Optimization of Cultivation Condition of Newly Isolated Strain Chlorella Sorokiniana pa.91 for CO2 Bio-Fixation and Nutrients Removal From Real Municipal Wastewater: Impact of Temperature and Light Intensity

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Optimization of cultivation condition of newly isolated strain *Chlorella sorokiniana pa.91* for CO$_2$ bio-fixation and nutrients removal from wastewater: Impact of temperature and light intensity

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ABSTRACT

The cultivation conditions of a newly isolated strain *Chlorella sorokiniana pa.91* were optimized for the first time by performing sixty batch cultivation experiments at various temperatures (20, 25, 30 and 35 °C) and light intensities (1000, 3000, 4000, 5000 and 7000 Lux) in three different culture mediums of BG-11, real settled municipal wastewater (RMWW) and synthetic wastewater (SWW). Additionally, to evaluate the capability of *C. sorokiniana pa.91* in CO$_2$ bio-fixation and wastewater treatment, the microalgae was cultivated in a flat-plate photobioreactor (CO$_2$ = 16% and 0.6 vvm aeration) under the optimal condition. The optimization results suggested that at the culture conditions of 30 °C, 4000 Lux and RMWW (COD 211 mgL$^{-1}$) microalgae had the best performance in growth and biomass productivity. Maximum biomass concentration and productivity of 3.21 gL$^{-1}$ and 0.31 gL$^{-1}$d$^{-1}$ were achieved, respectively, by cultivation of *C. sorokiniana pa.91* in the photobioreactor under the optimized condition. Experimental results showed that *C. sorokiniana pa.91* has a high capacity of CO$_2$ bio-fixation (0.59 mgL$^{-1}$d$^{-1}$) and CO$_2$ removal rate (35.6 %). Moreover, using *C. sorokiniana pa.91* could efficiently remove 74% of NH$_3$, 93% of NO$_3$-, 83% of PO$_4$$^{3-}$ and 76% of COD from real municipal wastewater after eight days of cultivation in the photobioreactor.

**Keywords:** Microalgae; *Chlorella sorokiniana pa.91*; Optimization; CO$_2$ bio-fixation, Wastewater nutrients removal,

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1
1. **Introduction**

The increasing use of water in urban area due to increased urbanization, industrialization and population growth has led to generate large amount of municipal wastewater. The discharge of untreated wastewater into the ecosystem has caused several environmental problems due to its high pollutants concentrations such as nitrate, phosphate and chemical oxygen demand (COD) \((\text{AlMomani and Órmecci, 2016; De Francisci et al., 2018; Wang and Lan, 2011})\). Currently, several methods have been comprehensively investigated for municipal wastewater treatment including chemical/physical treatments such as electrochemical or chemical oxidation \((\text{Shahid et al., 2020})\) which can remove high concentration of COD, and biological treatments such as anaerobic sequencing batch reactor, constructed wetlands and lagoon trickling filter \((\text{Yang et al., 2019})\). However, most of the chemical/physical methods are not environmentally friendly and in the case of biological methods, removal efficient in phosphorus may not be satisfactory \((\text{Li et al., 2019})\). Moreover, the current treatment methods produce high amount of sludge which required significant energy demand resulting in increased carbon dioxide \((\text{Javed et al., 2019})\). The increased emissions of CO\(_2\) in the atmosphere is another global crises leads to serious environmental problems \((\text{Yin et al., 2020})\). The total emission of CO\(_2\) has increased from 98 to 395 PPMV (parts per million volume) since 1990 and expected to reach 500 PPMV by 2050 if no reaction is taken \((\text{Shahid et al., 2020})\). Several attempts have been made to reduce CO\(_2\) concentration such as carbon capture, storage and utilization technologies \((\text{Nocito and Dibenedetto, 2020})\).

Meanwhile, microalgae has received great attention due to their enormous multiple applications for wastewater treatment, sustainable CO\(_2\) bio-fixation and producing high-valuable bio-produces \((\text{Anto et al., 2020; Pugazhendhi et al., 2020})\). Microalgae can reduce the nutrients such as nitrogen and phosphorus from wastewater by consuming them that promote microalgae biomass growth rate \((\text{Watsuntorn et al., 2019})\). In addition to the wastewater
treatment, microalgae have a great potential for carbon mitigation and CO₂ bio-fixation due to their higher photosynthetic efficiency (40–50% more than earthy plants) (Chang et al., 2016). It has been reported previously that microalgae contributed to almost 40% of global CO₂ sequestration. For example, 1.83 kg of CO₂ can be utilized by approximately 1 kg of microalgae (Ng et al., 2017).

Several research have investigated the performance of different microalgae strains for treatment of municipal and industrial wastewaters. Among them some microalgae species which grow rapidly have shown an excellent performance in nutrient removal from wastewater includes Chlorella sp. (Wang et al., 2010), Nannochloropsis sp. (Jiang et al., 2011) and Scenedesmus sp. (Xu et al., 2015). According to Kumar et al., (2019), Chlorella sp. microalgae could remove significant amounts of nitrate, phosphate and COD up to 67%, 88% and 71% from sewage wastewaters (Kumar et al., 2019). In another attempt, Wang et al. (2017) has reported that Neochloris aquatica CL-M1 microalgae could achieve a removal rate of 81% of COD and 96% of NH₄⁺ from swine wastewater (Wang et al., 2017). Novoveská et al. (2016) used scenedesmus dimorphus microalgae for raw municipal wastewater treatment. They could achieve 75% of total nitrogen, 93% of phosphorus and 84% of COD removal efficiency (Novoveská et al., 2016). However, still development of a new species of microalgae aiming to achieve high CO₂ bio-fixation and nutrient removal with lower energy consummation and capital investment is required. In this regard, a new microalgae strain chlorella sorokiniana pa.91 that was isolated from dairy wastewater has been introduced in our previous work (Asadi et al., 2019). According to Asadi et al., (2020), C. sorokiniana pa.91 showed a high capability of lipid production in comparison with chlorella vulgaris microalgae (Asadi et al., 2020). Considering the significant capability of C. sorokiniana pa.91 in converting pollutants into lipid production, it is hypothesized that this new microalgae may has a high wastewater pollutant ability and CO₂ bio-fixation potential.
Regardless the microalgae species, the complicated interactions between environmental factors such as temperature and light intensity have substantially influence on microalgae growth rate (Di Capua et al., 2016). Therefore, culture conditions for various microalgae strains needs to be optimized to achieving maximum biomass concentration at reasonable cost (Devi and Parthiban, 2020). The key factors that have significantly influenced on photosynthesis intensity and biomass productivity includes temperature, the light intensity, carbon dioxide and pH (Bazdar et al., 2018). Among them, temperature is an important factor that affects morphology and physiology of microalgae cell through changes in its metabolic rate (Qu et al., 2019). Therefore, finding optimal temperature for each microalgae species is vital to have a best performance of growth in culture medium (Brindhadevi et al., 2021; Nogueira et al., 2015). However, the most expensive environmental factor in microalgae cultivation is light illumination due to the usage of light emitting diodes (LEDs) (Devi and Parthiban, 2020). It has been reported that with an increase in light intensity the biomass growth rate increases up to a photo-inhibitory level (Nzayisenga et al., 2020). Optimization of light intensity in addition to aiming to enhancement of biomass productivity, can reduce of operational energy expenditure (Fan et al., 2020). To the best of our knowledge, the optimum culture condition of C. sorokiniana pa.91 and its capability for CO₂ bio-fixation and nutrient removal from municipal wastewater has not been investigated yet.

Therefore, in the present research, first the optimum condition for C. sorokiniana pa.91 growth were investigated by cultivation of the microalgae at variable temperatures and light intensities in different culture medium. Then, the performance of C. sorokiniana pa.91 for CO₂ bio-fixation and municipal wastewater treatment was comprehensively studied. To do this, the microalgae was cultivated in a flat plate photobioreactor using real municipal wastewater under optimum culture condition. Overall, this study provides an effective strategy for CO₂ bio-fixation and wastewater treatment by a new strain which had not been investigated before.
2. Materials and Methods

2.1. Microalgae strain and culture mediums

A new strain of *Chlorella sorokiniana pa.91* which was previously isolated from Gela dairy wastewater treatment plant (Amol, Iran) was selected and used in this study (Asadi et al., 2019). This strain was recognized and analyzed phylogenetically based on 18S rRNA gene sequence (Asadi et al., 2019). Because of the possibility of impurities and contamination, three purification phases were performed in plates containing agar and BG-11 culture medium. The microalgae cells were inoculated into several 500 ml Erlenmeyer flasks of BG-11 medium to prepare larger volumes of microalgae. Real raw municipal wastewater influent (RMWW) after preliminary settling tank was collected from wastewater treatment plant (WWTP), Sari, Iran. After pretreatment of the wastewater was used as culture medium to evaluate the microalgae capacity for nutrient removal from real municipal wastewater. Additionally, two synthetic media including BG-11 and synthetic wastewater (SWW) were considered in the present work. The physicochemical characteristics of the culture mediums are listed in Table 1.

2.2. Microalgae cultivation experiments

2.2.1. Batch experiments for optimization of cultivation condition

To investigate the optimum cultivation condition for new specie *C. sorokiniana pa.91*, series of batch cultivation experiments were carried out in 250 mL Erlenmeyer flasks containing 150 mL of the culture mediums which were aerated by HAIEAACKO-500 air pump. The effect of temperature and light intensities on microalgae growth in the three culture mediums (BG-11, RMWW and SWW) was evaluated by performing cultivation tests at different temperatures (20, 25, 30 and 35 °C) and light intensities (1000, 3000, 4000, 5000 and 7000 Lux). In each cultivation test, one parameter was varied while maintaining the other parameters constant. Finally, the results of the sixty batch cultivation experiments were used to plot 3D optimization graphs by Surfer 11.2 software. All the experiments were carried out in triplicate.
2.2.2. Photobioreactor experiments for CO$_2$ bio-fixation and nutrient removal

After optimizing the growth conditions of *Chlorella sorokiniana* pa.91, the microalgae was cultivated in a pilot-scale flat-plate photobioreactor (FP-PBR) at the optimum condition to investigate its capacity for CO$_2$ bio-fixation and nutrient removal from real municipal wastewater. The PBR used in this study was 8 L glass flat-plate photobioreactor (340 mm length×100 mm width×400 mm height), equipped with a gas sparger at the bottom of the PBR. On the top of the photobioreactor, three holes were placed to measure CO$_2$ output. Liquid samples were also collected from a sample point at the side of the photobioreactor daily to determined temperature, biomass concentration, pH and nutrient concentrations. The light intensity was provided by white fluorescent lamp that exposed to back side of photobioreactor with cycle of Light/dark 12/12h. In addition, CO$_2$ gas (16%) was aerated by the sparger at the rate of 0.6 vvm into the photobioreactor to providing mixing.

2.3. Analytical method

2.3.1. Determination of biomass concentration and productivity

The cell dry weight was measured by using the optical density at 680 nm in a UV spectrophotometer (2800 UV/VIS UNICO, China). The biomass concentration was determined by washing and drying the samples of microalgae cells in an oven at 70 °C for 24 h (Arbib et al., 2014). The relation between OD$_{680}$ and biomass concentration was obtained according to Eq. 1, as follows:

$$C (gL^{-1}) = 1.824 \text{OD}_{680} (gL^{-1}) + 0.043, (R^2 = 0.9889)$$  \hspace{1cm} (1)

Where, C and OD$_{680}$ are biomass concentration and optical density at 680 nm, respectively. Furthermore, the biomass productivity (P$_b$) were calculated from changes in biomass concentration over time according to Eq. (2) as follows (Nayak et al., 2016):
\[ P_B(gL^{-1}d^{-1}) = (C_f/C_0)/(t_f - t_0) \]  

(2)

Where, \( P_B \) is biomass productivity and \( C_f \) and \( C_0 \) are the final and initial biomass concentration at time \( t_f \) and \( t_0 \) (day), respectively.

2.3.2. Nutrient removal

During the cultivation period, the concentration of main nutrients from the municipal wastewater influent such as phosphate (Po\(^{-3}\)), ammonia (NH\(_3\)), nitrate (NO\(_3^-\)) and sCOD were determined based on standard methods (Association et al., 1912). 50 mL sample of microalgae medium (RMWW) from the photobioreactor was collected and centrifuged at 3500 rpm for 15 min, then, the supernatant was used for measuring the nutrients concentration. The nutrient removal percentage and removal rates were calculated as Eqs (3) and (4):

\[ \text{Removal percentage} \% = \left( C_0 - C_f \right)/C_0 \times 100 \]  

(3)

\[ \text{Removal rate} (gL^{-1}d^{-1}) = \left( C_0 - C_f \right)/(t_f - t_0) \]  

(4)

Where, \( C_0 \) and \( C_f \) are the initial and final nutrient concentrations at day \( t_0 \) and \( t_f \), respectively.

2.3.3 CO\(_2\) gas analysis

The CO\(_2\) gas concentration at the outlet of the photobioreactor were measured by using carbon dioxide analyzer gas detector RIKEN Model RX-515. During cultivation, the exhaust CO\(_2\) concentration was measured by the sensor of gas analyzer. The CO\(_2\) bio-fixation rate and CO\(_2\) removal percentage in the photobioreactor were calculated by the Eqs (5) and (6) (Basu et al., 2013):

\[ \text{CO}_2 \text{ fixation rate} (gL^{-1}d^{-1}) = 1.88 \times P_B \]  

(5)

\[ \text{CO}_2 \text{ removal} \% = \frac{C_{2\text{inlet}} - C_{2\text{outlet}}}{C_{2\text{inlet}}} \times 100 \]  

(6)

Where, \( P_{\text{overall}} \) is the biomass productivity (gL\(^{-1}\)d\(^{-1}\)), \( C_{2\text{inlet}} \) (\%), and \( C_{2\text{outlet}} \) (\%) are the
percentage of $\text{CO}_2$ concentration in the inlet and outlet gas, respectively. The pH and dissolved oxygen (DO) was measured daily by pH meter and dissolved oxygen meter (WA-2017SD LUTRON, Japan), respectively. Light intensity was measured in all experiments by a digital light meter (Mastech MS6610, USA).

**Results and discussion**

3.1. Optimization of cultivation conditions of *Chlorella sorokiniana pa.91*

To promote the growth rate of *Chlorella sorokiniana pa.91* for the purposes of $\text{CO}_2$ bio-fixation and nutrients removal from wastewater, optimization of the culture conditions are required. Among different cultures parameters, temperature and light intensity are the two most important environmental factors that influence the microalgae growth (Singh and Singh, 2015). Therefore, the present research focused on the optimization of temperature and light intensity to enhance microalgae growth rate at different culture mediums.

3.1.1. Effect of temperature on the growth of *Chlorella sorokiniana pa.91*

Temperature has a significant effect on microalgae growth through controlling the rate of chemical reactions, enzyme activities, diffusion rate in water and photosynthesis efficiency (Luo et al., 2017). According to previous studies, a range of 20-35 $^\circ\text{C}$ is an appropriate temperature for microalgae cultivation of many species (Khoo et al., 2019; Singh and Singh, 2015). Therefore, the effect of various temperatures (20 $^\circ\text{C}$, 25 $^\circ\text{C}$, 30 $^\circ\text{C}$ and 35 $^\circ\text{C}$) on the growth rate of *C. sorokiniana pa.91* in the BG-11 culture medium under light intensity 3000 Lux was investigated (Fig. 1). As shown in Fig. 1a, *C. sorokiniana pa.91* could grow well in a wide range of 20 – 35 $^\circ\text{C}$ temperature. The microalgae growth rate increased by increasing temperature from 20 to 30 $^\circ\text{C}$, then it started to detriment when the temperature further increased to 35 $^\circ\text{C}$. This might be related to the lower photosynthesis efficiency and metabolic rate at low temperatures and sever oxidative damages at high temperatures (Ali et al., 2005).
The maximum biomass concentrations were obtained as 1.05, 1.2, 1.4 and 0.9 gL\(^{-1}\) after 15 days of cultivation at temperatures of 20 °C, 25 °C, 30 °C and 35 °C, respectively (Fig. 1b). Therefore, the optimum temperature for cultivation of \textit{C. sorokiniana pa.91} in BG-11 at 3000 Lux was 30 °C and the temperatures order for cell growth were observed as follow 30°C > 25°C > 20°C > 35 °C. The results suggested that \textit{C. sorokiniana pa.91} has good temperature tolerance and is suitable for outdoor cultivation. Similarly, Qu et al. (2019), Ho et al. (2014) and Kitaya et al. (2005) obtained the optimum temperature of 30 °C, 35 °C and 29 °C for cultivation of \textit{Parachiorella kessleri QWY28}, \textit{Desmodesmus sp. F2} and \textit{Euglena gracilis} produced maximum biomass productivity of 646, 762 and 59 mgL\(^{-1}\)d\(^{-1}\), respectively (Ho et al., 2014; Kitaya et al., 2005; Qu et al., 2019).

3.1.2. Effect of light intensity on the growth of chlorella sorokiniana pa.91

It is well documented that light intensity has a direct influence on microalgae metabolic rate and microalgae growth (Brindhadevi et al., 2021; Qu et al., 2019). Generally, the range of applied light intensity for different microalgae cultivation was reported between 1000 – 8000 Lux (equivalent to 15 – 150 μmolm\(^{-2}\)s\(^{-1}\))(Devi and Parthiban, 2020). Accordingly, the effect of variable light intensities (1000, 3000, 4000, 5000 and 7000 lux) on the growth of \textit{C. sorokiniana pa.91} in BG-11 medium at 30 °C was investigated. As can be seen in Fig. 2a, during the two initial days \textit{C. sorokiniana pa.91} growth had a similar trend at all light intensities (approximately 0.16 g L\(^{-1}\)) except 4000 Lux which was bit higher (0.24 g L\(^{-1}\)). This might be attributed to the delay phase which was not observed at 4000 Lux. However, after the delay phase when the growth rate entered the exponential phase, the growth rate increased rapidly with an increase in the light intensity up to 4000 Lux with maximum biomass concentration of 1.61 gL\(^{-1}\). This might be due to the insufficient photons at low light intensities which cannot provide the light required for an appropriate photosynthesis process. However, further increase in light intensity leaded to a lower biomass concentration and decline in growth rate which
might be related to the photo-inhibition and cells damage due to heat accumulation (Devi and Parthiban, 2020; Ho et al., 2014). Fig. 2b shows the maximum biomass concentrations of *C. sorokiniana pa.91* obtained after 15 days of cultivation at different light intensities in BG-11 medium under 30 °C. The maximum concentrations of 0.99, 1.41, 1.61, 1.32, 1.18 gL⁻¹ were obtained at the light intensities of 1000, 3000, 4000, 5000 and 7000 Lux, respectively. The optimal condition for *C. sorokiniana pa.91* was achieved at 4000 Lux followed by 3000 > 5000 > 7000 > 1000 Lux at temperature of 30 °C. According to Devi & Parthiban. (2020), Maynard et al. (2015) and Khoeyri et al. (2012), maximum biomass concentration of 1.76, 1.9 and 2.05 mgL⁻¹ were obtained for *Nostoc ellipsosporumNCIM 2786, Nannochloropisis* and *Chlorella vulgaris* microalgae at optimal light intensity of 4500, 5000 and 4000 Lux, respectively (Devi and Parthiban, 2020; Khoeyi et al., 2012; Maynard et al., 2015).

### 3.1.3. Optimization of cultivation condition at different cultivation mediums

To investigate the practical application of the optimal condition for *chlorella sorokiniana pa.91* cultivation, it is essential to evaluate the maximum biomass production in different cultivation mediums, particularly in the real municipal wastewater (RMWW). Therefore, a series of *C. sorokiniana pa.91* cultivation experiments (60 tests) were carried out under various temperatures (20 °C, 25 °C, 30 °C and 35 °C) and light intensities (1000, 3000, 4000, 5000 and 7000 lux) in the three culture environments of BG-11, real municipal wastewater (RMWW) and synthetic wastewater (SWW) (Table 1) for 15 days. The maximum biomass concentration as a function of temperature and light intensity were plotted for the three culture mediums and presented in Fig. 3. According to Fig. 3, *C. sorokiniana pa.91* achieved the highest biomass concentration of 2.45 gL⁻¹ in RMWW, which was significantly higher than those obtained in BG-11 (1.61 gL⁻¹) and SWW (1.9 gL⁻¹) under the optimal condition of 30 °C and 4000 Lux. This might be related to the existence of the symbiotic bacteria such as Verrucomicrobium, Proteobacteria and Frimicutes in real municipal wastewater (RMWW) that can be favourable
to microalgae growth (Tandon and Jin, 2017; Toyama et al., 2018). The lower biomass concentration yield in the case of SWW (COD = 403 mgL\(^{-1}\)) might be attributed the low N/P rate (~ 1.8), high COD concentration (~ 403 mgL\(^{-1}\)) and turbidity which negatively affect the microalgae growth (Wen et al., 2017).

Moreover, a close monitoring of the interaction effects between temperature and light intensity on the biomass growth revealed a synergy between temperature and light intensity at higher values resulting in sub-optimal conditions. For instance at higher light intensity of 7000 Lux the optimal condition were obtained at 25 °C instead of 30 °C (Fig. 3). The results suggest that, in cold area with lower temperature microalgae cultivation at higher light intensity would be recommended. This synergistic effect might be related to the heat accumulation at higher light intensities which results in higher metabolic reaction of microalgae and carbon bio-fixation at lower temperature (Ho et al., 2014). However, at lower temperatures, the light intensity showed less effect on the growth rate, suggesting that C. sorokiniana pa.91 growth rate was light independent at low temperature. A similar phenomena was previously observed (Nogueira et al., 2015).

3.2. Cultivation of C. sorokiniana pa.91 in flat-plate photobioreactor

To further evaluate the capability of newly isolated strain C. sorokiniana pa.91 in CO2 bio-fixation and nutrient removal from real municipal wastewater, the microalgae was cultivated in a flat-plate photobioreactor under the optimized condition of 30 °C temperature and 4000 Lux light intensity, using RMWW as culture medium.

3.2.1. C. sorokiniana pa.91 growth rate and productivity in the photobioreactor

Fig. 4 shows the biomass concentration and productivity of C. sorokiniana pa.91 in the photobioreactor during 11 days of cultivation under optimal condition of 30 °C and 4000 Lux. According to Fig.4, C. sorokiniana pa.91 growth rate was very low (approximately 0.8 gL\(^{-1}\))
during the initial two days which might be due to being in adaptation phase. Similar behavior was observed by Hinterholz et al. (2019) and Cheng et al. (2020) when they cultivated *Chlorella PY-ZU1* and *P. Malhamensis* in the JTSP and FP photobioreactors, respectively (Cheng et al., 2019; Hinterholz et al., 2019). However, after the third day microalgae growth entered to an exponential phase, which biomass concentration gradually increased up to 2.83 gL$^{-1}$ until the eighth day when maximum biomass productivity of 0.30 gL$^{-1}$d$^{-1}$ was obtained. Then, microalgae concentration and biomass productivity slightly decreased due to the decay period of microalgae in the photobioreactor with might be related to the higher microalgae concentration that prevented equal light receiving by all microalgae cells in the photobioreactor. The results of *C. Sorokiniana pa.91* cultivation in the photobioreactor showed that this microalgae can growth rapidly and reach to 3.21 gL$^{-1}$ and 0.31 gL$^{-1}$d$^{-1}$ biomass concentration and productivity, respectively, within eight days of cultivation under optimal condition (30 °C and 4000 Lux). These results suggesting that *C. Sorokiniana pa.91* might has high potential for CO$_2$ bio-fixation and wastewater treatment.

### 3.2.2. CO$_2$ bio-fixation efficiency of *C. Sorokiniana pa.91* in the photobioreactor

The capability of *C. Sorokiniana pa.91* specie for CO$_2$ absorption and O$_2$ generation was investigated in this study for the first time. Fig. 5a shows the CO$_2$ removal rate and dissolved oxygen (DO) concentration during *C. Sorokiniana pa.91* cultivation in the photobioreactor under the optimal condition. Generally, sufficient supply of carbon is required for the growth of microalgae (Weissman et al., 1988). The maximum CO$_2$ removal percentage and the dissolved oxygen concentration after eight days of cultivation were 35.6% and 1.66 mgL$^{-1}$, respectively. It is well-known that in the presence of sufficient light, microalgae cells can convert CO$_2$ to oxygen by photosynthesis process (Lam et al., 2012). According to the results of the present work, *C. Sorokiniana pa.91* showed a great capability for CO$_2$ absorption when cultivated under optimal cultivation condition.
Fig. 5b shows the average CO₂ bio-fixation rate through *C. Sorokiniana pa.91* and pH variation in the photobioreactor. The maximum CO₂ fixation rate was obtained at 0.59 gL⁻¹d⁻¹ when the maximum biomass productivity of 0.31 gL⁻¹d⁻¹ was achieved at the eighth day. The results of CO₂ bio-fixation showed a good agreement with that of biomass productivity (Fig. 4) indicating the demand of CO₂ increase with increasing biomass concentration. Several research have been proposed previously for CO₂ bio-fixation through cultivation of different microalgae species in PBRs. Cheng et al. (2019) cultivated *Chlorella PY-ZU1* in a novel JTSP photobioreactor, resulting in a maximum CO₂ fixation rate of 0.52 gL⁻¹d⁻¹ (Cheng et al., 2019). Xia et al. (2018) achieved a maximum CO₂ fixation rate of 0.1 gL⁻¹d⁻¹ by cultivation *Chlorella vulgaris FACHB-31* in their new designed PBR. Additionally, Fig. 5b shows the variation pH of culture medium during *C. Sorokiniana pa.91*. A dramatically decrease in pH was observed from 7.8 to 5.8 at the initial three days (adoption period) which might be attributed to the higher H⁺ concentration as results of rapid dissolution of CO₂ in the culture medium (Cheng et al., 2019; Xia et al., 2018). However, the medium pH gradually began to increase again and stabilize at 7 due to the dissolved CO₂ saturated in the medium by microalgae cells.

3.2.3. *Nutrient removal efficiency of C. Sorokiniana pa.91 in the photobioreactor*

The consumption of nutrient from wastewater by microalgae cells promotes biomass growth rate and results in wastewater treatment. To assess the feasibility of microalgae based wastewater treatment using *C. sorokiniana pa.91*, the removal of nutrients such as ammonia (NH₃), nitrate (NO₃⁻), phosphate (PO₄³⁻) and chemical oxygen demand (COD) from real municipal wastewater influent (RMWW) was investigated in the photobioreactor under the optimized condition: 30 °C temperature and 4000 Lux light intensity (Fig 6).

Among different nutrients, nitrogen is the main source for microalgae growth (Daneshvar et al., 2019). Different forms of nitrogen in wastewater includes ammonium (NH₄⁺), ammonia (NH₃), nitrate (NO₃⁻), nitrite (NO₂⁻). It has been reported that ammonia (NH₃ = 34 mgL⁻¹) is
the main form of nitrogen in municipal wastewater influent. However, low concentration of
NO$_3^-$ (2.15 mgL$^{-1}$) and NO$_2^-$ (0.06 mgL$^{-1}$) were also identified in the wastewater influent
presumably due to the slightly nitrification as a result of accidental aeration. Fig. 6 (a) and (b)
show the evaluation of ammonia (NH$_3$), nitrate (NO$_3^-$) from the real municipal wastewater
(RMWW) by *C. sorokiniana pa.91*, respectively. As shown in Fig 6, *C. sorokiniana pa.91*
microalgae could remove ammonia and nitrate up to 74% and 93%, respectively after 10 days
day of cultivation. During the first two days, *C. sorokiniana pa.91* was almost ineffective in
removing ammonia and nitrate from wastewater due to the adoption period. However, after the
adoption period (the 3$^{rd}$ day) the removal efficiency was remarkably increased until the 8$^{th}$ day
in the photobioreactor. These results are in agreement with the maximum biomass
concentration of microalgae was obtained after eight days cultivation (Fig. 4b). According to
Fig 6a, NH$_3$ and NO$_3^-$ concentration decreased to less than 10 and 0.2 mg L$^{-1}$ within six days.
The removal of nitrate (NO$_3^-$) was almost completed after the 5$^{th}$ days in photobioreactor under
optimized condition. Although, in the presence of ammonia microalgae first consume NH$_3$ and
then utilize other forms of nitrogen, *C. sorokiniana pa.91* showed a good capacity for the
removal of nitrate even at low dosage which was not seen in the other species of microalgae.

Phosphate is another nutrient that can be used for microalgae growth while the current
treatment technologies cannot efficiently removed phosphate from wastewater (Watsuntorn et
al., 2019). According to Fig. 6c the consumption of phosphate (PO$_4^{3-}$) by *C. sorokiniana pa.91*
showed a similar trend with that observed for ammonia. However, the final removal rate of the
phosphate (83%) was higher than ammonia (73%) and less than nitrate (93%) which might be
attributed to the initial concentration of the nutrients in the culture medium. As showed in Fig.
6c, the PO$_4^{3-}$ concentration decreased markedly from 6.1 to less than 1 mg L$^{-1}$ within 8 days of
*C. sorokiniana pa.91* cultivation in the photobioreactor under the optimized condition. The
results suggested that *C. sorokiniana pa.91* has high capacity of orthophosphate storing from
the medium. It should be noted that the achieved final concentration of phosphate is below the acceptable level of the surface water quality standard (1 P-mgL^{-1}). (China, 2002)

Chemical oxygen demand (COD) has been reported as the most challenging problem in the treatment process due to its re-increasing in the wastewater (Lee et al., 2019). As illustrated in Fig 6d, the COD was eliminated gradually from 211 to 50.7 mgL^{-1} resulted in 76.3% removal rate of COD during 10 days cultivation of *C. sorokiniana pa.91* in the photobioreactor. In comparison with the other nutrients, the COD showed relatively lower removal efficiency. Generally, microalgae release organic matter after utilization of carbon sources from the COD components which leads to increased COD in the culture medium (Mujtaba and Lee, 2017). However, it seems that the wastewater in this study has provided sufficient sCOD to the microalgae cells for metabolism process. The results suggested that using *C. sorokiniana pa.91* for municipal wastewater could reduce COD concentration below the standard for agriculture reuse water (50 COD-mgL-1) (Shoushtarian and Negahban-Azar, 2020).

For comparison purposes, Table 2 presences the results of municipal wastewater treatment by *C. sorokiniana pa.91* used in this study and other microalgae species obtained from literature. Although there are many factors that affect microalgae based wastewater treatment, the removal efficiency of nutrients mainly depends on the specific adsorption capacity and cells yield of microalgae species (Yang et al., 2016). According to Table 2, when *C. sorokiniana pa.91* was used for the treatment of municipal wastewater, the removal efficiency of 73%, 93%, 83% and 76% was achieved for ammonia (NH₃), nitrate (NO₃⁻), phosphate (PO₄^{3-}) and COD, respectively, after 10 days of cultivation under optimal condition. In comparison with the other microalgae species, *C. sorokiniana pa.91* which has been investigated for municipal wastewater treatment for the first time in this study, showed higher utilization efficiency under optimized condition. The results suggested that the new specie of *C. sorokiniana pa.91* can be used as promising approach for the treatment of municipal wastewater.
Conclusion

The newly isolated microalgae *Chlorella sorokiniana pa.91* showed a great potential for CO₂ bio-fixation and nutrients removal from real municipal wastewater. After a comprehensive investigation on the effect of culture condition on the growth rate of C. *sorokiniana pa.91* in three different culture mediums, the optimal condition was achieved at 30 °C temperature and 4000 Lux light intensity in RMWW. Further, C. *sorokiniana pa.91* was cultivated in a flat-plate photobioreactor (16% CO₂ and 0.6 vvm) at the optimized condition which produced a maximum biomass concentration of to 3.21 gL⁻¹ after 10 days of cultivation. Moreover, a high CO₂ bio-fixation and removal rate of 1.66 mgL⁻¹ and 35.6% were achieved. Under optimal condition, C. *sorokiniana pa.91* showed an excellent nutrient removal ability from wastewater by achieving removal rates of 73%, 93%, 83% and 76% for ammonia (NH₃), nitrate (NO₃⁻), phosphate (PO₄³⁻) and COD, respectively. The results of this study suggested a promising method enhancing CO₂ bio-fixation and municipal wastewater treatment.

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Figures

Figure 1

The effect of temperature on (a) C. sorokiniana p. 91 biomass concentration and (b) maximum biomass concentration after 15 days of cultivation in BG-11 under 3000 Lux light intensity.

Figure 2
The effect of light intensity on (a) C. sorokiniana pa. 91 biomass concentration and (b) maximum biomass concentration after 15 days of cultivation in BG-11 under 30 °C temperature

Figure 3

Optimization of C. sorokiniana pa.91 cultivation condition in (a) BG-11, (b) RMWW and (c) SWW culture mediums
Figure 4

Biomass concentration and productivity of C. sorokiniana p.91 cultivated in flat-plate photobioreactor using real municipal wastewater under optimal condition of 30 °C temperature and 4000 Lux light intensity
Figure 5

Variation of (a) CO2 removal rate and dissolved oxygen concentration, (b) CO2 bio-fixation and pH variation during cultivation C. sorokiniana p.91 in flat-plate photobioreactor using real municipal wastewater under optimal condition of 30 °C temperature and 4000 Lux light intensity.
Figure 6

Removal rate and concentration of (a) NH3, (b) NO3-, (c) PO4-3 and (d) COD during cultivation of C. sorokiniana p.91 in flat-plate photobioreactor using real municipal wastewater under optimal condition of 30 °C temperature and 4000 Lux light intensity

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