

Phytoremediation of domestic wastewater using *Chara vulgaris* algae

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ABSTRACT

The current study aims at conducting the phytoremediation of sewage water using algae (*Chara vulgaris*) to identify the qualitative changes that occur in wastewater during the treatment period, and conducting periodic measurements of each: pH, EC₂₅, Total hardness, Phosphate and Nitrate ions and Bacterial contamination indicator, based on the methods universally adopted. The results of the study indicated a significant improvement in the transparency and quality of treated water, with percentage removal of organic load (BOD₅), 86%, PO₄⁻³ and NO₃⁻¹ ions were 89.5% to 50% respectively at the end of the treatment period. A sharp decline was also observed in the preparation of bacterial contamination indicator to reach the removal rate to 99.7 % of total numbers of bacteria and 100% for each of Fecal coliform and E. coli. Also, the study recommended using bioremediation to improve the wastewater before discharging them into the Tigris river to protect it from pollution.

Key word: Phytoremediation of wastewater. *Chara vulgaris*.

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INTRODUCTION

Wastewater contains large amounts of highly complex organic and inorganic compounds and phytonutrients, as well as vast numbers of microorganisms, and organic matter is subjected to biological degradation, resulting in the depletion of dissolved oxygen in water^[1, 2], and thus the possibility of creating anaerobic conditions that alter the chemical reaction pathways of decomposition processes and produce toxic substances such as the formation of sulfur forms H₂S, HS, S = Amines and other toxic substances to the aquatic ecosystem^[3, 4]. This is observed in many locations near the estuaries of wastewater discharge on the banks of the Tigris river in the city of Mosul^[5, 6].

Therefore, it is necessary to treat the wastewater in order to prevent deterioration the aquatic ecosystem of the Tigris river and the occurrence of the phenomenon of eutrophication, especially the poisonous species of algae, and thus the negative impact on biological diversity and water quality of Tigris river^[7].

Bioremediation, including phytoremediation, is one of the most promising solutions to solve these growing environmental problems using local plants and algae that are tolerant of contaminants to be treated^[8], to improve hazardous pollutants and toxic substances on ecosystem and human health or to convert them into less toxic forms^[9], as well as, the role of solar radiation in the activation of the photochemical reactions in the water and microorganisms already exist in wastewater, thereby enhancing biological treatment processes, Which is one of the best modern treatment methods, As well as being environmentally friendly and low cost compared to other technologies^[10, 11].

Phytoremediation include a variety of techniques such as pollutant extraction and concentration in plant tissues (Bioaccumulation) or blocking the movement of pollutants in the root zone or volatilizing pollutants from plants to the air through transpiration^[12], The study conducted by Kalin and Smith^[13], indicates the ability of *Chara vulgaris* and some types of Charophyta to absorb and concentrate toxic elements, also studies indicate^[14, 15] to a high susceptibility

for *Chara spp.* to improve water quality by absorbing most nutrients such as nitrates and phosphates, as well as, their allelopathic effects against the blue green algae (Cyanobacterium) known for its formation of many types of harmful toxins in aquatic life, which inhibits the growth of some toxic algae such as *Microcystic aeruginosa* through the production of types of fatty acids and then the possibility of controlling their growth.

So the current study came to treat wastewater using *Chara vulgaris*.

MATERIALS AND METHODS

Chara vulgaris green algae that exist in fresh and brackish water was used in the present study, the genus of Chara belongs to the family Characeae, order Charales, class Charophyceae and division Chlorophyta, its body is made up of an axis that contains sub-roots of branching and has a length of 20-30 cm^[16] as shown in (Photo 1).



Photo 1: alga of *Chara vulgaris*

Chara vulgaris was used in the treatment of 2,000 ml beakers and the following treatments were performed with three replicates:

Treatment 1 (t₂): Wastewater with the addition of 10 gm. L⁻¹ of algae.

Treatment 2 (t₂): Wastewater with the addition of 5 gm. L⁻¹ of algae.

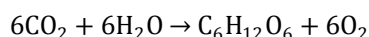
Treatment 3 (t₃): Diluted wastewater (50%) with the addition of 10 gm. L⁻¹ of algae.

Treatment 4 (t₄): Diluted wastewater (50%) with the addition of 5 gm. L⁻¹ of algae.

The beakers were placed in the laboratory near the windows exposed to solar radiation indirectly throughout the remediation period, the following measurements were performed: pH, Electrical conductivity, Total Hardness, Organic load (BOD₅), Phosphate and Nitrate ions (PO₄, NO₃), Total Plate Count (TPC), Fecal coliform and *E. coli* based on internationally approved methods^[17, 18] for all treatment and continued follow-up for (15) days with periodic measurements.

RESULTS AND DISCUSSION

A significant improvement in water transparency was observed in the study after it was of grey and black color due to the anaerobic conditions and formation of Sulfide forms, as a result of the impact of algae, which played a role in improving water aeration to produce oxygen as a byproduct of the process of photosynthesis and create aerobic conditions. Aerobic condition as it is shown in the following equation^[19]:



Thus changing the pathways of chemical reactions such as oxidation of sulphide forms to sulfates and increasing the degradation of organic matter leading to water infusion and the disappearance of unpleasant odours^[6]. As for the pH values, Table (1) shows a gradual increase in values to 8.8 during the remediation period, which may be due to the role of algae during the process of photosynthesis to take carbon either by CO₂ or from other images will result in the release of carbonate ions, this was confirmed^[20] in his study for the treatment of wastewater by algae (*Chlorella vulgaris*, *Spirulina platensis*) as shown in the following equation^[19, 21]:

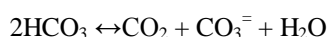


Table 1: Means* of pH values, nitrate and phosphate ions concentration during remediation.

Param.	Treatm	Before remediat	After remediation						
			1 day	5 days	7 days	11 days	13 days	15 days	% Removal
pH	t ₁	7.3	7.6	7.9	8.2	8.3	8.3	8.6	----
	t ₂		7.8	7.7	8.0	8.7	8.6	8.7	----
	t ₃	7.3	7.6	7.8	8.0	8.6	8.6	8.8	----
	t ₄		7.7	7.5	7.9	8.6	8.5	8.4	----
PO ₄ ³⁻	t ₁	4.13	1.64	2.44	2.48	0.42	0.40	0.46	% 88.8
	t ₂		2.50	2.85	2.92	0.56	0.45	0.43	% 89.5
	t ₃	1.24	0.84	1.31	1.08	0.12	0.44	0.42	% 66.1
	t ₄		1.26	1.17	1.29	0.43	0.45	0.43	% 65.3
NO ₃ ⁻¹	t ₁	0.86	0.27	0.75	0.52	0.56	0.57	0.47	% 45.3
	t ₂		0.55	0.71	0.65	0.52	0.53	0.50	% 41.8
	t ₃	0.4	0.20	0.37	0.22	0.29	0.20	0.20	% 50.0
	t ₄		0.34	0.51	0.37	0.30	0.32	0.27	% 32.5

*mg. l⁻¹

Or the possibility of extract oxygen from nitrates denitrification processes in anaerobic conditions for the oxidation of organic material biologically and the release of OH ions that raise the pH to the base as in the following equation^[22]:



The absence of higher pH levels is due to the high capacity of buffering for Iraqi water and environment^[23].

The concentration of nutrients (Table 1) indicates a decrease in the concentrations of phosphate and nitrate ions when remediated with *Chara vulgaris*, the phosphate removal ratio for t₁, t₂, t₃ and t₄ compared to phosphate concentrations before remediation (88.8%), (89.5%), 66.1%) and (65.3%), while the nitrate removal ratio for t₁ and t₂ and t₃ and t₄ compared to nitrate concentrations before remediation (45.3%) (41.8%) (50.0%) (32.5%), respectively, after 15 days of remediation of wastewater, this decrease in the concentrations may be due to the ability of the algae to absorb and aggregation of nutrients in large quantities, this is confirmed by^[20]. In a study conducted by Stephen et al.^[24] on the quality of water remediated with aquatic plants in the UK, they observed that the concentration of phosphate and nitrate decreased in the ponds where the Charophytes grow, the rate of phosphate and nitrate ions removal was (37% 81%) respectively, also the study of Al-Saffawi and Al-Sanjary^[25], showed that the *Limnaea minor* was able to remove phosphate and nitrate ions from sewage which reached to (81% and 54%) respectively, after nine days of remediation. The difference in nutrient removal ratio by plants used for remediation may be due to differences in abiotic factors and variations in the nature and characteristics of each plant.

As for the organic pollution, (Table 2) shows a decrease in the value of BOD₅, where the percentage of removal reached to (61.5%, 55.3%, 86.1% and 72.2%) for the t₁, t₂, t₃ and t₄, after 15 days of remediation compared to BOD₅ before remediation, this may be due to the fact that *Chara vulgaris* are used as a shelters for aquatic invertebrates, that reduce organic matter suspended by filter feeding processes^[26], as well as their ability to improve environmental conditions by providing oxygen dissolved in water and release enzymes that contribute to the activation of organic matter analysis and in some cases the use of carbon in the photosynthesis process, so the algae and aquatic plants as an environment Pollutants Scavenger^[20].

The mean values of electrical conductivity (EC₂₅) and total hardness as shown in (Table 2) indicates a relative increase in concentrations during remediation processes which may be attributed to biological degradation and chemical reactions that occur in the water leading to the formation of carbonic acid and some carboxylic acids that can interact with the non-dissolved calcium carbonate converted to the dissolved calcium bicarbonate, which leading to increased concentration of salts and total hardness as shown in the following reactions^[6]:

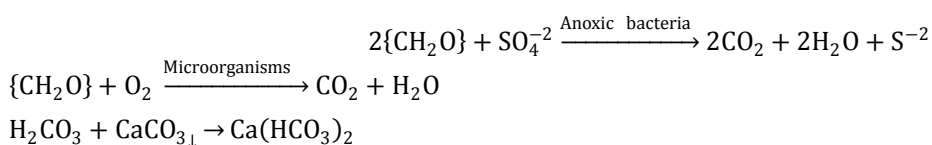


Table 2: Means results of EC₂₅, BOD₅and Total hardness* during the remediation period

Param.	Treatm	Before re mediat	After remediation						
			1 day	5 days	7 days	11 days	13 days	15 days	% Removal
BOD ₅	t ₁	65	63	45	37	----	29	25	% 61.5
	t ₂		65	43	34	----	30	29	% 55.3
	t ₃	36	33	15	12	----	11	5	% 86.1
	t ₄		23	16	14	----	11	10	% 72.2
EC ₂₅	t ₁	983	958	1100	1167	1141	1086	962	----
	t ₂		990	1140	1180	1169	1140	1021	----
	t ₃	600	587	657	697	687	684	609	----
	t ₄		594	671	697	701	711	655	----
T. Hard	t ₁	300	375	435	415	375	255	272	----
	t ₂		330	450	425	415	380	372	----
	t ₃	200	200	245	235	220	215	216	----
	t ₄		180	235	240	230	235	240	----

EC.: Electrical conductivity($\mu\text{S. cm}^{-1}$), BOD₅: Biological oxygen demand (mg. l⁻¹), *(mg. l⁻¹).

As for the total number of bacteria, fecal coliform, and *E. coli* as shown in (Table 3) indicates that the removal percentage of TPC at t₁ and t₂ and t₃ and t₄ reached to (99.0, 99.5, 99.4, 99.7)%, respectively, compared to the total number of bacteria before treatment after a week of remediation with *Chara vulgaris*, while the removal percentage of the number of fecal coliforms and *E. coli* are 100% for t₁ and t₃ and t₄, respectively, compared to the number before treatment after 5 days of treatment, these results approach the results of a study^[25], as the removal percentage of TPC bacteria reached between the percentage (99.4 - 99.1)%, after five days of wastewater remediation by the flora *Lemna minor* and attributed to the production of some compounds with inhibitory effect on bacteria, The picture (2) shows the results of the presumptive test of waste water before and after remediation with algae.

Table 3: results of the mean preparation of the bacteria studied during the during remediation period.

Param.	Treatm	Before re mediat	After remediation						
			1 day	5 days	7 days	11 days	13 days	15 days	% Removal
TPC ¹	t ₁	22×10 ⁵	33×10 ⁴	28×10 ⁴	2×10 ⁴	0.00	0.00	0.00	99.0%
	t ₂		44×10 ⁵	52×10 ⁴	1×10 ⁴	0.00	0.00	0.00	% 99.5
	t ₃	10×10 ⁵	204×10 ⁴	78×10 ³	6×10 ³	0.00	0.00	0.00	% 99.4
	t ₄		206×10 ⁴	36×10 ³	3×10 ³	0.00	0.00	0.00	% 99.7
F. colif. ²	t ₁	43×10 ⁴	4.0×10 ⁴	0.00	0.00	0.00	0.00	0.00	% 100
	t ₂		4.0×10 ⁴	3×10 ⁴	0.00	0.00	0.00	0.00	% 100
	t ₃	11×10 ⁴	11×10 ⁴	0.00	0.00	0.00	0.00	0.00	% 100
	t ₄		46×10 ³	0.00	0.00	0.00	0.00	0.00	% 100
<i>E. coli</i>	t ₁	43×10 ⁴	4.0×10 ⁴	0.00	0.00	0.00	0.00	0.00	% 100
	t ₂		4.0×10 ⁴	3×10 ⁴	0.00	0.00	0.00	0.00	% 100
	t ₃	11×10 ⁴	11×10 ⁴	0.00	0.00	0.00	0.00	0.00	% 100
	t ₄		46×10 ³	0.00	0.00	0.00	0.00	0.00	% 100

¹ cell. 1ml⁻¹, ² cell. 100ml⁻¹.



After remediation



Before remediation

Photo 2: Results of presumptive test of Fecal coliform during wastewater remediation.

The differences in the removal period of the Total number of bacteria (TPC) compared with *F. Coliform* and *E. coli* may be due to the changes in the environmental conditions of Faecal coliform and *E. coli* when they are transferred from the gut of the organisms to the aquatic environment, which is more sensitive than bacterial species and other harsh environmental conditions outside the body of the organism^[16], the ability to remove the studied bacteria species was attributed to the ability of many algae to produce inhibitory compounds for bacteria and pathogens^[27].

Also points^[28] to the viability of species of algae to produce antibiotics that work to eliminate species of bacteria such as derivatives Alklorlin, Chlorellin derivatives, Phenolic inhibitors, Acrylic acid and halogenated aliphatic compound, for example, *Chara spp.* produces an inhibitor and antibacterial materials and these materials are an enzyme Lipoxigenase, fatty acid, Stearic acid and Chlorellin derivatives. As it can be seen from the (Table 3) a clear reduction in the number of bacteria studied after one day of remediation.

CONCLUSIONS AND RECOMMENDATIONS

1. Improve the transparency of water when treated as a result of the activities of bio organism.
2. The phytoremediation of wastewater *Chara vulgaris* with good efficiency in the removal of nitrate and phosphate ions that cause the occurrence of the phenomenon of eutrophication in surface water.
3. The usage of algae and aquatic plants for wastewater remediation leads to improved water aeration, which promotes the activity of aerobic microorganisms to degrade organic material and composition of harmless products to the aquatic ecosystem.

Therefore, we recommend to develop and use of bioremediation because it works to remove contaminants and reduce toxic pollutants from wastewater before being discharged into rivers to reduce the pollution of the Tigris River.

These techniques are simple, natural and economical, as well as produced a suitable water, that can be used in many industrial and agricultural purposes like irrigation and thus reduce pollution loads of water resources.

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