Simultaneous Biological Nutrient Removal from Municipal Wastewater and CO2-biofixation using Chlorella kessleri

Mohammed Omar Faruque
Chemical Engineering Department, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

Kazeem Ayodeji Mohammed
Chemical Engineering Department, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

Mohammad Mozahar Hossain
Chemical Engineering Department, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

Shaikh Abdur Razzak (srazzak@kfupm.edu.sa)
King Fahd University of Petroleum & Minerals https://orcid.org/0000-0003-4316-7882

Research

Keywords: Wastewater Treatment, Nitrogen to Phosphorus Ratio, Nutrient Removal, Biomass, CO2 Bio-fixation, Kinetic Model

Posted Date: August 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41636/v1

Abstract

Growing microalgae in tertiary wastewater offers a prospective avenue to remove and re-use the nutrients N and P simultaneously. Moreover, CO_2 fixation via microalgae is a potential and promising approach of capturing and storing CO_2. The impacts of various nitrogen to phosphorous ratios on the growth, nutrient removal from municipal wastewater, and the bio-fixation of CO_2 using *Chlorella kessleri* were evaluated in this study. For this purpose, the microalgae was grown in synthetic wastewater, similar in composition to tertiary municipal wastewater, with NP ratios of 2:1, 4:1, 6:1, and 8:1 in batch photobioreactors for13 days. Biomass concentration increases at all NP ratios and the maximum biomass concentration is 606.79 mg/L at the NP ratio of 2:1. Nitrogen removal is more than 95% at all NP ratios except at 8:1, where it is only 72.4%. The removal efficiency of phosphorous is significantly affected by the NP ratio. The maximum phosphorous removal is about 97% for the NP ratio 6:1, whereas the lowest removal efficiency of about 20% is at the NP ratio of 2:1. The maximum CO_2 bio-fixation rate of 89.36 $mgL^{-1}d^{-1}$ at the end of the first 7 days of the cultivation period is at the NP ratio of 6:1. In this study, Monod growth kinetic model based on a single substrate factor was used and the experimental findings agree well with the predictions by the model.

Introduction

Rapid industrialization and urbanization are generating enormous quantities of wastewater that must be treated before safe disposal. The wastewater (WW) associated with over population and industrialization are the major wastes, which pose an immense challenge to ensuring environmental sustainability around the world (Eze et al. 2018). The presence of excessive nutrients, such as nitrogen and phosphorous in the wastewater, can lead to the eutrophication in receiving streams and disturb the stability of the ecosystem (Cai et al. 2013). Hence, the removal of nutrients such as nitrogen and phosphorous from tertiary wastewater is vital to reduce the oxygen requirement of the receiving streams, save aquatic life, and prevent eutrophication in lakes and streams to protect human health. At present, a broad range of methods, including filtration, membrane technology, precipitation, the advanced oxidation process (AOP), and biological nutrient removal (BNR) using activated sludge are available for the removal of nutrients from tertiary wastewater. However, these techniques generally entail higher energy and maintenance costs, hazards associated with the disposal of chemicals, and the production of a large volume of waste sludge (Rajasulochana and Preethy 2016). In addition, the inability to eliminate nitrogen (N) and phosphorus (P) from tertiary wastewater simultaneously during the treatment process is a major drawback of these processes (Arbib et al. 2014).

Several studies have confirmed that microalgae can remove nitrogen and phosphorous during the treatment of tertiary municipal wastewater, which have gained much interest in recent years (Xin et al. 2010a; Khan and Yoshida 2008). The most widely applied cultures of microalgae for nutrient removal are based on species of *Chlorella* (Hernandez et al. 2006; Moreno Osorio et al. 2019), *Scenedesmus* (Shi et al. 2007), *Botryococcus* (Yu and Kim 2017) and *spirulina* (Olguín 2003). As the absence of one nutrient

inhibits the removal of the other and vice versa, the nutrients N and P must be simultaneously present in culture media for microalgae growth. The concentration of nitrogen and phosphorous in wastewater varies, with nitrogen concentration varying between 15 and 90 ppm and phosphorous concentration varying between 4 and 20 ppm in municipal wastewater (Beuckels et al. 2015). As the main route of removal of nutrients by microalgae is through their uptake during growth, the microalgal growth rate directly influences the rate of removal of nutrients. Also, nitrogen and phosphorous can be concurrently consumed and removed effectively only if the nitrogen to phosphorous (NP) ratio of wastewater is in an appropriate range (Xin et al. 2010b).

Microalgae cultivation in wastewater through photosynthesis can overcome the aforesaid problems and offers many advantages, including the following: i) adds an economic value to tertiary wastewater effluents in terms of water and nutrient recovery (Arbib et al. 2014), ii) nitrogen and phosphorus are removed from wastewater simultaneously (Xin et al. 2010b), iii) microalgae grow about 10–50 times faster than terrestrial plants, resulting in the efficient conversion of CO_2 into organic compounds (Y. Li et al. 2008; Razzak et al. 2017), iv) can generate a large volume of water suitable for recycling on-site and off-site or safe to discharge to surface water bodies, and v) can produce algae biomass suitable for bioenergy generation (Driver et al. 2014).

Previous studies have demonstrated that concentration of nutrients has a great impact on the rate of growth of microalgae and bio-fixation of CO_2 (Razzak et al. 2017; Razzak et al. 2013). *Chlorella Kessleri* is a freshwater microalgae species, which is capable of removing nutrients from tertiary wastewater as well as capturing CO_2 from the atmosphere (Arbib et al. 2014; Lee and Lee 2002). Li et al. (2012) have investigated the ability of *Chlorella Kessleri* to remove nutrients based on the intensity of light. Wang et al. (2012) have studied the heterotrophic cultivation of *Chlorella Kessleri* for the production of fatty acids with varying amounts of carbon and nitrogen supplements (Wang et al. 2014). Even though De Morais and Costa (2007) studied the growth kinetics of *Chlorella kessleri* under different CO_2 concentrations, they did not conduct a detailed study of the bio-fixation capacity of CO_2 (de Morais and Costa 2007). However, to the best of our understandings, a detailed study of the removal of nutrients by the microalgae *Chlorella kessleri* sp. as a function of the nitrogen to phosphorous ratio and the bio-fixation of CO_2 under photoautotrophic condition s has not been conducted.

Finding the most appropriate nutrient ratio for the growth of microalgae is vital for the effective coupling of advanced wastewater treatment with bio-fixation of CO_2. Hence, this study was conducted using a set of synthetic tertiary municipal wastewater samples based on the modified BBM with varying NP ratios. In this study, *Chlorella kessleri* sp. was cultivated in modified BBM to evaluate the growth, nutrient uptake, and CO_2 bio-fixation at different nutrient ratios.

Materials And Methods

2.1 Microalgae strain

The experiments were conducted using the microalgae strain *Chlorella Kessleri* (UTEX 2229) found from the University of Texas, Austin, USA.

2.2 Culture medium

The modified BBM (MBBM) was utilized as the medium of microalgae growth in the experiments. A set of synthetic wastewater media was prepared with varying nitrogen to phosphorous (NP) ratios, with the purpose of analyzing the removal of nutrients by growing microalgae in them to apply the results for the treatment of municipal tertiary wastewater. Nitrate (NO_3-N) and phosphate (PO_4^{3-}-P) were utilized as the nitrogen and phosphorus supplies, correspondingly. Besides, nitrogen and phosphorus, the cultivation medium consisted of the following: 25 mg/L $CaCl_2.2H_2O$, 25 mg/L NaCl, 75 mg/L $MgSO_4.7H_2O$, 31 mg/L KOH, 50 mg/L Alkaline EDTA, 4.98 mg/L $FeSO_4.7H_2O$ (acidified), 11.45 mg/L H_3BO_3, and 1 mL/L of a solution of trace elements prepared according to the composition of the BBM. The solutions of trace elements were prepared separately by dissolving $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.\ 5H_2O$, $CoCl_2.6H_2O$, and MoO_3 together in 1 liter of distilled water.

2.3 Experimental procedure and design

Chlorella Kessleri was cultivated in batch photobioreactors (2 L Erlenmeyer flasks) and 1500 mL of the working liquid medium was added to each photobioreactor. The pre-cultured microalgae sp. was used to avoid the lag phase affecting the results. The flasks were capped with foam stoppers to protect the content from contamination. The photobioreactors were placed in a fume hood under continuous illumination with four perpendicularly oriented Grolux fluorescence (Sylvania F18W/T8/GRO) tube lights to cover a maximum area of the photobioreactor surfaces. The light intensity recorded at the reactor surface was in the range of 62–74 µmol/m^2/s, applying a Fisher Scientific™ Traceable™ Dual-Display Light Meter. Figure 1 depicts a schematic diagram of the experimental set-up employed in this experiment.

The air-CO_2 mixture was delivered to the bottom side of the photobioreactor precisely with the help of an air-CO_2 mixer device FC-SH (Live Cell Instruments, South Korea). Gas bubbles rise through the liquid medium and leave at the top side of the reactor, ensuring a well-mixed culture medium, thereby preventing cell sedimentation, and providing inorganic carbon. During photosynthesis, algae utilize carbon dioxide as the carbon source for cell growth. *Chlorella kessleri* is a photosynthetic microorganism with the capacity to grow in aqueous suspensions consuming nutrients from wastewaters, while simultaneously combining water and CO_2 photosynthetically to produce value added biomass.

The mechanism of conversion of CO_2 to biomass has been well explained by Sorensen et al.(Halling-Sørensen et al. 1996) as follows:

During the experiments, the temperature of the media was monitored daily applying a temperature sensor (Fisher Scientific™ Thermometers with a Stainless Steel Probe on Cable) in the Celcius scale and maintained at 25 ± 2 °C. In addition, the pH of the culture media was observed daily utilizing a pH meter

(Fisher Scientific Accumet^{R} Basic AB15 plus Meter) and recorded in the range of 6.7 to 7.9 in all experimental runs.

2.4 Analytical methods

2.4.1 Effect of the nitrogen to phosphorus (NP) ratios

Chlorella kessleri was cultured in media prepared with varying NP ratios to examine how the concentration of nitrogen and phosphorus influences the microalgae growth, the extent of nutrient removal, and bio-fixation of CO_2. The range of nitrogen and phosphorous concentration of the culture media was maintained in the range of observed concentrations of municipal wastewater with high and low waste content (Abdelaziz et al. 2013). The batch experiments were conducted in culture media with NP ratios of 2:1, 4:1, 6:1, and 8:1, while the carbon dioxide concentration in air supplied to the culture media was maintained at 2% for each experimental run. The same light intensity, temperature, and pH was used in all experiments and they are described in the section on experimental procedure and design. The nitrogen and phosphorous concentration were computed by determining the total nitrogen (TN) in the form of NO_3^--N and total phosphorus (TP) in the form of PO_4^{3-} in the culture media on a daily basis utilizing a spectrophotometer (DR 3900-HACH, USA) and a reactor (DRB 200-HACH, USA). In batch kinetics, the rate of TN and TP removal were determined using the following equation as percentage basis:

$$\%removal = \frac{S_0 - S_t}{S_0} \times 100$$

1

where S_0 and S_t is the substrate concentrations at the beginning, time t_0 and at the end of the cultivation time t_t correspondingly.

2.4.2 Biomass concentration, specific growth, and biomass productivity

Biomass concentration

The algal biomass in each photobioreactor was observed daily by computing the optical density (OD) at a wavelength of 690 nm (OD_{690}) (Wang et al. 2014) utilizing a UV-VIS spectrophotometer (Evolution 260 Bio-Thermo Scientific). At the same time, a 20-mL microalgae sample was withdrawn daily from the photobioreactors to determine the dry weight (mg/L) of the biomass. Hence, the magnitude of the dry weight represents the biomass concentration of the microalgae species (Razzak 2019). The relationship between the dry biomass weight and OD_{690} is linear, expressed as $y = mx_{OD690} + C$, with m = 0. 2228, C = 0.0333, and an R^2 of 0.989.

The specific growth rate, μ_g, described as the growth of the dry biomass weight per day, was computed applying the subsequent equation (Das et al.2011; Abreu et al.2012).

Specific growth rate,

$$Specific\ growth\ rate, \mu_g = \frac{\ln\left(\frac{X_2}{X_1}\right)}{t_2 - t_1} \qquad (2)$$

where,

where, X_1 and X_2 is the dry biomass weight at time t_1 and t_2 of the exponential growth phase.

Biomass productivity

The biomass productivity (P_b), which is also described as the rate of biomass production, was computed applying the following equation (Tang et al. 2011; Mohsenpour et al. 2012; Andruleviciute et al. 2014).

Biomass productivity,

$$Biomass\ productivity,\ P_b = \frac{X_t - X_0}{t_t - t_0} \qquad (3)$$

where, X_0 and X_t is the dry biomass weight at the beginning, time t_0 and at the end of the cultivation time, t_t

2.4.3 Growth kinetic model based on a single substrate factor

Growth of microalgae relies on the accessibility of nutrients such as N, P, and C in water environments under light saturation. Several kinetic models proposed for the growth are based on the concentration of a single nutrient (Lee et al. 2015). As the growth rate is regulated by the external nutrient concentration, it depends on the concentration of nutrients in the culture medium. The Monod model (Monod 1949) is a representative model, which considers only the limitation of nutrients. In this study, the Monod model expressed by the following equation is used to study the growth kinetic model:

$$\mu = \frac{\mu_m S}{K_s + S} \qquad (4)$$

where, μ is the specific growth rate, μ_m is the limiting nutrient concentration (mg/L), and $K_s$is the half saturation coefficient (mg/L).

Rearranging Eq.4), provides the following forms of the equation:

$$\frac{1}{\mu} = \frac{K_s + S}{\mu_m S}$$

5

$$\frac{1}{\mu} = \frac{K_s}{\mu_m} \bullet \frac{1}{S} + \frac{1}{\mu_m}$$

6

A linear plot of $\frac{1}{\mu}$ vs $\frac{1}{S}$ will provide the values of K_s and μ_m .

2.4.4 Determination of the carbon content and the bio-fixation rate of carbon dioxide

The bio-fixation rate of CO_2, R_{CO2}, was computed by determining the total carbon content of microalgae utilizing a TOC analyser (Teledyne Tekmar^R Torch Combustion TOC/TN Analyzer). The amount of carbon in microalgal biomass was calculated using the following equation:

$$\% \text{ C} = \frac{TOC_{biomass}}{X} \times 100 \qquad (7)$$

where, $TOC_{biomass}$ is the total organic carbon of microalgal biomass (mg/L), X is the biomass concentration (mg/L) on a given day, and % C represents the percent carbon content in dry biomass.

According to De Morais and Costa (de Morais and Costa 2007), the bio-fixation rate of carbon dioxide (R_{CO2}) was computed using the following equation:

$$R_{CO_2} = C_{carbon} P_B \left(\frac{M_{CO_2}}{M_C} \right)$$

8

where

where C_{carbon} carbon content of the microalgae species *Chlorella Kessleri*, M_{CO2} is the molecular weight of CO_2,M_c is the atomic weight of carbon, and P_B is the productivity of biomass.

Results And Discussion

3.1 Biomass concentration and the specific growth rate at different NP ratios

Healthy growth of microalgae determines the efficiency of the treatment process. Understanding and controlling the essential factors that directly impact microalgae growth is important to maintain a healthy growth of microalgae. Several environmental issues influence the rate of nutrient consumption by various species of microalgae. Together with other environmental issues, nutrient concentration and the NP ratios have a direct impact on the removal of nutrients, ultimately influencing the microalgal growth (Cai et al. 2013). Figure 2 depicts the time profiles of the biomass concentration and the specific growth rate of *Chlorella kessleri* cultivated at various NP ratios at a temperature of 25°C and a CO_2 concentration of 2% in air supplied to the culture media. Different NP ratios have a significant impact on the microalgae growth, which is confirmed by the biomass concentration and the specific growth rate depicted in Fig. 2. The biomass concentration increases at all investigated NP ratios of 2:1, 4:1, and 6:1 during the cultivation period of 13 days. However, the maximum biomass concentration of 606.79 mg/L was gained for the NP ratio of 2:1 at the end of the cultivation period. After reaching its highest value at each NP ratio in the first few days, the specific growth rate gradually decreases during the cultivation period. The specific growth rate at the NP ratio of 2:1 is higher than other two NP ratios (4:1 and 6:1) over the entire cultivation period, with a maximum specific growth rate of 1.74 d^{-1} attained on day 1.

3.2 Kinetic model based on a single substrate factor for microalgae growth

At constant temperature, light intensity, and homogeneous mixing, the substrate concentration and nutrient accessibility normally determines the rate of biomass accumulation during microalgae growth. The Monod model, as expressed in Eq. (4), is usually applied to analysis the microalgae growth restricted by a single nutrient (in this study nitrogen). This model has been used to predict and control algae cultivations in wastewater media. Figure 3 (a, b, and c) shows the experimentally determined specific growth rate and its Monod model predictions plotted as a function of the available nutrient (nitrogen) concentration in media for the cultivation of *Chlorella kessleri* at NP ratios of 2:1, 4:1, and 6:1 and a 2% concentration of CO_2 in the supplied air. The microalgae kinetic model was standardized utilizing experimental *Chlorella kessleri* sp. cultivation results to establish the maximum specific growth rate (μ_m) and half saturation constant (k_s). The kinetic model was validated utilizing supplementary experimental data for the cultivation of *Chlorella kessleri* in wastewater media.

3.3 Effect of the NP ratio on the inorganic nutrient removal

The nitrogen to phosphorous (NP) ratio has been found to influence the removal of nutrients from wastewater. In this study, the synthetic wastewater media with different NP ratios were prepared using a constant total nitrogen (TN) concentration and varying the total phosphorous (TP) concentration. Figure 4a shows the time profiles of the total nitrogen (TN) concentration and the percent total nitrogen (TN) removal by *Chlorella kessleri* cultivated under different NP ratios at a temperature of 25□C and a concentration of 2% CO_2 in the supplied air. Corresponding time profiles of the total phosphorus (TP) concentration and the percent total phosphorus (TP) removal are depicted in Fig. 4b. In general, the concentration of total nitrogen and total phosphorous decreases steadily during the cultivation period (13 days) at all evaluated NP ratios due to the consumption of N and P nutrients needed for the microalgae growth. The corresponding removal efficiency of nitrogen and phosphorous at the end of the 13-day cultivation period is shown in Table 1. The removal efficiency of nitrogen is more than 95% for all NP ratios evaluated except 8:1, at which the efficiency is only 72.4%. The lower nitrogen removal efficiency at an NP ratio of 8:1 may be due to the unavailability of the required amount of phosphorous in the culture media (Xin et al. 2010b). On the other hand, the NP ratio has a large effect on the removal efficiency of phosphorous. The maximum phosphorous removal efficiency at the NP ratio of 6:1 is about 97%. However, the maximum removal efficiency of phosphorous at the NP ratio of 2:1is about 20%, which is the lowest among the NP ratios evaluated. The significant variation of the removal efficiency of phosphorous at different NP ratios may be due the variation of the initial concentration of phosphorous and the limited availability of nitrogen in the culture media (Beuckels et al. 2015).

Table 1
Nutrient removal efficiency at different initial TN or TP concentrations at the end of the13-day cultivation period

Initial TN (mg/L)	**43.6**	**47.5**	**48.1**	**48.1**
NP ratio	2:1	4:1	6:1	8:1
TN removal efficiency (%)	99.54	96.46	95.45	72.4
Initial TP (mg/L)	18.1	9.7	5.6	5.7
NP ratio	2:1	4:1	6:1	8:1
TP removal efficiency (%)	19.92	88.85	96.26	50.87

3.4 Carbon content and biomass concentration under different NP ratios

The time-course profiles of the carbon content and biomass concentration of *Chlorella kessleri* cultivated under different nutrient levels at a temperature of 25□C and a concentration of 2% of CO_2 in the supplied air are presented in Fig. 5. The carbon content of the microalgae increases in the first five days of the cultivation period and starts decreasing after reaching a maximum at all NP ratios. On the other hand, the biomass concentration steadily increases during the entire cultivation period at all NP ratios. The increased percentage of carbon content in microalgae during the early stages of the cultivation period

may be due to the uptake of high C-rich metabolites during cultivation (Beuckels et al. 2015). The percent carbon content of microalgae reaches the highest value of 56% at the NP ratio of 2:1.

3.5 Effect of the NP ratio on the CO_2 bio-fixation rate (R_{CO2})

A lot of research and development studies is carried out around the world on a variety of methods to mitigate the effects of CO_2 on the environment. Among them, using microalgal photosynthesis for biological CO_2 fixation has been found to be an efficient method (Shaikh A. Razzak et al. 2013; Wang et al. 2008; Yanagi et al. 1995). During photosynthesis by microalgae, CO_2 is converted into lipids and other hydrocarbons, during which water acts as an electron donor and its hydrolysis emits the oxygen. The overall reaction for photosynthesis, resulting in CO_2 fixation, can be written as (Razzak et al. 2013)

$H_2O + CO_2 + photons\ [CH_2O]n + O_2$

In several studies, photobioreactors have been used to examine the influence of the concentration of nutrients on the growth of microalgae. The CO_2 fixation rate is clearly associated with the light consumption efficiency and the biomass concentration of microalgae. Morais and Costa (2007) (de Morais and Costa 2007) reported that CO_2 concentration has an impact on microalgae growth and CO_2 bio-fixation rate when *Chlorella kessleri* is cultured in a modified Bristol medium. However, in this study, the bio-fixation rate of CO_2 by *Chlorella kessleri* at different NP ratios was evaluated in synthetic wastewater media. Figure 6 shows the time-course profiles of carbon dioxide bio-fixation by *Chlorella kessleri* cultivated under different nutrient levels at a temperature of 25°C and with 2% CO_2 in the supplied air. The rate of bio-fixation of CO_2 increases in the first 7 days of the cultivation period at all NP ratios, reaching its highest value before gradually decreasing during the remainder of the cultivation period. The maximum CO_2 bio-fixation rate of 89.36 mg/L/d during the 7 days of cultivation period was obtained at an NP ratio of 6:1 and a concentration of 2% CO_2 in the air supplied to the culture media.

3.6 Comparison analysis

The bar chart in Fig. 7 depicts the overall productivity of the biomass (PB), rate of bio-fixation of CO_2 (R_{CO2}), and TN and TP removal by *Chlorella kessleri* under different NP ratios (2:1 to 6:1) in a cultivation period of 13 days at a temperature of 25°C and a concentration of 2% CO_2 in the supplied air. PB undergoes a minor variation when the NP ratio increased from 2:1 to 6:1, with the maximum PB observed at an NP ratio of 2:1. The rate of bio-fixation of CO_2 (R_{CO2}) also undergoes insignificant changes when cultivated at NP ratios ranging from 2:1 to 6:1. The removal of TN is more than 95% at all NP ratios, whereas the removal of TP significantly increases when the NP ratio is varied from 2:1 through 4:1 to 6:1.

Conclusion

The growth of the microalgae *Chlorella kessleri* sp. was evaluated in synthetic wastewater under different photoautotrophic conditions for the simultaneous CO_2 bio-fixation and the removal of nitrogen and

phosphorous. The synthetic wastewater is considered as equivalent to municipal tertiary wastewater for cultivating the microalgae sp. under different NP ratios and a concentration of 2% CO_2 in the supplied air. The maximum specific growth rate (μ_m) of *Chlorella kessleri* sp. and the concentration of the nutrients are in accordance with the Monod model. In this study, the NP ratio was maintained in a suitable range (2:1 to 6:1) to increase the removal efficiency of nitrogen by *Chlorella kessleri* sp. However, the removal efficiency of phosphorous is significantly affected by the variation of the NP ratio. According to the results of this study, the following conclusions can be drawn:

1. Biomass concentration increased at all studied NP ratios and the maximum biomass concentration of 606.79 mg/L was found at an NP ratio of 2:1.
2. The NP ratio and initial nutrient concentration are substantial factors affecting the efficiency of the removal of nutrients in wastewater. More than 95% of nitrogen is removed for at all evaluated NP ratios except 8:1, at which the percent removal is only 72.4%. On the other hand, the maximum 97% phosphorous removal efficiency is observed at an NP ratio of 6:1.
3. For all NP ratios, the bio-fixation rate of CO_2 gradually increases over the first seven days of the cultivation period and the maximum bio-fixation rate of CO_2 of 89.36 $mgL^{-1}d^{-1}$ is observed at an NP ratio of 6:1.

In terms of CO_2 bio-fixation rates as well as both TN and TP removal efficiency, *Chlorella kessleri* has the potential to be used for low-cost tertiary municipal wastewater treatment.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

King Fahd University of Petroleum and Minerals (KFUPM) Internal research grant under direct funding grant and financial support for this work through project No. DF191050.

Authors' contributions

Mr. Mohammed Omar Faruque

Acknowledgements

Authors would like to acknowledge the support received from King Fahd University of Petroleum & Minerals (KFUPM) along with internal direct funding grant and financial support for this work through project No. DF191050.

References

1. Abdelaziz AEM, Leite GB, Hallenbeck PC (2013) Addressing the challenges for sustainable production of algal biofuels: I. Algal strains and nutrient supply. Environ Technol (United Kingdom). https://doi.org/10.1080/09593330.2013.827748
2. Abreu AP, Fernandes B, Vicente AA et al (2012) Mixotrophic cultivation of Chlorella vulgaris using industrial dairy waste as organic carbon source. Bioresour Technol 118:61–66. https://doi.org/10.1016/j.biortech.2012.05.055
3. Andruleviciute V, Makareviciene V, Skorupskaite V, Gumbyte M (2014) Biomass and oil content of Chlorella sp., Haematococcus sp., Nannochloris sp. and Scenedesmus sp. under mixotrophic growth conditions in the presence of technical glycerol. J Appl Phycol. https://doi.org/10.1007/s10811-013-0048-x
4. Arbib Z, Ruiz J, Álvarez-Díaz P et al (2014) Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO2 bio-fixation and low cost biofuels production. Water Res 49:465–474. https://doi.org/10.1016/j.watres.2013.10.036
5. Beuckels A, Smolders E, Muylaert K (2015) Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. Water Res 77:98–106. https://doi.org/10.1016/j.watres.2015.03.018
6. Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew Sustain Energy Rev 19:360–369. https://doi.org/10.1016/j.rser.2012.11.030
7. Das P, Lei W, Aziz SS, Obbard JP (2011) Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. Bioresour Technol. https://doi.org/10.1016/j.biortech.2010.11.102
8. de Morais MG, Costa JAV (2007) Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers Manag 48:2169–2173.

https://doi.org/10.1016/j.enconman.2006.12.011

9. Driver T, Bajhaiya A, Pittman JK (2014) Potential of Bioenergy Production from Microalgae. Curr Sustain Energy Reports 1:94–103. https://doi.org/10.1007/s40518-014-0011-8
10. Eze VC, Velasquez-Orta SB, Hernández-García A et al (2018) Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration. Algal Res 32:131–141. https://doi.org/10.1016/j.algal.2018.03.015
11. Halling-Sørensen B, Nyholm N, Baun A (1996) Algal toxicity tests with volatile and hazardous compounds in air-tight test flasks with CO2 enriched headspace. Chemosphere. https://doi.org/10.1016/0045-6535(96)00059-8
12. Hernandez JP, De-Bashan LE, Bashan Y (2006) Starvation enhances phosphorus removal from wastewater by the microalga Chlorella spp. co-immobilized with Azospirillum brasilense. Enzyme Microb Technol 38:190–198. https://doi.org/10.1016/j.enzmictec.2005.06.005
13. Khan M, Yoshida N (2008) Effect of l-glutamic acid on the growth and ammonium removal from ammonium solution and natural wastewater by Chlorella vulgaris NTM06. Bioresour Technol 99:575–582. https://doi.org/10.1016/j.biortech.2006.12.031
14. Lee E, Jalalizadeh M, Zhang Q (2015) Growth kinetic models for microalgae cultivation: A review. Algal Res
15. Lee K, Lee CG (2002) Nitrogen removal from wastewater by microalgae without consuming organic carbon sources. J Microbiol Biotechnol
16. Li Y, Horsman M, Wu N et al (2008) Biofuels from Microalgae. Biotechnol Prog 0:0–0. https://doi.org/10.1021/bp070371k
17. Mohsenpour SF, Richards B, Willoughby N (2012) Spectral conversion of light for enhanced microalgae growth rates and photosynthetic pigment production. Bioresour Technol. https://doi.org/10.1016/j.biortech.2012.08.072
18. Monod J (1949) The Growth of Bacterial Cultures. Annu Rev Microbiol. https://doi.org/10.1146/annurev.mi.03.100149.002103
19. Moreno Osorio JH, Pinto G, Pollio A et al (2019) Start-up of a nutrient removal system using Scenedesmus vacuolatus and Chlorella vulgaris biofilms. Bioresour Bioprocess. https://doi.org/10.1186/s40643-019-0259-3
20. Olguín EJ (2003) Phycoremediation: Key issues for cost-effective nutrient removal processes. In: Biotechnology Advances. Elsevier Inc., pp 81–91
21. Rajasulochana P, Preethy V (2016) Comparison on efficiency of various techniques in treatment of waste and sewage water – A comprehensive review. Resour Technol 2:175–184. https://doi.org/10.1016/j.reffit.2016.09.004
22. Razzak SA (2019) In situ biological CO 2 fixation and wastewater nutrient removal with Neochloris oleoabundans in batch photobioreactor. Bioprocess Biosyst Eng 42:93–105. https://doi.org/10.1007/s00449-018-2017-x

23. Razzak SA, Ali SAM, Hossain MM, deLasa H (2017) Biological CO2 fixation with production of microalgae in wastewater – A review. Renew Sustain Energy Rev 76:379–390
24. Razzak SA, Hossain MM, Lucky RA et al (2013) Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing - A review. Renew Sustain Energy Rev 27:622–653
25. Shi J, Podola B, Melkonian M (2007) Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: An experimental study. J Appl Phycol 19:417–423. https://doi.org/10.1007/s10811-006-9148-1
26. Tang D, Han W, Li P et al (2011) CO2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO2 levels. Bioresour Technol 102:3071–3076. https://doi.org/10.1016/j.biortech.2010.10.047
27. Wang B, Li Y, Wu N, Lan CQ (2008) CO2 bio-mitigation using microalgae. Appl. Microbiol. Biotechnol
28. Wang Y, Chen T, Qin S (2014) Heterotrophic cultivation of Chlorella kessleri for fatty acids production by carbon and nitrogen supplements. Biomass Bioenerg 47:402–409. https://doi.org/10.1016/j.biombioe.2012.09.018
29. Xin L, Hong-ying H, Ke G, Jia Y (2010a) Growth and nutrient removal properties of a freshwater microalga Scenedesmus sp. LX1 under different kinds of nitrogen sources. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2009.11.003
30. Xin L, Hong-ying H, Ke G, Ying-xue S (2010b) Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. Bioresour Technol 101:5494–5500. https://doi.org/10.1016/j.biortech.2010.02.016
31. Yanagi M, Watanabe Y, Saiki H (1995) CO2 fixation by Chlorella sp. HA-1 and its utilization. Energy Convers Manag. https://doi.org/10.1016/0196-8904(95)00104-L
32. Yu JU, Kim HW (2017) Enhanced Microalgal Growth and Effluent Quality in Tertiary Treatment of Livestock Wastewater Using a Sequencing Batch Reactor. Water Air Soil Pollut. https://doi.org/10.1007/s11270-017-3547-6

Tables

Table 1: Nutrient removal efficiency at different initial TN or TP concentrations at the end of the13-day cultivation period

Initial TN (mg/L)	43.6	47.5	48.1	48.1
NP ratio	2:1	4:1	6:1	8:1
TN removal efficiency (%)	99.54	96.46	95.45	72.4
Initial TP (mg/L)	18.1	9.7	5.6	5.7
NP ratio	2:1	4:1	6:1	8:1
TP removal efficiency (%)	19.92	88.85	96.26	50.87

Figures

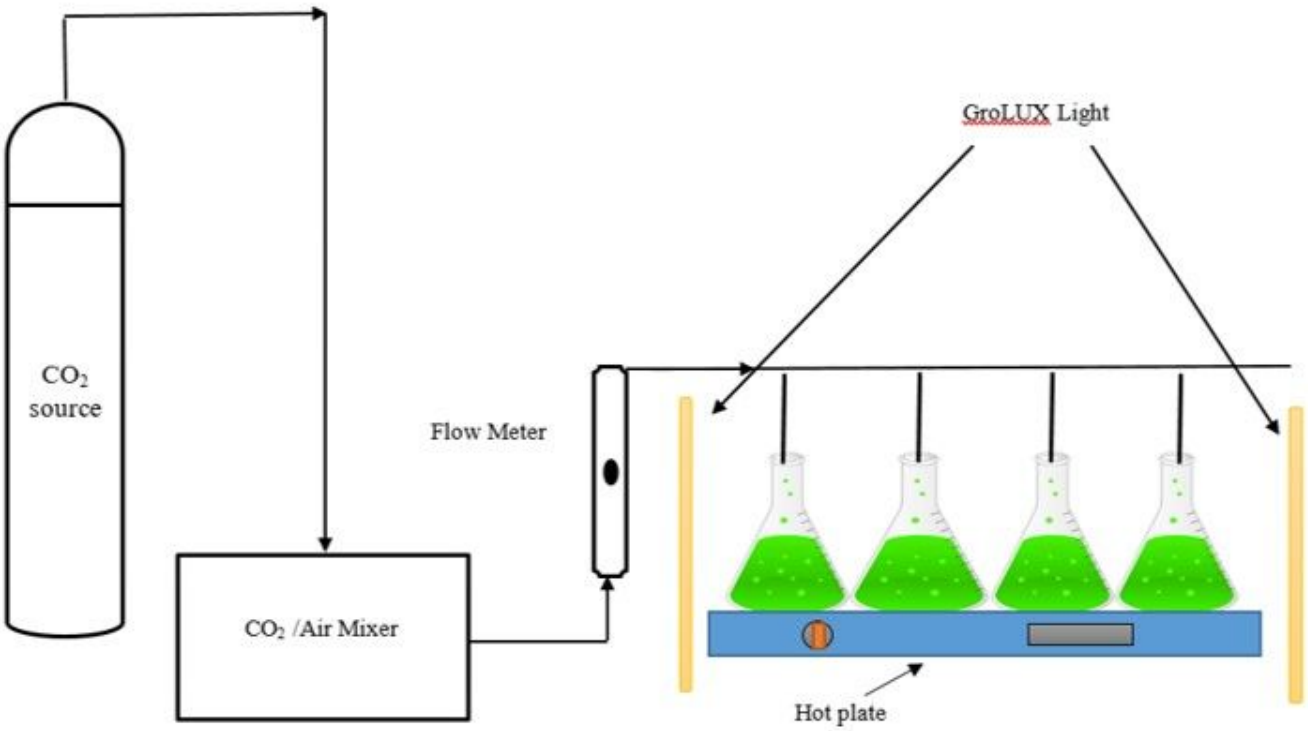

Figure 1

A schematic diagram of the experimental set-up.

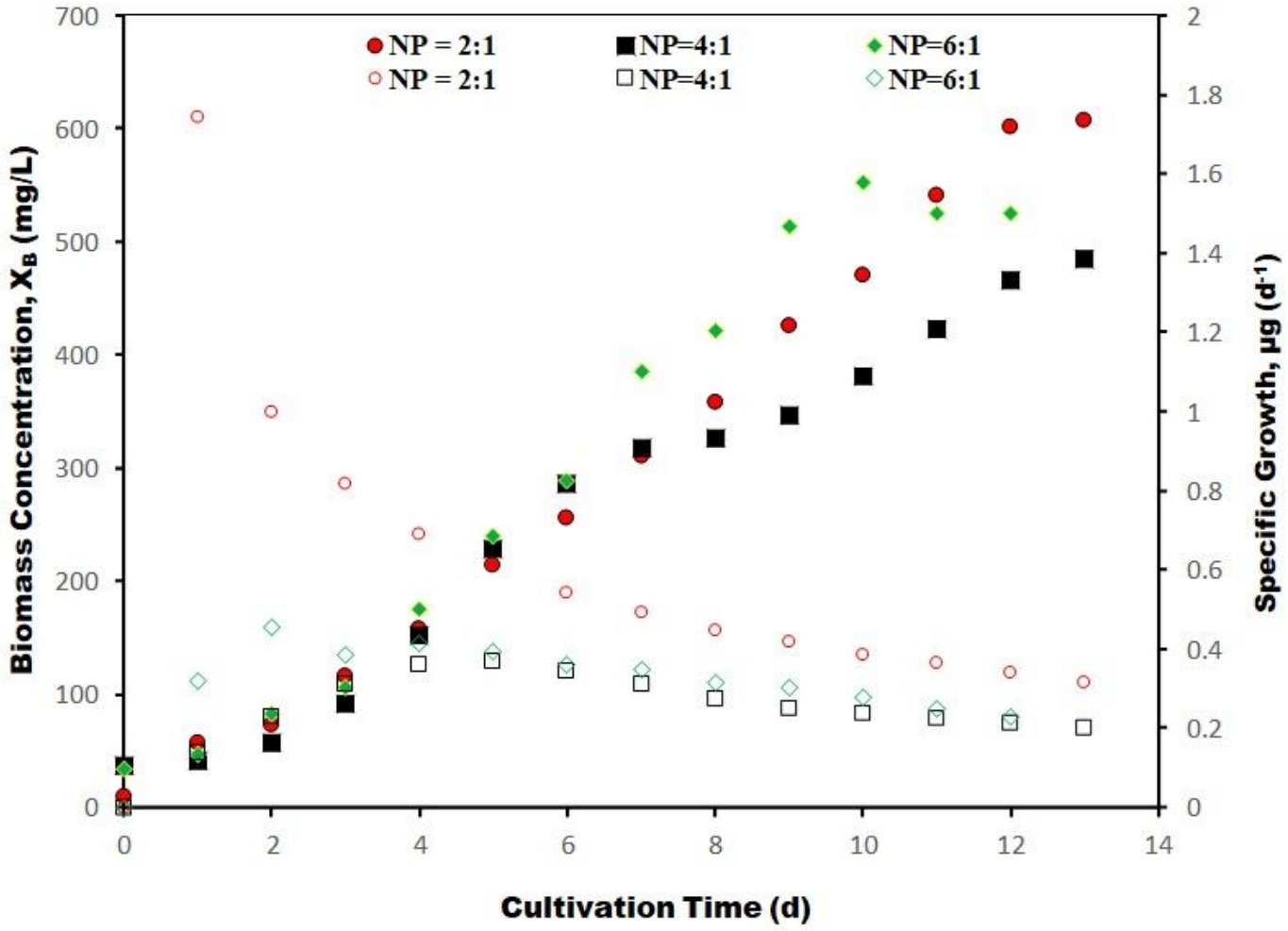

Figure 2

The time-course profiles of the biomass concentration (filled markers) and specific growth rate (empty markers) of Chlorella kessleri cultivated under different NP ratios at a temperature of 25°C and a CO2 concentration of 2% in the supplied air.

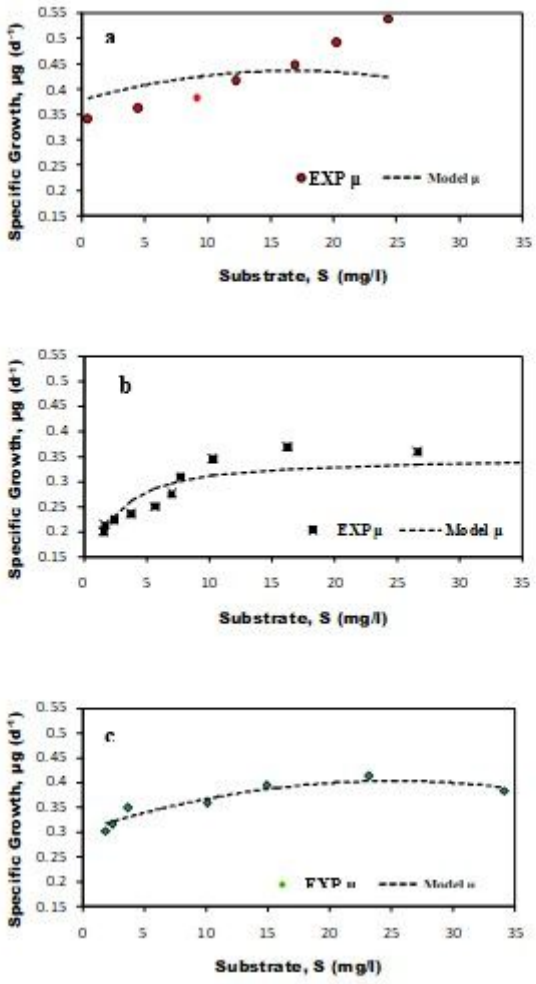

Figure 3

Experimental and Monod model predictions of specific growth rate, µg (d-1) as a function of the available substrate (nitrogen) concentration in media supplied with 2% CO2 in air during the cultivation of Chlorella kessleri at an NP ratio of (a) 2:1, (b) 4:1, and (c) 6:1.

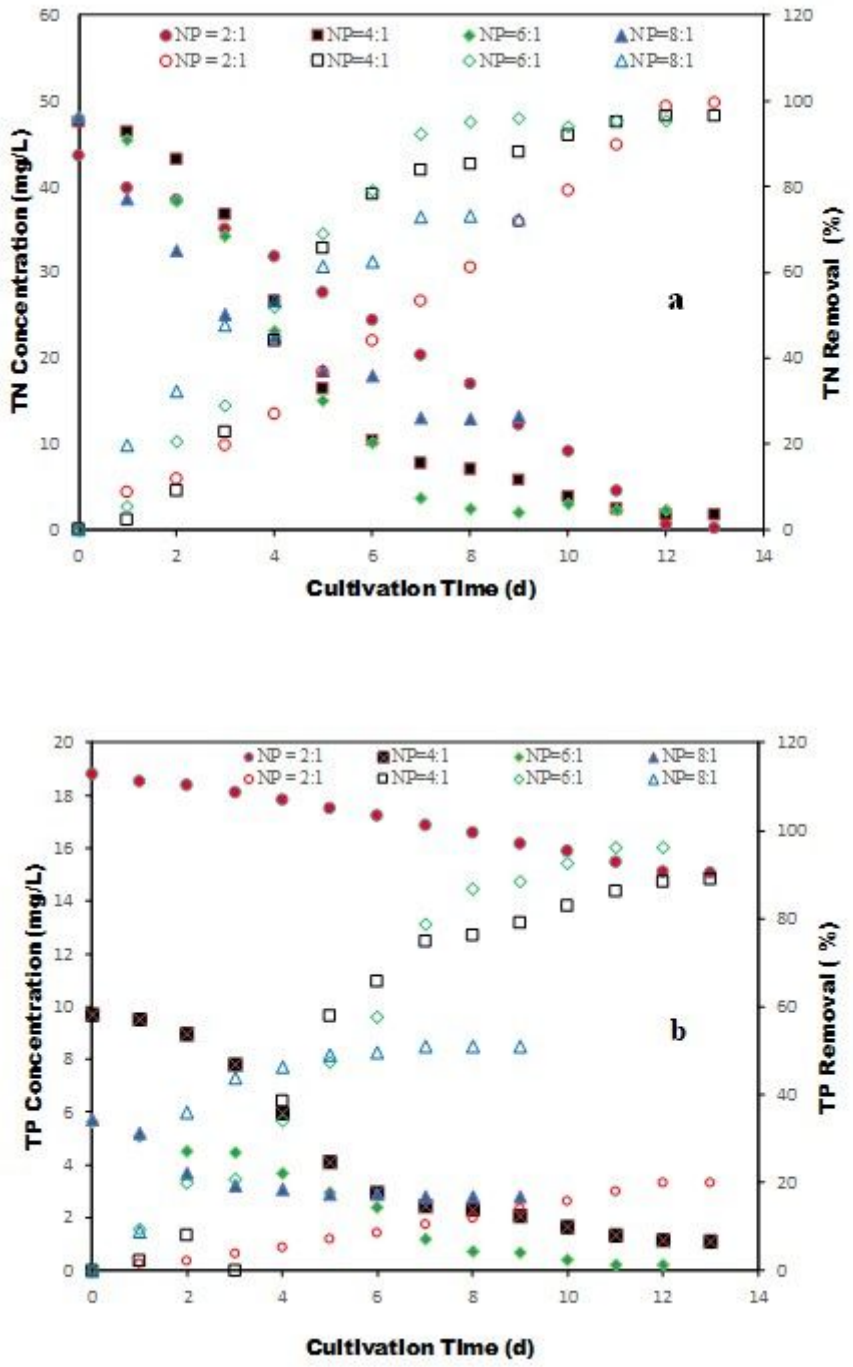

Figure 4

The time-course profiles of (a) total nitrogen (TN) concentration (filled markers) and total nitrogen (TN) removal (empty markers), (b) total phosphorus (TP) concentration (filled markers) and total phosphorus(TP) removal (empty markers) during cultivation of Chlorella kessleri under different NP ratios at a temperature of 25□C and a CO2 concentration of 2% in the supplied air.

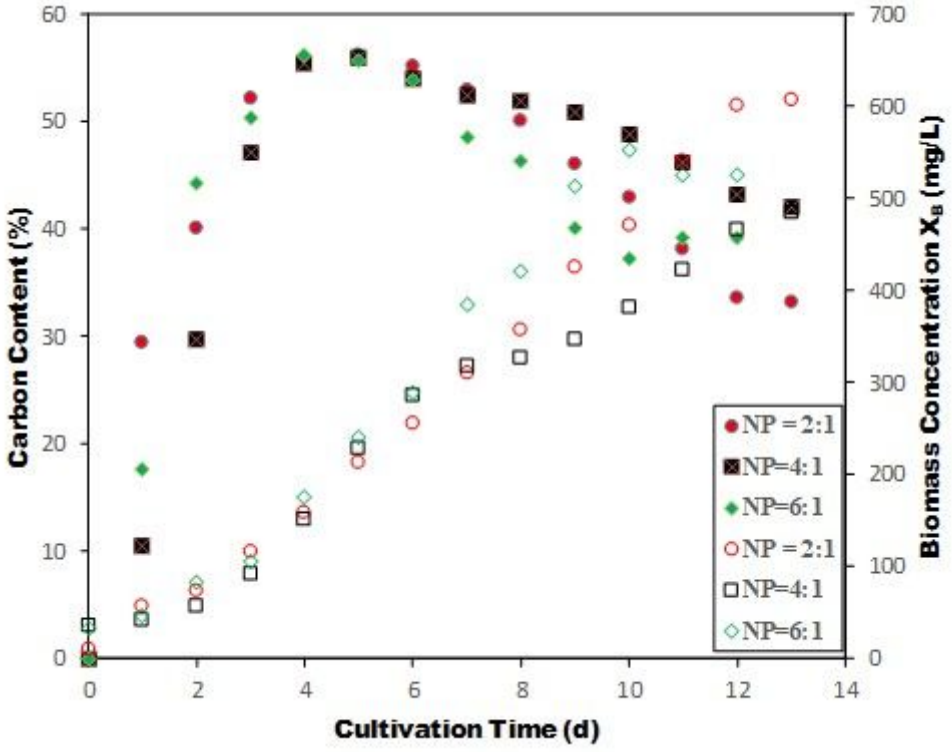

Figure 5

The time-course profiles of carbon content (filled markers) and biomass concentration (empty markers) during the cultivation of Chlorella kessleri under different NP ratios at a temperature of 25□C and a CO2 concentration of 2% in the supplied air.

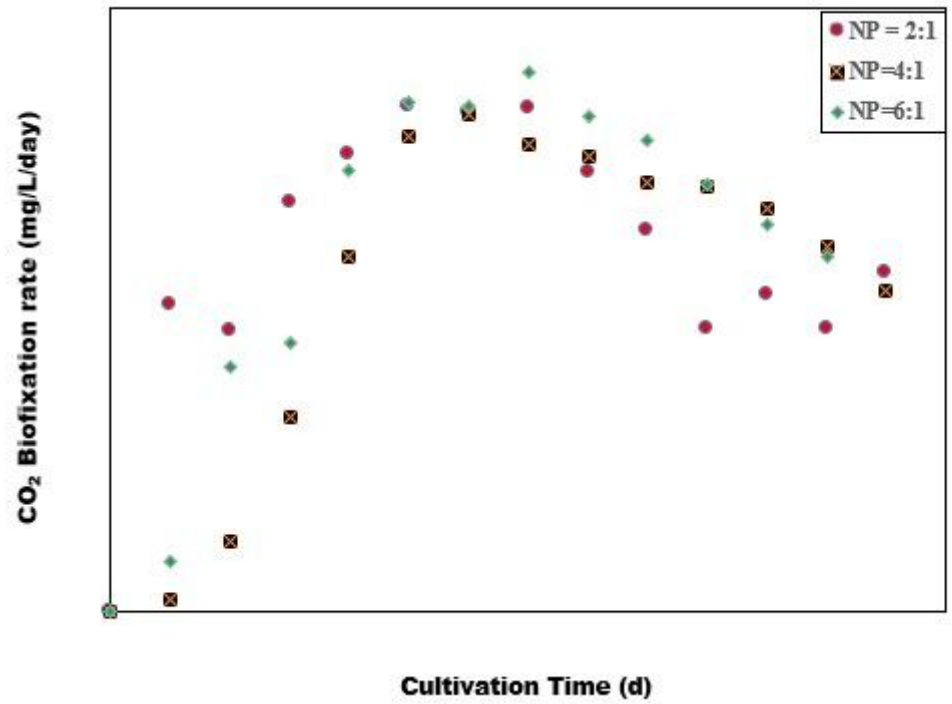

Figure 6

The time-course profiles of the capacity of bio-fixation of carbon dioxide during the cultivation of Chlorella kessleri under different NP ratios at a temperature of 25□C and a CO2 concentration of 2% in the supplied air.

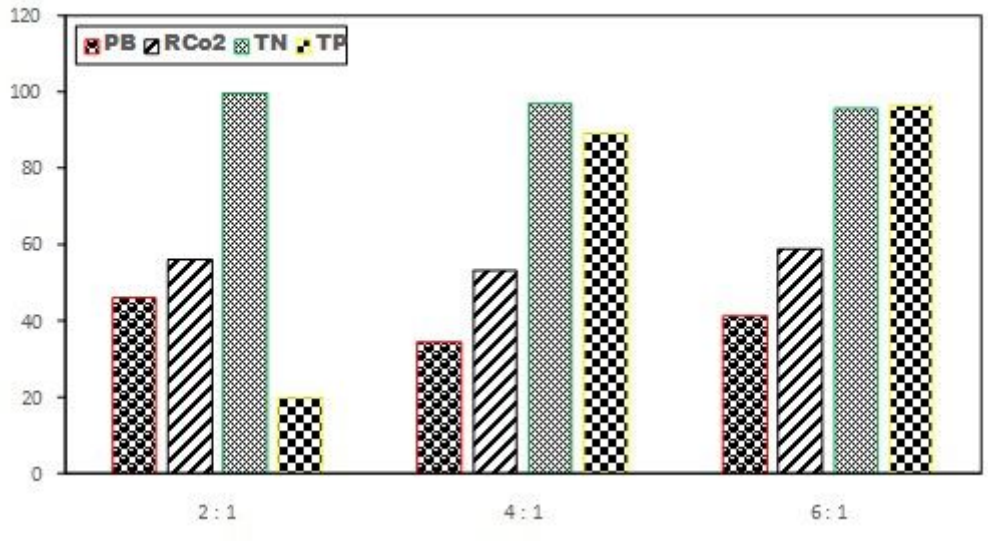

Figure 7

Biomass productivity (PB), rate of CO2 bio-fixation (RCO2), and total nitrogen (TN) and total phosphorus (TP) removal using Chlorella kessleri cultivated under different NP ratios at a temperature of 25 □C and a CO2 concentration of 2% in the supplied air.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx