ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889,
2023-5.384 ResearchBib IF: 8.848 / 2024

THE MULTIDISCIPLINARY JOURNAL OF SCIENCE AND TECHNOLOGY

VOLUME-4, ISSUE-6

Biotechnological Foundations of Treating Municipal Wastewater Using Higher Plants

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Abstract: This article presents the results of scientific research conducted on the biological treatment of municipal wastewater from urban utilities using higher aquatic plants. The experiments were carried out in biological ponds with Eichhornia crassipes (Solms) and Azolla caroliniana (Willd) grown in the wastewater. After 8 days, the biomass production of these plants was 200-760 g/m² and 200-485 g/m², respectively, and the water purification efficiency reached 88-90%. A comparative analysis showed that Eichhornia was 4% more effective in purifying water compared to Azolla.

Keywords: Biological treatment, Eichhornia, Azolla, BOD5, wastewater, hydrometer, semi-submerged plants, aerotank.

Introduction: Today, fresh water is one of the most scarce resources globally, and its depletion is not only due to the uneven distribution of fresh water resources but also due to pollution and inefficient purification of used water. The increasing pollution of water bodies with various wastes leads to ecological problems that affect both the environment and public health.

Despite the creation of modern new water purification devices, untreated wastewater from industrial enterprises, municipal utilities, agricultural enterprises, and residential areas is still being discharged into natural and artificial freshwater bodies in some places, leading to an increase in the amount of polluted wastewater.

ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2023-5.384 ResearchBib IF: 8.848 / 2024
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This polluted water contains many harmful substances that make it difficult for public consumption and pollute the environment. The most concerning is that such wastewater is increasing yearly, becoming a global problem.

Due to the high content of harmful compounds in polluted water worldwide, the development of opportunities for biological treatment of water and the reuse of treated water is one of the most urgent tasks today.

Literature Review: Both foreign and Uzbek scientists have conducted scientific research on the biological treatment of wastewater using lower and higher plants and achieved effective results.

In our republic, local scientists such as A.M. Muzaffarov (1972), R.Sh. Shoyakubov (1975), R.M. Alieva (1987), A. Abdukadirov (1990), E.E. Yunusov (1991), M.A. Kochkorova (1991), S.B. Boriev (1993), K.I. Aytmetova (1998), Yo.Q. Hayitov (2001), N.E. Rashidov (2001), M.I. Mustafoeva (2003), J. Qutliev (2004), A.T. Dosmetov (2004), S.O. Hojjiev (2010), T.N. Kholmurodova (2018), L.T. Yuldoshov (2021), and others have made significant contributions to analyzing the composition of wastewater and its biological treatment.

Among the scientists from the Commonwealth of Independent States (CIS), researchers like D.V. Dubina (1980), A.T. Dosmetov (2004), R.Sh. Shoyakubov (2004), N.V. Zagoskina (2009), L.V. Nazarenko (2009), K.E. Gulya (2012), B.K. Zayadan (2013), A.K. Sadvakasova (2013), D.K. Kirbaeva (2013), K.A. Bolatxan (2013), and others have conducted scientific research on the biological treatment of wastewater using lower and higher plants.

Foreign researchers such as Biplob Basak (2002), Hanife B (2009), Adebayo A.A (2011), Galal TM (2015), Shahabaldin Rezania (2016), Christopher O (2016), Dragos D (2018), Larisa S (2020), Elisabetta Bianchi (2020), Fadzlin A. A. (2020), Shambhvi (2020), A.M. Abdelatty (2021), and others have conducted extensive research in this area.

Materials and Methods: For scientific research on biological wastewater treatment, higher aquatic plants Eichhornia crassipes (Mart) Solms and Azolla caroliniana Willd were selected.

The genus Eichhornia originates from the tropical regions of South America and is a semi-submerged floating aquatic plant. This plant was first discovered in the 1960s in Lake Nyasa, South America, and today it naturally grows in many tropical and subtropical regions worldwide. Some sources state that this plant originates from the Amazon River. It was introduced as an ornamental plant in the late 19th century in the USA, Southeast Asia, and South Africa and is now cultivated in many tropical and subtropical regions.[1]

Currently, scientists have identified 11 naturally occurring species of the genus Eichhornia. Many aquarium varieties of this plant have been created worldwide. Eichhornia species grow 10-20 cm high, sometimes reaching 1 meter in favorable conditions.[4] It is a perennial aquatic plant with thick, shiny leaves 12-15 cm wide and 30-50 cm long. Its roots can grow up to 50-60 cm or more. The most common species is Eichhornia crassipes Solms.[3] Experiments were conducted with this species.

Azolla species naturally grow in the waters of North America, Western and Central Europe, South America, and the Galapagos Islands.[2] Only one species, the Nile Azolla, is found in the Nile River. The remaining species are widespread in tropical and temperate regions. Scientists have identified and studied seven natural species of Azolla. The natural species differ in leaf color, shape, size, and root length. Azolla species are small plants, with sizes up to 2,5 cm and leaf blades up to 1 mm.[4] Research was conducted on the Carolina Azolla (A. caroliniana Willd) species.

During the research, the physical-chemical composition of wastewater, both before and after planting the higher aquatic plants, was determined using international standard methods (Gost) and general hydrochemical methods by Yu.Yu. Lure and N.S. Strogonov.[5]

Results: Experiments were conducted in biological ponds established at the wastewater treatment facilities of Urgench city, operated by the Khorezm region water supply LLC (Limited Liability Company), where Eichhornia and Azolla were grown under field conditions. The experiments were carried out in 16 biological ponds arranged in two blocks, each with two rows of 8 ponds. Wastewater from the aerotanks was directed to the biological ponds in both blocks. Experiments were conducted using six biological ponds, with Eichhornia planted in the first block's 1st, 2nd, and 3rd ponds and Azolla in the second block's 1st, 2nd, and 3rd ponds. Eichhornia and Azolla were planted at a density of 200 g/m² of water surface.

In the first block, the growth and development of Eichhornia were monitored from the day it was planted in the wastewater. The average water temperature was 25-30°C, and light intensity was 15-25 thousand lux.

The daily growth and development of Eichhornia were recorded. After 8 days, the biomass was measured, showing that the 1st biological pond produced 8404 g, the 2nd pond 8001 g, and the 3rd pond 7602 g of Eichhornia. This indicated that Eichhornia grew well in wastewater (Table 1).

In the second block, Azolla was planted and grown under the same conditions for comparison. After 8 days, the biomass was measured, showing that the 1st biological pond produced 540,2 g, the 2nd pond 515,1 g, and the 3rd pond 485,4 g of Azolla. (Table 1,2,3)

The daily growth reached 42.5 g/m² in the 1st biological pond (1-BH), 39.3 g/m² in the 2nd biological pond (2-BH), and 35.6 g/m² in the 3rd biological pond (3-BH). The higher biomass in the 1st biological pond compared to the 2nd and 3rd ponds was due to the higher amount of organic and mineral nutrients present. (Table 1).

ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2023-5.384 ResearchBib IF: 8.848 / 2024
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Table 1

The Growth Dynamics of Eichhornia and Azolla in the Biological Ponds of the City Wastewater Treatment Facilities

№	Types of experience	Initial Biomass	Wet Biomass of Plants, g/m², g/m^2									
			Daily Growth				8-Day Biomass				Total biomass	
			Eichhornia		Azolla		Eichhornia		Azolla			
			g/m^2	%	g/m^2	%	g/m^2	%	g/m^2	%	э	a
1	1 -BH	200,0	80,0	40,0	42,5	21,2	640,0	320	340,0	170,2	840,4	540,2
2	2- BH	200,0	75,0	37,6	39,3	19,6	600,0	300	315,0	157,5	800,1	515,1
3	3- BH	200,0	70,0	35,0	35,6	17,8	560,0	280	285,0	142,5	760.2	485,4

At the same time, the physical-chemical composition of the wastewater was analyzed during the growth and development of the higher plants. Initially, the composition of the wastewater before planting Eichhornia and Azolla was determined. In the 1st biological pond (1-BH), the wastewater had a pH of 6.2, brown color, odor of 5.0, no oxygen, oxidation of 82.2 mg O2/l, COD of 119.4 mg O2/l, ammonia of 5.0 mg/l, nitrites of 0.09 mg/l, nitrates of 4.0 mg/l, chlorides of 65.4 mg/l, sulfates of 54.5 mg/l, phosphates of 7.5 mg/l, total salinity of 2.2 g/l, and suspended solids of 75.0 mg/l. In the 2nd biological pond (2-BH), the wastewater had a pH of 6.7, reddish color, odor of 4.0, oxygen of 1.4 mg/l, oxidation of 67.3 mg O2/l, COD of 95.3 mg O2/l, ammonia of 4.0 mg/l, nitrites of 0.07 mg/l, nitrates of 3.0 mg/l, chlorides of 51.5 mg/l, sulfates of 44.5 mg/l, phosphates of 5.6 mg/l, total salinity of 1.9 g/l, and suspended solids of 40.0 mg/l. In the 3rd biological pond (3-BH), the wastewater had a pH of 7.5, yellow color, odor of 3.0, oxygen of 2.5 mg/l, oxidation of 58.2 mg O2/l, COD of 71.4 mg O2/l, ammonia of 3.0 mg/l, nitrites of 0.06 mg/l, nitrates of 3.0 mg/l, chlorides of 40.5 mg/l, sulfates of 38.5 mg/l, phosphates of 4.2 mg/l, total salinity of 1.7 g/l, and suspended solids of 29.0 mg/l (Table 2).

Table 2

The Physical-Chemical Composition of Municipal Wastewater in Biological Ponds Before Planting Eichhornia and Azolla

	Indicators	Type of experience		
		I	II	III
		1 -BH	2- BH	3- BH
1	Temperature, ^{0}C	25,0±0,25	25,0±0,2	25,0±0,21
2	pH	6,2±0,07	6,5±0,06	6,7±0,08
3	Color	brown	reddish	yellow
4	The smell	5,0±0,03	4,0±0,02	3,0±0,04
5	Oxygen mg/l	no	1,4 ±0,05	2,5 ±0,12
6	BOD5 mg O_2/l	119,4 ±3,2	95,3 ±1,1	71,4 ±1,5
7	Oxidation mg O_2/l	82,2 ±3,3	67,3 ±1,5	58,2 ±0,62
8	Ammonia mg/l	5,0 ±0,14	4,0 ±0,04	3,0 ±0,08
9	Nitrite mg/l	0,09 ±0,001	0,07±0,001	0,06±0,001
10	Nitrate mg/l	4,0 ±0,12	3,0 ±0,9	3,0 ±0,14
11	Chlorides mg/l	65,4±3,6	51,5±1,9	40,5±1,6
12	Sulfates, mg/l	54,5±3,8	44,5±1,8	38,5±1,3
13	Phosphates, mg/l	7,5±0,46	5,6±0,33	4,2±0,28
14	General salinity, g/l	2,200±0,36	1,955±0,14	1,788±0,11
15	Suspended substances, mg/l	75,0±2,9	40,0±2,2	29,0±1,8
16	Plant biomass, g/m^2	0	0	0

After planting Eichhornia in the biological ponds and once the water surface was completely covered with the plants, the physical-chemical composition of the wastewater exiting the first, second, and third ponds in the first block was determined. At the end of the 8th day after planting Eichhornia, the composition of the wastewater was analyzed. In the 1st biological pond (1-BH), the wastewater had a pH of 7.0, yellow color, no odor, oxygen of 4.8 mg/l, oxidation of 33.0 mg O2/l, BOD5 of 49.3 mg O2/l, ammonia of 2.0 mg/l, nitrites of 0.02 mg/l, nitrates of 1.0 mg/l, chlorides of 48.4 mg/l, sulfates of 44.0 mg/l, phosphates of 4.8 mg/l, total salinity of 0.925 g/l, suspended solids of 30.0 mg/l, and plant biomass of 840 g/m². In the 2nd biological pond (2-BH), the wastewater had a pH of 7.4, clear color, no odor, oxygen of 6.4 mg/l, oxidation of 21.1 mg O2/l, BOD5 of 23.1 mg O2/l,

ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2023-5.384 ResearchBib IF: 8.848 / 2024
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ammonia of 1.0 mg/l, nitrites and nitrates were absent, chlorides of 34.5 mg/l, sulfates of 32.0 mg/l, phosphates of 2.5 mg/l, total salinity of 0.674 g/l, suspended solids of 15.6 mg/l, and plant biomass of 800 g/m². In the 3rd biological pond (3-BH), the wastewater had a pH of 7.5, clear color, no odor, oxygen of 9.2 mg/l, oxidation of 9.8 mg O2/l, BOD5 of 11.3 mg O2/l, ammonia, nitrites, and nitrates were absent, chlorides of 20.5 mg/l, sulfates of 18.5 mg/l, phosphates of 1.0 mg/l, total salinity of 0.445 g/l, suspended solids of 10.8 mg/l, and plant biomass of 760 g/m² (Table 3).

Table 3

The Physical-Chemical Composition of Wastewater After 8 Days of Planting Eichhornia

	Indicators	Type of experience		
		I	II	III
		1 -BH	2- BH	3- BH
1	Temperature, ^{0}C	26,0±0,39	26,0±0,28	26,0±0,21
2	pH	7,0±0,1	7,4±0,14	7,5±0,13
3	Color	Yellowish	Colorless	Colorless
4	The smell	no	no	no
5	Oxygen mg/l	4,8±0,11	6,4±0,9	9,2 ±0,8
6	BOD5 mg O_2/l	49,3 ±2,1	23,1 ±1,42	14,6 ±0,62
7	Oxidation mg O_2/l	33,0 ±1,8	21,1±0,92	9,8±0,11
8	Ammonia mg/l	2,0 ±0,25	1,0±0,13	no
9	Nitrite mg/l	0,02±0,02	no	no
10	Nitrate mg/l	1,0±0,26	no	no
11	Chlorides mg/l	48,4±1,8	34,5±0,98	20,5±0,60
12	Sulfates, mg/l	44,0±1,67	32,0±1,0	18,5±0,64
13	Phosphates, mg/l	4,8±0,12	2,5±0,12	1,0±0,14
14	General salinity, g/l	0,925±0,011	0,674±0,09	0,445±0,06
15	Suspended substances, mg/l	30,0±1,84	15,6±0,64	10,0±0,40
16	Plant biomass, g/m^2	840±3,8	800±4,1	760±3,6

In the experiments, Azolla plants were planted under the same conditions and at the same time in the BHs of the second block for comparison with Eichhornia. After aeration, 200 g of plant biomass per 1 m² of water surface was planted in the BHs where the municipal wastewater collected, and its development was monitored.

ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2023-5.384 ResearchBib IF: 8.848 / 2024
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At the end of the 8th day after planting Azolla in the biological ponds of the second block, the composition of the wastewater was analyzed. In the 1st biological pond (1-BH) of the second block, the wastewater had a pH of 7.0, clear color, no odor, oxygen of 4.0 mg/l, oxidation of 38.4 mg O2/l, BOD5 of 55.3 mg O2/l, ammonia of 3.0 mg/l, nitrites of 0.02 mg/l, nitrates of 1.0 mg/l, chlorides of 55.4 mg/l, sulfates of 39.3 mg/l, phosphates of 5.5 mg/l, total salinity of 1.3 g/l, suspended solids of 25.0 mg/l, and plant biomass of 540 g/m². In the 2nd biological pond (II-BH), the wastewater had a pH of 7.2, clear color, no odor, oxygen of 5.6 mg/l, oxidation of 21.1 mg O2/l, BOD5 of 23.1 mg O2/l, ammonia of 1.0 mg/l, nitrites and nitrates were absent, chlorides of 44.8 mg/l, sulfates of 35.5 mg/l, phosphates of 3.0 mg/l, total salinity of 0.945 g/l, suspended solids of 22.4 mg/l, and plant biomass of 515 g/m². In the 3rd biological pond (III-BH), the wastewater had a pH of 7.5, clear color, no odor, oxygen of 8.4 mg/l, oxidation of 9.8 mg O2/l, BOD5 of 11.3 mg O2/l, ammonia, nitrites, and nitrates were absent, chlorides of 28.4 mg/l, sulfates of 26.5 mg/l, phosphates of 2.0 mg/l, total salinity of 0.674 g/l, suspended solids of 14.5 mg/l, and plant biomass of 485 g/m² (Table 4).

Table 4

The Physical-Chemical Composition of Wastewater After 8 Days of Planting Azolla

№	Indicators	Type of experience		
		I	**II**	**III**
		1 -BH	2- BH	3- BH
1	Temperature, ^{0}C	26,0±0,28	26,0±0,22	26,0±0,26
2	pH	7,0±0,1	7,2±0,6	7,5±0,9
3	Color	Colorless	Colorless	Colorless
4	The smell	no	no	no
5	Oxygen mg/l	4,0±0,1	5,6±0,3	8,4±0,4
6	BOD5 mg O_2/l	55,3±3,2	23,1±2,1	11,3±1,41
7	Oxidation mg O_2/l	38,4 ±3,3	21,1 ±2,0	9,2 ±2,4
8	Ammonia mg/l	3,0±0,32	1,0±0,39	no
9	Nitrite mg/l	0,02±0,01	no	no

ISSN: 2582-4686 SJIF 2021-3.261,SJIF 2022-2.889, 2023-5.384 ResearchBib IF: 8.848 / 2024
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10	Nitrate mg/l	1,0±0,01	no	no
11	Chlorides mg/l	55,4±2,7	44,8±1,6	28,4±1,2
12	Sulfates, mg/l	39,3±1,7	35,5±1,113	26,5±0,89
13	Phosphates, mg/l	5,5±0,05	3,0±0,01	2,0±0,02
14	General salinity, g/l	1,375±0,13	0,945±0,09	0,674±0,07
15	Suspended substances, mg/l	25,0±1,54	22,4±1,32	14,5±0,76
16	Plant biomass, g/m^2	540±3,6	515±2,94	485±2,62

The research continued with experiments conducted by planting Eichhornia and Azolla in the biological ponds at the Urgench city municipal wastewater treatment complex. Comparative analysis of the results showed differences in the absorption of organo-mineral substances in the wastewater by the plants.

Conclusion.

Under field conditions, Eichhornia grown in municipal wastewater produced 200-760 g/m² biomass over 8 days. Azolla grown under the same conditions produced 200-485 g/m² biomass. The physical-chemical indicators showed that the water purification efficiency was 90% for Eichhornia and 88% for Azolla. Comparative analysis of the plants indicated that Eichhornia's purification efficiency was 2% higher than that of Azolla.

This demonstrates that various industrial wastewater can be treated using higher plants through ecologically safe biological methods. In the future, the results of this research can be used to utilize treated wastewater as secondary technical water and for irrigation in agriculture.

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