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Microalgae Cultivation Using Municipal Wastewater and Anaerobic Membrane Effluent: Lipid Production and Nutrient Removal

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Abstract: Microalgae cultivation using wastewater is a combined process for pollutant removal and lipid production that has been widely studied in recent years. In this study, the effects of anaerobic membrane effluent (AME) and municipal wastewater (MW) ratios on microalgae growth and pollutant removal processes were investigated, and the lipid production properties were also explored. Results show that microalgae can grow in all AME/WW ratios, and a 40% AME content is the optimal condition for microalgal biomass accumulation (52.9 mg/L·d) and lipid production (0.378 g/L). Higher AME addition would inhibit microalgae growth. In addition, high ammonia (approximately 97%) and phosphate (around 90%) removal efficiencies can be achieved in all AME/WW ratio conditions, while the total nitrogen removal efficiencies decreased with the addition of AME. Total nitrogen and phosphate are the limiting factors in treating water to meet the requirements of the integrated wastewater discharge standard. This study provided a new method for anaerobic digestion and municipal wastewater treatment and also realized green energy production based on the sustainable development principles.

Keywords: municipal wastewater; anaerobic digestate; nutrient removal; microalgae cultivation; green energy

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1. Introduction

Wastewater, as one of the dominant environmental pollution sources due to its significant accumulation and complicated components, can be divided into high-strength (e.g., livestock and industrial wastewater) and low-strength (e.g., municipal wastewater) types according to the pollutant content [1]. Although many treatment processes have been developed to manage wastewater and reduce its negative impacts, their high operation cost, energy input and excess sludge production necessitate new alternative technologies. Therefore, how to effectively reduce the operating cost, improve pollution control capacities and even wastewater treatment processes has been widely studied by researchers in recent years [2,3].

For high-strength wastewater, anaerobic digestion is a common pathway for energy recovery and pollution control [4–6]. In particular, the anaerobic membrane bioreactor (AnMBR), as a novel system, has been widely explored as a way to enhance methane production rate and improve the effluent quality [7,8]. With the assistance of membrane filtration, organic matter can be effectively intercepted in the reactor and degraded into methane by microorganisms. This is regarded as a promising technology for high-efficiency energy recovery [9,10]. However, a large amount of anaerobic digestate (AD) containing high contents of ammonia and phosphate is produced, which can seriously damage the environment without proper treatment [11]. Thus, how to effectively reduce the nutrients and organics from anaerobic digestate has become a hot research topic.

Anaerobic digestate is commonly separated into solid and liquid fractions to reduce the volume, decrease the transportation cost and alleviate storage equipment requirements [12]. Liquid digestate accounts for approximately 80–90% of the total digestate mass, containing a high content of macro elements, including carbon (C), nitrogen (N), phosphorous (P), potassium (K) and inorganics (e.g., Na, K, Ca, Mg, Fe and Al), and is thus suitable for nutrient recovery. However, contaminants such as heavy metals in digestate emphasize the necessity of appropriate management for digestate before its safe discharge to the receiving environment [13]. Digestate is commonly used for agricultural applications as a fertilizer and growing medium, which is a straightforward and economically feasible digestate valorization route [11]. However, the transportation cost is high; nutrient surplus may occur; and pathogens, recalcitrant organics and heavy metals may accumulate in the field in which it is applied, which has led to farmers decline the use of digestate owing to the competitively low prices of inorganic fertilizers. Valorizing digestate into high-value products has recently been proposed as a promising waste recycling strategy. Liquid digestate could be a renewable resource if adequately recovered and utilized. Strategies including the recovery of nutrients and cultivation of microalgae for biofuel production are of significant practical interest. But how to properly combine pollutant removal and nutrient recovery needs further investigation.

Municipal wastewater (MW), as a typical representative of low-strength wastewater, is produced in great quantities due to rapid urbanization and economic development, and has become the dominant environmental pollution source and a threat to human health. Although various wastewater treatment processes have been designed and applied for pollutant removal and risk reduction, their high operation cost and complicated operating processes restrict their practical application [7,14]. In one study, researchers attempted to withdraw the nutrients and energy from MW and reduce the operating cost or even improve the MW treatment processes by providing new treatment pathways [8,15]. By using integrated technologies, nutrients (nitrogen and phosphate) and organic matter can be effectively removed and utilized to produce high-value products through anaerobic processes [16,17]. However, due to the low content of organic matter in MW, AD processes for energy recovery cannot match the energy input, which is not very attractive or sustainable [17,18]. Additionally, nutrient concentrations in MW are also very low and exist as organic and inorganic forms, which makes high-efficiency nutrient recycling difficult. So, how to reduce nutrients from MW with a low-energy-consuming process has been widely explored by researchers.

Due to their high lipid content and short cultivation period, microalgae are regarded as the ideal feedstock for biodiesel production [13]. To cultivate microalgae, a large amount of nitrogen and phosphate and other nutrient elements are required, which hinders the large-scale application of this technology [3,19]. To reduce the cost of cultivation, researchers have attempted to search for an alternative medium for microalgae cultivation, such as food waste, cow manure and municipal wastewater [20,21]. Wastewater as a pollutant source contains a high content of nutrients (e.g., nitrogen, phosphorus and potassium), which can be utilized as a potential culture medium for microalgae [22,23]. During microalgae cultivation, the nutrients in wastewater can be effectively assimilated, organics can be partially reduced, and other micropollutants can be removed, which makes it an energy-saving wastewater treatment method [24]. In addition, microalgae can capture carbon dioxide (CO_2) and release the oxygen into wastewater, which contributes to alleviating global warming [25]. Recently, a variety of wastewater types have been successfully applied for cultivating microalgae, including municipal, livestock, agricultural and industrial wastewater [20,26–28]. Combining wastewater treatment and bioenergy production has thus become a hot research topic.

As was mentioned previously, anaerobic digestate contains a high content of nutrients, and can thus be used for microalgae cultivation. However, due to their high organics content and inhibition from nutrient imbalance, microalgae cannot grow well in raw anaerobic digestate [29,30]. Furthermore, the lipid productivity and content in microalgae cells cultured under high-nutrient-content conditions are always unsatisfying [30]. In one study, some researchers attempted to inoculate microalgae with diluted AD to reduce the negative impacts [30]. However, a large amount of clean water was wasted, and large volumes of wastewater were generated after microalgae collection. Therefore, understanding how to cultivate microalgae using digestate is crucial for both effective energy production and pollutant removal.

In this research work, effluent from an anaerobic membrane bioreactor (AnMBR) for food wastewater treatment and WW were used as nutrient sources for microalgae cultivation to simultaneously realize lipid production and pollutant removal. The microalgae growth and lipid production properties under different WW/AME ratios were analyzed, the ammonia, total nitrogen and phosphate removal performance during the cultivation processes was explored, and the relationships between pollutant removal efficiency and microalgae cultivation were evaluated. The results of this study provide a new method for high-efficiency wastewater treatment and green energy production through microalgae cultivation.

2. Materials and Methods

2.1. Municipal Wastewater and Anaerobic Digestate

The MW was collected from the influent of a local wastewater treatment plant (WWTP) in Chengdu, China. To avoid the negative impacts of particles in the MW on microalgae growth, the sampled MW was filtrated through filter paper (with a pore size of 1–3 μm) and stored at 4 °C for further use. The AME was obtained from a lab-scale AnMBR for food waste treatment. The AnMBR was stably operated for about 100 days using food wastewater as a substrate. The AME in this study was collected when the hydraulic retention time and sludge retention time of the AnMBR reached 10 days and 30 days, respectively. The characteristics of the MW and AME are shown in Table 1.

Table 1. Characteristics of the municipal wastewater and anaerobic digestate.

	Unit	MW	AME
Chemical oxygen demand (COD)	mg/L	180–290	500–800
Soluble COD (SCOD)	mg/L	60–150	480–570
Ammonia	mg/L	20–30	150–200
Total nitrogen (TN)	mg/L	30–35	160–230
Phosphate	mg/L	0.8–2	10–20
Total phosphate	mg/L	1.5–5	12–27

2.2. Microalgae

The microalgae (*Didymogenes* sp. CDU-W13) used in this study was a new species isolated by our research team from a natural lake in Chengdu characterized by a higher tolerance to high ammonia content, a fast growth rate and satisfactory lipid production. Characteristics and bioinformation of the *D.* CDU-W13 are described in the supplementary materials and can be found in the NCBI (https://www.ncbi.nlm.nih.gov/nuccore/MW812295 (accessed on 26 June 2023)). To begin the experiment, microalgae were firstly inoculated in the BG11 solution for 5 days at room temperature (25 °C) to achieve an optical density (OD) value higher than 1.0, and then acclimated using the MW for 10 days. When the final OD value was higher than 2.0, the acclimated microalgae were then collected and used as an inoculum for the following studies.

2.3. Microalgae Cultivation Processes

It has been reported that a high content of NH_4^+-N significantly affects microalgal vitality [31]. To investigate the effect of nutrient content on microalgae growth and lipid production, and also the pollutant removal performance, microalgae were cultivated in reactors with different AME contents (0 to 100%). The AME was added into the reactor with different volumes, and then the MW was added to reach a total volume of 1.5 L. Thereafter, microalgae (200 mL) were inoculated into each reactor. Algae grown in BG11 medium were used as the control. Air (0.1 L/min) was introduced using a pump to mix the culture liquid and provide carbon dioxide. The experiment was carried out for 21 days, and each group was set up in triplicate.

2.4. Analytical Methods

2.4.1. General Parameters

Samples were periodically obtained from each reactor and used to analyze the variations in pollutant concentration. The samples were centrifuged (4000 rpm) for 10 min at 4 °C and filtrated through 0.45 μm filters to analyze SCOD, ammonia and phosphate. The pH and COD measurements were performed in accordance with the American Public Health Association (APHA) standard methods [32]. All the analyses of individual samples were performed in triplicate.

2.4.2. Determination of Microalgae Growth

Microalgae culture liquid (3 mL) was taken from each reactor every day, and then measured with a spectrophotometer at a wavelength of 680 nm (OD_{680}). According to previous studies, OD was related to the suspension of algal cells due to chlorophyll absorption [29,33]. Therefore, the dry weight concentration (DWC, g/L) of microalgal biomass can be obtained according to the variations in the OD value.

2.4.3. Lipid Extraction and Analysis Processes

The lipid extraction and analysis processes were carried out according to previous studies [29]. At the end of cultivation, microalgae culture (50 mL) was collected and centrifuged at 10,000 rpm for 5 min. Thereafter, the supernatant was discarded and pelleted microalgae cells were stored at −80 °C. The microalgae pellets were then lyophilized with a freeze dryer for 12 h and re-suspended in 6 mL of a methanol/chloroform mixture (1:2, *v*/*v*) using a vortex mixer. Thereafter, the solution was centrifuged at 10,000× *g* for 10 min, and the supernatant was transferred to a new tube. The supernatant was then mixed with 1.25 mL of 0.1 M KCl and centrifuged at 10,000× *g* for 10 min. The bottom layer was extracted and inserted into a nitrogen blowing instrument (LC-DCY-24G, Lichen, Shanghai, China) for half an hour to one hour. The weight of dried lipids was obtained accordingly.

2.5. Statistical Analysis

All experiments were performed in triplicate, and the results were assessed using analysis of variance (ANOVA), with statistical significance designated as $p < 0.05$.

3. Results and Discussions

3.1. Microalgae Growth Properties

The properties of microalgae growth in cultures with different AME contents were firstly explored. Microalgal biomass (in terms of OD_{680}) increased with cultivation time and exhibited different tendencies under different MW/AME ratios (Figure 1a). During the first 2 days, the OD value in all reactors slightly increased from 0.9 to 1.1, which might be due to the fact that microalgae need time to acclimate to the growth conditions [34]. Thereafter, the OD value in all reactors gradually increased, indicating that MW and AME can be utilized as nutrient sources for microalgae cultivation after a short-term adjustment.

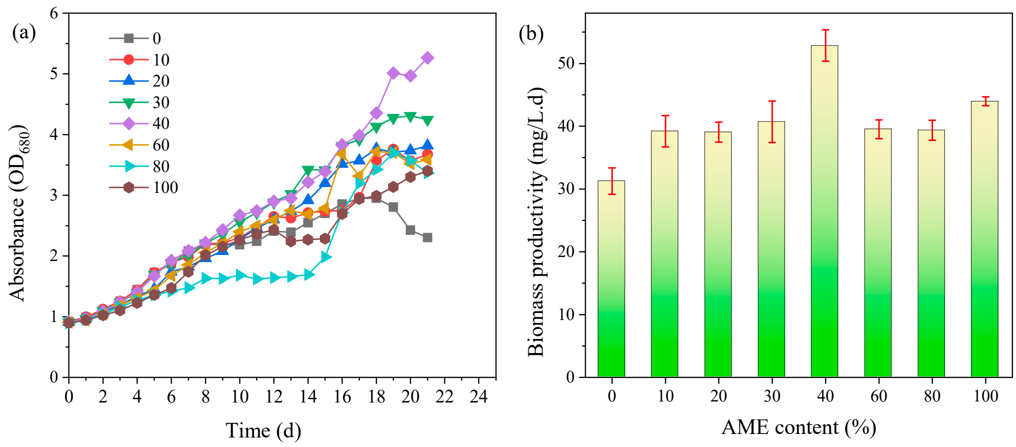

Figure 1. Properties of microalgae growth under different MW/AD ratios. (**a**) OD value, (**b**) biomass productivity.

In the reactor with only MW, the OD value continuously increased to 2.952 after 18 days, showing a biomass productivity of 31.3 mg/L·d (Figure 1b), which indicates that MW can be used for microalgae cultivation and realize a high algae biomass production rate. However, the OD value gradually decreased to 2.302 on Day 21, which might be due to the fact that the nutrients were exhausted at this stage and the microalgae cells self-degraded. A similar phenomenon was observed by Xu et al., who found that a nutrient shortage would result in microbial cell reduction [35]. However, when the AME content was increased to 10% (MW/AME ratio of 9:1), the final OD value increased to 3.675, showing that more microalgae biomass was produced when more liquid digestate was added, which is mainly due to the fact that with the addition of AME, more nutrients (ammonia and phosphate) were provided to microalgae cells, which promoted the microalgal growth rate. Further increasing the AME content to 20%, 30% and 40% led to increases in the final OD value to 3.819, 4.248 and 5.264, respectively, which further verified that the addition of AME can enhance microalgal growth and improve biomass production. However, further increasing the AME content to over than 60% (MW/AME ratio of 4:6) resulted in a lower final OD value. It was found that the final OD value was 3.588 at an AME content of 60%, but it slightly decreased to 3.420 when the AME content increased to 100% (only AME), which might be due to the fact that a high nutrient content would inhibit the microalgae growth. This is consistent with other studies showing that higher nutrient concentrations inhibit microalgae growth and biomass accumulation [28,36]. In addition, after 10 days, ammonia is almost exhausted, and microalgae biomass continuously increases after a stagnation period, which might be due to the fact that the microalgae adjusted to the use of other nitrogen compounds (e.g., nitrite or nitrate). Thus, the growth rate is different. For example, in the reactor with 60% AME, the OD was maintained at 2.5 for three days, and then gradually increased to 3.5 in five days. The relationships between microalgae and nitrogen sources will be further studied in the near future.

Biomass productivity showed increasing tendencies when the AME content was lower than 40% and then decreased at a higher AME content. At an AME content of 40%, the biomass productivity was 52.9 mg/L·d, which is much higher than that achieved with only MW (31.3 mg/L·d) and AME (43.9 mg/L·d), indicating that increasing the nutrient concentration to a certain level can promote the microalgae growth rate, but nutrient concentrations that are too high will inhibit the algal metabolisms. It has been widely reported that, beyond a certain concentration threshold, ammonia is toxic to microalgae growth, and the photosynthetic process occurs through the following mechanisms [31]: firstly, ammonia may cause damage to the oxygen-evolving complex (OEC) of the photosystem II, acting as an uncoupler of the Mn cluster of the OEC and displacing a water ligand; secondly, ammonia can diffuse through membranes and accumulate in algae cells, acting as an uncoupler and disrupting the ΔpH component of the thylakoid proton gradient. In this study, when the MW and AME ratio was 6:4, the microalgae exhibited the highest growth rate and biomass amount.

3.2. Lipid Production

Lipids are the main component of microalgae used for biodiesel production and are also an essential factor in the evaluation of the energy recovery from microalgae. It was found that the lipid production varied with the MW and AME ratios (Figure 2). In the reactor with only MW, the lipid production was around 0.278 g/L, while it increased to 0.291 g/L at an AME content of 10%. Further increasing the AME content to 40% (MW/AME ratio of 6:4) led to an increase in the lipid concentration to 0.378 g/L, which is 36% higher than that achieved in the reactor with only MW, which indicates that adding AME can effectively promote lipid accumulation and benefit bioenergy production through microalgae cultivation. Further increasing the AME content led to a decrease in lipid accumulation. In the reactor with MW/AME ratios of 4:6 and 2:8, the lipid concentration was 0.246 g/L and 0.298 g/L, respectively. Generally, lipid production depends on the accumulated microalgae biomass and lipid content in algal cells, which is influenced by cultivation conditions. According to a previous study [37], microalgae cells can accumulate large amounts of lipids under specific stress conditions, such as nutrient deficiency and unfavorable cultivation conditions. However, in these conditions, the growth rate of microalgae is always inhibited, which finally results in low lipid production. The significant increase in lipid productivity with digestate addition is consistent with previous studies on microalgae cultivation using wasted organics sources, such as cow manure digestate [38], garden waste [21] and food waste [33]. Therefore, anaerobic digestate of food wastewater from an AnMBR can be utilized as a promising nutrient source for lipid production.

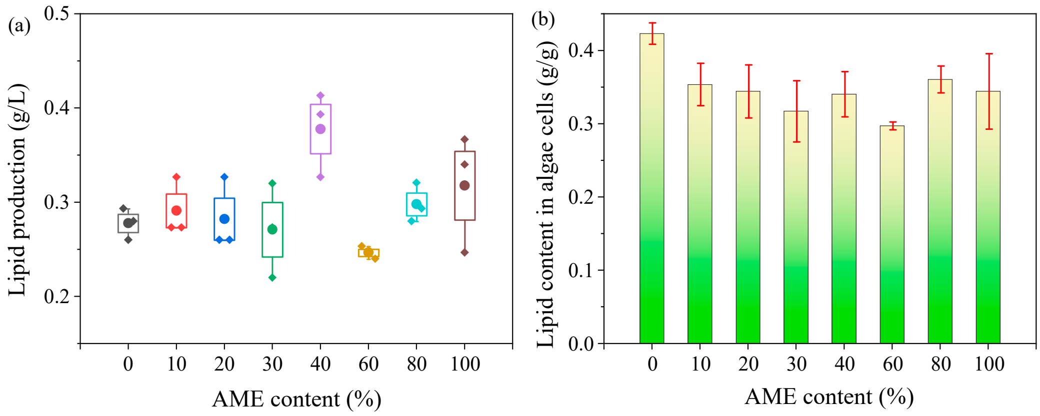

Figure 2. Lipid production through microalgae cultivation under different AME contents. (**a**) Lipid production, (**b**) lipid content in microalgae cells.

However, the lipid content in microalgae biomass was very similar. As is shown in Figure 2b, the lipid content in microalgal biomass ranged from 0.297 g/g to 0.361 g/g. It can thus be deduced that microalgae can accumulate lipids even under higher ammonia content conditions (around 100 mg/L). In addition, although the lipid productivity in the reactor with an AME content of 40% was the highest, the lipid content was relatively lower (0.34 g/g), which is consistent with other studies showing that the lipid content and saturation degree of fatty acids significantly decreased with sufficient nutrients [37,39]. The microalgae cultivated using only MW had the highest lipid content (0.42 g/g), which might be due to the fact that microalgae accumulate lipids under nutrient shortage conditions. The nutrients (nitrogen and phosphate) were used up in 8 days (Section 3.2), which may provide conditions for lipid accumulation in microalgae cells. Similarly, in the reactors with an AME content higher than 60%, the lipid content was also higher, which may result from the stress from high nutrient concentrations in the reactors. In addition, the COD/TN ratio may also change the microalgal metabolisms and affect the lipid production [23,26].

Therefore, it can be concluded that properly adding digestate can not only promote algae biomass accumulation, but also enhance lipid production.

3.3. Pollutant Removal through Microalgae Cultivation

3.3.1. Ammonia Removal

Ammonia is the main pollutant in MW and AME, but is also a favorable nutrient for microalgae growth. It can be clearly seen in Figure 3 that ammonia content decreased with the microalgae cultivation and was completely removed in all reactors. With the addition of AME, the initial NH_4^+-N concentration increased from 31.6 mg/L to 117.4 mg/L. In the reactor with only MW, due to the existence of microalgal assimilation and other processes (nitrification by nitrifiers, chemical reaction), the ammonia content decreased to 1.9 mg/L in just 6 days. In other reactors with an AME content lower than 40%, the ammonia concentration decreased to 1–2 mg/L after 9 days, but in the reactor with an AME content higher than 40%, the ammonia content sharply decreased during the first 12 days and was maintained at approximately 3.0 mg/L. During the cultivation process, high ammonia removal efficiencies (around 97%) were obtained in all conditions (Figure 3b), which indicates that ammonia removal was not influenced by the initial ammonia content or addition of digestate. AME can be effectively utilized as a nutrient source for microalgae cultivation. It was reported that a high content of ammonia and other metal ions in AME will negatively affect algae growth, or even cause damage to the microalgae cells [31,39]. However, in this study, NH_4^+-N removal efficiencies were almost the same in all reactors, showing that the negative influencing factors can be avoided.

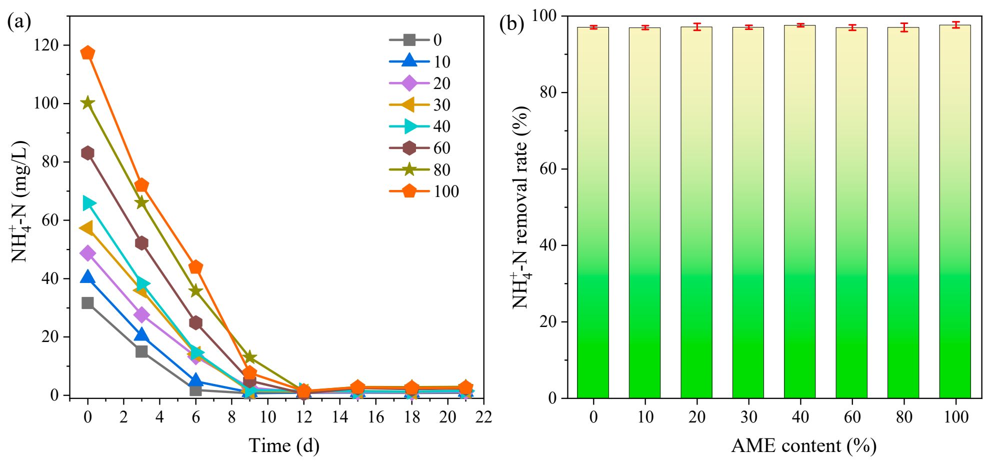

Figure 3. Ammonia removal properties under different AME contents. (**a**) Variations in NH_4^+-N content, (**b**) NH_4^+-N removal efficiency.

3.3.2. TN Removal

Although ammonia removal efficiencies were very high in all reactors, the TN removal showed significant differences under different MW/AME ratios. The TN content in the reactors with only MW sharply decreased from 25.6 mg/L to 1.6 mg/L in just 6 days (Figure 4), indicating that microalgae cultivation has high potential for TN removal. However, in the reactors with an AME addition, the rate of decrease of the TN concentration was relatively slower, and even resulted in high residual TN content in the reactors. In the reactors with an AME content lower than 20%, the final TN concentration was less than 5.0 mg/L, showing a removal efficiency higher than 90%, which indicates that microalgae cultivation can effectively remove TN under these conditions. Although TN content in these reactors slightly increased after 12 days due to the degradation of microalgae cells, it did not affect the TN removal efficiency. In other reactors, a high concentration of TN was detected after microalgae cultivation, and increased with the addition of AME. In the reactor with an AME content of 30%, the TN concentration was around 8.2 mg/L, but it increased to 101.2 mg/L in the reactor with only AME, exhibiting a decrease in TN removal efficiency from 88.4% to 42.6%. The high TN concentration and low removal efficiency may have resulted from two aspects: firstly, microalgae cultivation processes can effectively remove ammonia, but cannot assimilate other nitrogen compounds (e.g., nitrite or nitrate); secondly, part of the ammonia was not assimilated by microalgae, but transformed through other microbial metabolisms, such as nitrification processes by nitrifiers and heterotrophic bacteria [40]. As is shown in Figure 4c, a high content of other nitrogen-containing compounds was detected in the AME, which could not be easily removed by algae, and retained in the reactors. It has been reported that ammonia is a favorable nutrient for microalgae, but nitrogen can be removed by algae through complicated metabolism processes; thus, the TN removal efficiency is relatively lower. In addition, a higher content of nitrate was detected after microalgae cultivation, and its content increased with the increase in AME addition. It can thus be deduced that the nitrification process was activated and transformed the ammonia into nitrate. It has been reported that nitrification processes can coexist with algae cultivation and transform nitrogen into nitrite and nitrate. Therefore, it is important to consider the effect of nitrification and algal assimilation on nitrogen removal during microalgae cultivation.

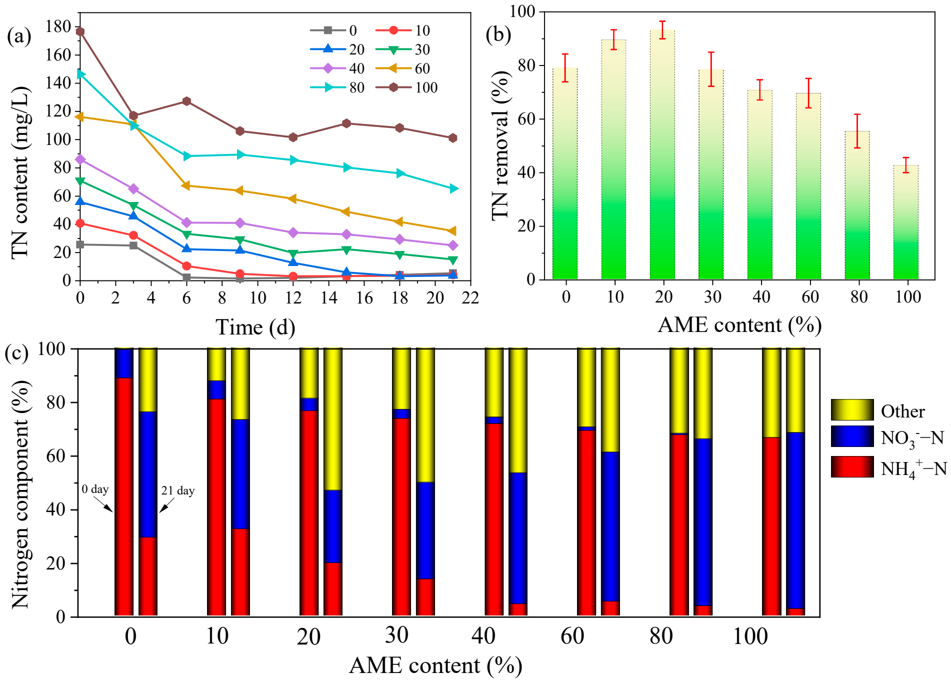

Figure 4. Variations in TN content and removal efficiency under different AME contents during microalgae cultivation. (**a**) Variations in TN content, (**b**) TN removal efficiency and (**c**) nitrogen components on Day 1 and 21.

3.3.3. Phosphate Removal

Similar to nitrogen removal, the phosphate content in the reactor with only MW gradually decreased to 0.02 mg/L in 6 days (Figure 5a), showing a removal efficiency higher than 99% (Figure 5b). In the reactors with an AME content lower than 30%, the phosphate concentration decreased to less than 0.3 mg/L in 15 days, which showed removal efficiencies higher than 96%, indicating that microalgae cultivation can realize high-efficiency phosphate removal. However, further increasing the AME content resulted in a high residual phosphate content, which indicated that the microalgae could not utilize all of the phosphate in the reactors. In the reactor with an AME content of 40% and 60%, the final phosphate concentration was around 0.4 mg/L, and it increased to 0.9 mg/L in the reactor with an AME content higher than 80%. Phosphate removal efficiencies were higher than 90% at an AME content lower than 80%, and slightly decreased to 86.5% in the reactor with only digestate, showing that phosphate can also be effectively removed through microalgae cultivation.

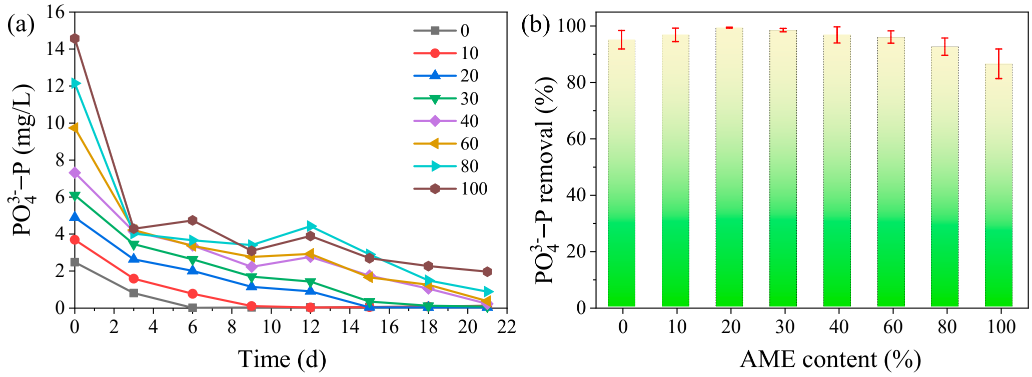

Figure 5. Variations in phosphate content (**a**) and removal efficiency (**b**) under different AME contents during microalgae cultivation.

3.3.4. COD Removal

COD removal properties are different from those of ammonia and phosphate. With the addition of AME, the initial SCOD content increased from 250 mg/L to 600 mg/L (Figure 6); however, the final SCOD content was much more stable and remained between 180 mg/L and 300 mg/L, showing a removal efficiency of around 40%. SCOD removal can be realized through three pathways: firstly, reduced inorganic matter in the wastewater, such as Fe^{2+} ions, can be oxidized during the cultivation processes; secondly, the organics may be consumed or utilized by heterotrophic bacteria in the reactors, which is a common process as a lot of bacteria are present in municipal wastewater and anaerobic digestate [29] and is consistent with previous studies; thirdly, some organic or inorganic soluble substances can be adsorbed by microalgae cells [23]. It has been reported that microalgae can adsorb a high content of organic or inorganic matter due to their high surface areas and the extracellular polymeric substances (EPSs) excreted by microalgae [41]. All of the three pathways can result in a decrease in COD. But it can be concluded that the microalgae cultivation exhibited a low capacity for COD removal, and a higher SCOD content was still retained in the reactors. The waste would thus need further treatment before its discharge into the surroundings.

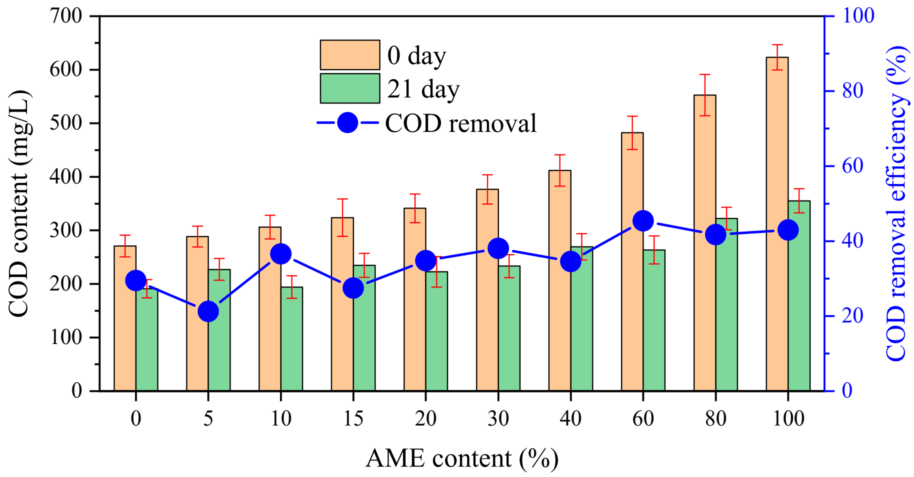

Figure 6. Variations in COD content and removal efficiency under different AME contents during microalgae cultivation.

3.4. Evaluation of Wastewater Treatment through Microalgae Cultivation

According to the former discussions, it can be concluded that nutrients can be effectively removed through microalgae cultivation. To meet the requirements of the Chinese wastewater discharge standard for ammonia (<5 mg/L), TN (<15 mg/L) and total phosphate (<0.5 mg/L) content, the relationships between cultivation time and AME content were further evaluated. It was found that with an increase in AME addition, reactors need more time to meet the requirement. For ammonia, the reactors needed more than 4 days to meet the requirement, and about 10.4 days if only AME was treated (Figure 7), indicating that, to meet the requirement of the discharge standard for ammonia, microalgae cultivation can be used to treat wastewater in all AME ratio conditions. For TN, reactors needed less time than they did for ammonia when the AME content was lower than 10%. However, if more digestate is added, a longer time should be provided. It was calculated that when the AME content was 40%, the TN content did not meet the required discharge level until 19.1 days. At an AME content over 60%, TN cannot be effectively removed through microalgae cultivation, and will result in excess TN pollution. Phosphate removal showed similar tendencies, and 4.5 to 19.1 days were needed to realize the safe discharge of phosphate when the AME content increased from 0% to 60%, which indicates that microalgae cultivation processes can achieve phosphate removal through algae cultivation processes. Based on the comprehensive requirements of all the indicators, it was found that when the AME content was lower than 10%, ammonia removal was the limiting factor for effluent discharge, but increasing the AME content to higher than 10%, TN and phosphate were the controlling factors for effluent quality. But if the AME content is higher than 80%, wastewater does not meet the requirement of the discharge standard, and additional treatment processes such as denitrification should be used to further treat the wastewater. Therefore, although nutrients can be removed through microalgae cultivation, the AME addition ratio is very sensitive to the final water quality. In this study, the highest biomass production and lipid recovery were obtained at an AME content of 40%, which meet the requirement of all indicators through microalgae cultivation.

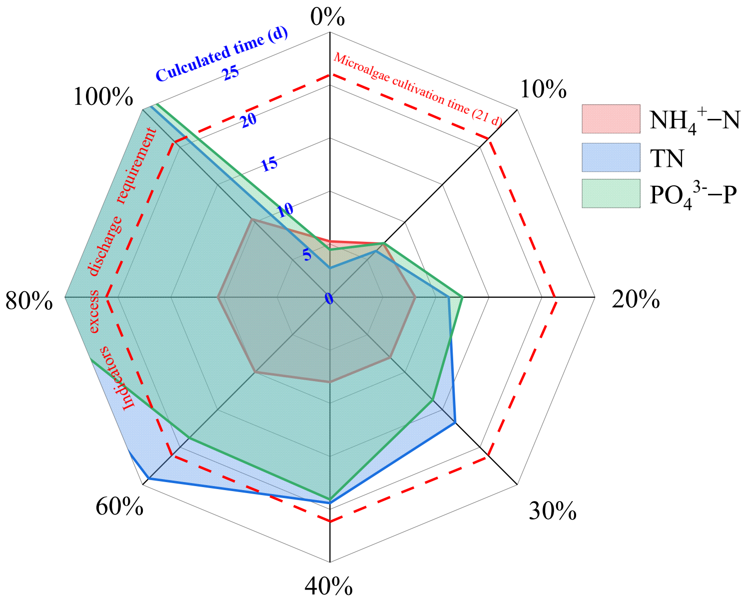

Figure 7. Relationships between the AME content and cultivation time needed to meet the requirement of discharge standard.

4. Conclusions

Microalgae were cultivated with AME and WW, and the effects of AME content on microalgae growth and pollutant removal were investigated, and the lipid production properties were also explored. It was concluded that an AME content lower than 40%

restricted the algae growth rate due to nutrient insufficiency, while a higher AME content (>40%) also inhibited algae biomass accumulation due to high nutrient concentrations. The highest microalgae growth rate (0.33/d) and lipid production (0.38 g/L) were obtained at an AME content of 40%. In addition, high ammonia (96.4–97.7%) and phosphate (86.5–99.4%) removal efficiencies were obtained, but the TN removal efficiency decreased with the addition of AME. TN and phosphate were the limiting factors in meeting the requirement of the wastewater discharge standard at a high AME content. This study provided a new method for wastewater treatment, and also realized green energy production based on sustainable development principles.

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