Sustainable Environmental Insight

Research Article
Volume 1(2), 2024, 58–69
https://doi.org/10.53623/sein.v1i2.425

Preliminary Investigation of Wastewater Phycoremediation and Biomass Productivity using Locally Isolated Green Microalgae from Ipoh, Malaysia

Pravin Muniandy[1], Leong Kong Yong[2]*, Siti Nor Aishah Mohd Salleh[1,*], Mirshayinee Muniandy[1], Chi Hien Lee[1]

[1]School of Applied Sciences (SAS), Faculty of Integrated Life Sciences, Quest International University, Malaysia.
[2]Department of Civil and Construction Engineering, Faculty of Engineering and Science, Curtin University Malaysia.

*Correspondence: yongleongkong@curtin.edu.my; noraishah.salleh@qiu.edu.my

SUBMITTED: 26 March 2024; REVISED: 17 May 2024; ACCEPTED: 21 May 2024

ABSTRACT: This study investigated the potential of microalgae, sourced from a pond in Gunung Lang, Ipoh, Malaysia, for the phycoremediation of domestic wastewater. Under laboratory conditions, identified and confirmed microalgae species were introduced to wastewater samples to assess their capacity for removing Chemical Oxygen Demand (COD), nitrogen, phosphorus, and heavy metals such as chromium and zinc. The study also examined the relationship between algae growth rates and nutrient absorption, alongside a detailed analysis of wastewater to determine the extent of pollutant reduction. Initial analyses revealed that the COD levels of the domestic wastewater stood at 158 mg/l, failing to meet the Effluent Standard requirements as per the Malaysia Environmental Quality (Industrial Effluents) Regulations 2009. nitrogen levels were measured at 11.16 mg/l, phosphorus at 5.56 mg/l, chromium at 1.53 mg/l, and zinc at 0.53 mg/l under the heavy metal category. The study demonstrates that phycoremediation significantly reduces pollutants and nutrients in wastewater samples. Remarkably, zinc removal achieved a 100% success rate, while the lowest pollutant removal was observed for COD at the 10^4 cell concentration in 100% wastewater concentration samples. The outcomes highlighted the efficacy of using microalgae for wastewater treatment, showing considerable promise in reducing environmental pollutants.

KEYWORDS: Green microalgae; wastewater; phycoremediation; nutrients; heavy metals

1. Introduction

Untreated wastewater discharge causes several environmental issues in natural water bodies. As mentioned above, untreated wastewater consists of nutrients and pollutants degrading water quality, affecting aquatic organisms. Eutrophication is one of the problems caused due to discharge of untreated wastewater, and the increased nutrient load can reduce the quality of water. Eutrophication minimizes the penetration of light into the water column and threatens aquatic life by lowering light essential for photosynthesis [1].

A high level of nutrients in wastewater promotes the growth of algae that covers the surface of the water. Thus it affects the whole ecosystem of the water body. A high amount of Biological Oxygen Demand (BOD) and COD level in wastewater reduces the oxygen availability in water sources. Dissolving oxygen is essential to maintain the ecosystem healthier [2]. Municipal wastewater contains nutrients, heavy metals, and microorganisms that harm the environment. Removal of ammonia, nitrates, and phosphorus is essential to maintain water quality. Moreover, the quality of treated wastewaters is evaluated by testing several parameters such as total dissolved solids, suspended solids, nitrogen, ammonia, Phosphorus, BOD, COD, pH, and colour. Municipal sewage wastewater alters all the parameters mentioned above, and thus the quality of water reduces drastically [3]. Wastewater management is needed compulsory in every place to prevent pollution and contamination on our natural water sources. Proper treatment and sanitation improve the quality of the environment and human life. Rapid development and urbanization increase the demand for a new wastewater treatment system that works more efficiently to treat the contaminated water produced daily [4].

According to Malaysia Environmental Quality (Sewage and Industrial Effluents) Regulation 2009, all the discharges should be within the limit set by the government. Standard A and B are the guidelines that mention the limit according to the discharge location. New treatment and remediation methods are needed to treat the growing amount of wastewater produced daily in our country. Microalgae treatment on wastewater is a sustainable practice and environmentally friendly [5]. Proper sanitation and clean water are also one goals in sustainable development. Poor sanitation systems are due to our country's lack of new techniques and treatment systems. The properties of different algae strains, as well as their wastewater treatment capacity, would differ. However, the properties of wastewater, the desired degree of treatment efficiency, the cost and energy demand of biomass harvesting, and the use of collected biomass would be the most important criteria in selecting a suitable strain or mix-consortia for wastewater treatment.

According to Abdelfattah et. al. [7], the robustness of microalgae species against wastewater pollutants, their ability to grow well or be known as high growth rate species, and their efficiency in assimilating nutrients from wastewater should all be considered when choosing microalgae species for wastewater treatment. There have been several investigations on various species of microalgae farmed in wastewater for the removal of nitrogen and phosphate. Microalgae studies help integrate the Phycoremediation method in the conventional wastewater treatment method. Some treatment methods generate a high volume of poisonous and complicated pollutants, making the biological agent ineffective at removing all kinds of pollutants. Thus, it is crucial to make advancements in the wastewater treatment plant to ensure the removal of COD, BOD, colour, heavy metal residues, ammonia, phosphorus, and other parameters without the high volume of electricity and chemicals [8]. Freshwater microalgae consume carbon dioxide, produce oxygen, and act as a base in food web in aquatic ecosystem.

Meanwhile, it also stabilizes sediments and maintains water temperature by blocking too much sunlight that penetrates water. Conventional wastewater treatment system needs more sludge retention time and high amount of sludge disposed of through the conventional method [9]. Besides that, a conventional treatment system suitable to operate in big scale can be a big disadvantage. Phycoremediation method in wastewater treatment systems reduces the sludge recovery and increases biomass production; thus, it becomes an added value for the treatment. Phycoremediation has high versatility and adaptability character in the wastewater treatment system. High microalgae concentration in the environment causes few effects like high dissolved oxygen in water; thus, it can inhibit the growth of photosynthetic microorganisms in the cycle of microalgae culture.

Phycoremediation technology in wastewater treatment aligns with several United Nations Sustainable Development Goals (SDGs), notably SDG 6 (Clean Water and Sanitation) by enhancing water quality and reducing sludge disposal, SDG 12 (Responsible Consumption and Production) through efficient resource use and waste reduction, and SDG 13 (Climate Action) by lowering energy use and potential carbon emissions. Additionally, it supports SDG 14 (Life Below Water) by improving aquatic environments and SDG 15 (Life on Land) by reducing land pollution through cleaner runoff. Phycoremediation not only presents a sustainable alternative to traditional wastewater treatments by increasing biomass production but also contributes broadly to environmental conservation and sustainable resource management.

2. Materials and Methods

3.1. Collection of microalgae sample.

Microalgae water samples were collected from a pond situated at Gunung Lang, Ipoh, Malaysia [10]. Upon arrival at the laboratory, each sample was transferred into a 50 ml centrifuge tube. These tubes were then subjected to refrigerated centrifugation at 10,000 rpm for 10 minutes to concentrate the microalgae. This process was repeated to ensure the purity of the strains obtained. Bold Basal Medium (BBM) broth was utilized for culturing the microalgae in the laboratory, facilitating the isolation of pure strains. Cultures grown in conical flasks were regularly monitored. Subsequently, a microalgae washing technique was employed to obtain a pure strain, which was then inoculated into wastewater for phycoremediation purposes.

3.2. Isolation of microalgae (micropipette washing technique).

The sample was introduced into BBM to facilitate the separation process. Specifically, it was mixed with 20 mL of sterile BBM and transported to the laboratory. Upon arrival, the laboratory preparation involved the setup of eight small sterile bottles, each containing 10 ml of sterile BBM. 10 drops of sterile solution were dispensed into the groove of a glass slide. Subsequently, microalgae cells were extracted from the stored sample using a micropipette under a microscope, ensuring the selection of individual cells. The extracted cells were then transferred into the prepared small bottles. This meticulous process was repeated to ensure the isolation of single cells. To prevent cross-contamination by microorganisms such as bacteria and viruses [11], centrifuge washing and streaking plating techniques were employed.

3.3. Inoculum culture (microalgae stocks).

An inoculum of the microalgae was prepared by introducing the chosen species into Bold Basal Medium nutrient broth. This medium solution was then exposed to sunlight for 12 days to facilitate the cultivation of the microalgae. Upon observation of growth, the species were harvested via centrifugation at 5,000 rpm for 10 minutes and subsequently washed with 10 ml of sterile saline solution with a pH of 7.0. The resulting sample was transferred using a sterilized pipette into sterilized bottles for suspension in 10 ml of sterile saline. To determine cell concentration, the cells were counted using a haemocytometer counter. The haemocytometer chamber and glass cover slip were cleaned with 70% ethanol to ensure sterility. A clean glass cover slip was then placed on the haemocytometer chamber, and the cell suspension was shaken for 1 minute. Following this, 10 µl of the cell suspension was transferred to each side of the haemocytometer counter, and the cells were counted under a light microscope. The cell count was expressed as cells/mL.

3.4. Phycoremediation efficacy experiments.

The pure sample of the selected microalgae species underwent testing in domestic wastewater samples within the laboratory setup. Various concentrations of microalgae cells were introduced into the wastewater and allowed to grow for a period of 14 days. Throughout this remediation process, the wastewater underwent testing for specific parameters including nitrogen concentration, phosphorus concentration, COD, total dissolved solids, and pH, as outlined in Table 1. Following the remediation period, the nutrient concentrations in the wastewater were reassessed to determine the efficacy and reduction rates of nutrients and pollutants.

Table 1. Parameters studies.

Type of Data	Source of Data	Analysis Method	Reference
Structure Color Shape	Isolated microalgae	- Microscope	[11]
COD Nitrogen Phosphorus Chromium Zinc TDS Turbidity Temperature pH Color	Phycoremediation study and Biomass productivity	- Method 8000 - Method 10031 - Method 8048 - Method 8023 - Method 8009 - DR 900 - Turbidity Meter - YSI Probe - pH Meter (YSI Probe) - Observation	[17]

In this study, the dependent variables included the values of COD, nitrogen, and phosphorus, while the independent variable was the concentration of microalgae, which was diluted to 10^4 cells/ml, 10^5 cells/ml, and 10^6 cells/ml, and inoculated into municipal wastewater collected from Indah Water treatment facilities in Ipoh. Phycoremediation efficiency was evaluated by comparing the initial and final readings of wastewater parameters, allowing for conclusive findings to be drawn.

The growth of microalgae follows distinct stages and phases, and the process of nutrient uptake requires time. Freshwater algae typically experience a stationary phase lasting 14 days, during which they grow and mature. Following this period, algae enter a death phase from day 15 onwards, rendering the cells inactive and unable to participate in further reactions. Consequently, it is advisable to conclude the phycoremediation process at this juncture and harvest the biomass for use as biofuel feedstock. Delays in harvesting may lead to algae decomposition, potentially impacting the dissolved oxygen concentration in the water.

The methodology employed in the study involves varying concentrations of microalgae (10^4, 10^5, 10^6 cells/ml) inoculated into municipal wastewater to assess phycoremediation efficiency in removing pollutants such as COD, nitrogen, and phosphorus. The research justifies the timing of microalgae harvesting by aligning it with their growth phases, specifically before the onset of the death phase to optimize nutrient uptake and prevent potential negative impacts from cellular decomposition. This approach not only enhances the efficiency of pollutant removal but also ensures that the harvested biomass is viable for use as biofuel feedstock, promoting sustainability. By using locally sourced wastewater from Indah Water treatment facilities in Ipoh, the study tailors its findings to improve local treatment practices, making the approach both scientifically robust and practically relevant for enhancing wastewater management and supporting sustainable resource recovery.

3.5. Determination of microalgae growth and biomass productivity.

Biomass productivity serves as a crucial aspect of this study, with biomass recovery conducted at the experiment's conclusion. To assess biomass productivity, the maximum growth rate and initial cell concentration are calculated. Previous research indicates that varying concentrations of microalgae cells enable the production of biomass with elevated productivity levels. The growth curve of microalgae typically exhibits a lag phase, exponential phase, and gradual increase over time, with the exception of samples featuring high initial cell concentrations. This anomaly occurs because an overpopulation of initial cells often fails to manifest significant growth or biomass production. Cell counting using a haemocytometer has been employed to determine the number of cells present in each concentration. Specifically, the average number of cells present in wastewater was determined by counting the cells within the outer four squares of the haemocytometer grid. This limited growth phenomenon could be attributed to constraints such as water, space, and nutrient availability, which typically stimulate cell growth. To extract algae from the domestic wastewater solution, a filtration process was conducted.

3.5. Statistical aAnalysis.

All the treatments were conducted in triplicates for each concentration. Data analysis of average, mean differences, standard deviation and the graph for each experiment were completed using Microsoft Office Excel.

3. Results and Discussion

3.1. Isolation and species identification.

The species of the microalgae was identified by observing under microscope repeatedly. The species was confirmed based on the morphology, structure and the colour of the cell. The cells were healthier and greener when observing under microscope. The species that was identified and used in this treatment process was *Chlorella Vulgaris* (Figure 1). The concentrated sample was observed under microscope and counted the number of cell to determine the average number of cell per mL in the sample. Haemocytometer was used to calculate the average number of cell and also used to detriment the growth of microalgae in the treatment process. The amount needed to inoculate in each flask was calculated based on the average number of cell. The amount calculated for 10^4 cell/ml was 17.3 µl and 173.4 µl was determined for 10^5 cell/ml. The amount decided for 106 cell/ml was 1734.1 µl.

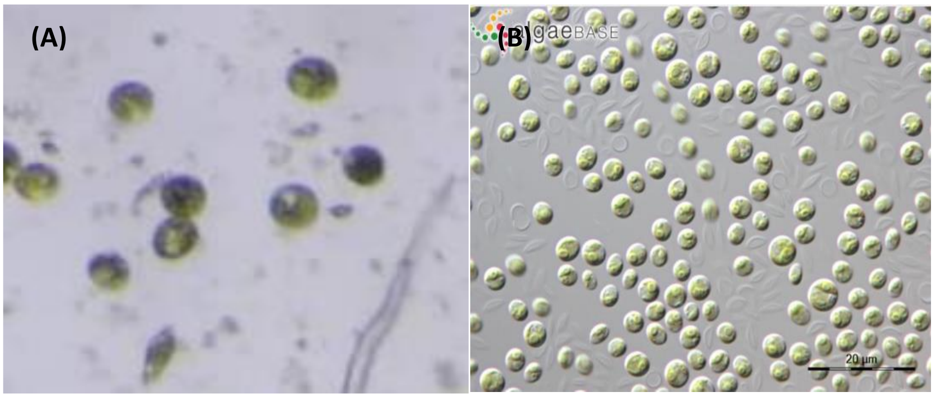

Figure 1. Isolated microalgae from pond (A) and picture retrieved from AlgaeBase (B).

The phycoremediation process was based on the concentration shown above to treat wastewater in laboratory condition. The species was confirmed with pictures shown in well-known website called AlgaeBase (https://www.algaebase.org/). The basic morphology of *Chlorella* was helpful to identify the species found in the sampling pond. *Chlorella* is a genus of roughly thirteen single-celled green algae that belongs to the Chlorophyta division. The cells are spherical in form, with a diameter of 2 to 10 m and no flagella. The green photosynthetic pigments chlorophyll –a and -b are found in their chloroplasts [12].

3.2. Characteristics of domestic wastewater.

Results obtained from the characterisation of domestic wastewater were tabulated as in Table 2 with three different readings are recorded to obtain average reading on each parameter. It is important to obtain these results in order to compare with results after phycoremediation process. Basically, the level of pollutant is higher due to anthropogenic activities by humans like cooking, washing and bathing. The main nutrients which was studied in this research are COD, nitrogen, phosphorus and heavy metal such as chromium and zinc.

Table 2. Characterisation of domestic wastewater (before treatment) and comparison of effluent standard 2009.

Parameters	Unit	Average Value	Effluent standard (mg/l)	
			Standard A	Standard B
Physicochemical				
COD	mg/l	158±2.00	120	200
Dissolve Oxygen, DO	mg/l	0.7±0.17	-	-
Turbidity	NTU	11.66±1.53	-	-
pH	-	6.5±0.30	6.0-9.0	5.5 – 9.0
Total Dissolved Solids, TDS	mg/l	45±1.73	-	-
Nitrogen	mg/l	11.16±0.65	10	20
Phosphorus	mg/l	5.56±0.35	-	-
Temperature	℃	28.33±0.57	40	40
Heavy metals				
Chromium,Cr	mg/l	1.53±0.05	0.05	0.05
Zinc, Zn	mg/l	0.53±0.05	2.00	2.00

The average reading of COD was 158 mg/l and 11.16 mg/l for nitrogen and 5.56 mg/l for phosphorus in the untreated wastewater. Moreover, the level of chromium and zinc was 1.53 mg/l and 0.53 mg/l, respectively in the wastewater. Besides that, very low amount of dissolve oxygen also recorded in the wastewater sample which was at 0.7 mg/l. This can be caused due to decomposition of organic matters in wastewater. The Table 2 also shows the between standard reading that had been set by Department of Environment, Malaysia to compare with wastewater condition. Based on the value of wastewater, COD, Nitrogen and Chromium exceeded the limit, thus it cause effects to environment especially for aquatic organisms [13]. This standard is important to main a balanced ecosystem.

3.3. Phycoremediation efficiency and microalgae growth.

Table 3 shows the results obtain from different concentration of cell. The cell concentration was fixed at 10^4 cell/ml and 100% wastewater concentration. The amount of COD is dropped to 58.66 mg/l and amount of nitrogen is dropped to 0.81 mg/l. Phosphorus also reduced to 1.71 mg/l. The removal of pollutant is not high compare to other concentration. This can be due to lower cell concentration which cause slow uptake of nutrients and pollutants. Zinc was recorded for 100% removal, thus it achieved untraceable level and chromium reduced to 0.18 mg/l. Dissolve oxygen has increased to 1.10 mg/l 1.43 mg/l for 75% and 100% wastewater concentration respectively, thus it shows that microalgae improve dissolve oxygen because of photosynthesis process.

Table 3. Effects of phycoremediation using microalgae cell concentration 10^4 cell/ml and different wastewater concentration.

Parameters	Initial concentration (mg/L)	After phycoremediation (mg/l)		Phycoremediation efficiency (%)	
		75% of WW	100% of WW	75% of WW	100% of WW
Physicochemical					
COD	158	46.66	58.66	70.47	62.87
DO	0.7	1.10	1.43	-57.14	-104.2
Nitrogen	11.16	0.93	0.81	91.67	92.74
Phosphorus	5.56	1.61	1.71	71.04	69.24
Heavy metals					
Chromium	1.53	0.15	0.18	90.20	88.24
Zinc	0.53	0.00	0.00	100	100

Figure 2 shows the growth rate of algae in 100% concentration of wastewater with cell concentration of 10^4 cell/ml or equivalent to 17.3 μl. The growth is very slow due to initial amount of cells that was inoculated. As we can observe in the graph the cells are keep on increasing even till day 14 due to duration of time to multiply themselves in the environment. The reduction of nutrients and pollutant has not reached to the level achieved by other concentration. The level of COD is still high if compare to 10^6 cell/ml and 10^5 cell/ml, thus the initial number of cells are important to have maximum reduction rate in 14 days of phycoremediation. Thus, optimizing the initial cell count is crucial for maximizing the efficacy of phycoremediation processes.

Table 3 shows the COD is reduced to 46.66 mg/l and nitrogen is reduced 0.93 mg/l. Phosphorus was at 1.61 mg/l. This experiment was conducted with cell concentration of 104 cell/ml and 75% of wastewater. The reduction is lower than other flask with same wastewater concentration. The reduction rate is less due to low initial cell concentration that reduces the uptake of pollutant by microalgae. Figure 2 shows the growth rate of algae in 75% wastewater flask with same cell concentration above. The algae cell number is even lower than Figure 2 in day 14 of the experiment. There are two main factors that affects the rate of absorption was initial cell concentration and nutrient availability [14]. This graph shows that very low initial cell concentration is not suitable for phycoremediation process.

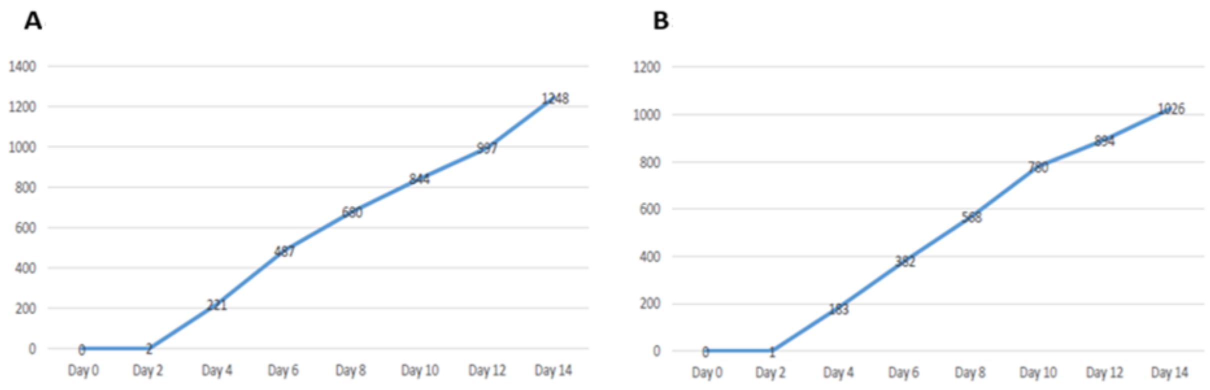

Figure 2. Microalgae growth (10^4 cell/ml): 100% of WW (A); and 75% of WW (B).

Table 4 is showing the results obtain after treatment with the cell concentration of 10^5 cell/ml and 100% concentration of wastewater. The results are better than 10^4 cell/ml concentration and has shown moderate reduction rate if compare to 10^4 cell/ml concentration. The COD is reduced to 45.33 mg/l from 158 mg/l and the nitrogen is dipped to 0.70 mg/l. The removal percentage for COD was 78.48% for 75% wastewater and 71.31% for 100% wastewater. Besides that, 1.40 mg/l is recorded for phosphorus. As usual Zinc is untraceable and chromium is dipped to 0.15 mg/l. The level of pollutant and nutrients shows good reduction rate. Figure 3 shows the growth rate of microalgae. This flask was inoculate with 173.4 µl or equivalent to 105 cell/ml in 300 mL of wastewater. The nutrients uptake will be at maximum rate on day 10 in this flask due to number of cells present in the flask. This growth pattern suggests that maximum nutrient uptake occurs around day 10, likely facilitated by the higher cell density in the flask, underscoring the importance of cell concentration in optimizing treatment efficacy.

Table 4. Effects of phycoremediation using microalgae cell concentration 10^5 cell/ml and different wastewater concentration.

Parameters	Initial concentration (mg/l)	After phycoremediation (mg/l)		Phycoremediation efficiency (%)	
		75% of WW	100% of WW	75% of WW	100% of WW
Physicochemical					
COD	158	34	45.33	78.48	71.31
DO	0.7	1.56	1.83	-122.86	-161.43
Nitrogen	11.16	0.63	0.70	94.35	93.73
Phosphorus	5.56	1.33	1.40	76.08	74.82
Heavy metals					
Chromium	1.53	0.12	0.15	92.16	90.20
Zinc	0.53	0.53	0.00	100	100

Table 4 also shows the results obtain with the treatment concentration of 10^5 cell/ml and 75% wastewater. The amount of COD is dropped to 34.00 mg/l and nitrogen dropped to 0.63 mg/l. Phosphorus also dropped to 1.33 mg/L. Dilution can be one of the factors that affects the reduction rate. The reduction rate is better for COD and nitrogen but dilution of wastewater could be the main factor of the poor reduction rate. Chromium also dropped 0.12 mg/l and Zinc is untraceable. Figure 3 shows the growth rate for cell concentration of 10^5 cell/ml or equivalent 173.4 µl in 75% wastewater concentration. The growth is slower compare to pervious graph due dilution of wastewater. The microalgae multiply steadily in this flask. As mentioned earlier, the nutrient availability and initial cell concentration is important to determine period of treatment is needed.

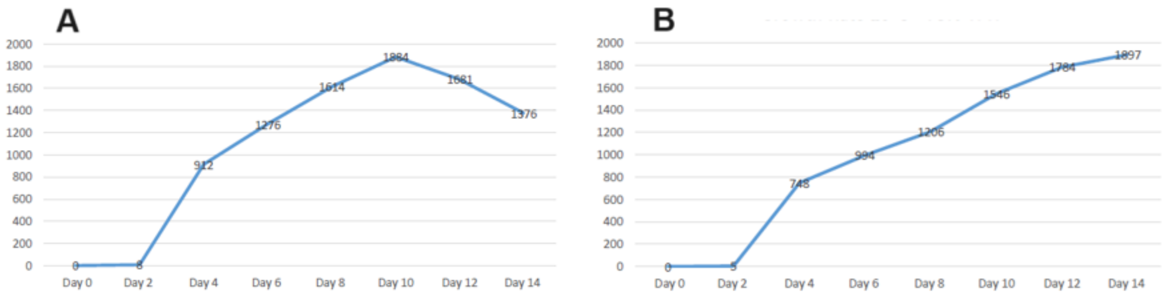

Figure 3. Microalgae growth (10^5 cell/ml): 100% of WW (A) and 75% of WW (B).

Table 5 shows the level of pollutant in wastewater after treatment process. This phycoremediation process was started with 10^6 cell/ml in 100% concentration of wastewater. The COD level dropped to 38.33 mg/l from 158 mg/l. Reduction of COD is 119.67 or equivalent to 75.74%. The amount of nitrogen and phosphorus also dropped to 0.26 mg/l and 0.30 mg/l respectively. Dissolve oxygen has increased more than 190% percentage in both concentration of wastewater under 10^6 cell/ml. During phototrophic growth, microalgae consume carbon dioxide and produce oxygen, thus the dissolve oxygen raise in water [15]. If compare to all other concentration, this concentration shows the maximum reduction of nutrients and pollutants in this 14 days of trial. The amount of zinc is untraceable which stood at 0.00 mg/l or 100% reduction after treatment. The amount of chromium is dipped to 0.04 mg/L from 1.53 mg/l which is equivalent to 99% reduction rate. As can be observed from Figure 4, the growth rate is steady due to the nutrient content and amount of cell that was inoculate in the wastewater. The cells are multiplying rapidly till day 8 and start to reduce from day 10. This shows the maximum lifespan of an algae is between 0 to 14 days. The uptake of nutrient and pollutant can be at maximum level on day 7 and 8. The quality of water would not improve much after day 14 due to decomposition reaction of death cells in the water. This graph shows the growth rate is triggered by high number of cells that was inoculate in the sample and the nutrient availability since 100% concentration of wastewater has been used in this sample. Moreover, the graph suggests that the growth rate of algae is influenced by the initial cell concentration and nutrient availability, particularly evident when maximum concentration of wastewater is utilized in the sample, further emphasizing the role of these factors in driving algae growth and remediation efficiency.

The concentration of microalgae is same as previous table which is at 10^6 cell/ml but the concentration of wastewater is changed to 75% of total amount of water. About, 225 ml of wastewater is mixed with 75 ml of distilled water. In total 300 ml of water is used in this research for each flask. The COD is reduced to 23.33 mg/l, 0.50 mg/l for nitrogen and 1.28 mg/l for phosphorus. The reduction rate of pollutant is higher if compare to the concentration of 105 cell/ml and 104 cell/ml experiment. The chromium level also dipped to 0.12 mg/l and Zinc is not traceable. Figure 4 shows the growth rate of algae in 75% of wastewater concentration. If we compare to Figure 3 the growth rate is lower due to less concentration of wastewater. The same amount of cell was inoculate in this sample but wastewater is diluted with 75 ml of distilled water, thus the nutrients are diluted. The peak day was day 10 in this concentration and the cells starts to reduce after day 12. Many microalgae species flourish in wastewater with high nitrogen and phosphorus concentrations, according to Yaakob et al. [16], and utilise them as a critical source of energy for their growth. This results in considerable nutrition absorption and nutrient concentration decrease.

Table 5. Effects of phycoremediation using microalgae cell concentration 10^6 cell/ml and different wastewater concentration.

Parameters	Initial concentration (mg/l)	After phycoremediation (mg/l)		Phycoremediation efficiency (%)	
		75% of WW	100% of WW	75% of WW	100% of WW
			Physicochemical		
COD	158	23.33	38.33	85.23	75.74
DO	0.7	2.03	2.33	-190	-232.86
Nitrogen	11.16	0.50	0.26	95.52	97.67
Phosphorus	5.56	1.28	0.30	76.98	94.60
			Heavy metals		
Chromium	1.53	0.12	0.04	92.16	97.39
Zinc	0.53	0.00	0.00	100	100

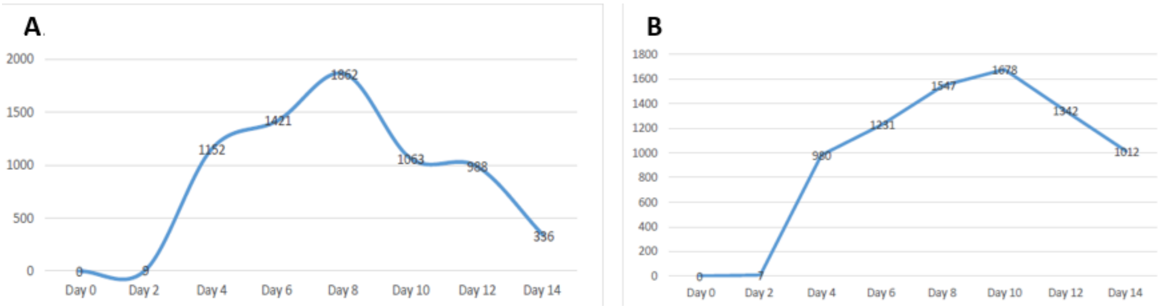

Figure 4. Microalgae growth (10^6 cell/ml): 100% of WW (A) and 75% of WW (B).

4. Conclusions

This study shows the ability of microalgae to absorb nutrients in wastewater. The initial cell concentration and percentage of wastewater play vital role in absorption capacity. The removal of nutrients and pollutant are lesser in low initial cell concentration flask due to the time taken for microalgae to multiply and grow. The concentration of pollutant is diluted when the wastewater is mixed with distilled water, thus the ability to absorb pollutant is getting weaker. Moreover, reducing wastewater concentration in conventional phycoremediation treatment process are not feasible. It may create more cost and energy to treat wastewater generally. Besides that, high initial cell concentration needs shorter period time to absorb nutrients due to abundance of microalgae in the wastewater. Their replicating time is shorter compare to other concentration. High absorption rate facilitate the growth of microalgae in wastewater, thus the growth curve is faster. Lower initial concentration flask samples has not reached the optimum growth due to time taken to replicate themselves in the environment. The highest removal rate achieved in the concentration of 10^6 cell/ml in this research and lowest was efficacy rate is 10^4 cells/ml. The growth is still observed in low initial cell concentration flask even till day 14. Growth phase will be slower after 14 days and death phase of algae occurs rapidly in wastewater, thus the quality of water deteriorate quickly. In this research the importance of duration of treatment and initial number of cells plays vital role in treating wastewater efficiently. As recommendation, optimize initial cell concentration, monitor treatment within

14 days, assess nutrient availability, evaluate cost-effectiveness, explore continuous treatment, enhance nutrient removal and invest in research for efficient phycoremediation of wastewater will be carried out in the future.

Acknowledgments

The authors thank the support of any parties involved in this project, especially Quest International University, for providing the equipment, research facilities and publication sponsorship for this study.

Competing Interest

All the authors declared no conflict of interest.

References

[1] Khan, M.N.; Mohammad, F. (2014). Eutrophication: challenges and solutions. In Eutrophication: Causes, Consequences and Control, Vol. 2; Ansari, A., Gill, S., Eds.; Springer: Dordrecht, Netherlands, pp. 1–15. https://doi.org/10.1007/978-94-007-7814-6_1.

[2] Bhateria, R.; Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, *2*, 161–173. https://doi.org/10.1007/s40899-015-0014-7.

[3] Nikuze, M.J.;Niyomukiza, J.B., Nshimiyimana, A.; Kwizera, J.P. (2020). Assessment of the efficiency of the wastewater treatment plant: a case of Gacuriro Vision City. *IOP Conference Series: Earth and Environmental Science*, *448*, 012046. http://doi.org/10.1088/1755-1315/448/1/012046.

[4] Silva, J.A. (2023). Wastewater treatment and reuse for sustainable water resources management: a systematic literature review. *Sustainability*, *15*, 10940. https://doi.org/10.3390/su151410940.

[5] Gani, P.; Sunar, N.M.; Matias-Peralta, H.; Parjo, U.K.; Razak, A.R.A. (2015). Phycoremediation of wastewaters and potential hydrocarbon from microalgae: a review. *Advances in Environmental Biology*, *9*, 1–9.

[6] Al-Jabri, H.; Das, P.; Khan, S.; Thaher, M.; AbdulQuadir, M. (2020). Treatment of wastewaters by microalgae and the potential applications of the produced biomass—A review. *Water*, *13*, 27. https://doi.org/10.3390/w13010027.

[7] Abdelfattah, A.; Ali, S.S.; Ramadan, H.; El-Aswar, E.I.; Eltawab, R.; Ho, S.H., Sun, J. (2023). Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environmental Science and Ecotechnology*, *13*, 100205. https://doi.org/10.1016/j.ese.2022.100205.

[8] Azimi, A.; Azari, A.; Rezakazemi, M.; Ansarpour, M. (2017). Removal of heavy metals from industrial wastewaters: a review. *ChemBioEng Reviews*, *4*, 37–59. https://doi.org/10.1002/cben.201600010.

[9] Hanum, F.; Yuan, L.C.; Kamahara, H.; Aziz, H.A.; Atsuta, Y.; Yamada, T.; Daimon, H. (2019). Treatment of sewage sludge using anaerobic digestion in Malaysia: Current state and challenges. *Frontiers in Energy Research*, *7*, 19. http://doi.org/10.3389/fenrg.2019.00019.

[10] Gani, P.; Muniandy, M.; Hua, A.K. (2024). Preliminary characterisation of physicochemical and heavy metals contents of algal blooms lake water. *AIP Conference Proceedings*, *2934*, 030003. https://doi.org/10.1063/5.0180588.

[11] Parvin, M.; Zannat, M.N.; Habib, M.A.B. (2007). Two important techniques for isolation of microalgae. *Asian Fisheries Science*, *20*, 117–124.

[12] Silva, J.; Alves, C.; Pinteus, S.; Reboleira, J.; Pedrosa, R.; Bernardino, S. (2019). Chlorella. In Nonvitamin and nonmineral nutritional supplements. Academic Press: Cambridge, USA; pp. 187–193.

[13] Aniyikaiye, T.E.; Oluseyi, T.; Odiyo, J.O.; Edokpayi, J.N. (2019). Physico-chemical analysis of wastewater discharge from selected paint industries in Lagos, Nigeria. *International Journal of Environmental Research and Public Health, 16*, 1235. https://doi.org/10.3390/ijerph16071235.

[14] Khan, M.I.; Shin, J.H.; Kim, J.D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories, 17*, 1–21. https://doi.org/10.1186/s12934-018-0879-x.

[15] Kazbar, A.; Cogne, G.; Urbain, B.; Marec, H.; Le-Gouic, B.; Tallec, J.; Pruvost, J. (2019). Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Research, 39*, 101432. https://doi.org/10.1016/j.algal.2019.101432.

[16] Yaakob, M.A.; Mohamed, R.M.S.R.; Al-Gheethi, A.; Aswathnarayana Gokare, R.; Ambati, R.R. (2021). Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: an overview. *Cells, 10*, 393. https://doi.org/10.3390/cells10020393.

[17] Rice, E.W.; Bridgewater, L. (2012). Standard methods for the examination of water and wastewater, Vol. 10. American Public Health Association: Washington DC, USA.