Enhanced microalgae cultivation using digested kitchen waste sewage treated with struvite precipitation

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Abstract: This study demonstrated the feasibility of using struvite for nutrient management in algae cultivation on sewage wastewater. The results showed that struvite precipitation treatment dramatically reduced the ammonia in the digested kitchen waste sewage. The untreated sewage was unable to sustain continuous growth of algae, while the algae growth on the treated sewage was comparable with that on an artificial algae culture medium (BG11). The rapid growth on the treated sewage was accompanied by substantial removal of nutrients. The struvite precipitate recovered from the treated sewage was proven to be an alternative source of nitrogen, phosphorus and magnesium. The struvite precipitate was evidenced that it could substitute culture media in algae growth. This study showed that struvite treatment is viable for nutrient management of algae cultivation on sewage wastewaters which do not have suitable nutrient profiles.

Keywords: digested kitchen waste sewage, microalgae, nutrients removal, struvite precipitation treatment

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1 Introduction

Microalgae wastewater cultivation is attracting tremendous attentions because it can produce algae biomass and treat wastewater simultaneously[1]. Several researchers have demonstrated that some microalgae strains can well adapt to different wastewaters, such as municipal wastewater, agricultural manure wastewater and industrial wastewater[2-4].

However, not all the wastewaters are suitable for microalgae cultivation. Microalgae usually cannot survive well in the anaerobic digested sewage because of high concentration of ammonium could greatly inhibit algae growth[5-7]. Digested kitchen waste sewage is a typical sewage which contains high concentration of ammonium as a result of food protein decomposition. It cannot be discharged directly for its high density nutrients and unpleasant smell. If treated improperly, it will lead to secondary contamination.

In order to make digested sewage suitable for microalgae growth, a common method is to dilute the sewage with water[8,9]. The biomass productivity and nutrient removal efficiency were influenced by the dilution ratio of water to sewage. Normally, a large amount of water is needed to dilute wastewater in order to provide conditions suitable for algae growth and high

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biomass accumulation[10]. A very high diluting water to sewage ratio will result in much higher volume of liquid to be treated, which is counter-productive in terms of sewage treatment. Therefore, an alternative method to mitigate the inhibition of high concentration of ammonium without adding excess water to the sewage is desirable.

Struvite is a mineral formed with $MgNH_4PO_4 \cdot 6H_2O$, which is widely used to remove nutrients (especially nitrogen and phosphorus) from sewage[11-13]. The nitrogen removal efficiency can be more than 90% under certain conditions such as at optimal pH and dosages of the precipitation agents[14]. Furthermore, natural struvite rocks or struvite recovered from black water and urine were tested as slow release fertilizer for plants[15,16]. Struvite supplemented with nutrients was tested as alternative nutrient source for microalgae cultivation[17].

In this study, digested kitchen waste sewage was treated with struvite to remove ammonium through precipitation. The growth of microalgae and further removal of nutrients from the treated sewage were studied and the inhibitive effect of ammonia on microalgae was evaluated. The struvite recovered from sewage was tested as nutrient source for algae cultivation in artificial medium.

2 Materials and methods

2.1 Microalgae strain and growth medium

The microalgae strain *Chlorella vulgaris* was purchased from the Freshwater Microalgae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, China. The algae culture used BG11 medium: $NaNO_3$ (1500 mg/L), K_2HPO_4 (40 mg/L), $MgSO_4 \cdot 7H_2O$ (75 mg/L), $CaCl_2 \cdot 2H_2O$ (36 mg/L), Na_2CO_3 (20 mg/L), Citric acid (6 mg/L), Ferric ammonium citrate (6 mg/L), EDTA-Na_2 (1 mg/L) and A_5 which contains H_3BO_3 (2.86 mg/L), $MnCl_2 \cdot 4H_2O$ (1.81 mg/L), $ZnSO_4 \cdot 7H_2O$ (0.222 mg/L), $CuSO_4 \cdot 5H_2O$ (0.079 mg/L), $CoCl_2 \cdot 6H_2O$ (0.050 mg/L), $NaMoO_4 \cdot 2H_2O$ (0.391 mg/L). Cultivation was done in 500 mL flasks with 300 mL of liquid culture at 25℃, an irradiance of 50-60 μmol photons/(m^2 s), and a 16/8 h day/night cycle. Cultures were kept suspended at 120 r/min using the shaking incubator.

2.2 Digested kitchen waste sewage and struvite treatment

Digested kitchen waste sewage was obtained from a fermentation plant of Beijing Sanitation Group. Large solid particles were removed through sedimentation and centrifugation (4000 r/min, 10 min).

Before struvite treatment, the pH of sewage was adjusted to 9.0 with NaOH. Based on the initial concentration of NH_4-N (1230 mg/L, 0.068 mol/L) in sewage, 0.082 mol/L of $Na_2HPO_4 \cdot 12H_2O$ and $MgSO_4 \cdot 7H_2O$ was added to adjust the molar ratio of NH_4^+: Mg_2^+: PO_4^{3-} to 1:1.2:1.2. Then the sewage was shaken at 100 r/min, 40℃ for 0.5 h, and settled at room temperature overnight to precipitate the struvite. The next day the sewage was centrifuged (4000 r/min, 10 min) again, and the supernatant was autoclaved at 121℃ for 20 min. Finally, the pH of the supernatant was adjusted back to 7.0±0.1 with HCl. Then the raw sewage was autoclaved and its pH was adjusted to 7.0±0.1. The struvite precipitate recovered was washed with distilled water twice and dried at 37℃ until constant weight was achieved.

2.3 Microalgae cultivation in untreated and treated sewage

The raw sewage (RS) and struvite treated sewage (STS) were both used as the culture media. Algae seeds in a linear growth phase were centrifuged (4000 r/min, 10 min) and the supernatant was removed and cell pellets were washed and re-suspended with distilled water to avoid the effect of remaining synthetic medium from the seed. Then the algae cells were inoculated into each flask containing 50 mL sewage. The initial algae cell concentration was approximately 3.4×10^6 cells/mL. The microalgae growing in BG11 was used as the control. Cultivation conditions were described above in 2.1. All experiments were carried out in duplicate.

2.4 Use of struvite precipitate as a nutrient source

Struvite precipitate recovered from the treated sewage was examined whether it could replace the nitrogen, phosphorus and magnesium sources in BG11. Component1 (C1) was positive control (BG11); Component2 (C2) was negative control (BG11 without

$NaNO_3$, K_2HPO_4 and $MgSO_4·7H_2O$); in Component3-Component5 (C3–C5), 630 mg/L, 1260 mg/L or 2520 mg/L struvite precipitate was added to negative control, respectively. All these components are listed in Table 1. The inoculation and cultivation conditions were the same with 2.3.

Table 1 Components for the tests

Chemicals	C1	C2	C3	C4	C5
$NaNO_3$/mg·L^{-1}	1500	0	0	0	0
K_2HPO_4/mg·L^{-1}	40	0	0	0	0
$MgSO_4·7H_2O$/mg·L^{-1}	75	0	0	0	0
$CaCl_2·2H_2O$/mg·L^{-1}	36	36	36	36	36
Na_2CO_3/mg·L^{-1}	20	20	20	20	20
Citric acid/mg·L^{-1}	6	6	6	6	6
Ferric ammonium citrate /mg·L^{-1}	6	6	6	6	6
EDTA-Na_2/mg·L^{-1}	1	1	1	1	1
A_5/mL·L^{-1}	1	1	1	1	1
Struvite precipitate/mg·L^{-1}	0	0	630	1260	2520

2.5 Analysis

2.5.1 Algae density

Algae samples were collected every 2 d and properly diluted, then the samples were added to the blood cell counting chamber (XBK25, Shanghai, China). Afterwards, the chamber was placed under a microscope and the algae cells were counted. Cell number was calculated using the following formula[18]:

$$\text{Number of cells} = \text{total counted cells} \times 10^4 \times \text{dilution factor} \quad (1)$$

2.5.2 Water quality and nutrients removal rates

Liquid samples in Experiment 1 were collected every 2 d and centrifuged (4000 r/min, 10 min), and the supernatants were properly diluted, and analyzed for total nitrogen (TN), ammonium (NH_4-N), chemical oxygen demand (COD) and total phosphorus (TP) concentration using the Spectroquant Pharo 300 (Merck, 2012). The major water quality parameters were measured according to the Monitoring Method of Water and Wastewater[19]. Nutrients removal efficiency was expressed by dividing the difference between the initial concentrations of the sewage and the harvested culture solution by initial concentrations.

2.5.3 Statistical analysis

The data were analyzed with Excel 2010 and SPSS 12.0 statistical software. One-way ANOVA and LSD tests were used to compare the results. Differences were considered significant when p values were below 0.05.

3 Results and discussion

3.1 Sewage nutrients removal by struvite treatment

The composition of sewage was listed in Table 2. After struvite treatment, TN and NH_4-N were dramatically decreased due to the addition and precipitation of struvite. In addition, the high temperature of autoclave also contributed to reduction in ammonium. The removal efficiencies for TN and NH_4-N were 84.1% and 87.9%, respectively. Meanwhile, 19.5% of COD was reduced. On the contrary, TP was increased because of the overuse of $Na_2HPO_4·12H_2O$.

Table 2 Water quality in different sewage

	RS/mg·L^{-1}	STS/mg·L^{-1}
TN	1431±112	228±11
NH_4-N	1230±0	149±6
COD	2185±47	1878±39
TP	23.5±0.7	111.0±1.4

The removal efficiency for TN and NH_4-N was below 90%, because the reaction conditions were not optimal (e.g., pH was relatively low). Moreover, the purpose of this study was to reduce rather than eliminate ammonium. According to Cai et al.[6], the residue ammonium was unlikely to inhibit algae growth but can be used by microalgae. Besides, the results showed that a fraction of COD could be removed in previous studies[20], indicating that part of organics can be precipitated along with struvite precipitation. In summary, the struvite treatment successfully made the raw sewage more favorable for microalgae cultivation.

3.2 Sewage nutrients removal by microalgae

Figure 1 shows the COD removal in STS during cultivation period. COD was decreased obviously in STS in the first 4 d. After that, COD concentration was maintained at the same level and fluctuated in a minor range during the culture period. It seems that there was a portion of organic sources can be utilized by microalgae, while the others remained unavailable, thus COD remained at a certain level. The final removal efficiency was 21.4%. This result was similar with previous study[21].

The removal of TN, NH_4-N is shown in Figure 2.

On day 4, the residual TN was 102 mg/L, more than half of the TN was removed. After 14 d, TN removal efficiency of STS was 66.7% finally. Similarly, NH_4-N was reduced along with cultivation time, especially in the first 4 d. At the end of the cultivation, NH_4-N was reduced to 2.5 mg/L, indicating a 98.3% removal. To sum up, because of its high efficiency and no lagging phase, *Chlorella vulgaris* had outstanding performance for removing ammonia.

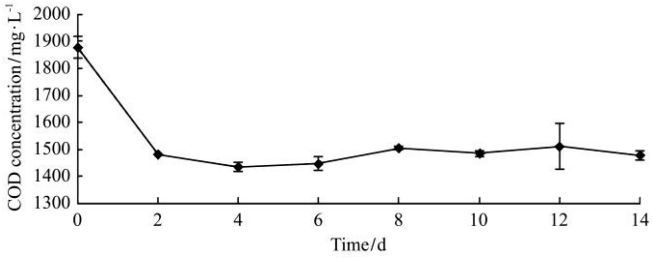
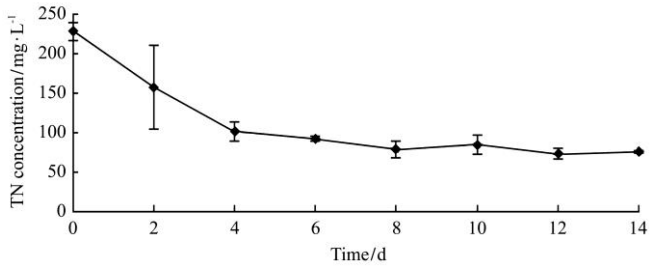
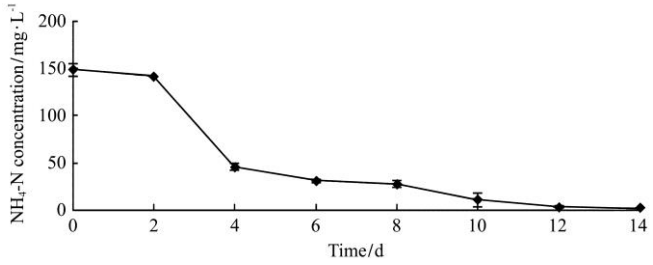


Figure 1 COD removal in STS for 14 d



a. TN



b. NH_4-N

Figure 2 TN (a) and NH_4-N (b) removal in STS for 14 d

Figure 3 shows that TP was removed in STS during culture time. In the first 8 d, TP decreased rapidly; after that, the removal tendency slowed down; and the residual TP was 24 mg/L and removal efficiency was 78.4% in day 14. According to Powell et al.[22], high temperature contributed to the accumulation of polyphosphate in microalgae. However, in this study, the temperature used was 25 ℃. If culture temperature was elevated, the TP residue in the STS may be less.

After two steps treatment, the removal efficiency of COD, TN and NH_4-N was 32.4%, 94.7% and 99.8%, respectively. A majority of the nutrients were removed, especially for the NH_4-H, which nearly reached the complete removal. Only TP maintained the original level, because there was extra PO_4^{3-} added in the first step. Through the second step, excessive TP was removed by microalgae. If the cultivation was prolonged, or the algae inoculum dosage was doubled, there will be less residual of TP.

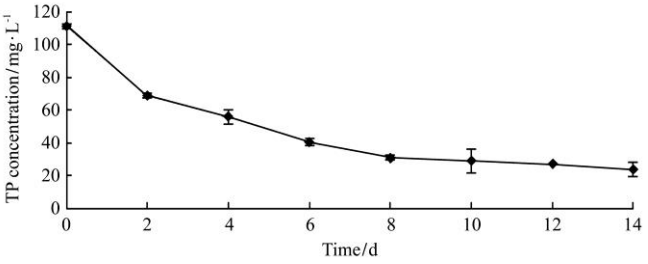


Figure 3 TP removal in STS for 14 d

3.3 Microalgae biomass production

Figure 4 shows the growth curves of *Chlorella vulgaris* in different culture media. A lag phase of 4 d was observed in STS. Then *Chlorella vulgaris* had a significant growth in the next 6 d and algae number was even higher than that in BG11 (from day 8 to day 10). The algae reached maximum microalgae density of 3.8×10^7 cells/mL on day 12 of cultivation. On the other hand, *Chlorella vulgaris* cannot survive in RS during the whole culture period. The algae density had merely a minor fluctuation and the maximum achieved on day 10 was 4.1×10^6 cells/mL.

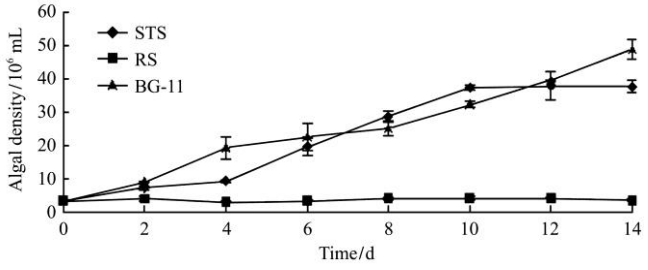


Figure 4 Microalgae growth curve in STS, RS and BG11.

Table 3 presents the multiple comparisons of biomass in different culture. Though *Chlorella vulgaris* grew better in STS for 4 d, the final density was less when compared to that in BG11. The reason may be the relatively less light penetration in STS. Unlike dilution, the turbidity in STS was still high, because there are still a lot of colored fine particles after the struvite treatment, which may suppressed microalgae photosynthesis and resulted in less biomass[23]. In addition, STS contains

little organic materials that microalgae cannot utilize. Therefore, microalgae could not grow in the more effective and efficient mixotrophic way. Furthermore, exogenous CO_2 supply and pH control would boost algae productivity.

Table 3 Multiple comparisons of STS with different components of biomass in different culture

Culture	Mean difference	Standard error	*p*-value	95% Confidence Interval	
				Lower Bound	Upper Bound
RS	685.500*	38.689	0.000	562.37	808.63
BG11	–225.000*	38.689	0.010	–348.13	–101.87

Note: *significant difference (p<0.05).

3.4 Use of struvite precipitate as a nutrient source for algae cultivation

Figure 5 shows the cell density after 14 d cultivation for algae grown on different culture media containing different levels of struvite added to BG11 recipe with nitrogen, phosphorus and magnesium removed. The algae density for different recipes is in the scending order of C2, C3, C4, C5 and C1. The lowest was in C2, indicating that absence of NO_3^-, PO_4^{3+} and SO_4^{2-} in the media was unable to sustain algae growth. The density increased with increasing struvite precipitate. The growth on C5 was slightly lower than C1.

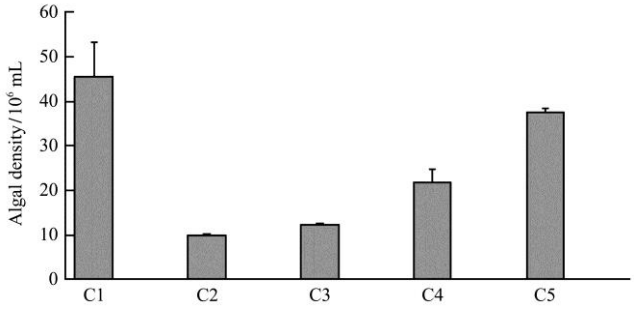


Figure 5 Microalgae density in C1-C5.

Table 4 Multiple comparisons of C1 with different components of biomass in different components

Components	Mean difference	Standard error	*p*-value	95% Confidence Interval	
				Lower Bound	Upper Bound
C2	35.60000*	3.73928	0.000	25.9879	45.2121
C3	33.30000*	3.73928	0.000	23.6879	42.9121
C4	23.80000*	3.73928	0.001	14.1879	33.4121
C5	8.12500	3.73928	0.082	–1.4871	17.7371

Note: *significant difference (p<0.05).

The algae cells in C5 were 3.74×10^7, which is slightly less than that in C1. However, the results from one-way ANOVA showed no statistical difference between C1 and C5, which mean struvite can replace $NaNO_3$, K_2HPO_4 and $MgSO_4\cdot7H_2O$ in BG11. Moreover, the struvite was recovered from sewage, and its price is much lower than BG11. Hence, the reuse of struvite can be significantly cheaper than the use of BG11 for algae cultivation.

4 Conclusions

Microalgae were unable to fully and potentially grow on raw digested kitchen waste sewage. The struvite treatment could increase microalgae growth on the undiluted the sewage rapidly, this enhanced growth coincided with ammonia reduction dramatically. Struvite precipitate recovered from the treated sewage capture a majority of ammonium and a fraction of COD present in the raw sewage. The algae also removed TN, NH_4-N, COD and TP simultaneously. Furthermore, struvite precipitate proved to be suitable substitutes of nitrogen, phosphorous and magnesium sources in the artificial algae medium, suggesting a great potential of using struvite to manage nutrient needs for microalgae cultivation.

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