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Cultivation of Green Algae *Chlorella* sp. in Different Wastewaters from Municipal Wastewater Treatment Plant

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Abstract The objective of this study was to evaluate the growth of green algae *Chlorella* sp. on wastewaters sampled from four different points of the treatment process flow of a local municipal wastewater treatment plant (MWTP) and how well the algal growth removed nitrogen, phosphorus, chemical oxygen demand (COD), and metal ions from the wastewaters. The four wastewaters were wastewater before primary settling (#1 wastewater), wastewater after primary settling (#2 wastewater), wastewater after activated sludge tank (#3 wastewater), and centrate (#4 wastewater), which is the wastewater generated in sludge centrifuge. The average specific growth rates in the exponential period were 0.412, 0.429, 0.343, and 0.948 day^{-1} for wastewaters #1, #2, #3, and #4, respectively. The removal rates of NH_4–N were 82.4%, 74.7%, and 78.3% for wastewaters #1, #2, and #4, respectively. For #3 wastewater, 62.5% of NO_3–N, the major inorganic nitrogen form, was removed with 6.3-fold of NO_2–N generated. From wastewaters #1, #2, and #4, 83.2%, 90.6%, and 85.6% phosphorus and 50.9%, 56.5%, and 83.0% COD were removed, respectively. Only 4.7% was removed in #3 wastewater and the COD in #3 wastewater increased slightly after algal growth, probably due to the excretion of small photosynthetic organic molecules by algae. Metal ions, especially Al, Ca, Fe, Mg, and Mn in centrate, were found to be removed very efficiently. The results of this study suggest that growing algae in nutrient-rich centrate offers a new option of applying algal process in MWTP to manage the nutrient load for the aeration tank to which the centrate is returned, serving the dual roles of nutrient reduction and valuable biofuel feedstock production.

Keywords Municipal wastewater · Centrate · Algae · Nutrients removal · Metal · *Chlorella*

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Introduction

With global shortages of fossil fuels, especially oil and natural gas, a major focus has developed worldwide on renewable biofuel production [1]. Algae, with a much higher unit area oil yield than terrestrial oilseed crops [2], are a promising biofuel feedstock. Another issue from burning of fossil fuels is the ever-increasing carbon dioxide (CO_2) emission [3], whose trend will continue with the fast pace of modern industry development if a feasible energy source replacement could not be found. Algae, which can assimilate CO_2 photoautotrophically or mixtrophically, is a perfect candidate for CO_2 sequestration and greenhouse gas reduction.

As mentioned in the close-out report for the Aquatic Species Program by the US Department of Energy, the concept of using wastewater as a medium and source of nutrients for algae production found a new life with the energy crisis of the 1970s, which had the benefit of serving multiple needs—both environmental and energy-related [2]. Compared to the conventional wastewater treatment process, which introduces activated sludge, a biological floc, to degrade organic carbonaceous matter to CO_2, algae can assimilate organic pollutants into cellular constituents such as lipid and carbohydrate, thus achieving pollutant reduction in a more environment-friendly way.

The research on using algae cultivation as a tertiary wastewater treatment process started as early as the 1970s [4–6]. While the initial purpose of introducing algae pond process was to further treat the secondary effluent in order to prevent from causing eutrophication [7, 8], it was observed that the treatment removed nutrients from settled domestic sewage more efficiently than activated sewage process did, suggesting that it would be more economical and desirable to employ the algal system as the secondary rather than tertiary treatment process [8]. Algae species *Chlorella* was widely applied for wastewater treatment and had proven abilities of removing nitrogen, phosphorus, and chemical oxygen demand (COD) with different retention times ranging from 10 h to 42 days, mixing with bacteria or not (Table 1), which shows the potential of replacing activated sludge process in a secondary or tertiary step in view of nutrient reduction and biomass production.

The present study was a part of the ongoing research to evaluate cultivation of green algae *Chlorella* sp. on municipal wastewaters taken from different points of the process flow of a local municipal wastewater treatment plant (MWTP). The wastewater before primary settling (#1 wastewater), wastewater after primary settling (#2 wastewater), wastewater after activated sludge tank (called "effluent" in latter context, although it is not the final effluent for the MWTP; #3 wastewater), and centrate (#4 wastewater), which is the wastewater generated in sludge centrifuge, contain different levels of inorganics and organics, which may be beneficial or inhibitory to the growth of microalgae. Algal cultivation in #2 and #3 wastewaters has been tested by several of the above-mentioned researchers [7–9]. Little work has been carried out in #1 and #4 wastewaters. Therefore, the main objective of the present research was to compare the growth of *Chlorella* sp. on these four wastewaters and their abilities of removing nitrogen, phosphorus, COD, and metals. The suitability of introducing algae cultivation into a conventional wastewater treatment process flow is also discussed.

Methods

Algae Strain and Culture Condition

Algae strain was wild-type *Chlorella* sp. isolated from local freshwater. It was preserved in Tris–acetate–phosphorus [13] media containing the following chemicals: NH_4Cl 400 mg/L,

Table 1 Summary of major nutrient removal efficiencies by algal cultivation.

Algae species	Wastewater characteristics	N (%)	P (%)	Carbon	Retention time	Literature
Algal–bacterial symbiosis (*Chlorella* + *Nitzchia*)	Settled domestic sewage	92	74	97% BOD, 87% COD	10 h	[7]
Chlorella pyrenoidosa	Settled domestic sewage	93.9	80	NA	13 days	[8]
Cyanobacteria	Secondarily treated domestic effluent + settled swine wastewater	95	62	NA	1 day	[9]
Chlorella vulgaris	Diluted pig slurry (suspended solids content to 0.2%)	54–98	42–89	BOD_5 98%	4.5 days	[10]
Chlorella pyrenoidosa	Domestic sewage and industrial wastewaters from a pig farm and a palm oil mill	60–70	50–60	80–88% of BOD, 70–82% of COD	15 days	[11]
Mixed culture of *Chlorella* and diatom species	Wood-based pulp and paper industry wastewater			58%	42 days	[12]

NA not applicable

$MgSO_4 \cdot 7H_2O$ 100 mg/L, $CaCl_2 \cdot 2H_2O$ 50 mg/L, K_2HPO_4 108 mg/L, KH_2PO_4 56 mg/L, Tris (hydroxymethyl)aminomethane 2,420 mg/L, glacial acetic acid 1 mL, and trace elements solution 1 mL. Trace elements solution consisted of Na_2EDTA 50 g/L, $ZnSO_4 \cdot 7H_2O$ 22 g/L, $CaCl_2 \cdot 2H_2O$ 0.05 g/L, H_3BO_3 11.4 g/L, $MnCl_2 \cdot 4H_2O$ 5.06 g/L, $FeSO_4 \cdot 7H_2O$ 4.99 g/L, $CoCl_2 \cdot 6H_2O$ 1.61 g/L, $CuSO_4 \cdot 5H_2O$ 1.57 g/L, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 1.10 g/L, and KOH 16 g/L (Fishersci, USA). Algae were inoculated at 10% ($v_{inoculation}/v_{media}$) in 250 mL Erlenmeyer flasks containing 100 mL liquid medium. The culture flasks were incubated under stationary condition at 25±2°C, 200 μmol m^{-2} s^{-1} continuous cool-white fluorescent light illumination on a shaker with 100 rpm rotation speed. All the experiments were carried out in triplicate and average values were recorded.

Characteristics of Wastewaters

Wastewaters were collected from four different points in the Metropolitan Wastewater Treatment Plant at Saint Paul, Minnesota. They are wastewaters #1, #2, #3, and #4, which were sampled from tanks before primary settling, after primary settling, secondary settling tank, and centrate line after sludge centrifuge, respectively. All wastewaters were filtered using glass microfiber filters (934-AH, Whatman, USA) to remove large particles and indigenous bacteria. Ammonia nitrogen (NH_3–N), nitrate nitrogen (NO_3–N), nitrite nitrogen (NO_2–N), total nitrogen (TN), total phosphorus (TP), and COD were determined for all samples following the Hach DR 5000 Spectrophotometer Manual [14].

Determination of Algal Growth

Samples were taken from the culture media every day for measurement of optical density at 680 nm (OD_{680}) using a spectrophotometer (Genesys 5, Spectronic Instruments, UK) as the algal density indicator. The growth rate (GR, per day) was calculated by fitting the OD for the first 3 days of culture to an exponential function:

$$GR = (\ln OD_t - \ln OD_0)/t$$

where OD_0 is the optical density at the initial day, OD_t is the optical density measured on day t. Each recorded OD_t was corrected by taking away that of the corresponding blank sample. Algae were centrifuged and harvested before the wastewater was discharged.

Analysis of Inorganic Compounds

Liquid samples for nutrient consumption analysis were collected every other day during the 9-day test period. The collected samples were centrifuged at 5,000 rpm for 15 min and the supernatants were collected for analyses of NH_3–N, NO_3–N, NO_2–N, TN, TP, and COD. The measurements of NH_3–N, NO_3–N, NO_2–N, TN, TP, and COD were performed following the Hach DR 5000 Spectrophotometer Manual [14]. The metal ion concentrations in all of the four wastewaters before and after algal cultivation were analyzed. Ten milliliters of centrifuged samples were acidified with 1% (*v*/*v*) nitric acid before analysis. A set of 15 elements including Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, and Zn was analyzed by a inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000, USA), which consists of an echelle polychrometer and two solid-state detectors. The detectors are referred to as segmented-array charged-coupled devices. One is used for the ultraviolet range (167–375 nm) and the other for the visible range (375–782 nm). The spectrometer is purged with nitrogen gas. Nutrient removal rates were calculated by dividing the difference between the first day and final day concentrations by the first day concentration, then multiplied by 100, and expressed as percentage.

Results and Discussion

Properties of the Four Wastewaters

The chemical compositions of the four wastewaters are listed in Table 2. Primary settling did not change the chemical characteristics of the wastewaters much but, however, other treatments that the effluent and centrate received resulted in significant changes in chemical composition of wastewaters. The effluent is stabilized by the activated sludge process in which ammonium had been oxidized to nitrate, phosphorus absorbed, and COD significantly reduced. The effluent is dischargeable with further disinfection. The centrate is generated from a physical process (sludge centrifuge) and thus retains high levels of ammonium, phosphorous, and COD.

The optimal inorganic N/P ratio for freshwater algae growth was suggested to be in the range of 6.8–10 [15–17]. While taking a close look at the four wastewaters, it is noticed that only wastewaters before and after primary settling had close-to-optimal inorganic N/P ratios, 5.9 and 4.7, respectively. The inorganic N/P ratio of the effluent was 53.2, much higher than the optimal ratio, indicating a high phosphorus limitation. In contrast, the inorganic N/P ratio of the centrate was 0.36, much less than the optimal ratio, indicating a high nitrogen limitation.

Table 2 Characteristics of the four wastewaters sampled from the St. Paul Metropolitan Wastewater Treatment Plant.

Parameters	Wastewater before primary settling	Wastewater after primary settling	Effluent from aeration tank	Centrate from sludge centrifuge
NH_3–N (mg/L)	33.4±0.6	32.2±0.4	ND	71.8±1.1
NO_3–N (mg/L)	ND	ND	16.95±0.07	ND
NO_2–N (mg/L)	ND	ND	0.074±0.003	ND
TP (mg/L)	5.66±0.08	6.86±0.05	0.32±0.04	201.5±10.6
TN (mg/L)	40.65±0.07	38.95±1.91	19.1±0.1	131.5±2.1
COD (mg/L)	231.0±4.2	224.0±4.2	42.2±1.9	2250.0±99.0
Inorganic N/P	5.9	4.7	53.2	0.36

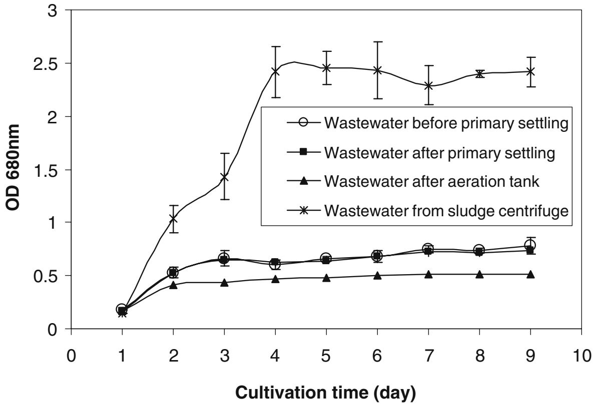
ND not detected

Algal Growth Curves in the Four Wastewaters

Algal growths in terms of optical density OD_{680} in the four wastewaters under axenic condition were plotted in Fig. 1. No lag phases were observed in all of the four curves, indicating that this wild-isolated algae *Chlorella* sp. could adapt well in all of the four wastewaters. Similar growth patterns, with exponential phases in the first 3 days followed by stationary phases in the next 6 days were present for all wastewaters, except the centrate, in which the exponential phase lasted 1 day more before entering into a stationary phase. Algae in wastewaters before and after primary settling had highly overlapped growth curves along the time, which coincided with the similar chemical composition of the two wastewaters, elucidating the strong relationship between the nutrient levels and algal growth [18].

Moreover, it can be found that the algal growth was significantly enhanced in the centrate because of its much higher levels of nitrogen, phosphorus, and COD than the other three wastewaters (Table 2). Therefore, the results show that the centrate is the best media for algal growth despite its highly unbalanced ratio of N/P. The stationary phase OD of centrate-grown algae was about five times of those grown on the other three wastewaters. The average specific GR in the first 3 days were 0.412, 0.429, 0.343, and 0.948 day^{-1} for wastewaters before and after primary settling, effluent, and centrate, respectively. The GR

Fig. 1 Algal growth curves in the four wastewaters



of algae grown in the effluent was also comparable to that found by Pouliot et al. [9] (0.34 day^{-1}) who used a mixture of settled manure and secondarily treated effluent with an ammonium nitrogen of 30 mg/L. Algal cells grew better in wastewater after primary settling than in the effluent because of the higher nutrients contained in the former one, which was also evidenced by Tam and Wong [8]. Thus, algal ponds with high inoculums might be more suitable to be installed as a secondary rather than a tertiary treatment process [8]. In addition, the centrate with very high nutrient level is found to promote rather than inhibit algal growth, which serves as the basis for applying algal process in MWTP in a new way to manage the nutrient load for the aeration tank to which the centrate is returned.

Inorganic Nitrogen, Total Nitrogen, Phosphorus, and COD

NH_4–N, the only inorganic nitrogen form in the wastewaters before and after primary settling and centrate, was significantly reduced. The removal rates for #1, #2, and #4 were 82.4%, 74.7%, and 78.3%, respectively (Table 3). Algae growth removed 62.5% of NO_3–N (the major inorganic nitrogen form in effluent) from the effluent with 6.3-fold of NO_2–N generated. The TN removal rates were 68.4%, 68.5%, 50.8%, and 82.8%, respectively (Table 3). This study shows that *Chlorella* sp. could use ammonium or nitrate, the two primary nitrogen sources for many organisms [19]. The relative constancy of uptake, irrespective of nitrogen source, is tentatively considered to be due to the saturation of the assimilator to the production of amino groupings for entry into nitrogenous metabolism. This homeostasis of nitrogen assimilation enables it to maximize growth in changing environmental conditions [20].

The increase in nitrite was accompanied by a decrease in nitrate in the effluent after cultivation of *Chlorella* sp. (Fig. 2b), which is an interesting phenomenon. Nitrate assimilation by plant involves two transport and two reduction steps to produce ammonium in the chloroplast [21, 22]. Therefore, nitrite is generated in the process of nitrate being reduced to ammonium and it is possible that part of the nitrite produced was excreted into the media [23].

Removal of phosphorus up to 90% (Table 3) from wastewater #1, #2, and #4 by algae growth was very effective. However, only 4.7% phosphorus was removed from the effluent (#3 wastewater). Table 4 shows the inorganic N/P ratios of the four wastewaters before and after algal cultivation. Compared to the optimal inorganic N/P ratio for algae growth which is in the range of 6.8–10 as mentioned before, effluent had a N/P ratio of 52.3, although it dropped to 20.8 at the end of the experiment, showing a severe phosphorus limitation for algal growth. However, the unbalanced N/P ratio of the centrate affected neither nitrogen

Table 3 Nutrient removal rates of growing algae in the four wastewaters.

Parameters removal rate	Wastewater before primary settling (%)	Wastewater after primary settling (%)	Effluent from aeration tank (%)	Centrate from sludge centrifuge (%)
NH_3–N	82.4	74.7	–	78.3
NO_3–N	–	–	62.5	–
NO_2–N	–	–	−6.297	–
PO_4–P	83.2	90.6	4.69	85.6
TN	68.4	68.5	50.8	82.8
COD	50.9	56.5	−22.7	83.0

Fig. 2 Nutrient evolution during the culture period. **a** Trends of ammonium nitrogen (NH_3–N) in wastewaters #1, #2, and #4. **b** Trends of NO_3–N and NO_2–N in effluent (#3). **c** Trends of TN in the four wastewaters. **d** Trends of TP in the four wastewaters. **e** Trends of COD in the four wastewaters

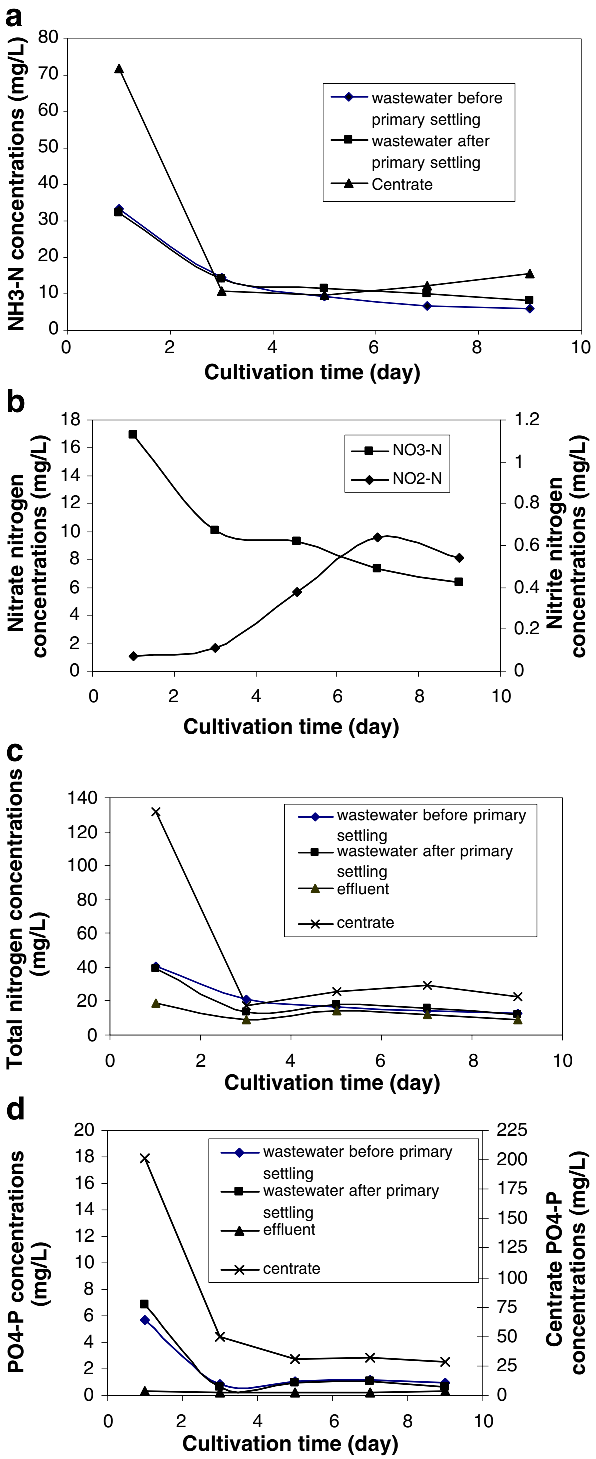
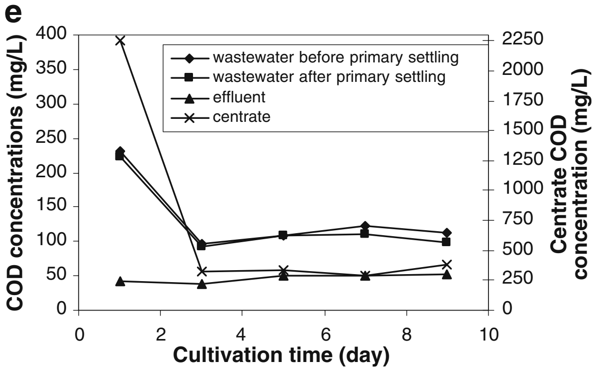


Fig. 2 (continued)



nor phosphorus removal, suggesting that both N/P ratios and the absolute levels of N and P must be considered in evaluating the effects of nutrient compositions on algal growth. Other studies [24, 25] also revealed that the nutrient removal efficiency is related to the level of nutrients in wastewater and the extent of nutrients utilized by algal growth or incorporated into algal tissues. Phosphorus in centrate, though around 30 times of that in wastewaters before and after primary settling, was removed at a comparable ratio, indicating that rapid and luxury uptake of phosphorus is an ability of the algae species *Chlorella* to synthesize and accumulate polyphosphates in their bodies [26].

COD removal efficiency varied much among different wastewaters. While removal rates of 50.9%, 56.5%, and 83.0% were achieved for wastewaters #1, #2, and #4, respectively, removal rate for wastewater #3 is a negative number, indicating that organics were excreted out instead of being taken up by algae grown in effluent. The two opposite results that happened to COD indicate two different metabolic pathways, i.e., heterotrophic and autotrophic growth of algae under different culture conditions. Eny [27] found that the metabolic pathway of *Chlorella* can alter with supply of organic substrates such as organic acids or glucose, which means that they can perform heterotrophic growth besides the common autotrophic one of using CO_2 as the sole carbon source. The organic substances may function directly as an essential organic nutrient [28] or act as an accessory growth factor [29]. Heterotrophic growth of *Chlorella* can proceed in a much faster way [30, 31] by directly incorporating organic substrate in the oxidative assimilation process for storage material production [32]. The carbon matters in effluent are mostly inert after activated sludge treatment and thus cannot be further utilized by algae. When the organic substrate is not available, autotrophic growth uses CO_2 as the carbon source, excreting small molecular organic substances such as glycolic acid to the environment as a product of photosynthetic carbon reduction cycle [33], which is the reason why COD in effluent increased after algal cultivation.

Table 4 Inorganic N/P ratios of the four wastewaters before and after algal cultivation.

Inorganic N/P	First day	Ninth day
Wastewater before primary settling	5.9	6.2
Wastewater after primary settling	4.7	12.7
Effluent	52.3	20.8
Centrate	0.356	0.538

Table 5 The initial and final concentrations of detectable metal ions.

	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
#1 W.W.											
First day	0.063±0.001	0.244±0.001	66.11±0.54	0.025±0.002	0.293±0.002	16.80±0.13	21.46±0.14	0.295±0.003	177.13±1.23	UD	0.046±0.000
Ninth day	0.008±0.002	0.495±0.211	51.17±1.45	0.022±0.003	UD	14.82±0.16	0.34±0.09	UD	164.8±1.3	UD	0.020±0.003
#2 W.W.											
First day	0.056±0.001	0.260±0.002	63.65±0.33	0.021±0.001	0.263±0.002	16.54±0.10	20.41±0.12	0.308±0.002	164.87±1.58	UD	0.046±0.002
Ninth day	0.012±0.005	0.640±0.050	47.31±0.51	0.019±0.001	UD	14.52±0.13	0.32±0.07	UD	151.47±0.83	UD	0.011±0.003
#3 W.W.											
First day	0.027±0.001	0.278±0.014	67.45±3.46	0.009±0.002	0.047±0.002	16.16±0.81	20.24±0.99	0.008±0.001	164.40±7.57	UD	0.064±0.006
Ninth day	0.008±0.003	0.498±0.041	36.69±0.29	0.010±0.002	UD	13.70±0.08	2.37±0.47	UD	147.3±1.8	UD	0.012±0.002
#4 W.W.											
First day	0.055±0.001	0.286±0.002	161.7±2.0	0.010±0.003	3.074±0.023	145.50±1.74	73.30±0.44	2.797±0.016	160.70±2.03	0.0273±0.0006	0.020±0.003
Ninth day	0.019±0.011	0.273±0.019	7.44±4.80	0.009±0.004	0.053±0.037	123.60±1.23	14.64±12.84	0.049±0.027	141.33±1.72	0.0187±0.0015	0.0087±0.0012

All units are in milligrams per liter

#1 W.W. wastewater before primary settling, *#2 W.W.* wastewater after primary settling, *#3 W.W.* effluent from aeration tank, *#4 W.W.* centrate, *UD* under detectable level

Figure 2 shows the dynamic changes in inorganic nitrogen, TN, phosphorus, and COD in the 9-day algal growth period. All nutrients decreased rapidly due to fast assimilation by algae in the first three culture days followed by slight increases as a result of release of cellular nutrients, suggesting that a retention time of 3 days is enough to achieve maximum nutrient reductions when using this strain under the current settings. Compared with other studies when maximum nutrient removals were achieved (Table 1), it is found that the retention time could be shortened to 10 h by introducing bacteria into the system, forming an algal–bacterial symbiosis, while the same treatment efficiencies were still obtained [7]. One bottleneck that hinders the widespread application of the algal treatment process is its relative long hydraulic retention time (HRT) to obtain efficient nutrient removal when compared with the conventional activated sludge process, which can achieve efficient overall reduction of COD, ammonium, and phosphorus within a much shorter HRT of 4–6 h [34]. Further research will focus on the algae–bacteria consortia on nutrient removal from wastewater and shortening the sampling interval to get better understanding of the nutrient dynamics during the exponential growth period.

Metal Ion Removal

The levels of Cd, Cr, and Pb, both before and after algal treatment, were under the detectable limits and, therefore, they are not included in Table 5. Al, Fe, Mg, Mn, and Zn were found to be removed from all the four wastewaters very efficiently, with removal rates ranging from 56.5% to 100% (Table 6). Microalgae were reported to be more efficient in sequestering metal species from solution than bacterial and fungal biomass [35]. The mechanism of the effectiveness in removing heavy metals from wastewater by microalgae is related to their large surface area and high binding affinity [36]. Different algal species have different sizes, shapes, and cell wall compositions, which affect their metal binding efficiency [37], and the cell wall, in particular, is the main binding site for metals [38]. A mathematical model was successfully applied by Khoshmanesh et al. [35] to describe the uptake of cadmium by algae, which includes two distinct steps in the process: an initial rapid uptake of metal ions due to attachment to the cell wall, followed by a relatively slow uptake due to membrane transport of the ion through the cell wall into the cell cytoplasm.

The distinguished removal (95.4%) of the high level of Ca in centrate by algae compared with those (22.6%, 25.7%, and 45.6%) in the other three wastewaters caught our attention, which needs further exploration of the possible causes. The computer program PHREEQC developed by Song et al. [39] suggested that, in a given solution with a certain phosphate concentration, the solution pH value and Ca/P ratio are two controlling factors for phosphate recovery by precipitation, an increase in either of which can enhance the thermodynamic driving force for the calcium phosphate precipitation. Therefore, besides the excessive uptake of phosphorus by algae cells as mentioned before, it is speculated that,

Table 6 Removal percentages for Al, Ca, Fe, Mg, Mn, and Zn in the four wastewaters after algal cultivation.

Metal	#1 W.W. (%)	#2 W.W. (%)	#3 W.W. (%)	#4 W.W. (%)
Al	87.3	78.6	70.4	65.4
Ca	22.6	25.7	45.6	95.4
Fe	100	100	100	98.3
Mg	98.4	98.4	88.3	80
Mn	100	100	100	98.2
Zn	56.5	76.1	81.2	56.5

with the high level of phosphorus and elevated pH observed in the centrate (final pH was approaching 10), calcium and phosphorus formed calcium phosphates, the precipitation of which helped remove both from the centrate significantly. Recovery of phosphorus as calcium phosphates than other forms is far more promising from the industry's viewpoint [40]. However, there are still a lot of obstacles from the technological side [41] because a lot of factors influence the precipitation of calcium phosphates, such as the concentrations of phosphate and calcium, the pH value [42], the ionic strength, the temperature, and the impurities of the solution [43, 44]. If the precipitation of calcium phosphorus by growing algae in the centrate is validated in the future, it would add one more side benefit to the centrate treatment by the algal process.

Conclusions

The results from this study demonstrated the feasibility of cultivating *Chlorella* sp. in four wastewaters sampled from different locations in MWTP. *Chlorella* sp. could adapt well in all of the four wastewaters with no lag phases observed. Algal growth was significantly enhanced in the centrate because of its much higher levels of nitrogen, phosphorus and COD than those in the other three wastewaters. The high phosphorus limitation in the effluent could not support a productive algal growth and efficient nutrient removal, opposing the idea of applying algal cultivation as a tertiary process. Although the treatment of wastewaters before and after primary settling is more efficient in nutrient reduction than that of effluent, nitrogen and COD could not be removed in a way comparable to current prevailing activated sludge process within a certain retention time. Thus, the proposal of applying it as a secondary wastewater treatment process is not ready for scale-up application either. However, the great growth in centrate offers a new option of applying the algal process in MWTP to manage nutrient load for the aeration tank where the centrate from sludge centrifuge is returned. The unbalanced N/P ratio of the centrate is found to affect neither nitrogen nor phosphorus removal, suggesting the importance of absolute abundance of both nutrients for algal growth, irrespective of the optimal relative ratio. Metal ions, especially Al, Ca, Fe, Mg, and Mn in centrate, were found to be removed very efficiently. Besides the luxury uptake and assimilation of phosphorus to polyphosphate by algae, the elevated pH after cultivating algae in centrate, causing the precipitation of calcium phosphates, may also contribute to a great extent of calcium removal.

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