Cultivation of Chlorella pyrenoidosainoutdoor open raceway pond using domestic wastewater as medium in arid desert region

Article *in* Bioresource Technology · August 2016

DOI: 10.1016/j.biortech.2016.08.019

CITATIONS
7

READS
229

5 authors, including:

Dehmani Siham
Université Kasdi Merbah Ouargla
1 PUBLICATION **7** CITATIONS
SEE PROFILE

Zerrouki Djamal
Université Kasdi Merbah Ouargla
16 PUBLICATIONS **392** CITATIONS
SEE PROFILE

Luveshan Ramanna
Durban University of Technology
5 PUBLICATIONS **169** CITATIONS
SEE PROFILE

Ismail Rawat
Durban University of Technology
47 PUBLICATIONS **1,615** CITATIONS
SEE PROFILE

Bioresource Technology 219 (2016) 749–752

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Cultivation of *Chlorella pyrenoidosa* in outdoor open raceway pond using domestic wastewater as medium in arid desert region

Siham Dahmani [a], Djamal Zerrouki [a,*], Luveshan Ramanna [b], Ismail Rawat [b], Faizal Bux [b]

[a] Univ. Ouargla, Fac. des sciences appliquées, Lab. dynamique interaction et réactivités des systèmes, BP 511, Route de Ghardaïa, Ouargla 30000, Algeria
[b] Institute for Water and Wastewater Technology, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa

HIGHLIGHTS

- Domestic wastewater was used as propagation medium for *Chlorella pyrenoidosa*.
- Successful outdoors culture of *C. pyrenoidosa* in desert area.
- Arid climate show a great potential for algae WW nutrient removal.
- High nutrient removal of algae treatment compared to lagoon process.

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form 3 August 2016
Accepted 5 August 2016
Available online 8 August 2016

Keywords:
Microalgae
Wastewater
Nutrient removal
Secondary treatment
Desert
High irradiance

ABSTRACT

Chlorella pyrenoidosa was cultivated in secondary wastewater effluent to assess its nutrient removal capabilities. Wastewaters were obtained from a wastewater treatment plant located in Ouargla, Algeria. The experiments were conducted in winter under natural sunlight in an outdoor open raceway pond situated in the desert area. The highest biomass of the microalgae was found to be 1.71 ± 0.04 g/L. Temperatures ranged between 18 and 31 °C. The average annual insolation was no less than 3500 h with an annual solar irradiance of more than 2000 kWh/m^2. Analyses of different parameters including COD, NH_4^+-N and TP were conducted throughout the cultivation period. Their average removal efficiencies were 78%, 95% and 81% respectively. The results demonstrated the potential of nutrient removal by microalgae grown on secondary wastewater in arid areas.

1. Introduction

Large-scale microalgal production is limited by various factors that would otherwise ensure an economically feasible process. These include selection of a cultivation system (open or closed system), utilizing efficient microalgal strains, the quantity and quality of light, the availability of nutrients and carbon dioxide (CO_2) to the algae and a reliable source of water that has little or no environmental impact (Lam and Lee, 2012).

Nevertheless, production processes are still under development and there is considerable scope to reduce costs and improve efficiency with this technology. Microalgal cultivation requires large amounts of water (Rawat et al., 2013). The impact of large-scale algae production on water utilization has generated great debate. Using seawater, brackish water or WW would reduce the need for freshwater (Komolafe et al., 2014).

Microalgae have been previously used for the treatment of WW (Komolafe et al., 2014; Ma et al., 2014). In turn, WW has been shown to be economically viable and sustainable for the production of microalgal biomass (Yang et al., 2015). Recent findings have shown that microalgae are able to grow and utilize the N and P present in WW, thereby, removing these nutrients before WW discharge (Ma et al., 2014).

Ammonia-N and P in secondary-treated WW are generally in the range of 20–40 mg/L and 10 mg/L, respectively (Grady et al., 2011). These nutrient concentrations are deemed sufficient to support the growth of most freshwater microalgae (Olguín, 2012). Coupling WW treatment with biomass production is a very attractive option for energy, freshwater and fertilizer reduction. This would reduce the cost of WW treatment incurred for nutrient removal by conventional methods (Lam and Lee, 2012). Cultivation of microalgae in WW has the potential to reduce the N requirement by up to 97% (Olguín, 2012). Wastewater utilization as a

* Corresponding author.
E-mail address: Djamal.zerrouki@gmail.com (D. Zerrouki).

http://dx.doi.org/10.1016/j.biortech.2016.08.019
0960-8524/

replacement for freshwater can totally negate the need for additional potassium, magnesium and sulphur (Rawat et al., 2013).

Despite the favorable outlook for the use of WW mediated biomass production, the real potential must be explored practically. Wastewater is susceptible to viral and bacterial contamination and inconsistent nutrient compositions (Zhang et al., 2012). These factors as well as the presence of inhibitory substances such as cadmium, mercury, or organic chemicals could retard the growth of microalgae (Ma et al., 2014). High nutrient concentrations are also known to be inhibitory to certain microalgal strains. For instance, an oversaturation of ammonium in medium is toxic to microalgae. Excess intracellular ammonium will inhibit the formation of adenosine triphosphate in the chloroplast resulting in substrate activation ultimately leading to cellular inhibition (Ramanna et al., 2014). These factors can be controlled by close monitoring and adjustment of nutrient levels by augmentation or dilution.

This work assessed the suitability of domestic WW as a medium for cultivating *Chlorella pyrenoidosa*. The experiments were conducted in the desert area in open pond systems. The study also aimed to evaluate and compare the nutrient removal efficiencies of algal secondary treatment and lagoon process using bacteria for nutrient removal.

2. Materials and methods

2.1. Strain and culture conditions

Chlorella pyrenoidosa was used in this study. Cultures were grown in the laboratory. All inoculations were performed under sterile conditions. 6 ml (7.5×10^6 cells/ml) of *C. pyrenoidosa* was inoculated in 500 ml of autoclaved BG11 medium in 1000 ml Erlenmeyer flasks. Once a sufficient density was reached, they were inoculated into three 1000 ml conical flasks. The composition of BG 11 medium consists of: (g/L) $NaNO_3$ (1.5), K_2HPO_4 (0.04), $MgSO_47H_2O$ (0.075), $CaCl_2H_2O$ (0.036), Na_2CO_3 (0.02), Citric acid (0.006), EDTA (0.001), and 1 mL of trace elements solution having the following composition (g/L): H_3BO_3 (2.86); $MnCl_24H_2O$ (1.81); $ZnSO_47H_2O$ (0.222); $NaMoO_42H_2O$ (0.39); $CuSO_45H_2O$ (0.079); $Co(NO_3)_26H_2O$ (0.0494). All cultures were placed in a culture closet equipped with blue and red light-emitting diode (LED) (Light intensity 100 µmol photons m^{-2} s^{-1}) as a light source. Mixing was achieved by continuous bubbling with air. The temperature was maintained between 24 and 26 °C and pH 7–8 under aseptic conditions.

2.2. Outdoor culture in open raceway ponds

Open raceways were inoculated with 6% algal suspensions (v/v) of the working volume (165 L). The initial inoculum cell concentrations were 7.5×10^6 cells/ml. Experiments were run for 19 days. Outdoor experiments were conducted in a galvanized open raceway pond with a 360 L capacity (1.5 m length, 0.6 m width, and 0.4 m depth). The mixing and recirculation of the culture medium was achieved by a stirring system, which consisted of one paddle wheel made of galvanized steel and fixed to an axle. This was powered by a 70 W electric motor.

2.3. Experimental site and wastewater

The study was carried out at a domestic WW treatment plant situated in the northeast of the Capital of Ouargla, Algeria (geographic coordinates: latitude: 31°59′46, 23″ N; Longitude: 5°21′55, 77′ E). The experiments were carried out in the winter period using sunlight as a light source. The temperatures ranged between 18 and 31 °C during the day and 6–15 °C at night. The duration of insolation during this season was approximately 9 h a day (7 a.m.–5 p.m.); the maximum irradiance was approximately 1100 W/m^2. This was measured using a solarimeter (SL200, KIMO instrument, France). The WW used in this study was obtained directly from the aerated lagoon station basins after primary screening. The characteristics of the raw WW (RW) are summarized in Table 1. Experiments were also conducted to investigate the bacterial born WW on nutrient removal. Raw WW and autoclaved (AW) samples were inoculated with algal culture. Nutrient removals were compared. The WW was sterilized by autoclaving at 121 °C for 30 min before the introduction of microalgae. These experiments were run in the lab for a 7-day period.

Table 1
Wastewater characteristic before/after algae and lagoon process treatment.

Characteristics	Raw WW	Algae treated WW	Lagoon treated WW
pH	7.82	9.36	7.43
DO (mg/L)	0.2	9.4	4.5
Conductivity (mS/cm)	7.97	10.88	10.99
Salinity (g/L)	4.4	6.1	6.8
COD (mg/L)	426	90	110.75
NH_4-N (mg/L)	46.2	2.1	32
TP (mg/L)	3.22	0.596	2.41
NO_3^--N (mg/L)	1.156	16.9	0.57

2.4. Analytical methods

Algae biomass were calculated by subtracting Total suspended solid (TSS) of wastewaters measured before inoculation from total volatile suspend solids (TVSS) of wastewaters with algae measured daily at the same time based on the standard method (Ma et al., 2014). Dissolved oxygen (DO) and pH were measured using Multi-parameter analysers (Consort C3020, Belgium). Chemical oxygen demand (COD), was determined with cuvette tests kits LCK 314 (15–150 mg COD/L) and LCK 514 (100–2000 mg COD/L), following DIN 38049-4. Ammonium-N (NH_4-N) and Nitrate-N (NO_3-N) were obtained using the cuvette tests kits (LCK303 (2.0–47.0 mg/L NH_4-N), and LCK 339 (0.23–13.50 mg NO_3-N/L), following standards DIN38406-E 5-1 and DIN 38402-A51. Total phosphorous (TP) was measured according to ISO 6878-1-1986 standards, DIN 38405 D11-4 Hach Lange test kit LCK 349 ranges of PO_4-P (2–20 mg TP/L). The conversion of absorbance to concentration (mg/L) was done using a spectrophotometer DR 2800 (Hach Lange, Germany). For COD analysis all sample were heated for 2 h at 148 °C before being read on the spectrophotometer. For TP analysis the samples were heated for 60 min at 100 °C in a high-temperature thermostat (HT 200S, Germany).

2.5. Data analysis

All the experiments were performed in triplicate. All algal pond experimental samples were taken at three different points from the same pond. Analysis of variance (ANOVA) test was employed ($p = 0.05$). Graph were done using GraphPad Prism version 6.

3. Results and discussion

3.1. Algal growth in wastewater

From the WW characteristics (Table 1), it can be seen that the amount of nutrient elements in WW is more than that of the prepared BG11 medium. The WW contains abundant N, available as NO_3-N (1.15 mg/L) and NH_4-N (46.2 mg/L) and P as TP (3.22 mg/L). From the TP and NH_4-N concentrations, it was hypothesized

that this WW stream would sustain microalgal growth. Several studies have demonstrated efficient microalgal growth on municipal and agricultural WW (de Godos et al., 2009; Wang et al., 2010; Yang et al., 2015). Algal biomass showed typical growth with the exponential phase lasting approximately 3–15 days. This was followed by a stationary phase. The results showed a lag phase; however, this only lasted 3 days and can be considered insignificant. This indicated that *C. pyrenoidosa* adapted well to the WWs used. Similar growth patterns have been observed by (Cabanelas et al., 2013) and (Hongyang et al., 2011). In the Cabanelas et al. (2013) study, the maximum biomass concentration reached was 2.05 ± 0.12 g/L. Hongyang et al. (2011), found that the growth of *C. pyrenoidosa* increased from 2.09 g/L to 6.20 g/L when cultivated on soybean processing WW with an additional glucose (10 g/L) as carbon source. Yang et al. (2015) evaluated the potential of pure and combining anaerobically digested starch WW (ADSW) and alcohol WW (AW) for *C. pyrenoidosa* cultivation. The best growth performance (3.01 ± 0.15 g/L) was achieved using a combination of both these mixed WWs [AW:ADSW = 0.053:1(v/v)].

The variation of temperature during the experiment ranged between 18 and 31 °C (Fig. 1). Zhao et al. (2015) showed that at a temperature of 35 °C, *C. pyrenoidosa* was able to achieve a biomass productivity of 0.16 g/L.d. From Fig. 1 it can be seen that the maximum temperature during the culture period reached as high as 31 °C. This can be considered low when compared to other seasons. During the summer (July–August) the temperature can reach 50 °C. The latitudes and the absence of could cover is the desert area let the annual levels of solar radiation significantly higher than in other areas. High and uniform incident irradiance levels present an advantage for microalgal cultivation by increasing the photosynthetic active radiation (PAR) (Garcia-González et al., 2003), which represent around 43% of the solar radiation. Firstly, the intensity of solar radiation is a major factor that influences the growth of algae in outdoor culture. The annually uniform incident irradiance is an important to maintain significant productivity (Garcia-González et al., 2003). The average maximum solar irradiation per day was 992 ± 40 W/m^2 which was measured between 12 a.m and 1 p.m. From the current study it was concluded that the optimal lighting duration was between 8 a.m. and 5 p.m.

At the start of the experimentation, the pH was 7.8 (Fig. 2). This gradually increased to 9.2. This was mostly related to photosynthetic activity. As microalgae use CO_2, the pH increases from acidic to alkaline due to the CO_2 depletion. The pH optimum for most algae range between 7 and 12.

Fig. 2 shows the variation of the DO during the experimental period. It can be seen that the lowest DO was on day 1. This was potentially attributed to organisms such as bacteria that required the DO to decompose any organic material in the WW. It then

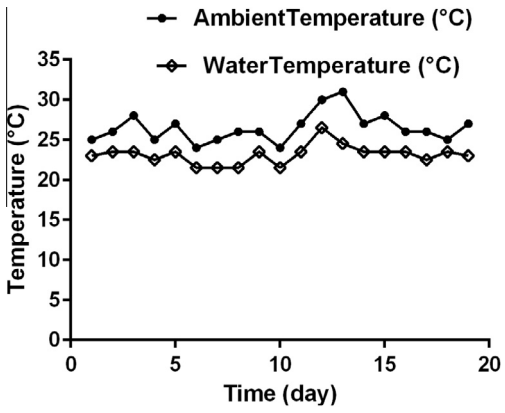

Fig. 1. Ambient and water temperature during the culture period.

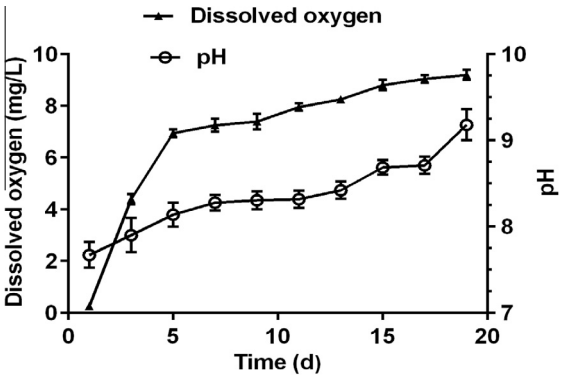

Fig. 2. Dissolved oxygen and pH variation during the culture period. Data are expressed as a mean ± SEM (n = 3).

increased gradually to an approximate concentration of 8 mg/L during the cultivation period. From the results, it can be seen that the microalgal growth increase with DO. Microalgae perform photosynthesis in which they release DO into the culture medium. As the culture density increased there was an increase in the DO levels in the medium.

3.2. Nutrient removal from waste water

Nitrogen and P are known algal growth limiting factors. The optimal value of N:P ratio for freshwater algae was suggested to be in the range of 6.8–10 (Wang et al., 2010). However, the N:P ratio in this study was approximately 14.74. This higher ratio may induce a limitation (Wang et al., 2010).

Nitrogenous matter is mainly composed of inorganic NH_4–N in WWs. the initial NH_4–N concentration was, approximately 46.2 mg/L. Algae have considerable intracellular capacity for storing soluble and organic N molecules. Nitrogen is rapidly taken up as it is indispensable for the regulation of the metabolic pathways. It is used to produce the amino acids, and other organic N-containing macromolecules. At too high concentrations the NH_4–N becomes toxic which thereby inhibits algal growth. Nitrogen limitation decreases microalgal protein content. The limitation stress decreases the synthesis of pigments and photosynthetic proteins as involved in the biosynthesis, therefore affects the growth rates of microalgal biomass (Ramanna et al., 2014).

The removal of NH_4–N was accomplished by the direct utilization by the algae. The initial concentration was significantly reduced to 2.1 mg/L (Table 1). At the end of the culture period, the average removal rates were 95%.

The concentration of the NO_3-N in WW can also be seen in Table 1. It has been found that algae preferentially utilize NH_4–N and other reduced forms of N before NO_2^- and NO_3^-. From the current results in Table 1 it can be seen that *C. pyrenoidosa* preferred NH_4–N rather than NO_3^-. The NO_3^- Nisonly utilized by algal cells after the NH_4-N concentration depletes. A high NH_4 concentration directly influences the NO_3^- uptake by microalgae (Komolafe et al., 2014). This explained why the NO_3-N concentration in WW never showed a significant decrease. In fact, by the end of the study this concentration increased. A reduction of NO_3^- was only noticed toward the end of the culture period where almost 90% of NH_4-N was removed.

The TP was considerably reduced from 3.22 mg/L to 0.59 mg/L. *C. pyrenoidosa* cells assimilated P as inorganic orthophosphate which was used for the production of phospholipids, ATP and nucleic acids.

De Godos et al. (2009) reported that both P uptake and precipitation accounts for the removal of P at alkaline pH levels between 9 and 11. The pH only exceeded 9 on the last day (Fig. 2); therefore,

the precipitation effect might have been neglected. The main mechanism of P removal was accumulation into the algal biomass (Ma et al., 2014). The kinetic uptake of P was slightly slow at the start culture compared to NH_4–N, the final TP removal efficiencies were 81%.

Chemical oxygen demand expresses the overall organic load (dissolved and suspended matter) of the WW. The initial COD concentration in WW was approximately 426 mg/L (Table 1). High carbon concentrations affect the metabolic pathway of *C. pyrenoidosa* (Yang et al., 2015). Initially, the COD removal efficiently was slightly high but dropped as the experiment proceeded (data not shown). Almost 60% of COD was removed in the two first days. This caused a nutrient deficiency for further algal growth. Similar results was observed by (Ma et al., 2014) where 80% of COD was removed in the first 2 days of cultivation. This can be explained by the participation of WW born bacteria using organic compounds as carbon source in the degradation process (Ma et al., 2014).

By the end of cultivation, the COD concentration was 90 mg/L (Table 1). The average removal efficiency of COD was 78%. Aziz and Ng (1992) reported that the COD removal rate of *C. pyrenoidosa* in domestic sewage and industrial WWs from a pig farm and palm oil mill was 70% and 82% respectively with a retention time of 15 days, this is in accordance to our result with a retention time of 19 days.

3.3. Post algal and lagoon treatments

From Table 1 it can be seen that the algal secondary treatment removed 95% of the NH_4–N while the lagoon process only removed 30%. The same trend can be observed with COD (algae, lagoon) (78%, 75%), and TP (81%, 25%). The current results, therefore, demonstrate the efficiency of algal nutrient uptake.

The effects of bacteria born WW on nutrient removal were also investigated in this study. Wastewater treatment entails the removal of wastes by using physical processes in the initial stages to remove the solid materials and fine organic particles. This is followed by a biological treatment (secondary treatment) where water-born microorganisms use oxygen to convert the organic matter into biomass.

Zhang et al. (2012) reported that bacteria break down complex organic compounds into smaller molecules as nutrients available for algal usage. Microalgae cause detrimental effects on bacterial growth and activity by increasing the pH, DO concentration or by excreting inhibitory metabolites (Ma et al., 2014). To investigate the bacterial effects of algae grown on WW, raw and autoclaved WW were used to culture *C. pyrenoidosa*. The COD removal efficiencies in RW were higher than the AW, 77.54% and 55.46% respectively. This signified that the WW born bacteria did participate in the degradation process by consuming organic compounds as carbon source.

The TP in both RW and AW was reduced. The final TP removal efficiencies in RW and AW were 83% and 69%, respectively. The changes of NH_4–N concentration were also investigated in this study. The removals of COD, TN and TP were significantly ($P < 0.05$) different among RW and AW. The removal rate in RW was slightly higher than that in AW, which indicated that WW borne bacteria participated in degradation and assisted in the removal of N.

4. Conclusion

The results demonstrate the success of cultivating *C. pyrenoidosa* in desert regions on WW in open pond systems using direct sunlight. The average biomass productivity showed a high yield of 1.71 ± 0.04 g/L. Algal treatments show higher efficiencies for nutrient removal (95% NH_4–N, 78% COD and 81% TP) when compared to lagoon treatments process (30% NH_4–N, 75% COD and 25% TP). These results could provide a guideline for efficient microalgal based domestic WW treatment in arid areas where sunlight irradiance is high.

Acknowledgements

Authors hereby acknowledge the General Directorate for Scientific Research and Technological Development (Algeria) (DGRSDT) and the National Research Foundation (South Africa) (NRF), for financial contribution.

References

Aziz, M.A., Ng, W.J., 1992. Feasibility of wastewater treatment using the activated-algae process. Bioresour. Technol. 40, 205–208.
Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour. Technol. 131, 429–436.
de Godos, I., Blanco, S., García-Encina, P.A., Becares, E., Muñoz, R., 2009. Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresour. Technol. 100, 4332–4339.
Garcia-González, M., Moreno, J., Cañavate, J.P., Anguis, V., Prieto, A., Manzano, C., Florencio, F.J., Guerrero, M.G., 2003. Conditions for open-air outdoor culture of Dunaliella salina in southern Spain. J. Appl. Phycol. 15, 177–184.
Grady Jr., C.P.L., Daigger, G.T., Love, N.G., Filipe, C.D.M., 2011. Biological Wastewater Treatment, third ed. CRC Press, Boca Raton, Florida.
Hongyang, S., Yalei, Z., Chunmin, Z., Xuefei, Z., Jinpeng, L., 2011. Cultivation of *Chlorella pyrenoidosa* in soybean processing wastewater. Bioresour. Technol. 102, 9884–9890.
Komolafe, O., Velasquez, S.B., Monje-ramirez, I., Yáñez, I., Harvey, A.P., Orta, M.T., 2014. Biodiesel production from indigenous microalgae grown in wastewater. Bioresour. Technol. 154, 297–304.
Lam, M.K., Lee, K.T., 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol. Adv. 30, 673–690.
Ma, X., Zhou, W., Fu, Z., Cheng, Y., Min, M., Liu, Y., Zhang, Y., Chen, P., Ruan, R., 2014. Effect of wastewater-borne bacteria on algal growth and nutrients removal in wastewater-based algae cultivation system. Bioresour. Technol. 167, 8–13.
Olguín, E.J., 2012. Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. Biotechnol. Adv. 30, 1031–1046.
Ramanna, L., Guldhe, A., Rawat, I., Bux, F., 2014. The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. Bioresour. Technol. 168, 127–135.
Rawat, I., Ranjith Kumar, R., Mutanda, T., Bux, F., 2013. Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl. Energy 103, 444–467.
Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R., 2010. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Appl. Biochem. Biotechnol. 162, 1174–1186.
Yang, L., Tan, X., Li, D., Chu, H., Zhou, X., Zhang, Y., Yu, H., 2015. Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater. Bioresour. Technol. 181, 54–61.
Zhang, Y., Su, H., Zhong, Y., Zhang, C., Shen, Z., Sang, W., Yan, G., Zhou, X., 2012. The effect of bacterial contamination on the heterotrophic cultivation of *Chlorella pyrenoidosa* in wastewater from the production of soybean products. Water Res. 46, 5509–5516.
Zhao, F., Tan, X., Zhang, Y., Chu, H., Yang, L., Zhou, X., 2015. Effect of temperature on the conversion ratio of glucose to *Chlorella pyrenoidosa* cells: reducing the cost of cultivation. Algal Res. 12, 431–435.