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Sewage Water Treatment Using Chlorella Vulgaris Microalgae for Simultaneous Nutrient Separation and Biomass Production

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Abstract: Recovery of wastewater is essential for better management of water resources and can aid in reducing regional or seasonal water shortages. When algae were used to clean wastewater, amazing benefits were guaranteed, such as a decrease in the formation of dangerous solid sludge and the creation of valuable algal biomass through recycling of the nutrients in the wastewater. The trace elements nitrogen, phosphorus, and others that microalgae need for cell development are frequently present in contaminated wastewater. Hence, microalgal bioremediation is used in this study as an effective technique for the simultaneous treatment of COD, NH_3-N, and orthophosphate from domestic wastewater and biomass production. Different concentrations of wastewaters were used. The maximum removals attained were: 84% of COD on the fifth day using the lowest mixing ratio of 50%, 95% of ammoniacal nitrogen, and 97% of phosphorus. The highest biomass production was achieved at day 12, except for the mixing ratio of 80% where the growth rate increased until day 14 at 400 mg/L.

Keywords: bioremediation; wastewater treatment; circular economy; biomass; microalgae

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1. Introduction

The concentration of substance rises or undesired mixtures are discharged into the environment, which are both examples of man-made activities that cause contamination [1]. The creation of sewage is a reflection of innovations and ways of life that are publicly practiced. Wastewater treatment typically only reaches secondary levels in underdeveloped nations. However even after secondary treatment, wastewater still has a large quantity of untreated phosphorus and nitrogen in the effluents, which leads to eutrophication, increased turbidity, and poisonous lakes [2,3].

When such wastes are disposed of in nearby bodies of water, they cause a change in pH and a decrease in dissolved oxygen concentration, resulting in the death of aquatic life forms. Excess nitrate concentration also interferes with water disinfection capacity. Thus, removing nutrients and toxic substances to meet acceptable limits is critical in wastewater treatment prior to discharge or reuse [4]. The most common category of contaminants in the world today is heavy metals (HMs). Even at extremely low concentrations, these hazardous compounds can pose serious health risks to people and environmental deterioration [5–9].

Water and wastewater remediation become necessary and thus selecting the optimum treatment approach is required to achieve the required treatment levels [10,11]. Conventional methods including different physical and chemical processes have previously been widely used in several studies [12]. Chemical treatment methods are considered the most effective wastewater treatments, and include: chlorination, coagulation/flocculation, UV radiation, and ozonation [13]. Despite the fact that these methods are more effective when they are employed in wastewater treatment, the main drawbacks of these technologies are their high costs, dewatering restrictions, and high maintenance requirements [14]. Contrarily, biological methods rely on the metabolic processes of microorganisms to break down and transform wastewater contaminants into biomass and related gases (CO_2, CH_4, N_2, and SO_2). As a result, BOD and COD levels in the effluents are reduced, and their quality is increased [15].

Biological treatments use a variety of microorganisms including bacteria, fungus, yeast, and microalgae for biodegradation bleaching [16–18]. Under aerobic or anaerobic conditions, nearly all biological methods are inexpensive. However, several limitations associated with biological methods such as the ineffective removal of pollutants, pollutants with complex chemical structures are resistant to degradation using conventional methods, and biological wastewaters only provide a limited amount of flexibility in terms of design and operation over the long run, have been reported [19]. Biological treatments are often applied as a secondary treatment in order to reduce the BOD levels, while in the wastewater in which the contaminants can mainly be removed by chemical treatment, the biological treatment is applied as a post-treatment step to principally remove contaminants that are still present after the main treatment process [11].

Nitrogen, phosphorus, and carbonaceous pollutants have been removed from wastewater by microalgae. In addition, advanced and post-treatment procedures that aim to enhance the quality of water after the biological treatment can also include algae [20]. While algae require phosphorus and nitrogen for development, they may also quickly treat wastewater. In addition, a high-value product can be made from the algal biomass that is created as a result of the treatment [21]. Another benefit is the ability to use the expanding microalgae mass as an energy source while also removing phosphate and nitrogen [22].

Numerous microalgal species, including *Scenedesmus*, *Chlorella*, *Botryococcus*, *Phormidium*, *Limnospira*, and *Chlamydomonas*, have been shown to be effective in the bioremediation of nutrients, emerging contaminants, and pathogens from wastewater [21,23,24]. Usually, several microalgae species have grown successfully in wastewater, including *Scenedesmus*, *Chlorella*, *Euglena*, *Oscillatoria*, *Chlamydomonas*, and *Ankistrodesmus* [25]. As a result, treatment methods based on microalgae have recently attracted a lot of interest in treating municipal, industrial, and agro-industrial wastewater. Through the removal of phosphorus and nitrogen constituents, microalgae can reduce the danger of eutrophication [21,26]. These are regarded as multipurpose biological treatment solutions that convert undesired inorganic and organic elements into valuable biomass [27]. In addition, the microalgae that may be harvested from specific treatment ponds can be utilized as a source of food and feedstock for several things as well as play a variety of functions in a wide range of sectors.

During the last few decades, several studies have reported that different algae species are powerful in removing different parameters of wastewater such as COD, BOD, nitrogen, and phosphorus [28–30]. The main mechanism in algal nutrient removal from wastewater is cell uptake and ammonia stripping via increased pH [31]. Several studies related the removal of phosphorus, nitrogen, and ammonia by algae with the quantized fixation of CO_2. For example, the treatment of industrial wastewater using *Chlorella* sp. was reported by Tarlan et al. [32]. The authors revealed that *Chlorella* sp. was able to treat 80% of absorbable organic xenobiotics, 58% of COD, and 84% of color. Silambarasan et al. [33] used a combination of *Chlorella* sp. and *Scenedesmus* sp. for domestic wastewater treatment. The results showed that the microalgal consortium exhibited a greater potential in nutrient removal (78–98%) from domestic wastewater. However, optimal microalgae cultivation and biomass production should be further investigated to identify its suitability to treat sewage water at different concentrations and the best time for harvesting.

The main objective of this study was to evaluate the optimal microalgae cultivation and biomass production using *Chlorella vulgaris* and different concentrations of sewage water.

The ideal microalgae growing period for achieving the maximum biomass output and best treatment effectiveness for COD, NH_3-N, and orthophosphate were also determined using comparable data.

2. Materials and Methods

2.1. Microalgae Culture

The *Chlorella vulgaris* microalgae stock were maintained in a 500 mL conical flask filled with Bold's Basal Medium (BBM) [34]. The BBM culture media was autoclaved at a temperature of 121 °C for 15 min. Before the study on the wastewater treatment, the *Chlorella vulgaris* microalgae were pre-cultivated in 10 L water medium that added in 5 mL fertilizer. The microalgae were cultivated under continuous light illumination and an air-bubbling system of ~1.5 L/h at room temperature. After pre-cultivation, the stock was used for the experiment in 500 mL conical flasks. The initial biomass for the stock solution was 489 mg/L.

2.2. Wastewater Sample Collection and Characterization

Raw wastewater samples were collected from a domestic sewage plant facility and placed into high-density polyethylene (HDPE) plastic bottles that had already been cleaned. In order to reduce biodegradation and chemical reactions in the environmental laboratory, the sample was held at 4 °C. Prior to conducting experiments, general characteristics were carried out to investigate the main parameters of wastewater. COD was measured in mg/L using DR 890 Portable Colorimeter (Hach, CO, USA) following the USEPA Reactor Digestion Method 8000 [18]. pH was measured using the HI2550 pH/ORP and EX/TDS/NaCl Benchtop Meter (Hanna Instruments, London, UK). The pH meter was calibrated each time before use, using standard solutions of pH 4.01, 7.01, and 10.01, respectively. Ammoniacal nitrogen (NH_3-N) concentrations were tested using DR3900 Spectrophotometer (Hach, USA) following the Salicylate Method 10023 and 10031 [35]. Reactive phosphorus, orthophosphate, was also measured using DR3900 Spectrophotometer (Hach, USA) following the USEPA PhosVer 3 (Ascorbic Acid) Method 8048 [35]. After wastewater treatment, the same parameters were tested to investigate the efficiency of microalgae in wastewater treatment. The characteristics of collected sewage are displayed in Table 1.

Table 1. The characteristics of collected sewage.

Parameter	Unit	Value
COD	mg/L	93
NH_3-N	mg/L	6.2
Color	Pt Co	82
Orthophosphate	mg/L	12.8
pH	mg/L	7.45

2.3. Wastewater Sample Preparation

Borosilicate conical flasks, 500 mL, were used for the experiment. Each conical flask contained a total of 250 mL solution consisting of varying amounts of wastewater, microalgae stock solution, and distilled water (in case the sample was diluted). Prior to being used, the collected wastewater sample and the microalgae stock solution were, respectively, mixed thoroughly via stirring to ensure homogeneity. In this experiment, 5 groups of different dilutions were used, resulting in wastewater concentrations of 50%, 60%, 70%, 80%, and 90% by volume. A constant amount of 25 mL microalgae stock solution was used in each sample as presented in Table 2. The amount of wastewater and distilled water varied in each sample to create the desired dilution. For each sample, duplicates were applied to determine the harvested biomass on the initial day and desired days. The experiment was carried out for 19 days; thus, to harvest biomass on days 0, 2, 5, 7, 9, 12, 14, 16, and 19, a total of 9 duplicates of the same sample were applied.

Table 2. Wastewater concentration and its proportions.

Wastewater Concentration (%)	Wastewater Sample (mL)	Microalgae (mL)	Distilled Water (mL)	Total Volume (mL)
50	125	25	100	250
60	150	25	75	250
70	175	25	50	250
80	200	25	25	250
90	225	25	0	250

2.4. Experimental Setup

Figure 1 shows the setup of each conical flask. Each conical flask was supplied with air bubbling with an airflow of ~0.3 L/min.

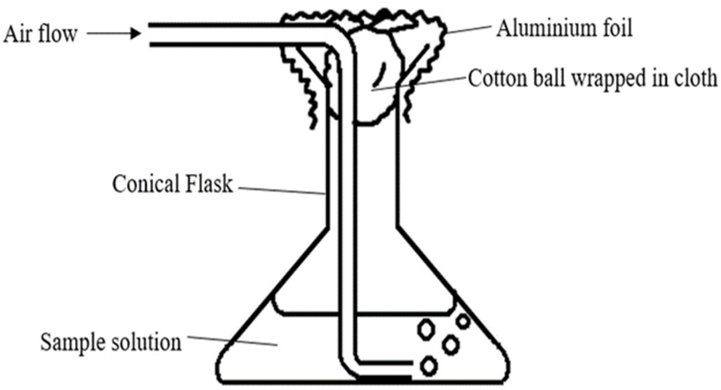

Figure 1. Setup of each conical flask.

The mouth of the conical flask was stoppered and wrapped with a layer of aluminum foil. The overall setup of the experimental area is as shown in Figure 2. A rack was used to accommodate the apparatus, where each layer was installed with a fluorescent lamp.

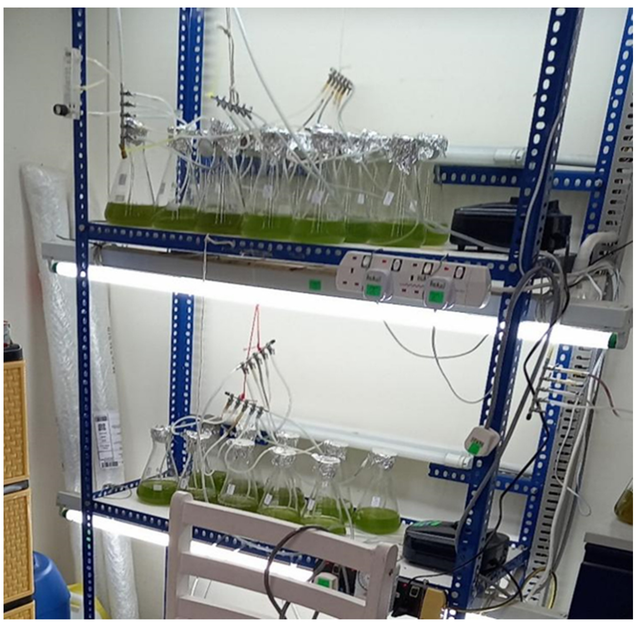

Figure 2. Experimental setup.

Air flow was supplied using aquarium air pumps (SOBO SB-948), where each pump can supply 4 air flows through major air tubes having an inner diameter of 4.5 mm, where each major tube supplies air flow to 5 conical flasks using a 5-way air flow control splitter (Figure 3) into 5 minor tubes with an inner diameter of 2 mm. Each minor tube connection was wrapped with parafilm to ensure a tight connection with minimum air escape.

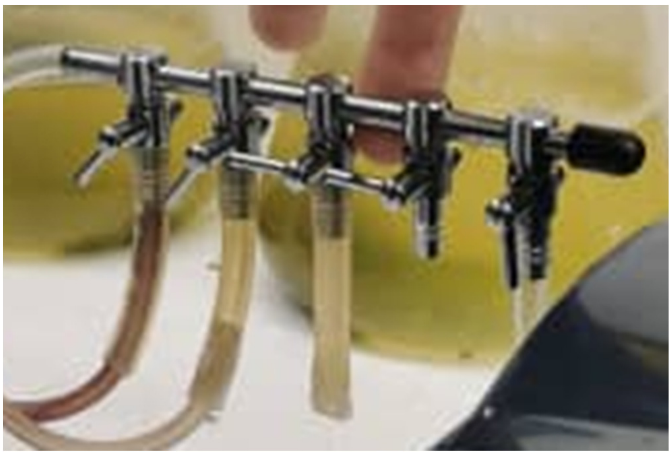

Figure 3. A 5-way air flow control splitter.

2.5. Biomass Collection and Characterization

Biomass was collected using centrifugation for 10 min at 3000 rpm in 50 mL centrifuge tubes. The centrifuge machine model was Z326K Laboratory Centrifuge (Hermle Labortechnik, Wehingen, Germany). The conical flasks were shaken and stirred to ensure the homogeneity of the sample and that biomass did not adhere to the flask walls or base. A 200 mL of sample, otherwise the whole amount if insufficient, was centrifuged for each sample. Part of the sample was centrifuged each round, and the drained supernatant was collected for characterization, subsequently adding the same sample into the same centrifuge tube to accumulate all of the biomass. The accumulated biomass was dried in an oven at 60 °C for ~16 h using the XU032 Universal Oven (France Etuves, Chelles, France) and weighed on the AUX320 Analytical Balance (Shimadzu, Kyoto, Japan). The temperature should not exceed 60 °C as it is the optimum temperature to retain the best concentration of triacylglycerol (TAG) and lipid yield [36,37]. The obtained dried biomass was used for characterization to obtain more data on the biomass.

3. Results and Discussion

3.1. Sewage Water Treatment

By using laboratory-scale air-bubbling reactors, it was determined how well microalgae performed in eliminating COD, NH_3-N, and orthophosphate over the course of 19 days. To assess their impact on the effectiveness of the targeted parameters' elimination, several wastewater mixing ratios, including 50%, 60%, 70%, 80%, and 90%, were used. Substantial COD removal was noted on day two of aeration, and the highest removal (84%) was attained on day five utilizing the lowest mixing ratio of 50%; then the removal declined after day five of aeration as can be seen from Figure 4. The negative removal for COD at day 19 may be attributed to the decay of the accumulated biomass and led to an increase in the organic concentration [38].

Due to the substantial amount of oxygen given by microalgae and the heterotrophic growth of bacteria present in wastewater, COD may have been removed [39,40]. Biological oxidation of the carbon atoms can result in the production of CO_2 [41]. In order to remove COD from concentrated wastewater, Rani et al. [42] observed synergistic connections between a microalgae strain and bacteria. According to the study, two days of incubation with 700 mg/L microalgae and 200 mg/L sludge resulted in a 67% elimination of COD.

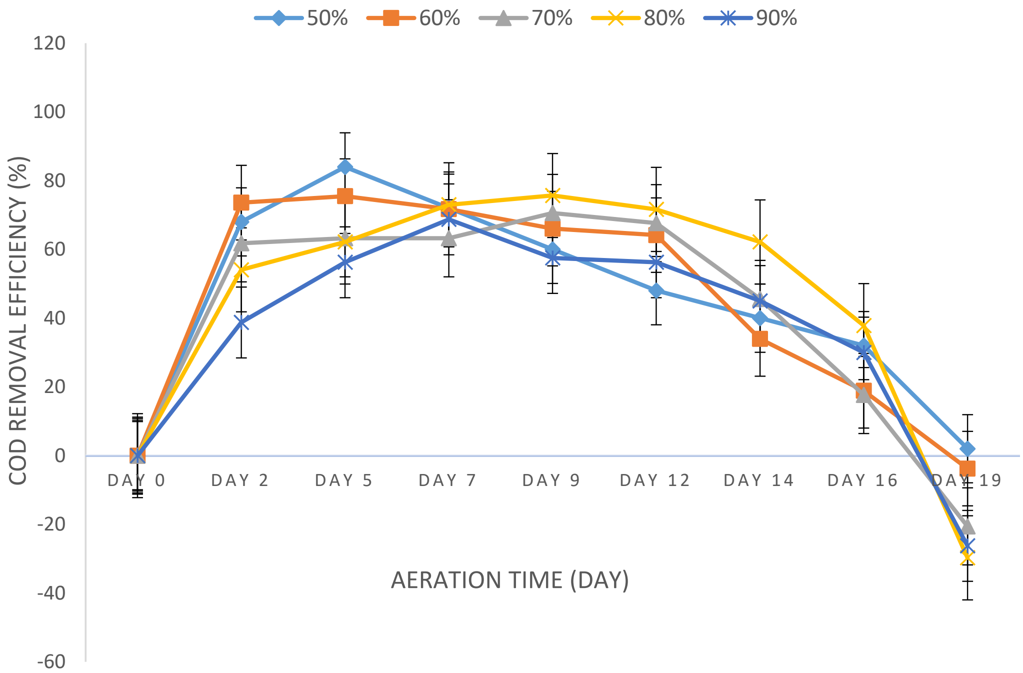

Figure 4. The effect of wastewater mixing ratio on COD removal efficiency.

Using microalga-assisted wastewater, about a 25% reduction in COD was reported by Halfhide et al. [40]. Nevertheless, microalgae's ability to remove COD from wastewater is limited by the amount of light, aeration, and initial concentration of the wastewater. Although highly concentrated wastewater was used in earlier research to boost COD removal [42], greater mixing ratios did not increase the removal efficiency in the current study. This may be attributed to the high concentration of suspended particles and turbidity, which decreased light intensity and inhibited the growth of biomass [43].

Even when utilizing varying wastewater mixing ratios, the removal of phosphate and ammoniacal nitrogen was not seen during the initial incubation days as shown in Figures 5 and 6. After the second day of incubation, their removal was first noticed, and the highest removal of ammoniacal nitrogen of 95% was reported on the seventh day with a sewage ratio > 50% as illustrated by Figure 5. During the 12th day of incubation, the maximum clearance for phosphorus was achieved at 97% as shown in Figure 6. A substantial amount of ammonia that is not removed completely during aeration may become volatile.

Concalves et al. [44] observed that 2–3 mg/L of ammonia was volatile during 30 h of microalgae cultivation for wastewater treatment. The influence of the mixing ratio was not reported as making a significant difference to the phosphorus removal at day 12, but some significant differences in the removal were observed during the incubation periods between the 2nd and the 12th day. The concentration and availability of N and P may have a significant impact on the microalgal metabolism and increase biomass production, which may enhance the nutrients' uptake from wastewater [45]. There is a correlation between the intake of N and P. Phosphorus increases nitrogen intake, and when there is an excess of phosphorus available, algae can absorb it (luxury uptake) in the presence of nitrogen [46]. The pH value affects the nutrients' availability. It influences the solubility of ammonium or phosphate as well as a precipitate formation at a pH value higher than 8 [47]. In the current study, the pH level was monitored daily. The initial pH varied between 7.38 and 7.78 using all wastewater mixing ratios (50%, 60%, 70%, 80%, and 90%). Between the 2nd and 7th day of aeration, the pH rose to 8.03 and 8.38 utilizing a wastewater mixing ration higher than 60%, while the highest pH value (8.64) was reported at day 14 of aeration using the 80% mixing ratio. Luo et al. [48] reported around 78% and 91% removal of nitrogen and phosphorus from wastewater using microalgae. Duan et al. [49] evaluated the performance of eight microalgae species and confirmed the nutrient absorption capability of microalgae from wastewater. The reduction in the removal of COD, NH_3-N, and orthophosphate after the 12th day of incubation is attributed to the die-off process in microalgae due to the oxygenation and food limitation [42], and led to the release of nutrients, which may affect the pH level.

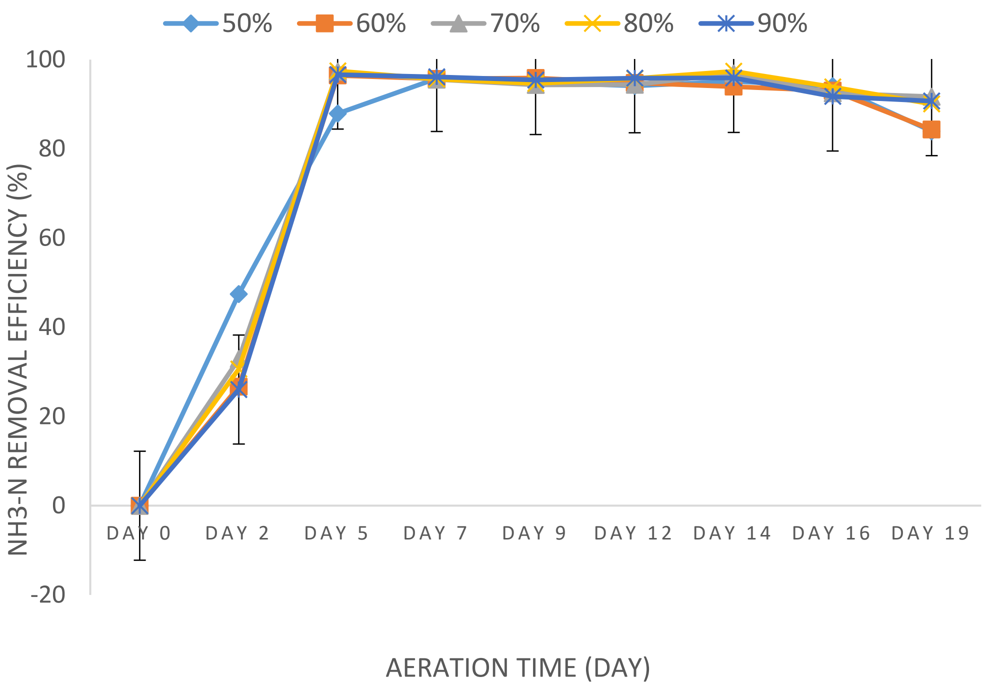

Figure 5. The effect of wastewater mixing ratio on NH_3-N removal efficiency.

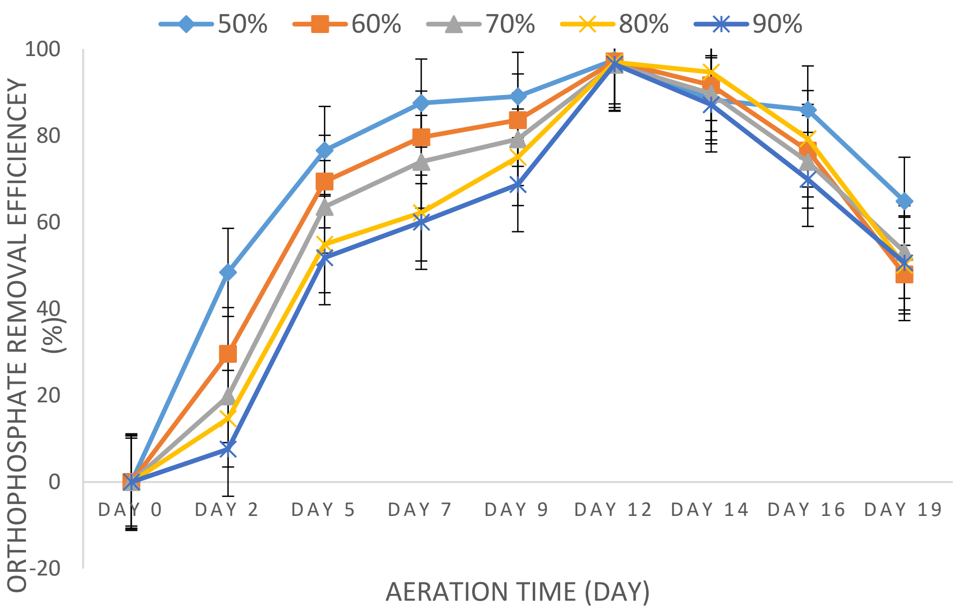

Figure 6. The effect of wastewater mixing ratio on orthophosphate removal efficiency.

3.2. Biomass Production

The influence of different wastewater mixing ratios on the microalga growth rate was observed and evaluated (Figure 7). During the 1st and 2nd day of aeration, a limited growth of biomass was observed utilizing all wastewater mixing ratios. Most wastewater ratios reported the highest biomass rate at day 12, except in ratio 80% where the growth rate increased until day 14 at 400 mg/L. The growth rate of microalga depends on several factors such as the lighting period and intensity, temperature, pH, and nutrient concentration [42].

However, continuous lighting may affect biomass growth due to the diurnal light–dark cycle [50]. Moreover, the limitation in inorganic nitrogen may cause cessation of the microalga growth rate [42]. On the other hand, light penetration can be limited due to the high density of microalga.

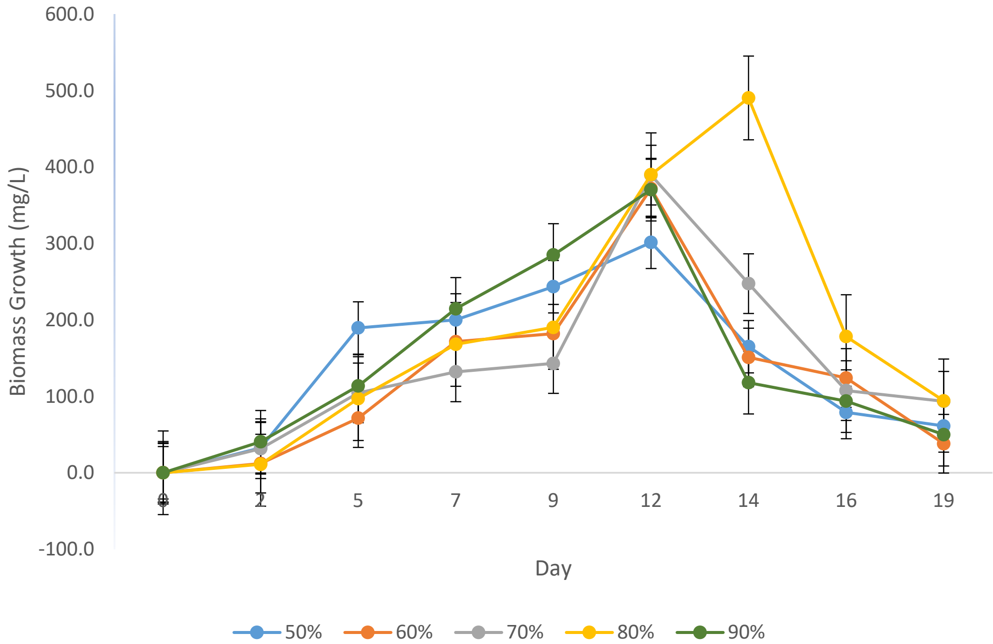

Figure 7. The effect of wastewater mixing ratio on biomass growth.

Subsequently, utilizing bead mill disruption and extraction with isopropanol solvents, this biomass can be transformed to yield fatty acids high in poly-unsaturated fatty acids. As an alternative, the hydrolyzed microalgae biomass can be used as useful fish feed. Several microalgal genera and species produce secondary metabolites that have biocontrol, pesticidal, or insecticidal qualities that are used in agriculture [51].

4. A Large-Scale Microalgae-Based Wastewater Treatment Plant

Microalgae have been utilized to treat wastewater because of their ability to utilize both organic and inorganic carbon, nitrogen, and phosphorus while also accumulating biomass and reducing N, P, and chemical oxygen demand (COD) in the wastewater [52].

Since algae's photosynthesis produces a lot of oxygen without using any electricity and also absorbs nutrients, algae ponds are a desirable solution for wastewater treatment (WWT) to eliminate impurities. Microalgae are particularly appealing for bio-treatment because they are capable of photosynthesizing, transforming solar energy into useful biomasses, and incorporating nutrients that cause eutrophication [53].

There are only a few illustrations of industrial systems based on suspended microalgae cultures. For the treatment of smaller amounts, immobilized systems are also commercially available. Unfortunately, the use of large-scale microalgae-based wastewater treatment plants is not yet widespread. This may be the result of a number of technical and engineering difficulties with large-scale algal culture systems and harvesting techniques, all of which are crucial for the design and operation of high-rate algae cultures to create high-value products. Indeed, various elements influence the removal of nutrients from wastewater and the growth of microalgae [51,53]. Furthermore, since microalgae are photosynthetic organisms, environmental elements such as light, temperature, and pH have a big impact on how effectively they can treat sewage. Major difficulties include maintaining optimal temperature, pH range, and light intensity and duration.

These elements should be carefully managed and controlled in large-scale systems to produce the intended product in the best possible way.

4.1. Environmental Conditions

In order to make the process cost-effective, the placement of the algal-based wastewater reclamation system is crucial. Seasonal variations in day length and light levels depend on the environment and have a significant impact on algal communities and their macromolecular structures [51]. To grow at their fastest rate possible, microalgae need to be exposed to a certain amount of light, known as the saturating light level. If the light intensity is significantly higher than the saturation point, growth will be inhibited (photoinhibition). Conversely, growth will be light-limited if the light intensity is substantially below the saturation level (light limitation) [53].

One of the most significant environmental elements influencing the growth rate and biochemical makeup of algae is temperature because of its impact on microalgal development. The ideal temperature for mesophilic species growing outside in a wastewater treatment facility was determined to be between 20 and 25 °C. For thermophilic strains (*Chaetoceros* sp., *Anacystis nidulans*), this temperature increased up to 40 °C, while for psychrophilic strains, it decreased to 17 °C (*Asterionella formosa*) [54]. According to Wang et al. [55], lowering the temperature below the ideal range may result in more lipids becoming unsaturated.

In warmer areas, using wastewater reclamation based on algae has its own difficulties. Growth and productivity were significantly reduced when temperatures were above the ideal range for growth [56]. Long-term exposures to excessive light levels and direct sunlight can also result in photodamage and photoinhibition, which eventually impede cell growth in algal cultures [57]. It is vital to keep in mind that evaporation increases in hotter climates, which causes increased salinity, which causes osmotic and cellular ionic stress [51].

4.2. Concentration of Wastewater Parameters

Wastewater-specific effects on microalgal metabolism result from variations in nitrogen or phosphorus concentration and availability. During N starvation, many microalgae will still produce biomass, but they will produce more lipids and/or carbohydrates and less protein [45]. There is a correlation between the intake of N and P. Phosphorus facilitates the intake of nitrogen, and an excess of phosphorus can be absorbed by the algae in the presence of nitrogen [58]. Due to its impact on nutrient removal and microalgal development, wastewater color plays a significant role in wastewater cleansing. Dark brownish-grayish and opaque wastewater (such as animal waste, pulp, and paper) exhibit significant levels of light absorption, which restricts the growth and uptake of nutrients by microalgae [52]. The pH value affects the nutrients' availability [47]. For instance, it influences the solubility of ammonium or phosphate as well as precipitate formation. Calcium phosphate can develop at pH levels of 9 or higher, which is inaccessible to microalgae. Thus, before wastewater may be utilized as a growth medium, it must be corrected for its low pH and salinity. The growth of some microalgae in raw wastewater, however, may be restricted by a high organic content and heavy metals, leading to a longer retention time [51,53].

4.3. Harvesting

One of the primary barriers to an effective, sustainable, and inexpensive operation still exists in the fact that promising downstream process applications (such as harvesting) must assure cost-effective wastewater treatment for large-scale operations [51,59].

One of the main challenges for reasonably priced wastewater treatment and its downstream processing has been recognized as algae biomass extraction [59]. Both upstream and downstream processes are impacted by the harvesting method in terms of system design and operation. Due to the small size of microalgal cells, and their negative surface charge, which prevents them from forming larger and easily harvestable particles, more than one stage of harvesting is typically needed to separate the microalgal biomass from its aqueous environment [59,60]. The entire cost of harvesting algal biomass and using it to clean wastewater is greatly increased by these factors. According to estimates, the cost of harvesting algae can account for up to 30% of overall production costs [51]. This is because the process requires a lot of energy, depending on the method used. Moreover, harvesting and dewatering equipment accounts for up to 90% of the expenditures associated with inventory [61]. A variety of methods for harvesting algae have been created and are extensively utilized, but each one has advantages and disadvantages that motivate research toward more practical, affordable, and straightforward procedures [51,59].

5. Conclusions

The demand for food, energy, technology, and fresh water all rise as the world's population expands. This causes both home and industrial sources to produce more wastewater as a result. These various wastewaters contain a wide range of organic and inorganic substances that, if released untreated, can have serious negative effects on the environment. Conventional treatment methods are frequently pricy, energy-intensive, and unable to address all problems caused by the produced wastewater. Microalgae are potential candidates for wastewater reclamation because they can reduce the levels of nitrogen, phosphate, and other harmful substances such as organic compounds. Microalgae are widespread, microscopic organisms that flourish in bodies of water that contain essential nutrients. Sewage water is frequently contaminated with nitrogen, phosphorus, and other trace elements that are essential for cell growth. In order to effectively treat wastewater, microalgal bioremediation could be combined with current treatment techniques or used alone. In this work, the method of growing microalgae for concomitant sewage treatment and biomass production was examined. In order to simultaneously produce biomass and treat COD, NH_3-N, and orthophosphate from domestic wastewater, microalgae were used as an efficient technology. Various sewage water concentrations and cultivation times were employed. The highest removals were: 95% ammoniacal nitrogen, 97% phosphorus, and 84% COD. The maximum biomass production rate was obtained at day 12 with the exception of an 80% mixing ratio, where the growth rate increased until day 14 at 400 mg/L when the maximal biomass production was reached.

Notwithstanding the efforts made in this subject, there are still a lot of uncertainties in large-scale cultivation systems. This also highlights the necessity to broaden and intensify the research into the specifics of the affecting parameters. When compared to the traditional treatment system, more research is needed to identify the underlying causes that contribute to the treatment and removal of nutrients from the system because of the process's multiplicity of components.

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Conflicts of Interest: The authors declare no conflict of interest.

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