Environmental Science
Processes & Impacts

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Processes Impacts*, 2014, **16**, 2018

Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae

Guang-Jie Zhou, Guang-Guo Ying,* Shan Liu, Li-Jun Zhou, Zhi-Feng Chen and Fu-Qiang Peng

Received 13th February 2014
Accepted 22nd May 2014

DOI: 10.1039/c4em00094c

rsc.li/process-impacts

Batch experiments were carried out for 7 days to investigate the simultaneous removal of various organic and inorganic contaminants including total nitrogen (TN), total phosphorus (TP), metals, pharmaceuticals and personal care products (PPCPs), endocrine disrupting chemicals (EDCs), and estrogenic activity in wastewater by four freshwater green microalgae species, *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella pyrenoidosa* and *Chlorella vulgaris*. After treatment for 7 days, 76.7–92.3% of TN, and 67.5–82.2% of TP were removed by these four algae species. The removal of metals from wastewater by the four algae species varied among the metal species. These four algae species could remove most of the metals efficiently (>40% removal), but showed low efficiencies in removing Pb, Ni and Co. The four algae species were also found to be efficient in removing most of the selected organic compounds with >50% removal, and the estrogenic activity with removal efficiencies ranging from 46.2 to 81.1% from the wastewater. Therefore, algae could be harnessed to simultaneously remove various contaminants in wastewater.

Environmental impact

Domestic wastewater contains various inorganic and organic contaminants, which could pose risks to the environment and public health. To avoid these adverse effects, wastewater must be treated prior to its final discharge into the receiving environment. Unfortunately, incomplete removals have often been reported for these contaminants in wastewater by conventional wastewater treatment technologies. The use of microalgae in the treatment of wastewater has gained great interest over the past few years. However, previous studies on wastewater treatment by algae have been limited and most of them are focused on the removal of inorganic or organic pollutants in artificial wastewaters. This study investigated the simultaneous removal capacity of inorganic (nitrogen, phosphorus and metals) and organic pollutants (PPCPs and EDCs) by four freshwater microalgae, and also evaluated the elimination of estrogenic activity in the wastewater by these algae species. The results demonstrated that simultaneous removal of various contaminants in wastewater was achieved, which showed potential application of these algae species in the wastewater treatment.

1. Introduction

Domestic wastewater contains cocktails of contaminants such as nutrients (N and P), metals, pharmaceuticals and personal care products (PPCPs), and endocrine disrupting chemicals (EDCs).[1–4] Thus discharge of untreated wastewater should be avoided as it could pose risks to the environment and public health. In many countries, domestic wastewater has often been treated by various types of conventional wastewater treatment technologies such as activated sludge process and oxidation ditch by making use of microbial processes. However, incomplete removals have been reported for these contaminants in wastewater.[5–8] Therefore, alternative or advanced treatment is needed to improve the water quality of the effluents from sewage treatment plants (STPs).

The use of microalgae in the treatment of wastewater has gained great interest over the past few years. The microalgae used in the treatment can not only effectively assimilate inorganic nitrogen and phosphorus for growth, but also remove heavy metals and organic substances. There have been some studies on the removal of pollutants, including nitrogen and phosphorus,[9,10] heavy metals,[11] EDCs and PPCPs,[12,13] using various microalgae. But most of these experiments were only conducted to remove inorganic or organic pollutants in artificial wastewaters. Little information is available about the simultaneous removal of inorganic (nitrogen, phosphorus and metals) and organic pollutants (PPCPs and EDCs) by algae. Given that inorganic and organic contaminants occur simultaneously in wastewaters and aquatic environments, it would be necessary to understand the applicability of algal treatment

State Key Laboratory of Organic Geochemistry, CAS Centre for PRD Environmental Pollution and Control Research, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, P. R. China. E-mail: guangguo.ying@gmail.com; guang-guo.ying@gig.ac.cn; Fax: +86 020 85290200; Tel: +86 020 85290200

technology in the removal of multiple contaminants in wastewater.

Freshwater green microalgae such as *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella pyrenoidosa* and *Chlorella vulgaris* have been employed in studying the removal of various contaminants due to their high removal efficiencies for pollutants and potential feedstock for bioenergy production or other high value added products.[11,13–18] In this study, we aimed to further assess the capability of these four green microalgae species in the simultaneous removal of various contaminants (nitrogen, phosphorus, metals, organic compounds) in real wastewater. Estrogenic EDCs such as nonylphenol possess the ability to disrupt the endocrine systems of higher organisms by interacting with the estrogen receptor.[19] Thus, in addition to chemical analysis, a recombinant yeast-based estrogen screen bioassay (YES) was also used to evaluate the elimination of estrogenic activity in the wastewater by the four algae species. Since green microalgae are widely distributed in most types of freshwater ecosystems, it is also helpful for us to understand the interaction between algae and aquatic contaminants. The raw wastewater used in the experiment was collected from a sewage treatment plant (STP). The removal efficiencies for various contaminants by the four algae species were compared with those in the STP.

2. Materials and methods

2.1 Algal strain and culture medium

Four freshwater green algae species *C. reinhardtii* (FACHB-479), *S. obliquus* (FACHB-416), *C. pyrenoidosa* (FACHB-9) and *C. vulgaris* (FACHB-31) were kindly provided by Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). BG11 medium was used as the growth medium for these algal species. Pre-cultivated microalgae were centrifuged at 9391*g* for 10 min, and after the supernatant was discarded the pelleted microalgal cells were washed twice with sterile Milli-Q water and re-suspended in sterile Milli-Q water for inoculation into the wastewater.

2.2 Algae treatment experiment

The raw wastewater (influent) used for the algae treatment experiment was obtained on May, 2012 from the Xintang sewage treatment plant (STP), which is located in Guangzhou, South China. This treatment plant serves a population of 410 000 equivalent inhabitants and treats up to 100 000 m^3 per day of municipal wastewater. The wastewater treatment process consists of pre-treatment (screens), a grit chamber, an anoxic–anaerobic–anoxic tank, an aerobic tank, and followed by a second clarifier. The secondary effluent is further treated with UV-C before discharge as the final effluent. In order to assess the removal efficiencies of various contaminants in the STP, both influent and effluent samples were collected for chemical analysis.

The influent sample (30 L) collected from the STP was sterilized through filtering using sterile 0.22 μm pore-size Whatman GF/F glass-fiber filters. The wastewater treatment systems were set up in 1000 mL Erlenmeyer flasks containing 500 mL wastewater volume, and inoculated with algae by adding each of the microalgae cultures at the exponential growth phase to give a chlorophyll a concentration of 0.05 mg L^{-1}. Culturing in these flasks was performed in an incubator (SKY-211BG, China) at 150 rpm and 25 ± 1 °C. Light was provided by continuous cool white fluorescent lamps at 60 μmol m^{-2} s^{-1} with a dark–light cycle of 12 h : 12 h. Each set of the experiment lasted for 7 days, and all tests were carried out in triplicate. In order to have enough wastewater for analysis of organics, additional six experimental replicates were included. At the beginning and end of the incubation, the concentrations of various contaminants (total nitrogen (TN), total phosphorus (TP), metals, PPCPs and EDCs) in the flasks were measured.

2.3 Algal density and chlorophyll a analysis

Algal density (cells per mL) was determined daily using a haemocytometer under a light microscope. Chlorophyll a measurement was carried out daily according to the procedure described by Zhou *et al.*[11] Briefly, samples withdrawn from the incubation flasks were centrifuged at 5445*g* for 10 min to separate algae, and after the supernatant was discarded the concentrated algal cells were frozen (−20 °C) for 20 min and thawed (25 °C) for 5 min. This procedure was repeated three times, then the algal cells were frozen (−20 °C) overnight until the cell wall was broken. The algae were suspended in 95% ethyl alcohol at 80 °C and heated for 2 min in a water bath. Then chlorophyll a was extracted for 6 h at room temperature, centrifuged for 10 min at 5445*g* and analyzed spectrophotometrically.

The content of chlorophyll a (C_A, mg L^{-1}) was calculated from the absorbance values at 665 and 649 nm according to the following eqn (1):

$$C_A = 13.95A_{665} - 6.88A_{649} \quad (1)$$

Growth rates (μ, h^{-1}) of the four algae were calculated using the following eqn (2):

$$\mu = (\ln C_t - \ln C_0)/(t_t - t_0) \quad (2)$$

where C_t is the algal density at time t_t, and C_0 is the initial algal density at the beginning of the test.

2.4 Analysis of inorganic compounds (TN, TP and metals)

After filtration through a 0.45 μm pore-size membrane filter, the contents of TN and TP in the samples were measured daily by spectrophotometric methods according to Methods of Monitoring and Analysis for Water and Wastewater.[20] The removal rate (R_i, mg per L per day) and specific removal rate (R_{xi}, mg per mg chl a per day^1) of total nitrogen and total phosphorus by the algal biomass were calculated using the following eqn (3) and (4):[21]

$$R_i = (C_0 - C_t)/(t_t - t_0) \quad (3)$$

$$R_{xi} = R_i/(\text{chl a})_0 \quad (4)$$

where C_0 is the initial concentration of a substrate in the solution (mg L^{-1}); C_t is the corresponding substrate concentration

(mg L^{-1}) at "t_t" which is the time at which the concentration of the substance did not change significantly; and $(Chl\ a)_0$ is the chlorophyll a concentration (mg L^{-1}) at the beginning of the experiment.

At the end of treatment, the concentrations of 15 metal elements (Ag, Al, As, Au, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sn and Zn) in the samples after filtration were measured by inductively coupled plasma mass spectrometry (ICP-MS: ELAN 6000, PerkinElmer Co., Ltd, USA). In addition, the concentrations of TN, TP and metals in the influent and effluent samples of the STP were also determined.

2.5 Analysis of organic compounds

Considering the serious adverse effects on the aquatic environment of emerging organic compounds, fifty organic compounds including EDCs and PPCPs were selected in the analysis of wastewater samples. The extraction and instrumental analysis followed the authors' previous methods.[22–25] In brief, the collected water samples (1 L each) from the STP and the laboratory experiment were filtered through prebaked glass fiber filters (GF/F, Whatman 0.45 μm pore-size) before extraction. Then 50 mL methanol and 200 μL 4 M H_2SO_4 were added immediately to each 1 L bottle to adjust the pH to 3.0. After addition of the internal standards (100 ng), the collected water samples were extracted using Waters Oasis HLB cartridges (6 cm^3, 500 mg sorbent). The SPE cartridges were preconditioned with 10 mL methanol and 10 mL Milli-Q water and then water samples were introduced to the cartridges at a flow rate of 5–10 mL min^{-1}. After loading of the water samples, the cartridges were dried for approximately 2 h to remove excess water under vacuum, and eluted with 12 mL methanol. The eluates were dried under a gentle stream of nitrogen and redissolved in 1 mL of methanol. After filtration through a 0.22 μm membrane to remove particles, the final extract was transferred to a 2 mL amber vial and stored at −18 °C until analysis. The concentrations of the target compounds in the samples were determined by rapid resolution liquid chromatography-tandem mass spectrometry (RRLC-MS/MS). The instrument used for the analysis of the target compounds was an Agilent 1200 series RRLC system coupled to an Agilent G6460A triple quadrupole detector (Agilent, Palo Alto, USA). The tandem mass spectrometer was operated with an electrospray ionization (ESI) source in both negative and positive modes. The quantitative analysis of the target compounds was performed with multiple reaction monitoring (MRM) mode. Detailed instrumental conditions can be found in the previous studies.[22–25]

2.6 Yeast estrogen screen (YES) assay

Samples for YES bioassay were prepared in the same procedure as for the organic analysis but without addition of the internal standards. The recombinant yeast was donated by J. P. Sumpter (Brunel University, Uxbridge, United Kingdom). The YES assay was conducted according to the method described by Zhao *et al.*[26] In brief, a sample extract was diluted by twofold in series, then 10 μL sample of each concentration was transferred to the corresponding wells on a 96-well plate. The solvent was allowed to dry in a laminar flow cabinet, and then 200 μL yeast solution in growth medium was added to each well to obtain a yeast density of 4×10^7 cells per mL and chlorophenol red-β-D-galactopyranoside concentration of 0.1 mg mL^{-1} in the well. The microplate was sealed and packed with foil. Then 72 h of static incubation at 32 °C in darkness was needed, and the absorbance at 620 nm and 540 nm was finally measured on a BMG microplate reader (BMG Lab Technologies).

Methanol was used as a blank control and estradiol (E2) was used as the positive control of YES with an initial concentration of 54.48×10^3 ng L^{-1}. The potency of estrogenic activity *versus* concentration follows the dose–effect relationship and shows a sigmoid curve. The curve is fitted with a four-parameter logistic model using Origin 8.0 software. The potency of estrogenic activity of a sample was calculated from the ratio of the median effective concentration (EC50) of the sample and the EC50 of the corresponding positive control. The estrogenic activity of a sample measured by the YES assay was then expressed as an E2 equivalent concentration (EEQ).

2.7 Statistical analysis

Statistical analysis was carried out using the SPSS 16.0 package. One way-ANOVA followed by LSD was used to check the significance of treatments. Levels of significance used were 5% and 1%, described as "significant" and "highly significant", respectively. Data are presented as mean ± standard deviation unless otherwise stated.

3. Results and discussion

3.1 Removal of nutrients

The concentrations of dissolved TN and TP in the STP influent were 6.64 and 0.15 mg L^{-1}, respectively. The algae species of *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris* all grew well in the influent (Fig. 1), with an average growth rate of 0.016, 0.011, 0.014 and 0.015 h^{-1}, respectively. These values of the algal growth rate are slightly lower than the reported data in other urban wastewater and mineral medium at the same experimental temperature,[9,27] which may be attributed to the relatively low dissolved nutrient level or carbon supply in influent wastewater than those in the literature. Considering the significant difference in cell volume occurred between four algal species, the chlorophyll a concentration (not algal density) was mainly used as the biomass indicator in the following analysis to add the comparability of different algal species in the present study. Without algae, the concentrations of TN and TP in the control remained unchanged with 7 days of the experiment (Fig. 2). With algae, the TN and TP levels in the wastewater decreased with incubation time, and became stable 5 days (except for *S. obliquus*) and 2 days after treatment, respectively (Fig. 2). Microalgae could accumulate large amounts of phosphorus within their cells in the form of polyphosphates upon starvation of the cells.[28] After the available total phosphorus in the influent was exhausted, algal growth was still sustained until the available total nitrogen concentration was also depleted. Although these algae species removed efficiently TN and TP in the present study,

the removal rates (0.73–0.88 mg per L per day for TN and 0.05 mg per L per day for TP) were significantly lower than the previously reported values in the artificial wastewater treatment by algae.[29] The nitrogen and phosphorus depletion rates by *C. vulgaris* were reported to be 5.44 mg per L per day and 1.30 mg per L per day.[29] A possible reason for the slow removal of TN and TP in the present study is that algal growth was limited due to the low levels of biologically available microelements and carbon sources.[30] For example, copper ion concentration detected in the influent in the present study was below 0.568 μg L^{-1} (Table 2), which is far lower than the value (20 μg L^{-1}) used in the growth medium (BG11 medium) for the algal species. In the present study, the specific TP removal rates varied between 0.81 and 1.07 mg per mg chl a per day, which are higher than the values (0.2–0.52 mg per mg chl a per day) reported by Aslan and Kapdan.[21] In addition, the specific TN removal rates (12.13–17.53 mg per mg chl a per day) were also found to be significantly higher than the specific NH_4–N removal rates (peak value: 3.0 mg per mg chl a per day) in which NH_4Cl was used as the nitrogen source.[21] The observed high specific TP and TN removal rates in the present study are due to the low initial chl *a* concentration (0.05 mg L^{-1}), much lower than the value (3.5 mg L^{-1}) reported by Aslan and Kapdan.[21] The specific removal rate was seldom reported in most of the previous studies, but this parameter can reflect very well the effect of the initial inoculated algal biomass on the removal efficiencies of the total nitrogen and total phosphorus in wastewater. The recorded high R_{xi} values in this study showed clearly that these four green microalgae species had application potential in wastewater treatment.

The concentrations of dissolved TN and TP in the STP effluent were 7.08 and 0.36 mg L^{-1}, respectively, which are higher than those detected in the influent. This indicates that the activated sludge process is not very efficient for removing dissolved TN and TP contained in the wastewater, which was mainly attributed to the release of dissolved TN and TP accumulated in sewage sludge. After 7 days of experiment, 76.7%, 88.9%, 91.7% and 92.3% of TN, and 67.5%, 70.5%, 81.2% and 82.2% of TP were removed by *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*, respectively. The nitrogen and phosphorus removal efficiencies achieved in the present study are comparable to those in some previous studies.[9,31] No obvious difference in TP removal was observed among four algae species. However, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris* showed higher TN removal rates and specific TN removal rates than *C. reinhardtii* (Table 1).

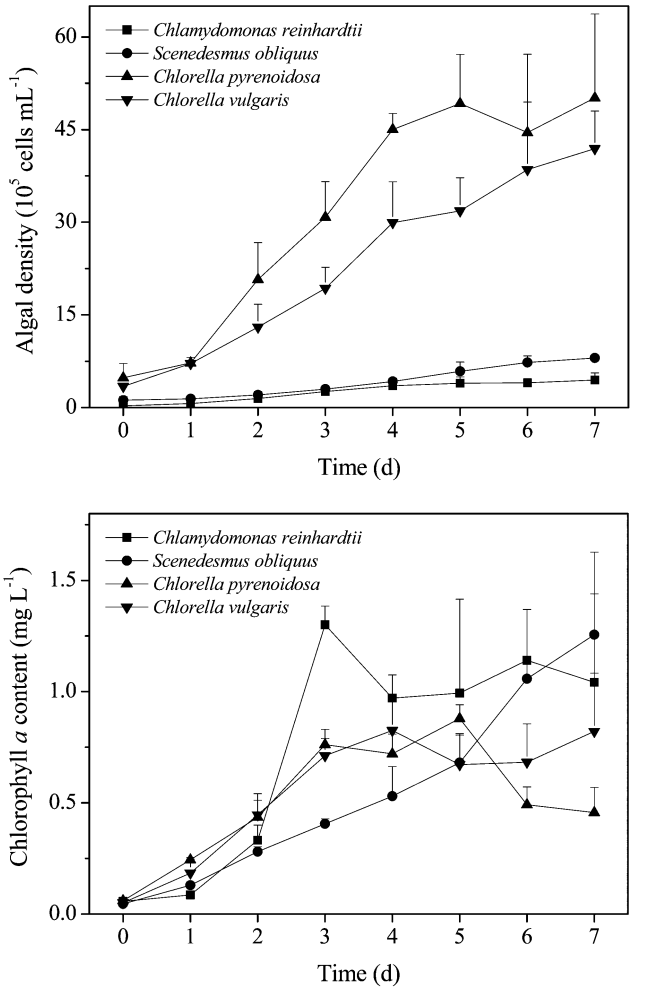

Fig. 1 Changes of algal density and chlorophyll a concentration in the wastewaters with growth of *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*. Data are presented as mean ± S.D. ($n = 3$).

3.2 Removal of metals

The influent contained various metals with their concentrations ranging from 0.03 μg L^{-1} for Ag to 540.6 μg L^{-1} for Mn (Table 2). The concentration levels for the 15 elements in the influent are comparable to the reported values for wastewaters in previous studies.[1,3] High concentrations in the influent were found for Mn, Zn, Fe and Al with the values being 540.6, 149.7, 39.1 and 38.8 μg L^{-1}, respectively.

Fig. 3 shows the removal efficiencies for the 14 elements by the STP. The removal efficiencies of these metals from wastewater by the STP were not affected by their initial metal concentrations, which is not consistent with the previous findings that the metal removal is directly proportional to the metal concentrations in the influent.[1,32] The removal efficiencies for 14 metals by the STP varied widely between 2 and 99%. Nine metals (Ag, Al, Au, Co, Fe, Hg, Mn, Pb and Zn) had their removal efficiencies more than 50%, while the other elements (As, Cd, Cr, Ni and Sn) showed low removal efficiencies (<50%). The metals were removed through a combination of several processes such as adsorption and biological assimilation by bacteria, and the removal efficiencies were affected by many factors including metal species and concentration, bacteria abundance and the pH of the process.[1,32–34] Additionally, a possible reason for high removal efficiencies for some metals such as Co, Fe, Mn and Zn is that these metals can be used by microorganisms in a wastewater treatment system to maintain their functions and activities. Low removal efficiencies for Ni and Cd had also been reported previously.[33,34] Chipasa[1] studied the removal of heavy metals (Cd, Cu, Pb and Zn) by a biological wastewater treatment system, and found that Cd removal (<20%) was the lowest, whereas zinc removal (>80%) was the

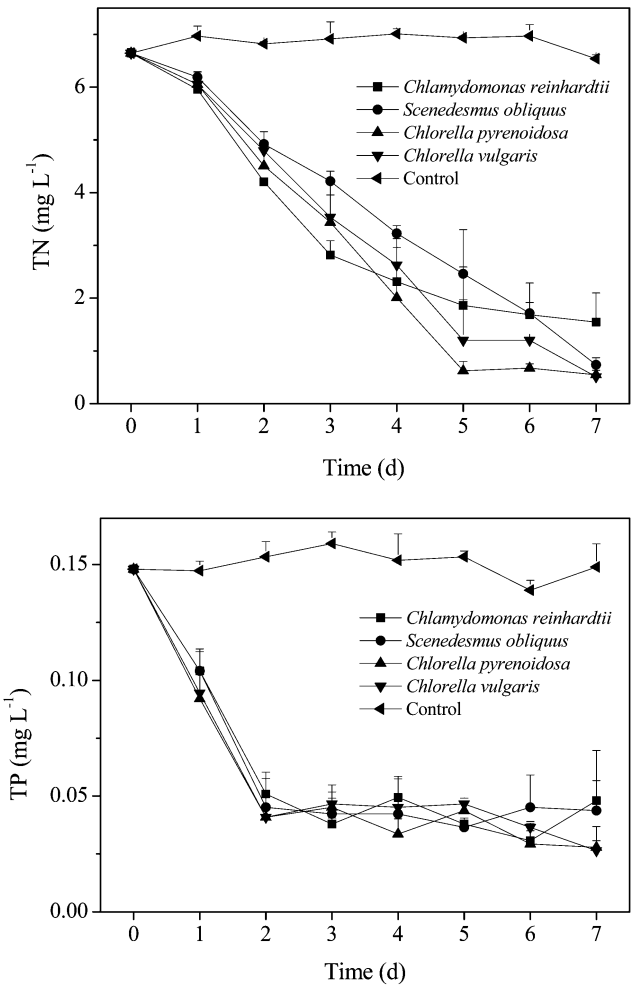

Fig. 2 Removal of total nitrogen (TN) and total phosphorus (TP) by *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*. TN: total nitrogen; TP: total phosphorus. Data are presented as mean ± S.D. ($n = 3$).

highest. The concentrations for Cu in the present study were found to be 2.5 μg L^{-1} in the effluent and lower than the limit of detection in the influent. The increase in the Cu concentration in the effluent in this system might be attributed to the release of Cu accumulated in the sewage sludge.

Fig. 3 also shows the removal efficiencies for the 14 elements by the four algae species. High removal efficiencies (>50%) were found for seven elements (Al, Ag, Au, Fe, Hg, Mn and Zn), but low efficiencies (<50%) were found for seven elements (As, Cr, Cd, Co, Ni, Pb and Sn). In addition, for most of the metals there was no significant difference in removing metals among the four algae species. For Zn, the four algae species exhibited different removal efficiencies ranging from 38.7% by *C. reinhardtii* to 93.3% by *S. obliquus*. High removal capacity for Zn and Hg was observed with the green alga *Cladophora fracta*.[35] In addition, *C. fracta* also showed a low removal efficiency of 20% Cd when the initial Cd concentration was 0.1 mg L^{-1}.[35] In the present study, except for 47.0% Cd removal by *S. obliquus*, the removal efficiencies of Cd by the other three algae species were below 20%. One of the possible reasons for different removal efficiencies between different algal species is the different biological requirement for the specific metal species. Similar patterns in metal removal between algae and activated sludge treatment were recorded in the present study, indicating that similar mechanisms might have occurred including adsorption and complexation, as mentioned by Hu *et al.*[36] Our previous study also suggested that algal adsorption played an important role in the removal of heavy metals Cu and Zn.[11] The removal of metals from wastewater by the four algae species was also not affected by initial metal concentrations, but determined by metal species. Thus, bioremoval of metals by microalgae can be an alternative option to activated sludge treatment. In the present study, most of the metals exhibited obvious decrease in concentration in the control (Table 2), which was mainly attributed to chemical precipitation in the experimental period. However, the removal of metals by four algae species should not be affected by chemical precipitation as the algal sorption of metals occurred in a very short time, which is indicated by our previous study.[11]

3.3 Removal of organics

The concentrations of 50 organic compounds (PPCPs and EDCs) in the influent are presented in Table 3. The concentrations of PPCPs and EDCs ranged between 1.6 ± 0.7 ng L^{-1} for sulfapyridine and 62 510.2 ± 5557.1 ng L^{-1} for salicylic acid. High concentrations in the influent were detected for some organic compounds such as salicylic acid, clofibric acid and bisphenol A. The concentration levels of these compounds are comparable with the reported data in STPs in China,[24,25] but relatively different for some compounds from the data in STPs in other countries.[4,37] For example, the BPA concentration was higher than 20 000 ng L^{-1} in this study, but lower than 1000 ng L^{-1} in those STPs.[4,37]

Table 1 The removal rates and specific removal rates (mean ± S.D., $n = 3$) of total nitrogen (TN) and total phosphorus (TP) by the four algae species

Species	TN removal rate[a] (mg per L per day)	Specific TN removal rate (mg per mg chl a per day)	TP removal rate (mg per L per day)	Specific TP removal rate (mg per mg chl a per day)
C. reinhardtii	0.73 ± 0.09[a]	12.13 ± 1.45[a]	0.05 ± 0[a]	0.81 ± 0.06[a]
S. obliquus	0.84 ± 0.03[b]	16.87 ± 0.51[b]	0.05 ± 0.01[a]	1.03 ± 0.15[a]
C. pyrenoidosa	0.87 ± 0.03[b]	14.51 ± 0.49[c]	0.05 ± 0[a]	1.07 ± 0.11[a]
C. vulgaris	0.88 ± 0.03[b]	17.53 ± 0.60[b]	0.05 ± 0.01[a]	1.07 ± 0.11[a]

[a] Values with different letters (a, b and c) are significantly different at the $p = 0.05$ level.

Table 2 Concentrations (μg L^{-1}) of selected metals in wastewaters after treatment by the sewage treatment plant and four algae

Element	Influent[a]	Effluent	*C. reinhardtii*	*S. obliquus*	*C. pyrenoidosa*	*C. vulgaris*	Control
Ag	0.03 ± 0.02[a]	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0[a]
Al	38.83 ± 3.94[a]	12.25 ± 2.18	14.67 ± 3.34	20.00 ± 4.18	18.35 ± 5.66	18.78 ± 5.28	14.25 ± 2.26[b]
As	2.65 ± 0.17[a]	2.45 ± 0.25	2.08 ± 0.36	1.94 ± 0.61	1.39 ± 0.28	1.61 ± 0.39	1.70 ± 0.01[b]
Au	12.29 ± 12.43[a]	0.94 ± 0.11	0.66 ± 0.10	0.58 ± 0.07	0.47 ± 0.06	0.39 ± 0.05	1.90 ± 0.70[b]
Cd	0.04 ± 0.01[a]	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0	0.03 ± 0	0.01 ± 0[b]
Co	0.82 ± 0.06[a]	0.23 ± 0.03	0.60 ± 0.10	0.62 ± 0.19	0.53 ± 0.09	0.60 ± 0.15	0.25 ± 0[b]
Cr	1.66 ± 0.04[a]	1.12 ± 0.20	1.15 ± 0.32	0.94 ± 0.30	0.97 ± 0.17	1.16 ± 0.28	0.68 ± 0.02[b]
Cu	<0.568[a]	2.53 ± 0.51	<0.568	<0.568	<0.568	<0.568	<0.568[a]
Fe	39.11 ± 0.44[a]	3.45 ± 0.35	11.35 ± 6.46	6.04 ± 1.45	8.62 ± 1.78	8.73 ± 0.26	5.38 ± 0.11[b]
Hg	0.92 ± 0.59[a]	0.16 ± 0.04	0.10 ± 0.02	0.08 ± 0.02	0.05 ± 0.01	0.04 ± 0	0.34 ± 0.07[b]
Mn	540.63 ± 33.57[a]	3.39 ± 0.27	110.13 ± 24.16	9.84 ± 2.84	21.48 ± 4.00	14.78 ± 3.52	16.22 ± 9.09[b]
Ni	6.21 ± 0.27[a]	6.10 ± 0.82	4.47 ± 0.93	4.25 ± 1.30	3.79 ± 0.16	4.27 ± 0.34	3.06 ± 0.10[b]
Pb	1.03 ± 0.13[a]	0.49 ± 0.14	0.92 ± 0.35	0.77 ± 0.11	0.87 ± 0.20	0.85 ± 0.40	0.48 ± 0.14[b]
Sn	0.66 ± 0.08[a]	0.60 ± 0.11	0.35 ± 0.11	0.43 ± 0.23	0.42 ± 0.15	0.54 ± 0.25	0.17 ± 0.02[b]
Zn	149.66 ± 51.55[a]	48.00 ± 4.76	91.74 ± 20.51	10.05 ± 2.18	52.17 ± 45.72	39.49 ± 7.58	24.85 ± 2.47[b]

[a] Mean ± S.D. ($n = 3$). Values with different letters are significantly different at the $p = 0.05$ level.

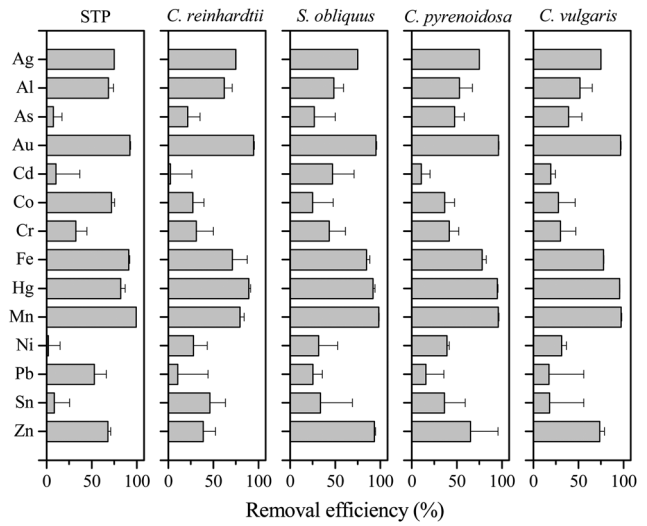

Fig. 3 Removal of metals by activated sludge treatment (STP) and algal treatments with *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*. Data are presented as mean ± S.D. ($n = 3$).

Fig. 4 shows the removal efficiencies for the organic compounds by the STP and four algae species. The removal efficiencies for these organic compounds varied widely in the STP. Among the 50 organic compounds, 32 compounds were efficiently removed (>70%) by an activated sludge treatment process, 7 compounds experienced intermediate removal (38.0–63.8%), and 5 compounds (sulfamethazine, sulfamonomethoxine, roxithromycin, carbamazepine and carbendazim) experienced poor removal (8.3–22.1%), but other 6 compounds (clarithromycin, fluconazole, diclofenac, sulfamethoxazole, sulfapyridine and ibuprofen) showed increasing concentrations in the treated wastewater. The increase in concentration in the effluent for the STP could be attributed to the biotransformation of compounds due to microbial processes or release from sludge.[2,19,38] For example, the estrogenic alkylphenols and steroid estrogens found in the effluent were the breakdown products of incomplete biodegradation of their respective parent compounds.[2,19] Auriol *et al.*[39] summarized a number of treatment processes of EDCs, and indicated that not all chemicals were completely removed by the activated sludge process, which is consistent with the results from the present study.

Although only 14 compounds (28%) were found no significant variations in the concentration during the experiment in the control ($p > 0.05$, Table 3), more than 50% of the compounds had little changes (<25%), and only 28% of the compounds had >50% variations in concentration. The variation in concentration of the compounds in the control should be attributed to photo-degradation or transformation in the experiment. Similar removal patterns were observed for the four algae species, but with some differences from the activated sludge treatment (Fig. 4). For example, five compounds (lincomycin, trimethoprim, 2,4-D, MCPA and clofibric acid) with >80% removal efficiency in the STP showed very low removal (<50%) in the algal treatment. However, more than 80% removal was observed for clarithromycin, roxithromycin and triclocarban by the four algae species, but <50% removal was found in the STP. These results suggested that coupling the STP and algal treatment would be more beneficial than anyone only for the removal of organic compounds. The difference in removal efficiencies for these compounds was attributed to the different biological requirements for them between bacteria (heterotrophic) in the STP and green microalgae species (mixotrophic). Among the 50 selected compounds (EDCs and PPCPs) in the present study, 32, 30, 28 and 31 compounds were removed efficiently (>50%) by *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*, respectively (Fig. 4). The present study clearly demonstrated the capability of the four algae to remove efficiently most of the selected organic contaminants in wastewater. In fact, limited previous studies have also found that algal strains could induce biotransformation or biodegradation of chemicals.[13,40] Bisphenol-A can be metabolized to BPA glycosides in the presence of some freshwater green microalgae such as *Pseudokirchneriella subcapitata*, *S. acutus*, *S. quadricauda*, and *Coelastrum reticulatum*.[40] Nonylphenol and octylphenol were found to be

Table 3 Concentrations (ng L^{-1}) of selected organic compounds in wastewaters after treatment by the sewage treatment plant and four algae

Compound	Influent[a]	Effluent	*C. reinhardtii*	*S. obliquus*	*C. pyrenoidosa*	*C. vulgaris*	Control
17α-Boldenone	19.2 ± 0.3[a]	1.1 ± 1.9	3.5 ± 0.2	3.4 ± 0	3.3 ± 0	3.4 ± 0.1	16.5 ± 0[b]
17β-Boldenone	16.9 ± 0.4[a]	1.9 ± 0	4.3 ± 0.4	2.5 ± 0.1	3.7 ± 0.1	2.4 ± 0.1	8.3 ± 0.2[b]
2,4-D	432.8 ± 12.7[a]	20.5 ± 6.6	423.6 ± 21.1	406.2 ± 8.9	426.8 ± 31.9	375.3 ± 19.3	381.6 ± 13.0[b]
4-Nonylphenol	1785.8 ± 420.3[a]	930.5 ± 115.9	2750.2 ± 313.6	1696.0 ± 109.8	2348.9 ± 123.2	2700.0 ± 114.5	1866.0 ± 147.5[a]
4-OHA	52.5 ± 12.9[a]	6.8 ± 1.8	18.4 ± 1.0	15.5 ± 3.9	66.6 ± 4.2	25.2 ± 1.1	30.0 ± 7.3[a]
ADD	160.0 ± 6.1[a]	1.5 ± 0	1.6 ± 0.1	1.6 ± 0	1.8 ± 0.1	1.8 ± 0.2	7.5 ± 0[b]
AED	25.6 ± 0.2[a]	2.2 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	10.3 ± 0.3[b]
Bentazone	80.5 ± 8.2[a]	36.9 ± 1.5	50.6 ± 5.5	39.1 ± 4.3	49.1 ± 5.2	43.5 ± 8.3	75.8 ± 7.2[a]
Bisphenol A	20 145.6 ± 3135.7[a]	85.1 ± 12.8	287.1 ± 38.1	225.5 ± 22.7	172.3 ± 19.7	192.0 ± 6.7	130.7 ± 7.8[b]
Carbamazepine	130.0 ± 5.5[a]	110.9 ± 2.9	114.7 ± 11.2	123.2 ± 5.7	125.4 ± 2.9	116.9 ± 5.5	125.0 ± 4.5[a]
Carbendazim	185.4 ± 2.8[a]	164.2 ± 2.8	130.1 ± 1.3	152.6 ± 1.5	137.3 ± 4.4	158.9 ± 0.5	204.7 ± 8.7[b]
Ciprofloxacin	29.3 ± 1.6[a]	5.6 ± 0.1	6.2 ± 1.9	6.4 ± 2.0	7.5 ± 0.7	6.8 ± 0.4	29.0 ± 0.8[a]
Clarithromycin	17.3 ± 1.1[a]	17.5 ± 0.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14.0 ± 0.7[b]
Climbazole	146.7 ± 2.7[a]	57.2 ± 1.0	91.8 ± 3.2	43.3 ± 1.1	101.9 ± 3.4	102.6 ± 2.9	109.3 ± 3.5[b]
Clofibric acid	21 036.4 ± 419.3[a]	1966.4 ± 17.0	21 143.1 ± 674.5	19 259.3 ± 113.4	19 620.2 ± 491.1	14 774.3 ± 41.4	21 470.8 ± 568.5[a]
DEET	81.4 ± 3.0[a]	50.5 ± 2.5	109.4 ± 6.8	113.4 ± 3.2	129.7 ± 18.3	116.2 ± 15.1	98.0 ± 3.9[b]
Diclofenac	138.4 ± 27.4[a]	185.0 ± 38.2	706.3 ± 22.7	1280.6 ± 53.7	896.2 ± 108.7	1134.9 ± 44.3	2448.0 ± 70.8[b]
Enrofloxacin	26.2 ± 1.0[a]	5.1 ± 0.1	6.6 ± 0.5	6.2 ± 0.2	6.0 ± 0.1	5.9 ± 0.2	26.9 ± 0.5[a]
Erythromycin–H_2O	1025.1 ± 53.6[a]	301.1 ± 8.9	144.4 ± 9.4	370.1 ± 6.6	262.8 ± 7.3	338.5 ± 3.2	849.6 ± 11.4[b]
Estrone	91.7 ± 7.8[a]	11.8 ± 1.2	14.2 ± 1.7	11.7 ± 0.6	11.4 ± 0.3	12.3 ± 0.8	60.6 ± 3.7[b]
Ethylparaben	26.6 ± 5.1[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0[b]
Fluconazole	44.0 ± 1.1[a]	44.9 ± 0.7	32.6 ± 0.9	31.7 ± 0.8	33.1 ± 0.8	32.0 ± 4.4	41.0 ± 0.4[b]
Gemfibrozil	4.2 ± 2.1[a]	1.5 ± 0.5	43.8 ± 6.2	70.3 ± 2.8	55.4 ± 17.2	67.0 ± 4.4	109.0 ± 5.6[b]
Ibuprofen	70.3 ± 42.1[a]	389.4 ± 93.5	977.3 ± 74.1	7875.6 ± 60.4	4197.1 ± 713.0	4486.9 ± 219.1	7912.3 ± 366.6[b]
Lincomycin	362.9 ± 14.7[a]	9.0 ± 0.1	260.9 ± 6.8	326.6 ± 4.7	374.3 ± 49.7	430.6 ± 64.0	312.1 ± 3.9[b]
Lomefloxacin	22.9 ± 0.7[a]	4.4 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0	4.4 ± 0.1	24.5 ± 0.5[b]
MCPA	1512.9 ± 14.5[a]	11.1 ± 1.0	1517.7 ± 54.1	1135.7 ± 20.0	1501.7 ± 98.3	1476.3 ± 92.7	1528.5 ± 182.0[a]
Methylparaben	47.4 ± 2.0[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13.2 ± 3.9[b]
Monensin	18.0 ± 0.1[a]	3.6 ± 0	3.7 ± 0.1	3.6 ± 0	3.6 ± 0	3.6 ± 0	18.0 ± 0.1[a]
Narasin	28.0 ± 0.2[a]	5.7 ± 0.1	6.1 ± 0.2	5.7 ± 0.1	5.8 ± 0.1	5.8 ± 0.2	27.8 ± 0.1[a]
Norfloxacin	32.3 ± 2.1[a]	8.6 ± 0.1	15.1 ± 1.4	19.0 ± 0.9	18.4 ± 1.5	15.7 ± 0.5	30.3 ± 1.7[a]
Ofloxacin	53.6 ± 10.7[a]	15.4 ± 0.7	25.8 ± 1.1	29.6 ± 0.6	29.7 ± 0.9	30.4 ± 0.9	32.2 ± 2.0[b]
Paracetamol	1673.7 ± 618.1[a]	7.5 ± 3.7	193.8 ± 7.5	183.5 ± 43.7	108.5 ± 82.9	116.1 ± 74.7	57.5 ± 14.9[b]
Progesterone	11.5 ± 0.4[a]	2.4 ± 0.8	1.9 ± 0	1.9 ± 0.8	1.8 ± 0.5	1.5 ± 0.1	6.6 ± 0[b]
Propylparaben	3.8 ± 1.6[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.6 ± 0.8[a]
Roxithromycin	49.2 ± 2.7[a]	38.3 ± 2.3	3.0 ± 0.1	6.4 ± 0.5	3.9 ± 0.2	5.0 ± 0.2	39.9 ± 1.2[b]
Salicylic acid	62 510.2 ± 5557.1[a]	793.2 ± 281.0	1397.7 ± 193.7	1071.3 ± 79.5	647.9 ± 41.5	1560.2 ± 25.4	3018.1 ± 220.5[b]
Salinomycin	23.0 ± 0.2[a]	5.1 ± 0.2	6.7 ± 0.5	4.9 ± 0.1	5.1 ± 0.1	5.0 ± 0.2	22.1 ± 0.2[b]
Sulfadiazine	28.6 ± 2.9[a]	16.2 ± 0.8	7.3 ± 0.1	13.6 ± 0.6	12.3 ± 0.7	9.5 ± 0.4	16.3 ± 0.4[b]
Sulfadimethoxine	6.8 ± 0.4[a]	1.3 ± 0	3.0 ± 0.3	1.5 ± 0.1	2.4 ± 0.2	1.9 ± 0.1	6.4 ± 0.1[a]
Sulfameter	27.9 ± 0.8[a]	7.5 ± 0.2	5.0 ± 0	3.5 ± 3.0	5.1 ± 0.2	4.9 ± 0.1	25.5 ± 0.3[b]
Sulfamethazine	56.7 ± 1.5[a]	52.0 ± 0.8	29.3 ± 0.8	46.0 ± 0	43.9 ± 0.3	37.9 ± 0.8	44.0 ± 1.0[b]
Sulfamethoxzole	50.7 ± 2.1[a]	96.9 ± 0.5	40.0 ± 2.4	60.9 ± 2.6	56.4 ± 13.3	66.6 ± 5.7	37.9 ± 1.1[b]
Sulfamonomethoxine	258.8 ± 6.9[a]	227.1 ± 12.7	501.3 ± 32.6	351.2 ± 2.3	535.7 ± 46.9	420.2 ± 24.9	138.8 ± 3.0[b]
Sulfapyridine	1.6 ± 0.7[a]	5.7 ± 0.2	0 ± 0	1.1 ± 0.2	0.4 ± 0	0.1 ± 0.1	0 ± 0[b]
Testosterone	11.3 ± 0.8[a]	1.9 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9.5 ± 0.1[b]
Triclocarbon	57.1 ± 4.7[a]	29.8 ± 2.3	9.9 ± 7.2	0.8 ± 0.3	9.9 ± 1.2	11.0 ± 1.8	35.5 ± 2.0[b]
Triclosan	41.7 ± 4.4[a]	7.3 ± 0.7	17.4 ± 2.1	28.6 ± 3.9	24.0 ± 0.9	19.6 ± 0.1	19.1 ± 1.4[b]
Trimethoprim	27.0 ± 0.5[a]	4.4 ± 0	17.1 ± 1.4	22.0 ± 1.1	19.4 ± 3.0	27.8 ± 1.1	21.6 ± 0.7[b]
Tylosin	19.9 ± 0.1[a]	4.4 ± 0.1	4.6 ± 0	4.7 ± 0	4.7 ± 0.1	4.8 ± 0.1	19.9 ± 0.2[a]

[a] Mean ± S.D. ($n = 3$). Values with different letters are significantly different at the $p = 0.05$ level. Abbreviations: ADD, androsta-1,4-diene-3,17-dione; AED, 4-androstene-3,17-dione; 4-OHA, 4-hydroxy-androst-4-ene-17-dione; 2,4-D, 2,4-dichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; DEET, *N*,*N*-diethyl-3-methylbenzamide.

completely degraded by *S. obliquus* within 5 days' incubation.[13] In comparison with the activated sludge treatment process, similar algal removals were found for 23 organic compounds with high efficiencies (>50%) and 8 organic compounds with low efficiencies (<50%). Therefore, algal treatment appears to be effective for removing some organic contaminants from wastewater and could be used as alternative treatment technology.

3.4 Estrogenic activity

As shown in Fig. 5, estrogenic activities were detected in the influent and effluent of the STP, and the final wastewaters after algal treatment by the four algae species. The high estrogenic activity in the STP influent is consistent with the detection of some estrogenic compounds such as nonylphenol, bisphenol-A and estrone by RRLC-MS/MS. The

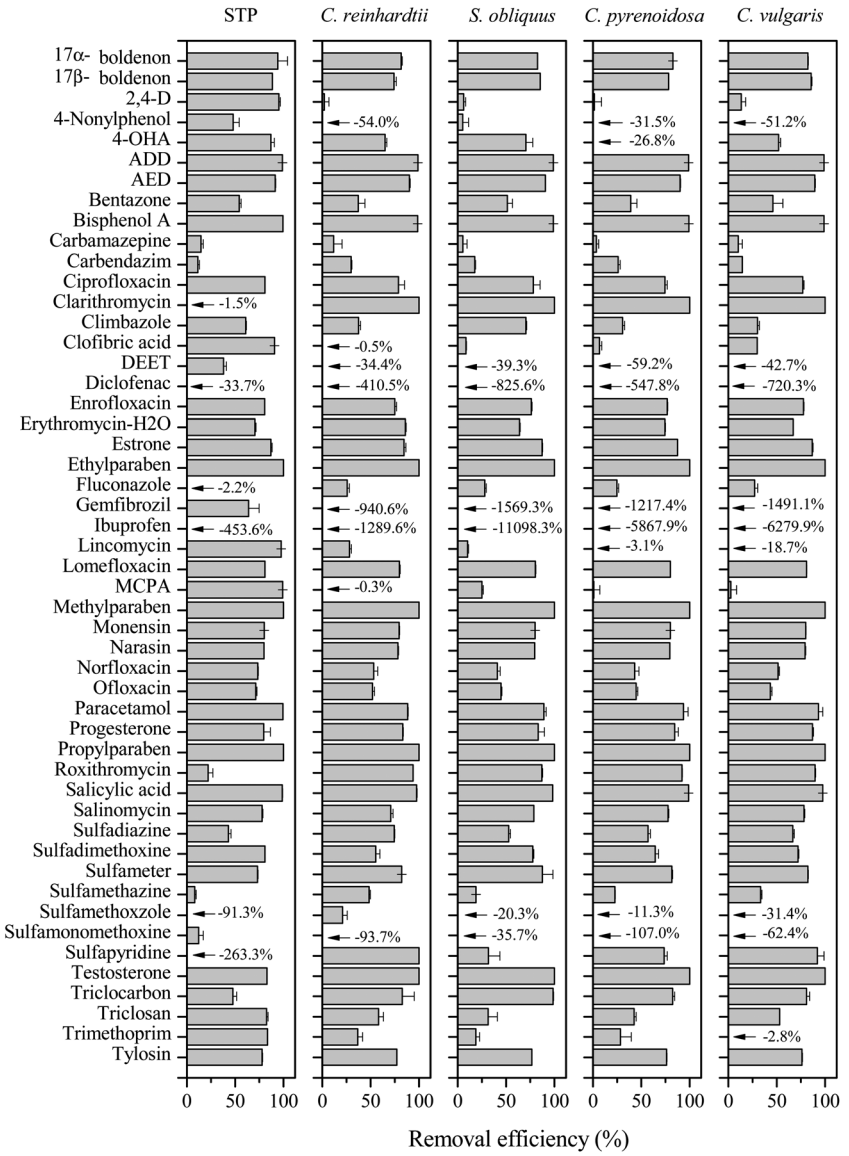

Fig. 4 Removal of PPCPs and EDCs by activated sludge treatment (STP) and algal treatments with *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*. Abbreviations: ADD, androsta-1,4-diene-3,17-dione; AED, 4-androstene-3,17-dione; 4-OHA, 4-hydroxy-androst-4-ene-17-dione; 2,4-D, 2,4-dichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; DEET, *N*,*N*-diethyl-3-methylbenzamide. Data are presented as mean ± S.D. ($n = 3$).

estrogenic activity expressed as EEQ in the influent was 11.7 ng L^{-1}, but reduced to 2.7 ng L^{-1} in the STP effluent, and to 6.3, 2.2, 4.2 and 5.1 ng L^{-1} in the final wastewater after algal treatment by *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris*. The removal efficiencies by the activated sludge treatment and by the four algae species were 76.8%, 46.2%, 81.1%, 64.5% and 56.5%, respectively (Fig. 5). Among the four algae species, *S. obliquus* is the most efficient in removing the estrogenic activity in the influent. Hirooka *et al.*[30] also reported that the estrogenic activity originated from bisphenol-A could be completely removed after treatment by green alga *Chlorella fusca*, which is consistent with the present study. Thus, algal treatment is quite effective in the removal of estrogenic activity in wastewater.

3.5 Environmental implications

Microalgae can be cultivated in open or closed reactors, and closed photobioreactors offer higher photosynthetic efficiencies and better control than open systems.[14] The results of the present study provided new knowledge about the simultaneous removal of various pollutants in the closed algal treatment systems. Different algal species sometimes showed different removal efficiencies for pollutants, indicating that the monocultures might remove limited pollutants from wastewater. Considering the enhancement of removal efficiencies of various pollutants, mixed culture with numerous algal species in real sewage treatment plants will be expected in the further studies. After harvesting of microalgae with high biomass productivities and in some cases high lipid productivities from wastewater,

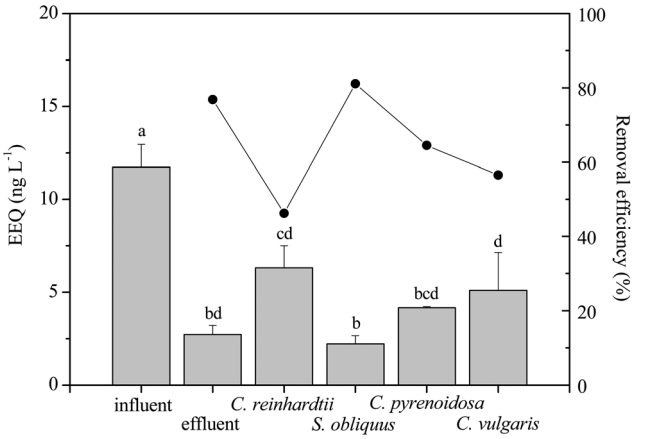

Fig. 5 Elimination of estrogenic activity in wastewater after activated sludge treatment (STP) and algal treatments with *C. reinhardtii, S. obliquus, C. pyrenoidosa* and *C. vulgaris*. Data are presented as mean ± S.D. ($n = 3$).

there is real potential in the utilization of these high nutrient resources for cost-effective biofuel production, which has been indicated in previous studies.[15–17]

This study also has environmental implications in terms of interaction between various contaminants and algae as these green algae are widely present in different aquatic environments. Algae could play an important role in the degradation of some contaminants in the environment. Actually, in the natural environment, it is possible that the contaminant removal might be enhanced due to the presence of bacteria. Bacteria have the ability to degrade organic pollutants to a form that algae are capable of utilizing.[41] Also, algae can provide the O_2 necessary for aerobic bacteria to biodegrade organic pollutants, consuming in turn the CO_2 released from bacterial respiration.[14]

4. Conclusions

The four freshwater green microalgae *C. reinhardtii*, *S. obliquus*, *C. pyrenoidosa* and *C. vulgaris* exhibited simultaneous removal of total nitrogen and total phosphorus, metals and some organic compounds in wastewater. The estrogenic activity in wastewater was also significantly reduced after treatment by these algal species. Similar removal patterns were observed between the activated sludge treatment and algal treatment due to having similar removal mechanisms. This implies that algae species can be applied in the treatment of wastewater containing cocktails of inorganic and organic contaminants.

Acknowledgements

The authors would like to acknowledge the financial support from the National Natural Science Foundation of China (NSFC 41103057, 41273119 and U1133005), CAS Key Project (KZZD-EW-09), and China Postdoctoral Science Foundation funded project (Grant no. 20110490932). This is a Contribution No. 1919 from GIG CAS.

References

1 K. B. Chipasa, *Waste Management*, 2003, **23**, 135–143.
2 A. C. Johnson and J. P. Sumpter, *Environ. Sci. Technol.*, 2001, **35**, 4697–4703.
3 M. Karvelas, A. Katsoyiannis and C. Samara, *Chemosphere*, 2003, **53**, 1201–1210.
4 N. Nakada, T. Tanishima, H. Shinohara, K. Kiri and H. Takada, *Water Res.*, 2006, **40**, 3297–3303.
5 M. A. Barakat, *Arabian J. Chem.*, 2011, **4**, 361–377.
6 R. Buzier, M. H. Tusseau-Vuillemin, C. M. Meriadec, O. Rousselot and J. M. Mouchel, *Chemosphere*, 2006, **65**, 2419–2426.
7 G. G. Ying, R. S. Kookana and D. W. Kolpin, *J. Environ. Monit.*, 2009, **11**, 1498–1505.
8 P. Verlicchi, M. A. Aukidy and E. Zambello, *Sci. Total Environ.*, 2012, **429**, 123–155.
9 M. E. Martínez, S. Sánchez, J. M. Jiménez, F. E. Yousfi and L. Muñoz, *Bioresour. Technol.*, 2000, **73**, 263–272.
10 A. Ruiz-Marin, L. G. Mendoza-Espinosa and T. Stephenson, *Bioresour. Technol.*, 2010, **101**, 58–64.
11 G. J. Zhou, F. Q. Peng, L. J. Zhang and G. G. Ying, *Environ. Sci. Pollut. Res.*, 2012, **19**, 2918–2929.
12 Y. Liu, Y. T. Guan, Q. T. Gao, N. F. Y. Tam and W. P. Zhu, *Chemosphere*, 2010, **80**, 592–599.
13 G. J. Zhou, F. Q. Peng, B. Yang and G. G. Ying, *Ecotoxicol. Environ. Saf.*, 2013, **87**, 10–16.
14 R. Muñoz and B. Guieysse, *Water Res.*, 2006, **40**, 2799–2815.
15 DOE, *US national algal biofuels technology roadmap*, US Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, 2010.
16 J. K. Pittman, A. P. Dean and O. Osundeko, *Bioresour. Technol.*, 2011, **102**, 17–25.
17 G. W. Roberts, M. O. P. Fortier, B. S. M. Sturm and S. M. Stagg-Williams, *Energy Fuels*, 2013, **27**, 857–867.
18 A. F. Clarens, E. P. Resurreccion, M. A. White and L. M. Colosi, *Environ. Sci. Technol.*, 2010, **44**, 1813–1819.
19 G. G. Ying, B. Williams and R. Kookana, *Environ. Int.*, 2002, **28**, 215–226.
20 State Environmental Protection Administration (SEPA), *Methods of Monitoring and Analysis for Water and Wastewater*. 2002.
21 S. Aslan and I. K. Kapdan, *Ecol. Eng.*, 2006, **28**, 64–70.
22 F. Chen, G. G. Ying, J. F. Yang, J. L. Zhao and L. Wang, *J. Environ. Sci. Health, Part B*, 2010, **45**, 682–693.
23 Z. F. Chen, G. G. Ying, H. J. Lai, F. Chen, H. C. Su, Y. S. Liu, F. Q. Peng and J. L. Zhao, *Anal. Bioanal. Chem.*, 2012, **404**, 3175–3188.
24 S. Liu, G. G. Ying, J. L. Zhao, F. Chen, B. Yang, L. J. Zhou and H. J. Lai, *J. Chromatogr. A*, 2011, **1218**, 1367–1378.
25 L. J. Zhou, G. G. Ying, S. Liu, J. L. Zhao, F. Chen, R. Q. Zhang, F. Q. Peng and Q. Q. Zhang, *J. Chromatogr. A*, 2012, **1244**, 123–138.
26 J. L. Zhao, G. G. Ying, B. Yang, S. Liu, L. J. Zhou, Z. F. Chen and H. J. Lai, *Environ. Toxicol. Chem.*, 2011, **30**, 2208–2215.

27 M. E. Martínez, J. M. Jiménez and F. El. Yousfi, *Bioresour. Technol.*, 1999, **67**, 233–240.
28 L. E. de-Bashan, M. Moreno, J. P. Hernandez and Y. Bashan, *Water Res.*, 2002, **36**, 2941–2948.
29 P. S. Lau, N. F. Y. Tam and Y. S. Wong, *Bioresour. Technol.*, 1998, **63**, 115–121.
30 T. Hirooka, H. Nagase, K. Uchida, Y. Hiroshige, Y. Ehara, J. I. Nishikawa, T. Nishihara, K. Miyamoto and Z. Hirata, *Environ. Toxicol. Chem.*, 2005, **24**, 1896–1901.
31 P. S. Lau, N. F. Y. Tam and Y. S. Wong, *Environ. Technol.*, 1996, **17**, 183–189.
32 G. E. Üstün, *J. Hazard. Mater.*, 2009, **172**, 833–838.
33 P. Chanpiwat, S. Sthiannopkao and K. W. Kim, *Microchem. J.*, 2010, **95**, 326–332.
34 E. Lipczynska-Kochany and J. Kochany, *Chemosphere*, 2003, **77**, 279–284.
35 L. Ji, S. L. Xie, J. Feng, Y. H. Li and L. Chen, *J. Appl. Phycol.*, 2012, **24**, 979–983.
36 Z. Hu, K. Chandran, D. Grasso and B. F. Smets, *Environ. Sci. Technol.*, 2003, **37**, 728–734.
37 Y. Zhang and J. L. Zhou, *Chemosphere*, 2008, **73**, 848–853.
38 J. Reungoat, B. I. Escher, M. Macova and J. Keller, *Water Res.*, 2011, **45**, 2751–2762.
39 M. Auriol, Y. Filali-Meknassi, R. D. Tyagi, C. D. Adams and R. Y. Surampalli, *Process Biochem.*, 2006, **41**, 525–539.
40 N. Nakajima, T. Teramoto, F. Kasai, T. Sano, M. Tamaoki, M. Aono, A. Kubo, H. Kamada, Y. Azumi and H. Saji, *Chemosphere*, 2007, **69**, 934–941.
41 Y. Zhou, L. Schideman, G. Yu and Y. Zhang, *Energy Environ. Sci.*, 2013, **6**, 3765–3779.