Nitrogen and Biomass Recovery from Low Carbon to Nitrogen Ratio Wastewater by Combing Air Stripping and Microalgae Cultivation

MA SHANSHAN1, LU HAIFENG1,*, ZHANG YUANCHUI2,*, LI BAOMING1, DONG TAILI3 and ZHANG DONGMING3
1College of Water Resource and Civil Engineering, China Agriculture University, Beijing 100083, China
2Agricultural Engineering Sciences, University of Illinois at Urbana-Champaign, 332N AESB, MC-644 1304 W, Pennsylvania Avenue, Urbana IL 61801, USA
3Minhe Animal Husbandry Incorporated Company, Penglai 265607, China

ABSTRACT: An air stripping-microalgae cultivation system was proposed to recover nitrogen and produce biomass from biogas fertilizer production wastewater. Air stripping was firstly used for reducing the nitrogen to low levels. Then microalgae were used for absorbing the residual nitrogen. Results showed that the nitrogen removal reached 95.0%, with a microalgae productivity and biomass yield of 0.21 (g/L/d) and 4.29 (g-biomass/g-nitrogen), respectively. For the system, 87.7% of the total nitrogen was recycled, from which 83.9% came from air stripping and 3.8% from microalgae absorption. This system enables carbon-emission reduction, wastewater treatment and the promotion of nitrogen recovery.

1. INTRODUCTION

POULTRY litter digested effluent is one type of nutrient-rich wastewater [1]. It contains a high concentration of nitrogen, phosphorus, trace elements, ammonia acid, organic acid, humic acid and auxin [2], which can be processed for use in foliar fertilizer, animal feed and culture solution [3]. The use of membrane technology as a potential method for condensing anaerobic-digested streams to produce foliar fertilizer has been recently developed [4]. However, the effluent generated from membrane-condensing processing, called biogas fertilizer production wastewater (BFPW), still contains a relatively high concentration of nutrients. After filtration, the total organic carbon (TOC) of the effluent is relatively low (600–650 mg/L), whereas its ammonia (NH₄⁺) concentration (2200–3000 mg/L) and pH value (8.4-8.5) are very high [5]. This effluent is a special type of high ammonia/nitrogen-containing and low carbon to nitrogen ratio (C/N) wastewater which is difficult to dispose using traditional biotechnology methods [5]. If not properly disposed, this effluent will lead to nitrogen resource loss and environmental pollution.

Microalgae is a type of eukaryotic micro-organism that has been attracting significant worldwide attention for wastewater treatment, as it has the ability to capture CO₂ and meanwhile absorb nitrogen and phosphorus from wastewater through various metabolic pathways. Since the 1950s, researchers have studied the use of microalgae to treat anaerobic-digested effluents such as swine effluent, dairy effluent and poultry litter digested effluent both to recover their nutrients and to generate useful biomass [6]. However, the initial total nitrogen (TN) and total phosphorus (TP) of anaerobic-digested effluent were approximately 802–3213 (mg/L) and 50–987 (mg/L), respectively [6], which exceeds the nutrient demand for microalgae growth. Especially for ammonium nitrogen; the tolerance concentration of ammonium for different algae species varies from 450 (mg/L) to 18000 (mg/L) [7]. Excess ammonium has a repressive effect on microalgae [7]. Thus, the nutrients concentration must be controlled at a proper level for microalgae growth. Common methods used for ammonia nitrogen removal include physiochemical processes such as dilution [8], air stripping [9], struvite precipitation [10] and chlorination, membrane filtration [11,12] along with biological methods such as nitrification and denitrification [13]. Air stripping has successfully been used to remove nitrogen from various wastewaters, such as dairy manure [9] and landfill leachate [10]. Air stripping of the anaerobic-digested stream in a biogas
plant is more feasible than other technologies for nitrogen removal because of its low economic investment, the availability of heat from biogas utilization, and the basic pH of an anaerobic-digested stream [9,14].

Therefore, in this work, a system combining air stripping and microalgae cultivation was proposed to realize nitrogen resource recovery, wastewater purification and microalgae biomass production from the low C/N BFPW. First, nitrogen in BFPW was reduced by air stripping to proper levels. Then microalgae were used for absorbing the residual-nitrogen and producing biomass. During the above processing, the wastewater treatment efficiency, nitrogen resource recovery efficiency, biomass production and nitrogen footprint of the system were all evaluated.

2. MATERIAL AND METHODS

2.1. The Characteristics of Wastewater

The BFPW was provided by Minhe Animal Husbandry Incorporated Company, where ultra-and nanofiltration membranes were used for the production of water soluble foliar fertilizer. The characteristics of the BFPW were as follows: chemical oxygen demand (COD) was 1800–3000 (mg/L), total organic carbon (TOC, mg/L) was 500–1000 (mg/L), TN was 2500–3500 (mg/L), ammonia nitrogen (NH3-N) was 2000–3000 (mg/L), TP was below 30 (mg/L), potassium was 1341 (mg/L), colour was 5.0, turbidity was 0.30, pH was 8.3–8.5. The C/N ranges from 1:7 to 1:2.5, which represents a low C/N wastewater.

2.2. Microalgae Strain

The microalgae was Chlorella vulgaris 1067 (FACHB-1067). It was obtained from the Chinese Academy of Science’s Institute of Hydrobiology (Wuhan, China). It was cultivated in a standard BG-11 medium [5]. A previous literature review showed that Chlorella sp. is highly resistant to inhibitors in wastewaters [15]. Therefore, Chlorella vulgaris 1067 was chosen as the test strain. The enrichment cultivation of the Chlorella vulgaris 1067 was carried out in 500 (mL) flasks. All of the flasks were placed in a light incubator with a light intensity of 200 (µmol/(photons·m·s)). The incubator was maintained at 26°C with a daily lighting schedule of 12 h on:off. No CO2 was supplied during the cultivation. The carbon source came from sodium bicarbonate in the BG-11 medium. All of the Chlorella vulgaris 1067 used in the experiments was in the logarithmic growth phase (approximately 4–6 days after the starting day).

2.3. Experiment Setup and Procedures

2.3.1. The Feasibility Study of using BFPW to Cultivate Microalgae

The BFPW was diluted to four NH3-N concentration levels. There was a BG-11 medium run as well, which was made using BG-11 medium. The experiments were carried out in 1000 (ml) batch reactors (flasks). The inoculum size varied from 0.07 to 0.10 (g/L). The cultivation conditions were the same as pure cultivation (Section 2.2).

2.3.2. The Experimental Setup and Procedures of the Combination System

The experimental setup is primarily composed of five parts (Figure 1): the main reactor (1000 mL), the absorption system (1000 mL), the pure cultivation system (1000 mL), the air stripping system, and the CO2 supplementation system. The ammonia nitrogen stripping, pH regulation and microalgae cultivation by BFPW were carried out in the main reactor. The absorption system was used for the absorption of the ammonia nitrogen that was stripped from the main reactor. The pure cultivation system was used for the pure cultivation of microalgae. The air stripping system was used for nitrogen stripping. The combination of an air stripping system and a CO2 supplementation system was used to carry out both pH regulation and microalgae cultivation. The operation steps and the experimental procedures of each step are shown as follows.

The first step was air stripping for ammonia nitrogen removal and adsorption. For air stripping, 600 (mL) BFPW was poured into the main reactor. Air was blown through the air stripping system into the main reactor. The ammonia nitrogen was blown out and flowed into the absorption system. When the ammonia nitrogen concentration decreased to a suitable level (below 600 mg/L), the air stripping was stopped. Moreover, the stripped ammonia nitrogen was absorbed by 1 (mol/L) H2SO4 in the adsorption system.

The second step was pH regulation using CO2. This step was carried out in the main reactor. Following ammonia nitrogen stripping, the air stripping system and the CO2 supplementation system were simultaneously opened to form the mixed gas that ultimately contained 4% CO2. Next, the 4% CO2 mixed gas was blown into
the main reactor to regulate the pH to 7.2 for 30 (min) with a flow rate of 1.0 (L/min).

The third step was microalgae cultivation. This step can be divided into two parts, each of which was carried out in different reactors. The first part involved microalgae cultivation in the BFPW (in the main reactor). When the pH regulation step finished, microalgae were inoculated into the main reactor and cultivated with a continuous supplementation of the mixture gas (4% CO₂). The light and temperature conditions were the same as the pure cultivation system (Section 2.2). The microalgae and the BFPW were collected from the main reactor for biomass and water analysis every two days.

The second part involved the pure cultivation of microalgae (in the pure microalgae cultivation reactor). The ammonia nitrogen was absorbed by 1.0 (mol/L) H₂SO₄ to form the (NH₄)₂SO₄ solution in the absorption system. Next, the (NH₄)₂SO₄ solution was used for Chlorella vulgaris 1067 cultivation in the pure cultivation system.

2.4. Analysis Methods

The fate of nitrogen can be analysed and described as Figure 2.

The calculation of each part of the nitrogen can be seen as follows:

\[
A = \text{TN in the BFPW before air stripping (mg/L) } \times \text{ the total volume of the BFPW before air stripping (L)}
\]

\[
B = B_1 + B_2
\]

\[
B_1 = \text{TN in the } (\text{NH}_4)^2\text{SO}_4 \text{ solution (mg/L) } \times \text{ the } (\text{NH}_4)^2\text{SO}_4 \text{ solution volume (L)}
\]

\[
C = \text{TN in the BFPW after the air stripping step is finished (mg/L) } \times \text{ the total volume of the BFPW after the air stripping step is finished (L)}
\]

\[
C_1 = \text{Nitrate (NO}_3\text{-N) in the BFPW after air stripping step is finished (mg/L) } \times \text{ the total volume of the BFPW after the air stripping step is finished (L)}
\]

Figure 1. The combined system of air stripping-microalgae cultivation used for the BFPW treatment, biomass production and nitrogen recovery.
$C_2 = \text{NH}_3\text{-N in the BFPW after the air stripping step is finished (mg/L) } \times \text{the total volume of the BFPW after the air stripping step is finished (L)}$

$C_3 = \text{dry cell weight of Chlorella vulgaris 1067 at the end of the cultivation process (mg/L, 10 d) } \times \text{the total volume of the BFPW after the air stripping step is finished (L)} \times \text{N element content in the harvest Chlorella vulgaris 1067 at the 10th day (%)} - \text{dry cell weight of Chlorella vulgaris 1067 at the beginning of the cultivation process (mg/L, 0 d) } \times \text{the volume of the BFPW after Chlorella vulgaris 1067 cultivation finished (L)} \times \text{N element content in the inoculated Chlorella vulgaris 1067 (%)}$

$C_4 = \text{TN in the BFPW after microalgae cultivation is finished (mg/L) } \times \text{the volume of the BFPW after Chlorella vulgaris 1067 cultivation is finished (L)}$

COD, TN, TP, NH$_3$-N, NO$_3$-N, NO$_2$-N, colour and turbidity were tested using the standard methods for water and wastewater examination [16]. Dry cell weight was measured according to Lee and Shen [17]. Representative aliquots of algal cultures were taken and the cells were separated using a 0.22 (µm) membrane. The filter membranes were then pre-weighed. The cells were normally washed with diluted medium or buffer several times, followed by rinsing with distilled water. The drying temperature was 100°C. After the filter membrane cooled in a desiccator at room temperature for about 15–30 (min), the dried sample was weighed immediately once taken out of the desiccator. TOC was analyzed using a Torch Combustion TOC analyzer (TOC-VCNP, Shimadzu Co., Tokyo, Japan). The pH was measured with a previously calibrated pH meter (FE20, Mettler Toledo Co., Inc., Germany). The potassium was measured with a Perkin Elmer Optima 5300 DV ICP (Perkin Elmer Inc., America).

The nitrogen and carbon of the dry cell biomass were measured through elemental analysis (LECO CHNS-932 with oxygen furnace VTF 900, LECO Corporation, St. Joseph, MI, USA). Samples were placed in the oven’s capsules for combustion at 950°C using pure oxygen as the combustion gas and pure helium as the carrier gas. Carbon was determined through infrared absorption, and nitrogen was measured as N$_2$ using a thermal-conductivity detection system [18].

All of the measurements were carried out three times and all of the results were reported as the average values. The data was statistically analysed using one-way ANOVA (SPSS 17.0) based on the bottles as replicates ($n = 3$). After checking the data for homoscedasticity and normal distribution of the variances, the Duncan test was used for multiple average comparisons and to detect any differences between pairs of variables at a significance level of $p < 0.05$.

![Figure 2. The nitrogen footprint in the air stripping-microalgae cultivation system.](image-url)
3. RESULTS AND DISCUSSION

3.1. Appropriate Ammonia Nitrogen Concentration for Chlorella vulgaris 1067 Growth in BFPW

High ammonia nitrogen is toxic to microalgae [19], therefore, the proper nitrogen concentration for Chlorella vulgaris 1067 growth was firstly investigated. In practical pure cultivation of microalgae, BG-11 medium is often used as a supplementation. Therefore, biomass production from the BFPW and regular pure cultivation were compared. Chlorella vulgaris 1067 could grow in all diluted BFPW runs (Figure 3). The suitable ammonia nitrogen concentration of the BFPW for Chlorella vulgaris 1067 growth was from 200 to 600 (mg/L). The final dry cell weights of N2 and N3 were all higher than that of the BG-11 medium run. The highest dry cell weight appeared in the N3 run with 0.38 (g/L) at 6 d. The final dry cell weights of N1 and N4 runs were both lower than that of the BG-11 medium run.

The BG-11 medium run showed a very different performance compared to the other BFPW runs. The logarithmic growth phase appeared from the beginning of the test to the end. The initial nitrogen concentration in the BG-11 medium run was 363.2 (mg/L), which was higher than the N2 run (280.4 mg/L) and lower than the N3 run (560.7 mg/L). However, the biomass was lower than both the N2 and N3 runs at the end of the test. They are three main reasons for the above phenomenon. The carbon limitation might be one reason. In the BG-11 medium, the carbon source was Na2CO3 with the concentration of 2.26 (mg/L). In N2 and N3 run, the TOC concentration was 118.2 and 230.9 (mg/L), respectively, which provided more carbon sources for Chlorella vulgaris 1067. The different metabolic mode might be another reason. In the BG-11 medium run, the carbon source was CO3\(^2-\). Chlorella vulgaris 1067 carried out in autophotosynthetic mode [17]. While in BFPW runs, organic and inorganic carbon both existed, Chlorella vulgaris 1067 was carried out in mixotrophic mode [17]. For Chlorella vulgaris, the maximum specific growth rate of mixotrophic growth was higher than that of the photosynthetic and heterotrophic growth [17]. In addition, the initial phosphorus concentration in N1 was very low, just 1.0 (mg/L), which might lead to the phosphorus limitation for microalgae growth compared to the BG-11 medium run.

The NH3-N removal ratio was also measured to investigate the nitrogen recovery from the BFPW. The highest NH3-N removal ratio appeared in the N1 run (47.3%); ultimately, the NH3-N concentration was 44.3 (mg/L). In other words, the utilization efficiency of microalgae with respect to nitrogen was very low. That might have been caused by the low C/N ratio. The ratio of carbon, nitrogen and phosphorous was very important for microalgae growth and nutrients uptake. For this, the Redfield ratio of 106C: 16N: 1P is widely used, as a point of departure, to quantify possible nutrient limitations [17]. In the BFPW, the C/N ratio was 0.3, which showed the severe deficiency of the carbon source. Hence, a supplemental carbon source might promote biomass production and nitrogen utilization.

Figure 3. (a) Dry cell weight, and (b) NH3-N removal ratio changes at different dilution times with the initial NH3-N concentration for N1, N2, N3, N4 of 84.1, 280.4, 560.7, 1120 mg/L, respectively; inoculum size was from 0.07 to 0.10 (g/L) dry cell weight, 200 (µmol/(photons·m·s)), 26°C, no CO2 was supplemented.
Therefore, as the above results, the nitrogen concentration must be cut down firstly. Then the carbon source must be supplemented and the pH value must be reduced to realize the promotion of biomass production and nitrogen recovery.

3.2. Air Stripping for Ammonia Removal

Air stripping is always used for treating wastewater which contains a high concentration of ammonia (> 500 mg/L). Previous works showed that the pH value, temperature, flow rate and flow time influenced the nitrogen removal in air stripping [15, 20]. In this work, the initial pH was 8.3–8.5, which can be regulated to 9.0 or 10.5 by NaOH and HCl; however, because of the very high initial potassium concentration in the wastewater, the addition of NaOH would lead to the microalgae experiencing salinity stress. Therefore, in this work, air stripping without pH correction was performed. Figure 4 showed that the NH₃-N concentration decreased with time in all three flow rate runs. There was no substantial difference between the 6.0 (L/min) run and the 8.0 (L/min) run with respect to NH₃-N concentration changes. To save energy, 6.0 (L/min) was the optimal choice. Without pH regulation, the NH₃-N concentration degraded below 500 (mg/L) after 3.5 hours of air stripping.

However, the pH value in the BFPW decreased to 7.8 when the air stripping finished, which was still not suitable for microalgae growth. Accordingly, the pH value had to be regulated to a more suitable level for microalgae cultivation.

3.3. CO₂ Supplementation for Biomass Production and Nitrogen Recovery

After air stripping, the NH₃-N concentration in the BFPW ranged from 470 to 490 (mg/L), and the pH value was 7.8. Firstly, the pH value was regulated by CO₂. With the supplementation of the mixture gas that contained 4% CO₂, the pH in the BFPW dropped to 7.2 after 30 (min). Secondly, different flow rate runs of the mixture gas with 4% CO₂ for Chlorella vulgaris 1067 cultivation was carried out to investigate the dry cell weight and the NH₃-N concentration changes.

Figure 5 showed that the dry cell weight and NH₃-N removal ratio increased with time. In addition, a high flow rate led to rapid biomass accumulation and NH₃-N degradation. The highest biomass, daily productivity, specific growth rate and biomass yield all occurred in the 1.0 (L/min) run with the values of 2.38 (g/L), 0.2113 (g/L/d), 0.2203 (d⁻¹), 4.287 (g-biomass/g-nitrogen), respectively. Except for the 0.2 (L/min) run, the final NH₃-N concentration in all of the other runs met the discharge standard of pollutants for livestock and poultry breeding (< 80 mg/L) [21].

The optimal flow rate ranged from 0.6 to 1.0 (L/min). For the 0.6, 0.8 and 1.0 (L/min) runs, after 3 days, Chlorella vulgaris 1067 entered the logarithmic growth and lasted to the end. In 0.6, 0.8 and 1.0 (L/min) runs, after 10 days’ treatment, the final NH₃-N removal ratio of all samples was above 95%.

It can be seen from the above results that the daily productivity and NH₃-N removal ratio were approximately 3-fold or 2-fold times of that without CO₂ supplementation (Figure 3). Especially, the elements analysis also showed that a higher CO₂ supplementation quantity led to a higher carbon and nitrogen content in the microalgae. With different flow rates of mixture gases, the carbon and nitrogen elements in Chlorella vulgaris 1067 were all promoted 1.8 to 2.0-fold, and 1.6 to 1.8-fold, respectively. Researchers have found that there are some relationships between the carbon and nitrogen metabolism [22]. Carbon’s metabolism produces ATP, which affects the assimilation of nitrogen. Flynn [22] had found that the adjustment of algae to nitrogen stress can be realized through changing the proportions of the key metabolites of carbon and nitrogen. It has been supposed that there are two key metabolites: α-ketoglutaric acid and glutamic acid. When the α-ketoglutaric acid/glutamic acid ratio decreased, the intensity of nitrogen transportation, nitrogen metabolism and the nitrogen cycle were enhanced. Thus, CO₂ supplementation will also increase the inorganic carbon in the BFPW, which will promote nitrogen re-
Nitrogen and Biomass Recovery from Low Carbon to Nitrogen Ratio Wastewater

covery by microalgae through the metabolic pathway of autophotography.

Compared to Singh’s and Bhatnagar’s work [1, 23], the biomass daily productivity in this study is higher, and the tolerance of Chlorella sp. to wastewater was promoted approximately 16.8-fold. This might be caused by the higher light intensity, lower colour and turbidity, and extra carbon source supplementation in this work, which enhanced the mixotrophic metabolism of microalgae [17]. In Liang’s work, the highest biomass was obtained as 0.7 (g/L/d) [24], which was approximately 3.5-fold times the value presented in this work. The supplementation of glucose as a carbon source and the continuous cultivation mode in Liang’s work might be the main reason that leads to the high biomass production. Therefore, in order to promote biomass productivity from BFPW, the CO2 supplementation quantity, the carbon source addition and cultivation mode need to be investigated in the future.

3.4. Nitrogen Footprint of the Whole System

In the BFPW, nitrogen was recovered from the combined system through two pathways: air stripping and microalgae absorption. In order to investigate the efficiency of nitrogen resource recovery from the combined system, the nitrogen footprint was studied. Figure 6 showed that most of the nitrogen was absorbed by H2SO4 (83.9%) and then formed (NH4)2SO4. 3.8% of the nitrogen was synthesized into microalgae cells. It could be confirmed that the nitrogen disused from the combined system took 3.3%, which came from two parts. One part was the nitrogen that was lost during the air stripping or H2SO4 adsorption processing (2.4%) and other part was the nitrogen that stayed in the residual wastewater (after microalgae cultivation processing finished, 0.9%). In addition, the specific fate of the 9.0% nitrogen in the whole system cannot be confirmed. It might be lost or completely transformed into N2, or both exist. During microalgae cultivation

Absorbed by H2SO4 83.9%

Lost during air stripping 2.4%

Emitted as N2 or lost 9.0%

The residual N in wastewater 0.9%

Figure 6. Nitrogen footprint of the air stripping-microalgae cultivation system.
with CO₂ supplementation, the CO₂ flow might strip some of the ammonia from the wastewater. Meanwhile, *Chlorella vulgaris* 1067 utilized nitrogen during the cultivation processing, in which some of the nitrogen was assimilated as the cell components, and the other might be totally oxidized into N₂ that was then released into the atmosphere [25]. However, this hypothesis needs to be further investigated. If 9.0% of nitrogen was totally lost because of the stripping effect, the nitrogen resource recovery efficiency from the combined system would reach up to 87.7%. If this part was totally transformed into N₂ by *Chlorella vulgaris* 1067, then the harmless efficiency of the nitrogen to the environment of this system would reach up to 96.7%.

In addition, the nitrogen recovered by air stripping can be used for ammonium fertilizer production and pure microalgae cultivation, thus realizing the generation of multi-utilization by-products. For the purification of the initial fertilizer production wastewater, the collected microalgae can be used as chicken feed and raw materials for biocrude oil production. However, there are some points of caution that need to be considered with regard to the collected microalgae. For example, after being collected after membrane filtration, the ash content of the collected microalgae reached up to 23.1%, which lead to low oil production and a low heating value of biocrude oil. Therefore, pre-treatment methods that can remove ash from the biomass is very important.

4. CONCLUSIONS

A combined air stripping and microalgae cultivation system was used to treat BFPW and recover nitrogen. The final effluent of the system met the discharge standard of pollutants for livestock and poultry breeding, with a microalgae daily productivity and biomass yield of 0.2113 (g/L/d) and 4.287 (g-biomass/g-nitrogen) respectively. The nitrogen resource recovery efficiency reached up to 87.7% and the harmless efficiency of nitrogen to the environment reached up to 96.7%. The nitrogen recovered by air stripping can be used for ammonium fertilizer production and pure microalgae cultivation, thus ensuring multi-utilization of the process by-products. The collected microalgae can be used as chicken feed and raw materials for biocrude oil production as well. In future, for the biogas products from biogas engineering, if the CO₂ that was purified from the biogas can be used in microalgae cultivation, the enabling carbon-emission reduction and nitrogen recovery promotion could be realized.

5. ACKNOWLEDGEMENTS

The authors thank the National Natural Science Foundation of China (51576206 and 51308535) and Minhe Animal Husbandry Incorporated Company for their financial support. Thank Jamison Watsons from the University of Illinois at Urbana-Champaign for revising the language.

6. REFERENCES