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FACULTY OF ENVIRONMENTAL SAFETY,
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ECOLOGY DEPARTMENT

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MASTER THESIS

(EXPLANATORY NOTE)

SPECIALTY 101 "ECOLOGY",
TRAINING PROFESSIONAL PROGRAM
"ECOLOGY AND ENVIRONMENTAL PROTECTION"

Theme: «**Research of biogenic compounds removal from sewage waters by microalgae**»

Done by: student of the ЕК– 202 ма group, Iryna O. Syrotina
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Supervisor: Ph. D. in Technological Sc., Associate Professor, Lesia I. Pavliukh
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KYIV 2020

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

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової кафедри
_____ В.Ф. Фролов
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ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ВИПУСКНИКА ОСВІТНЬОГО СТУПЕНЯ МАГІСТРА

ЗА СПЕЦІАЛЬНІСТЮ 101 «ЕКОЛОГІЯ»,
ОСВІТНЬО-ПРОФЕСІЙНОЮ ПРОГРАМОЮ
«ЕКОЛОГІЯ ТА ОХОРОНА НАВКОЛИШНЬОГО СЕРЕДОВИЩА»

Тема: «Дослідження видалення органічних сполук зі стічних вод за допомогою мікроводоростей»

Виконавець: студентка групи ЕК- 202 ма, Сиротіна Ірина Олегівна
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Faculty of Environmental Safety, Engineering and Technologies

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«____» _____ 2020

QUALIFICATION PAPER ASSIGNMENT

Iryna O. Syrotina

1. Theme: «Research of biogenic compounds removal from sewage waters by microalgae» approved by the Rector on September 06, 2020, № 1937/ст.

2. Duration of work: from 05.10.2020 to 31.12.2020.

3. Output work: The research data from the Institute of Hydrobiology of the National Academy of Sciences of Ukraine.

4. Content of explanatory note: to analyze the various types of sewage waters composition; to study perspectives of biological treatment; to prepare of algae sowing material (suspensions); to prepare of cultural environment for microalgae; to investigate of the growth kinetics of Chlorella vulgaris and Euglena gracilis in sewages under different cultivation conditions; to study of the kinetics of changes in the concentration of nutrient compounds in wastewater under different conditions of purification; to estimate economic benefits from microalgae application.

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

6. Schedule of thesis fulfillment

№ з/п	Task	Term	Advisor's signature
1	Receive themes task, search the literature and legislation	06.09.2020-15.09.2020	
2	Preparing the main part (Chapter I)	16.09.2020-30.09.2020	
3	Preparing the main part (Chapter II)	01.10.2020-15.10.2020	
4	Preparing the main part (Chapter III)	16.10.2020-26.10.2020	
	Preparation of the main part (Chapter IV)	27.10.2020-04.11.2020	
	Consultation on section V (Occupational safety)	05.11.2020	
	Preparation of the main part (Chapter V)	05.11.2020-20.11.2020	
5	Formulating conclusions and recommendations of the thesis	21.11.2020-25.11.2020	
6	Making an explanatory note to the previous presentation of the department, consultation with the norms controller	26.11.2020-30.11.2020	
7	Presentation of the work at the department	01.12.2020	
8	Taking into account the comments and recommendations and training to protect	01.12.2020-20.12.2020	
9	Thesis defense at the department	21.12.2020	

7. Consultant(s) of certain chapter(s):

Chapter	Consultant (academic rank, S.N.P)	Date, signature	
		Given by	Accepted by
Labor Precaution	Olena V. Konovalova, Associate professor Chair of Civil and Industrial Safety		

8. Date of task issue: «06» September 2020

Qualification Paper Advisor: _____ Lesia I. Pavliukh
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Task is taken to perform: _____ _____
(graduate's signature) (S.N.P.)

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
Факультет екологічної безпеки, інженерії та технологій
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ЗАТВЕРДЖУЮ
Завідувач кафедри
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«____» _____ 20__ р.

ЗАВДАННЯ
на виконання дипломної роботи
Сиротіни Ірини Олегівни

1. Тема роботи «Дослідження видалення органічних сполук зі стічних вод за допомогою мікроводоростей» затверджена наказом ректора від 06 вересня , 2020, № 1937/ст.

2. Термін виконання роботи: з 05.10.2020 р. по 31.12.2020 р.

3. Вихідні дані роботи: дані досліджень Інституту гідробіології НАН України.

4. Зміст пояснювальної записки: проаналізувати різні типи стічних вод; вивчити перспективи біологічного очищення; підготувати культурне середовище для мікроводоростей; підготувати посівний матеріал для водоростей (суспензії); дослідити кінетику росту Chlorella vulgaris та Euglena gracilis у стічних водах за різних умов вирощування; вивчити кінетику змін концентрації поживних сполук у стічних водах за різних умов очищення; оцінити економічні вигоди застосування мікроводоростей.

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	06.09.2020-15.09.2020	
2	Підготовка основної частини (Розділ I)	16.09.2020-30.09.2020	
3	Підготовка основної частини (Розділ II)	01.10.2020-15.10.2020	
4	Підготовка основної частини (Розділ III)	16.10.2020-26.10.2020	
5	Підготовка основної частини (Розділ IV)	27.10.2020-04.11.2020	
6	Консультація щодо розділу V(Охорона праці)	05.11.2020	
7	Підготовка основної частини (Розділ V)	05.11.2020-20.11.2020	
8	Формулювання висновків та рекомендацій дипломної роботи	21.11.2020-25.11.2020	
9	Оформлення пояснювальної записки до попереднього представлення на кафедрі, консультація з нормоконтролером	26.11.2020-30.11.2020	
10	Представлення роботи на кафедрі	01.12.2020	
11	Урахування зауважень, рекомендацій та підготовка до захисту	01.12.2020-20.12.2020	
12	Захист роботи на кафедрі	21.12.2020	

7. Консультація з окремого(мих) розділу(ів):

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ABSTRACT

Explanatory note to thesis « Research of biogenic compounds removal from sewage waters by microalgae»: 75 pages, 22 figures, 8 tables, 13 formulas, 47 references.

Object – sewage treatment.

Subject – investigation of Chlorella vulgaris and Euglena gracilis application to remove phosphorous and nitrogen compounds.

Aim of work –to research of biogenic compounds removal from sewage waters by microalgae.

Methods of research - laboratory testing, observations, analysis, data comparison, statistical data processing (using IBM SPSS Statistics Base v.2), mathematical modeling, conventional methods of hydrochemistry.

SEWAGE TREATMENT, BIOGENIC COMPOUNDS, CHLORELLA VULGARIS AND EUGLENA GRACILIS, MICROALGAE METABOLISM, REMAIN BIOMASS, BIOFUEL, BIOFERTILIZER.

CONTENT

INTRODUCTION

Relevance. Due to the high rate of urban development there is an increase in wastewater. This situation leads to the fact that existing treatment systems become inefficient. Discharge of insufficiently treated wastewater from nutrients(P and N elements) into water bodies is one of the most common causes of eutrophication. Aquatic ecosystems respond to nutrient enrichment primarily by the intensive development of cyanobacteria. Their rapid reproduction causes "blooming" or scientifically named eutrophication of water, which leads to oxygen deficiency and mass death of fish and other aquatic organisms. This problem is especially relevant for small rivers and lakes. Water eutrophication, makes impossible to use water for recreation, fishing and domestic needs. The phytoplankton toxins can cause health problems through exposure to the human body after contact with the skin or the use of contaminated water for drinking. Therefore, there is a problem of more efficient wastewater treatment from phosphorous and nitrogen compounds.

Aim and tasks of graduate work. The aim of the work is to research of biogenic compounds removal from sewage waters by microalgae.

Tasks of work:

1. To analyze the various types of sewage waters composition;
2. To study perspectives of biological treatment;
3. To investigate Chlorella vulgaris and Euglena gracilis potential to remove biogenic elements;
4. To estimate economic benefits from microalgae application.

Object – sewage treatment.

Subject – investigation of microalgae application to remove phosphorous and nitrogen compounds.

Methods of research. Laboratory testing, observations, analysis, data comparison, statistical data processing (using IBM SPSS Statistics Base v.2), mathematical modeling, conventional methods of hydrochemistry.

Scientific novelty of the results.

1) Has further developed the study of microalgae metabolism.

2) For the first time the use of Euglena gracilis for treatment of wastewater with concentration 30 mg L−1 of N-NH4+ and 7 mg L−1 of P-PO43-, 50 mg L−1 of N-NH4+ and 14 mg L−1 of P-PO43-, and 90 mg L−1 of N-NH4+ and 26 mg L−1 of P-PO43- of model solutions is offered.

3) For the first time the efficiency of using chlorella algae on non-model solutions was confirmed.

The practical value of the results obtained. Confirmation of efficiency of use of microalgae class Chlorella vulgaris and Euglena gracilis in purification processes.

Personal contribution of the graduate. Experimental studies on microalgae (Chlorella vulgaris and Euglena gracilis) potential to remove phosphorous and nitrogen compounds.

Approval of the results.

1. The cascade photobioreactor for microalgae cultivation/Syrotina I.O., Todorovych O.S. - All-Ukrainian Scientific and Practical Conference of Young Scientists and Students "Ecological Safety of the State". – K., 2020. – p.52.

2. Bioreactor for microalgae cultivating/ Pavliukh L., Shamansky S., Syrotina I., Todorovych O. - IX International Scientific and Practical Conference Osaka, Japan, 2020.- p.158.

Publications.

1. Cascade photobioreactor for waste water treatment by microalgae/Lesia Pavliukh, Sergii Boichenko, Sergii Shamanskyi, Iryna Syrotina, Olena Todorovych.- Modern Management Review (dawna nazwa: Zarządzanie i Marketing). - Oficyna Wydawnicza Politechniki Rzeszowskiej, al. Powstańców Warszawy 12, 35-959 Rzeszów, Poland, 2020. -MMR, vol. XXV, 27 (3/2020), p. 17-29. DOI: 10.7862/rz.2020.mmr.19

2. Syrotina I., Pavliukh L., Todorovych O. Improvement of waste water treatment technology by microalgae// Problemy eksploatacji I diagnostyki: Systemy i srodki transportu samochodowego, 18-20 September 2019 : monograph. – Rzeszow, Poland, 2019. – P. 71-77.

CHAPTER 1
ANALYSIS OF WASTE WATER COMPOSITION

1.1. Municipal sewage

The general composition of municipal wastewater may be classified into the following five categories:

· Organic matter (measured as biochemical oxygen demand or BOD),

· Disease-causing microorganisms (pathogens),

· Nutrients (nitrogen and phosphorus),

· Toxic contaminants (both organic and inorganic),

· Dissolved minerals.

Wastewater is a complex matrix containing significant concentrations of solids (total solids 350–1200mg/l), dissolved and particulate matter (chemical oxygen demand 250–1000mg/l), microorganisms (up to 109 number/ml), nutrients, heavy metals and micro-pollutants [1]

According to the Water Code of Ukraine, wastewater is water formed in the course of household and production activities (except for mine, quarry and drainage water), as well as taken from the built-up area where it was formed due to precipitation.

Depending on the origin and composition of pollutants (impurities), wastewater is divided into four main categories: domestic, industrial (industrial), agricultural and rainwater, which flows from the territory of industrial facilities and settlements as a result of precipitation. or watering the streets. The sewage systems are removed from the territories of production facilities and settlements. Wastewater is contaminated with various impurities - mineral, organic, and also contains pathogenic (pathogenic) microorganisms.

Wastewater is considered treated if it meets the following requirements [2]:

- Biochemical oxygen consumption does not exceed 50 mg / l;
- No floating organic pollutants;

- The content of suspended particles does not exceed 60 mg / l;
- The oil (condensate) content during extraction with ether does not exceed 5 mg / l;
- Chromaticity - more than 20 units (on a platinum-cobalt scale);
- Hydrogen pH = 6-9.

Discharges of formation waters that come with gas are complicated by the presence of emulsified oil (condensate), various corrosive substances, sand, dissolved sulfides, which then precipitate, a large number of dissolved mineral salts, and sometimes salts of weak organic acids. In addition, in formation waters in measured quantities may be present toxic substances - barium compounds, strontium, heavy metals. Discharge of industrial wastewater from compressor and pumping stations, gas processing plants and underground gas storage facilities is complicated by the presence of dissolved (with subsequent precipitation) sulfides, salts, acidic and alkaline solutions, mono- and polyhydric alcohols [2].

Municipal wastewater is mainly comprised of water (99.9%) together with relatively small concentrations of suspended and dissolved organic and inorganic solids. Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from the process industries [3].

Municipal wastewater also contains a variety of inorganic substances from domestic and industrial sources, including a number of potentially toxic elements such as arsenic, cadmium, chromium, copper, lead, mercury, zinc, etc. Even if toxic materials are not present in concentrations likely to affect humans, they might well be at phytotoxic levels, which would limit their agricultural use. However, from the point of view of health, a very important consideration in agricultural use of wastewater, the contaminants of greatest concern are the pathogenic micro- and macro-organisms [3].

Pathogenic viruses, bacteria, protozoa and helminths may be present in raw municipal wastewater and will survive in the environment for long periods. Pathogenic bacteria will be present in wastewater at much lower levels than the coliform group of bacteria, which are much easier to identify and enumerate (as total coliforms/100ml).

Escherichia coli are the most widely adopted indicator of faecal pollution and they can also be isolated and identified fairly simply, with their numbers usually being given in the form of faecal coliforms (FC)/100 ml of wastewater [3].

The following types of wastewater are distinguished by origin: [4] [5] [6].

- Domestic wastewater - is formed in residential premises, as well as in domestic premises in the workplace (for example, showers, toilets), is discharged through the system of domestic sewerage or general. Contaminated mainly with detergents and excrement. Most suspended solids are cellulosic in nature, and other organic contaminants include fatty acids, carbohydrates, and proteins. The unpleasant smell of domestic wastewater is due to the decomposition of proteins under anaerobic conditions.
- The composition of domestic wastewater is relatively constant and is characterized mainly by organic pollutants (about 60%) in the undissolved, colloidal and dissolved state, as well as various bacteria and microorganisms, including pathogenic [7].
- Industrial wastewater - is formed as a result of the use of water in technological processes at industrial enterprises or mining, discharged through a system of industrial or alloy sewerage. The most characteristic and dangerous pollutants of industrial wastewater are extractives (mainly petroleum products), phenols, synthetic surfactants, heavy metals, organic substances with a long decomposition period, including various pesticides. Emit contaminated and relatively clean industrial wastewater. An example of conditionally pure wastewater is water used for cooling in heat exchangers.
- Agricultural wastewater - are divided into wastewater from livestock complexes and surface wastewater from fields. The first type of wastewater contains a large amount of organic pollutants, the second contains agrochemicals used as fertilizers and plant protection products against pests.
- Rainwater wastewater - is formed by rain, melt (snow, hail) and irrigation water. Drained usually through a storm sewer system. Divided into rain and thaw. Contaminated usually with suspended solids of organic and mineral origin, petroleum products, nutrients and heavy metals.

- Mine and mine wastewater - are formed in the process of extraction and processing of minerals, so they often have a high mineralization, acid reaction of the environment, a large number of mine elements that are in dissolved and suspended forms.
- Sewage from concentrators - flotation waste, thickener showers, vacuum filter filtrates.
- Wastewater from the oil and gas industry.

To determine the composition of wastewater requires a significant number of laboratory tests - chemical, physico-chemical, sanitary and bacteriological. The main tasks that are solved on the basis of the obtained analysis data are:

- assessment of sanitary and toxicological condition of wastewater;
- determining the suitability of wastewater for a particular type of use, the degree and nature of wastewater pollution;
- search for a method of water treatment, as well as the definition of methods for managing wastewater treatment processes and control of facilities;
- assessment of the efficiency of individual facilities and the technological scheme of wastewater treatment in general;
- ecological control of the state of the water body into which the wastewater is discharged after treatment, in order to prevent its pollution and compliance with water protection requirements.

1.2. Investigation of various types of sewage

Dairy production waste water. The composition of dairy wastewaters is also quite variable, but some common features can be identified, such as the high concentrations of organic matter (especially made of lactose, oil and grease, proteins), nitrogen and suspended solids [7], and the presence of various trace soluble organics. Various residues of cleaning products, including alkaline and acidic chemicals, are also often present. So, COD is high (80–95 g/L), but BOD is high too (40–48 g/L) due to the presence of biodegradable substances [6]. The concentrations of suspended solids, TKN, and total

phosphorus range between 0.1 and 22.0 g/L, 0.01 and 1.7 g/L, and 0.006 and 0.5 g/L, respectively [8].

According to Ahmad et al. [9], pH also varies within a very wide range (4.7–11). Dairy effluents often include milk or milk products lost during processing (skimmed milk, spoiled milk, spilled milk, and curd pieces), by-products of processing operations (whey permeates, whey, and milk), starter cultures used in manufacturing of fermented products, reagents used in CIP (cleaning in-place) procedures, contaminants used for washing trucks, cans, equipment tanks, bottles and floors, and different additives used in the manufacturing process [10].

Airports Sewages.Water consumed by airports can be broadly divided into the water consumed by the landside activities undertaken at the airport as well as the water consumed by the key stakeholders involved in the provision of the airport's airside area activities (operations). Landside means those parts of an airport as well as the adjacent terrain and buildings or portions thereof that are not in the airside precinct. The airside means the movement area at an airport, adjacent terrain and buildings/infrastructure, or portions, the access to which is restricted [11].

There are a number of key stakeholders, for example, airlines, ground handling agents and the airport authorities themselves that are involved in the facilitation and handling of passengers within an airport's landside precinct. Additionally, at some airports, air cargo is handled in cargo terminals that are located on the airport's landside. Many airports around the world also have hotels located in the landside precinct. These actors require and consume water for a variety of purposes. These include drinking water for passengers, meeters and greeters, airport staff, washing of vehicles, the flushing of toilets, personal hygiene (for example, washing of hands and in showers provided for passengers and airport-related staff), cooking and beverages provided by concessionaires operating at the airport and by flight catering firms. Water is also used for landscaping and lawn and plant watering.

The various key stakeholders that are typically involved in the facilitation and handling of aircraft (passenger and freighter aircraft), passenger and air cargo movements, aircraft maintenance and air traffic control (ATC) on the air side of an airport also require

and consume water for a variety of Infrastructures purposes. In addition to the actors involved with servicing and maintaining aircraft (and ground service equipment), many airports also often have concessionaires located in the airside section of the terminal, for example, duty-free shops and restaurants [12,13].

These actors require drinking water for staff working on the airside, water is required for the washing of aircraft and ground service vehicles, the flushing of toilets, personal hygiene (for example, the washing of hands and in showers provided for airport-related staff), cooking and beverages provided by concessionaires operating restaurants and coffee shops in the airside terminal precinct and by flight catering firms operating on the airside at the airport.

Most countries have set water quality standards. However, the water quality standards set by airports are normally much more stringent [14]. Around the world, the organic water pollution indicators that are commonly used to measure water pollution are chemical oxygen demand (COD), total nitrogen (T-N), and total phosphorus (T-P) [15]. The chemical oxygen demand (COD) test measures the total amount of oxygen that is required to completely oxidize organic matter to carbon dioxide (CO2) and water. Hence, the COD test provides an indication of its potential maximum oxygen demand [16].

Because water sources are often connected to each other, any adverse impacts on the local deterioration of water quality arising from airport operations can have an impact in regions that are located quite a distance from the airport itself [17].

Brewery waste water. There are three main issues to address with regards to the wastewater — pH, concentration of BOD and TSS. Biochemical oxygen demand (BOD) is a measure of the nutrient value of wastewater. Total suspended solids (TSS) is a measure of suspended solids in wastewater. Brewery wastewater is higher in sugar and alcohol compared to normal domestic wastewater which most treatment plants were designed to treat [18].

The pH swings from high to low as well. Some less common restrictions may be for temperature, ammonia or an instantaneous or daily flow limit. Some towns apply reasonable surcharges for wastewater, other towns are highly excessive. Fats, oils and greases (FOG) is a big deal with municipalities these days, but brewery wastewater has

almost no FOG. Each one of these characteristics can be major issues, and to make matters even more fun these vary state to state as well as town to town [18].

In a brewery's case, most BOD cannot be filtered out, we're talking about sugar and alcohol. The TSS can be filtered out, but in both cases the best option is to not put it down the drain in the first place. Side stream those high BOD and TSS loads, things like trub, spent yeast and waste beer, and truck that stuff off site as fertilizer [18].

1.3. Waste water biotreatment

Sewage treatment, or domestic wastewater treatment, is the process of removing contaminants from wastewater, both runoff (effluents) and domestic. It includes physical, chemical and biological processes to remove physical, chemical and biological contaminants [17]. Its objective is to produce a waste stream (or treated effluent) and a solid waste or sludge suitable for discharge or reuse back into the environment [19]. This material is often inadvertently contaminated with many toxic organic and inorganic compounds.

Contaminated wastewater from industrial enterprises significantly reduces drinking water volumes. Fish is grown in such an environment, crops are watered with polluted water, and animals are watered. These are all foods that can have a direct negative impact on human health. There are many ways to treat wastewater and different types of their classification. Among the methods of cleaning the most common: mechanical, physico-chemical and biological. Let`s consider the biological type of treatment.

Biological treatment takes place in natural conditions: in irrigation fields, filtration fields, biological ponds or in artificial conditions - biological filters. The method of biological wastewater treatment is based on the ability of microorganisms to use various substances contained in wastewater as a source of nutrition in the process of life. Thus microorganisms release water from pollution. Biological filters are facilities in which wastewater is filtered through a loading substance covered with a biological film formed by colonies of microorganisms. The classification of bio filters can be carried out on

many grounds, the main of which are the type of loading used the method of contact of the biofilm with treated wastewater and the method of air supply to the body of the bio filter.

The efficiency of biological wastewater treatment processes depends on a number of factors, some of which can be regulated in a wide range, and others, such as the composition of wastewater, are virtually uncontrollable.

Temperature is one of the main factors that ensures the efficiency and high productivity of biological treatment plants. The optimum temperature for aerobic processes occurring in biological oxidants is 20-30 °C, and the biocenosis should be represented by various and well-developed organisms under other favorable conditions. It should be noted that for different species of organisms, in particular bacteria, the optimal temperature varies from 4 to 85 ° C [20].

The development of organisms is also influenced by the active reaction of the environment (pH), because a significant part of living beings thrive best in a neutral or slightly alkaline environment. The environment with pH = 6.5-7.5 is considered optimal for biological treatment. Deviation of pH outside 6 and 8.5 reduces the rate of oxidation due to the slowing down of metabolic processes in the cell [20].

Thus, the normal course of biological wastewater treatment processes from organic pollutants must be ensured by certain conditions. If these conditions are not met, they must be adjusted:

- change the temperature regime due to heating or cooling of wastewater;
- to carry out neutralization of sewage;
- in the absence of nutrients in wastewater, they should be added artificially in the form of superphosphate, ammonia water, ammophos, etc.

In aerobic biological treatment plants, the concentration of dissolved oxygen must be maintained at least 2 mg / l, otherwise there is a decrease in the rate of utilization of organic compounds [21]. The required oxygen concentration in the buildings is maintained by the supply of air or technical oxygen through aeration systems and aerators.

During the operation of biological treatment plants, the concentration of toxic components, which should not exceed the MPC, is constantly monitored. In the process of biological treatment, the amount of wastewater having a certain concentration of organic

pollutants is fed so as not to exceed the daily load of these pollutants in terms of 1 m3 of treatment plant, 1 g of dry biomass or 1 g of ashless biomass [21]. Virtually all organic matter can be oxidized under aerobic conditions, although the rate of their oxidation varies widely.

Biological wastewater treatment is called complete if the BSC (biochemical oxygen demand) of treated wastewater is less than 20 mg / l and incomplete with BSC - more than 20 mg / l [21].

Aerobic Wastewater Treatment. Aerobic wastewater treatment processes include simple septic or aerobic tanks, and oxidation ditches; surface and spray aeration; activated sludge; oxidation ditches, trickling filters; pond and lagoon-based treatments; and aerobic digestion. Constructed wetlands and various types of filtration are also considered biological treatment processes. Diffused aeration systems may be used to maximize oxygen transfer and minimize odors as the wastewater is treated. Aeration provides oxygen to the helpful bacteria and other organisms as they decompose organic substances in the wastewater [22].

A time-honored example of an aerobic biological treatment method is the activated sludge process, which is widely used for the secondary treatment of both domestic and industrial wastewater. It is well suited for treating waste streams high in organic or biodegradable content and is often used to treat municipal sewage; wastewater generated by pulp and paper mills or food-related industries such as meat processing; and industrial waste streams containing carbon molecules[23].

The membrane aerated biofilm reactor (MABR) treatment [19]. In recent years, technological advances have been transforming biological processes. One example is the membrane aerated biofilm reactor (MABR), which refines this process to use 90% less energy for aeration, typically the most energy-intensive stage of traditional biological treatment. In Fluence's MABR treatment, air at atmospheric pressure is gently blown into a spirally wound membrane in a tank, with air on one side of the membrane and mixed liquor on the other in a single tank [24]. Nitrification-denitrification is achieved by a biofilm that forms on the membrane. The result is an effluent suitable for irrigation or release into the environment.

Most legacy plants around the world use activated sludge treatment or other older aerobic treatment processes. Such plants are time-consuming and expensive to replace, or don't have necessary space for expansion. To address this need, Fluence has created SUBRE MABR modules. SUBRE submerges arrays of MABR membranes in existing wastewater treatment plant tanks to increase energy efficiency, capacity, and effluent quality — all on the plant's existing footprint.

Fluence has also packaged complete Aspiral™ MABR wastewater treatment plants inside standard shipping containers, which allows efficient transportation and fast commissioning in virtually any region. The plug-and-play units can be used in tandem to increase capacity, and are designed for low maintenance and remote monitoring.

In just a few years, MABR has developed into a mature technology, with extensive projects underway in China in compliance with the country's strict Class 1A effluent standards. In the United States, Fluence MABR proved itself in compliance with California Title 22 effluent standards during a yearlong demonstration at Stanford University.

Anaerobic Treatment. In contrast, anaerobic treatment uses bacteria to help organic material deteriorate in an oxygen-free environment. Lagoons and septic tanks may use anaerobic processes, but the best-known anaerobic treatment is anaerobic digestion, which is used for treating effluent from food and beverage manufacturing, as well as municipal wastewater, chemical effluent, and agricultural waste [23].

Anaerobic digestion drives one of the most robust areas of resource recovery: energy recovery. In this form of energy recovery, also known as waste-to-energy, anaerobic digestion is used to produce biogas, which is composed primarily of methane. Operators can use it to generate energy to help fuel operations on the way to become energy net zero, or even turn waste streams into revenue streams.

Further Treatment. The type of biological treatment selected for wastewater treatment, whether aerobic or anaerobic, depends on a wide range of factors, including compliance with environmental discharge quality regulations.

Biological treatments often are supplemented with additional treatment stages, including chlorination and UV treatment, as well as a range of filtration options including carbon filtration, reverse osmosis, and ultrafiltration.

Researchers continue to look for ways to optimize conventional biological wastewater treatment. In one example, Finnish researchers added iron sulfate to wastewater before biological treatment to reduce phosphorous in tough-to-treat pulp mill wastewater. Other researchers have used UV light to remove challenging substances such as chemical residues and pharmaceutical compounds. And, MABR's groundbreaking aeration model saves so much energy that it makes treatment possible in remote areas on alternative energy sources [23].

So, while biological treatment has a long history, it's continuing to evolve in ways that make it more effective, efficient, and available. Contact Fluence for information on MABR (membrane aerated biofilm reactor) products or to take advantage of our 30 years of experience in waste-to-energy solutions.

The treatment of wastewater subsequent to the removal of suspended solids by microorganisms such as algae, fungi, or bacteria under aerobic or anaerobic conditions during which organic matter in wastewater is oxidized or incorporated into cells that can be eliminated by removal process or sedimentation is termed biological treatment [25].

Biological treatments rely on bacteria, nematodes, or other small organisms to break down organic wastes using normal cellular processes. Wastewater typically contains a buffet of organic matter, such as garbage, wastes, and partially digested foods. It may also contain pathogenic organisms, heavy metals, and toxins [26].

The goal of biological wastewater treatment is to create a system in which the results of decomposition are easily collected for proper disposal. Scientists have been able to control and refine both aerobic and anaerobic biological processes to achieve the optimal removal of organic substances from wastewater [26].

Biological treatment can be divided into aerobic and anaerobic treatment. Aerobic treatment occurs with the presence of oxygen, and in the result of metabolic processes of bacteria, fungi or algae we received water, carbon dioxide and remain biomass. And after anaerobic metabolic processes we received carbon dioxide, remain biomass and methane.

Algae are ubiquitous in the environment and can be found in all types of water bodies, including freshwater, brackish waters and marine environments.

Since the term "algae" is used to define such a vast and diverse group of organisms, scientists usually distinguish macroalgae, multicellular algae and microalgae, microscopic algal organisms.

Microalgae include types of unicellular algal organisms that can live individually or in colonies. Microalgae can be free-floating or attached to the surface of any underwater formations. Algae can vary in size from 1 to several hundred micrometers (μm) depending on the species. For reference: 1 micrometer (μm) = 1/1000 millimeter (mm).

Microalgae are one of the most important groups of organisms on our planet. It has been found that they produce almost half of the atmospheric oxygen on Earth by absorbing huge amounts of the greenhouse gas carbon dioxide.

Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals [27].

First of all, need to define microalgae and which organisms belong to. Microalgae are unicellular species which exist individually, or in chains or groups. Depending on the species, their sizes can range from a few micrometers (μm) to a few hundred micrometers. Unlike higher plants, microalgae do not have roots, stems, or leaves. They are specially adapted to an environment dominated by viscous forces. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen [28] and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically. Microalgae, together with bacteria, form the base of the food web and provide energy for all the trophic levels above them. Microalgae biomass is often measured with chlorophyll a concentrations and can provide a useful index of potential production. The standing stock of microphytes is closely related to that of its predators. Without grazing pressures, the standing stock of microphytes dramatically decreases [29].

Nowadays, the strictly arose the problem of eutrophication of water bodies. Eutrophication or hypertrophication, is when a body of water becomes overly enriched

with minerals and nutrients which induce excessive growth of algae [30]. This process may result in oxygen depletion of the water body [31].

One example is an "algal bloom" or great increase of phytoplankton in a water body as a response to increased levels of nutrients. Eutrophication is often induced by the discharge of nitrate or phosphate-containing detergents, fertilizers, or sewage into an aquatic system. But with the controlled “blooming” on the treatment plant the eutrophication potential can be decreased.

The history of the commercial use of algal cultures spans about 75 years with application to wastewater treatment and mass production of different strains such as Chlorella and Dunaliella. Currently significant interest is developed in some advanced world nations such as Australia, USA, Thailand, Taiwan and Mexico [32]. These are due to the understanding of the biologists in these nations for the biology and ecology of large-scale algal cultures, as well as in the engineering of large-scale culture systems and algal harvesting methods, all of which are important to the design and operation of high rate algal cultures to produce high-value products, such as Pharmaceuticals and genetically engineered products [33].

Bio-treatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporating nutrients such as nitrogen and phosphorus causing eutrophication [34]. This fascinating idea launched some fifty-five years ago in the U.S. by Oswald and Gotaas (1957) has since been intensively tested in many countries [35]. Palmer (1974) surveyed microalgal genera from a wide distribution of waste stabilization ponds. In order of abundance, and frequency of occurrence the algae found were Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium and Golenkinia [36].

1.4. Conclusions to the Chapter

We can conclude that:

- Wastewater is containing significant concentrations of solids, dissolved and particulate matter, microorganisms, nutrients, heavy metals and micro-pollutants.

- The content and volume of pollutants in waste water differ from the type of production in which waste water produced.
- The most significant parameters which observed for waste water are BOD (biological oxygen demand), COD (chemical oxygen demand), pH of water, total nitrogen (T-N), total phosphorus (T-P), total suspended solids (TSS).
- In food industry the content of biogenic elements (phosphorous and nitrogen compounds) which influence on aquatic flora and fauna are the highest.
- Sewage treatment, or domestic wastewater treatment, is the process of removing contaminants from wastewater, both runoff (effluents) and domestic. It includes physical, chemical and biological processes to remove physical, chemical and biological contaminants.
- Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals.

CHAPTER 2
METHODOLOGY OF BIOGENIC ELEMENTS DETECTION

2.1. Equipment

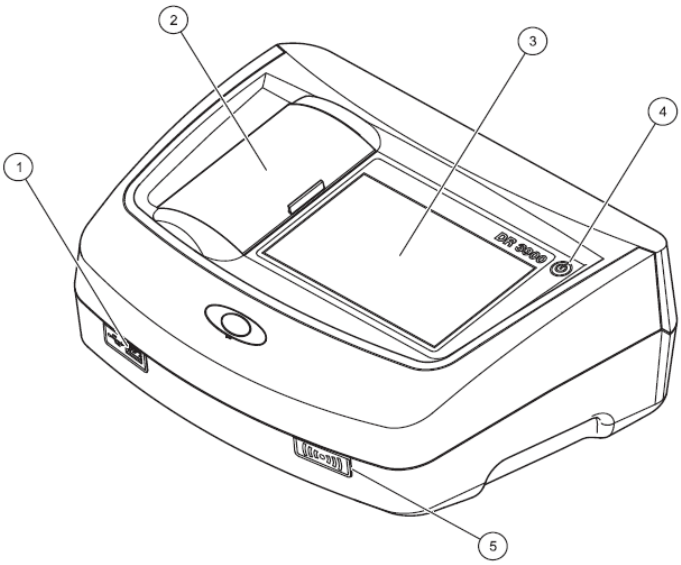
For investigation the microalgae potential we made certain experimental sessions which include the hypothesis approval and observation of bioreactor work.

For the sessions of experiments, we used the following equipment:

1. Spectrophotometer. (*Present on figure 2.1).* The DR 3900 is a VIS spectrophotometer with a wavelength range from 320 to 1100 nm. Device comes with a complete set of applications and support for multiple languages. The DR 3900 spectrophotometer offers the following methods and methods of operation:

- Methods in memory (preset tests);
- Techniques with a barcode;
- User techniques;
- Selected techniques;
- Single wave mode;
- Multi-wave mode;
- Spectral analysis;
- Kinetic analysis.

The DR 3900 spectrophotometer has an output result in units of concentration, optical density or as a percentage of transmission. When you select custom or programmed methods, menus and tips are displayed to help take a measurement. The menu system also allows you to create reports, statistical estimates of generated gauges curves and display the results of device diagnostics.



1 USB module	**4** Switch
2 Sample compartment cover	**5** Module RFID
3 Touch screen	

Fig. 2.1. VIS spectrophotometer DR 3900. *1* –USB module, *2* – sample compartment cover, *3* – touch screen, *4* – switch, *5* – module RFID

2. Cuvettes for the spectrophotometer.

3. Reagents for phosphates, nitrates and nitrites.

4. Distilled water.

5. Paper filters.

6. Funnels and flasks for filtration.

7. CO_2 generator.

8. Culture of microalgae Chlorella vulgaris.

9. Bottles for growing algae.

10. Wastewater from the apartment building before entering the treatment plant was taken at 10.00 in the morning in Kyiv.

11. Waste water from a utility company from Novograd-Volynsky before treatment was taken at 8.30 in the morning.

12. Waste water from a communal enterprise from Novograd-Volynsky after treatment was taken at 8.30 in the morning.

2.2. Methods of biogenic elements observation

Ammonia nitrogen

For ammonia nitrogen we use the Nessler method for 0.02…2.5 ml/L NH_3-N. The procedure schematically presented in the figure 2.2-2.4.

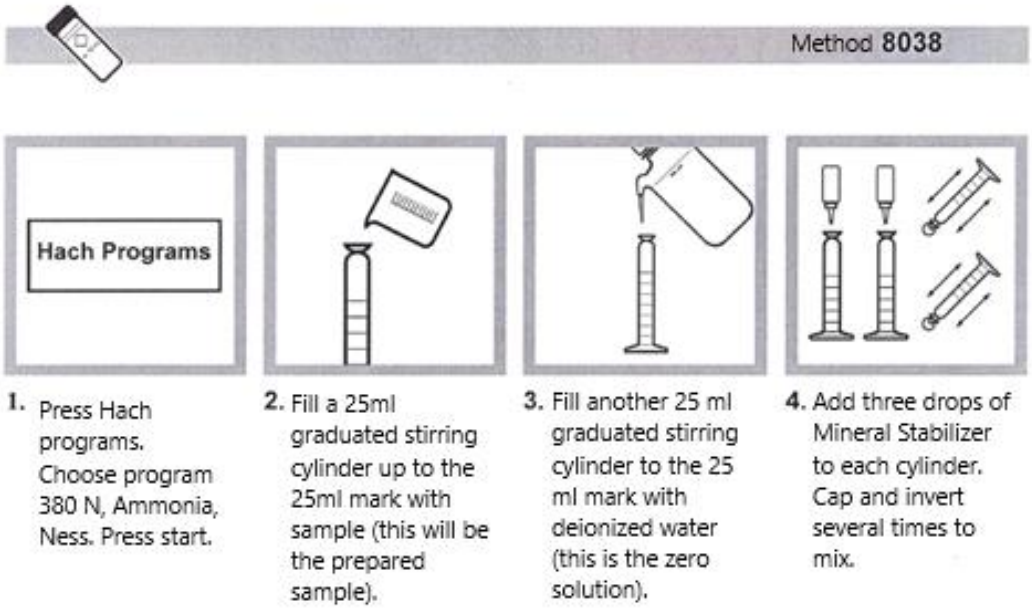


Fig. 2.2. Ammonia nitrogen testing procedure on spectrophotometer (part 1)

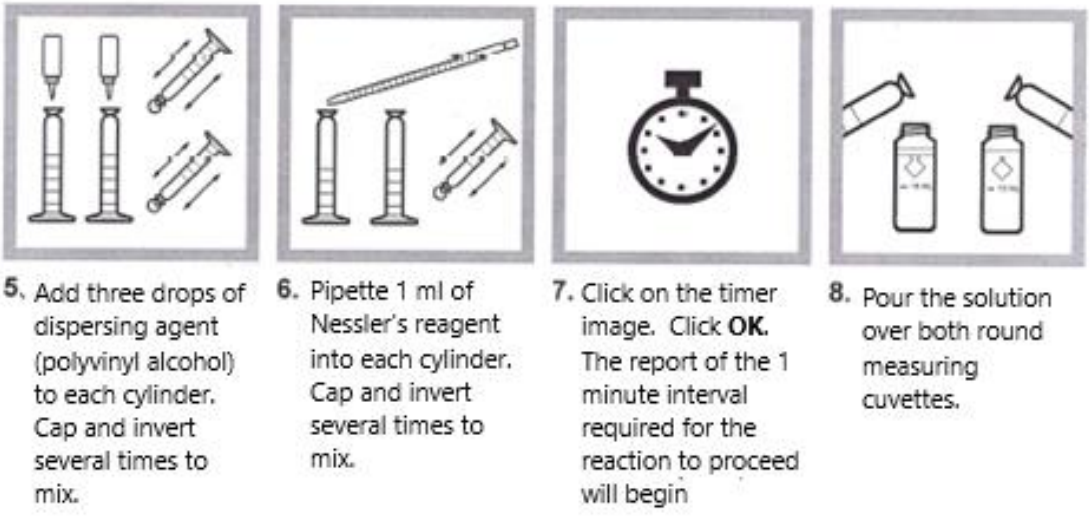


Fig. 2.3. Ammonia nitrogen testing procedure on spectrophotometer(part 2)

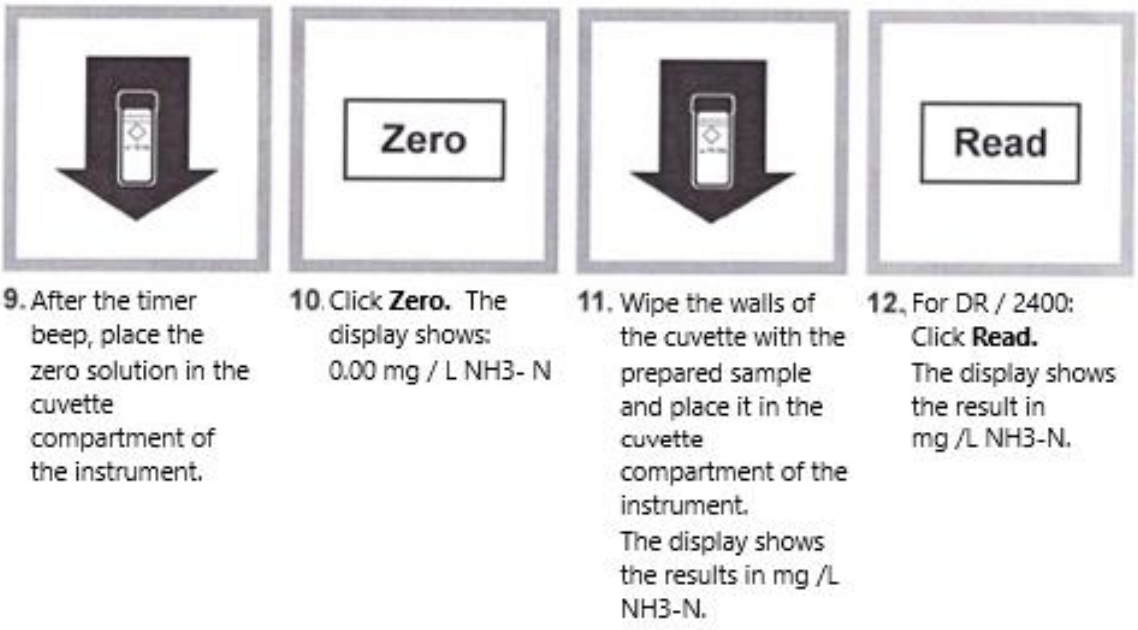


Fig. 2.4. Ammonia nitrogen testing procedure on spectrophotometer(part 3)

Nitrate

The method used was cadmium reduction for 0.3...30 mg/L NO_3^-N.

The procedure schematically presented in the figure 2.5-2.7.

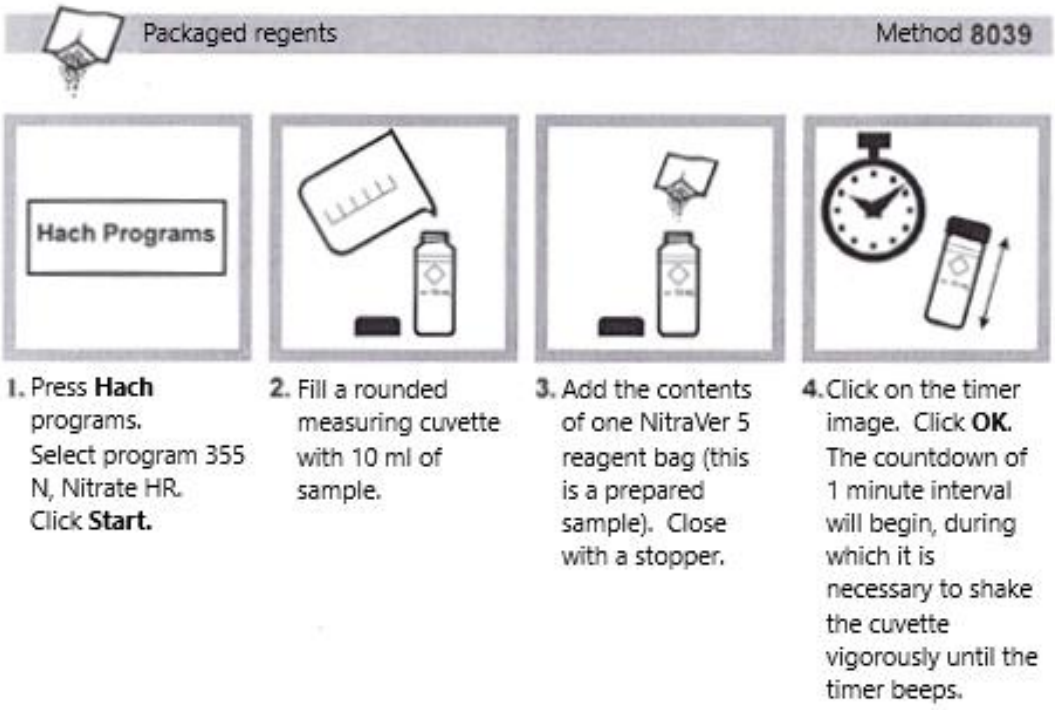


Fig. 2.5. Nitrate testing procedure on spectrophotometer(part 1)

5. After the timer beep, tap the timer icon again. Click **OK**. The countdown will begin to find the minute interval required for the reaction to proceed. In the presence of nitrate, an amber color will develop.

6. After the timer beep, remind the second round 10ml sample cuvette (zero solution).

7. Place the zero solution in the sample compartment of the instrument.

8. Click **Zero.** The display shows 0.0 mg / L NO3- -N

Fig. 2.6. Nitrate testing procedure on spectrophotometer (part 2)

9. Within 1 minute after the timer beep, place the prepared sample in the cuvette compartment of the instrument. The display shows the result in mg / l NO3- - N.

10. For DR / 2400: Click **Read**. The display shows the result in mg / l NO3- - N.

Fig. 2.7. Nitrate testing procedure on spectrophotometer (part 3)

Nitrite

The method used is diazotization method for 0.002…0.300 mg/L NO_2–N.

The procedure schematically presented in the figure 2.8-2.10.

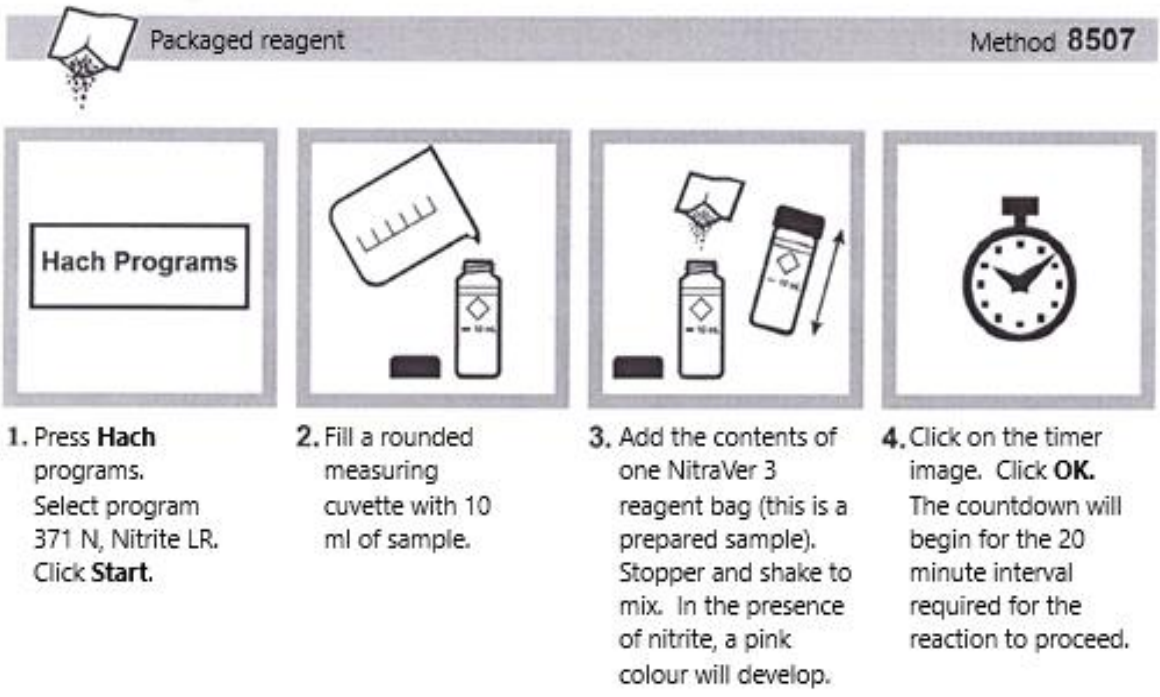
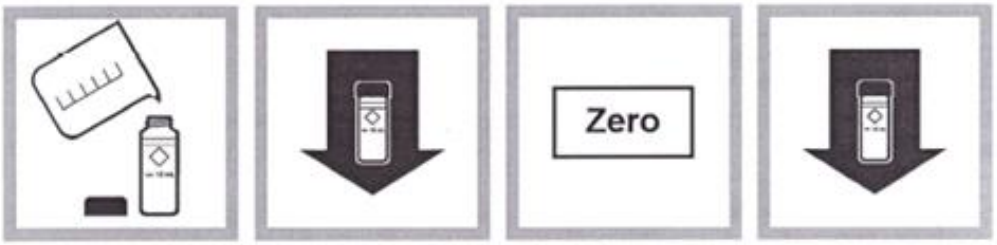


Fig. 2.8. Nitrite testing procedure on spectrophotometer (part 1)

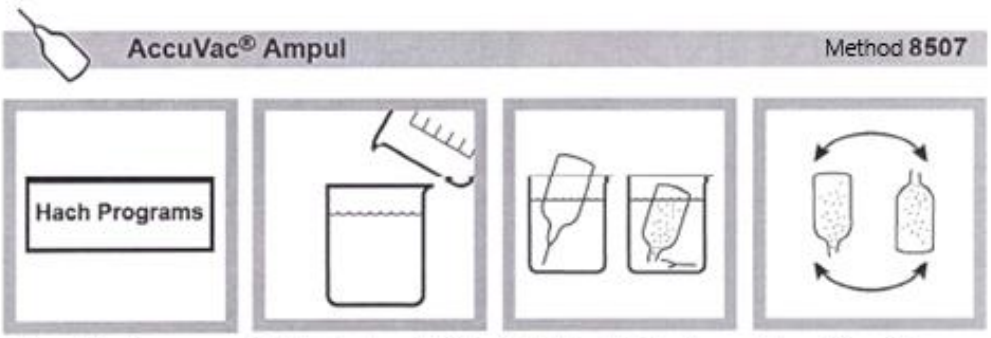


5. After the timer beep, remind the second round 10ml sample cuvette (zero solution).

6. Wipe down the walls of the cuvette and place the zero solution in the cuvette compartment of the instrument.

7. Click **Zero**. The display will show 0.0 mg / L NO2- -N.

8. Wipe the prepared sample and place it in the instrument. For DR / 2400: Click **Read.** The display shows the result in mg / l NO2- -N.



1. Press **Hach** programs. Select program 375 N, Nitrate LR AV. Click **Start.**

2. Collect at least 40 ml of sample into a 50 ml beaker.

3. Fill the NitraVer 5 ampule with sample. Keep the tip submerged until the ampoule is full. Place the rubber stopper on the tip of the ampoule.

4. Invert the mixing ampoule several times. In the presence of nitrite, a pink color will develop.

Fig.2.9. Nitrite testing procedure on spectrophotometer (part 2)

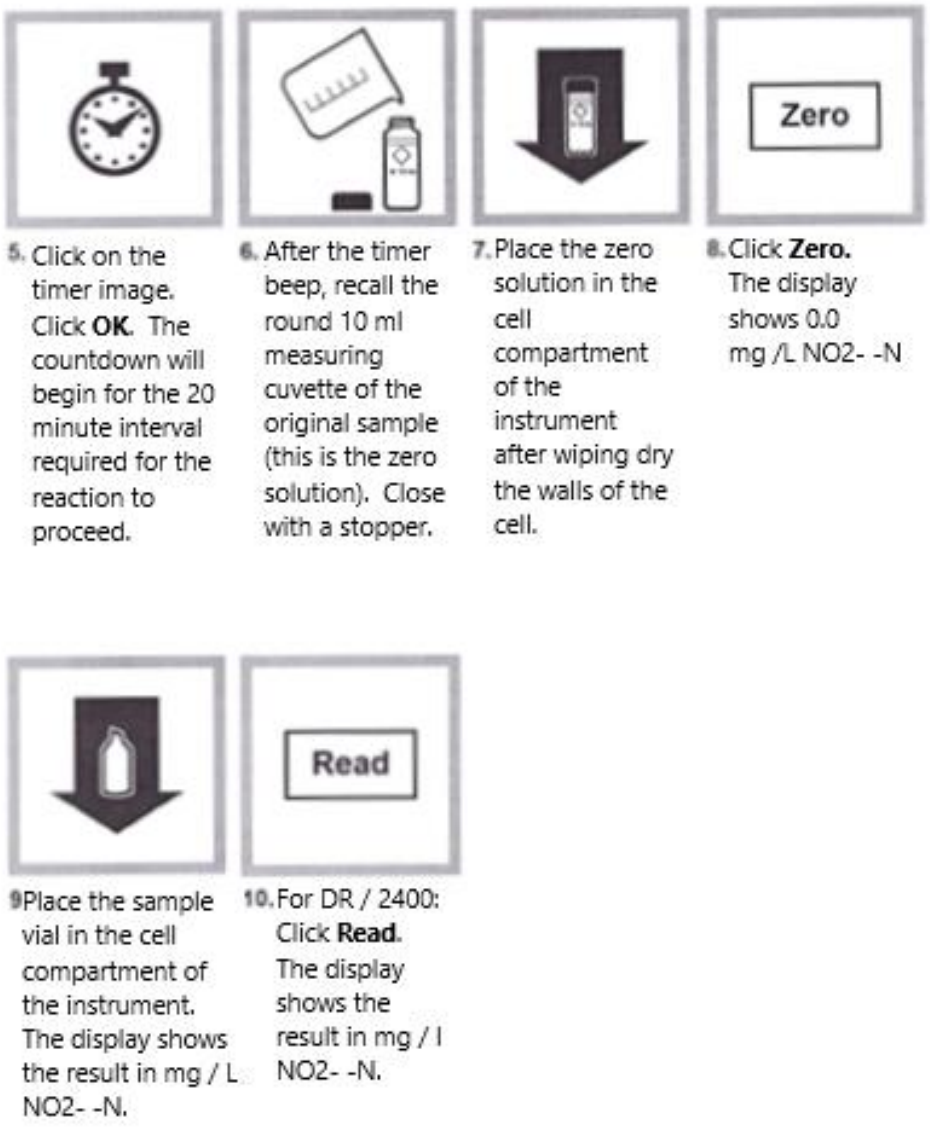


Fig.2.10. Nitrite testing procedure on spectrophotometer (part 3)

Phosphate

The method used is Amino Acid method for 0.23…30.0 ml/L PO_4^{3-}.

The procedure schematically presented in the figure 2.11-2.12.

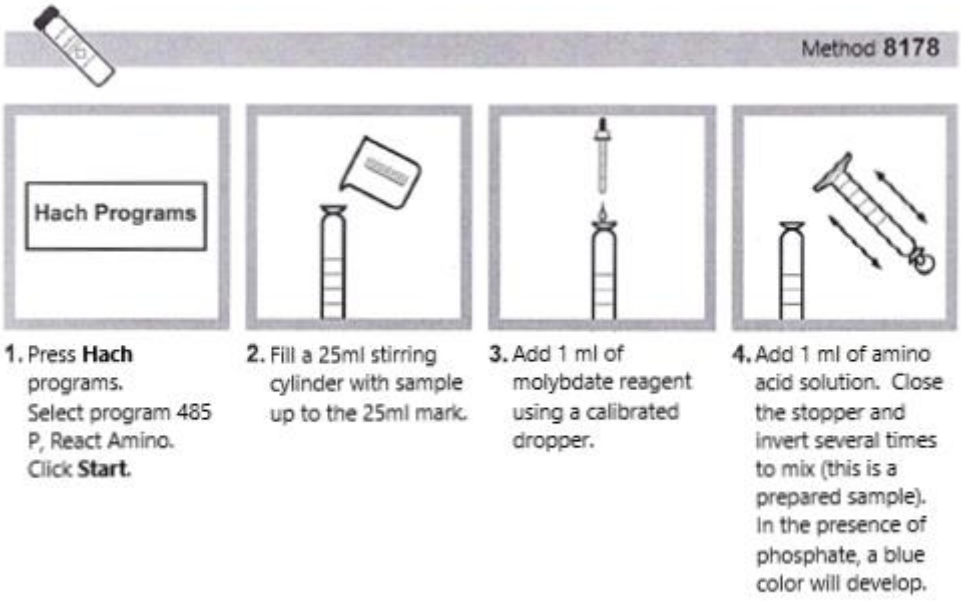


Fig.2.11. Phosphate testing procedure on spectrophotometer (part 1)

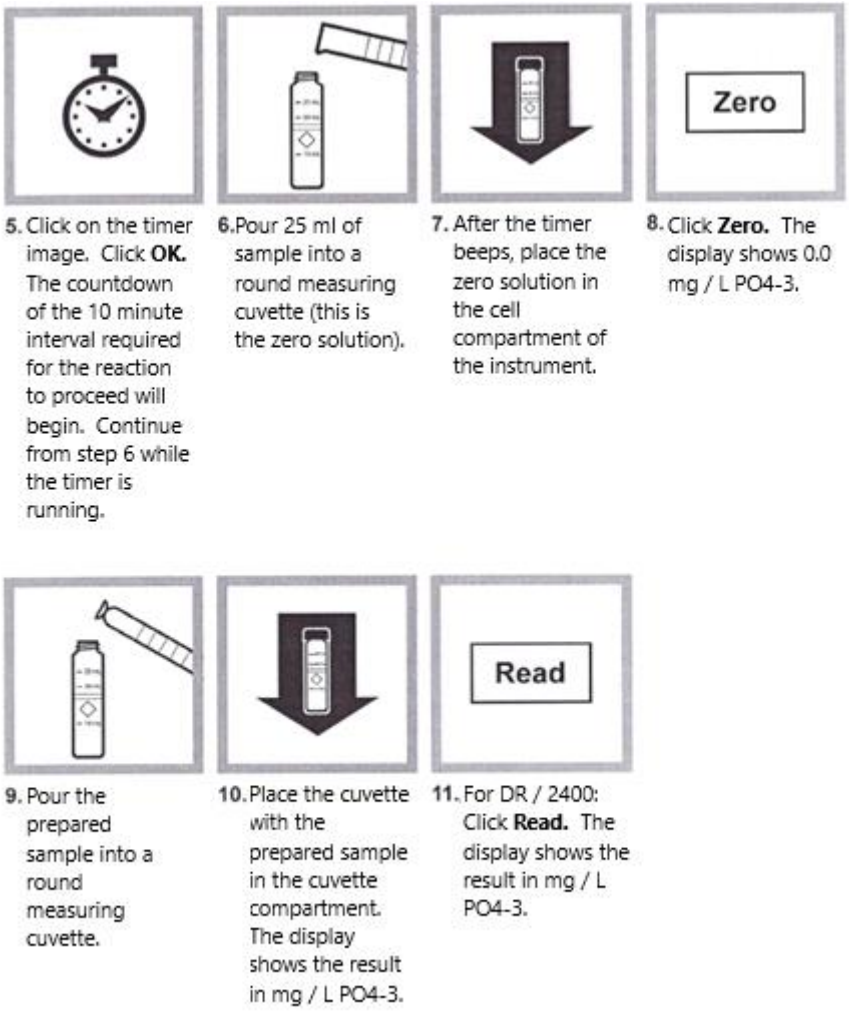


Fig.2.12. Phosphate testing procedure on spectrophotometer (part 2)

2.3. Experiment progress of Chlorella vulgaris cultivation

The necessary characteristics of the experiment that we took into account are:

1. Time of experimental sessions.

2. Conditions for growing microalgae

3. Measurement of nutrient levels

Time and frequency of experimental sessions.The laboratory of the National Aviation University Department of Environmental Safety, Engineering and Technology was chosen as the site of the experiment. For the experimental sessions, the summer period was chosen, since warm temperatures are more favorable for the growth of algae. The first experimental session was held from July 9, 2019 to July 17, 2019. The second experimental session was held from August 1, 2019 to August 12, 2019.

Conditions for growing microalgae. It was decided to use transparent bottles for growing microalgae in order to create the most favorable conditions for growing. The average temperature in the laboratory is 22 degrees. The average pressure on the dates of the experiment was 746 millimeters of mercury. The concentration of microalgae is equal to 1 liter of water per 200 ml of suspension of microalgae.

The first experimental session included three parallel experiments for each type of water: one control without microalgae, one with wastewater and microalgae, one with a connected CO_2 generator in wastewater with microalgae. The tanks were in a well-lit laboratory and had the same conditions.

The second experimental session included four parallel experiments for one type of wastewater: the first control, the second in wastewater with microalgae, 3 and 4 identical samples with a connected CO_2 generator.

Measurement of nutrient levels. To measure the levels of nutrients (phosphates, nitrates and nitrites), the following measurement frequency was chosen:

1) in the first session - the first three days every day, then every two days;

2) in the second session - every 6 days.

Since spectrophotometric equipment requires a sample free of particles, double filtering was carried out with paper filters.

For the first experimental session, three reservoirs (bottles) were installed for each type of water (wastewater from a residential building in Kyiv before treatment, wastewater from a utility in Novograd-Volynsk before and after treatment). Total 9 samples.

The initial hypothesis was that the bulk of nutrients will go away in the first three days, so measurements of nutrient levels were carried out for the first three days every day, and then every two days.

Immediately after the installation of all tanks, phosphate, nitrate and nitrite levels were measured in order to record the initial data.

To take measurements, it is necessary to follow this order:

1) to take an equal sample volume for all experiments;

2) carry out a double filtration with paper filters (first time through a funnel with a filter, and separately a second time through a funnel with a filter);

3) gently wash the cuvettes with distilled water and wipe the cuvettes without touching the walls with your fingers so as not to create an error in the measurement;

4) take the necessary program on the spectrophotometer, and following the instructions add the reagent to the control sample;

5) record the result;

6) rinse the cuvettes with distilled water and take measurements for other samples of the experiment, also record the result;

7) at the end of the measurements, rinse all tubes, funnels and cuvettes with distilled water and dry.

For the second experimental session, four tanks were installed for one type of wastewater (wastewater from a residential building in Kyiv before entering the treatment plant): 1) control, 2) with microalgae, 3) and 4) identical with the connected CO_2 generator.

Since the initial hypothesis was not confirmed, it was decided to take measurements every 6 days. The measurement progress remained the same as in the first session.

2.4. Experiment progress of Euglena gracilis cultivation

Strain *Euglena gracilis* Klebs HPDP-114 was obtained from the collection of microalgae cultures of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine.

Euglena gracilis is a freshwater species of single-celled alga in the genus *Euglena*. It has secondary chloroplasts, and is a mixotroph able to feed by photosynthesis or phagocytosis. It has a highly flexible cell surface, allowing it to change shape from a thin cell up to 100 µm long, to a sphere of approximately 20 µm. Each cell has two flagella, only one of which emerges from the flagellar pocket (reservoir) in the anterior of the cell, and can move by swimming, or by so-called "euglenoid" movement across surfaces. *E. gracilis* has been used extensively in the laboratory as a model organism, particularly for studying cell biology and biochemistry [37].

A morphological and molecular study of the Euglenozoa put *E. gracilis* in close kinship with the species *Khawkinea quartana*, with *Peranema trichophorum* basal to both,[38] although a later molecular analysis showed that *E. gracilis* was, in fact, more closely related to *Astasia longa* than to certain other species recognized as *Euglena*. The transcriptome of *E. gracilis* was recently sequenced, providing information about all of the genes that the organism is actively using. They found that *E. gracilis* has a whole host of new, unclassified genes which can make complex carbohydrates and natural products.

The experiments used synthetic domestic wastewater, which was prepared by adding to the culture medium different concentrations of ammonium nitrogen (N-NH4 +) and phosphorus phosphates ($P\text{-}PO_4^{3-}$) (Table 2.1). NH_4Cl was used as a source of $N\text{-}NH_4^{+}$, KH_2PO^4 – as a source of $P\text{-}PO_4^{3-}$. Algae were kept at a temperature of 24 ± 2 ^oC and illumination of 3500 lux (with alternating light and dark periods 16:8). The duration of cultivation of *Euglena gracilis* in wastewater was 7 days. Materials for analysis were selected on 0 and 7 days of the experiment.

Table 2.1

Composition of domestic wastewaters

Variant of the experiment	Concentration, mg L^{-1}	
	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$
1	30.00±1.12	7.00±0.18
2	50.00±2.25	14.00±0.56
3	90.00±2.68	26.00±0.67

The concentration of ammonium nitrogen and phosphorus phosphates in wastewater was investigated by conventional methods in hydrochemistry.

Dry mass of the culture was determined by weight (Nezbrytskaya, Kureyshevich, 2015). The algal cell suspension was filtered through a pre-dried and weighed membrane filter (pore diameter 0.45 μm). Filters with precipitated algae were dried in a thermostat at a temperature of 105 oC to constant weight.

Biomass productivity was calculated by the formula:

$$BP = \frac{N - N_0}{T} \tag{2.1}$$

where N – the dry matter content at the end of cultivation, (mg $L^{-1}day^{-1}$);

N_0 – dry matter content at the beginning of cultivation, (mg $L^{-1}day^{-1}$);

T – duration of cultivation (7 days).

The content of chlorophyll *a* and the sum of carotenoids was determined by extract-spectrophotometric method (Nezbrytskaya, Kureyshevich, 2015). For extraction, the algae filter was placed in a porcelain mortar and thoroughly ground with the addition of quartz sand and 90% acetone. The obtained extract was separated by centrifugation during 15 min at 5000 thousand rpm. Spectrophotometry was performed at wavelengths of 664, 647 and 480 nm, corresponding to the maxima of light absorption in 90% acetone, respectively, chlorophyll *a, b* and carotenoids. Non-specific absorption of the light extract at a wavelength of 750 nm was also measured.

The chlorophyll *a* content was calculated by the formula (Jeffrey and Humphrey, 1975):

$$Chlorophylla = (11.93E664 - 1.93E647) \times \frac{V_1}{V_2}, \quad (2.2)$$

where V_1 – extract volume, cm^3; V_2 – the volume of the filtered sample, dm^3; $E664$ and $E647$ – experimentally determined the optical densities of the extract at wavelengths 664 and 647, nm.

The total carotenoid content was calculated by the formula (Parsons and Strickland, 1963):

$$Carotenoids = 10(E480 - 3E750) \times \frac{V_1}{V_2} \times 1, \quad (2.3)$$

where V_1 – extract volume, cm^3; V_2 – the volume of the filtered sample, dm^3; 1– the thickness of the spectrophotometric cell, sm; $E480$ and $E750$ – optical densities of the extract at wavelengths 480 and 750 nm.

The content of photosynthetic pigments was expressed in terms of dry weight of algae.

All measurements were performed in triplicate. Statistical processing of the results was performed using IBM SPSS Statistics Base v.2

2.5. Conclusions to Chapter

To sum up all above:

- For investigation the microalgae potential we made certain experimental sessions which include the hypothesis approval and observation of bioreactor work.
- Two experimental sessions were prepared in laboratory in National Aviation University, and one session in Institute of Hydrobiology NAN of Ukraine. The cultivation of Chlorella vulgaris in the laboratory of NAU and the cultivation of Euglena gracilis in Institute of Hydrobiology NAN of Ukraine.
- First experiment (two experimental sessions) done in summer 2019. Second in autumn 2020.

- In experiment with Chlorella vulgaris used 1) wastewater from the apartment building before entering the treatment plant was taken at 10.00 in the morning in Kyiv; 2) waste water from a utility company from Novograd-Volynsky before treatment was taken at 8.30 in the morning; 3) waste water from a communal enterprise from Novograd-Volynsky after treatment was taken at 8.30 in the morning.

- In experiment with Euglena gracilis used synthetic domestic wastewater, which was prepared by adding to the culture medium different concentrations of ammonium nitrogen (N-NH4 +) and phosphorus phosphates ($P\text{-}PO_4^{3-}$)

- In experiment with Chlorella vulgari the concentration of ammonium nitrogen and phosphorus phosphates in wastewater was investigated by the DR 3900 spectrophotometer.

- In experiment with Euglena gracilis the concentration of ammonium nitrogen and phosphorus phosphates in wastewater was investigated by conventional methods in hydrochemistry and the statistical processing of the results was performed using IBM SPSS Statistics Base v.2

CHAPTER 3
EXPERIMENTAL STUDY OF BIOGENIC COMPOUNDS REMOVAL

3.1. Result of Chlorella vulgaris cultivation

During the Chlorella vulgaris cultivation we have some issues and further solution of it. The main issues and solutions are:

1) Issue: investigation of the growth kinetics of microalgae in sewages under different cultivation conditions.

Solution: usage of CO_2 aeration for metabolic processes of microalgae speeding-up.

2) Issue: study of the kinetics of changes in the concentration of nutrient compounds in wastewater under different conditions of purification.

Solution: take into account different stages of waste water treatment (before and after treatment at the municipal sewage plant, sewage out flow from the residential house).

On figures 3.1 – 3.6 presented the results of chlorella vulgaris application on waste water treatment.

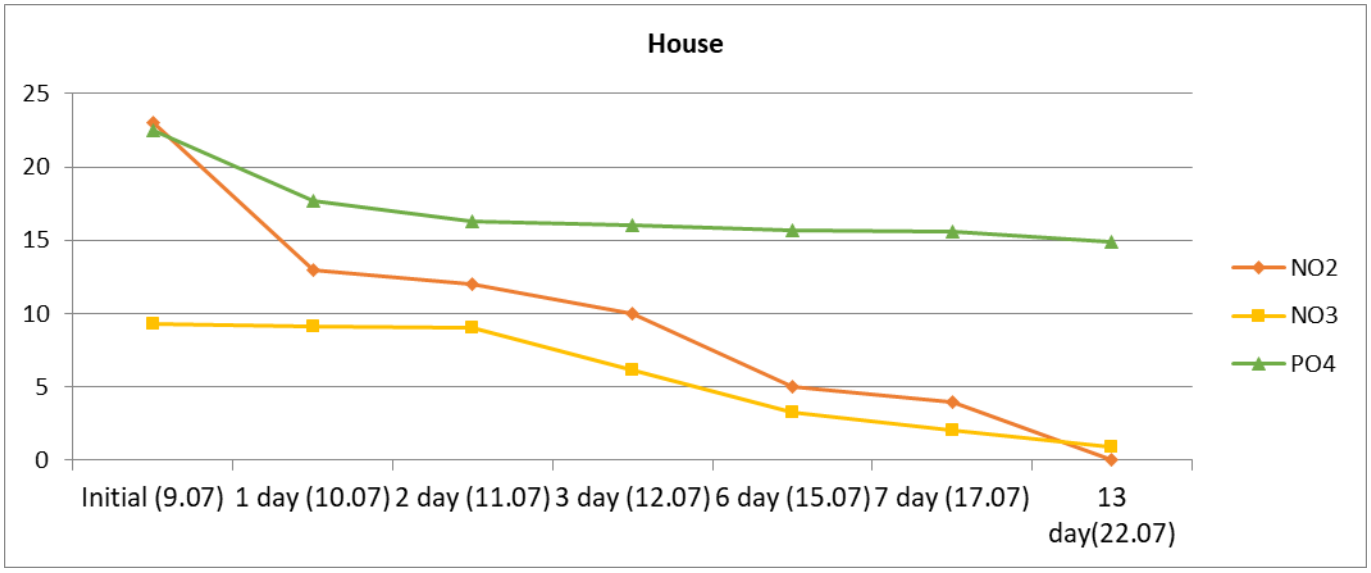


Fig.3.1. Dynamic of PO_4 ,NO_2, NO_3
in the water from kyiv city residential house before treatment
with the CO2 aeration applying

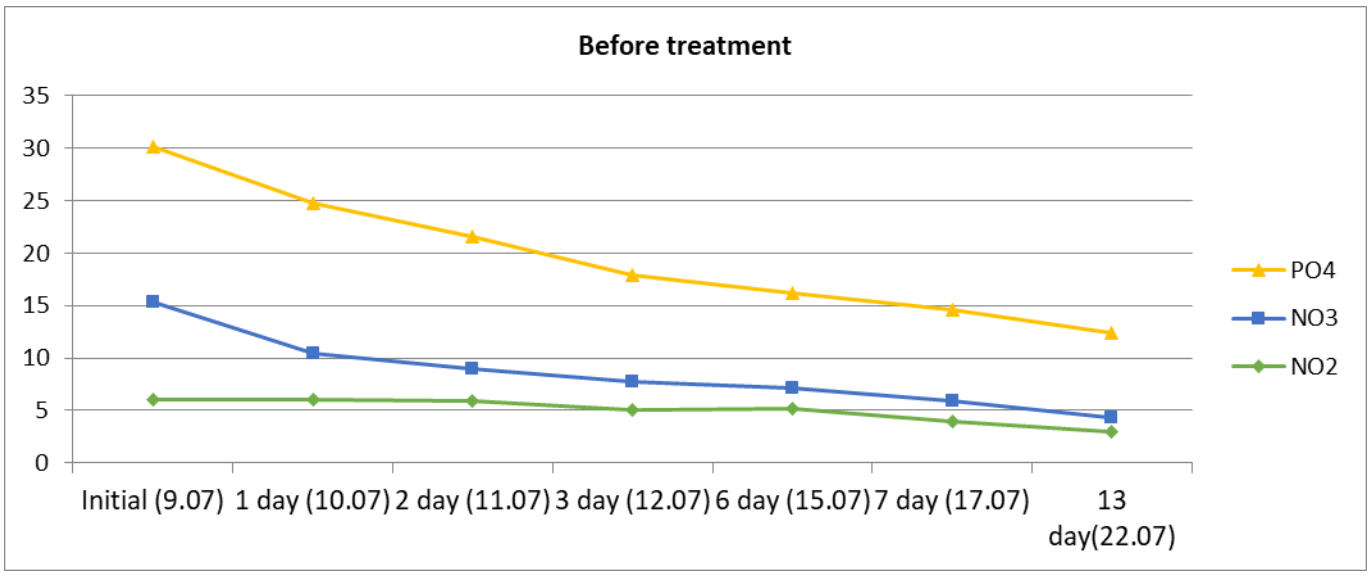


Fig.3.2. Dynamic of PO_4 ,NO_2, NO_3
in the water from municipal sewage treatment plant of Novograd-Volynsky city before treatment with the CO2 aeration applying

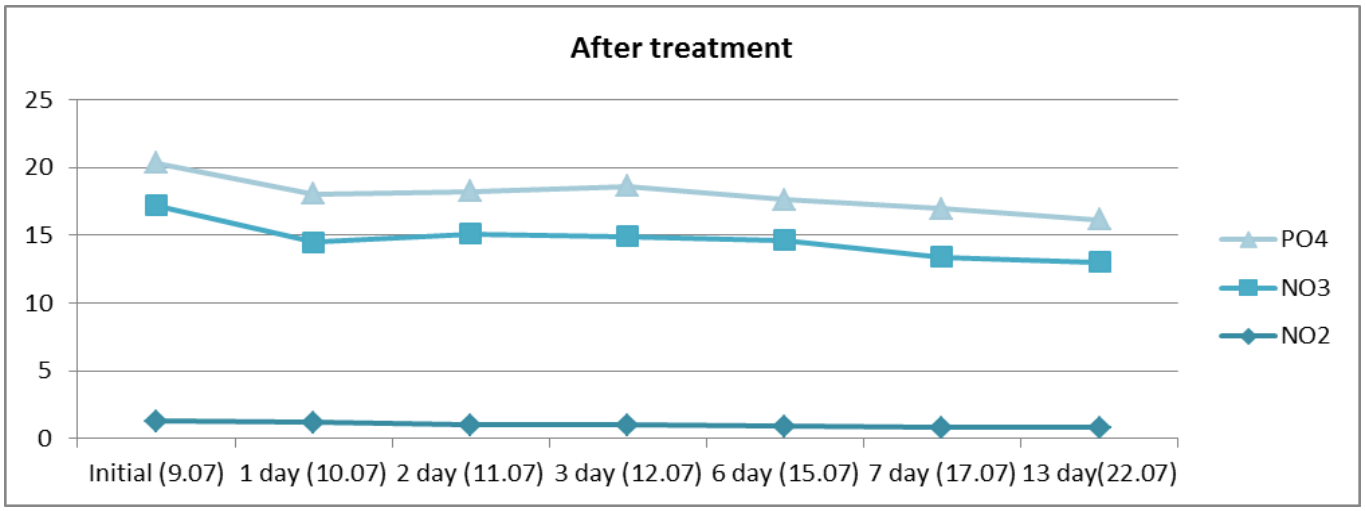


Fig.3.3. Dynamic of PO_4 ,NO_2, NO_3
in the water from municipal sewage treatment plant of Novograd-Volynsky city after treatment with the CO2 aeration applying

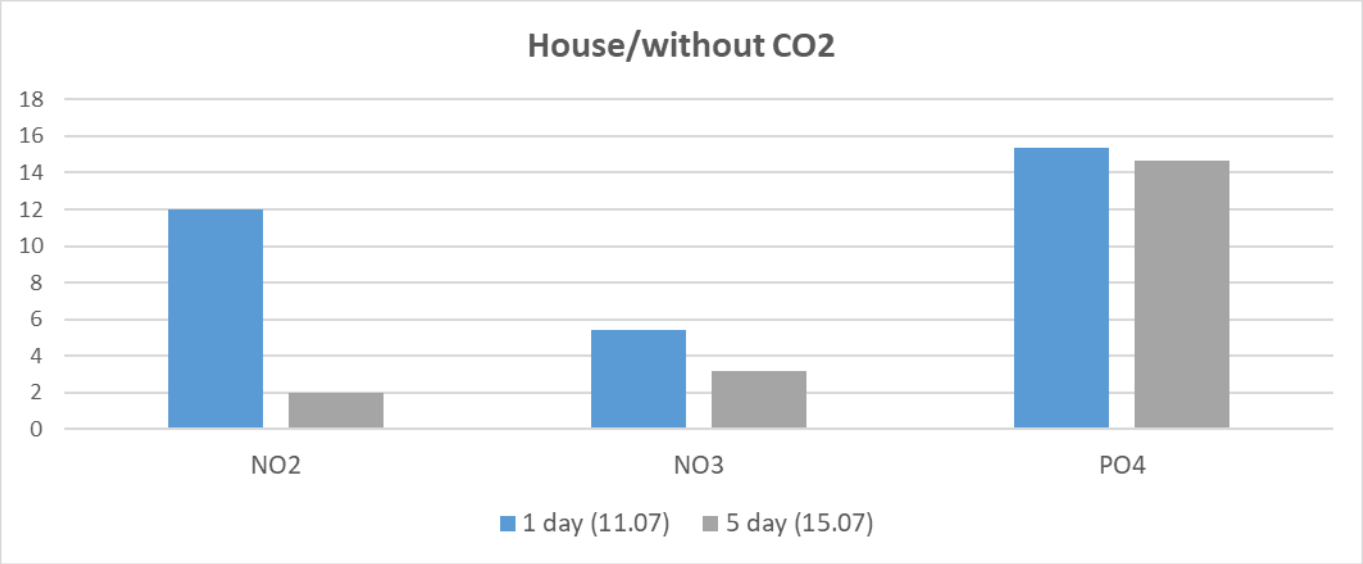


Fig.3.4. Dynamic of PO_4 ,NO_2, NO_3
in the water from Kyiv city residential house before treatment
without the CO2 aeration applying

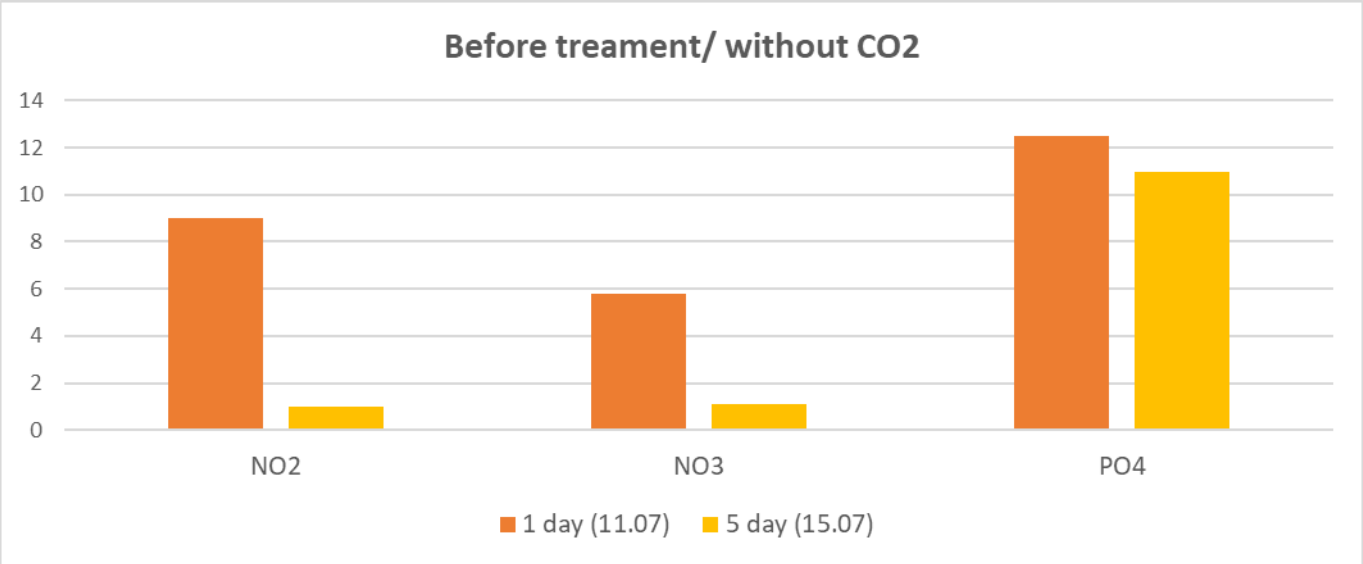


Fig.3.5. Dynamic of PO_4 ,NO_2, NO_3
in the water from municipal sewage treatment plant of Novograd-Volynsky city before
treatment without the CO2 aeration applying

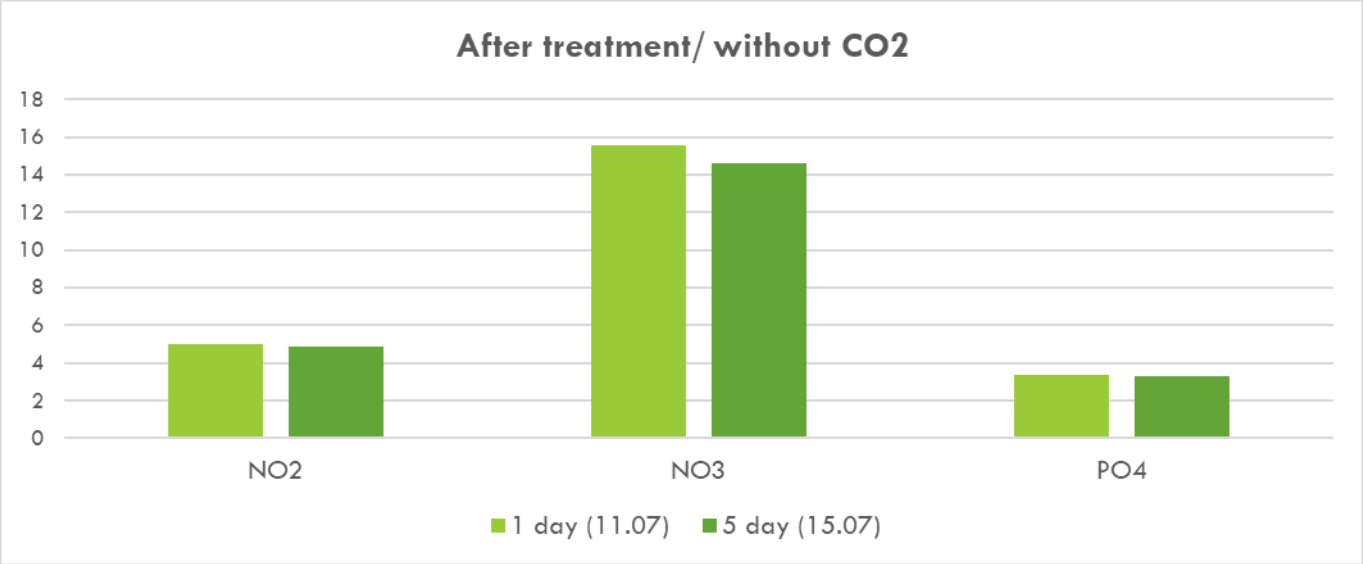


Fig.3.6. Dynamic of PO_4, NO_2, NO_3
in the water from municipal sewage treatment plant of Novograd-volynsky city after treatment without the CO2 aeration applying

As a results of our experiments the ability of microalgae to reduce biogenic elements wastewater waters was confirmed. On the figure 3.7 the results of joint measurements are presented.

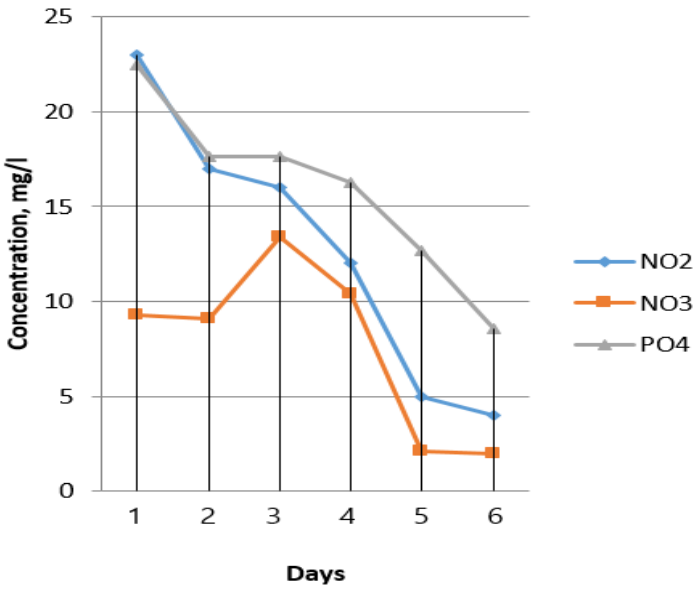


Fig.3.7. Nitrogen and phosphorus compounds concentration changes in wastewater with connection to CO_2 generator

Table 3.1 demonstrates positive effect of microalgae in the case of nitrogen and phosphorous concentration after 6 days.

Table 3.1

Decreasing of biogenic compounds in wastewater

Compounds	Concentration, mg/l		Decreasing, %
	Initial	Final (after 6 days)	
NO_2	23	4	82,6
NO_3	9,3	2	78,5
PO_4	22,49	15,55	30,9

The results of the study show that the content of nitrogen and phosphorus compounds significantly decreased, indicating the effectiveness of post-treatment of effluents using chlorella microalgae. Prospects of the microalgae use for wastewater treatment from nutrients are obvious. They are specially adapted to an environment dominated by viscous forces. The use of microalgae, which is grown under controlled environmental conditions, is wastewater treatment technology that does not require large initial investments and operating costs. It can be used in addition to or instead of mechanically aerated wastewater treatment systems.

The introduction of microalgae-based wastewater treatment systems is particularly beneficial in areas with low-cost land plots and a fair number of clear and warm days.

The use of waste for the cultivation of microalgae allows to address the issues of their utilization and reduction of anthropogenic pressure on the environment. The cultivation of microalgae and the production of biodiesel or other fuels from them contributes to solving the problems of alternative energy from renewable raw materials. In addition, biologically active substances can be obtained from microalgae.

The remain biomass can be used as raw material for biofertilizers production or raw material for biofuel production. The calculation of economic benefit from usage of Chlorella vulgaris as sewage treatment is presented on Chapter 4.

3.2. Results of Euglena gracilis cultivation

According to the results of the studies, *Euglena gracilis* for 7 days almost completely removes ammonium nitrogen and phosphorus phosphates from the studied wastewater (Table 3.2). It has been shown (Madkour et al. 2017) that the ability of microalgae to remove nitrogen and phosphorus compounds from the aquatic environment is determined primarily by the physiological characteristics of species, including metabolic rate and the need for nutrients to maintain their viability. In the process of bioremediation of microalgae, a large amount of nitrogen and phosphorus is used for the synthesis of proteins, nucleic acids, phospholipids and other important organic compounds.

Table 3.2

Changes in the concentration of nutrients in wastewater, M±m, n=3

Variant of the experiment	Initial concentration (0 days), mg L^{-1}		Final concentration (7 days), mg L^{-1}	
	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$
1	30.00±1.12	7.00±0.18	traces	traces
2	50.00±2.25	14.00±0.56	traces	traces
3	90.00±2.68	26.00±0.67	0.44±0.01	0.05±0.02

On figures 3.8-3.10 presented the changes in concentrations of biogenic elements after Euglena gracilis cultivation. Variants of the experiment mean the different initial concentration of model cultivation environment.

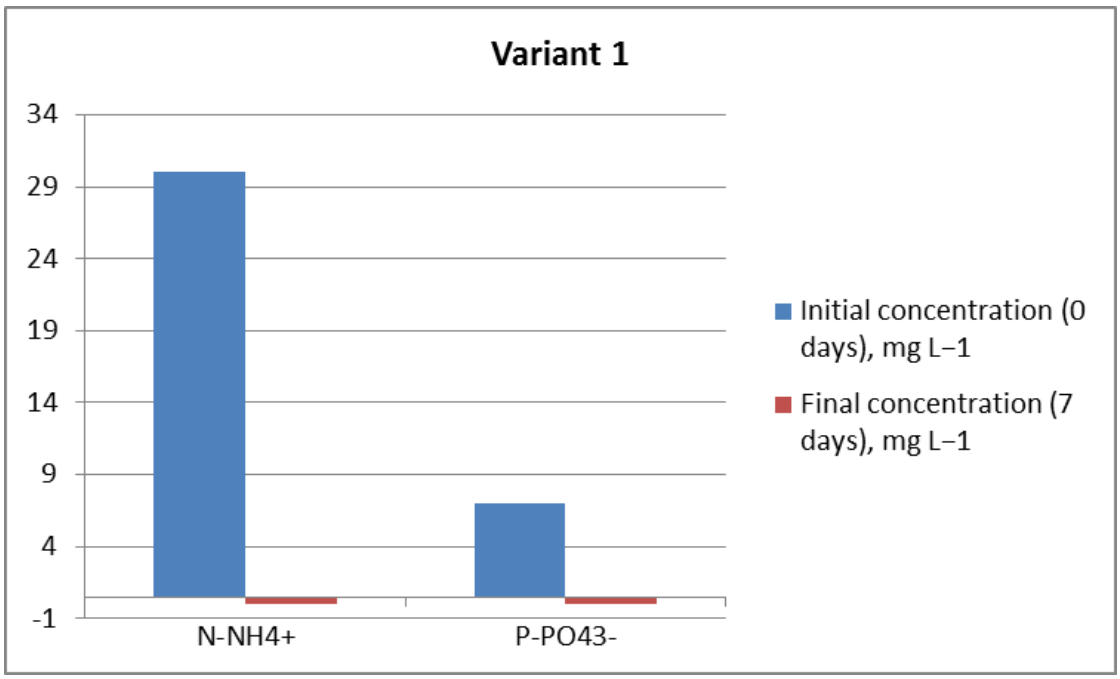


Fig.3.8. First measuremement with initial concentration 30 mg L^{-1} of $N\text{-}NH_4^+$ and 7 mg L^{-1} of $P\text{-}PO_4^{3-}$

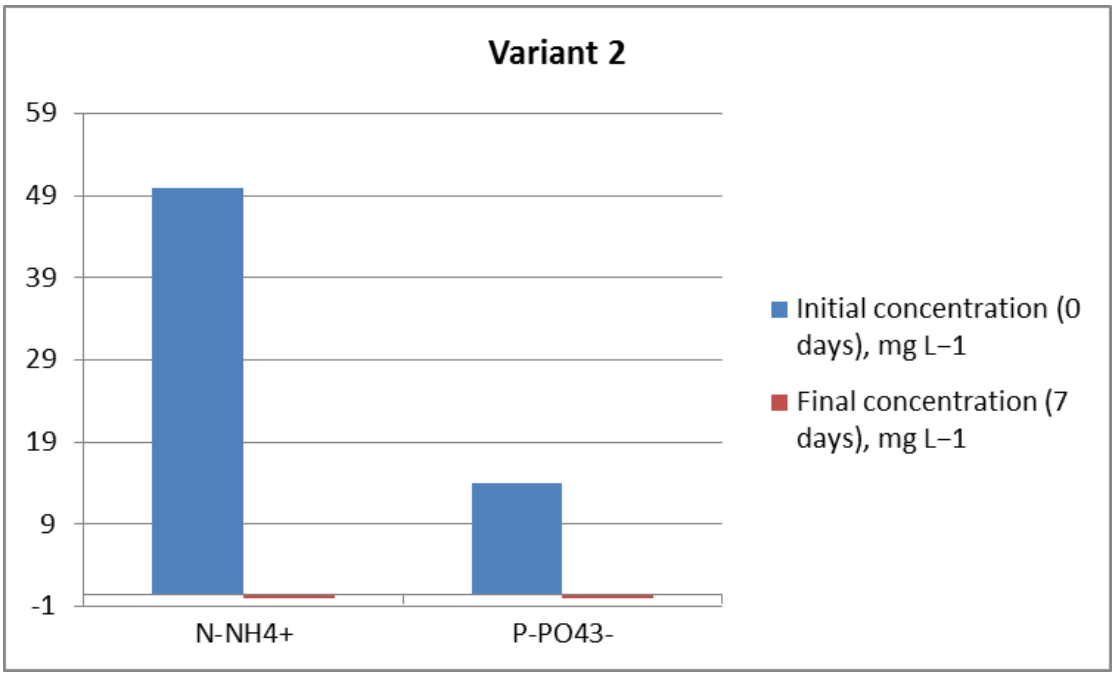


Fig.3.9. Second measuremement with initial concentration 50 mg L^{-1} of $N\text{-}NH_4^+$ and 14 mg L^{-1} of $P\text{-}PO_4^{3-}$

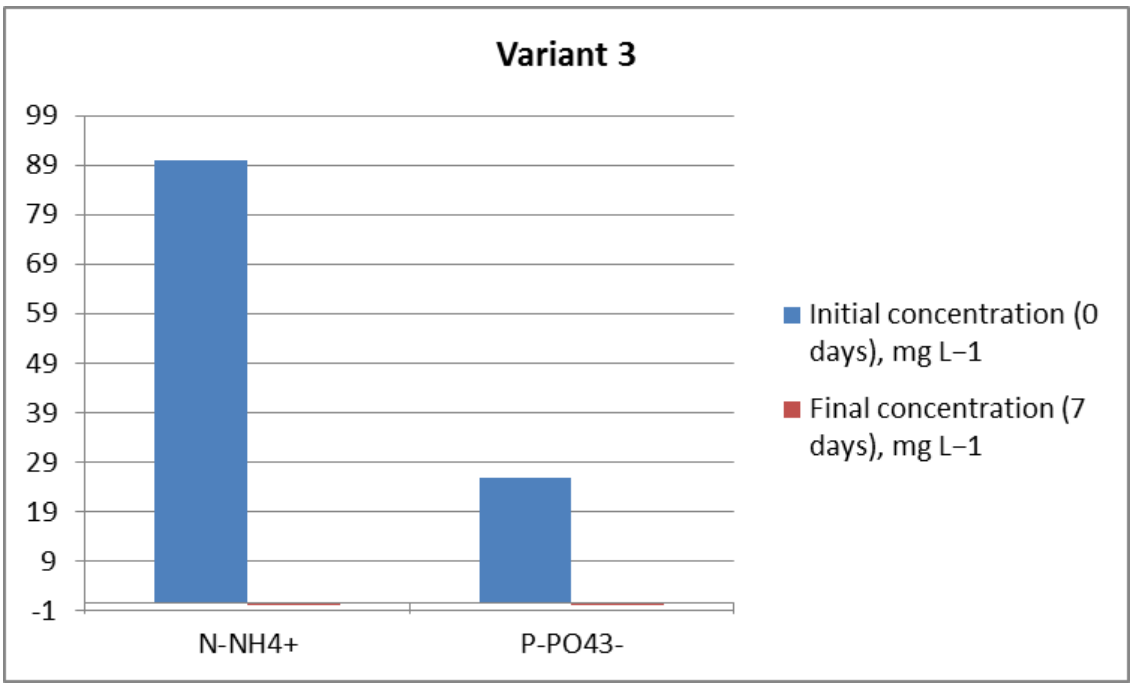


Fig.3.10. Third measuremement with initial concentration 90 mg L^{-1} of $N\text{-}NH_4^+$ and 26 mg L^{-1} of $P\text{-}PO_4^{3-}$

The growth intensity of *Euglena gracilis* was assessed by the accumulation of dry mass (Table 3.3). At concentrations of $N\text{-}NH_4^+$ 30 mg L − 1 and $P\text{-}PO_4^{3-}$ 7 mg L^{-1} the increase in biomass was 120 mg L^{-1} day^{-1}, at concentrations of ammonium nitrogen 50-90 mg L^{-1} and phosphorus phosphate 14-26 mg L^{-1} it was 151-156 mg $L^{-1}day^{-1}$, which indicates a positive dynamics of dry mass accumulation during the experiment. Mahapatra et al. (2013) obtained similar results on *Euglena sp.* During short-term cultivation of this alga in domestic wastewater, 98% removal of $N\text{-}NH_4^+$ (initial concentration 25 mg L^{-1}) and 85% removal $P\text{-}PO_4^{3-}$ (initial concentration 16 mg L^{-1}) was achieved, the biomass productivity index was 132 mg L^{-1} day^{-1}.

Table 3.3

Dry weight of Euglena gracilis (mg L^{-1}), M±m, n=3

Variant of the experiment	0 days	7 days
1	70.00±3.56	909.00±32.68
2	70.00±3.56	1127.00±51.25
3	70.00±3.56	1162.00±51.90

The photosynthetic activity of Euglena gracilis under the conditions of the experiment was evaluated by the change in the content of pigments - chlorophyll a and the sum of carotenoids (Table 3.4).

During the cultivation of Euglena gracilis in wastewater with a concentration of N-NH_4^+ and P-PO_4^{3-} 30 and 7 mg L^{-1}, respectively, the content of chlorophyll a in the biomass doubled. At the concentration of ammonium nitrogen 50-90 mg L^{-1} and phosphorus phosphate 14-26 mg L^{-1} at the end of the experiment, the content of basic photosynthetic was 2.5 times higher than the initial values. A similar trend was observed in the case of carotenoids, their content increased more than 2 times, indicating high photosynthetic activity of algae. However, it should be noted that the dry weight changed to a greater extent than the content of photosynthetic pigments. During seven days of cultivation, the dry weight increased 13-16 times. According to (Mahapatra et al. 2013), an important feature that allows euglenae algae to develop intensively in wastewater is the ability to mixotrophic type of food.

Table 3.4

Changes in the content of photosynthetic pigments in Euglena gracilis, M±m,n=3

Variant of the experiment	*Chlorophyll a, mg/g DW*		Carotenoids, *mg/g DW*	
	0 days	7 days	0 days	7 days
1	3.84±0.14	8.12±0.36	0.89±0.03	*2.15*±0.06
2	3.84±0.14	9.53±0.44	0.89±0.03	*2.47*±0.11
3	3.84±0.14	9.69±0.60	0.89±0.03	*2.53*±0.15

Biomass Euglena gracilis is considered a promising raw material for biofuel production, as it is characterized by a high content of lipids (Toyama et al. 2019). Cultivation of Euglena gracilis in domestic wastewater can provide not only an environmentally friendly way to extract nutrients from them, but also a cost-effective way to obtain biomass of microalgae for biodiesel production.

3.3. Conclusions to Chapter

- As a results of our experiments the ability of microalgae to reduce biogenic elements wastewater waters was confirmed.

The results of the study show that the content of nitrogen and phosphorus compounds significantly decreased, indicating the effectiveness of post-treatment of effluents using *Chlorella vulgaris* microalgae. Prospects of the microalgae use for wastewater treatment from nutrients are obvious. They are specially adapted to an environment dominated by viscous forces.

- The remain biomass can be used as raw material for biofertilizers production or raw material for biofuel production.

- According to the results of the studies, *Euglena gracilis* for 7 days almost completely removes ammonium nitrogen and phosphorus phosphates from the studied wastewater

- Cultivation of Euglena gracilis in domestic wastewater can provide not only an environmentally friendly way to extract nutrients from them, but also a cost-effective way to obtain biomass of microalgae for biodiesel production.

CHAPTER 4
ECONOMIC BENEFIT

4.1. Market analysis

The current commercial applications of microalgae mainly include direct human uses as food, cosmetic and nutraceuticals, in addition to speciality feeds for niche markets as aquaculture and pets [39]. However, there are several emerging markets on which microalgae also can contribute as on (i) the production of bio-fibres and commodities for the chemical industry, replacing that from fossil origin, (ii) the production of biofertilizers, (iii) the bioremediation of liquid and gaseous effluents, or (iv) including the production of biofuels [40].

The food-related markets are actually consuming most of microalgae biomass worldwide produced, up to 20.000 t/year, but including this production capacity is low for these medium size markets [41]. This is one of the raisons because the production of microalgae is growing up to 10% annually, to satisfy the large demand of microalgae based products, and the price of the biomass is not decreasing. In spite of this high demand, the actual market is dominated by small and medium size companies, close to the production step, most of them located in Asia and USA, in addition to Europe.

The biofertilizers market is at a prime focus in the agriculture industry for its multi-functional benefits and functional properties. Biofertilizers are used to improve the availability of nutrients for plants and enhance the plant uptake of phosphorus and zinc [41]. They also act as a physical barrier against pathogens and protect plants from pests, decompose organic residues, and stimulate the overall plant growth and development. Besides the expected increase in soil fertility, this technology would increase the amount of stabilized carbon stored (C-sequestration) in soils and thus contribute significantly to the reduction of greenhouse gas emissions.

Moreover, further contribution to reduce fuel usage in agriculture can be expected by the improved workability of soils (better water retention, less use of mineral fertilizers and pesticides, and reduced release of nitrous oxide).

Microalgae biomass contains valuable compounds that are ideally suited to be used as biostimulants, biopesticides, and to produce biofertilizers. Green microalgae like Chlorella are able to produce plantgrowth regulating substances, including auxins and cytokinins among others, which enhance the growth of higher plants; fast growing green microalgae belong to the best plant-growth enhancing strains.

Purification of wastewater from nutrients is a means of protecting the environment, that is, the need to use this technology is prescribed in the Environmental Law. But also, using the microalgae treatment technology, an enterprise can receive financial benefits from the sale of raw materials for the creation of biofertilizers and biofuels.

This technology can be applied in the brewery industry, food industry and in the municipal waste water treatment plant for the elimination of environmental adverse impact on the aquatic environment. Also, after technology implementation the remain microalgae biomass can be used by the customer or can be sold (as a material for biofertilizers of biofuel production) for the extra financial benefit.

Information about market validation:

What market validation have you done?

We conducted a study on Ukraine from publicly available sources and concluded that many enterprises do not have additional stages of purification from nutrients, and based on laws on Environmental Protection in Ukraine, we concluded that such a technology as ours is of great relevance due to tightening fines for exceeding indicators of substances that pollute water bodies. International market research is still under study.

What market are you operating in?

We are focused on enterprises that have an increased indicator of phosphate and nitrate elements in wastewater, often these are municipal wastewater treatment plants, a brewery and food industry enterprises.

What is the size of the market? Is it growing? Is the industry growing, stable or in decline?

In today's world, more and more are turning to environmental methods of wastewater treatment, and we can say the market for environmental protection is also growing. Preventive environmental practices are also valued for their cleanliness and their safety, which is why our product has a stable niche in the market.

What are the current trends of your target market?

With the tightening of fines for violation of laws on environmental protection, the need to use additional methods for wastewater treatment is obvious. Therefore, more and more enterprises are choosing to install additional environmental measures instead of paying fines.

Who are your competitors and how do you differentiate in the market?

Based on the Ukrainian market, our technology has no working analogue in biological treatment from biogenic elements, although research in this topic has rarely been traced since the 2000s, but they all stopped at the research stage. On the international market, we can see several types of working bioreactors for growing microalgae used for wastewater treatment, but we could not find obvious competitors with a stable business proposal. Our major difference from other bioreactors is the cascade type of reservoir which allow to locate it in the smaller territory and don`t lose the lightning capacity.

4.2. Revenue model

Let`s calculate the *economic benefit from the biomass utilization as biofertilizer production:*

The water consumption in Ukrainian breweries is 55 million cubic meters per year.

In the waste water of the brewery, the highest emissions of phosphorus compounds are 19 ml /l. That means 1045000 kg (1045 tons) of phosphorus is thrown annually.

Microalgae will hold 869 tons, which will reduce emissions by 6 times.

With 1 ton of phosphate - 2 tons of fertilizer. 1 ton of phosphate fertilizer costs 25000 UAH. UAH 43 450 000 - profit for the year.

A person consumes 200 liters / day of water for different needs [42].

In Ukraine, 42 million inhabitants, of which 69% - urban population [43] → 29 million people who use municipal wastewater treatment facilities.

Hence, in a day in Ukraine, the urban population uses 5.8 × 109 l. After clearing the water now it contains 4.64 × 1010 mg/l, and after clearing the algae will be 8.7 × 109 mg/l. That is, for a day in Ukraine, irreversibly lost 37, 7 tons of phosphates, causing irreparable damage to the environment, this year 13760 tons.

In phosphate fertilizers 50% phosphates, this means that from 13760 tons of phosphates we get 27520 tons of fertilizers.

1 ton of phosphate fertilizer costs 25000 UAH → 27520 t × 25000 UAH.

688,000,000 UAH - this is the profit that the state can receive by applying this technology to municipal wastewater treatment facilities.

The economic benefit from the biomass utilization as biofuel production:

The average annual productivity of the growth of microalgae by biomass, when cultivated in a photobioreactor in the weather conditions of Ukraine, can accept 11.5 kg / m^2 of the surface of the photobio-reactive zone (culture medium) [44, 45]. In this case, the average lipid productivity will be 4.1 kg / m^2.

Provided that the waste water is in the working area of the photobioreactor as a medium for an average of 3 days, the total volume of photobioreactor working areas for cleaning the effluent of the urban population of Ukraine will be:

$$5{,}8\times10^9 \times 3 = 17{,}4\times10^9 \text{ l}, \quad (4.1)$$

$$\text{or } 17{,}4\times10^6 \text{ m}^3.$$

If the thickness of the layer of effluents as a culture medium in the photobioractor zone does not exceed 0.2 m, then the total area of all photobioreactors should be:

$$\sum S_{bioreactor} = \frac{17{,}7\times10^6}{0{,}2} = 87\times10^6 m^2 \quad (4.2)$$

Thus, the annual growth of biomass of algae can be

$$M_{biomass} = 87 \times 10^6 \times 11{,}5 = 1000{,}5 \times 10^6 \text{ kg}. \quad (4.3)$$

Revenue model presented on the Table 4.1.

Table 4.1

Revenue model

Expenses		**Revenue**					**Break even point, years**
Title	**Total, EU**	**Title**	**Units**	**Amount**	**Price, EU**	**Total, EU**	
1	15 000	Biodiesel	l	109000	28	101400	
2	18300	Methane	m3	600000	8,98	27200	
3	172 700	Carbon dioxide	kg	310000	15	7600	
4	14 400	Phosphate fertilizers	kg	130000	25	21800	
5	7 500						
6	7 400						
7	4000						
8	3 300						
9	10700						
10	54 300						
Total expenses	307 600					158000	1,95

Where –

1. Design works (project preparation, complete set of technological equipment, author's supervision)
2. Preparatory works and landscaping (site preparation, temporary buildings and roads)
3. Photobioreactors (base plates, cultivation tanks, bubbling systems complete with compressors and pressure valves, documentation)
4. Centrifuge OGSh 321 K5

5. Installation for oil extraction

6. Installation for biodiesel production (site preparation, temporary buildings and roads, site improvement)

7. Technical and administrative buildings (pumping, buildings for electrical equipment, building for centrifuges and oil extraction plants, building for biodiesel production)

8. Equipment of technical premises (pumps, devices for mixing MKV with JI, pipes, fittings, measuring devices, lightning protection, internal heating)

9. Costs for obtaining permits, approvals (development of a detailed plan of the territory, change of purpose of the land plot, commissioning)

10. Costs of biodiesel production

Micro-algae wastewater treatment is a method that allows waste processing to be separated into clean liquid and solid fractions. The hard part contains a significant amount of pollutants, therefore it is disposed of, and in the case of microalgae (after purification), the latter can be used as biofuel (phosphate) and biofuels raw material.

4.3. Conclusions to Chapter

We conclude that:

- Microalgae teratment can be applied in the brewery industry, food industry and in the municipal waste water treatment plant for the elimination of environmental adverse impact on the aquatic environment.
- After technology implementation the remain microalgae biomass can be used by the customer or can be sold (as a material for biofertilizers of biofuel production) for the extra financial benefit.
- Break-even point of the remain biomass usage equal less than 2 years.

CHAPTER 5
LABOR PRECAUTION

5.1. Analysis of harmful and dangerous production factors

According to the theme of my diploma project “Research of biogenic compounds removal from sewage water by microalgae”, the microalgae growth was observed in laboratory conditions. We need to pay attention to requirements for the area of the workplace, safe usage of equipment and reagents.

In the process of performing work, the laboratory tester may be affected by the following hazardous and harmful production factors according to standard ГОСТ 12.0.003-74 “Occupational safety standards system. Dangerous and harmful production effects. Classification”:

1. Factors which belong to physical harmful factor

- increased or decreased temperature of surfaces of equipment, materials;
- sharp edges, burrs and roughness on the surfaces of workpieces, tools and equipment;
- increased noise level;
- insufficient illumination of the working area.

2. Factors which belong to chemical harmful factor

by the nature of the impact on the human body on:

- toxic;
- sensitizing;
- skin and mucous membranes.

3. Factors which belong to psychophysiological dangerous and harmful production factor

- physical overload;
- neuropsychiatric overload.

5.2. Measures to reduce the impact of harmful and dangerous production factors

Proper ventilation of laboratory settings is required to promote and maintain laboratory safety and protection to life and property. Items such as fume containment, worker safety, proper cleanliness through pressure relationships, filtration, air changes per hour (ACH), point of fume capture, temperature, and relative humidity requirements are elements necessary to design the ventilation system depending on the laboratory type [46]. Codes identify ventilation measures to provide minimum requirements for the protection of life and property through prevention and control of fumes and containment of hazardous fumes and contaminants for worker safety.

Calculation of the ventilation system for laboratory in which the experiments were occurred done by the regular scheme of ventilation choice [47].

The calculation of the ventilation system begins with determining the air capacity (air exchange), measured in cubic meters per hour. For calculations, we need a plan of the facility, where the names (purposes) and areas of all premises are indicated.

After calculating the air exchange for people, we need to calculate the air exchange rate (this parameter shows how many times a complete air change occurs in the room during one hour). To prevent the air from stagnating in the room, at least one air exchange must be provided.

Thus, in order to determine the required air flow rate, we need to calculate two values of air exchange: by the number of people and by frequency, and then select the larger of these two values:

1. Calculation of air exchange by the number of people:

$$L = N * L_{norm}, \text{ where} \quad (5.1)$$

L - required capacity of supply ventilation, m^3 / h;

N is the number of people;

Lnorm is the rate of air consumption per person: typical value - 60 m^3 / h;

2. Calculation of air exchange rate:

$$L = n * S * H, \quad (5.2)$$

where L is the required capacity of the supply ventilation, m^3 / h;

n - standardized rate of air exchange:

for residential premises - from 1 to 2, for offices - from 2 to 3;

S is the area of the room, m^2;

H - room height, m;

Having calculated the required air exchange for each serviced room, and adding the obtained values, we find out the overall performance of the ventilation system.

Air exchange (by number of people) for laboratory in which 3 people working simultaneously: L= 3*60=180 m^3 / h.

Air exchange (by rate) L= 3*21*3= 189 m^3 / h.

To calculate the dimensions (cross-sectional area) of the ducts, we need to know the volume of air passing through the duct per unit of time, as well as the maximum permissible air velocity in the duct. As the air speed increases, the dimensions of the ducts decrease, but the noise level and network resistance increase. In practice, for apartments and cottages, the air speed in the ducts is limited to 3-4 m / s, since at higher air speeds the noise from its movement in the ducts and distributors may become too noticeable.

It should also be borne in mind that it is not always possible to use "quiet" low-speed air ducts with a large cross-section, since they are difficult to place in the ceiling space. Reducing the height of the ceiling space allows the use of rectangular air ducts, which, with the same cross-sectional area, have a lower height than round ones (for example, a round air duct with a diameter of 160 mm has the same cross-sectional area as a rectangular air duct measuring 200 × 100 mm). At the same time, it is easier and faster to install a network of round flexible ducts.

So, the estimated cross-sectional area of the duct is determined by the formula:

$$Sc = L * 2.778 / V, \quad (5.3)$$

where Sc - calculated cross-sectional area of the duct, cm^2;

L - air flow through the duct, m^3 / h;

V is the air velocity in the duct, m / s;

2.778 is a coefficient for reconciling different dimensions (hours and seconds, meters and centimeters).

We get the final result in square centimeters, since in such units it is more convenient for perception.

The actual cross-sectional area of the duct is determined by the formula:

$$S = \pi * D^2 / 400 \text{ - for round ducts}, \quad (5.4)$$

$$S = A * B / 100 \text{ - for rectangular ducts}, \quad (5.5)$$

where S is the actual cross-sectional area of the duct, cm^2;

D is the diameter of the round duct, mm;

A and B - width and height of a rectangular duct, mm.

The table 5.1 shows data on air consumption in round and rectangular air ducts at different air speeds.

Table 5.1

Air consumption in air ducts

Duct parameters			**Air consumption (m^3 / h) at air speed:**				
Round duct diameter	**Dimensions of rectangular duct**	**Duct cross-sectional area**	**2 м/с**	**3 м/с**	**4 м/с**	**5 м/с**	**6 м/с**
	80×90 мм	72 см²	52	78	104	130	156
Ø 100 мм	63×125 мм	79 см²	57	85	113	142	170
	63×140 мм	88 см²	63	95	127	159	190
Ø 110 мм	90×100 мм	90 см²	65	97	130	162	194
	80×140 мм	112 см²	81	121	161	202	242

The *estimated cross-sectional area of the duct* for laboratory:

Sc= (189* 2.778)/ 4= 131,3 cm²

The *actual cross-sectional area of the duct* for laboratory:

S = (3,14 * 110²) / 400= 95 cm²

The calculation of the dimensions of the air duct is carried out separately for each branch, starting from the main channel to which the ventilation unit is connected. Note that the air velocity at its outlet can reach 6–8 m / s, since the dimensions of the connecting flange of the ventilation unit are limited by the size of its housing (the noise that occurs inside it is damped by a silencer). To reduce the air velocity and reduce the noise level, the dimensions of the main air duct are often chosen larger than the dimensions of the ventilation unit flange. In this case, the connection of the main air duct to the ventilation unit is made through an adapter.

Knowing the air flow rate, it is possible to select air distributors from the catalog, taking into account the ratio of their sizes and noise level (the cross-sectional area of the air distributor, as a rule, is 1.5–2 times larger than the cross-sectional area of the air duct).

After determining the ventilation capacity, we can calculate the required power of the air heater. To do this, we need the values of the air temperature at the outlet of the

system and the minimum outside temperature during the cold season. The temperature of the air entering the dwelling must not be lower than + 18 ° C. The minimum outside temperature depends on the climatic zone and for Kyiv is taken to be -26 ° C. Thus, when the heater is turned on at full power, it must heat the air flow by 44 ° C. Since severe frosts in Kyiv are short-lived, you can use a lower power heater, provided that the ventilation system has capacity control: this will allow maintaining a comfortable air temperature during the cold period by reducing the fan speed.

The power of the heater is calculated by the formula:

$$P = \Delta T * L * Cv / 1000, \quad (5.6)$$

where P - heater power, kW;

ΔT is the difference in air temperatures at the outlet and inlet of the heater, ° C.

For Kyiv, ΔT = 44 ° C;

L - ventilation capacity, m³ / h.

Cv - volumetric heat capacity of air, equal to 0.336 W · h / m³ / ° C. This parameter depends on pressure, humidity and air temperature, but we neglect this in the calculations.

The *power of the heater* for laboratory:

P= (44*189*0,336)/1000= 2,8 kW

After calculating the power of the heater, you need to select the supply voltage (for an electric heater): 220V / 1 phase or 380V / 3 phases. If the heater power is over 4–5 kW, it is advisable to use a 3-phase connection. The maximum current consumed by the heater can be calculated using the formula:

$$I = P / U, \quad (5.7)$$

where I is the maximum consumed current, A;

P - heater power, W;

U - supply voltage:

220V - for single-phase power supply;

660V (3 × 220V) - for three-phase power supply (when heaters are connected with a "star" between 0 and phase).

The *maximum current consumed by the heater* for laboratory:

I= 2800/660 = 4,2 A

Table 5.2

Parameters of ventilation system for laboratory

Parameter	**Result**
Air exchange (by number of people)	180 m^3 / h.
Air exchange (by rate)	189 m^3 / h.
The estimated cross-sectional area of the duct	131,3 cm^2
The actual cross-sectional area of the duct	95 cm^2
The power of the heater	2,8 kW
The maximum current consumed by the heater	4,2 A

5.3. Occupational Safety Instruction

5.3.1. General safety requirements

Requirements for the working area. Each laboratory (or suite of combined laboratories) should have its own Laboratory Safety Manual or set of manuals. Contents should include:

- Standard Operating Procedures for commonly shared equipment
- Standard Risk Assessments for commonly performed tasks
- Register of Equipment and Chemical and Biological Agents within the laboratory
- Material Safety Data Sheets for Chemical and Biological agents within the laboratory
- Working Rules appropriate to the particular laboratory including
- Statutory Obligations such as for the Office of the Gene Regulator (OGTR)/radioactive/dangerous goods/ prescribed Chemical and Biological Agents/Australian Quarantine and Inspection Service (AQIS) etc compliance

- Emergency Procedures for fire/smoke, personal injuries/spills
- Transport Requirements for materials being brought into or taken out of the laboratory
- Waste Management and Disposal procedures.

Each person within the laboratory needs to sign (and date) that they have read, understood and will abide by this manual before being permitted to commence work. This should also be countersigned (and dated) by the Laboratory Manager or Supervisor.

Safety instructions for the spectrophotometer

Before unpacking the device, adjusting it and putting it into operation, read the complete operating instructions supplied with it. Observe all safety instructions and warnings. Failure to do so can result in serious injury to the operator or damage to the instrument.

To ensure that the protection provided by this product is not impaired, do not use or install this product in any manner other than that specified in manual.

Warning notices

Read all tags and labels on the instrument. Failure to do so creates a risk of injury or damage to the device. The symbols on the device correspond to the warnings in manual.

Indicates a possible or imminently hazardous situation which, if not avoided, will result in death or serious injury.

- Indicates a possible or imminently hazardous situation which, if not avoided, could result in death or serious injury.
- Indicates a potentially hazardous situation that could result in minor or moderate injury.

Chemical and biological safety

Normal use of this device may require the use of hazardous reagents or bio-toxic samples.

- Before handling such substances, read all warnings and safety notes that appear on the original solution packaging and on the MSDS.

- Dispose of all used solutions in accordance with national regulations and laws.
- Select the type of protective equipment according to the concentration and quantity of hazardous materials used.

Potential hazard in contact with chemical / biological materials. Handling chemical samples, standards and reagents can be hazardous.

Familiarize yourself with the relevant safety procedures and reagent handling before starting work, read all relevant safety data sheets and follow the instructions.

5.3.2. Safety Requirements before starting work

- Know locations of laboratory safety showers, eyewashstations, and fire extinguishers. The safety equipment may be located in the hallway near the laboratory entrance.
- Know emergency exit routes.
- No horseplay will be tolerated.
- Post warning signs when unusual hazards, hazardous materials, hazardous equipment, or other special conditions are present.
- All laboratory personnel should place emphasis on safety and chemical hygiene at all times.
- All containersmust have appropriate labels. Unlabeled chemicals should never be used.
- Long hair and loose clothing must be pulled back and secured from entanglement or potential capture.
- Laboratory safety glasses or goggles should be worn in any area where chemicals are used or stored. They should also be worn any time there is a chance of splashes or particulates to enter the eye. Closed toe shoes will be worn at all times in the laboratory. Perforated shoes or sandals are not appropriate.
- Determine the potential hazards and appropriate safety precautions before beginning any work.

- Computers and instrumentation should be labeled to indicate whether gloves should be worn or not. Inconsistent glove use around keyboards/keypads is a source of potential contamination.
- Avoid wearing jewelry in the lab as this can pose multiple safety hazards.

5.3.3. Safety Requirements during operation

- Avoid skin and eye contact with all chemicals.
- Minimize all chemical exposures.
- Assume that all chemicals of unknown toxicity are highly toxic.
- Avoid distracting or startling persons working in the laboratory.
- Use equipment only for its designated purpose.
- Combine reagents in their appropriate order, such as adding acid to water.
- Avoid adding solids to hot liquids.
- Never leave containersof chemicals open.
- Do not taste or intentionally sniff chemicals
- Never consume and/or store food or beverages or apply cosmetics in areas where hazardous chemicalsare used or stored.
- Do not use mouth suction for pipetting or starting a siphon.
- Wash exposed areas of the skin prior to leaving the laboratory.
- No contact lenses should be worn around hazardous chemicals – even when wearing safety glasses.
- Procedures should be developed that minimize the formation and dispersion of aerosols.
- If an unknown chemical is produced in the laboratory, the material should be considered hazardous.
- Keep all sink traps (including cup sink traps and floor drains) filled with water by running water down the drain at least monthly.
- Perform work with hazardous chemicals in a properly working fume hoodto reduce potential exposures.

- Avoid working alone in a building. Do not work alone in a laboratory if the procedures being conducted are hazardous.
- The PEL and the Threshold Limit Values (TLV) will be observed in all areas. If exposure above a PEL/TLV is suspected for an ongoing process, please contact EHS immediately.
- Laboratory employees should have access to a chemical inventory list, applicable SDSs, Department Laboratory Safety Manual, and relevant SOPs.
- Access to laboratories and support areas such as stockrooms, specialized laboratories, etc. should be limited to approved personnel only.
- Equipment should be maintained according to the manufacturer's requirements and records of certification, maintenance, or repairs should be maintained for the life of the equipment.
- No cell phone or ear phone usage in the active portion of the laboratories, or during experimental operations.
- Clothing made of synthetic fibers should not be worn while working with flammable liquids or when a fire hazard is present as these materials tend to melt and stick to exposed skin.

5.3.4. Safety Requirements after work

- Do not pour chemicals down drains. Do NOT utilize the sewer for chemical waste disposal.
- Do not utilize fume hoods for evaporations and disposal of volatile solvents.
- All equipment should be regularly inspected for wear or deterioration.
- Designated and well-marked waste storage locations are necessary.
- Laboratory coats should not be stored in offices or break rooms as this spreads contaminates to other areas.
- Turn off all equipment, clean the working area.

5.3.5. Safety Requirements at emergency situations

It is the responsibility of all supervisors, lecturers and demonstrators to ensure that persons working in a laboratory know the location of:

- the nearest fire extinguishers/fire blankets
- fire / emergency escape routes
- first aid box
- emergency shower/eye wash facilities
- isolation devices for gas, water and power (where fitted)
- emergency spill containment equipment and procedures
- emergency personal protective equipment
- any special substances that require antidotes.

Wash skin immediately with plenty of water if contaminated with acids and alkalis (if required seek medical attention).

Eyes splashed with any chemical must be washed with water for 15 mins and medical advice obtained immediately.

All breakages and spills must be reported to the supervisor and dealt with immediately. Spills should be cleaned up and bins provided for broken glass and spill clean up materials etc.

When a fire source appears, you must:

- stop working;

- turn off electrical equipment;

- organize the evacuation of people;

- start extinguishing the fire immediately.

When electrical equipment catches fire, use only carbon dioxide or dry powder fire extinguishers.

If it is impossible to carry out extinguishing on his own, the design engineer should, in accordance with the established procedure, call the fire brigade and inform the immediate supervisor about it.

In the event of injury or deterioration of health, the design engineer must stop work, notify the management and seek medical help.

5.4. Conclusions to Chapter

- According to the fact that the experiment sessions were conducted in laboratory conditions the working place was used under the rules of Laboratory manual.
- Equipment was prepared and controlled according to their manual.
- Ventilation system was calculated for the given laboratory in National Aviation University where experiment sessions were conducted.

CONCLUSIONS

As a result of research scientific and applied task was solved to minimize negative impact on the environment.

1. In this scientific work was analyzed the various types of sewage waters composition. Wastewater is containing significant concentrations of solids, dissolved and particulate matter, microorganisms, nutrients, heavy metals and micro-pollutants. The content and volume of pollutants in waste water differ from the type of production in which waste water produced. The most significant parameters which observed for waste water are BOD (biological oxygen demand), COD (chemical oxygen demand), pH of water, total nitrogen (T-N), total phosphorus (T-P), total suspended solids (TSS).

In food industry the content of biogenic elements (phosphorous and nitrogen compounds) which influence on aquatic flora and fauna are the highest.

2. However, were studied perspectives of biological treatment. Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals.

3. The Chlorella vulgaris and Euglena gracilis potential to remove biogenic elements were investigated.

For investigation the microalgae potential we made certain experimental sessions which include the hypothesis approval and observation of bioreactor work. Two experimental sessions were prepared in laboratory in National Aviation University, and one session in Institute of Hydrobiology NAN of Ukraine. The cultivation of Chlorella vulgaris in the laboratory of NAU and the cultivation of Euglena gracilis in Institute of Hydrobiology NAN of Ukraine. First experiment (two experimental sessions) done in summer 2019. Second in autumn 2020.

In experiment with Chlorella vulgaris used 1) wastewater from the apartment building before entering the treatment plant was taken at 10.00 in the morning in Kyiv; 2) waste water from a utility company from Novograd-Volynsky before treatment was taken

at 8.30 in the morning; 3) waste water from a communal enterprise from Novograd-Volynsky after treatment was taken at 8.30 in the morning. In experiment with Euglena gracilis used synthetic domestic wastewater, which was prepared by adding to the culture medium different concentrations of ammonium nitrogen (N-NH4 +) and phosphorus phosphates (P-PO43-)

In experiment with Chlorella vulgari the concentration of ammonium nitrogen and phosphorus phosphates in wastewater was investigated by the DR 3900 spectrophotometer. In experiment with Euglena gracilis the concentration of ammonium nitrogen and phosphorus phosphates in wastewater was investigated by conventional methods in hydrochemistry and the statistical processing of the results was performed using IBM SPSS Statistics Base v.2.

As a results of our experiments the ability of microalgae to reduce biogenic elements wastewater waters was confirmed.

The results of the study show that the content of nitrogen and phosphorus compounds significantly decreased, indicating the effectiveness of post-treatment of effluents using *Chlorella vulgaris* microalgae. Prospects of the microalgae use for wastewater treatment from nutrients are obvious. They are specially adapted to an environment dominated by viscous forces. The remain biomass can be used as raw material for biofertilizers production or raw material for biofuel production.

According to the results of the studies, *Euglena gracilis* for 7 days almost completely removes ammonium nitrogen and phosphorus phosphates from the studied wastewater. Cultivation of Euglena gracilis in domestic wastewater can provide not only an environmentally friendly way to extract nutrients from them, but also a cost-effective way to obtain biomass of microalgae for biodiesel production.

4. The economic benefits from microalgae application was estimated.

Microalgae treatment can be applied in the brewery industry, food industry and in the municipal waste water treatment plant for the elimination of environmental adverse impact on the aquatic environment.

After technology implementation remain microalgae biomass can be used by the customer or can be sold (as a material for biofertilizers of biofuel production) for the extra financial benefit. Break-even point of the remain biomass usage equal less than 2 years.

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