ISSN: 3067-8366
Research Article
Journal of Environmental Science, Sustainability, and Green Innovation

Coelastrella-Scenedesmus Consortium for Protein & Bioindicator

Kamalendu De*

Assistant Professor, Midnapore City College, Department of Biological Sciences (Botany), Midnapore, West Bengal, India

***Corresponding author:** Kamalendu De, Assistant Professor, Midnapore City College, Department of Biological Sciences (Botany), Midnapore, West Bengal, India

Submitted: 09 May 2025 **Accepted:** 16 May 2025 **Published:** 23 May 2025

https://doi.org/10.63620/MKJESSGI.2025.

Citation: *De, K. (2025). Coelastrella-Scenedesmus Consortium for Protein & Bioindicator. J Environ Sci & Sustain & Green Innov, 1(2), 01-14.*

Abstract

The handling of wastewater has long been a contentious issue. Microalgal consortia are realistic and sustainable options for advanced wastewater treatment since they produce single-cell proteins in addition to high-value commodities. Unavoidably, this process is already in motion, but it proceeds too slowly to allow for prompt recycling. Microalgae consortia can be more effective at detoxifying organic and inorganic pollutants and removing nutrients from wastewater than single microorganisms. The "bioaugmentation" process entails adding nutrients or other microorganisms with specific degrading abilities to microbial populations that clean wastewater. In this experimental setup of the algal consortium, two strains of algae, Scenedesmus and Coelastrella sp., which are members of Chlorophyceae, were employed. The effluent wastewater of Bengal Chemical, Khardaha, 24 Pgs N, W. B. facility was used as the nutritional media in this investigation. A consortium created with these two alga strains at a 1:1 ratio of biomass achieved maximum growth on the 6th day of cultivation. According to the results, biomass, protein, and chlorophyll contents of 3.71 mg/ml, 500.50 µg/mg, and 111.26 µg/mg, respectively, were produced by this synergistic combination. However, antagonistic effects occurred when both the 2:1 and 1:2 combinations of primary inoculums were used, which resulted in less of the desired product. At a 1:1 ratio, the Coelastrell and Scenedesmus consortia outperformed the other combinations in the removal of phosphate and ammonium from wastewater, with removal rates of 65% and 82%, respectively.

Keywords: Biomass, Biomolecules, Wastewater, Consortium, Coelastrella Sp. Scenedesmus Sp.

Introduction

Large amounts of wastewater have been produced in recent decades, primarily as a result of anthropogenic activities such as agricultural practices, urbanization, and industry [1, 2]. More than 300 km3 of wastewater is produced globally each year due to the ongoing acceleration of urbanization and industrialization processes; this amount is equivalent to one-seventh of the volume of the entire world's rivers [3]. Without proper treatment, the continuous discharge of wastewater can cause serious pollution problems. Microalgae can effectively absorb nitrogen and phosphate from wastewater according to several published experimental results, and these microorganisms require these nutrients to grow. Hence, it is possible to remediate biodegradable waste products utilizing a microbial regime, which is more cost-effective and environmentally benign than chemical treatment is. The definition of consortia for bioremediation has evolved as a result of the vast amount of research on wastewater treatment that has been conducted in recent decades. The ability to remediate contaminated soil and water using a method that encourages the biodegradation and bioremediation of contaminants is perhaps the most comprehensive definition of microbial consortia. Interestingly, biodegradable waste products connect-

ed to organisms behave physiologically differently from their comparable planktonic counterparts. According to recent studies, this method is the best at removing phosphates and ammonium from contaminated water while also using algal biomass to produce commodities with high economic value. The so-called eutrophication phenomenon, which is the enrichment of water resources in nutrients, primarily nitrogen and phosphorus, is one of the key issues connected to the continual discharge of effluents into water bodies [4]. This phenomenon causes algal blooms, aquatic plant growth, oxygen depletion, and the extinction of important species, which completely degrade freshwater ecosystems. In addition to harming freshwater ecosystems, the growth of these blooms is a public health concern. However, a number of regional organizations and countries have also enforced phosphorus restrictions in a number of water bodies to prevent eutrophication. The United States Environmental Protection Agency (USEPA, 1986) states that the total P concentration should not exceed 50 µg/L in streams that reach lakes or reservoirs or 25 µg/L inside lakes or reservoirs [5]. Nitrates can harm fish by causing a disease known as "brown blood illness." The hemoglobin present in human blood as well as the blood of other warm-blooded animals interacts directly with nitrates to form methemoglobin. Methemoglobin reduces the oxygen-carrying capacity of red blood cells. Sickness is especially dangerous for infants younger than three months of age. Methemoglobinemia, popularly known as "blue baby disease," is the outcome. Nitrate is commonly recorded as nitrate nitrogen (NO3-N) in the United States; a concentration of 3 mg/L or more indicates contamination. The preparation of infant formula or other foods using water containing nitrate or nitrite concentrations greater than 10 mg/L or 1 mg/L, respectively, is not recommended for children younger than one [6]. Total N and P levels in wastewater from cities, farms, and agricultural effluent ranges from 10 to 100 mg/L, >1000 mg/L, and 500 to 600 mg/L, respectively [7]. This demonstrates the urgent need for efficient treatment processes that can lower nitrogen and phosphorus concentrations in wastewater before releasing them into natural bodies of water [8]. The removal of nitrogen and phosphorus can also be accomplished chemically, for example, by precipitation using alum and iron salts. However, these techniques are expensive. Biological treatment utilizing microalgal consortia has been extensively studied in recent decades to overcome the drawbacks associated with commonly used tertiary treatment methods [9]. With respect to consortium partnerships, there are three different types of partnerships: algae-algae, algae-bacteria, and algae-fungi. However, an algae-algae consortium is more efficient at removing heavy metals, phosphate, and nitrogen from wastewater. Several published experimental results revealed that microalgae need a large amount of nitrogen and phosphate for development, and these microorganisms are capable of efficiently absorbing these nutrients from wastewater. Indeed, microalgae have been shown to have high nitrogen and phosphorus removal efficiencies (80–100%) from a variety of wastewater sources, including agricultural, industrial, and municipal sources [10]. The water and nutrients needed to grow algae are readily available in wastewater. Microalgae offer an affordable and sustainable method for advanced wastewater treatment while simultaneously producing goods with high economic value [11]. Because the nutrients (ammonia, nitrate, phosphate, urea, and trace elements) contained in different wastewaters are crucial for microalgal growth, microalgae exhibit greater effectiveness in nutrient removal than other microorganisms [12]. Microalgae can thrive in a variety of environmental circumstances, making them a viable source of sustainable biomass feedstock for the production of single-cell proteins and other value-added products [13]. Additionally, there are a number of benefits to using microalgae to remove nutrients from wastewater: (i) nitrogen and phosphorus ingested by microalgae can be recycled when algal biomass is used as a fertilizer; (ii) generated biomass is used as a raw material in biorefinary systems for the production of food, feed, fuel, pharmaceutical, and nutraceutical products; and (iii) less polluted and oxygenated wastewater is released into natural water bodies [14, 15]. Potent microalgal consortia have been documented for a variety of applications, including biomass generation and nutrient removal. Moreover, algae can grow in three different ways, namely, autotrophy, heterotrophy and mixotrophy, considering the nature of their energy and carbon sources. Algal farming is economical and not climate dependent. Growing bacteria and fungi require a specific climate and are expensive. Again, fungi are connected to the production of mycotoxins. Consequently, the pelleted biomass of a fungal and algae mixture should not be taken into account for SCP creation. Chlorophyll molecules, which are important pharmaceutical and natural colorants, can be found in abundance in algal consortia. Microalgae are an incredibly appealing method for treating environmentally friendly and reasonably priced wastewater because they can grow well in nutrient-rich conditions, utilize nutrients effectively, and gather metals from wastewater. The benefit of the algae–algae consortium is outlined in Fig. 1 for clarity. The use of polycultures for nutrient removal is advantageous because mixing microorganisms with various metabolic capabilities and environmental adaptations enables the development of a strong biological system that can function under a variety of environmental conditions and nutrient loads. Additionally, the microorganisms that form consortia can establish cooperative contacts with one another, which may boost nutrient uptake rates [2]. The "bioaugmentation" procedure includes supplementing microbial communities that clean wastewater with nutrients or microorganisms with particular degradation skills. Additionally, bioaugmentation improves the effectiveness of treating industrial and municipal wastewater. Therefore, in this research, the choice of potential microalgal species that may flourish in sewage and produce a significant amount of biomass was taken into account. Several experimental results have shown that Scenedesmus, Chlorella sp., and Chlamydomonas can grow and actively deplete inorganic molecules present in wastewater [16, 17]. The alga Coelastrella sp. has special adaptive characteristics, such as being capable of withstand extreme dehydration, salt stress, and thermotolerance, as well as powerful wastewater valorization. However, none of the researchers studied consortia using a combination of Coelastrella sp. and Scenedesmus sp. This research article provides a systematic description of the algal consortia of two strains, Scenedesmus and Coelastrella sp., both of which are Chlorophyceae. These strains were grown under laboratory conditions in the effluent wastewater of 'Bengal Chemical's' Khardaha, 24 Pgs N, W. B., which is used as a nutrient medium for biomass production for single-cell protein generation and successful removal of nutrients from wastewater. There were three limitations in this research strategy. The selection of the algal strains for consortium preparation and their interactions posed the first limitation, followed by their combination and, finally, the absence of prior data from the Scenedesmus and Coelastrella

consortium studies. The main goal of this work was to simultaneously remove phosphate and ammonium while producing single-cell proteins and high-value commodities using microalgal consortia, which is a realistic and long-lasting solution for advanced wastewater treatment.

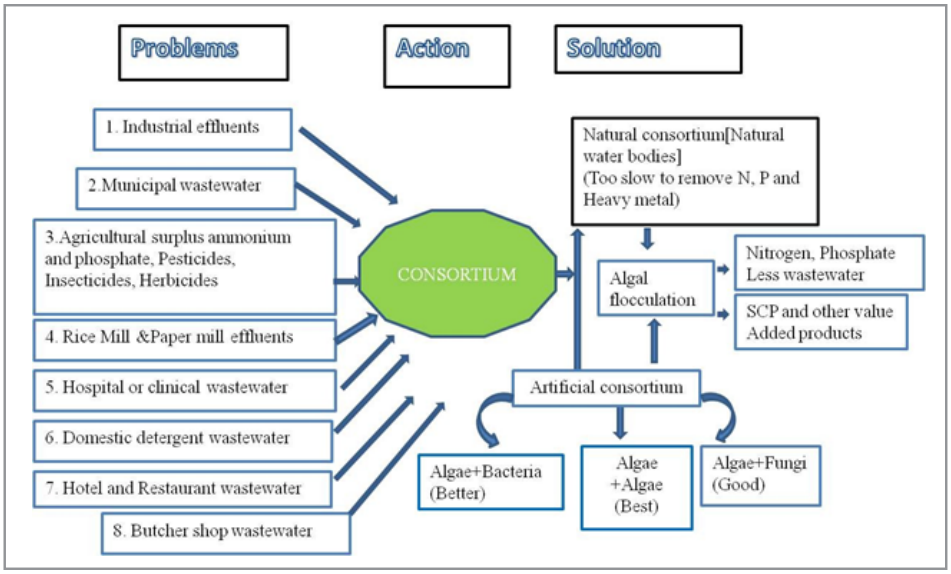


Figure 1: Conceptualization of consortium benefits for wastewater treatment

Materials and Methods

Wastewater Collection

The wastewater used in this study was collected from the industrial effluent wastewater of Bengal Chemical's Khardaha, 24 Pgs N, W. B (Latitude-22.71861 and Longitude-88.37806) and stored immediately at 4°C to minimize substrate decomposition. After that, the collected sample water was sent to the laboratory of JIS UNIVERSITY, Agarpara, Kolkata, West Bengal, India, prior to starting the experiment.

Algal Strain

For this experiment, the algal strain Coelastrella sp. (DK1) was previously collected from the K-ecosystem (Kangsabati River). Through serial dilution and repeated streak plate methods, the algal strains were isolated. Another algal strain, the Scenedesmus sp., was collected from the Molecular Engineering and Biotechnology (MEAB) laboratory of the Biotechnology Department. JIS University, Agarpara, Kolkata, and West Bengal, India. Proper identification of these algal strains was performed by the 18S rRNA sequencing method. These two algal strains were grown in BBM media on a rotary shaker at 120 rpm and 24±2°C under continuous illumination with white LED light. These algal strains were subsequently used to study the algal consortium.

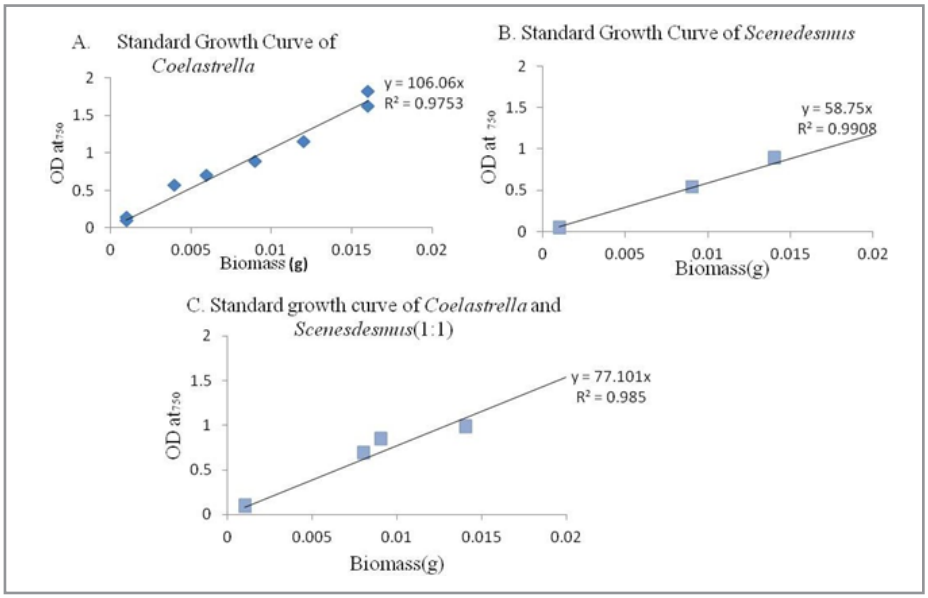


Figure 3. A: Standard growth curve of Coelastrell sp. B. Standard growth curve of Scenedesmus sp. and C. Standard growth curve of Coelastrella + Scenedesmus sp.(1:1)

OD-vs Biomass Standard Curve and Preparation of Algal Consortium

OD vs Biomass Standard Curve: To establish the standard curve of OD vs biomass of Coelastrella sp., Scenedesmus and equal volumes of both Coelastrella and Scenedesmus algal strains were cultivated under batch cultivation in a 250 ml conical flask with 100 ml of BBM broth on a rotary shaker at 120 rpm and 24±2°C under continuous illumination using white LED light. After that, a standard curve of OD vs. biomass (dry weight) was created for culture; OD750 was greater than 1 at a particular stage of the development cycle of algae. The empty 2 ml Eppendorf tubes were measured and recorded before the algal isolates were added. The algal strains were divided into two groups of 2 ml Eppendorf tubes and centrifuged at 12000 rpm for 10 minutes. Volumes of 400, 800, 1200, and 1600 µl were used for isolation. The pelleted Eppendorf tube was then dried in a dryer at 40°C, after which the supernatant was discarded. The weight of the biomass was determined by measuring the Eppendorf tube again after the pellet had dried. The dry algal biomass was then diluted in an Eppendorf tube with 1 ml of distilled water to a final volume of 2 ml, after which an extra 1 ml of distilled water was added. The absorbance of the isolates was then determined using a spectrophotometer at a wavelength of 750 nm (UV–Vis spectrophotometer from Shimadzu, Model No: UV-1800) [18]. With the use of these typical linear equations, the amount of biomass was estimated using spectrophotometric data (Figure 3. A. B and C).

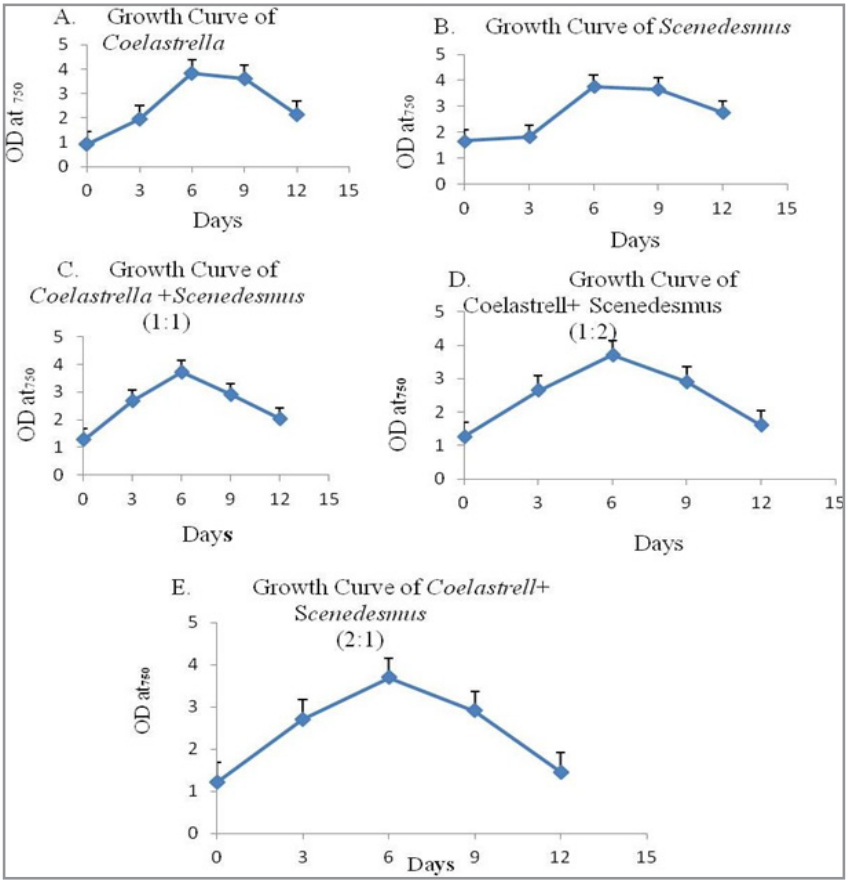


Figure 4: Set-A. Growth curve of Coelastrell Set-B. Growth curve of Scenedesmus Set-C.Growth curve of Coelastrell+Scenedesmus(1:1); Set-D. Growth curve of Coelastrell+Scenedesmus(1: 2) and Set- E. Growth Coelastrell+Scenedesmus (2:1)

Preparation of Algal Consortium: Coelastrella sp. and Scenedesmus sp., two different species of algae, were used to create the algal consortium. This study was conducted in five 100 ml conical flasks, and each flask was filled with 50 ml of disinfected wastewater containing different concentrations of algal biomass cultures as primary inoculums. Of the five sets, Set-A and Set-B were considered the control set, and the remaining three sets, namely, Set-C, Set-D and Set-E, were considered the algal consortium. Set-A: 4 mg monocultures of Coelastrella sp.; Set-B: 4 mg monocultures of Scenedesmus sp.; Set-C: an equal mixed concentration (1:1) of both Coelastrella and Scenedesmus microalgae species; Set-D: 2 mg of Coelastrella sp. and 4 mg of Scenedesmus sp. (1:2); and Set-E: 4 mg of Coelastrella sp. and 2 mg of Scenedesmus sp. (2:1) were inoculated separately into each set using the seed culture of algae. The labeled flasks were illuminated with 16 W/m2 wavelengths (73.6 µmole m2/s) of RGB (red-622, green-528 and blue-467) LED light for 12 days on a rotary shaker at 120 rpm and 24±2°C. Scenedesmus and Chlorella sp. performed well in an experiment using a light intensity of 16 w/m2 for a consortium study on the treatment of wastewater [19]. This was the cause behind the selection of this light intensity. For the quantification of biomolecules, 1 ml of biomass was taken from the consortium at three-day intervals for analysis starting on day 0 and then on the 3rd, 6th, 9th, and 12th days of algal culture. The supernatant was collected after centrifuging the samples and examined for dissolved ammonia and phosphate concentrations, and the pellet was examined for biomolecules.

Quantification of Biomass and Bomolecules at the Common Parameters

Growth curve analysis of the algal consortium

Algal biomass concentrations were measured using a UV–Vis spectrophotometer at 750 nm every three days (using a UV–Vis spectrophotometer from SHIMADZU, Model No: UV-1800). Then, using the OD vs. biomass standard equation, we calculated the amount of biomass (mg/ml) needed for algal growth [18] (Fig-4. Set-A, Set-B, Set-C, Set-D and Set-E).

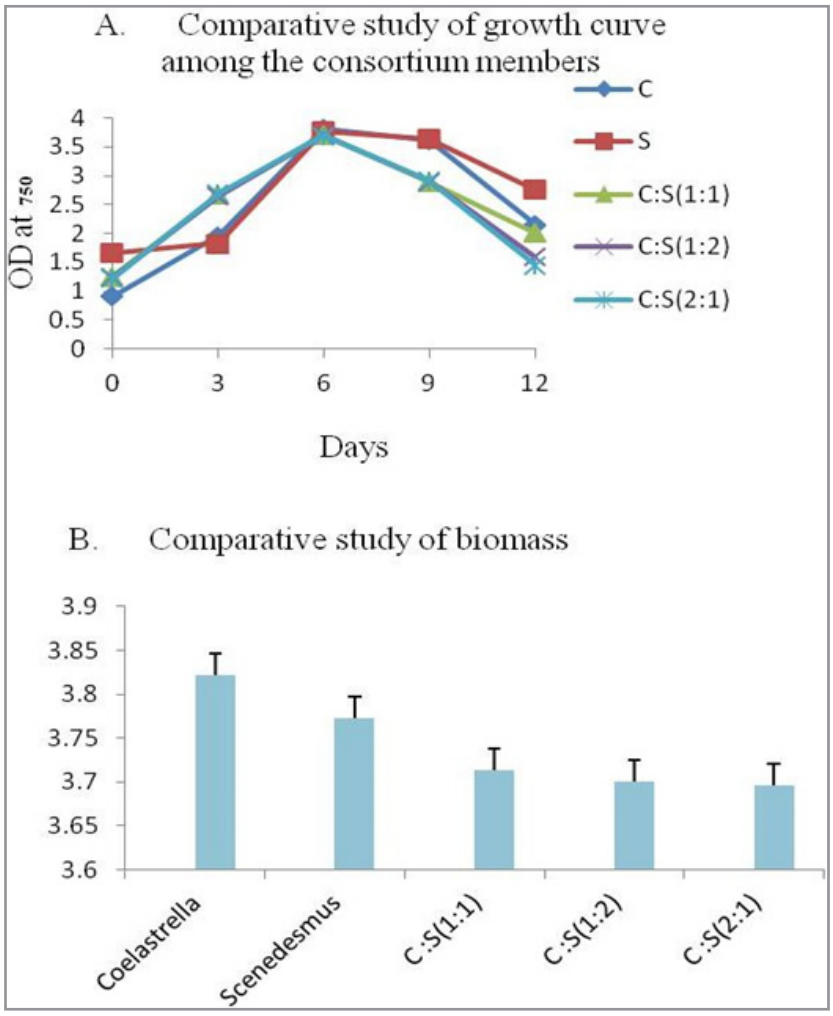


Figure 6. A: Comparative study of growth curve in each consortium. B. Comparative study of biomass generation in each consortium on 6th day of cultivation. [C=Coelastrella and S=Scenedesmus]

Protein quantification of the algal isolates

We used the methodology of Slocombe et al. (2013) for the extraction of protein from the algal isolates, and the Bradford method was used for protein quantification [20, 21]. A known concentration of BSA was standardized by using the Bradford method, and a standard protein curve was generated (Supplementary Figure 2.a.) was prepared first.

Extraction and Estimation of Total Chlorophyll (a & b) Pigments

Each separated, labeled Eppendorf tube was filled with 1 ml of the algal culture from the batch cultivation, which was centrifuged for 10 minutes at 12000 rpm. The supernatant was collected to determine the concentrations of ammonium and phosphate. The pelleted material was dried at a temperature of 50°C in a dryer. Then, 1 ml of 90% acetone was added as a solvent, and the mixture was repeatedly sonicated until the residue was colorless [22]. An ice bath was used after two straight sonications. The mixture was then centrifuged once again for 8 minutes at 8000 rpm. A 2 ml cuvette was filled with 1 ml of isolate extract and 1 ml of distilled water to measure the absorbance of these samples at 645 nm and 663 nm wavelengths (against the solvent blank) [23].

I used the following methods to measure the total chlorophyll (a+b): (mg/mg) = □(([20.21 (A645) + 8.02(A663)] x volume of extraction(ml))/(weight of the sample (mg)))…… (Equation…. 1)

DNA Extraction and Quantification

Thermo Fisher Scientific's DNAzol makers provided the DNA extraction methodology, which was used to extract the cellular DNA; a guanidine thiocyanate- and detergent-based DNA isolation reagent that is still in the patent process [24, 25]. The diphenylamine (DPA) method was used to estimate the concentration of DNA in the algal biomass [26]. The OD was taken at a wavelength of 595 nm. All these data were compared with a DNA standard curve to calculate the unknown concentration of

DNA in µg/mg. The known concentration of DNA was standardized by using the DPA method (diphenylamine) (Supplementary Figure 2.b.) The concentrations of DNA used were compared to determine the concentration of DNA in µg/mg.

RNA Extraction and Quantification

Thermo Fisher Scientific's TRIzol extraction technique was used to extract cellular RNA. This procedure is based on the method of phenol–chloroform extraction [27]. To quantify the total RNA concentration, we used the standard orcinol reagent method [28]. The OD was taken at a wavelength of 665 nm. A known concentration of RNA was standardized using the Orcinol method, and a standard RNA curve was prepared at the earliest time point (Supplementary Figure 2.c.). The concentration of RNA used was compared to the known concentration of RNA in µg/mg.

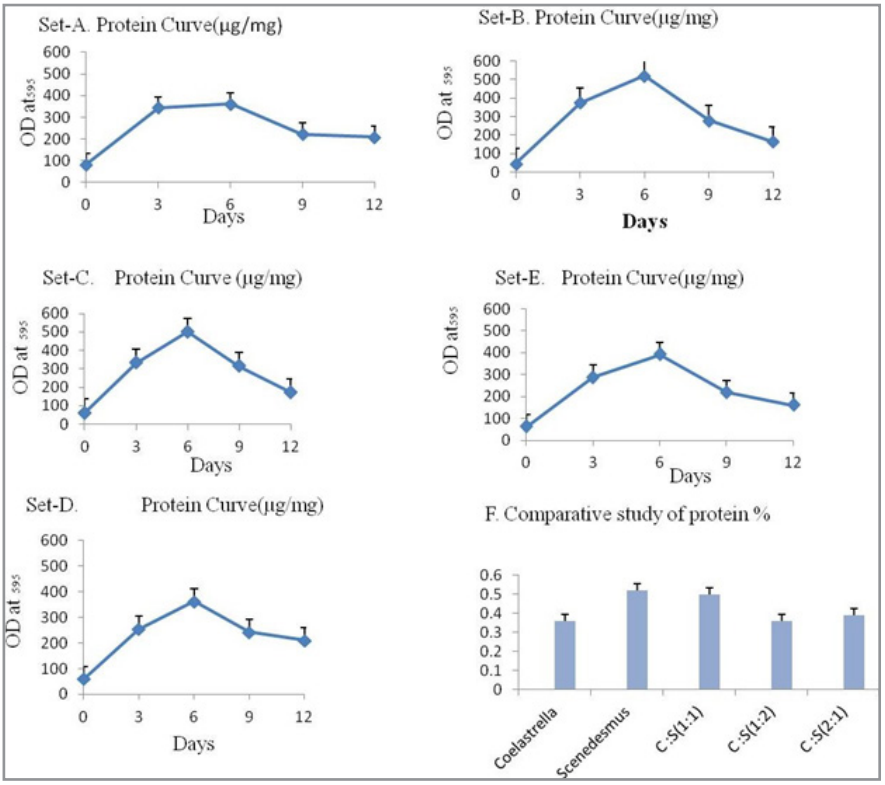


Figure 7: Set-A, Set-B, Set-C, Set-D and Set-E; Protein concentration in (µg/mg) F. comparative study of protein % on 6th day of algal biomass

Ammonium Standard Curve and Estimation

Ammonium Standard Curve: A stock solution of ammonia was prepared by dissolving 0.3819 g of anhydrous NH4 C1 in one liter (L) of distilled water after drying at 100°C. The solution concentration was 100 ppm. Working solutions of 10, 20, 40, 60, 80, and 100 ppm were prepared from the stock solution by adding distilled water, and absorbance was measured at 630 nm. By using a known concentration of ammonium, a standard curve of ammonium was prepared against the blank containing distilled water (Supplementary 5.a.). A calibration curve was prepared to determine the relationship between the absorbance and day.

Ammonium Estimation: To estimate the residual amount of ammonium in the wastewater after algae culture, we examined the supernatant every three days. For the measurement of dissolved ammonium in wastewater, we adhered to the guidelines provided by Zhou and Boyd (2016) and the Bulletin of the Korean Chemical Society (2009) [29, 30]. The first solution was a stock solution of ammonium. The second solution was sodium phenate solution, which consisted of 200 ml of water, 25 g of phenol, 20% NaOH (1.76 g), and 6 ml of acetone. The third solution contained 0.15 g of sodium nitroprusside dissolved in 1 L of distilled water. The fourth solution was sodium hypochloride solution, which had a 10% concentration. After centrifuging the algal culture for 10 minutes at 12000 rpm, 70 µl of algal supernatant was placed in a 2 µl Eppendorf tube. Then, for 30 minutes at 20 to 30°C, 700 µl of distilled water, 345 µl of sodium phenate solution, 35 µl of sodium nitroprusside, and 350 µl of sodium hypochloride solution (10%) were added. The final volume was 1500 µl. Absorbance was measured at 630 nm. This procedure was followed on days "0", 3, 6, 9 and 12. All these data were compared with the standard curve of ammonium to calculate the concentration of ammonium in µg/mg.

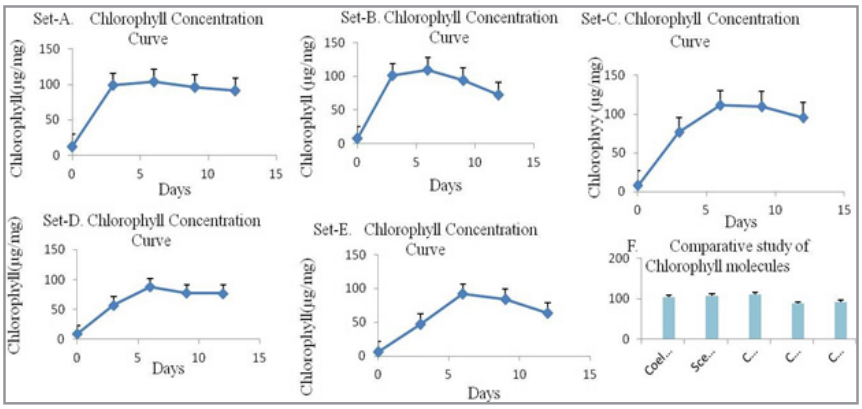


Figure 8: Chlorophyll concentration (µg/mg) of Set-A, Set-B, Set-C, Set-D and Set-E algal consortia on the 6th day of cultivation; F. Comparative study of chlorophyll concentration (µg/mg) in each set of biomasses on the 6th day of cultivation

Phosphate Standard Curve and Estimation

Phosphate Standard Curve: First, a stock solution of phosphate was prepared by dissolving 0.439 g of KH 2PO4 in 1 L of distilled water. By incorporating distilled water into the stock solution, working solutions of 2, 4, 6, 8 and 10 mg/L were produced. At 840 nm, absorbance measurements were taken. This concentration was used to produce a phosphate standard curve versus distilled water as the blank (Supplementary. 5.b.). A calibration curve was prepared to determine the relationship between the absorbance and day.

Phosphate Estimation: For the estimation of dissolved phosphate in the wastewater, we followed the protocol of Pradhan and Pokhrel (2013) [31]. To quantify the amount of dissolved phosphate in the wastewater, five different stock solutions were made. For stock solution-1, 136 ml of H2SO4 was dissolved in 1 liter of distilled water. Three grams of potassium sodium tartrate was dissolved in 1 liter of distilled water to make stock solution -2. Forty grams of ammonium heptamolybdate was dissolved in one liter of distilled water to create stock solution 3. To create stock solution-4, 18 g of ascorbic acid was dissolved in 1 liter of distilled water. For the final stock solution-5, a 100 ml conical flask was filled with 25 ml of stock solution-1, 2.5 ml of stock solution-2, 7.5 ml of stock solution-3, and 15 ml of stock solution-4, resulting in a final volume of 50 ml, which was light yellowish in color. After centrifuging the algal culture for 10 minutes at 12000 rpm, 500 µl of algal supernatant, 80 µl of stock solution-5 and 920 µl of distilled water were added to an Eppendorf tube. The final volume reached 1500 µl. A blue color formed after the entire mixture was kept for 20 minutes. The absorbance was finally measured at 840 nm. This procedure was followed on days "0", 3, 6, 9 and 12. All these data were compared with the standard curve of phosphate to calculate the concentration of dissolved phosphate in µg/mg.

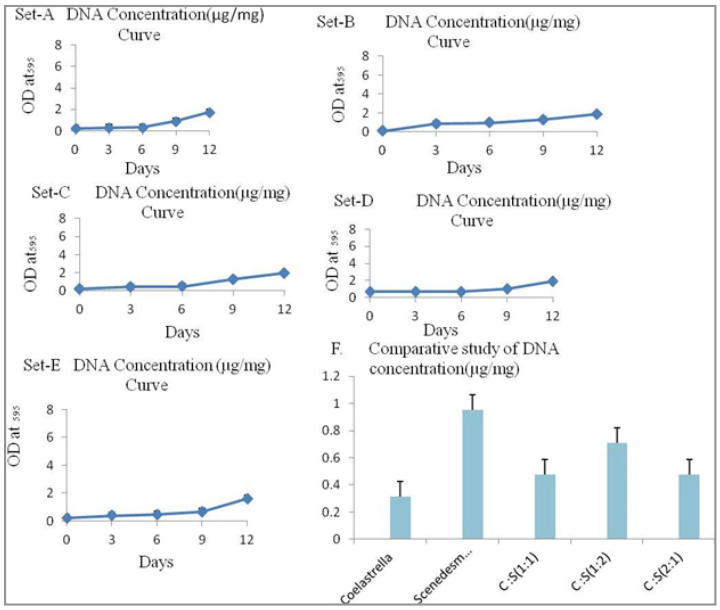


Figure 9: Total DNA generation curve, Set-A, Set-B, Set-C, Set-D and Set-E; F. Comparative study of DNA (µg/mg)

Results and Discussion

Trial and error approach successfully overcame all of the limitations related to this research design that were first outlined. Scenedesmus and Coelastrella, two chosen algal strains, were employed to create the algal consortium. Numerous studies have shown that both algae are highly effective at removing phos-

phate and nitrogen. By altering the biomass concentration of the primary inoculum, the potential of each consortium partner was assessed. However, using each partner in a 1:1 ratio produced good results for removing phosphate and ammonium from wastewater, as did using each partner, which had the highest amount of biomass, protein, and chlorophyll in comparison with the other consortia used in this experiment.

Growth Curve Analysis

The growth of the algal isolates was very and the maximum growth on the 3rd to 6th days during the log period. All the algal isolates reached the exponential phase after 6 days in wastewater. On the sixth day, the growth curve declined sharply. The maximum biomass was formed on the 6th day for all the consortia. However, the highest biomass was generated on day 6 in the Set-C (Coelastrell and Scenedesmus sp.; 1:1) consortium. The total amount of biomass was 3.71 mg/ml, which was greater than that of the other consortia, both Set-D and Set-E (Fig. 6.B.). Most likely, the combination of a 1:1 ratio of Coelastrella and Scenedesmus in Set-C had synergistic effects on the highest biomass generation. In contrast, unequal concentrations of primary inoculums from both the Set-D (2:1) and Set-E (1:2) consortia of Coelastrella and Scenedesmus had antagonistic effects on reducing biomass generation, which was 3.70 and 3.69 mg/ml, respectively (Fig. 6.B.). A comparative study of the growth curves showed that the growth rates of both the Set-A and Set-B monocultures were more or less equal and resulted in the highest biomass generation compared with that of the other consortia (Fig. 6.A.).

Protein Content

On day 6 in Set-A, Coelastrell sp. generated 36% of the protein. These findings were consistent with previously published data in which the percentage of protein in Coelastrell was 35.3 ± 0.3% [32]. On the same day, Set-B Scenedesmus generated 52% of the proteins that were also correctly matched with previously published data [33]. However, in Set-C, the percentage of protein was 50%, which was greater than that in both Set-D and Set-E. In Set-D and Set-E, the percentages of protein were 36 and 39%, respectively (Fig. 7.F). Set-A itself generated 36% of the protein, but in combination with Scenedesmus, an equal cell density had synergistic effects on 50% of the protein in Set-C. However, in the consortium, Set-D and Set-E were antagonistic to each other. Therefore, Set-C had synergistic effects on the two partners of this consortium. The gradual decrease in nutrient element levels in the wastewater might be the main cause of reduced protein generation after the 6th day onward (Fig. 7.Set-C). For Set-C, the growth rate declined suddenly after six days, possibly because all the essential nutrients for growth were fully utilized on or before six days, but in Set-D and Set-E, the growth rate gradually decreased (Fig. 7. Set- D, and Set-E). For algae, phosphorus is a critical macronutrient for new cell generation. This process helps with electron transfer and the formation of energy-rich molecules (ATPs), which are a type of nucleic acid. Therefore, the levels of macronutrients, namely, phosphate and ammonium, in the culture medium need to be measured every three alternative days.

Chlorophyll Content

The total chlorophyll estimation results showed that the highest amount of chlorophyll was found in Set-C on day 6 among the consortia, even in the other two monocultures, Set-A and Set-B. On the same day, in Set-D and Set-E, the concentrations were 88.00 and 91.68 µg/mg respectively. A comparative study showed that the highest concentration of chlorophyll (111.26 µg/mg) was detected in the Set-C algal consortium (Figure 8.F). This result again confirmed the synergistic effect of the algal partner Set-C. The total chlorophyll concentration curves are shown in Fig. 8 (Set-A; Set-B; Set-C; Set-D and Set-E). In algal growth media, nitrogen plays a pivotal role in regulating all metabolic activities that result in growth and different biochemical compositions, mainly protein, DNA, RNA and chlorophyll [34]. Therefore, the reduced level of nitrogen in the culture medium after six days retarded chlorophyll synthesis.

Total Nucleic Acid Quantification

On day 6, the total amount of DNA was quantified and found to be 0.47, 0.71 and 0.47 µg/mg of dry biomass from the Set-C, Set-D, and Set-E algal consortia, respectively (Figure 9. F.). The total amounts of RNA in Set-C, Set-D and Set-E were 4.48, 4.07 and 4.93 µg/mg dry biomass, respectively (Figure-10.F.). The total amount of nucleic acid (DNA+RNA) was 4.96, 4.78 and 5.41 µg/mg of the dry biomass of the Set-C, Set-D and Set-E consortia, respectively, on day 6 of culture (Fig-11.A.). In Set-D, the total amount of nucleic acid was less than that in the other consortium algal biomasses, namely, Set-C and Set-E, but the total protein and chlorophyll contents were very low compared to those in the Set-C algal biomass. In contrast, Set-C algal biomass contained a high amount of protein, and the amount of chlorophyll and residual amount of nucleic acid were low, which was equivalent to what is known for conventional sources of protein.

Phosphate Removal Rate from the Wastewater

By dividing the difference between the first-day and final-day concentrations by the first-day concentration and multiplying by 100 and expressed as a percentage, the removal of phosphate from the wastewater was estimated [35]. The highest percentage of phosphate was removed by consortium Set-C, which was nearly 65% (Figure-12.F.), which led to the generation of the highest amount of protein and chlorophyll molecules. In contrast, Set-D and Set-E removed very poor percentages of phosphate 41% and 39%, respectively, resulting in poor generation of biomass, protein and chlorophyll. Whereas both the Set-A and Set-B monocultures removed 58% of the phosphate, this was greater than that of the Set-D and Set-E consortia but less than that of Set-C. Therefore, the Set-C consortium is an ideal combination of two microalgae for removing wastewater phosphate in a realistic way. Microalgae are unable to utilize both organic and inorganic phosphate equally; instead, they take inorganic phosphate from the culture medium as H2PO4- and (HPO4)2 [36]. An experimental result demonstrated that the generation of essential photosynthetic pigments and photosynthetic activity were reduced by phosphorus depletion from the growing medium [37]. Phosphate limitation in Selenastrum minutum growing media decreased the rate of cellular respiration and photosynthetic activity as well as the concentration of protein [38]. It has been reported that algal communities are under stress when the ratio of nitrates to phosphates in microalgal culture is less than 16:1, which is known as the Redfield ratio [39]. The optimal phosphorus concentration in the growth medium for Chlorella vulgaris was 6 g/l, which increased the protein content to 75.56% of the dry biomass. According to another experimental finding,

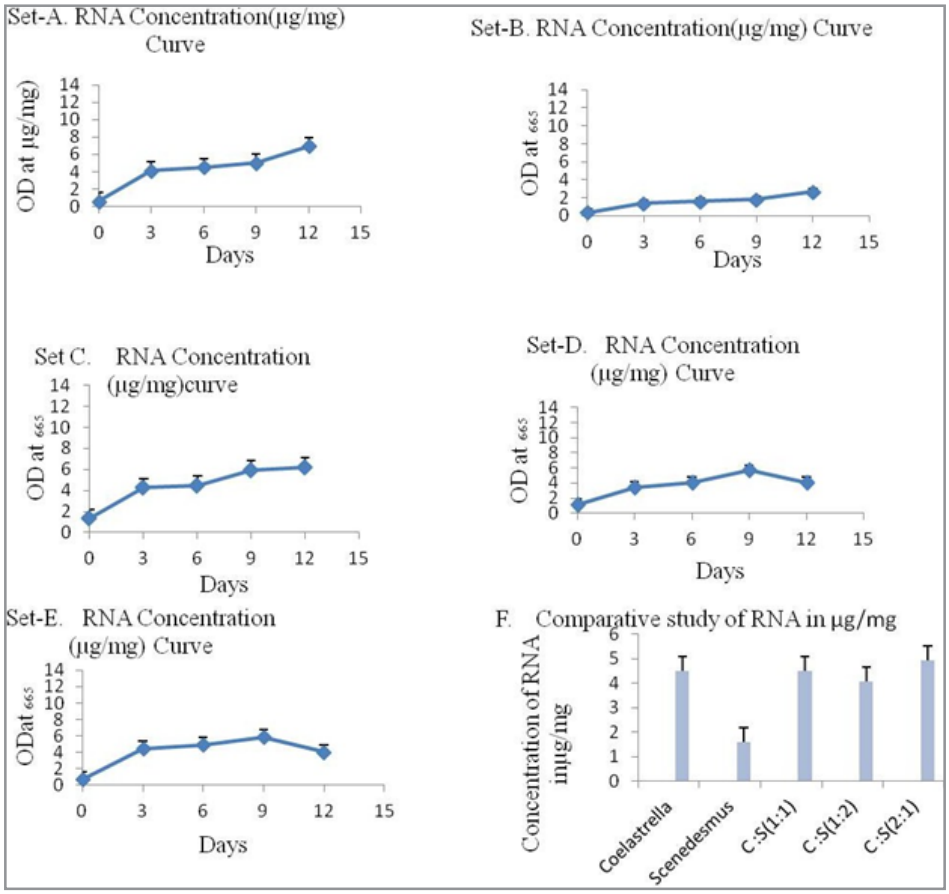


Figure 10: Total RNA generation curve, Set-A, Set-B, Set-C, Set-D and Set-E; F. Comparative study of RNA (µg/mg)

if the phosphorus concentration is less than 6 g/l, which is equivalent to 2 g or 4 g/l, the percentage of protein will decrease, and the protein content will reach 31.90% and 51.17%, respectively [40]. Therefore, the scarcity of phosphate in this consortium wastewater after six days of algal culture reduced the algal biomass and biomolecules generation and proved that algae consortia are a realistic and sustainable option for advanced wastewater treatment. A comparative study of the phosphate removal percentage is shown in Fig. 12F.

Ammonium Removal Rate from Wastewater

After cultivating the algae, we looked at the supernatant portion every three days to assess the remaining amount of ammonium in the wastewater. By dividing the difference between the first-day and final-day concentrations by the first-day concentration and multiplying by 100 and expressed as a percentage, the removal of ammonium from the wastewater was estimated [35]. The highest percentage of ammonium was removed by the Set-C consortium, at nearly 82% (Figure 13. F), which led to the generation of the highest amount of protein and chlorophyll

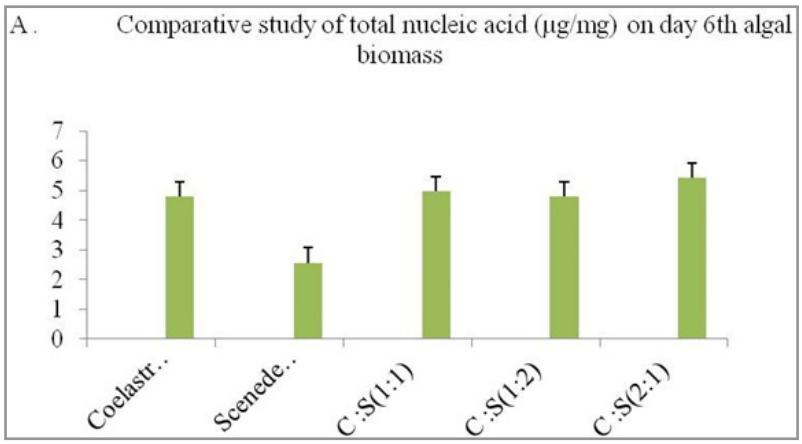


Figure 11. A: Total nucleic acid (µg/mg)

molecules. In contrast, Set-D and Set-E removed 76% and 78%, respectively, of the ammonium, resulting in poor generation of biomass, protein and chlorophyll. The monoculture Set-B removed 80% of the ammonium, which was greater than that of the Set-D and Set-E consortia but less than that of Set-C. As a result, the Set-B monoculture generated the highest amount of protein and chlorophyll compared to the Set-A monoculture.

Table 1: Comparative study of different consortium studies with the current study

Sl. No.	Types	Consortium Members	Initial Biomass	Cultured medium	Total % of Ammonium/ nitrogen removal	Total % of Phosphate removal	References
1	Algae+ Fungi	Scenedesmus sp., Trichoderma reesei	-	Sea food processing plant	44	93	(Srinuanpan et al. 2018).
2	Algae+ Algae	Chlorella sp., Scenedesmus sp., Sphaerocystis sp., and Spirulina sp	10^5 cells mL−1 equivalent to DBW 0.13 g/L	Domestic wastewater	39	59	(Bhakta et al. 2015).
3	Algae+ Algae	C.vulgaris; C prototheocoides	40 g/L	Municipal wastewater	35.4	74.4	(Oberholster et al. 2021).
4	Algae+ Bacteria	Chlorella sp.,and Bacillus firmus and Beijerinckia fluminen	Algae-1.0 × 10^5 cells/mL andBacteria- 1% (v/v) or 10% (v/v)	Vinegar production wastewater	20	18	(Huo et al. 2020).
5	Algae+Fungi	Chlorella vulgaris and Aspergillus sp.,	-	Swine manure wastewater	44	84	(Zhou et al. 2012).
6	Algae+Algae	Coelastrella sp., + Scenedesmus sp.,	1:1 (4 mg:4 mg)	Bengal chemical wastewater	82.26	64.7	Current study

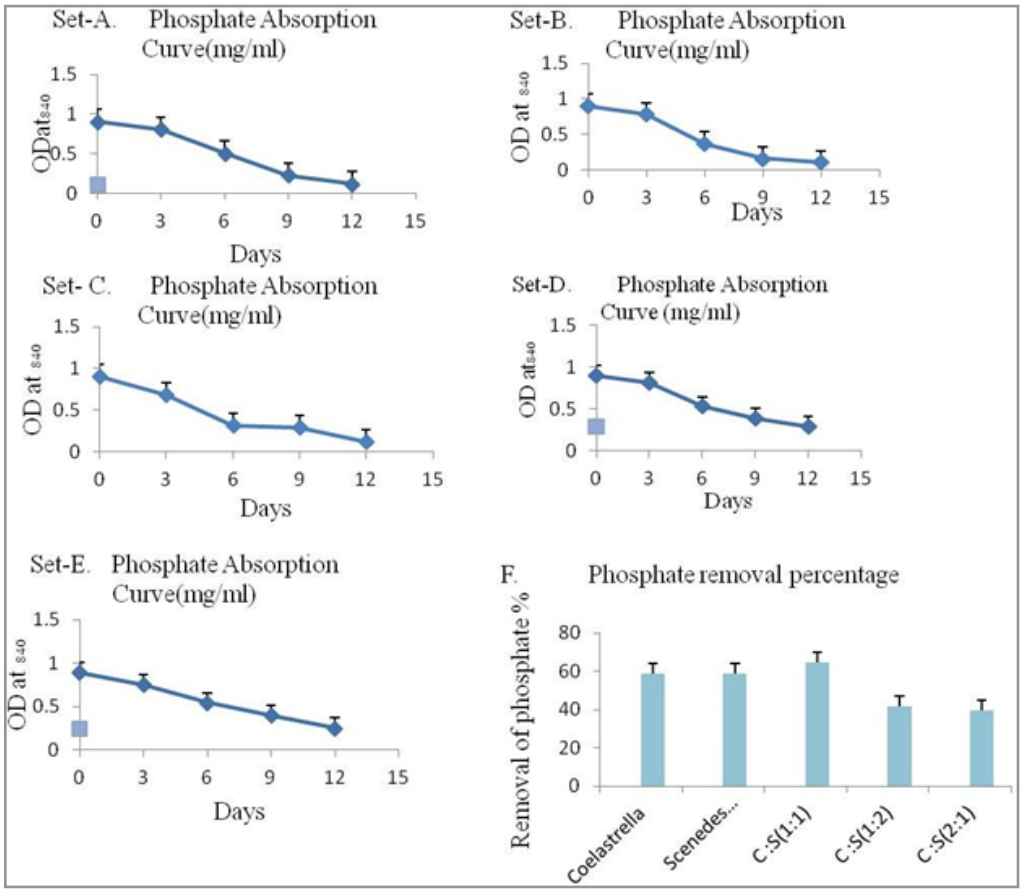


Figure 12: Phosphate removal curve, Set-A, Set-B, Set-C, Set-D and Set-E; F. Comparative study of phosphate removal %

Although ammonium is the preferred nitrogen source, microalgae can assimilate nitrogen from a wide range of nitrogen sources, including nitrate, nitrite, and urea. Since less energy is required for its reception, ammonia is the most energy-efficient nitrogen source. As predicted, ammonium (NH4+) took priority over nitrogen dioxide (NO2) in regard to the utilization of nitrogen sources. Compared to those in NH4+, oxidized N species, especially nitrate (NO3-), are more prevalent. The two-electron reduction of NO3 to NO2 is always the first step in NO3 assimilation (Glass 2011).

$$NO3- +2e- +2H+ \rightarrow NO2- +H2O$$

N (Nitrogen) inclusion requires a six-electron reduction of NO2- to NH4+ after NO3- reduction. The total process, which is reduced by six electrons to produce NH4+, is as follows [41].

$$NO2- +6e- + 8H+ \rightarrow NH4 +2H2O$$

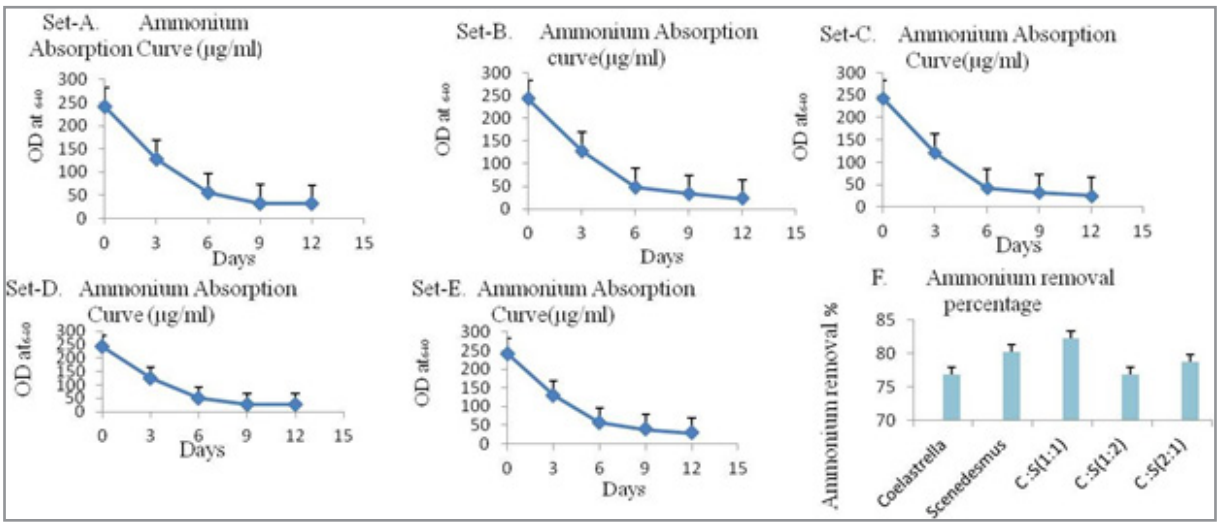
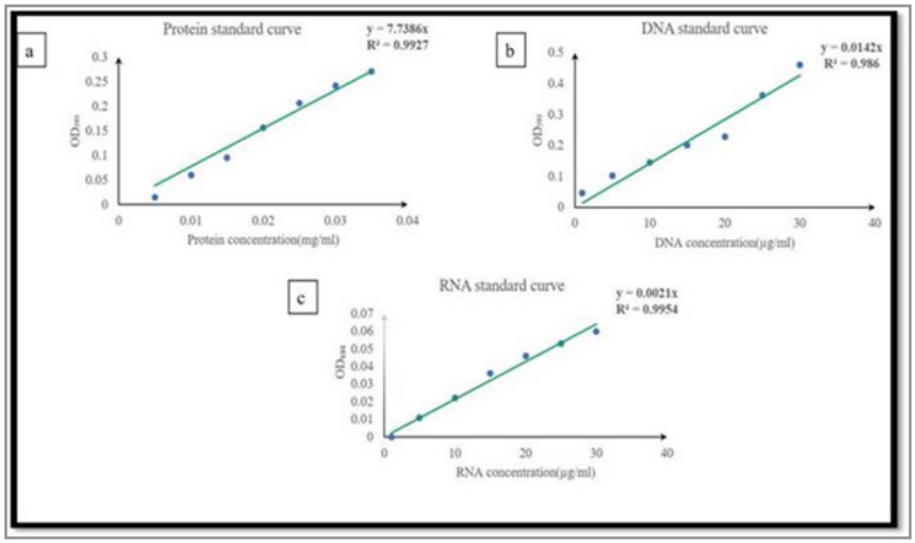


Figure 13: Ammonium removal curve, Set-A, Set-B, Set-C, Set-D and Set-E; F. Comparative study of ammonium removal %

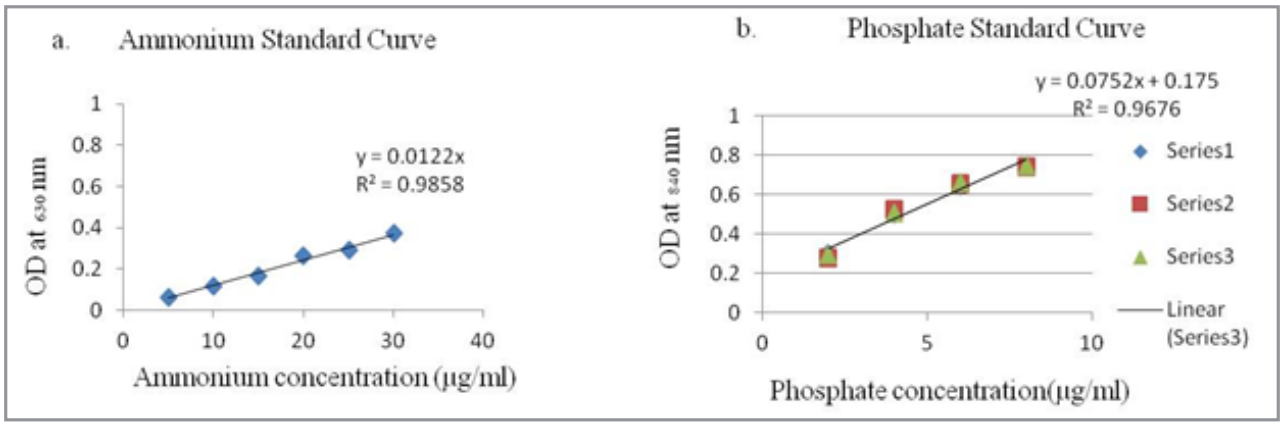
Since reduction is not necessary for ammonium before it is converted into amino acids, microalgae can quickly assimilate ammonium. Therefore, electrons must be supplied for NO3- and NO2- to be reduced to NH4+. Therefore, in our algal consortium, first, algae utilized easily accessible ammonium, and after six days of growth, they reached the highest level of biomass and other biomolecules. After six days, the algae were switched to an obvious nitrate-based alternate source of nitrogen. Nitrate was first transformed into nitrite and then ammonium. Algal development was slowed down by a time-consuming technique. If we compare our results with previously published consortium results when cultured in seafood processing plants, a combination of algae and fungal strains, namely, Scenedesmus sp. and Trichoderma reesei can remove 44% and 93%, respectively, of total nitrogen and total phosphate [42]. Other experimental findings demonstrated that four different algal strains, Chlorella sp., Scenedesmus sp., Sphaerocystis sp., and Spirulina sp., when cultivated in domestic wastewater can remove 59 and 39% of phosphate and nitrogen, respectively [43]. Another study showed that, when grown in municipal wastewater, Chlorella protothecoides and Chlorella vulgaris can remove 35.4% and 74.4%, respectively, of nitrogen and phosphate [44]. Another group of bacteria, called Beijerinckia fluminen, Bacillus firmus and the alga Chlorella sp., was cultured in vinegar manufacturing wastewater, and 20% and 18% of the total nitrogen and phosphate, respectively, were removed [45]. Chlorella vulgaris was cultured in swine manure wastewater supplemented with the fungal partner Aspergillus sp. and could remove 44.68% and 84.70% of total nitrogen and phosphate, respectively [46]. However, our experimental results showed that the Set-C consortium could remove 82% and 65% of total nitrogen and phosphate from wastewater, respectively (Fig. 13.F and 12.F). A comparative table was constructed (Table 1) to compare our results with those of other consortia.

Conclusion

This study demonstrated the ability of mixed algal consortia to bioremediate (remove nutrients) phosphates and ammonia, with the potential to produce single-cell proteins and other products of added value. The efficient treatment of wastewater and algal harvest were made possible by mixotrophy and the self-flocculating capabilities of the algal consortia. This method facilitates the decentralized treatment of water while creating goods with value added if utilized in sewage-fed ponds and lagoons. This approach would reduce GHG emissions while preventing starvation or nutritional deficiencies. As a result, bioremediation using mixed algal consortia satisfies a number of sustainable development goals, aiding in the treatment of wastewater and preventing water scarcity. Microalgal consortia are among the finest reported methods for removing harmful pollutants and toxins from the environment. However, the use of microalgal consortia is still in its infancy in this field. This is mainly due to the wide range of practical combinations that are possible. Additionally, relatively little research has been conducted on the interactions that occur between photosynthetic microorganisms. Previous studies have shown that microalgal-bacterial and microalgal-fungal consortia are more effective than algal-algal consortia because they can be used to replace the secondary and tertiary treatment stages



Supplementary Figure 2. a. Protein Standard curve b. DNA standard curve. c. RNA standard curve
(a) [Protein standard curve at 595 nm with linear regression equation Y = 7.7386x, R^2 = 0.9927.
b) DNA standard curve at 595 nm (DPA method–Diphenylamine) with linear regression equation Y = 0.0142x, R^2 = 0.9860.
c) RNA standard curve at 665 nm (Orcinol method) with linear regression equation Y = 0.0021X, R^2 = 0.9054



Supplementary Figure 5. a. Ammonium standard curve b. Phosphate standard curve
(a) Ammonium standard curve at 630 nm with linear equation Y = 0.0122x and R^2 = 0.9858
(b) Phosphate standard curve at 840 nm (Series3) with equation Y = 0.0752x + 0.175 and R^2 = 0.9676.

of wastewater treatment, respectively, while microalgal consortia can be used only for wastewater polishing (as a replacement for tertiary treatment). However, only a small number of studies have shown the identification of specific symbiotic strains and the establishment of a distinct and well-designed symbiotic system. Because of the complexity of the microorganisms in co-culture systems created by mixed flora, it is difficult to monitor the dependability of these systems, which ultimately affects the efficacy of wastewater treatment. This approach is unfavorable for the study of the mechanisms underlying interactions between microalgae and symbiotics; however, this consortium system requires additional research and work so that this unique technology can be used and marketed on an industrial scale, for example.

Acknowledgments

The author would like to thank JIS University, Kolkata, W.B., and JIS Group Educational Initiatives and is deeply indebted to my research supervisor, Dr. Dipankar Ghosh, associate professor, "Microbial Engineering and Algal Biotechnology Laboratory," Department of Biosciences, JIS University, Kolkata, West Bengal, 700109, India.

Author Contribution

Kamalendu De has made a direct intellectual contribution to the research methodology and conceptualization and drafted the original manuscript for the publication.

Funding

This study did not receive any kind of financial support

Data Availability

All the datasets were obtained during this study and were incorporated into the manuscript.

Declarations of Ethics Approval and Consent to Participate
Not applicable.

Competing Interests
The author declares no competing interests.

Consent to Participate Declaration
Not applicable'.

Clinical Trial Number
Not applicable

Consent to Publish Declaration
Not applicable

Publisher's Note
This journal remains neutral about jurisdictional claims in published institutional affiliation.

References

1. Akhtar, N., Syakir Ishak, M. I., Bhawani, S. A., et al. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. Water, 13(19), 2660. https://doi.org/10.3390/w13192660
2. Gonçalves, A. L., Pires, J. C. M., & Simões, M. (2017). A review on the use of microalgal consortia for wastewater treatment. Algal Research, 24, 403–415. https://doi.org/10.1016/j.algal.2016.11.008
3. Wu, L., Ning, D., Zhang, B., et al. (2019). Global diversity and biogeography of bacterial communities in wastewater treatment plants. Nature Microbiology, 4(7), 1183–1195. https://doi.org/10.1038/s41564-019-0426-5
4. Glibert, P. M. (2017). Eutrophication, harmful algae and biodiversity—Challenging paradigms in a world of complex nutrient changes. Marine Pollution Bulletin, 124(2), 591–606. https://doi.org/10.1016/j.marpolbul.2017.04.027
5. Mardamootoo, T., Du Preez, C. C., & Barnard, J. H. (2021). Agricultural phosphorus management for environmental protection: A review. Journal of Geoscience and Environment Protection, 9(8), 48–81. https://doi.org/10.4236/gep.2021.98004
6. Woolf, A. D., Stierman, B. D., Barnett, E. D., et al. (2023). Drinking water from private wells and risks to children. Pediatrics, 151(2), e2022060645. https://doi.org/10.1542/peds.2022-060645
7. Hena, S., Fatimah, S., & Tabassum, S. (2015). Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resources and Industry, 10, 1–14. https://doi.org/10.1016/j.wri.2015.02.002
8. Cai, T., Park, S. Y., & Li, Y. (2012). Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renewable and Sustainable Energy Reviews, 19, 360–369. https://doi.org/10.1016/j.rser.2012.11.030
9. Mohsenpour, S. F., Hennige, S., Willoughby, N., et al. (2020). Integrating microalgae into wastewater treatment: A review. Science of the Total Environment, 752, 142168. https://doi.org/10.1016/j.scitotenv.2020.142168
10. Torres-Franco, A., Passos, F., & Figueredo, C. (2021). Current advances in microalgae-based treatment of high-strength wastewaters: Challenges and opportunities to enhance wastewater treatment performance. Reviews in Environmental Science and Bio/Technology, 20(1), 209–235. https://doi.org/10.1007/s11157-020-09556-8.
11. Kalra, R., Gaur, S., & Goel, M. (2021). Microalgae bioremediation: A perspective toward wastewater treatment along with industrial carotenoids production. Journal of Water Process Engineering, 40, 101794. https://doi.org/10.1016/j.jwpe.2020.101794
12. Nagarajan, D., Lee, D. J., Chen, C. Y., et al. (2020). Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective. Bioresource Technology, 302, 122817. https://doi.org/10.1016/j.biortech.2020.122817
13. 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), 36. https://doi.org/10.1186/s12934-018-0879-x
14. De, K., & Ghosh, D. (2023). Isolation and biochemical characterization of natural algal isolates from Kangsabati River, West Bengal toward single-cell proteins production. Journal of Applied Biology and Biotechnology, 11, 168–177. https://doi.org/10.7324/JABB.2023.131653
15. Ahmad, A., Banat, F., Alsafar, H., et al. (2022). Algae biotechnology for industrial wastewater treatment, bioenergy production, and high-value bioproducts. Science of the Total Environment, 806(2), 150585. https://doi.org/10.1016/j.scitotenv.2021.150585
16. Doria, E., Longoni, P., Scibilia, L., et al. (2012). Isolation and characterization of a Scenedesmus acutus strain to be used for bioremediation of urban wastewater. Journal of Applied Phycology, 24(3), 375–383. https://doi.org/10.1007/s10811-011-9759-z
17. Dayana Priyadharshini, S., Suresh Babu, P., & Manikandan, S. (2021). Phycoremediation of wastewater for pollutant removal: A green approach to environmental protection and long-term remediation. Environmental Pollution, 290, 117989. https://doi.org/10.1016/j.envpol.2021.117989
18. Griffiths, M. J., Garcin, C., & van Hille, R. P. (2011). Interference by pigment in the estimation of microalgal biomass concentration by optical density. Journal of Microbiological Methods, 85(2), 119–123. https://doi.org/10.1016/j.mimet.2011.02.005
19. Patil, N. S., Tidke, S. A., Kiran, S., et al. (2019). Phycoremediation of carton box industry effluent using consortia of green microalgae Chlorella sp. and Scenedesmus sp. and phytotoxicity assessment.
20. Slocombe, S. P., Ross, M., Thomas, N., et al. (2013). A rapid and general method for measurement of protein in microalgal biomass. Bioresource Technology, 129, 51–57.
21. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999
22. Oo, Y. Y. N., Su, M. C., & Kyaw, K. T. (2017). Extraction and determination of chlorophyll content from microalgae. International Journal of Advanced Research Publications, 1(5), 298.
23. Schagerl, M., & Künzl, G. (2007). Chlorophyll a extraction from freshwater algae—a re-evaluation. Biologia, 62(3), 270–275.

24. Ismail, M. M., & Osman, M. E. H. (2016). Seasonal fluctuation of photosynthetic pigments of most common red seaweeds species collected from Abu Qir, Alexandria, Egypt. Revista de Biología Marina y Oceanografía, 51(3), 515–525. https://doi.org/10.4067/S0718-19572016000300004
25. Chomczynski, P., Mackey, K., & Drews, R. (1997). DNAzol®: A reagent for the rapid isolation of genomic DNA. BioTechniques, 22(3), 550–553. https://doi.org/10.2144/97223pf01
26. Li, X., Wu, Y., Zhang, L., et al. (2014). Comparison of three common DNA concentration measurement methods. Analytical Biochemistry, 451, 18–24. https://doi.org/10.1016/j.ab.2014.01.016
27. Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Analytical Biochemistry, 162(1), 156–159. https://doi.org/10.1006/abio.1987.9999
28. Almog, R., & Shirey, T. L. (1978). A modified orcinol test for the specific determination of RNA. Analytical Biochemistry, 91(1), 130–137. https://doi.org/10.1016/0003-2697(78)90823-0
29. Zhou, L., & Boyd, C. E. (2016). Comparison of Nessler, phenate, salicylate and ion selective electrode procedures for determination of total ammonia nitrogen in aquaculture. Aquaculture, 450, 187–193. https://doi.org/10.1016/j.aquaculture.2015.07.022
30. Park, G. E., Oh, H. N., & Ahn, S. Y. (2009). Improvement of the ammonia analysis by the phenate method in water and wastewater. Bulletin of the Korean Chemical Society, 30(9), 2032–2038. https://doi.org/10.5012/bkcs.2009.30.9.2032.
31. Pradhan, S., & Pokhrel, M. R. (2013). Spectrophotometric determination of phosphate in sugarcane juice, fertilizer, detergent and water samples by molybdenum blue method. Scientific World, 11(11), 58–62. https://doi.org/10.3126/sw.v11i11.9139
32. Dimitrova, P., Marinova, G., & Pilarski, P. (2016). Preliminary studies on the growth and biochemical composition of a promising carotenoid producing strain Coelastrella sp. Natural and Mathematical Sciences, 6(3), 139–149.
33. Kent, M., Welladsen, H. M., & Mangott, A. (2015). Nutritional evaluation of Australian microalgae as potential human health supplements. PLOS ONE, 10(2), e0118985. https://doi.org/10.1371/journal.pone.0118985
34. Zarrinmehr, M. J., Farhadian, O., Heyrati, F. P., et al. (2020). Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, Isochrysis galbana. Egyptian Journal of Aquatic Research, 46(2), 153–158. https://doi.org/10.1016/j.ejar.2019.11.003
35. Li, Y., Horsman, M., Wang, B., et al. (2008). Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Applied Microbiology and Biotechnology, 81(4), 629–636. https://doi.org/10.1007/s00253-008-1681-1
36. Rausch, C., & Bucher, M. (2002). Molecular mechanisms of phosphate transport in plants. Planta, 216(1), 23–37. https://doi.org/10.1007/s00425-002-0921-3
37. White, S., Anandraj, A., & Bux, F. (2011). PAM fluorometry as a tool to assess microalgal nutrient stress and monitor cellular neutral lipids. Bioresource Technology, 102(2), 1675–1682. https://doi.org/10.1016/j.biortech.2010.09.097
38. Theodorou, M. E., Elrifi, I. R., Turpin, D. H., et al. (1991). Effects of phosphorus limitation on respiratory metabolism in the green alga Selenastrum minutum. Plant Physiology, 95(4), 1089–1095. https://doi.org/10.1104/pp.95.4.1089
39. Geider, R. J., & La Roche, J. (2002). Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. European Journal of Phycology, 37(1), 1–17. https://doi.org/10.1017/S0967026201003456
40. Baiee, M. A., & Salman, J. M. (2016). Effect of phosphorus concentration and light intensity on protein content of microalga Chlorella vulgaris. Mesopotamian Environment Journal, 2(2), 75–86. ISSN: 2410-2598
41. Glass, J. (2011). Molybdenum biogeochemistry in an evolutionary context: Nitrogen assimilation, microbial storage and environmental budgets [Doctoral dissertation, Arizona State University].
42. Srinuanpan, S., Chawpraknoi, A., Chantarit, S., et al. (2018). A rapid method for harvesting and immobilization of oleaginous microalgae using pellet-forming filamentous fungi and the application in phytoremediation of secondary effluent. International Journal of Phytoremediation, 20(10), 1017–1024. https://doi.org/10.1080/15226514.2018.1452187
43. Bhakta, J. N., Lahiri, S., & Pittman, J. K. (2015). Carbon dioxide sequestration in wastewater by a consortium of elevated carbon dioxide-tolerant microalgae. Journal of CO2 Utilization, 10, 105–112. https://doi.org/10.1016/j.jcou.2015.02.001
44. Oberholster, P. J., Steyn, M., & Botha, A. M. (2021). A comparative study of improvement of phycoremediation using a consortium of microalgae in municipal wastewater treatment pond systems as an alternative solution to Africa's sanitation challenges. Processes, 9(9), 1677. https://doi.org/10.3390/pr9091677
45. Huo, S., Kong, M., Zhu, F., et al. (2020). Co-culture of Chlorella and wastewater-borne bacteria in vinegar production wastewater: Enhancement of nutrients removal and influence of algal biomass generation. Algal Research, 45, 101744. https://doi.org/10.1016/j.algal.2019.101744
46. Zhou, W., Cheng, Y., Li, Y., Wan, Y., Liu, Y., Lin, X., & Ruan, R. (2012). Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. Applied biochemistry and biotechnology, 167(2), 214–228. https://doi.org/10.1007/s12010-012-9667-y.