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Evaluating the Efficiency of Chlorella Vulgaris and Spirulina Microalgae in Wastewater Remediation Under Different Light Conditions



Ouhood Hassan Saleh¹⁽¹⁰⁾, Shahad A. Raheem²⁽¹⁰⁾, Shahad L. Abbas³⁽¹⁰⁾, Maryam Jawad Abdulhasan^{4*}⁽¹⁰⁾, Ali Muhsen Mohammedhassan¹⁽¹⁰⁾, Rusul Jabbar Lafta¹⁽¹⁰⁾

¹Ministry of Environment, Department of Protection and Improvement of the Environment in Middle Euphrates Region, Directorate of Babylon Environment, Babylon 00965, Iraq

² Civil Engineering Department, College of Engineering, Al-Qasim Green University, Babylon 51002, Iraq

³ College of Dentistry, Al-Farahidi University, Baghdad 00965, Iraq

⁴ Chemical Engineering and Petroleum Industries Department, College of Engineering and Engineering Technologies, Al-Mustaqbal University, Hillah 51001, Iraq

Corresponding Author Email: mm893505@gmail.com

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ABSTRACT

Microalgae have emerged as a pivotal component of wastewater treatment paradigms, offering an environmentally friendly, sustainable, and cost-effective approach. Beyond the purification of wastewater from diverse sources, microalgae exploit these effluents as a nutrient matrix, facilitating the biosynthesis of valuable bioproducts, bioenergy, and biomaterials. The present study evaluates the pollutant remediation capabilities of Chlorella vulgaris and Spirulina in the presence of common wastewater contaminantschemical oxygen demand (COD), nitrate (NO3⁻), and cadmium (Cd²⁺). These pollutants were selected due to their prevalence in wastewater, with nitrates and COD representing primary organic and inorganic pollutants, respectively, and cadmium being recognized for its acute toxicity. Experimental setups involved two flasks, each containing a mixture of the aforementioned contaminants, with one flask exposed to sunlight and the other placed in a darkroom to simulate varied lighting conditions. The removal efficiencies of Spirulina in the sunlit flask reached 86.6% for COD, 99.1% for NO₃, and 84.5% for Cd²⁺, while the darkroom condition yielded lower efficiencies of 54.3% for COD, 64.4% for NO3, and 61.8% for Cd²⁺. Conversely, Chlorella vulgaris exhibited removal efficiencies of 50.5% for COD, 52.3% for NO3⁻, and 74.6% for Cd²⁺ under sunlight, and 25.4%, 33.01%, and 53.3% for the respective contaminants in darkness. These findings underscore the crucial influence of sunlight and temperature on algal photosynthesis, thereby enhancing the bioremediation potential of wastewater contaminants. The study substantiates the significant role of microalgae in the reduction of contaminants, affirming their utility as an effective and economical treatment option.

1. INTRODUCTION

The provision of clean water, an imperative for global health, is increasingly challenged by the relentless urban expansion and concomitant escalation in demand. This growth engenders a vast spectrum of wastewater streams, encompassing domestic, industrial, and food-related effluents, each imbued with nutrients such as phosphorus and nitrogen, heavy metals, and contemporaneous pollutants [1-3]. wastewater Contemporary treatment paradigms predominantly employ sequential aerobic/anaerobic frameworks, designed to transmute pollutants into inert derivatives, thus facilitating the water's safe discharge or reuse. These systems effectively curtail the levels of carbon, nitrogen, and phosphorus: however, they are not without limitations, manifesting in substantial capital and operational expenditures, intricate maintenance, nutrient depletion, and the inadvertent emission of greenhouse gases (CH4, CO2, N2O, etc.) [4-7].

Delineated into physicochemical and biological categories, traditional remediation methods, while proficient in contaminant abatement, are marred by fiscal burdens and excessive sludge production [8]. Conversely, biological strategies, albeit cost-effective and eco-congenial, are susceptible to operational vicissitudes driven by temperature, pH, oxygen levels, and salinity [9]. These constraints necessitate the exploration of facile, economical, and efficacious technologies for water resource augmentation, thereby underscoring the potential of algae in biological wastewater remediation.

Algae, encompassing eukaryotic and prokaryotic singlecelled organisms, are broadly categorized as microalgae and macroalgae, with the former identifiable solely through microscopy [10]. Predominant microalgal taxa include Bacillariophyceae sp., Chlorophyceae sp., Chrysophyceae sp., and Cyanophyceae sp. [11]. Compared to traditional treatment modalities, microalgae confer multiple advantages, such as energy conservation, cost reduction, nutrient recovery, diminished sludge generation, and the mitigation of greenhouse gas emissions [12]. These organisms not only sequester substantial quantities of nutrients like nitrogen and phosphorus but also oxygenate the water through photosynthesis, thereby facilitating the bacterial oxidation of organic matter [13]. Currently, microalgae are deployed in the remediation of diverse wastewater types, including industrial, municipal, food, dairy, and pharmaceutical [14-16].

Microalgae utilize carbonaceous substrates and macronutrients for biomass accrual while simultaneously yielding dissolved oxygen, augmenting bacterial degradation processes [13, 17]. As autotrophic entities, they harness inorganic nutrients to synthesize organic molecules. The anthropogenic release of phosphorus and nitrogen, through waste, agricultural runoffs, and effluents, has positioned algal biomass as an effective vector for nutrient reclamation, attributed to its nutrient absorption efficacy [18, 19]. Albeit the pursuit of microalgal biofuel production is constrained by nutrient availability, efforts to recuperate and recycle nitrogen and phosphorus are imperative to forestall diffuse pollution during downstream biofuel processing [20]. Algal biomass is thus lauded for its potential in sustainable biofuel and bioproduct generation, given its rapid growth kinetics, biochemical composition, and lipid content [21]. Sustainable algal biomass production mandates the integration of nutrient recovery from residual biomass, employing both biological (anaerobic digestion) and hydrothermal treatments [22, 23].

The burgeoning interest in utilizing microalgae for environmental remediation has yielded promising results in the context of nutrient recovery from wastewater [24]. demonstrated that Spirulina exhibits a remarkable capacity for mitigating the load of total phosphorus and ammonia nitrogen in malodorous and black water systems, achieving remediation rates of 100%. Similarly, the studies [25, 26] reported that food wastewater treated with Scenedesmus obliquus and Chlorella vulgaris achieved phosphorus (P) and nitrogen (N) removal efficiencies of up to 54%. Additionally, study [26] assessed seven microalgal species for their proficiency in extracting phosphorus and nitrogen from municipal wastewater, noting that nitrogen and total dissolved phosphorus concentrations were reduced by 87% and greater than 80%, respectively.

The scourge of heavy metal contamination presents a formidable challenge to environmental health and biodiversity. Metals such as lead, arsenic, cadmium, and mercury pose significant risks to human health, as illustrated by study [1]. In response to this issue, study [27] evaluated the efficacy of four microalgae in remediating Mn and Fe from groundwater in a mining area, with Microcystis aeruginosa demonstrating superior removal rates for these metals. Furthermore, study [28] reported that Chlorella sorokiniana was capable of removing 99.6793% of Chromium (Cr) after 72 hours of exposure to a concentration of 100 mg/l Cr (VI).

Microalgae also contribute to the reduction of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in wastewater through dual mechanisms. As reported by study [29], microalgae can concurrently assimilate organic and inorganic substances in wastewater while utilizing light energy. Concurrently, photosynthesis by microalgae releases oxygen, thereby diminishing oxygen demand and effectuating a reduction in COD and BOD [30, 31], employed Chlorella vulgaris in the treatment of wastewater from sugarcane alcohol distillation, achieving BOD and COD reductions of 70% and 49%, respectively. Chandra et al. [32] compared various

microalgae in dairy wastewater treatment and noted that Scenedesmus abundans and Chlorella minutissima reduced COD and BOD by 56% and 70%, respectively. In contrast, Spirulina sp. and Nostoc muscorum exhibited minimal impact, with removal rates of approximately 24% and 38%, respectively. The study [29] explored the nutrient removal capabilities of Chlorella vulgaris and the bacterium Pseudomonas putida in wastewater, documenting significant reductions in ammonium and COD, with rates reaching approximately 80% after four days.

This study aims to extend the body of knowledge on wastewater treatment by examining the performance of two microalgae species, Chlorella vulgaris and Spirulina, in the removal of nitrates, cadmium, and phosphates. The influence of two distinct light conditions, namely natural sunlight and darkness, on the remediation efficacy of these microalgae is also investigated.

2. MATERIALS AND METHODS

2.1 Material

Chlorella vulgaris and Spirulina are microalgae species employed in our study to treat wastewater as shown in Figure 1. Chlorella vulgaris are a single-celled species of green microalgae in the group of Chlorophyta, these algae found in fresh water [33]. Spirulina are multicellular and cylindrical cyanobacteria (blue-green microalgae) found in tropical and subtropical lakes [34-36]. Chlorella vulgaris and Spirulina were obtained from laboratories of Ministry of Science and Technology/ Iraq. The algae culture was multiplied by planting them in the culture medium (Chu 10). EDTA (ethylene diamine tetra acetate) was purchased from HISEA chem. Co., Ltd.

2.2 Solutions preparation

EDTA is prepared by addition 50 mg from EDTA powder to 500 mL of distilled water, EDTA employed in this study to facilitate the sorption of contaminates by algae.

The standard solutions of Cadmium and nitrates ions were prepared with a concentration of 1000 mg/L in distilled water by dissolving cadmium acetate $Cd(CH_3COO)_2$ and Sodium nitrate NaNO₃, respectively, where the solutions were prepared at a concentration of 0.5 g/L.

2.3 Methods

We prepared two flasks filled with 500 mL of distilled water solution, contained EDTA at a concentration of 100 mg/L. After that, we added contaminates (NO₃, COD, Cd⁺²) at a concentration of 0.5 g/L for each one, then we stirred the flasks for two minutes. Then we added 20 mL of Chlorella vulgaris and Spirulina for each flask with 989,156 cell/liter. The first flask put under sunlight, and the second flask put in darkroom as show in Figure 2. The first flask put outdoor under sunlight at ambient temperture of 26°C, while the second flask put in dark room at temperture 20°C. The reason for choosing two different conditions was to determine the suitable conditions for the growth of microalgae and their effect on the wastewater treatment process. The duration of the experiment was ten days.

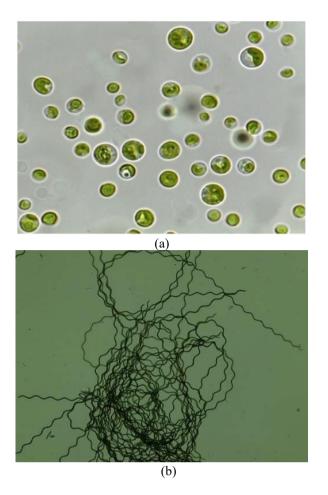


Figure 1. Microalgae under microscope (a) Chlorella vulgaris (b) Spirulina



Figure 2. The samples prepared in the present study

3. RESULTS AND DISCUSSION

3.1 Nitrate (NO₃) removal

The concentration of nitrate was continuously reduced with increasing in time. In the first days of experience, materials are not available to algae, but over time these materials will be easy to absorption by algae [37]. We observed that the best removal efficiencies were 52.3% and 99.1% at 10th day for Chlorella vulgaris and Spirulina, respectively for the flask put under the sunlight. While the second flask that put in darkroom

got the removal efficiencies of 33.01% and 64.4%, respectively for Chlorella vulgaris and Spirulina for the flask put in dark room at the same day as shown in Figure 3. This result is because that sunlight and temperature have the strongest effect on algal photosynthesis and therefore enhancing bioremediation of wastewater contaminants [38-41]. Furthermore, Spirulina algae form irregular aggregates surrounded by a gelatinous substance, which increases its surface area and enhances its adsorption capacity for various contaminates [42].

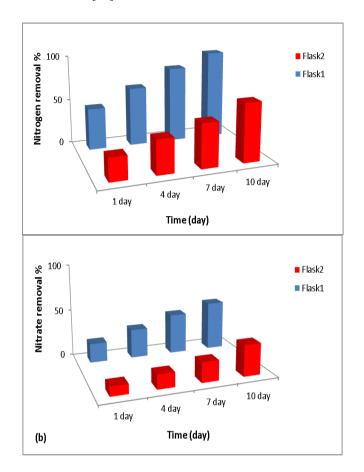


Figure 3. The removal efficiency of NO₃ for (a) Spirulina (b) Chlorella vulgaris

3.2 COD removal

It was found through research, that the effectiveness of Spirulina for removal of COD at 10th day was (86.6%), which was higher than the effectiveness of Chlorella vulgaris (50.5%)for first flask and this efficiency was increased with increasing the contact time. While the second flask that put in darkroom got the removal efficiencies of 25.4% and 54.3%, respectively for Chlorella vulgaris and Spirulina at the same day as shown in Figure 4. As previously mentioned, the environmental parameters (e.g., temperature, light period and light intensity) play a key role on the function and efficiency of algal treatment mechanisms [13, 39]. Also, the temperature has important effect on the absorption of organic materials by microalgae [43]. Spirulina is better in removal of COD than Chlorella vulgaris, this can be attributed to its large surface area of the adsorbent resulting from its undifferentiated and filamentous structure, or perhaps to its high ability for the adsorption process and the difference in the composition of cellular membranes [44].

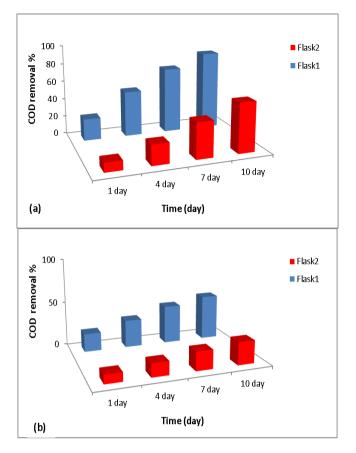
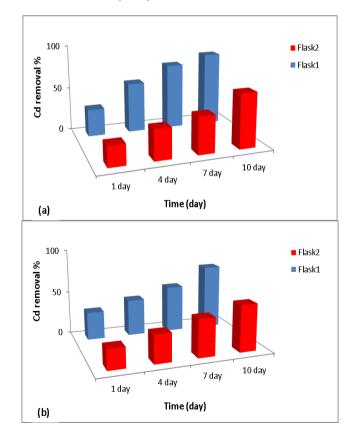


Figure 4. The removal efficiency of COD for (a) Spirulina (b) Chlorella vulgaris



3.3 Cadmium ion (Cd⁺²) removal

Figure 5. The removal efficiency of Cd⁺² for (a) Spirulina (b) Chlorella vulgaris

Figure 5 displays that the removal efficiency of Spirulina to cadmium was higher than Chlorella vulgaris for the first flask. This result shows the best removal rates are 84.5% for Spirulina and 74.6% for Chlorella vulgaris under sunlight condition at 10th day, while the second flask gets the removal efficiencies of 61.8% and 53.3%, respectively for Chlorella vulgaris and Spirulina at the same day. Algae employ biosorption and bioaccumulation for heavy metals reduction. Biosorption defined as the ability of biological materials (e.g., bacteria, algae, yeast and fungus) to adsorb metals onto their cell membrane [45]. In spite of this, bioaccumulation known as the capacity of algae to accumulate heavy metals inside their living cells [46]. Also, these mechanisms increase with time and optimum condition such as sunlight and optimum temperature [13, 45, 47] Spirulina algae are more effective in reducing Cadmium than Chlorella vulgaris algae. This may be attributed to the fact that the cell wall may contain various polysaccharides, proteins and other high-complexity components, which in turn have a high affinity for divalent positive ions, thereby increasing the biological absorption process [48].

4. CONCLUSION

In the present study, it was noticed that the Spirulina microalgae showed more removal efficiency of contaminants than Chlorella vulgaris microalgae in first flask, where the best removal efficiencies were (86.6%, COD; 99.1%, NO3 and 84.5%, Cd⁺²) and (50.5%, COD; 52.3%, NO₃ and 74.6%, Cd⁺²) for Spirulina and Chlorella vulgaris, respectively. On the other hand, Spirulina microalgae exceeded on Chlorella vulgaris microalgae in contaminants bioremediation at second flask, but with removal efficiencies slightly less than first flask. The best removal rates in second flask of Spirulina and Chlorella vulgaris to COD, NO₃ and Cd⁺² were (54.3%, 64.4%, and 61.8%) and (25.4%, 33.01% and 53.3%), respectively. It can be noticed that the removal efficiencies of microalgae are significantly affected by the surrounding condition, where the removal efficiencies increase with sunlight and suitable temperature. The response of the algae to the contaminants impact differs from one type to another, depending on the differences in the composition of the cell wall and the positive and negative ion exchange sites in the cell wall and biomass membrane. From the results, it is clear that algae have the ability to accumulate large amounts of cadmium, nitrates, and carbon within their biomass, making them a sustainable, effective and environmentally friendly option that can be used as an additional stage in wastewater treatment plants. Further studies could be conducted to optimize the suitable conditions for maximum contaminants removal, other species of microalgae could be tested to remove different contaminants.

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