphorus in particularly polluted areas.

5.Guarantee the development of scientific research and financial support from the states to create safe detergents.

Some European Union countries such as Belgium, Germany, Austria, Ireland, Denmark, France, the Netherlands, Finland and Italy have developed legislation that helps control the addition of phosphates to detergents. These measures have helped reduce the amount of phosphates in wastewater [13] . Thus:

– In Belgium in 2001 a law was passed banning washing powders containing more than 0.5% phosphorus.

– In Germany in 1984 the law was adopted to reduce the content of phosphates in powders by 50%, and since 1986. the Law prohibiting the use of phosphate washing powders was adopted. At the same time, the use of phosphate fertilizers was reduced by 30%.

– Austria passed two legislative acts in 1985. - limited phosphorus content to < 6% in washing powders, in 1987. - up to < 5%, and since 1995. - no phosphates.

– In Ireland, by voluntary consent of the parties since 2002. produce only phosphate-free synthetic detergents.

– In Denmark by voluntary agreement in 1992 50% of phosphate-free detergents were used in the country, and in 2002. — 90%.

– France was the world leader in the production of sodium tripolyphosphate, so the Environmental Protection Secretariat legally limits the content of phosphates in powders to 6.2% in 1991. and up to 5% from 01.07.1991. In 2002 he became a member of the National University of Kyiv. 66% of phosphate-free detergents were used in the country.

– In Finland in 1992 industry voluntarily stopped the production of phosphate synthetic detergents and today 95% of phosphate-free synthetic detergents are used in the country.

– In the Netherlands since 1990. only phosphate-free synthetic detergents are used and an effective wastewater treatment system from phosphorus is used

– In Italy in 1981 passed a law, which indicated the content of phosphates in powders no more than 5%, and since 1988. - no more than 1% phosphorus in synthetic detergents is indicated. 3 2002. in Italy, synthetic detergents containing phosphates are not used.

Also in Japan, due to a combination of legislative acts and public voluntary refusal to use phosphate synthetic detergents since 1986. completely limited use of phosphate detergents.

Today, the content of phosphates in powders in Ukraine is 5.4% (22% in terms of P_2O_5). Environmental regulations on the content of phosphates in wastewater and the amount of fines for exceeding them have been developed, but they cannot be used for unknown reasons. As a result, in recent years there has been a massive eutrophication of reservoirs and reservoirs, no less tense was the situation in the Black and Azov Seas. Ukraine ranks 87th in the world in the quality of drinking water. At the same time, in 2012, the draft law on state regulation of synthetic detergents and household chemicals will come into force with a gradual reduction in the use of phosphate-based synthetic detergents in Ukraine and a complete termination until 2020.

Laws include standards for detergents and phosphates, but they do not give guidance on other components of the cleaner formula.

Thus, companies producing detergents can use about 80% of the total number of components that are not biodegradable and toxic. These include antioxidants, antiseptics, fungicides, chlorine, chloramines, organochlorine substances, and heavy metals such as mercury. Thus, they were not considered completely safe for human health and the environment. Large manufacturers of detergents in the EU have recognized that they cannot supply safe and highly efficient household chemicals to enterprises. Although the program to reduce the supply of phosphates to water resources has yielded very positive results in some countries, the problem continues to exist throughout the world.

Creating legislation that controls these other components is important and necessary for manufacturers [14].

3.3. Introduction to effective system's water purification from phosphates with the use of microalgae

Wastewater treatment is a complex process consisting of several stages. In particular, water purification involves reducing or eliminating pollution: the destruction of organic substances, colloidal or insoluble solids, as well as pathogenic and other bacteria.

In the technology of water purification from phosphorus, biological methods are most commonly used [15]. The disadvantage of this method is the complexity and duration of the process. In addition, its use is limited by high capital and operational costs. But among all the modern methods of processing industrial and household waste, the biological method is the most environmentally friendly. First, it is based on natural processes, such as the ability of heterotrophic microorganisms to use to feed organic matter in wastewater (alcohol, protein, carbohydrates, etc.), as well as some inorganic substances (ammonia, nitrates, phosphates, salts). As for these compounds, energy-producing microorganisms partially destroy them by converting them into water, carbon dioxide, anions (sulfate ions), and some metal cations, using a fraction of these substances for their own reproduction. Secondly, microorganisms quickly accumulate and form colonies, which makes it easy to separate them from purified water [16].

Microalgae play a particularly important role in the biological purification of water. Taking into account the economic efficiency, the most promising is the use of algae for the wastewater treatment of food industry enterprises, fish farms, livestock farms, poultry farms. Microalgae, including eukaryotic algae and cyanobacteria, have demonstrated to be an environmentally friendly and sustainable alternative to the energy-intensive and traditional biological purification processes widely used today[17]. It is a renewable source of biomass, and the use of microalgae for wastewater treatment is a cost-effective and feasible method of biofixation CO_2 [18].

Studies on the removal of phosphorus compounds using microalgae have been evaluated several studies, and they were performed on different types of sewage (municipal, agricultural, brewery, and industrial drains) by different types of algae[19]. For example, Scendesmus obliquu strain successfully removed phosphorus compounds from agricultural wastewater [20]. Chlorella pyrenoidosa has successfully removed the contamination of milk production sewage, and other species of Chlorella, such as Chlorella vulgaris white, presented for purification from phosphorus compounds of municipal wastewater[21,22].

Wastewater treatment with microalgae is a method that allows waste treatment, dividing them into pure liquid and solid fractions. The solid part contains a significant amount of pollutants, so it is disposed of, and in the case of microalgae (after purification), the latter can be used as biofuels (phosphate) and raw materials for biofuels. Thus, the technology is waste-free and cyclic (Fig.3.9.)

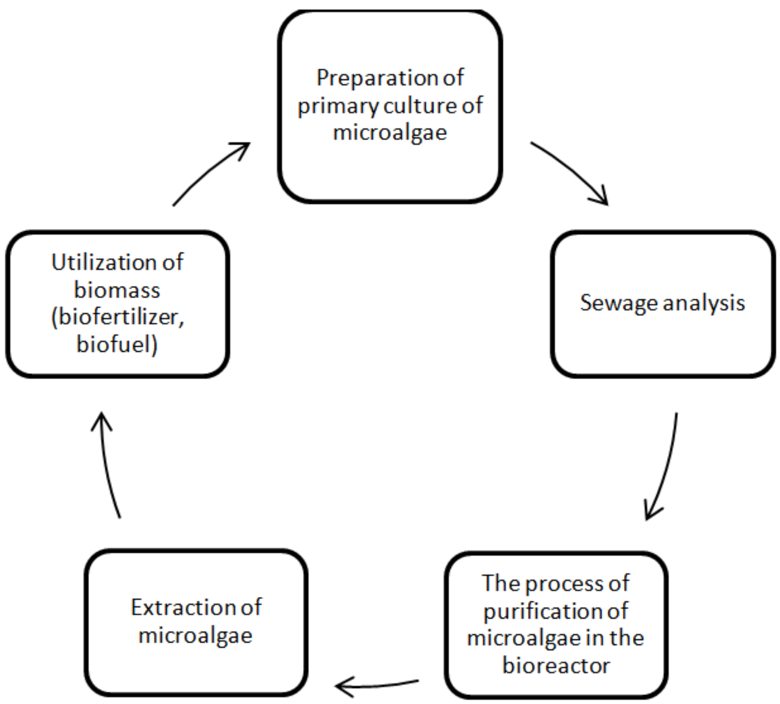

Fig.3.9. Technology of wastewater treatment by microalgae

The classic wastewater treatment scheme, includes primary and secondary treatment facilities, as well as additional ultraviolet treatment, which is sufficiently high-cost, and, practically not applicable.

Currently, Ukraine does not have water purification systems that are able to completely purify it from pollution. The classic wastewater treatment scheme consists of primary and secondary treatment facilities, as well as additional ultraviolet treatment, which is sufficiently high-cost, and practically does not apply. In my opinion, it should be added tertiary pre-purification, the use of microalgae, which, as a result of their metabolism, absorb biogenic compounds.

3.4. Conclusion to chapter

The use of microalgae for wastewater treatment as a perspective has been viewed by mankind for a very long time since algae is very well absorbed by organic and inorganic compounds. Therefore, the integration of microalgae as an alternative biological wastewater treatment option is technologically and environmentally appropriate. Also, the use of microalgae to reduce phosphorus content will potentially work with lower energy consumption and the formation of greenhouse gases when compared with conventional processes of biological wastewater treatment.

CONCLUSIONS

1. Sources of phosphate pollution of aquatic ecosystems on average are: municipal sewage - 1.1%; detergents - 38.8%; agricultural activity (fertilizers, plant protection products) - 18.0%; soil erosion - 4.7%; seasonal regeneration from bottom mineralized organic deposits - 12.0%; industrial activity - 3.1%; other sources of income - 5.5%.

2. Phosphate contamination of surface water is largely associated with the entry of phosphate-containing components of synthetic detergents containing domestic wastewater. Washing powders sold in Ukraine contain 5-40% of phosphate substances that are not removed by outdated cleaning technologies. To address this issue, it is necessary to impose restrictions on the use of phosphorus-containing detergents at the state level, as in most developed countries.

3. The analysis of modern perspective methods of phosphate extraction from wastewater by determining the possibility of further use of phosphorus compounds according to foreign scientific literature.

4. In the cultivation of algae of the species Desmodesmus brasiliensis and Microcystis aeruginosa in environments containing phosphate and phosphate-free detergents, rapid growth of cells and mass building was observed; at the same time, there is no reliable difference between them within the studied concentrations.

5. Development and implementation of modern phosphate-free detergents containing sodium gluconate, sodium cecarbonate, polycarboxylate, ethylenediaminetetraacetic acid derivatives, which reduce water hardness, is a promising way to improve their environmental performance and increase safety for aquatic ecosystems.

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