BIOREMEDIATION TREATMENT OF WASTEWATER FROM OIL AND GAS PRODUCTION ENTERPRISES

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Abstract. *This research work highlights the importance of using higher aquatic plants such as Lemna minor, Azolla caroliniana, and Eichhornia crassipes in the bioremediation of wastewater generated by oil and gas production enterprises. The study assesses their ecological, toxicological, and biotechnological potential and explores the implementation of co-cultivation biotechnology for the biological treatment of industrial and domestic wastewater.The study found that domestic wastewater (mg/l: phosphates—5.66; chlorides—651.74; sulfates—664.33; nitrates—531.6; nitrites—16.36) and industrial wastewater (mg/l: phosphates—8.34; chlorides—577.94; sulfates—641.62; nitrates—664.01; nitrites—40.06) from oil and gas production enterprises were heavily contaminated with primary biogenic elements. Additionally, domestic wastewater contained iron at 0.38 mg/l, while industrial wastewater had iron levels of 0.96 mg/l.*

The research demonstrated that macrophytes significantly reduce the levels of salts and biogenic elements in industrial and domestic wastewater by assimilating these substances as nutrients during their growth. Notably, co-cultured E. crassipes and L. minor, which adapt well to the wastewater composition and reproduce synchronously, were found to absorb 79.42% of iron, 57.15% of chromium, and 66.67% of Cr in domestic wastewater. The practical outcomes of the study revealed that, compared to the traditionally used E. crassipes macrophyte in biological wastewater treatment, the co-cultivation of E. crassipes and L. minor in wastewater significantly enhances the removal of biogenic elements and heavy metals. The bioremediation purification biotechnology based on this co-cultivation method has been successfully implemented, moderating the chemical composition of industrial and domestic wastewater discharged from the enterprise to meet established regulatory standards.

Keywords: *Macrophyte, E. crassipes, L. minor, bioremediation method, biogenic element, oil and gas.*

Introduction. Scientific research is being conducted to prevent serious environmental damage caused by incomplete purification of toxic chemical compounds, oil and petroleum products, and various organic substances present in industrial and domestic wastewater discharged by global oil and gas extraction enterprises. In this regard, particular attention is being paid to improving the purification processes of biogenic elements, heavy metals, salts, oil, and petroleum products in industrial and domestic wastewater, implementing advanced biotechnological methods for biological treatment, and especially the effective use of higher aquatic plants (macrophytes).

Due to the rapid development of global industrial production and the increasing water demands of technological production processes (such as oil and gas extraction, oil refining, mining of heavy and rare metals, heavy and light industries, food and feed production, pharmaceuticals,

and cosmetics), as well as agricultural crop cultivation and livestock farming (including animal husbandry, poultry farming, fish farming, etc.), the demand for clean water is growing daily.

One of the global ecological challenges is the increasing scale of environmental pollution through wastewater. According to data from UNESCO's World Water Assessment Programme (established in 2002, report 2017), approximately 80% of wastewater worldwide is discharged into the environment without adequate treatment [1; pp. 1-2]. However, recent systematic studies have found these figures to be insufficiently substantiated, indicating that about 50% of globally generated wastewater is discharged completely untreated into the environment [2; pp. 237-254].

Due to the global expansion of urban areas and the sharp increase in municipal wastewater volumes, current projections indicate that by 2030, urban wastewater generation will reach 470 billion m^3 (a 24% increase), and is expected to grow to 574 billion m^3 by 2050, representing a 51% surge compared to current levels [3; pp. 40-51]. Globally, obtaining scientifically validated and systematically organized statistical data on the total volume of wastewater generated and the proportion that undergoes adequate treatment according to established standards remains a significant challenge [4; pp. 1-13]. The World Business Council for Sustainable Development identifies the primary reason for this as the inherent complexity and high cost associated with global monitoring of wastewater generation and its treatment compliance [5; p. 32].

Consequently, UNESCO's World Water Assessment Programme has identified as its primary objective the achievement of treating at least 50% of annually generated wastewater worldwide by 2030 [6; pp. 501-513].

Despite these efforts, the growing global population, urban expansion, and rapid development across nearly all economic sectors have made it impossible to achieve comprehensive wastewater treatment and full-scale reuse worldwide. Consequently, numerous scientific-practical and innovative projects are being implemented to address emerging challenges related to: the negative impacts of wastewater generation, systematic wastewater treatment technologies, and the widespread practical application of advanced technologies for effective reuse of treated wastewater [7; p. 515, 8; pp. 1-10, 9; p. 131, 10; pp. 792-800].

Scientific sources and statistical data indicate that industrial wastewater discharged from production facilities contains high concentrations of various toxic chemical compounds, petroleum products, and diverse organic substances. These effluents not only harm the environment but also directly and severely impact human health [11; pp. 488-896]. The release of industrial wastewater contaminated with various chemical compounds into the environment without adequate treatment causes a process of eutrophication in nature. As a result, it has a strong negative impact on the ecosystem of zooplankton and phytoplankton in water bodies, and as a result of the excessive increase in biogenic elements in the water, it leads to the growth of various pathogenic microorganisms (bacteria: E.coli O157:H7, Legionella pneumonia, Helicobacter pylori, Vibrio cholera, Campylobacter, Yersinia, Salmonella, Cyanobacter, Leptosporosis), viruses: Hepatitis viruses, Adenoviruses, Enchoviruses), parasites: Giardia lamblia, Cryptosporidium, Acanthamoeba) [12; 2379-2387-b] and algae (Cyanophyceae, Chlorophyceae, Bacillariophyceae, Chrysophyceae-Heterokontophyta) [14; 123-129-b, 15; 363-381-b].

Furthermore, this situation—specifically, the contamination of water with excessive nitrogen sources-leads to changes in the concentrations of ammonia (NH_4^+) and nitrate (NO_3^-) as well as their relative ratios [18; pp. 6363–6369]. This, in turn, adversely affects the survival of zooplankton and phytoplankton populations in the water [19; pp. 18–26]. Excess nitrogen (N_2) inputs entering rivers or riverbeds via wastewater can be identified by monitoring specific

physiological indicators in aquatic plants. These include measuring either:Reductase enzyme activity in plants, or Concentrations of nutrient elements (biogenic substances) [20; pp. 655-671, 21; pp. 64-70].

Scientific literature extensively documents that the efficacy of macrophytes in purifying diverse contaminants from wastewater is dependent on multiple factors and biochemical processes [22; pp. 754-781].

Typically, plants absorb nitrogen for growth and development and actively use it during the assimilation process, and nitrogen also has a significant impact on the productivity and species composition of ecosystems [29, pp. 1-21].

Aquatic plants absorb and utilize nitrogen primarily in three forms—nitrate ions (NO_3^-), urea, and ammonium ions (NH_4^+)—through processes of absorption, assimilation, and translocation. This metabolic activity enhances nitrate reductase enzyme activity in plant tissues, facilitating nitrogen conversion.

Key Microalgae Groups in Nitrogen Removal from Wastewater:

1. Cyanophyceae (Blue-Green Algae/Cyanobacteria):

Notable genera:

- Unicellular: Aphanothece, Gloeocapsa, Synechococcus
- Filamentous: Anabaena, Nostoc, Oscillatoria,
- Nitrogen-fixers: Cylindrospermum, Nodularia

2. Chlorophyceae (Green Algae):

- Prasinophytes, Trebouxiophyceae (e.g., Chlorella),
- Ulvophyceae (e.g., Ulva), Charophytes (e.g., Chara),

3. Bacillariophyceae (Diatoms):

- Centric: Thalassiosira, Skeletonema
- Pennate: Navicula, Nitzschia

4. Chrysophyceae (Golden-Brown Algae/Heterokontophyta):

- Dinobryon, Mallomonas, Synura

Scientific Significance:

These microalgae play critical roles in:

- Nitrogen assimilation via enzymatic pathways,
- Biofilm formation that enhances nutrient uptake,
- Symbiotic relationships with nitrogen-fixing bacteria [14, pp. 123–129].

The use of Cyanophyceae (Cyanobacteria/blue-green algae) for monitoring and regulating chemical pollutants in wastewater is widely practiced [15, pp. 363–381; 32, pp. 2–17]. Modern molecular genetic studies have reclassified these organisms, which are now also referred to as cyanoprokaryotes [18, pp. 43–64].

Extensive research confirms that effluents from numerous manufacturing facilities are heavily saturated with organic and inorganic pollutants that pose severe risks to human health [19, pp. 275–286]. Ruiz et al. conducted comprehensive studies identifying primary pollutants in industrial wastewater:

Nutrients: Nitrogen (N) and phosphorus (P) compounds, Hydrocarbons: Petroleum derivatives and organic solvents,Toxic Metals: Cadmium (Cd), lead (Pb), mercury (Hg), Pathogens: Fecal coliforms, E. coli, Salmonella, Critical Findings: Ammonia (NH_3) exhibits extreme toxicity, even at low concentrations. Nitrate (NO_3^-) contamination in drinking water causes acute poisoning (e.g., methemoglobinemia). The microalga Chlorella vulgaris demonstrates

high efficacy in biological treatment, removing: 85–92% of NH_3/NH_4^+, 78% of NO_3^-, 60–70% of heavy metals via biosorption [20, pp. 884–896].

Scientific sources indicate that livestock farming is considered one of the sectors emitting a very large amount of carbonate anhydride into the environment. In addition, a very large amount of urea is also released into the environment from the livestock sector. It has been proven that small duckweed can be effectively used to treat wastewater heavily contaminated with urea, which is generated in large quantities in the livestock sector [21, pp. 1-14.]. Jones and his colleagues noted that the treatment of industrial wastewater using small jets is progressing day by day, but the presence of high ammonia concentrations in the water, and the addition of additional water to dilute the strong ammonia concentration in this water, hinder the widespread development of this technology [22, pp. 1-8-].

The presence of two forms of ammonia in wastewater from industrial enterprises, namely ammonium ion (NH^{4+}) and unionized free ammonia (NN^{3-}), results in a high pH of wastewater. Reducing this environmental parameter using macrophytes (*L. gibba*) and purifying high concentrations of ammonia in wastewater provides an opportunity to use water for technical purposes [23, pp. 71-78].

Method: The Importance of Macrophytes in Biological Wastewater Treatment

Typically, in the biological treatment of wastewater contaminated with various chemical components, the initial process is followed by biological treatment (after aeration tanks and activated sludge) based on high-flow algae. Russian scientists have proven that using the same method, ammonia nitrogen can be removed up to 99.9% and phosphates up to 80% [24, pp. 20]. Ukrainian scientists have created a technology for year-round cultivation of the macrophyte *L. minor*, and it has been shown that adjusting light and temperature regimes for year-round use is of great importance in the purification of nitrogen and phosphorus compounds, the main biogenic elements, from wastewater [25, pp. 79-84].

Chinese scientists studied the year-round cultivation of macrophytes and their seasonal chemical composition and the effect of seasonal characteristics of their chemical composition and, accordingly, the absorption of various biogenic elements, and found that the absorption rate of biogenic elements such as NH_4^+–N, NO_3—N, NO_2—N has a seasonal nature [26, pp. 82-91].

Russian scientists have found that wastewater heavily contaminated with phosphates contained 9.9 mg/l at the time of entering the biological treatment plant, but increased to 14.32 mg/l at the time of leaving the treatment plant, and also noted that wastewater taken from the inlet to the aeration tank contained 10.8 mg/l of phosphates, but increased to 12.92 mg/l at the time of leaving the aeration tank [26, pp. 311-314]. They also noted that the ability of the small duckweed to treat wastewater containing phenol depends on the amount of its biomass in the wastewater and its greater contact with water [28, pp. 58-65].

Results: During the study, domestic wastewater samples with consistent pollutant composition were collected from the industrial outlet. Cultivation materials were applied at a rate of 200 g per m². Results demonstrated varying pollutant uptake capacities among macrophyte species, with all three cultures showing high phosphate removal efficiency. Specifically:

Eichhornia crassipes (Water Hyacinth) achieved:

- 37.85% phosphate assimilation on Day 2
- 51.08% by Day 7
- 7.38% by Day 12

Teble 2

The study evaluated the average assimilation efficiency of aquatic plants (*E. crassipes, Azolla* spp., *L. minor*) in treating domestic wastewater contaminants over a two-year period (2022–2023).

№	Oqova suv tarkibidagi moddalar	Dastlabki miqdor, mg/l	Kulturalar va o'stirish kunlari								
			Eyxorniya			Azolla			Kichik ryaska		
			2-kun	7-kun	12-kun	2-kun	7-kun	12-kun	2-kun	7-kun	12-kun
1	Fosfatlar	6,50	2,46±0,11	3,32±0,23	0,48±0,17	1,33±0,08	2,21±0,18	1,08±0,41	1,73±0,26	3,21±0,18	0,82±0,04
		O'zlashtirish, %	37,85±0,05	51,08±0,42	7,38±0,33	20,46±0,11	34,00±0,31	16,62±0,03	26,62±0,14	49,38±0,31	12,62±0,15
		Jami o'zlashtirish, %			96,31			71,08			88,62
2	Xloridlar	651,0	175,12±0,21	262,16±0,18	108,14±0,42	116,42±0,03	208,34±0,08	153,13±0,02	168,33±0,42	226,13±0,11	138,23±0,14
		O'zlashtirish, %	26,90±0,11	40,27±0,03	16,61±0,56	17,88±0,17	32,00±0,12	23,52±0,23	25,86±0,14	34,74±0,27	21,23±0,03
		Jami o'zlashtirish, %			83,78±0,23			73,41±0,17			81,83±0,14
3	Sulfatlar	664	172,12±0,13	231,24±0,43	127,36±0,18	158,24±0,13	166,44±0,32	108,12±0,12	182,12±0,24	213,03±0,22	117,89±0,11
		O'zlashtirish, %	25,92±0,27	34,83±0,27	19,18±0,08	23,83±0,11	25,07±0,51	16,28±0,28	27,43±0,36	32,08±0,17	17,75±0,23
		Jami o'zlashtirish, %			79,93			65,18			77,27
4	Nitratlar	621	132,13±0,08	241,33±0,24	112,02±0,36	118,24±0,52	201,34±0,08	107,42±0,12	128,14±0,23	213,21±0,11	117,31±0,03
		O'zlashtirish, %	21,28±0,04	38,86±0,13	18,04±0,11	19,04±0,21	32,42±0,23	17,30±0,17	20,63±0,44	34,33±0,26	18,89±0,13
		Jami o'zlashtirish, %			78,18			68,76			73,86
5	Nitritlar	16,34	4,21±0,08	6,42±0,17	1,16±0,08	3,21±0,16	4,15±0,24	1,02±0,08	3,56±0,23	4,74±0,14	2,14±0,03
		O'zlashtirish, %	25,76±0,03	39,29±0,12	7,10±0,02	19,65±0,23	25,40±0,08	6,24±0,14	21,79±0,21	29,01±0,11	13,10±0,08
		Jami o'zlashtirish, %			72,15			51,29			63,89

$p<0,05$

Cumulative removal: 96.31% (See **Table 2**). In studies conducted with Azolla spp., the following phosphate assimilation rates were observed:

Day 2: 20.46% removal;

Day 7: 34.0% removal;

Day 12: 16.62% removal, total phosphate remediation capacity: 71.08% over 12 days.

When monitoring the nitrate uptake rate of higher algae cultures, it was found that on the 12th day of observation, eichhornia absorbed 78.18% of nitrates, azolla 68.76%, and small duckweed 73.86%. When comparing between cultures, it was found that azolla absorbed 9.42% less nitrates than eichhornia, and small duckweed 4.32% less. The results showed that the nitrites contained in household wastewater are not absorbed equally by different cultures. In particular, it was noted that eichhornia absorbed 72.15% of nitrates over 12 days of observation, while azolla absorbed 51.29%, and small duckweed absorbed 63.89%. When comparing the results obtained, it was noted that each of the higher algae assimilates biogenic elements in its own way. In particular, it was noted that Eichhornia showed the highest levels of absorption of salts contained in household wastewater, while the small duckweed showed higher indicators than Azolla.

In our scientific research, studies were conducted to determine the importance of purifying biogenic elements in wastewater through the co-cultivation of macrophytes, which was theoretically advanced. In these studies, the amounts of the main pollutants, the amounts recorded in the wastewater discharged from the enterprise's treatment plant, were taken as controls and as initial chemical quantities. During the research, it was revealed that macrophytes absorb phosphates from domestic wastewater Figure 1.

Figure 1. Phosphate uptake by macrophytes in domestic wastewater (initial concentration, 6.5 mg/l, $p<0.05$)

During our research, we attempted to improve the traditional biological treatment process, i.e. the eichhornia-based treatment process, which was introduced into the production facility. In this, along with eichhornia, small duckweed was used in a ratio of 1:10.

Conclusion. The conducted research confirms the critical importance of phytoremediation using macrophytes for treating industrial and domestic wastewater generated by oil and gas extraction facilities.

It was found that the recommended method *(E. crassipes + L. minor)* reduced the Na content of industrial wastewater by 23.63% compared to the traditional method, and the Na content of domestic wastewater by 43.01%, compared to the traditional method, the recommended method removed 4.78% more Mg in industrial wastewater, and 5.91% more Mg in domestic wastewater. The recommended method removed 32.32% more K in industrial wastewater by 21.25% compared to the traditional method, and the recommended method removed 3.21% more Ca in industrial wastewater and 20.83% more Ca in domestic wastewater compared to the traditional method. It was shown that the recommended method (*E. crassipes + L. minor)* can remove 11.69% more Cr in industrial wastewater than the traditional method, and 3.38% more Cr in domestic wastewater, and 21.08% more Fe in industrial wastewater than the traditional method, and 2.22% more Fe in domestic wastewater, respectively.

The research revealed that, compared to macrophytes grown separately, macrophytes grown together have the ability to effectively purify wastewater generated from oil and gas production facilities from major biogenic elements and heavy metals.

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