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Treatment of Nutrient-rich Municipal Wastewater Using Mixotrophic Strain *Chlorella kessleri* GXLB-9

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Abstract.

Growing algae on wastewaters offers a promising way for effective N and P recycling as well as low-cost algal biofuel feedstock accumulation. In this study, a locally isolated microalgae strain *Chlorella kessleri* GXLB-9 (*C. kessleri* GXLB-9), was evaluated for growth and nutrient removal efficiency grown in nutrient-rich wastewater centrifuged from activated sludge (NWCAS). And 3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea (DCMU), one chemical that could block microalgae-based photosynthetic pathway, was used to evaluate the growth mode (autotrophy, heterotrophy or mixotrophy) of *C. kessleri* GXLB-9. The results showed that *C. kessleri* GXLB-9 was a facultative heterotrophic strain and 7-day batch cultivation idicated that the maximal removal efficiencies for total nitrogen, total phosphorus, and chemical oxygen demand (COD) were over 59%, 81%, and 88%, respectively, with high growth rate (0.490 d^{-1}) and high biomass productivity (269 mg L^{-1} d^{-1}). In addition, the impact of light-dark cycle on algae growth and nutrient removal was minimal while pH has significant impact on both algae growth and nutrient removal efficiency.

Keywords*:* *Chlorella kessleri*; Facultative heterotrophy; Light-dark cycle; Municipal wastewater; Nutrient removal; pH.

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1. Introduction

Growing algae in wastewaters for effective nutrients removal as well as algal biomass accumulation can contribute a lot to the management of freshwater ecosystem by providing an environmentally sustainable approach. Moreover, the harvested algal biomass can be used as feedstock for biofuel and high-value byproducts purpose, further reduce the costs of such algae-based wastewater treatment system [1,2,3,4].

However, although there are numerous publications available on the topic of wastewater-based algae cultivation in recent years, yield of wastewater-grown algal biomass and nutrient removal efficiency is still unsatisfactory due to the unique properties of unbalanced nutrient profiles, potential toxic compounds and turbidity for different types of wastewaters [5,6]. Thus, the ideal wastewater streams selected for fast algae growth as well as improved nutrient removal rate should satisfy following aspects: (1) enough macronutrients such as nitrogen (N), phosphorus (P), and other essential trace elements; (2) less toxic compounds; (3) enough organic carbons (e.g., glucose, acetate acid, glycine,) favorable to some mixotrophic algae strains for high algal biomass and lipid accumulation [4, 7]; (4) Large available all year around and easy handle for pretreatment. Therefore, in this study, nutrient-rich wastewater centrifuged from activated sludge (NWCAS) (Fig. 1) was used to cultivate locally isolated microalgae strain *Chlorella kessleri* GXLB-9 (*C. kessleri* GXLB-9) and growth mode of *C. kessleri* GXLB-9 (obligate autotrophy or facultative heterotrophy) was identified. In addition, some key growth parameters such as pH, light-dark cycle were evaluated for algae growth and nutrient removal in the experiment design.

The specific objectives of this study were 1) to evaluate the impact of pH and light-dark recycle on growth and nutrient removal efficiency of *C. kessleri* GXLB-9 grown in organic-rich municipal wastewater; 2) to examine trophic conversion property of *C. kessleri* GXLB-9 and identify the main growth mode grown on NWCAS.

2. Materials and methods

2.1. Algae strain and culture condition

Microalgal strain GXLB-9 was isolated from a local river, downstream area of Anning Starch Processing Plant, Nanning, Guangxi Zhuang Autonomous Region, China and was identified as *Chlorella kessleri* subsequently. The strain was preserved in a typical algae culture medium-BG-11 medium as described previously [8].

2.2. Determination of growth type of *Chlorella kessleri* cultivated on activated sludge wastewater

3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea (DCMU) was added in the broth to determine the algal growth mode (autotrophic, heterotrophic or mixotrophic growth mode). DCMU is commonly used as photosynthesis inhibitor to interrupt the photosynthetic electron transport chain in photosynthesis and thus blocks the ability of the organism to turn light energy into chemical energy (ATP and reductant potential) [9, 10]. Each experiment was carried out in triplicate and average values were reported.

2.3. Wastewater resource

NWCAS used in this study was obtained from Jiangnan Municipal Wastewater Treatment Plant in Nanning, Guangxi Zhuang Autonomous Region, China (Fig. 1). Prior to use, the wastewater was pretreated through filter cloth to remove large solids and autoclaved at 121 °C for 15 min. After cooled to room temperature, and then stored in cold room (4 °C) for a few days, the upper water phase was collected and used in the subsequent experiments.

2.4. Growth and chemical analysis

2.4.1. Determination of algal growth

The algal biomass concentration was measured daily by the total volatile suspended solids (TVSS) method [11]. Usually the growth rate of the cells is proportional to the biomass of cells during the exponential growth phase. Therefore, the growth rate is calculated using the following equation:

$$R = Ln(TB_t)-Ln(TB_0)/t \qquad (1)$$

where R is growth rate based on TVSS. TB_t and TB_0 are the TVSS at day t and day 0. t is the time interval (days) between TB_t and TB_0.

2.4.2 Nutrient analysis

Samples were centrifuged and soluble nutrients in the supernatant including total nitrogen, total phosphorus, chemical oxygen demand (COD) and total organic carbon (TOC) were measured by a local authoritative testing organization.

3. Results and discussion

3.1 Characterization of NWCAS

The characteristics of the supernatant after centrifugation of activated sludge are listed in Table 1. It was obvious that the concentrations of COD, TOC, nitrogen and phosphorus in NWCAS were much higher than in any other wastewater streams collected from different stages of the municipal wastewater treatment plant [6], thus this type of wastewater might provide sufficient nutrients for algae growth. Furthermore, the TOC content was around 1000 mg/L, which could stimulate fast growth for some facultative heterotrophic microalgal strains with two different carbon metabolic pathways (autotrophy and heterotrophy). The characteristics of NWCAS indicated that algae cultivation on this type of wastewater could serve the dual role of effective wastewater nutrient reduction and low cost biofuel feedstock production.

3.2 Identification of heterotrophy property of *Chlorella kessleri* GXLB-9

It is known that a large amount of heterotrophic algae species exist in nature. These species are more capable of utilizing organic carbons under light condition than under dark condition [12, 13]. Moreover, it was estimated that the growth rates of facultative heterotrophic microalgae cultivated in mixotrophic growth mode were approximate the sum of those cultivated in autotrophic and heterotrophic growth mode [14]. Since the total organic carbon was as high as around 1000 mg/L in NWCAS (Table 1), it is better to select microalgae with facultative heterotrophy for effective treatment this type of wastewater, thus the locally isolated microalgal strain *C. kessleri* GXLB-9 was further evaluated for heterotrophy property.

In order to confirm facultative heterotrophic characterictics of *C. kessleri* GXLB-9, DCMU, a photosynthesis inhibitor, was used to block photosynthetic metabolic pathway and investigate the contribution of heterotrophy on algal biomass. Firstly, different DCMU concentrations were added to evaluate the optimized concentration to inhibit photosynthesis. The results were clearly shown that 40μM DCMU was best among all concentraions (Fig. 2). Therefore, 40μM DCMU was used in the next experiment. From Fig. 3, it was apparent that *C. kessleri* GXLB-9 had lower growth rate than that without DCMU addition in culture broth (Fig. 3), which further proved that the tested strain was capable of both heterotrophic and autotrophic growth.

3.3 Effect of pH on algae growth and nutrient removal efficiency

The growth curve of *C. kessleri* GXLB-9 cultivated on sterilized NWCAS is shown in Fig 4. It was observed that *C. kessleri* GXLB-9 cultivated on NWCAS showed faster growth rate and higher biomass yield during the first 3-day cultivation. And algae followed a similar stationary phase and declined accordingly after 6 days cultivation, when algae cultivated in a condition without pH adjustment. However, when cultivated in pH controlled conditions, the algae continued to grow during the first 5 days and kept stationary phase until the end of experiment. In addition, the microalgae concentration under pH adjustment was more than 2 times higher than that without pH adjustment (Fig. 4), which suggested that pH had significant impact on algae growth for this type of wastewater. As shown in Fig 5, pH in the broth reached over 9 during the first 3 days and maintained similar level until the end of experiment under condition without pH adjustment. However, under controlled condition, the pH maintained around 7, which is ideal condition for fast algae growth. Li et al. [15] compared the growth rate and biomass concentration of *Chlorella. sp.* grown on both Centrate and artificial medium (TAP medium) and found that algae grown on Centrate achieved higher biomass yield compared with those on artificial medium, which are in agreement with our results. It was obvious that this strain is very competitive to those reported in previous studies in terms of algal growth rate when growing on different types of wastewater resource (data not shown) [16, 17, 18].

The data in Fig.6 summarized the variation of ammonia, phosphorus and chemical oxygen demand (COD) in NWCAS at initial and after 7-day batch cultivation in both pH control and non-control condition.

As indicated in Fig 6a, for condition without pH control, the ammonia content was greatly reduced from 136 to 57 mg/L in the first three days and followed by a short stationary phase in which the ammonia content stayed at a similar level and slightly increase till the end of 7-day batch culture experiment, resulting in a maximal ammonia removal efficiency of 58%. The slight increase for ammonia probably was due to partial decay of microalgae under environment stress. For pH control condition, the ammonia concentration was reduced from 136 to 52 mg/L in the first 5 days and followed by a stationary phase until the end of experiment. The maximal ammonia removal efficiency was 62%, only slight higher than that without pH control. Considering the algal biomass yield grown under pH control is much higher than that with pH control, it is reasonable to conclude that there might be other way for ammonia removal besides algae uptake for condition without pH control. It was reported that ammonia stripping occurred under high pH condition, considering the fact the high pH for the broth after 3 days cultivation (pH >9) (Fig. 5), the ammonia stripping should contribute a lot for ammonia removal in this study.

Phosphorus (P) removal pattern was similar to ammonia removal. For algae grown under pH controlled condition, in the first three days P content dropped dramatically from 142 to 48 mg/L and increased accordingly until the end of experiment. The maximal phosphorus removal efficiency was 66%. However, for algae grown under non-controlled condition, the continuous reduction for phosphorus concentration was observed even after the cease of algae growth, till the end of the experiment (Fig. 6b). Maximal removal rate reached over 90%, which showed much higher removal efficiency compared with other studies using other municipal wastewaters [5, 18]. The reason for high P removal rate in this study was both of algae uptake and phosphate precipitation through coagulation with metal ion in supernatant under high pH condition (Fig. 2) [15, 19, 20].

For algae grown under pH controlled condition, the COD concentration was reduced from 3050 to 321 mg/L in the first 2 days and followed by a stationary phase until the end of experiment (Fig. 6c). The COD removal efficiency reached maximal (89%) at first 2 days, much higher than that without pH control (71%). Higher COD removal properties suggested that the algal strain could utilize different organic compounds as carbon sources besides CO_2, which coincided with our previous findings [4, 21].

Inhibiting factors that affect algae growth were expressed by the slow algal growth and the algal cells density kept constant after the 4th day cultivation (Fig. 6). Factors affecting algae growth are numerous. The main factors are limited nutrients supply, pH level, light saturation/penetration, temperature, etc. In this study, it is clear that the pH variation was the main limiting factors affecting algae growth and nutrient removal efficiency.

3.4 Effect of light-dark cycle on algae growth and nutrient removal efficiency

The data in Fig.7 summarized the growth pattern and variation of total nitrogen, phosphorus and chemical oxygen demand (COD) in NWCAS at 7-day batch cultivation under 24/0 and 12/12 light-dark cycle condition.

As indicated in Fig. 5a, the growth pattern for algae grown on 24/0 and 12/12 light cycle conditions was similar. And algae concentration grown under continuous light condition was slightly higher compared than that under 12/12 light-dark cycle, which suggested light-dark cycle did not have significant impact on algae growth. And the nutrient profiles for TN, TP and COD also followed the similar patterns as growth curve. Our results coincided with results of Elsold et al. (Elsold et al., 2009) that the impact of light-dark cycle on the algae growth for mixotrophic strain was minimal. Considering the fact that NWCAS is one type of organic-rich wastewater, the algae strain mainly utilized the organic carbon compounds to support their fast growth in this study [4, 21].

4. Conclusions

The strain *C. kessleri* GXLB-9 was proven to be facultative heterotrophic microalgae strains and effective for organic-rich wastewater nutrient removal like NWCAS. pH was considered to play significant role in algae growth and nutrient removal efficiency in this study. However, the effect of light-dark cycle on algae growth and nutrient removal was minimal. The strain has great potential to be used in other organic carbon-rich wastewater sources to achieve dual purpose of effective wastewater treatment and economically viable and environmentally friendly production of biofuel and bio-based products in the near future.

Acknowledgements

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Table 1. Nutrient Profiles of supernatant of activated sludge after centrifugation

Parameter	Concentration (mg L^{-1})	Parameter	Concentration (mg L^{-1})
COD	3050 ± 53	PO_4^{3-}-P	142± 5.7
TOC	1009±29	NH_3-N	136±6.3
TVSS	0.14 ± 0.11	TKN	227± 9.1

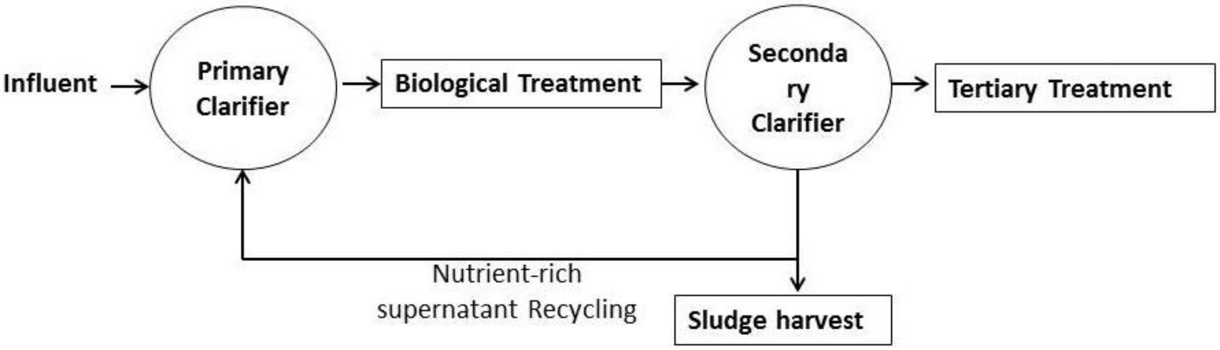


Figure 1. Simple schamatic of municipal wastewater treatment processs.

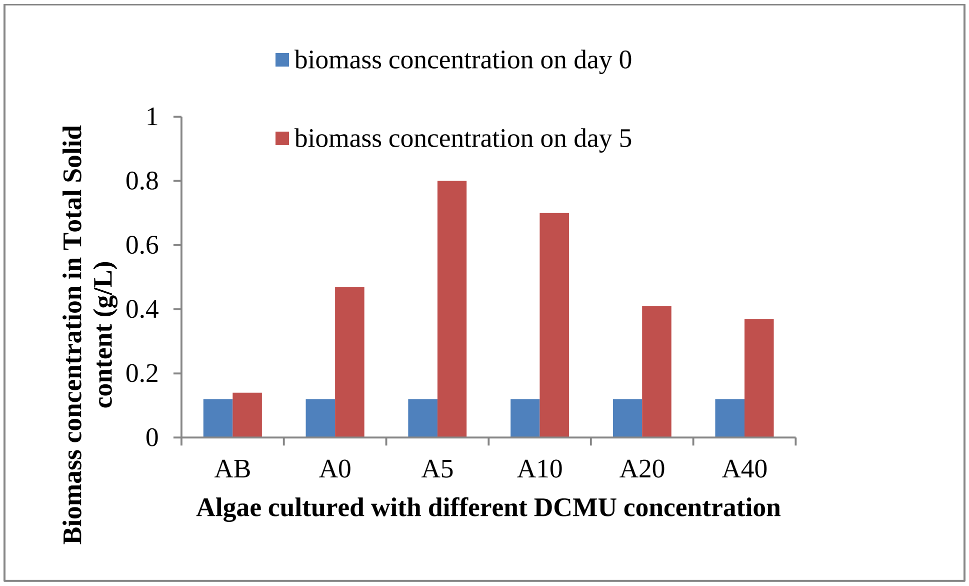


Figure 2. Effect of DCMU concentration on algae growth. (AB): Black control; (A0): 2mL ethanol added; (A5): 5μM DCMU added; (A10): 10μM DCMU added; (A20): 20μM DCMU added; (A40): 40μM DCMU added

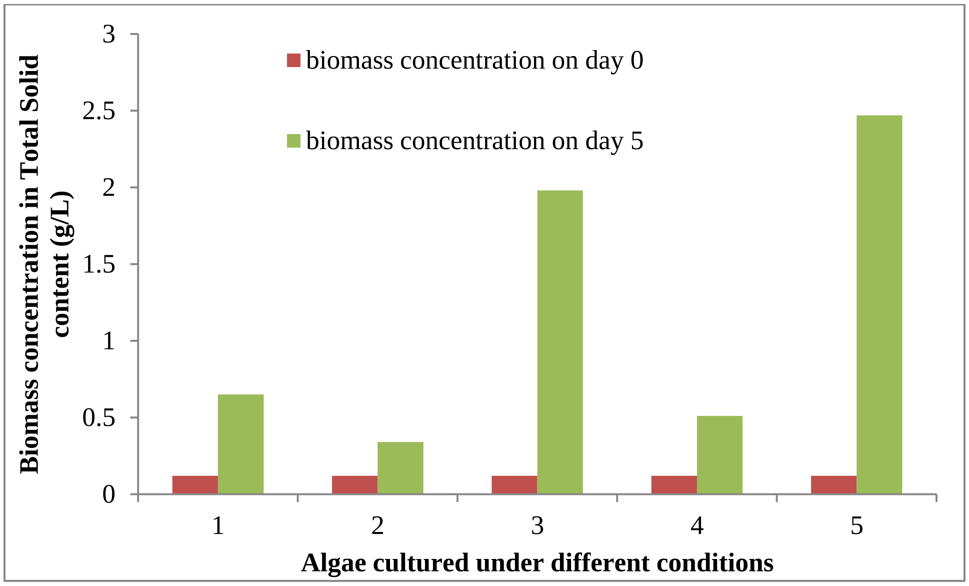


Figure 3. Evaluation of growth profile of targeted microalgae grown in different cultivation conditions. (1): Light and CO_2 was added; (2): Light, CO_2 and 10μM DCMU ertr added; (3): Light, CO_2 and glucose were added; (4) Light, CO_2, glucose and 10μM DCMU were added; (5) Light and glucose were added;

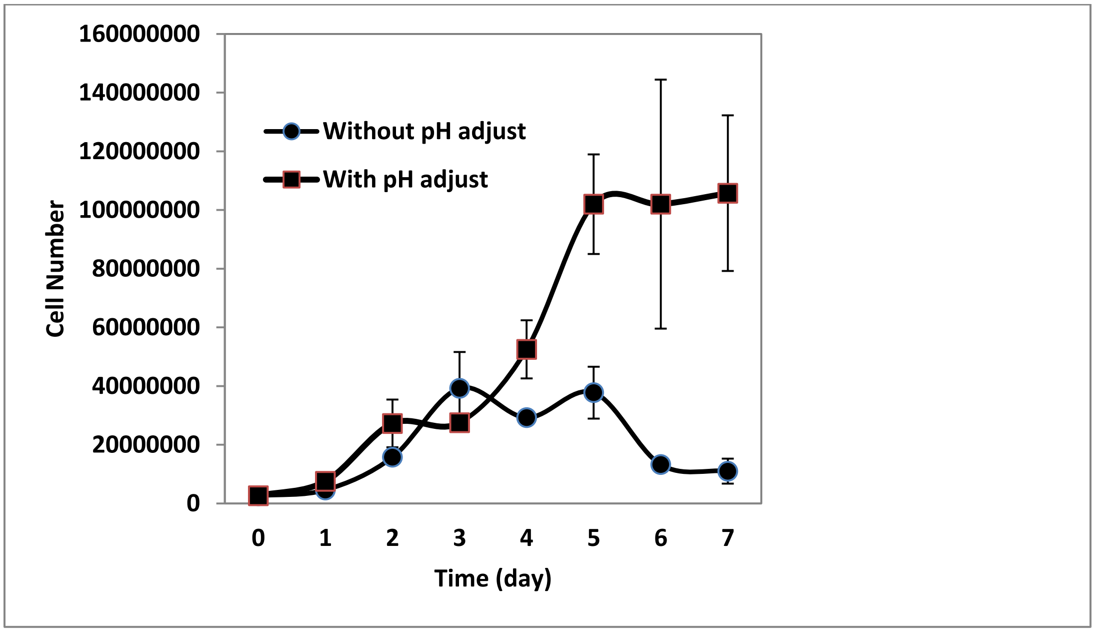


Figure 4. Growth curve of microalgae GXLB-9 grown in SWCAS. (Square): with pH adjust; (Cirle): without pH adjust

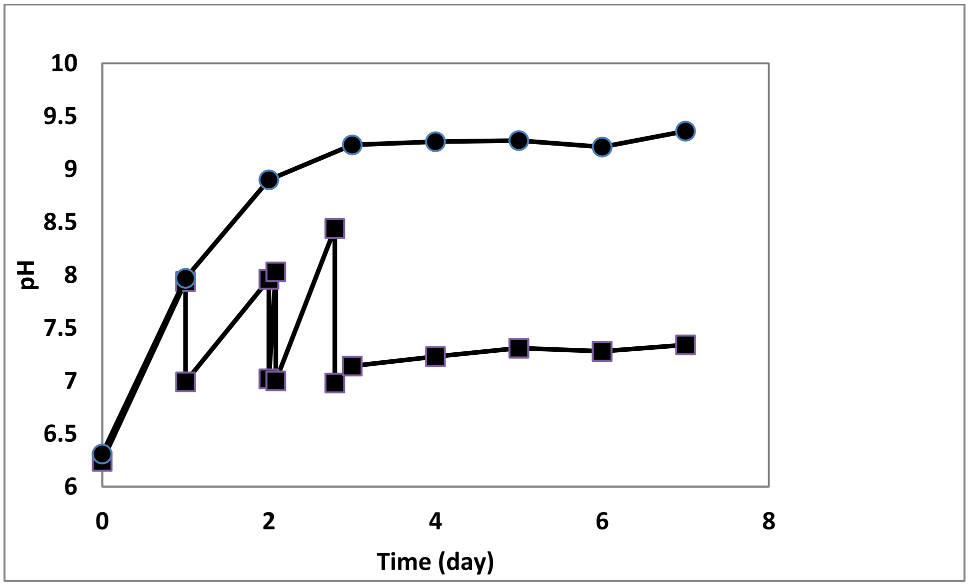


Figure 5. pH varietion during growth curve of microalgae GXLB-9 in NWCAS. (Square): with pH adjust; (Cirle): without pH adjust.

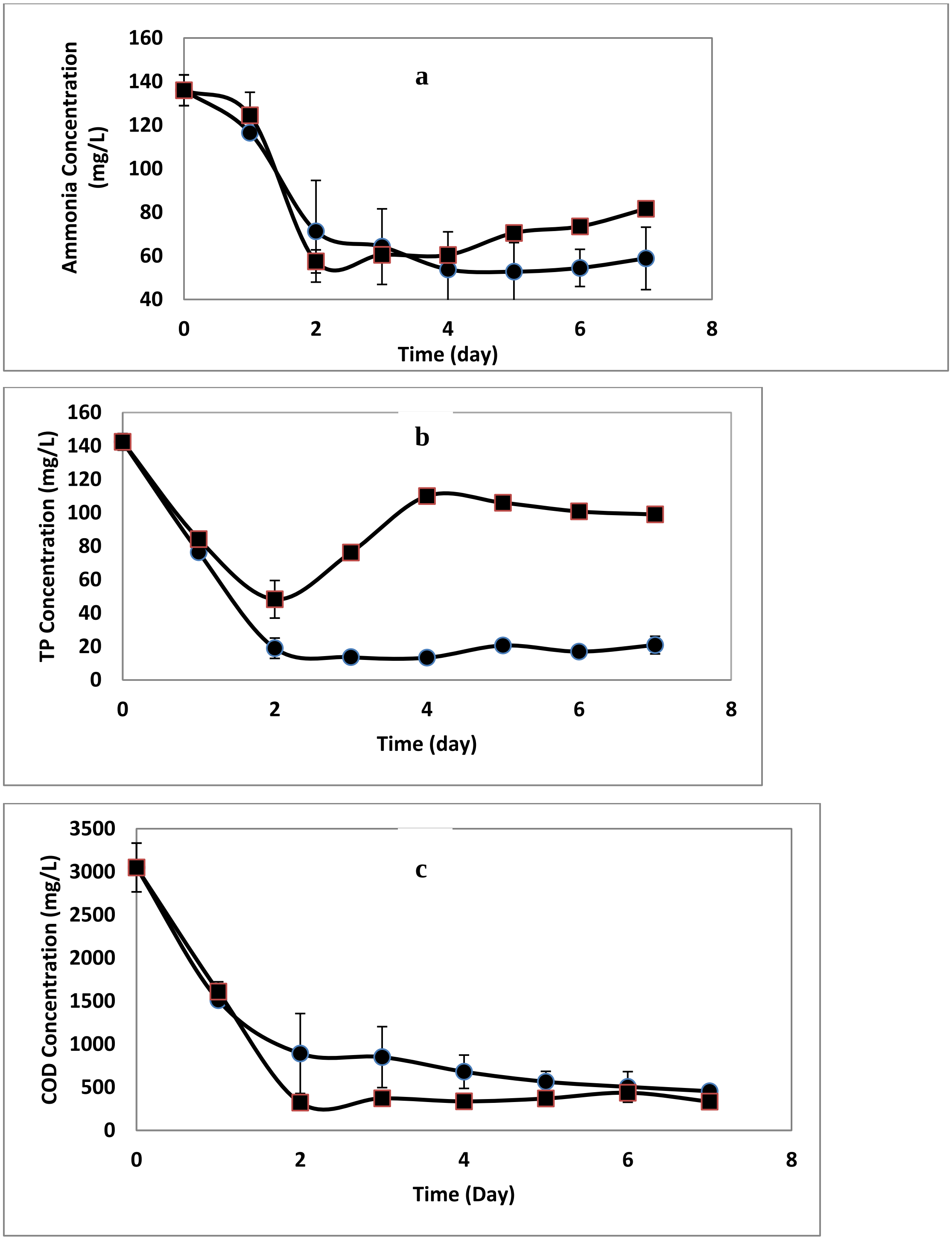
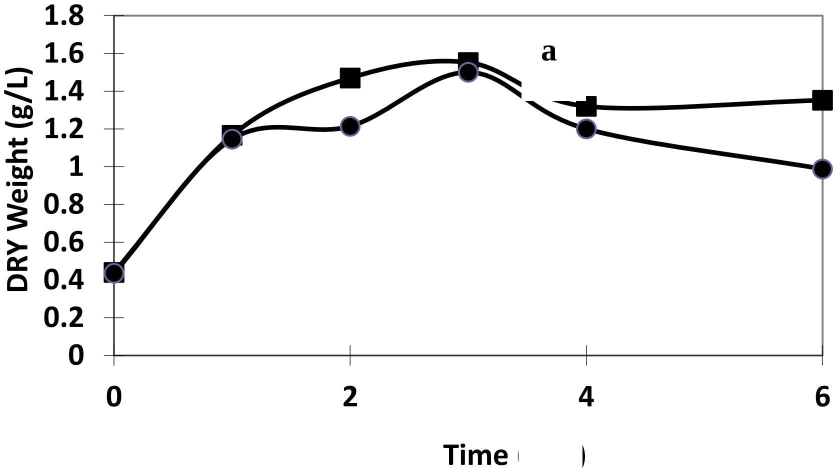
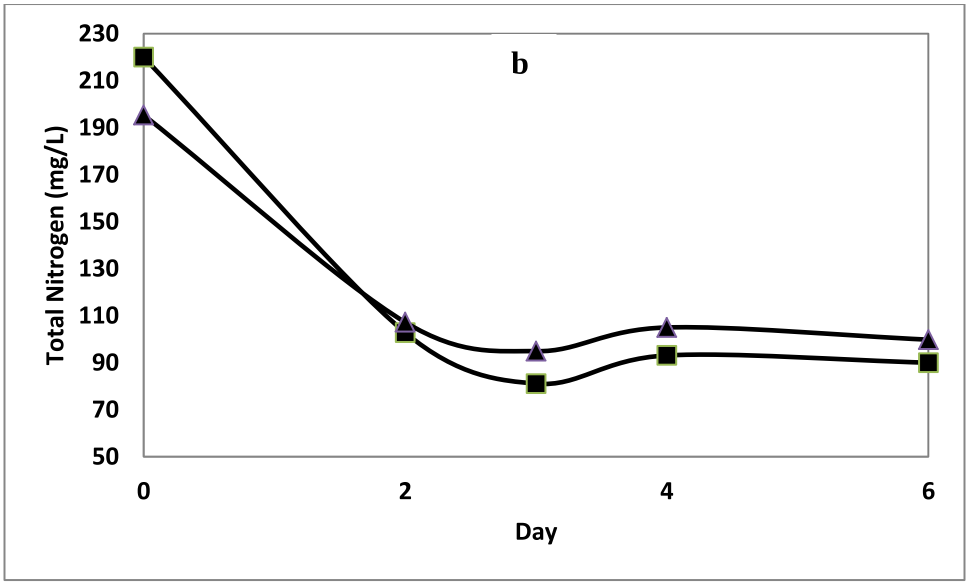


Figure 6. Nutrient removal efficiency of microalgae GXLB-9 grown in NWCAS. (a): Ammoniae removal profile; (b): Total phosphorus removal profile; (c) COD remoal profile; Note: Square stands for with pH adjust; Cirle stands for without pH adjust.


a
DRY Weight (g/L)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
Time ( )


b
Total Nitrogen (mg/L)
230
210
190
170
150
130
110
90
70
50
0
2
4
6
Day

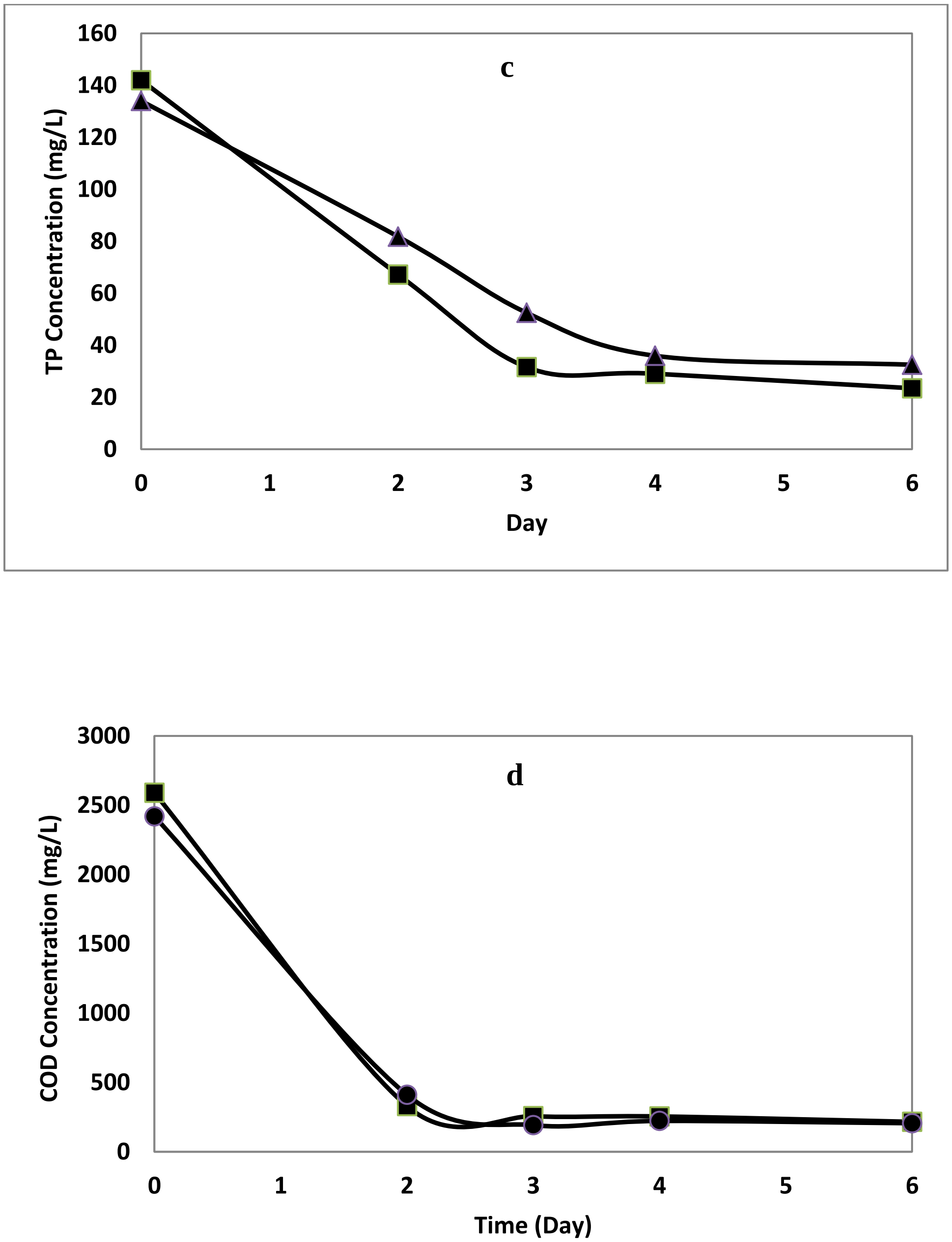


Figure 7. Growth and nutrient removal profiles of microalgae GXLB-9 grown in NWCAS. (a): algae growth curve grown in supernatent wastewater; (b): Total nitrogen removal profile; (c): Total phosphorus removal profile; (d) COD remoal profile; Note: Square stands for continuous light; Cirle stands for 12/12 light/dark.