Cultivation of *Chlorella sorokiniana* in a bubble-column bioreactor coupled with cooking cocoon wastewater treatment: effects of initial cell density and aeration rate

Chunye Xue, Kun Gao, Pingkang Qian, Jingwei Dong, Zheng Gao, Qiaoqiao Liu, Biao Chen and Xiangyuan Deng

ABSTRACT

Previous studies have documented that *Chlorella sorokiniana* could grow well on cooking cocoon wastewater (CCW) with a maximum biomass of 0.49 g/L. In order to further enhance the biomass production and nutrient removals, a bubble-column bioreactor was designed and performed to cultivate *C. sorokiniana* in CCW, and two main cultivation parameters were investigated in this work. Results showed that a maximum algal biomass, specific growth rate, and biomass productivity of 2.83 g/L, 0.854 d^{-1}, and 476.25 g/L/d, respectively, were achieved when this alga was cultivated in the bioreactor with an initial cell density of 0.8 g/L and an aeration rate of 3.34 L air/L culture/min; meanwhile, removal efficiencies of ammonium, total nitrogen, total phosphorus, and chemical oxygen demand reached 97.96, 85.66, 97.96, and 86.43%, respectively, which were significantly higher than that obtained in our previous studies. Moreover, chemical compositions in the algal cells varied with the changes of cultivation conditions (i.e., initial cell density and aeration rate). Thus, it is concluded that (1) the bubble-column bioreactor was suitable for cultivation of *C. sorokiniana* coupled with the CCW treatment and (2) initial cell density and aeration rate affected the biomass production, nutrient removals and chemical compositions of this alga.

Key words | aeration rate, *Chlorella sorokiniana*, cooking cocoon wastewater, initial cell density, photobioreactor

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HIGHLIGHTS

- A bubble-column bioreactor was designed and performed in this work.
- The bioreactor was suitable to cultivate *C. sorokiniana* using CCW.
- Initial cell density and aeration rate were two key factors during the cultivation.
- Biomass production and nutrient removal were enhanced under the optimal conditions.
- Algal chemical compositions varied with the changes in cultivation conditions.

INTRODUCTION

Microalgae are one of the most underutilized aquatic organisms, and have attracted much attention worldwide because their biomass could be used as a reliable and sustainable feedstock for production of biofuels and a variety of value-added products (Chew *et al.* 2017; Koyande *et al.* 2019). However, large-scale commercial production of algal biomass is potentially more costly than that of traditional crops because algae cultivation requires an abundance of water

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and nutrients, such as carbon, nitrogen, and phosphorus (Chen *et al.* 2015). It has been demonstrated that algae cultivation using wastewater is an ideal scenario for sustainable production of algae-based biofuels and bio-based chemicals, since large quantities of freshwater and nutrients required for algae growth could be saved and the associated life cycle burdens could be reduced significantly (Zhou *et al.* 2014).

A large amount of wastewater containing high organic matter is generated during silk production, which would deplete the dissolved oxygen in the receiving water bodies and threaten the aquatic life (Capar *et al.* 2008). It has been reported that 800–1,000 t wastewater would be generated when 1 t raw silk was produced at the industrial scale, and the cost of wastewater treatment was huge (Zhang 2002). The integration of algae cultivation and wastewater treatment is likely to be one of the most viable strategies for sustainable production of algal biomass and bio-based products because wastewater provides not only water source but also most of the necessary nutrients for algal growth. According to the industrial process of raw silk, silkworm cocoons are cooked firstly in hot water (95–100 °C) for 30–40 min to separate fibroin from sericin. Cooking cocoon wastewater (CCW) will be generated during this process, which is nearly sterile because few microbes could survive at such high temperatures. Additionally, the CCW is rich in sericin, pupa oil, pigments, carbohydrates, and inorganic substances, such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}, and meets the nutrient requirements of microalgae (Li *et al.* 2019). Thus, the CCW could be used as a potential medium to cultivate microalgae, and the feasibility of growing *C. sorokiniana* on CCW was investigated in a previous study, which showed that CCW could be used as culture media to cultivate *C. sorokiniana* directly (Li *et al.* 2019). Thus, it is concluded that the CCW could be used as a good-quality medium for algal growth.

A photobioreactor (PBR) is an open, closed or semi-closed vessel, which could provide an ideal growing environment for photosynthetic microorganisms (Han *et al.* 2017). Since the first PBR was reported in the late 1940s, many different types of PBRs have been invented and produced for microalgae cultivation during the past decades and some of them have achieved large-scale commercial production (Ugwu *et al.* 2008; Wang *et al.* 2012). To date, a common understanding has been accepted that large-scale cultivation and commercial application of microalgae are limited due to the development of PBRs. Thus, the design and engineering of PBRs is still a very hot topic in the field of algae cultivation. The PBRs for the integration of microalgae cultivation coupled with wastewater treatment need to be redesigned and improved because the compositions of wastewater are quite complicated, and the growth conditions differ with various algal strains (Han *et al.* 2017). Based on the existing PBRs, some types of PBRs have been developed to cultivate microalgae coupling with wastewater treatment. For example, Tan *et al.* (2014) designed an airlift circulation PBR to cultivate *C. pyrenoidosa* in anaerobically digested starch processing wastewater, and found that this alga could remove 65.99% of chemical oxygen demand (COD), 83.06% of total nitrogen (TN), and 96.97% of total phosphorus (TP). As mentioned above, microalgae cultivation using wastewater in PBRs is considered as one of the most promising routes for producing algal biomass in an economically viable and environmentally friendly way.

On the basis of the above analysis, a bubble-column bioreactor was designed and performed in this work to cultivate *C. sorokiniana* in CCW, and two main cultivation parameters (initial cell density and aeration rate) were optimized for producing algal biomass and nutrient removal. The specific objectives of this work were: (1) to design a simple bubble-column bioreactor, which was used for the cultivation of *C. sorokiniana* in CCW; (2) to evaluate the feasibility of microalgae cultivation coupled with CCW treatment in the bioreactor; and (3) to investigate the effects of initial cell density and aeration rate on the algal growth characteristics, chemical compositions, and capability of removing nutrients when this alga was cultivated in CCW within the bioreactor. It is hoped that these research efforts would help to obtain the optimal conditions of microalgae cultivation coupled with wastewater treatment in the bubble-column bioreactors, and realize the large-scale cultivation of microalgae for biomass production and nutrient removal in the future.

MATERIALS AND METHODS

Collection, pretreatment, and analysis of the wastewater

Cooking cocoon wastewater was collected from a local silk processing plant in Jiangsu Province, China. Prior to use, the CCW was firstly settled in a plastic bucket, and then centrifuged (5,000 × *g*, 10 min) to remove the solids. As shown in Table 1, the supernatant had a high pH value of about 8.95, which was adjusted to around 7.00 using 3 mol/L hydrochloric acid before being autoclaved at 121 °C for 20 min.

Table 1 | Physicochemical properties of cooking cocoon wastewater (CCW) before and after autoclaving

Characteristics	Raw CCW	Autoclaved CCW
TN (mg/L)	87.16 ± 2.12	97.32 ± 1.69
NO_3-N (mg/L)	2.73 ± 0.15	2.52 ± 0.23
NO_2-N (mg/L)	0.05 ± 0.00	0.08 ± 0.12
NH_4^+-N (mg/L)	17.11 ± 0.32	15.17 ± 0.47
TIN (mg/L)	19.89 ± 0.58	17.77 ± 0.54
TON (mg/L)	67.27 ± 4.01	79.55 ± 0.43
TC (mg/L)	244.10 ± 3.01	252.33 ± 0.32
TOC (mg/L)	216.81 ± 2.21	210.53 ± 1.23
TIC (mg/L)	27.29 ± 0.21	41.80 ± 0.32
TP (mg/L)	9.83 ± 0.35	10.23 ± 0.15
COD (mg/L)	780 ± 5.21	983.85 ± 4.74
N/P ratio	8.87:1	9.51:1
pH value	8.95 ± 0.03	7.80 ± 1.01
Salinity (‰)	2.94 ± 0.03	2.96 ± 0.03
Nephelometric turbidity units (NTU)	5.73 ± 0.07	5.67 ± 0.05

All measurements were performed in triplicate, and results were expressed as mean value ± standard deviation.

Physicochemical characteristics of the CCW before and after autoclaving were determined, and are presented in Table 1.

Algal species and culture conditions

In the current work, *Chlorella sorokiniana* (FACHB-275) was selected as an experimental algal strain because it could grow well on the CCW with a maximum biomass of 0.49 g/L, and could remove nutrients effectively from the CCW (Deng *et al.* 2020). This alga was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Prior to the experiment, this alga was pre-cultivated in 300 mL autoclaved Tris-Acetate-Phosphorus (TAP) medium (Ma *et al.* 2016) using 1,000 mL Erlenmeyer flasks. The flasks were continuously shaken at 100 rpm using an orbital shaker (SPH-211B, Shiping Laboratory Equipment Co. Ltd, Shanghai, China) at 25 ± 2 °C under a light intensity and photoperiodicity of 50 μmol m^{-2} s^{-1} and 12 L:12D, respectively.

Bioreactor design and set-up

In this work, a bubble-column bioreactor (Figure 1) was designed and constructed according to the methods described in a previous study (Deng *et al.* 2009), and its geometrical dimensions are presented in Table 2. The bioreactor was placed in a light incubator (Jiangnan Instrument Factory, Ningbo, China), and illuminated by ten vertical cool-white fluorescent lamps (28 W) mounted in a direction parallel to both sides of the bioreactor. The light intensity was measured using a luxmeter (Jiading Xuelian Meter Factory, Shanghai, China) in 12 spots equally distributed around the bioreactor. The autoclaved CCW was loaded into the bioreactor, and then *C. sorokiniana* was inoculated into the CCW at initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L. To ensure a well-mixed culture and inorganic carbon resource for microalgae photosynthesis, an aquarium pump (Xixin Electromechanical Factory, Raoping, China) was used to provide ambient air, which was sterilized with a Pall autoclavable air filter of 0.2 μm in pore size, and then bubbled through a self-designed and sterilized humidifier before being introduced into the culture

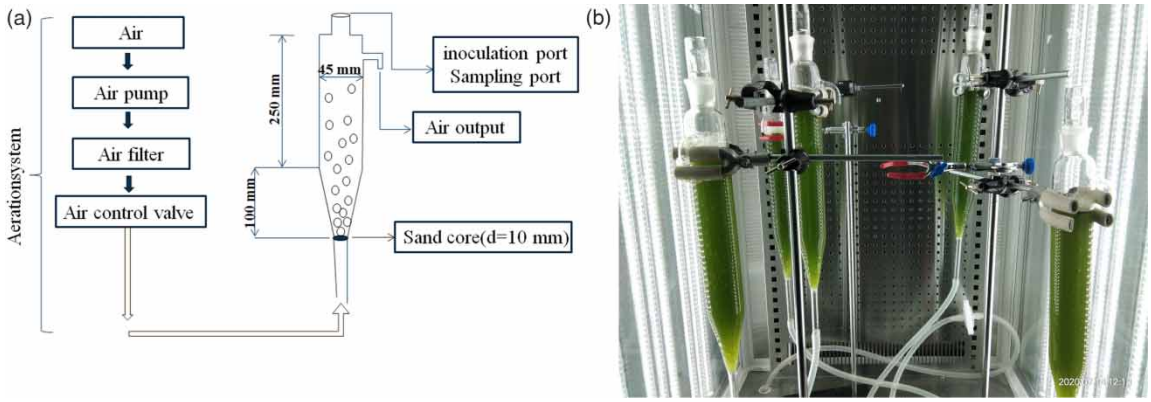

Figure 1 | Schematic diagram (a) and photograph (b) of a bubble-column bioreactor for cultivating *Chlorella sorokiniana* in cooking cocoon wastewater (CCW). Its geometrical dimensions are described in Table 2.

Table 2 | Geometrical dimensions of the bubble-column bioreactor and the cultivation conditions of *Chlorella sorokiniana* grown on cooking cocoon wastewater (CCW) in the bioreactor

Parameters (unit)	
Total volume (mL)	465
Working volume (mL)	300
Column diameter (mm)	45
Straight-section height (mm)	250
Riser-section height (mm)	100
Culture period (days)	7
Temperature (°C)	25 ± 2
Photoperiod (h light/h dark)	12:12
Incident light intensity (μmol/m^2/s)	50
Initial cell density range (g/L)	0.05–1.0
Aeration rate range (L air/L culture/min)	0–6.68

through a sparger. The sparger with a pore size of 20 μm (Haimen Huaxing Glass Instrument Factory, Nantong, China) was installed at the bottom of the bioreactor to supply ambient air (containing about 380 ppm of CO_2) at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 L air/L culture/min (vvm). To avoid bacteria contamination, 75% ethanol was sprayed in the light incubator once a day during cultivation, and sterile serological pipettes were used for sampling in this work. In addition, opening of the air outlet pipe in the bioreactor was downward for maintaining a pure culture of *C. sorokiniana* in the 7-day cultivation. At the end of algae cultivation, plate cultivation method was used to detect other microorganisms in the bioreactor. Ambient air was bubbled into the CCW in a bioreactor without algae to detect the effects of air stripping on the removal of ammonium (NH_4^+-N). Batch cultivation of *C. sorokiniana* was performed in three replicates within the bioreactor according to the conditions described in Table 2. Samples were taken at the designated times for determination of algal growth, nutrient removals, and chemical compositions as described below.

Analytical methods

Algal biomass determination

The algal biomass (g/L) was determined in triplicate according to the method of Zhou *et al.* (2012). Moreover, a logistic kinetic model was used to describe the relationship between algal growth and biomass under different culture conditions according to the following equation (Tan *et al.* 2016):

$$X = \frac{X_{\max}}{1 + e^{m-\mu \times t}} \quad (1)$$

where X is the algal biomass (g/L) at time t (d), $X_{\max}$ is the carrying capacity (the maximum algal biomass (g/L) reached in the medium), m is a constant in the logistic model which indicates the relative position from the origin, and μ is the specific growth rate (d^{-1}).

Based on the algal biomass, productivity (P, mg/L/d) was calculated using Equation (2), which was more suitable for calculating productivity during the batch cultivation because it excluded the lag phase ($X < 1.1 \times X_0$) and the stationary phase ($X > 0.9 \times X_{\max}$) (Ruiz *et al.* 2013):

$$P = \mu \times \frac{0.9 \times X_{\max} - 1.1 \times X_0}{\ln\left[\frac{9 \times (X_{\max} - 1.1 \times X_0)}{1.1 \times X_0}\right]} \times 1000 \quad (2)$$

where X_0 is the algal biomass (g/L) at initial time (t_0).

Analysis of physicochemical characteristics

Physicochemical characteristics of the CCW before and after autoclaving were determined according to the methods described previously (Deng *et al.* 2020). During algae cultivation, 5 mL of well blended cultures were collected daily from the bubble-column bioreactor, and their pH values were measured with a pH meter (MP511, San-Xin Instrumentation Inc., Shanghai, China). Before physicochemical analysis, the cultures were firstly centrifuged at 12,000 × g for 5 min, and then the supernatants were collected and diluted to analyze the concentrations of NH_4^+-N, TN, total phosphorus (TP), and COD according to the methods described previously (Deng *et al.* 2020).

Removal efficiency of nutrient i ($R_{e,i}$, %) was calculated according to the following equation (Deng *et al.* 2017a):

$$R_{e,i} = \frac{C_{i,0} - C_{i,t}}{C_{i,0}} \times 100 \quad (3)$$

where $R_{e,i}$ is the removal efficiency (%) of nutrient i (NH_4^+-N, TN, TP or COD); $C_{i,0}$ and $C_{i,t}$ are the initial and final concentrations (mg/L) of i during algae cultivation, respectively.

The biomass yield based on nutrient i consumption (Y_i, mg biomass/mg nutrient i) was calculated according

to Equation (4):

$$Yi = \frac{\Delta X}{\Delta C} = \frac{X_{i,\max} - X_{i,0}}{C_{i,0} - C_{i,t}} \times 1000 \quad (4)$$

where Y_i is the yield of biomass linked with the consumption of nutrient i (TN, TP or COD) (mg biomass/mg nutrient i); $X_{i,o}$ and $X_{i,max}$ represent the initial and maximum algal biomass (g/L) with respect to the nutrient i during the 7-day cultivation.

Measurements of photosynthetic parameters

In this work, the ratio of the variable to maximum fluorescence (F_v/F_m) was measured using a pulse-amplitude modulation (PAM) fluorometer (AquaPen AP-C100, Photon Systems Instruments, Drasov, Czech Republic) after samples of the cultures were kept in the dark for 15 min. According to the methods described previously (Deng *et al.* 2017b), the F_v/F_m was calculated using the following equation:

$$F_v/F_m = \frac{F_m - F_0}{F_m} \quad (5)$$

where F_0 and F_m are the initial and maximum chlorophyll fluorescence, respectively. F_v represents the variation in chlorophyll fluorescence between F_m and F_0.

Determination of chemical compositions in algal cells

Contents of photosynthetic pigments, carbohydrates, lipids, and proteins were extracted and determined according to the spectrophotometry method, phenol–sulfuric acid method, chloroform–methanol method, and elemental analysis method, respectively, which were described by Li *et al.* (2019) and Deng *et al.* (2020).

Statistical analysis

Results were presented as the means ± standard deviation (SD) of three independent tests to check the reproducibility of the data. One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests was used to evaluate the statistical significance of the experimental data using an SPSS software (version 18.0) for Windows (Statistical Product and Service Solutions, distributed by SPSS Inc., Chicago, IL, USA). The results were considered statistically significant if the p-values were 0.05 or less.

RESULTS AND DISCUSSION

Effects of initial cell densities on the algal growth and nutrient removals

Algal growth and photosynthetic performance

As shown in Figure 2(a), *C. sorokiniana* could grow well on CCW in the bioreactor without obvious lag phase at different initial cell densities, indicating that the bioreactor was suitable for microalgae cultivation using the CCW. The algal biomass increased dramatically within the first 3 days, and then increased gradually to 0.91, 1.15, 1.35, 1.69, 2.46, and 2.23 g/L when the initial cell densities were

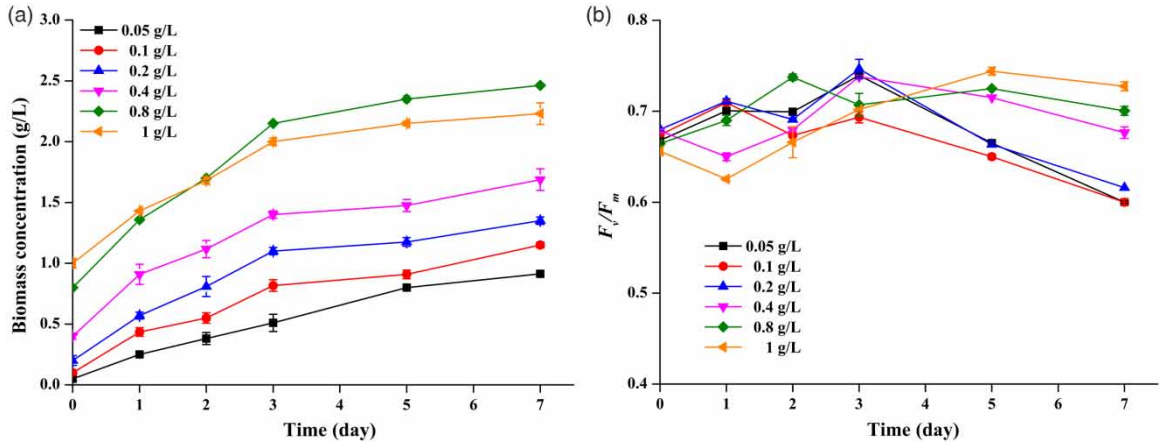

Figure 2 | Growth profiles (a) and daily changes in the maximum quantum yield of photosystem II (PSII) (F_v/F_m) (b) of *Chlorella sorokiniana* when it grew on cooking cocoon wastewater (CCW) in the bubble-column bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1 g/L for 7 days. Each data point represents the mean of three replicates; and the error bars represent the standard deviation.

0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L, respectively. The corresponding specific growth rates and biomass productivities were 0.801, 0.847, 0.917, 0.796, 0.817, 0.726 d^{-1} and 124.23, 176.31, 238.01, 265.08, 392.13, and 296.06 g/L/d, respectively. These data suggested that the optimal initial cell density was 0.8 g/L for the cultivation of *C. sorokiniana* using CCW in the bubble-column bioreactor. Similar results were obtained by Deng *et al.* (2009), who found that the final cell density increased first and then decreased with the increase of initial cell density when transgenic gametophytes of *Laminaria japonica* were cultivated in an illuminated bubble-column bioreactor. Additionally, Zhi & Rorrer (1996) observed that length of lag phase significantly decreased with increase in the initial cell density from 30 to 117 mg/L when they cultivated the filamentous gametophyte life phase of the complex brown alga *Laminaria saccharina* in a bubble-column bioreactor, and reported that increasing the initial cell density would affect the final biomass. This phenomenon could be explained by the fact that algal cells could obtain sufficient nutrients and light conditions when the initial cell density was low; however, increasing initial cell density would intensify the competition among algal cells for nutrients uptake, light, and other conditioning factors, resulting in the decrease of algal biomass. Thus, the initial cell density would be an important factor affecting algal growth and biomass accumulation, which should be optimized during microalgae cultivation to obtain the maximum algal biomass.

It has been reported that values of F_v/F_m are around 0.83 for healthy plants and somewhat lower for algae (0.55–0.80) (Maxwell & Johnson 2000). As presented in Figure 2(b), values of F_v/F_m were in the range of 0.60–0.75 when *C. sorokiniana* was grown on the CCW in the bubble-column bioreactor at different initial cell densities for 7 days, suggesting that this alga was in a good physiological state and had a strong photosynthetic performance to support its autotrophic growth. The data were in line with that reported by Li *et al.* (2019), who found that values of F_v/F_m remained stable at about 0.72 when *C. sorokiniana* grew on autoclaved CCW in 250 mL Erlenmeyer flasks. Based on these data, it was concluded that (1) microalgae would be in a good physiological state when they were cultivated in CCW within the bioreactor and (2) the bioreactor could provide good light conditions to this alga, which would help to enhance the algal growth and biomass.

Nutrient removals and pH variation

As illustrated in Figure 3(a), concentrations of NH_4^+-N in the cultures increased slightly followed by a rapid decrease within the first 3 or 5 days, and then decreased slowly or leveled off in the remaining days of the experiments. By the end of algae cultivation, the corresponding R_e values of NH_4^+-N were 85.94, 86.78, 89.16, 89.33, 92.67, and 90.84%, respectively, when this alga grew on the CCW in the bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L. As we know, the silkworm cocoon is composed of 20–25% of sericin, 70–75% of fibroin, and 5% of other impurities (Wang & Zhang 2017). The sericin is a water-soluble globular protein with a molecular mass of 10–310 kDa, and consists of 18 kinds of amino acids, such as serine, aspartic acid, and glutamic acid (Li *et al.* 2019). In the silk industry, the sericin is separated from the fibroin for obtaining the raw silk by cooking the cocoons in hot water, resulting in CCW. Thus, the CCW is rich in proteins and amino acids, which would be converted to NH_4^+-N during microalgae cultivation. This conversion may be the main reason responsible for the increase in NH_4^+-N concentrations at the beginning of algae cultivation. Along with the growth of microalgae, large quantities of NH_4^+-N would be consumed by the algal cells, which would lead to a rapid decrease in the NH_4^+-N concentrations in the cultures observed in this work. Furthermore, nutrients in the CCW were exhausted at the end of algae cultivation, which caused the algal cells to grow in a stationary phase. As shown in Figure 3(b), a significant decrease in TN concentrations was observed within the first 3 or 5 days, and then leveled off or decreased gradually during the 7-day algae cultivation. When *C. sorokiniana* grew on the CCW in the bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L for 7 days, the corresponding R_e and Y values of TN by the end of cultivation were 79.68, 82.84, 84.21, 85.07, 87.40, 81.69%, and 11.36, 13.34, 14.38, 15.89, 19.95, 16.22 mg biomass/mg N, respectively. The obtained R_e values were significantly higher than that reported by Li *et al.* (2019) and Deng *et al.* (2020), who used an Erlenmeyer flask to cultivate *C. sorokiniana* in the CCW, indicating that the bioreactor was more suitable than the flask for microalgae cultivation. Additionally, the obtained Y values were close to the theoretical biomass yield on N of 15.8 mg biomass/mg N, which was calculated according to the stoichiometric formula for the most common elements in an average algal cell ($C_{106}H_{263}O_{110}N_{16}P$) (Redfield *et al.* 1963). Based on the obtained data, it was concluded that: (1) some nitrogenous compounds in the CCW would be converted into ammonium nitrogen to support the algal growth during microalgae cultivation, but some compounds could not be assimilated by the algal cells because the TN could

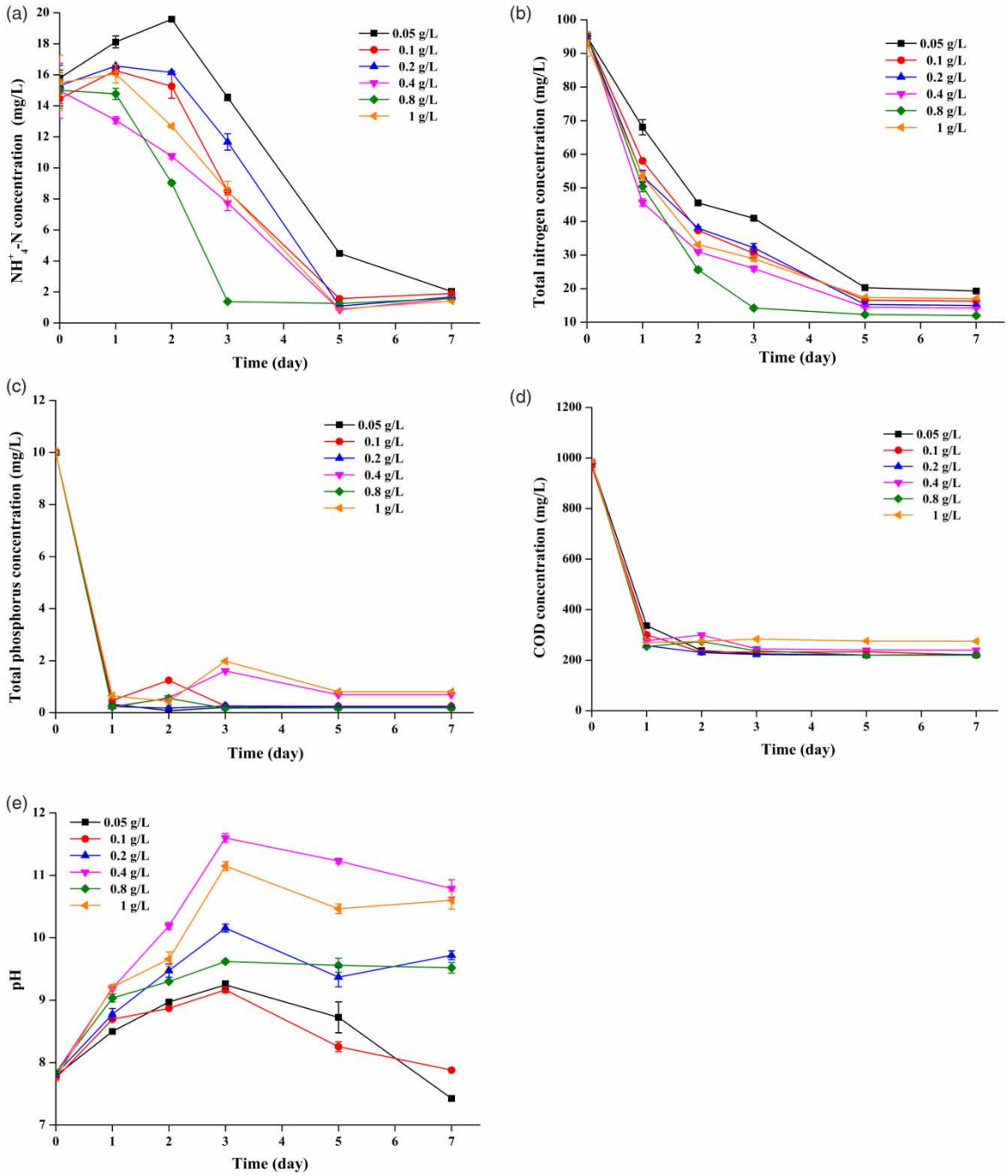

Figure 3 | Variations in nutrient concentrations and the culture's pH when *Chlorella sorokiniana* was cultivated in cooking cocoon wastewater (CCW) within the bubble-column bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1 g/L for 7 days. (a) NH_4^+-N removal; (b) total nitrogen (TN) removal; (c) total phosphorus (TP) removal; (d) chemical oxygen demand (COD) removal; and (e) culture's pH. Each point represents the mean of three replicates, and the error bars represent the standard deviation.

not be completely removed; and (2) initial cell density had impacts on the algal growth and ability to remove TN in the CCW.

As shown in Figure 3(c), concentrations of TP decreased dramatically from 10.01, 10.32, 10.11, 10.35, 10.4, and 10.21 mg/L to 0.33, 0.47, 0.24, 0.21, 0.24, and 0.65 mg/L, respectively, when *C. sorokiniana* grew on the CCW within the bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L from the beginning to Day 1. After Day 1, its concentrations increased slightly or leveled off. By

the end of algae cultivation, the corresponding R_e and Y values of TP were 97.99, 97.59, 97.66, 93.37, 98.18, 92.11%, and 87.68, 104.25, 116.48, 133.49, 162.58, 130.78 mg biomass/mg P, respectively, indicating that phosphorus in the CCW could be removed effectively by *C. sorokiniana* and converted into the algal biomass concurrently. Compared with the results of Li *et al.* (2019), higher R_e values of TP were observed in this work because the bioreactor could provide more optimal growth conditions to the alga than the Erlenmeyer flask. In addition, the obtained Y values of TP in this work were similar to the theoretical biomass yield on P of 114.7 mg biomass/mg P, which was calculated on the basis of the stoichiometric formula ($C_{106}H_{263}O_{110}N_{16}P$) (Redfield *et al.* 1963). Thus, it is concluded that phosphorus in the CCW was a proper form of phosphorus source for the algal growth and biomass accumulation, which would be removed effectively from the CCW during algae cultivation.

As illustrated in Figure 3(d), concentrations of COD decreased significantly from 980.21, 980.56, 984.44, 989.01 990.01, and 984.36 mg/L to 336.52, 300.51, 257.53, 277.22, 254.02, and 270.00 mg/L within the first day when *C. sorokiniana* grew on the CCW in the bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L, respectively, and then leveled off. The corresponding R_e and Y values of COD by the end of algae cultivation were 76.74, 77.46, 77.35, 75.73, 77.68, 72.06%, and 1.14, 1.38, 1.51, 1.72, 2.17, 1.73 mg biomass/mg COD, respectively, which indicated that this alga was capable of utilizing organic carbon in the CCW for their growth and metabolism. It has been reported that *C. sorokiniana* had the ability to use organic substrates as carbon source for heterotrophic or mixotrophic growth to synthesize chemical compositions, such as carbohydrates, proteins, pigments, and lipids (Petrovič & Simonič 2015). The demand for carbon source is large for microalgae biomass production because carbon content in the biomass accounts for 42.5–50.3% of the total biomass (Tan *et al.* 2014). However, COD concentrations in the cultures at the end of algae cultivation remained relatively high, suggesting that some organic substrates could not be utilized by this alga. In conclusion, the bioreactor designed and assembled in this work was suitable to cultivate microalgae for nutrient removals and biomass production based on the above results.

During the 7-day algae culture, the culture's pH was not controlled, and changes over time are presented in Figure 3(e). It can be seen that values of the culture's pH increased dramatically from about 7.81 to 9.25, 9.17, 10.16, 11.60, 9.62, and 11.15 within the first 3 days, and then gradually decreased to 7.42, 7.88, 9.72, 10.79, 9.52, and 10.60 at the end of cultivation, respectively, when *C. sorokiniana* grew on the CCW in the bioreactor at different initial cell densities of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 g/L. The range of pH values for the growth of most microalgae is between 7.0 and 9.0, with an ideal range between 8.2 and 8.7, although some species inhabit more acidic or basic environments, the latter of which can reach values above pH 9.5 during growth (Piiparinen *et al.* 2018). In this work, *C. sorokiniana* could grow on cultures with high pH values (>9.5), meaning that this alga was an alkali-resistant strain. Additionally, the algal photosynthesis growth was reported to be an alkalization process, which could be described simply as the consumption of inorganic carbon and the release of basic bioreaction metabolites ($CO_2 + H_2O \rightarrow CH_2O + O_2$) or HCO_3^- in water ($HCO_3^- + H_2O \rightarrow CH_2O + O_2 + OH^-$) (Pedersen *et al.* 2013). Thus, the changes in culture's pH values may be related to the photosynthetic activity, CO_2 delivery and the growth of this alga.

Effects of aeration rates on the algal growth and nutrient removals

Algal growth and photosynthetic performance

As shown in Figure 4(a), there was only a slight increase in algal biomass from the initial level of 0.80 g/L to 1.81 g/L after 7 days of cultivation when *C. sorokiniana* grew on the CCW in the bioreactor without aeration. In aeration conditions, the maximum algal biomass and biomass productivity of 2.83 g/L and 476.25 g/L/d, respectively, were obtained when *C. sorokiniana* was cultivated in the bioreactor with an aeration rate of 3.34 vvm. Interestingly however, further increase in the aeration rate (above 3.34 vvm) did not show benefits for algal growth. For example, the maximum algal biomass and biomass productivity were only 2.57 g/L, 416.27 g/L/d, and 2.42 g/L, 417.15 g/L/d, respectively, when *C. sorokiniana* grew on the CCW in the bioreactor with aeration rates of 5.01 and 6.68 vvm. As mentioned earlier, increasing aeration rate generally induces mixing, liquid circulation, and mass transfer between gas and liquid phases in the bubble-column systems. However, too high an aeration rate will cause excessive turbulence of the algal cultures, resulting in too high a shear stress, which would destroy the algal cells. Moreover, at the gas–liquid interface, the stress generated by bubble breaking will splash the algal cells onto the wall of the bioreactor, resulting in a reduction of algal biomass

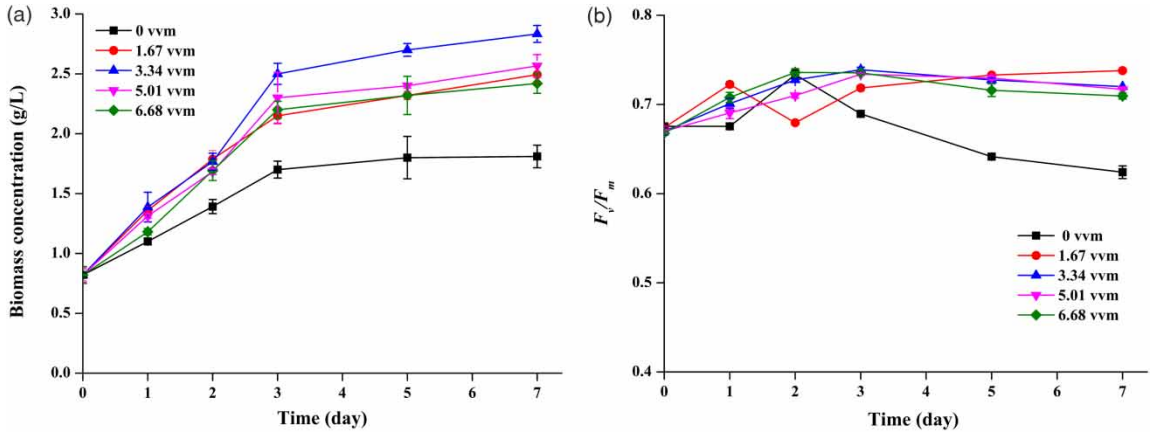

Figure 4 | Growth profiles (a) and daily changes in the maximum quantum yield of photosystem II (PSII) (F_v/F_m) (b) of *Chlorella sorokiniana* when it grew on cooking cocoon wastewater (CCW) in the bubble-column bioreactor at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 L air/L culture/min (vvm) for 7 days. Each data point represents the mean of three replicates; and the error bars represent the standard deviation.

(Barbosa *et al.* 2004; Dasgupta *et al.* 2010). Therefore, it was concluded that aeration is crucial for a suitable growth of this alga in the bubble-column bioreactor, but it must be maintained at low levels to minimize shear stress. In addition, the method of plate cultivation was used in this work to detect the possible microorganisms in the algal cultivation system at the end of operation stage. As shown in Fig. S1, only *C. sorokiniana* colonies were observed in the plates, indicating that this alga was a pure culture in the cultivation system over a 7-day operation, and there was no other microbial pollution.

Figure 4(b) shows the changes in values of F_v/F_m when *C. sorokiniana* grew on the CCW in the bubble-column bioreactor at different aeration rates for 7 days. As presented in the figure, F_v/F_m values of *C. sorokiniana* were greater than 0.55, suggesting that photosynthesis of this alga was not affected by aeration under the cultivation conditions used in the current work. After Day 3, the F_v/F_m values of *C. sorokiniana* grown in aeration conditions were greater than that with no aeration, indicating that aeration had a beneficial effect on algal photosynthesis. It is not surprising to obtain these results because aeration can facilitate mixing or turbulence of the algal cultures, which will prevent sedimentation of the cells and formation of nutritional and gaseous gradients, and therefore ensure that all cells are equally exposed to nutrients and light (Vidyarathna *et al.* 2014). Additionally, the changes in F_v/F_m values were in line with that of algal biomass in the current work, suggesting that the F_v/F_m value was a sensitive parameter to reflect algal growth when *C. sorokiniana* grew on the CCW in the bioreactor at different aeration rates.

Nutrient removals and pH variation

As presented in Figure 5(a), concentrations of NH_4^+-N decreased rapidly from 14.64, 14.46, 14.90, 14.87, and 15.01 mg/L to 8.53, 2.80, 1.88, 2.06, and 2.1 mg/L within the first 3 days when *C. sorokiniana* grew on the CCW in the bioreactor at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 vvm, respectively. After Day 3, its concentrations decreased slightly and then leveled off. By the end of microalgae cultivation, the corresponding R_e values of NH_4^+-N were 88.78, 94.83, 97.96, 95.12, and 95.24%, respectively. However, a significant increase in NH_4^+-N concentrations was observed when the alga was not inoculated into the CCW within the bioreactor at 3.34 vvm (Figure 5(a)). The increase in NH_4^+-N concentrations may be due to the fact that proteins and amino acids in the CCW could be converted into NH_4^+-N as described above. Thus, it is concluded that more NH_4^+-N would be removed from the CCW when *C. sorokiniana* was cultivated in aeration conditions than that with no aeration. Similar to the changes in NH_4^+-N concentrations, concentrations of TN decreased rapidly from 99.01, 95.22, 95.81, 98.19, and 98.39 mg/L to 39.93, 20.01, 13.38, 17.18, and 17.58 mg/L within the first 3 days, and then decreased slightly, when *C. sorokiniana* was cultivated in the bioreactor at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 vvm, respectively (Figure 5(b)). The corresponding R_e and Y values of TN were 62.06, 81.10, 85.66, 83.32, 81.37%, and 16.44, 21.93, 24.78, 21.59, 20.23 mg biomass/mg N, respectively, by the end of algae cultivation. Although a maximum R_e value of TN (85.66%) was achieved when the aeration rate was 3.34 vvm in this work, no significant differences in the R_e values of TN were observed when

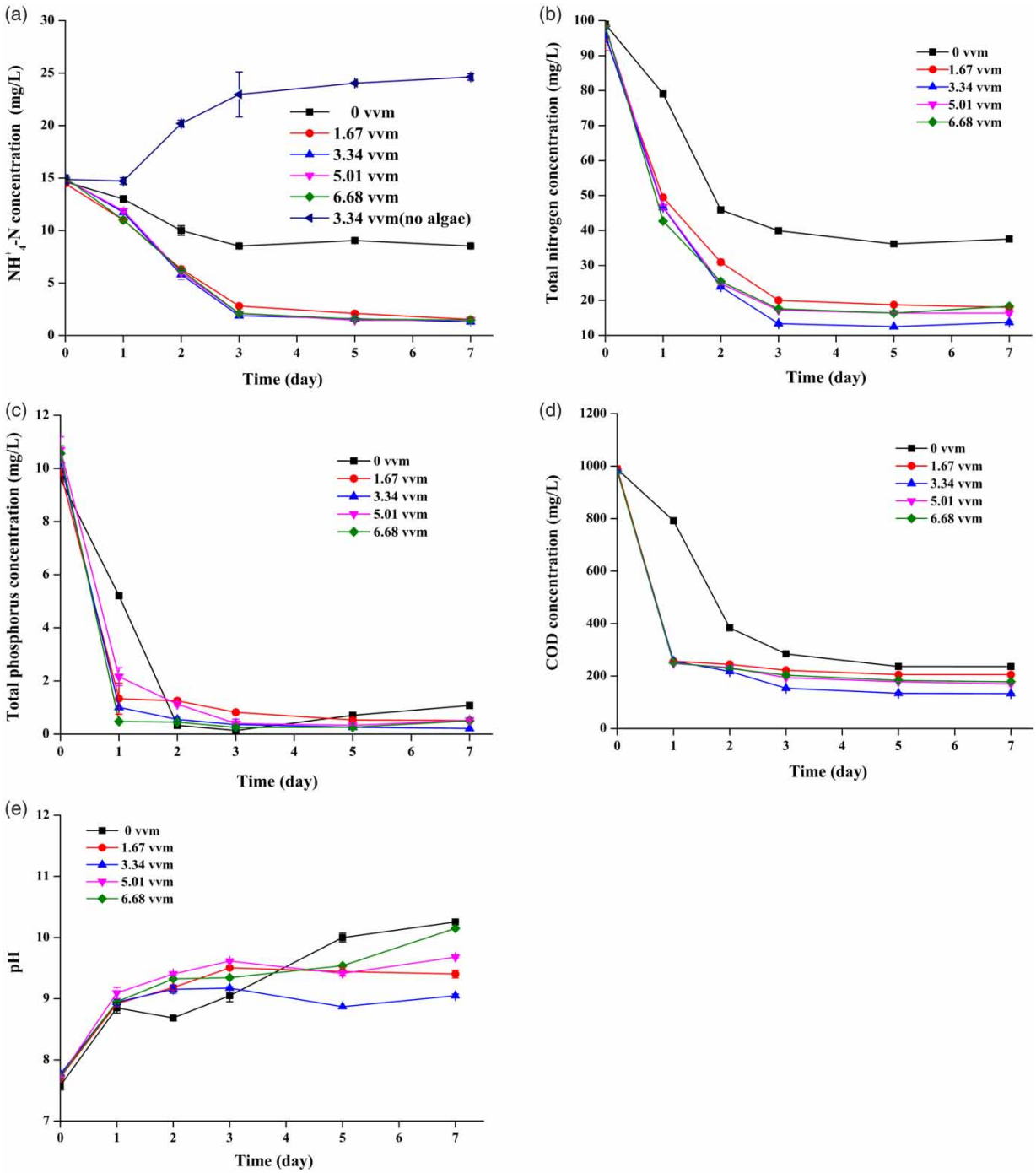

Figure 5 | Changes in nutrient concentrations and the culture's pH when *Chlorella sorokiniana* was cultivated in cooking cocoon wastewater (CCW) within the bubble-column bioreactor at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 L air/L culture/min (vvm) for 7 days. (a) NH_4^+-N removal; (b) total nitrogen (TN) removal; (c) total phosphorus (TP) removal; (d) chemical oxygen demand (COD) removal; and (e) culture's pH. Each point represents the mean of three replicates, and the error bars represent the standard deviation.

C. sorokiniana was cultivated in aeration conditions, indicating that aeration rates could not affect the removal of TN dramatically. However, when *C. sorokiniana* was cultivated in aeration conditions, the R_e values of TN was significantly higher than that with no aeration, suggesting that aeration was helpful to remove TN in the cultures. These results could be explained by the fact that aeration can improve the algal cultivation conditions as described

above for enhancing the algal growth and biomass accumulation (Figure 4(a)), resulting in higher consumption of nitrogen.

As seen in Figure 5(c), TP was removed markedly from the beginning to Day 1 when *C. sorokiniana* was cultivated in aeration conditions, whose concentrations decreased from 9.87, 10.29, 10.70, and 10.56 mg/L to 1.33, 1.01, 2.16, and 0.47 mg/L, respectively, when the aeration rates were 1.67, 3.34, 5.01, and 6.68 vvm. After Day 1, its concentrations decreased gradually and then leveled off. However, the TP concentrations decreased from 9.60 mg/L to 0.33 mg/L when *C. sorokiniana* was cultivated in the conditions with no aeration for 2 days, and then increased slightly. By the end of algae cultivation, the corresponding R_e and Y values of TP were 88.80, 94.83, 97.96, 95.12, 95.24%, and 118.53, 180.86, 201.80, 173.59, 161.05 mg biomass/mg P, respectively, when the aeration rates were 0, 1.67, 3.34, 5.01, and 6.68 vvm. The obtained R_e values of TP were significantly higher than that reported by Li *et al.* (2019), who cultivated *C. sorokiniana* in the CCW within an Erlenmeyer flask under the conditions without aeration. These data suggested that aeration was beneficial to the removal of TP in the cultures because it could improve the cultivations conditions of microalgae as described earlier.

As illustrated in Figure 5(d), concentrations of COD decreased significantly from 989.50, 980.33, 980.33, and 980.54 mg/L to 254.01, 257.03, 248.22, and 251.08 within the first day, and then decreased gradually or leveled off when *C. sorokiniana* grew in the CCW within the bioreactor at different levels of aeration rates of 1.67, 3.34, 5.01, and 6.68 vvm for 7 days. Concentrations of COD decreased significantly from 988.56 to 384.03 mg/L within the first 2 days, and then decreased slightly to 236.01 mg/L when the alga was cultivated in the conditions with no aeration for 7 days. After 7-day algae cultivation, the corresponding R_e and Y values of COD were 76.13, 78.78, 86.43, 82.66, 81.85%, and 1.34, 2.17, 2.40, 2.18, 2.02 mg biomass/mg COD, respectively, when the aeration rates were 0, 1.67, 3.34, 5.01, and 6.68 vvm. It was reported that *C. sorokiniana* could grow mixotrophically using organic compounds and CO_2 simultaneously as carbon sources (Deng *et al.* 2020). Thus, the usable organic carbon in the CCW could be utilized effectively by *C. sorokiniana* during the mixotrophic culture phase, but some residual organic carbon cannot be consumed by the alga because only a portion of organic compounds can be directly used as a carbon source for algae growth. In the photoautotrophic culture phase, CO_2 in the atmosphere was mainly used as inorganic carbon to maintain the algal growth. Therefore, it was concluded that aeration was favorable to the algal growth and biomass accumulation, and help to remove the COD in the cultures based on the results of R_e and Y values of COD.

From the beginning to Day 1, the culture's pH increased rapidly from 7.71 to 8.85, 8.92, 8.95, 9.10, and 8.95, respectively, when *C. sorokiniana* grew on the CCW in the bioreactor at different aeration rates of 0, 1.67, 3.34, 5.01, and 6.68 vvm (Figure 5(e)). As described earlier, *C. sorokiniana* could grow mixotrophically using organic compounds and CO_2 simultaneously as carbon sources. Thus, the increase in culture's pH was mainly attributed to the consumption of inorganic carbon and the release of basic bioreaction metabolites during algal photosynthesis (Gonzalez *et al.* 2008). In addition, the culture's pH changed slightly after Day 1 because of the buffer capacity of CO_2 in the atmosphere aerated into the bioreactor.

Chemical compositions of algal biomass

Generally, chemical compositions of algal biomass mainly consist of protein (6–52%), lipids (7–23%), and carbohydrates (5–23%) (Zhu 2015). As shown in Table 3a, it was found that protein was a predominant biochemical composition in the algal cells, whose contents maintained at a relatively stable level (43.18–48.39%), suggesting that the initial cell density had no significant effects on its contents. Under the cultivation conditions with aeration, contents of protein in the algal cells were at the same level (46.07–48.86%), but dramatically higher than that with no aeration ($p < 0.05$) (Table 3b). In agreement with this study, the same range of protein contents of *C. sorokiniana* (36.97–50.80%) was observed previously when the alga grew on mixed wastewaters (Deng *et al.* 2020). However, the contents of protein were lower than that reported by Xie *et al.* (2020), who cultivated the original and mutant strains of *C. sorokiniana* TX in artificial medium. Thus, it can be inferred that the medium and culture conditions would affect the contents of protein in algal cells, which should be optimized in the future for further improving biomass and protein contents because protein is the primary nutrient element in foods with a unique nutritional function.

As presented in Table 3a, with the increase of initial cell density from 0.05 to 1 g/L, contents of lipids decreased from 25.91 to 23.30%, while that of carbohydrates and pigments increased from 21.73 to 25.08% and from 0.56 to 2.27% when *C. sorokiniana* grew on the CCW in the bioreactor at different initial cell densities. In addition, when *C. sorokiniana* was cultivated in conditions with different aeration rates, contents of lipids in the algal cells were

Table 3 | Changes in chemical compositions of *Chlorella sorokiniana* when it was grown on cooking cocoon wastewater (CCW) in the bubble-column bioreactor at different initial cell densities (a) and aeration rates (b) for 7 days

(a)

Initial cell densities (g/L)	Protein (%)	Lipids (%)	Carbohydrates (%)	Pigments (%)
0.05	43.18 ± 1.21	25.91 ± 2.01	21.73 ± 1.01	0.56 ± 0.02
0.1	48.39 ± 1.68	24.74 ± 1.88	23.08 ± 1.23	0.81 ± 0.01
0.2	46.31 ± 1.11	24.51 ± 1.68	24.58 ± 1.53	1.49 ± 0.02
0.4	47.38 ± 2.12	24.30 ± 1.12	24.88 ± 0.89	2.12 ± 0.02
0.8	46.27 ± 2.02	23.32 ± 1.98	25.32 ± 1.98	2.10 ± 0.01
1	47.29 ± 1.98	23.30 ± 2.08	25.08 ± 1.81	2.27 ± 0.02

(b)

Aeration rates (L air/L culture/min)	Protein (%)	Lipids (%)	Carbohydrates (%)	Pigments (%)
0	41.48 ± 2.21	30.87 ± 1.01	16.41 ± 1.01	1.46 ± 0.02
1.67	46.07 ± 2.02	26.02 ± 1.98	23.32 ± 1.98	2.10 ± 0.01
3.34	48.86 ± 1.11	22.84 ± 1.18	24.63 ± 1.53	2.21 ± 0.02
5.01	47.08 ± 2.12	24.71 ± 1.12	23.07 ± 0.89	2.01 ± 0.02
6.68	46.74 ± 2.02	25.44 ± 2.08	23.03 ± 1.98	2.03 ± 0.01

All measurements were performed in triplicate, and results were expressed as mean value ± standard deviation.

significantly lower ($p < 0.05$), while the contents of carbohydrates and pigments were markedly higher than that with no aeration ($p < 0.05$) (Table 3b). Microalgae were reported to be able to undergo programmatic changes in photosynthetic carbon partitioning, and thus cellular biochemical compositions, particularly in the relative amounts of lipids, carbohydrates, and pigments, were in response to changes in environmental and cultivation conditions (Wang *et al.* 2013). Generally, biosynthesis of lipids in the algal cells is a process competitive to that of carbohydrates because their synthesis requires more ATP and NAD(P)H per carbon than that of carbohydrates (Ren *et al.* 2016). Based on the data obtained in this study, it was concluded that more carbon and energy would be used for the biosynthesis of carbohydrates and pigments when *C. sorokiniana* was grown on the CCW in the bioreactor under aeration conditions. Recently, pigments in microalgae have been used as one kind of nutraceuticals because they have multiple therapeutic properties, such as antioxidant activities, a protective effect against retina degeneration, regulate blood cholesterol, and fortify the immune system (Nam *et al.* 2016; Hu *et al.* 2018). Microalgae were reported to have the ability to change their pigment contents under different cultivation conditions. Pigments are closely related to algal photosynthesis because they allow the algal cells to obtain energy from light, and their contents could reflect the level of photosynthesis to some extent (Seyfabadi *et al.* 2011). In the current work, high algal biomass was obtained when *C. sorokiniana* was grown on the CCW in the bioreactor under aeration conditions, which would result in shading effects between the algal cells. The shading effects would cause a decrease in light intensity during algae cultivation, where the algal cells should increase the contents of pigments for absorbing more light energy. Therefore, chemical compositions in the algal cells varied with changes in cultivation conditions.

CONCLUSIONS

The following conclusions were drawn from this research. (1) The bubble-column bioreactor designed and constructed in this work was suitable to cultivate *C. sorokiniana* in the CCW for nutrient removals and biomass production. (2) Nutrients (i.e., N, P, C, and other elements) in the CCW could support the growth and biomass accumulation of *C. sorokiniana*. (3) Initial cell density and aeration rate were two important factors affecting the nutrient removal and biomass production when *C. sorokiniana* was grown on the CCW in the bioreactor, which should be further optimized during microalgae cultivation to obtain the maximum algal biomass and removal efficiency. (4) Chemical

compositions in the algal cells varied with changes in cultivation conditions, indicating that a desirable chemical composition could be obtained by regulating the conditions (i.e., initial cell density and aeration rate). Therefore, data obtained in this study would help to realize the large-scale cultivation of microalgae in future studies for biomass production and nutrient removals.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Barbosa, M. J., Hadiyanto & Wijffels, R. H. 2004 Overcoming shear stress of microalgae cultures in sparged photobioreactors. *Biotechnology and Bioengineering* **85** (1), 78–85.

Capar, G., Aygun, S. S. & Gecit, M. R. 2008 Treatment of silk production wastewaters by membrane processes for sericin recovery. *Journal of Membrane Science* **325** (2), 920–931.

Chen, G., Zhao, L. & Qi, Y. 2015 Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: a critical review. *Applied Energy* **137**, 282–291.

Chew, K. W., Yap, J. Y., Show, P. L., Suan, N. H., Juan, J. C., Ling, T. C., Lee, D. J. & Chang, J. S. 2017 Microalgae biorefinery: high value products perspectives. *Bioresource Technology* **229**, 53–62.

Dasgupta, C. N., Gilbert, J. J., Lindblad, P., Heidorn, T., Borgvang, S. A., Skjanes, K. & Das, D. 2010 Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. *International Journal of Hydrogen Energy* **35**, 10218–10238.

Deng, X. Y., Qin, S., Zhang, Q., Jiang, P., Cui, Y. L. & Li, X. K. 2009 Microprojectile bombardment of *Laminaria japonica* gametophytes and rapid propagation of transgenic lines within a bubble-column bioreactor. *Plant Cell, Tissue and Organ Culture* **97**, 253–261.

Deng, X. Y., Gao, K., Zhang, R. C., Addy, M., Lu, Q., Ren, H. Y., Chen, P., Liu, Y. H. & Ruan, R. 2017a Growing *Chlorella vulgaris* on thermophilic anaerobic digestion swine manure for nutrient removal and biomass production. *Bioresource Technology* **243**, 417–425.

Deng, X. Y., Li, D., Wang, L., Hu, X. L., Cheng, J. & Gao, K. 2017b Potential toxicity of ionic liquid ($[c_{12}mim]BF_4$) on the growth and biochemical characteristics of a marine diatom *Phaeodactylum tricornutum*. *Science of the Total Environment* **586**, 675–684.

Deng, X. Y., Li, D., Xue, C. Y., Chen, B., Dong, J. W., Tetteh, P. A. & Gao, K. 2020 Cultivation of *Chlorella sorokiniana* using wastewaters from different processing units of the silk industry for enhancing biomass production and nutrient removal. *Journal of Chemical Technology and Biotechnology* **95** (1), 264–273.

Gonzalez, C., Marciniak, J., Villaverde, S., Garcia-Encina, P. A. & Munoz, R. 2008 Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. *Applied Microbiology and Biotechnology* **80**, 891–898.

Han, T., Lu, H. F., Ma, S. S., Zhang, Y. H., Liu, Z. D. & Duan, N. 2017 Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: a review. *International Journal of Agricultural and Biological Engineering* **10** (1), 1–29.

Hu, J. J., Nagarajan, D., Zhang, Q. G., Chang, J. S. & Lee, D. J. 2018 Heterotrophic cultivation of microalgae for pigment production: a review. *Biotechnology Advances* **36**, 54–67.

Koyande, A. K., Chew, K. W., Rambabu, K., Tao, Y., Chu, D. T. & Show, P. L. 2019 Microalgae: a potential alternative to health supplementation for humans. *Food Science and Human Wellness* **8** (1), 16–24.

Li, D., Amoah, P. K., Chen, B., Xue, C. Y., Hu, X. L., Gao, K. & Deng, X. Y. 2019 Feasibility of growing *Chlorella sorokiniana* on cooking cocoon wastewater for biomass production and nutrient removal. *Applied Biochemistry and Biotechnology* **188**, 663–676.

Ma, X. C., Zheng, H. L., Addy, M., Anderson, E., Liu, Y. H., Chen, P. & Ruan, R. 2016 Cultivation of *Chlorella vulgaris* in wastewater with waste glycerol: strategies for improving nutrients removal and enhancing lipid production. *Bioresource Technology* **207**, 252–261.

Maxwell, K. & Johnson, G. N. 2000 Chlorophyll fluorescence–a practical guide. *Journal of Experimental Botany* **51** (345), 659–668.

Nam, K., Lee, H., Heo, S. W., Chang, Y. K. & Han, J. I. 2016 Cultivation of *Chlorella vulgaris* with swine wastewater and potential for algal biodiesel production. *Journal of Applied Phycology* **29**, 1171–1178.

Pedersen, O., Colmer, T. D. & Sand-Jensen, K. 2013 Underwater photosynthesis of submerged plants – recent advances and methods. *Frontiers in Plant Science* **4**, 140.

Petrovič, A. & Simonič, M. 2015 The effect of carbon source on nitrate and ammonium removal from drinking water by immobilised *Chlorella sorokiniana*. *International Journal of Environmental Science and Technology* **12**, 3175–3188.

Piiparinen, J., Barth, D., Eriksen, N. T., Teir, S., Spilling, K. & Wiebe, M. G. 2018 Microalgal CO_2 capture at extreme pH values. *Algal Research* **32**, 321–328.

Redfield, A. C., Ketchum, B. H. & Richards, F. A. 1963 The influence of organisms on the composition of sea-water. *The Sea* **2**, 26–77.

Ren, X. J., Chen, J. K., Deschênes, J. S., Tremblay, R. & Jolicoeur, M. 2016 Glucose feeding recalibrates carbon flux distribution and favours lipid accumulation in *Chlorella protothecoides* through cell energetic management. *Algal Research* **14**, 83–91.

Ruiz, J., Álvarez-Díaz, P. D., Arbib, Z., Garrido-Pérez, C., Barragán, J. & Perales, J. A. 2013 Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: prediction from a batch experiment. *Bioresource Technology* **127**, 456–463.

Seyfabadi, J., Ramezanpour, Z. & Khoeyi, Z. A. 2011 Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology* **23**, 721–726.

Tan, X. B., Chu, H. Q., Zhang, Y. L., Yang, L. B., Zhao, F. C. & Zhou, X. F. 2014 *Chlorella pyrenoidosa* cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. *Bioresource Technology* **170**, 538–548.

Tan, X. B., Zhang, Y. L., Yang, L. B., Chu, H. Q. & Guo, J. 2016 Outdoor cultures of *Chlorella pyrenoidosa* in the effluent of anaerobically digested activated sludge: the effects of pH and free ammonia. *Bioresource Technology* **200**, 606–615.

Ugwu, C. U., Aoyagi, H. & Uchiyama, H. 2008 Photobioreactors for mass cultivation of algae. *Bioresource Technology* **99** (10), 4021–4028.

Vidyarathna, N. K., Fiori, E., Lundgren, V. M. & Granéli, E. 2014 The effects of aeration on growth and toxicity of *Prymnesium parvum* grown with and without algal prey. *Harmful Algae* **39**, 55–63.

Wang, X. F. & Zhang, Y. Q. 2017 Effects of alkyl polyglycoside (APG) on *Bombyx mori* silk degumming and the mechanical properties of silk fibroin fibre. *Materials Science and Engineering C* **74**, 152–158.

Wang, B., Lan, C. Q. & Horsman, M. 2012 Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances* **30** (4), 904–912.

Wang, L., Li, Y. G., Sommerfeld, M. & Hu, Q. 2013 A flexible culture process for production of the green microalga *Scenedesmus dimorphus* rich in protein, carbohydrate or lipid. *Bioresource Technology* **129**, 289–295.

Xie, F. X., Zhang, F. F., Zhou, K., Zhao, Q., Sun, H. B., Wang, S., Zhao, Y. J. & Fu, J. R. 2020 Breeding of high protein *Chlorella sorokiniana* using protoplast fusion. *Bioresource Technology* **313**, 123624.

Zhang, Y. Q. 2002 Applications of natural silk protein sericin in biomaterials. *Biotechnology Advances* **20** (2), 91–100.

Zhi, C. X. & Rorrer, G. L. 1996 Photolithotrophic cultivation of *Laminaria saccharina* gametophyte cells in a bubble-column bioreactor. *Enzyme and Microbial Technology* **18**, 291–299.

Zhou, W. G., Li, Y. C., Min, M., Hu, B., Zhang, H., Ma, X. C., Li, L., Cheng, Y. L., Chen, P. & Ruan, R. 2012 Growing wastewater-born microalga *Auxenochlorella protothecoides* UMN280 on concentrated municipal wastewater for simultaneous nutrient removal and energy feedstock production. *Applied Energy* **98**, 433–440.

Zhou, W. G., Chen, P., Min, M., Ma, X. C., Wang, J. H., Griffith, R., Hussain, F., Peng, P., Xie, Q. L., Li, Y., Shi, J., Meng, J. Z. & Ruan, R. 2014 Environment-enhancing algal biofuel production using wastewaters. *Renewable and Sustainable Energy Reviews* **36**, 256–269.

Zhu, L. D. 2015 Biorefinery as a promising approach to promote microalgae industry: an innovative framework. *Renewable and Sustainable Energy Reviews* **41**, 1376–1384.

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