Journal of Environmental Management 244 (2019) 453–461

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Influence of irrigation with microalgae-treated biogas slurry on agronomic trait, nutritional quality, oxidation resistance, and nitrate and heavy metal residues in Chinese cabbage

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ARTICLE INFO

Keywords:
Biogas slurry irrigation
Nutrient and metal removal by microalgae
Chinese cabbage
Nutritional quality
Nitrate and heavy metal residue

ABSTRACT

Biogas slurry (BS) is a main byproduct of biogas production that is commonly used for agricultural irrigation because of its abundant nutrients and microelements. However, direct application of BS may cause quality decline and nitrate and heavy metal accumulation in crops. To address this issue, a microalgae culture experiment and an irrigation experiment were performed to evaluate the removal efficiencies of nutrients and heavy metals from diluted BS by microalgae *Scenedesmus* sp. and to investigate the effects of irrigation with microalgae-treated BS (MBS-25, MBS-50, MBS-75, and MBS-100) on nutritional quality, oxidation resistance, and nitrate and heavy metal residues in Chinese cabbage. After 8 days of continuous culture, a ratio of 1/1 for BS/tap water mixture (BS-50) was the optimal proportion for microalgal growth (3.73 g dry cell L^{-1}) and efficient removal of total nitrogen (86.1%), total phosphorus (94.3%), COD (87.5%), Cr (50%), Pb (60.7%), and Cd (59.7%). The pH in MBS-50 medium recovered to the highest level in a shorter period of time and accelerated the gas stripping of ammonia nitrogen and the formation of insoluble phosphate and metals, which partly contributed to the high removal efficiencies. MBS irrigation significantly promoted crop growth; improved nutritional quality, edible taste, and oxidation resistance; and reduced nitrate and heavy metal residues in Chinese cabbage at a large scale. Therefore, microalgae culture was beneficial to reduce negative impacts of BS irrigation in crop growth and agricultural product safety. This study may provide a theoretical basis for the safe utilization of BS waste in agricultural irrigation.

1. Introduction

The biogas-linked ecological agriculture has experienced a rapid development over the past few decades. An 800 m^3-volume operational biogas system needs to discharge approximately 15 tons of biogas slurry (BS) daily (Chen et al., 2017). The subsequent disposal for considerable quantities of BS is confronted with dire challenges. In many countries and regions, BS as the chief byproduct has been encouraged to apply in irrigating vegetable plots instead of chemical fertilizers due to its low cost-effectiveness and rich nitrogen, phosphorus, potassium, other microelements, and microorganisms (He et al., 2017a; Lu et al., 2012; Zhao et al., 2015). Increasingly, however, researches are suggesting that long-term irrigation of untreated BS in farmlands not only reduces crop yield and quality (Singla et al., 2014; Wu et al., 2013; Yang et al., 2018), but also accelerates the accumulation of heavy metal or antibiotic resistance genes in soil and crop (Bo et al., 2014; Pu et al., 2017) and the migration of excess nutrients into surface water and groundwater via surface runoff or soil percolation (Bian et al., 2015; Lu et al., 2012). Thus, studies must be performed to find some proper techniques for BS preprocessing before its extensive application in agricultural irrigation.

In recent years, some methods for nutrients and heavy metals removal from BS have been reported sporadically (Zhou et al., 2018; Zhu et al., 2016), such an integrated membrane technology with

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https://doi.org/10.1016/j.jenvman.2019.04.058
Received 16 November 2018; Received in revised form 25 February 2019; Accepted 16 April 2019
Available online 30 May 2019

microfiltration (MF), ultra filtration (UF), and reverse osmosis (RO) membranes (Ruan et al., 2015) and biological molecular technology (Hailei et al., 2017; Hidaka et al., 2018). Chemical precipitation was also applied to remove dissolved nutrients by adding a struvite or poly aluminum chloride to wastewater (Abeldenee et al., 2018; Galvagno et al., 2016). Meanwhile, these technologies have brought about some new problems that how to deal with a huge amount of waste membrane materials and precipitated sludge with high moisture content (Wang et al., 2017a). A microalgae-based technology with environmentally friendly and low-cost input begins to be concerned in dealing with BS waste because of its high photosynthetic efficiency, strong environmental adaptability, short growth cycle, high biological yield, and high calorific value (ideal raw materials for biodiesel production) (Jain et al., 2017; Mohan et al., 2015). Abundant easily absorbed nutrients and microelements from BS perfectly satisfy the need of microalgal growth, which simultaneously reduce the risks of water eutrophication and heavy metal accumulation posed by untreated BS irrigation (Suresh et al., 2015; Wang et al., 2017b). This type of cultivation also greatly reduces the productive expenditure of microalgal products (Tan et al., 2016a). The carbo-hydrate production and purifying capacity of microalgae are largely influenced by the amount and ratio of nutrients in BS (Lee et al., 2016; Lu et al., 2015). Lenzi et al. (2013) pointed out that N:P > 30 indicated P limitation, and N:P < 10 suggested N limitation for algal growth and nutrition removal. Therefore, the optimal nutritional status for culturing microalgae in the present BS for the nitrogen, phosphorus and heavy metal removal needs to be investigated. Moreover, microalgal assimilation may synthesize and release auxin, abscisic acid, and cytokinin by directly utilizing organic matters in BS via functional plant hormone metabolic pathways (Lu and Xu, 2015), which may be beneficial to plant growth, can improve soil fertility, and can enhance farm production. Even so, the effects of irrigation with microalgae-treated BS (MBS irrigation) on crop growth, yield, and environment and human health have yet to be rarely studied.

Chinese cabbage (*Brassica campestris* L. *ssp. chinensis* Makino var. *communis* Tsen et Lee), which is abundant in vitamins and minerals and high in dietary fiber, is widely cultivated in southeast Asia and has attracted as a new delicious dish across North America in recent years (Cao et al., 2006). It is worthwhile to investigate the effects of MBS irrigation on the consumption risks of Chinese cabbage. In specific, the objectives of the present work are to: (i) investigate the optimal nutrient strategy startup parameters for microalgal growth in the present BS; (ii) evaluate the abilities of microalgae cultured in different concentrations of BS to remove nitrogen, phosphorus, COD, and heavy metals; and (iii) analyze the effects of MBS irrigation on the agronomic traits, nutritional quality, oxidation resistance, and nitrate and heavy metal residues of Chinese cabbage. The results of this study may provide basic data for the safe application in agricultural irrigation of BS as a byproduct and address the demands in energy and environmental sustainability.

2. Material and methods

2.1. Microalgal growth in diluted biogas slurry

Microalgae *Scenedesmus* sp. (Fig. 1b) was selected for BS purification because of its high pollution tolerance levels, removal efficiencies (REs) of nitrogen and phosphorus, and growth rate (Duangjan et al., 2016; Prandini et al., 2016), which was provided and preserved by the Guangzhou Institute of Energy, Chinese Academy of Sciences. The selection of the tested microalgae was mainly based on the comparison results of biomass production, total lipid content, and nutrient REs of the various microalgae reported by Gim et al. (2014) and Zhang et al. (2015). The BS used for microalgal culture was prepared by the volume ratios of 1:3 (BG-25), 1:1(BG-50), 3:1(BG-75), and 1:0 (BG-100) mixtures of the digested effluent of energy grass and tap water. These diluted BS was adjusted the pH to 7 with a 2.5 M NaOH solution. Several reported diluents may not be suitable for microalgae growth such as deionized water (Ledda et al., 2016) and seawater (Sepúlveda et al., 2015), and low-cost tap water used as diluents has not been reported and is worth studying. Medium for blue green algae (BG11) was served as a control treatment according to the nutritional ingredient provided by (Wang et al., 2017a).

Microalgal culture was carried out in the 150 mL of transparent-glass bubble column reactors at 26 ± 1 °C (Fig. 1a). Roughly 2% CO_2 (by volume) was injected in the culture medium via plastic conduct on top of the reactor (Kumari et al., 2014). The light intensity required for microalgal growth was set as 380 μmol photon·m^{-2}·s^{-1} under a light/dark cycle of 16:8 h for 12 days. Nearly equal microalgal density (0.6×10^7 cell L^{-1}) in logarithmic growth was inoculated to each glass reactor with 120 mL of prepared diluted BS (a total of 10 glass tubes; 5 treatments × 2 tubes). During a 12-day cultivation period, culture solution was obtained every other day to determine cell density with a conventional hemocytometer (Boonstra et al., 1983) and pH change by oxidation–reduction potentiometer. The cell density of the microalgae was calculated by the following formula:

$$\text{Cell density (cells } L^{-1}) = N/5 \times 25 \times 10 \times 10^6 \times 200, \quad (1)$$

where N represents the total number of cells in five squares.

After 8-day cultivation, about 5 mL of microalgal fluid was filtered and retained in a dried hybrid fiber filter membrane and then dried at 80 °C in an oven until constant weight was reached. The microalgal biomass was calculated by the following formula:

$$\text{Dry cell weight } (g{\cdot}L^{-1}) = (m_2\text{-}m_1)\ /0.005, \quad (2)$$

where m_2 and m_1 represent the membrane weight before and after filtration, respectively, g.

2.2. Nitrogen, phosphorus, COD, and heavy metals removal from biogas slurry by microalgae

The culture solution before inoculation (Day 0) and 8 days after inoculation (Day 8) was used for chemical analysis. The total nitrogen (TN), nitrogen from NH_4^+ ($N\text{-}NH_4^+$), total phosphorus (TP), and phosphorus from PO_4^{3-} ($P\text{-}PO_4^{3-}$) contents were measured using a water quality analyzer (DR2700, HACH, USA) (Borba et al., 2014). The chemical oxygen demand (COD) was determined using the potassium permanganate acidic method (Di et al., 2013). Heavy metal contents in BS were digested with HNO_3/H_2O_2 in a microwave digestion system (CEM-MARS5, USA) and analyzed using an inductive coupled plasma emission spectrometer (ICP-MS, PE, USA) (Xu et al., 2017). The Cr, Pb, and Cd were selected based on the five mandatory heavy metal indicators (Cr, Pb, Cd, Hg, and AS) in standards for irrigation water quality (GB5084-2005; China). In the preliminary experiment, Hg and AS contents were determined too low in BS, only average 0.02 and 3.15 μg L^{-1}, respectively. The pH change in BS was determined. The removal efficiencies (REs) of nutrients and heavy metals were calculated by the following formula:

$$\text{REs } (\%) = M_{before}\text{-}N_{after} \ / \ M_{before} \times 100, \quad (3)$$

where M_{before} and M_{after} represent the M content in the culture solution before inoculation and the N content in the culture solution after 8-day cultivation, respectively, mg L^{-1}/μg L^{-1}.

2.3. Agronomic traits, nutritional quality, and heavy metal contents of Chinese cabbage

A total of 1 kg soil was placed in a PVC pot with three holes in the bottom. The seeds were soaked in 0.2% hydrogen peroxide solution for 5 min and then washed with tap water before they were finally sown into surface soil (sown on 25 December 2016). At 9 days after germination, untreated BS (BG-25, BG-50, BG-75, and BG-100), BS treated by

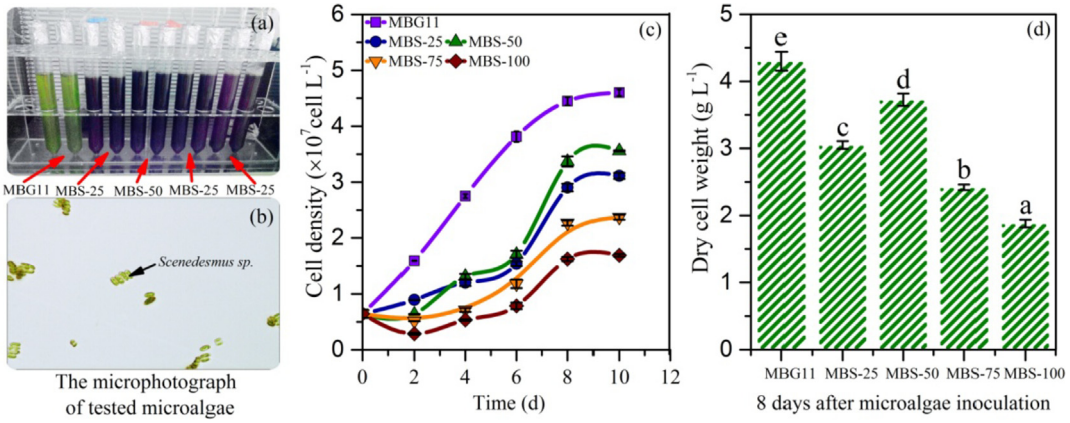

Fig. 1. Microphotograph (b), growth curve (c) and an eight-day biomass (d) of microalgae cultivated in BG-11 medium and diluted biogas slurry with tap water (biogas slurry/tap water = 1/3 (MBS-25), 1/1 (MBS-50), 3/1 (MBS-75), and 1/0 (MBS-100) in volume). Different lowercase letters on bar diagram denote significant differences at $p < 0.05$ among treatments. Values are means (± SD; n = 4).

microalgae for 8 days (MBG-25, MBG-50, MBG-75, and MBG-100), and tap water were used to irrigate these potted plants (a total of 36 PVC pots; 9 treatments × 4 repetitions) at an intensity of about 50 mL pot^{-1} every 5 days. Namely, 200 mL of solution was required for each treatment during a single irrigation process. To avoid the change in irrigation solution composition caused by long time placement, irrigation solution was freshly prepared before each use in same reactors under the same culture condition. Chinese cabbage was harvested days after 35 days based on optimum planting cycle. The harvested fresh plant samples were used for agronomic traits analysis. The relative growth rates (RGR) and Net assimilation rate (NAR) were calculated by the following formulas:

$$RGR\ (g\ g^{-1}\ d^{-1}) = (\ln Q_1\text{-}\ln Q_2)\ /T_1\text{-}T_2, \quad (3)$$

$$NAR\ (mg\ m^{-2}\ d^{-1}) = (\ln S_2\text{-}\ln S_2)/\ (S_2\text{-}S_1) \times (Q_2\text{-}Q_1)/\ (T_1\text{-}T_2), \quad (4)$$

Where Q_1 and Q_2 were the dry weight at time T_1 and T_2, respectively, g; S_1 and S_2 were the dry weight at time T_1 and T_2, cm^2.

Some plant samples used for food nutrition analysis by determining the TN, TP, nitrate (NO_3^-), soluble sugar, crude fiber, and vitamin C contents in edible parts (Fabre et al., 1999; Nozawa et al., 2006; Yuan et al., 2014). The heavy metal content in edible parts were determined using graphite furnace atomic absorption spectrometry (GFAAS, Shimadzu AA-7000, Japan) after digesting with 8 mL of concentrated HNO_3 at 120°C–180 °C in a microwave digestion system (CEM MARs5, USA) (Xu et al., 2018a).

2.4. Antioxidant enzyme and nitrate reductase (NR) activities and malondialdehyde (MDA) content of Chinese cabbage

About 0.3 g of fresh edible parts was placed in a pre-cooled mortar, ground to homogenate with 2 mL of phosphate buffer solution (PBS; 50 mmol/L; pH 7.8), transferred to centrifuge tubes, and then centrifuged at 12,000 × g for 20 min at 4 °C (Liu and Lin, 2014). The supernatant obtained (enzyme extracting solution) was used to determine the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Some fresh edible parts were used to determine nitrate reductase (NR) activity through sulfanilamide method (Chen et al., 2004). The MDA content in edible parts was determined using the trichloroacetic acid–thiobarbital method. Antioxidant enzymatic activity was calculated by the following formula (Kang and Saltveit, 2010):

$$A\ activity\ (U\ min^{-1}{\cdot}g^{-1}) = (OD_{ck}\text{-}OD_{sample}) \times V_{total}\ /\ (OD_{ck} \times N \times W \times V_{sample} \times T), \quad (5)$$

where A is SOD, POD, CAT, or NR; OD_{ck} and OD_{sample} are the absorbance values of buffer solution and sample, respectively; V_{total} is the total volume of the enzyme extracting solution, mL; N is 0.5 in the calculation of SOD activity and 0.01 in the calculation of POD and CAT activities; W is the weight of the used plant sample, g; V_{sample} is the tested volume of the enzyme extracting solution, mL; and T is the color reaction time, min.

2.5. Rhizospheric soil pH after Chinese cabbage harvesting

The rhizospheric soils were separated through shaking the root method. The collected soil samples were used for chemical analysis to investigate the effects of MBS irrigation on the rhizospheric soil pH. The pH was measured in a 1:5 mixture of water and dry soil using a pH meter (Xu et al., 2017).

2.6. Data analysis

Experimental data were recorded in Microsoft Excel 2010 and analyzed with SPSS19.0 (Chicago, IL, USA). Differences among treatments and cultivars were analyzed using one-way ANOVA (independent sample t-test) and two-way ANOVA. All graphs were charted using Origin 9.2 (MA, USA).

3. Results and discussion

3.1. Optimal nutrition strategy for microalgae growth and pH change in biogas slurry over culture time

The growth curve and an eight-day biomass of microalgae cultivated in BG-11 medium and diluted BS with tap water are presented in Fig. 1c and d. The cell density increased slowly in the first 2 days of microalgal culture in BS medium, and then the microalgal growth rate significantly increased until 8th day; finally, their growth rate successively entered the stagnate and decline phases after 8th day (Fig. 1c). Therefore, microalgae at the 8th day of the culture had high reproductive rate and biomass accumulation, which was in a transition period from logarithmic phase to stationary phase. Microalgae from this time period exhibited thriving anabolism and high pollutant removal efficiency (Tan et al., 2016a). In all BS mediums, the microalgae cultivated in BS-50 medium (MBS-50) had the highest cell density (3.37×10^7 cells L^{-1}) and dry biomass (3.73 g L^{-1}) after 8 days of continuous culture (Fig.1d; $p < 0.05$). Apart from BG-11 medium (Fig. 1c), BS-50 medium (TN: 101 mg L^{-1}; $N\text{-}NH_4^+$: 81.0 mg L^{-1}; TP: 77.8 mg L^{-1}; $P\text{-}PO_4^{3-}$: 61.5 mg L^{-1}) was more appropriate for the cell proliferation and biomass accumulation of microalgae than the three other BS mediums (BS-25, BS-75, and BS-100). Uggetti et al. (2014) found that *Scenedesmus* sp. had good growth rate under 50 mg L^{-1} N-

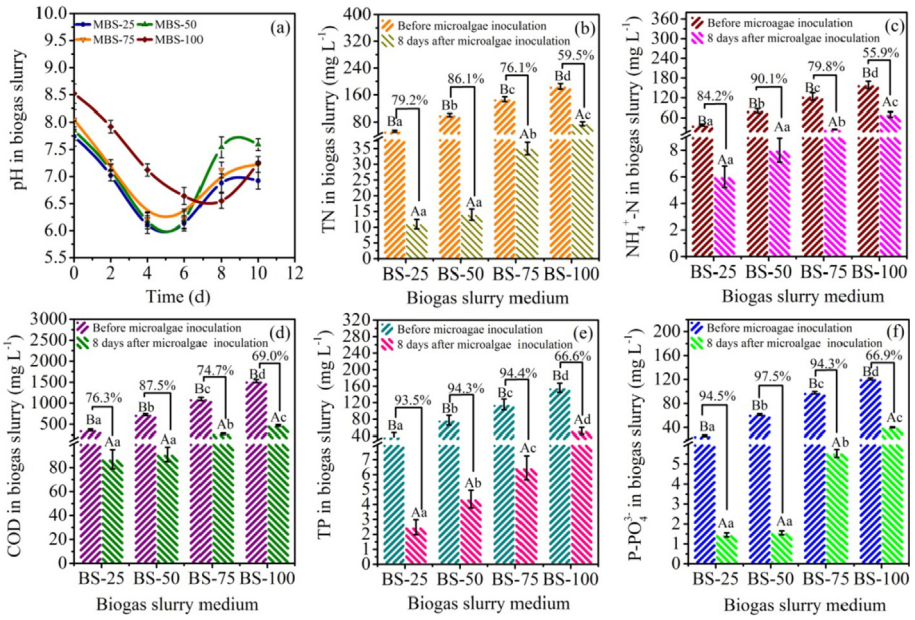

Fig. 2. pH change (a) over time in biogas slurry and TN (b), NH_4^+-N (c), COD (d), TP (e), and P-PO_4^{3-} (f) contents (after 8 days) of microalgae cultivated in diluted biogas slurry with tap water (BS-25, BS-50, BS-75, and BS-100). Percentage values on the broken line denote removal efficiency. Different capital letters on bar diagram denote significant differences at $p < 0.05$ between "before inoculation" and "8 days after microalgae inoculation" in the same culture medium, and different lowercase letters denote significant differences at $p < 0.05$ among culture mediums under the same inoculation treatment. The same below. TN: total nitrogen, TP: total phosphorus, NH_4^+-N: nitrogen from NH_4^+, P-PO_4^{3-}: phosphorus from PO_4^{3-}, COD: chemical oxygen demand. Values are means (± SD; n = 4).

NH_4^+ condition. Park et al. (2010). found the optimal N-NH_4^+ concentration (100 mg L^{-1}) in favor of green alga *Scenedesmus accuminatus* growth. A favorable nutrient level (N-NH_4^+: 76.2 mg L^{-1}; P-PO_4^{3-}: 9.7 mg L^{-1}) for *Scenedesmus dimorphus* growth was also observed by González et al. (2018). Therefore, the optimal nutrient strategy startup parameters for microalgal cultivation varied with nutrient composition in the medium and microalgal species. Before scale-up cultivation and water purification application, the optimization of culture conditions should be investigated for specific microalgae and medium composition. In the present study, *Scenedesmus* sp. exhibited good environment adaptability and maintained strong reproduction ability, and a relatively optimal nutrition strategy (BS-50) for microalgal growth was found. The microalgae with optimal growth condition might possess a strong BS purification ability (Zhao et al., 2015).

The pH change over time in the BS mediums is depicted in Fig. 2a. As the culture time progressed, the pH values in the MBS-mediums initially decreased, increased, and then stabilized at the 8th and 10th days (Fig. 2a). The initial pH drop was mainly due to NH_4^+ deprotonation on the plasmalemma induced by the preferential utilization of ammonia nitrogen (N-NH_4^+) in BS by microalgae (Elreedy et al., 2017; Tan et al., 2016b). This phenomenon may also be explained by the theory of ionic balance in which the H^+ ions released by proton pump may maintain the electric neutrality of cells due to excess NH_4^+ absorption (Curtin and Wen, 2004; Xu et al., 2017). In the present study, the pH values had been reduced to a minimum (mean pH 6.17) in the MBS-25, MBS-50, and MBS-75 mediums 4–5 days after culture but arrived the minimum (mean pH 6.55) in the MBS-100 medium until the 8th day (Fig. 2a). This result suggested that the microalgae grown in the diluted BS–mediums basically removed the vast majority of N-NH_4^+ from BS faster than those grown in the undiluted BS-medium. With the depletion of N-NH_4^+, microalgae could assimilate free carbon dioxide converted by carbonic anhydrase or soluble carbonate, gradually increasing the medium pH by pushing the reaction $HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$ (Aboushanab et al., 2013; Cai et al., 2013). Furthermore, the continuous degradation of organic acids caused by abundant microorganisms in BS may further increase the medium pH. However, the organic acids in BS of this experiment were not determined and need to be investigated in future studies. In the present study, the pH value in MBS-50 medium was the fastest to increase (mean pH 7.54) at the 8th day (Fig. 2a). Thus, the microalgae grown in BS-50 medium (MBS-50) had high REs for ammonia nitrogen and inorganic carbon in BS. In addition, the microalgae grew the fastest when the pH in BS was about 7.5 (Fig. 2a), indicating that pH change was closely associated with the growth and metabolism of *Scenedesmus* sp. The best pH condition for culturing microalgae agreed with the results (pH 7.0–9.0) reported by Zhang et al. (2014).

3.2. Removal efficiencies of nutrients and heavy metals from biogas slurry by microalgae

The REs of TN, N-NH_4^+, COD, TP, P-PO_4^{3-}, and heavy metals in BS at the 8th day of the culture are depicted in Figs. 2 and 3. Microalgae in this period had strong assimilation and the largest number of alive cells, and over time, growth ratio gradually stagnate and decline. Assimilated

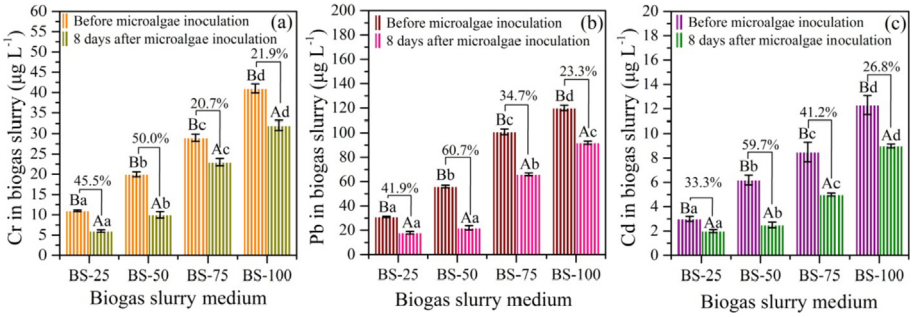

Fig. 3. Cr (a), Pb (b), and Cd (c) contents (after eight days) of microalgae cultivated in diluted biogas slurry with tap water (BS-25, BS-50, BS-75, and BS-100). Values are means (± SD; n = 4).

pollutants may be released back into the BS due to the disintegration of dead microalgae cells. Therefore, 8 days of continuous culture was most consistent with the purpose of the study. In the present study, the microalgae removed TN and N-NH_4^+ from diluted BS with tap water, with average REs of 80.5% and 84.7%, respectively (Fig. 2b and c). The microalgae grown in BS-50 medium (MBS-50) shown the highest REs of TN and N-NH_4^+ (up to 86.1% and 90.1%, respectively) (Fig. 2b and c; $p < 0.05$). Nitrogen in BS were removed by synthesizing into amino acids, protein, enzyme, chlorophyll, and genetic materials through autotrophic action in microalgal cells (He et al., 2017b). The N-NH_4^+ could be directly absorbed, stored in cytosol, and then assimilated using the glutamine synthetase by NH_4^+ + ATP + glutamic acid→L-glutamine + ADP + PO_4^{3-} (Luze et al., 2008; Yang et al., 2013). Without the oxidoreduction participation, the assimilation process has a small demand for energy (Wang et al., 2009), which partly explained why the microalgae utilized ammonium nitrogen (N-NH_4^+) preferentially. Moreover, a significant positive correlation between culture medium pH and nitrogen RE was observed in the present study ($p < 0.05$), This may because the high pH could change the balance between ammonium and ammonia, thereby accelerating the gas stripping (NH_3) of ammonia nitrogen (Tan et al., 2016a; Wang et al., 2017b).

As shown in Fig. 2d, COD RE was the highest (87.5%) in MBS-50 medium but the lowest (36.2%) in MBS-100 medium ($p < 0.05$), indicating that high concentrations of BS largely inhibited microalgal growth. The main mechanisms for COD removal include COD decomposition and assimilation in microalgal metabolism (González et al., 2018), and intake air during microalgal culture improves directly the activity of aerobes and facultative aerobes, enhances organic matter degradation, and decreases COD content in BS. In the present study, *Scenedesmus* sp. presented strong removal abilities for TP and P-PO_4^{3-} in diluted BS, with an average RE of over 94.0% (Fig. 2e and f), which were significantly higher than the results of similar studies (Cheng et al., 2015; Han et al., 2014). The P-PO_4^{3-} could be directly absorbed in microalgal cells and then converted into ATP, phospholipid, and nucleic acid through the level phosphorylation, oxidative phosphorylation, and photophosphorylation pathways (Ekholm and Krogerus, 1998). Moreover, a significant positive correlation was observed in our results between culture medium pH and phosphorus RE ($p < 0.05$). The high pH in BS could facilitate the formation of insoluble metal phosphate (Xu et al., 2014), and the RE of phosphate by precipitation is highly satisfactory. This may explains why the microalgae grown in BS-50 medium had a stronger ability to remove phosphate.

In the present study, microalgae exhibited different removal abilities for Cr, Pb, and Cd in BS, which was consistent with the results reported by Suresh et al. (2015). On average, the microalgae grown in BS-75 and BS-100 mediums removed only 21.3% of Cr, 29.0% of Pb, and 34.0% of Cd (Fig. 3). Compared with Cr RE, the Pb and Cd REs in BS-50 medium were relatively higher (up to 60.7% and 59.7%, respectively) (Fig. 3; $p < 0.05$). This result implied that there was an order of selective biosorption for various metal ions in microalgae. Carboxyl (−COOH), hydroxyl (−OH), and amidogen ($-NH_2$) in microalgal cell walls and extracellular metabolites (polysaccharide and mucus) could chemically adsorb large amounts of metal ions through ion exchange, coordination, complexation, chelation, micro-precipitation (Andrade et al., 2010; Gélabert et al., 2006; Monteiro et al., 2012), thus decreasing heavy metals in BS. Therefore, the capacities of functional groups to adsorb metal ions may greatly decide the RE of heavy metals by microalgae (Chojnacka et al., 2005). FT-IR studies of the present experiment were not been tried and had been included in the next work. Apart from metal-binding capacities of microalgae, high medium pH may also facilitate the formation of insoluble heavy metal hydroxides and thus achieve heavy metal removal from BS. In conclusion, the purification efficiency of BS mainly depended on microalgal cell density involved in nutrients assimilation and metal ion biosorption, and the volume ratio of 1:1 (BG-50) mixtures of the digested effluent and tap water was suitable for *Scenedesmus* sp. to remove pollutants.

3.3. Growth, nutritional quality, and oxidation resistance of Chinese cabbage under microalgae-treated biogas slurry irrigation

Some studies expounded that irrigation with suitable concentrations of BS could effectively increase crop yield and quality (Lu et al., 2012; Wu et al., 2013). Before microalgal inoculation, COD contents were 367, 729, 1100, and 1526 mg L^{-1} in BS-25, BS-50, BS-75, and BS-100 mediums, respectively, which considerably exceed the maximum permissible level (100 mg L^{-1}) of the standards for irrigation water quality of China (GB5084-2005, China) (Fig. 2d). The Cd content in BS-100 medium also reached 1.23-fold of national standard (0.01 mg L^{-1}) (Fig. 3c). Therefore, directly using such BS for crop irrigation may lead to crop failure and even health risk posed by heavy metals accumulation in agricultural products (Bian et al., 2015; Pu et al., 2017). In the present study, microalgal culture distinctly improved the water quality of BS for agricultural irrigation, especially for Cd removal (Fig. 3c). Only the COD contents in MBS-75 (278 mg L^{-1}) and MBS-100 (473 mg L^{-1}) still exceeded the irrigation water threshold (Fig. 2d).

The biomass, root and shoot lengths, leaf number, max leaf width and length, leaf area, relative growth rate (RGR), and net assimilation rate (NAR) of Chinese cabbage increased slightly under tap water and BS-25 irrigation and then decreased significantly under BS-50, BS-75 and BS-100 irrigation (Table 1; $p < 0.05$). The TN, TP, soluble sugar, and vitamin C contents in edible parts presented the similar trend of first increasing and then decreasing, but crude fiber contents shown a noticeable increase along with the BS concentration increased (Table 2; $p < 0.05$). These results may largely be due to the facts that BS had liquidity, high pH, high nutrient content, and potentially high metal content (Chen et al., 2017). Direct application of BS may reduce the soil water potential in the rhizosphere resulting in extravasation of the cell sap, inhibit N and P uptake by roots, facilitate metal accumulation, and eventually obstruct carbohydrate synthesis and metabolism (Lampurlanés et al., 2016; Ma et al., 2017). Interestingly, MBS irrigation significantly improved yield, RGR, NAR, edible taste, and nutritional quality in contrast with BS irrigation (Tables 1and 2; $p < 0.05$). During microalgae culture, lots of organics in BS were mineralized into dissolved inorganic micromolecules that were directly uptake by plants (González et al., 2018), and microalgae metabolism also may release multiple auximones facilitating crop growth such as indoleacetic acid and cytokinin (Lu and Xu, 2015). The BS concentration loss caused by microalgae culture directly avoided the negative effects of over-concentration on plant growth and nutrient uptake. Overall, these reasons basically explained why MBS irrigation may improve biomass accumulation and nutritional quality in Chinese cabbage.

The excess reactive oxygen species (ROS) accumulation may cause plasmalemma oxidative damage, disrupt intracellular hormonal balance, result in potassium loss, and even cause programmed cell death (Bethke and Jones, 2010; Demidchik, 2015). The antioxidant enzymes (SOD, POD, and CAT) may work synergistically to scavenge the environmental stress generating ROS in plant cells (Srivastava et al., 2017). The SOD, POD, and CAT activities enhanced under tap water, BS-25, and BS-50 irrigation and then markedly weakened under BS-75 and BS-100 irrigation (Table 3; $p < 0.05$). By contrast, these antioxidant enzymes exhibited significantly stronger activities under MBS irrigation (Table 3; $p < 0.05$). The stronger SOD activity broken more superoxide anion (O^{2-}) down into hydrogen peroxide (H_2O_2), and then stronger POD and CAT activities further removed the generated H_2O_2 in order to alleviate membrane damage (Kim et al., 2018). The result of MDA content further confirmed that MBS irrigation definitely enhanced the oxidation resistance in Chinese cabbage (Table 3; $p < 0.05$). Therefore, high concentrations of BS without any pretreatment were unsuitable for agricultural irrigation because of the otherwise damaged rather than improved oxidation resistance of the plants, and MBS as agricultural irrigation water was a good choice.

Table 1
Agronomic traits of Chinese cabbage under irrigation with microalgae-treated biogas slurry.

Treatments	Relative growth rate	Net assimilation rate	Biomass allocation		Plant height		Photosynthetic organ character			
	RGA (mg g^{-1} d^{-1}; DW)	NAR (mg cm^{-2} d^{-1}; DW)	Root biomass (g $plant^{-1}$; FW)	Shoot biomass (g $plant^{-1}$; FW)	Root length (cm)	Shoot length (cm)	Leaf number (each plant)	Maximum leaf width (cm)	Maximum leaf length (cm)	Leaf area (cm^2)
Before microalgae inoculation										
Tap water	43.20 ± 2.70c	0.09 ± 0.00c	0.04 ± 0.00c	1.19 ± 0.04c	4.56 ± 0.10c	6.19 ± 0.32c	8 ± 1a	2.90 ± 0.17c	4.08 ± 0.16c	9.86 ± 0.21d
BS-25	53.56 ± 4.52Ad	0.11 ± 0.01Ad	0.05 ± 0.00Bd	1.56 ± 0.09Be	5.75 ± 0.10Be	6.73 ± 0.20Bd	11 ± 1Ab	3.42 ± 0.14Bd	4.62 ± 0.23Ad	13.17 ± 0.86Ae
BS-50	47.07 ± 2.84Acd	0.10 ± 0.01Ad	0.04 ± 0.00Ac	1.32 ± 0.06Ad	4.94 ± 0.11Ad	6.18 ± 0.25Ac	9 ± 1Ba	2.72 ± 0.12Ac	3.86 ± 0.10Ac	8.75 ± 0.24Ac
BS-75	26.66 ± 1.71Ab	0.06 ± 0.00Ab	0.03 ± 0.00Ab	0.77 ± 0.05Ab	4.25 ± 0.13Ab	4.93 ± 0.08Ab	8 ± 1Aa	2.26 ± 0.10Ab	3.42 ± 0.11Ab	6.44 ± 0.19Ab
BS-100	12.25 ± 1.02Aa	0.03 ± 0.00Aa	0.02 ± 0.00Aa	0.53 ± 0.03Aa	3.55 ± 0.15Aa	3.95 ± 0.17Aa	7 ± 1Aa	1.88 ± 0.19Aa	2.87 ± 0.15Aa	4.50 ± 0.15Aa
8 days after microalgae inoculation										
MBS-25	54.03 ± 0.00Aa	0.11 ± 0.00Aa	0.05 ± 0.00Aa	1.58 ± 0.08Aa	5.60 ± 0.10Aa	6.43 ± 0.15Aa	10 ± 1Aa	3.56 ± 0.08Aa	4.54 ± 0.18Aa	13.47 ± 0.87Aa
MBS-50	56.10 ± 2.17 Bab	0.10 ± 0.01Aa	0.06 ± 0.00Aa	1.66 ± 0.04Ba	6.16 ± 0.20Ba	7.64 ± 0.16Ba	10 ± 1Aa	4.23 ± 0.13Ba	5.12 ± 0.18Ba	18.05 ± 0.99Bb
MBS-75	58.06 ± 3.90 Bab	0.12 ± 0.00Bb	0.07 ± 0.00Bb	1.74 ± 0.03Bb	6.96 ± 0.21Bb	8.23 ± 0.21Bb	13 ± 1Bb	4.75 ± 0.06Bb	5.62 ± 0.10Bb	22.25 ± 1.04Bc
MBS-100	61.32 ± 5.62Bb	0.12 ± 0.00Bb	0.08 ± 0.00Bc	1.89 ± 0.07Bc	7.58 ± 0.18Bc	8.79 ± 0.17Bc	15 ± 1Bc	5.12 ± 0.07Bc	6.55 ± 0.08Bc	27.95 ± 1.12Bd

Different capital letters denote significant differences at $p < 0.05$ between "Before microalgae inoculation" and "8 days after microalgae inoculation" in the same culture medium, and different lowercase letters denote significant differences at $p < 0.05$ among culture mediums under the same inoculation treatment. The same below. Values are means (± SD; n = 10).

3.4. Nitrate and heavy metal residues of Chinese cabbage under microalgae-treated biogas slurry irrigation

Nitrate (NO_3^-) residue in crops was a key indicator influencing nutritional quality (Yao et al., 2018). Under BS-75 and BS-100 irrigation, the nitrate contents (3.55 and 4.88 g kg^{-1}, respectively) in edible parts exceeded the danger threshold (3.00 g kg^{-1} for leaf vegetable) of safety qualification for agricultural products (GB18406-2001, China) (Table 4; $p < 0.05$). Once such vegetables were consumed, nitrate ingested was easily reduced to toxic nitrites in the human digestive tract, resulting in hemoglobin hypoxemia (Chen et al., 2017; Hall, 2018). Therefore, reducing nitrate residue was a top priority for nutrition quality improvement. The nitrate reductase (NR) was a rate-limiting enzyme involved in nitrate reduction in the cytoplasm (Rosales et al., 2011). Our present study showed that BS-75 and BS-100 irrigation significantly decreased NR activity in edible parts (Table 3; $p < 0.05$), and a significantly negative correlation was found between nitrate content and NR activity ($p < 0.05$). In contrast, the higher NR activity in edible parts under MBS irrigation may accelerate nitrate transformation, resulting in nitrate content considerably below what was normally considered dangerous (GB18406-2001, China) (Table 4; $p < 0.05$). NR activity in plants was closely associated with photosynthetic intensity, illumination intensity, and nitrate concentration (Hippler et al., 2018). Many studies also reported that increasing the ratio of $N\text{-}NH_4^+$ and TN was beneficial to reduce nitrate residue in crops, but too high concentrations of $N\text{-}NH_4^+$ was toxic to plant roots (Chen et al., 2017; Kronzucker, 2010). In our present study, a continuous decline in nitrate content under the irrigation with from MBS-25 to MBS-100 may partly due to the increased ratios of $N\text{-}NH_4^+$ and TN from 0.54 to 0.93. The effective measures to improve NR activity and reduce nitrate residue need to be studied further.

Heavy metal residue in crops was a serious concern because of the high health risk associated with food chain transfer (Xu et al., 2018b). Before microalgal inoculation, the Cr and Pb contents in BS-25, BS-50, BS-75, and BS-100 mediums accorded with the irrigation water quality requirement (Fig. 3a and b), and only the Cd contents (12.3 μg L^{-1}) in BS-100 medium exceeded the maximum permissible level (10 μg L^{-1}) (GB5084-2005; China) (Fig. 3c). Namely, BS-100 irrigation may lead to overmuch Cd uptake by crops, thereby triggering serious health risks. After microalgae culture, the Cr, Pb and Cd contents in MBS-25, MBS-50, MBS-75, and MBS-100 mediums were well below their respective maximum allowable concentrations. Thus, except for the tap water dilution effect, microalgal culture greatly reduced the safety risk of heavy metal residues in crops (Kumar et al., 2015). A continuous significant increase in the Cr, Pb and Cd contents of edible parts was observed under BS-25, BS-50, BS-75, and BS-100 irrigation (Table 4; $p < 0.05$). Under MBS irrigation, the Cr, Pb and Cd contents of Chinese cabbage initially decreased from MBS-25 to MBS-50 and then increased from MBS-50 to MBS-100, and significantly lower than those under BS irrigation (Table 4; $p < 0.05$). The Cr, Pb, and Cd contents of all tested Chinese cabbage did not exceed the national standards for food safety (GB2762-2017; China). No significant correlation was found between heavy metal contents in edible parts and rhizosphere soil pH (Table 4; $p > 0.05$). Rhizospheric soil pH was an important factor controlling the heavy metals uptake via desorption of metals from the solid phase by competition with H^+ (Xu et al., 2017). However, the consumption risk of agricultural products may not only depend on the rhizospheric soil pH but also on heavy metal contents in BS, irrigation methods, and crop varieties (Bo et al., 2014; Wentzel et al., 2015). Overall, MBS irrigation may considerably reduce nitrate and heavy metal residue in Chinese cabbage. These results may provide a new idea and theoretical basis for the safe utilization of BS resources in agricultural irrigation.

4. Conclusions

The disposal of BS had become an environmental issue due to thin

Table 2
TN, TP, soluble sugar, vitamin C, and crude fiber contents in edible parts of Chinese cabbage under irrigation with microalgae-treated biogas slurry.

Treatments	TN (g kg^{-1}; FW)	TP (g kg^{-1}; FW)	Soluble sugar (g kg^{-1}; FW)	Vitamin C (g kg^{-1}; FW)	Crude fiber (g kg^{-1}; FW)
Before microalgae inoculation					
Tap water	3.97 ± 0.01d	0.42 ± 0.01c	21.12 ± 0.02c	0.10 ± 0.00b	45.15 ± 0.02c
BS-25	4.97 ± 0.01Be	0.57 ± 0.01Bd	26.35 ± 0.01Ae	0.16 ± 0.01Ad	53.19 ± 0.02Ba
BS-50	3.42 ± 0.02Ac	0.43 ± 0.01Ac	22.13 ± 0.01Ad	0.12 ± 0.01Ac	60.13 ± 0.02Bb
BS-75	2.69 ± 0.03Ab	0.34 ± 0.01Ab	18.36 ± 0.02Ab	0.10 ± 0.00Ab	78.56 ± 0.02Bd
BS-100	2.20 ± 0.04Aa	0.20 ± 0.01Aa	12.79 ± 0.02Aa	0.08 ± 0.01Aa	85.87 ± 0.03Be
8 days after microalgae inoculation					
MBS-25	3.99 ± 0.03Aa	0.43 ± 0.00Aa	28.89 ± 0.02Ba	0.15 ± 0.01Ba	55.75 ± 0.02Aa
MBS-50	4.02 ± 0.01Bb	0.45 ± 0.00Bb	35.15 ± 0.02Bb	0.18 ± 0.01Bb	43.13 ± 0.03Ab
MBS-75	5.83 ± 0.07Bc	0.69 ± 0.01Bc	38.12 ± 0.02Bc	0.21 ± 0.01Bc	40.12 ± 0.03Ac
MBS-100	6.31 ± 0.09Bd	0.74 ± 0.01Bd	42.13 ± 0.03Bd	0.24 ± 0.01Bd	31.13 ± 0.03Ad

Values are means (± SD; n = 4).

Table 3
SOD, POD, CAT, and NR activity and MDA content in edible parts of Chinese cabbage under irrigation with microalgae-treated biogas slurry.

Treatments	SOD (U $min^{-1}{\cdot}g^{-1}$; FW)	POD (U $min^{-1}{\cdot}g^{-1}$; FW)	CAT (U $min^{-1}{\cdot}g^{-1}$; FW)	MDA (g kg^{-1}; FW)	NR (μg $h^{-1}{\cdot}g^{-1}$; FW)
Before microalgae inoculation					
Tap water	148 ± 1.12b	35.12 ± 0.88b	48.12 ± 1.56b	0.35 ± 0.00c	22.12 ± 1.04c
BS-25	165 ± 2.55Ad	47.51 ± 0.98Ad	59.10 ± 1.38Ad	0.32 ± 0.01Bb	29.13 ± 1.01Ad
BS-50	187 ± 2.87Ae	56.13 ± 1.08Ae	68.31 ± 1.69Ae	0.27 ± 0.01Ba	28.21 ± 1.07Ad
BS-75	154 ± 2.48Ac	41.40 ± 1.48Ac	51.10 ± 1.55Ac	0.41 ± 0.02Bd	21.45 ± 1.18Ab
BS-100	118 ± 1.69Aa	30.50 ± 0.98Aa	42.05 ± 1.78Aa	0.49 ± 0.01Be	15.17 ± 1.05Aa
8 days after microalgae inoculation					
MBS-25	179 ± 3.18Bb	55.50 ± 1.28Bb	67.04 ± 1.12Bb	0.28 ± 0.01Ab	35.15 ± 1.42Bb
MBS-50	198 ± 2.16Bc	68.50 ± 1.34Bc	78.05 ± 1.16Bc	0.26 ± 0.02Ab	39.18 ± 1.17Bc
MBS-75	227 ± 3.07Bd	77.21 ± 1.49Bd	82.12 ± 1.18Bd	0.21 ± 0.01Aa	42.23 ± 1.14Bd
MBS-100	263 ± 3.15Ba	85.20 ± 1.08Ba	89.12 ± 1.12Ba	0.19 ± 0.02Ab	51.35 ± 1.08Ba

SOD: superoxide dismutase, POD: peroxidase, CAT: catalases, NR: nitrate reductase, MDA: malondialdehyde. Values are means (± SD; n = 4).

Table 4
Nitrate, Cr, Pb, and Cd contents in edible parts of Chinese cabbage and rhizospheric soil pH under irrigation with microalgae-treated biogas slurry.

Treatments	Nitrate (g kg-1; FW)	Cr (μg kg^{-1};FW)	Pb (μg kg^{-1};FW)	Cd (μg kg^{-1};FW)	Rhizospheric soil pH
Before microalgae inoculation					
Tap water	0.82 ± 0.01b	6.45 ± 0.05a	5.56 ± 0.04a	0.15 ± 0.02a	6.81 ± 0.02a
BS-25	0.72 ± 0.01Ba	10.43 ± 0.04Bb	8.35 ± 0.05Bb	0.21 ± 0.02Bb	7.62 ± 0.01Bb
BS-50	2.11 ± 0.01Bc	12.61 ± 0.03Bc	8.90 ± 0.01Bc	0.23 ± 0.00Bb	7.86 ± 0.02Bd
BS-75	3.55 ± 0.01Bd	14.57 ± 0.05Bd	9.49 ± 0.03Bd	0.26 ± 0.01Bc	7.81 ± 0.03Bc
BS-100	4.88 ± 0.01Be	15.78 ± 0.04Ae	9.92 ± 0.04Ae	0.29 ± 0.01Ad	8.12 ± 0.02Be
8 days after microalgae inoculation					
MBS-25	0.69 ± 0.01Aa	8.48 ± 0.01Aa	6.86 ± 0.01Aa	0.18 ± 0.01Aa	7.11 ± 0.01Ac
MBS-50	0.67 ± 0.01Aa	8.05 ± 0.05Ab	5.67 ± 0.03Ab	0.16 ± 0.00Aa	7.30 ± 0.03Ad
MBS-75	0.39 ± 0.00Ac	9.12 ± 0.02Ac	7.39 ± 0.04Ac	0.19 ± 0.01Ab	7.08 ± 0.05Ab
MBS-100	0.15 ± 0.01Ad	11.23 ± 0.06Bd	8.26 ± 0.05Bd	0.22 ± 0.01Bc	6.31 ± 0.14Aa

Values are means (± SD; n = 4).

fluidity, high pH, high nutrient contents, and potentially high metal content. The crop failure, soil and water eutrophication, and excessive nitrate and heavy metal accumulation caused by BS irrigation led to a rethinking of the safe utilization of BS resources in agricultural irrigation. Our results indicated that *Scenedesmus* sp. culture in the volume ratio of 1:1 (BG-50) mixtures of the BS and tap water can not only obtain the most lipid-rich microalgal cells for biodiesel production, but also efficiently remove nutrients and heavy metals from BS. Furthermore, MBS irrigation significantly improved crop yield, nutritional quality, and oxidation resistance and decreased the consumption risks of nitrate and heavy metal accumulation in edible parts. This study set up an inexpensive and efficient whole-process method from pollutant removal to agricultural irrigation for BS resource utilization. In particular, the relatively integrated effects of BS irrigation before and after microalgae culture on crop yield, growth, nutrient use efficiency agricultural product quality, and safety, as well as the potential consumption risk had been contrasted and clarified.

Acknowledgments

We are grateful to the Sub-project of National Key R&D Plan Project (No. 2017YFD0801305) and National Natural Science Foundation of China (Grant 41701349) for their financial support. We are profoundly grateful to Dr. P. Yang, whose expert advice has guided us through every step of writing this work. Finally, we thank numerous individuals who participated in this study.

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