Ammonium nitrogen removal in batch cultures treating digested piggery wastewater with microalgae

Oedogonium sp.

Haiping Wang, Zhiquan Hu, Bo Xiao, Qunpeng Cheng and Fanghua Li

ABSTRACT

Due to the nutrient characteristics of the high concentration of available ammonium in digested piggery wastewater (DPW), microalgae can be used to treat DPW before its final discharge. Four green microalgae (Hydrodictyaceae reticulatum Lag, Scenedesmus obliquus, Oedogonium sp. and Chlorella pyrenoidosa) and three blue-green algae (Anabaena flos-aquae, Oscillatoria amoena Gom and Spirulina platensis) were used to remove the nutrients (N, P, C), especially ammonium nitrogen (NH₄⁺-N), from diluted DPW with 300 mg/L algae density in batch tests. The microalgae with the best NH₄⁺-N nutrient removal was then selected for further optimization of the variables to improve NH₄⁺-N removal efficiency using a central composite design (CCD) experiment. Taking into account the nutrient removal efficiency, Oedogonium sp. showed the best performance (reduction of 95.9% NH₄⁺-N, 92.9% total phosphorus (TP) and 62.5% chemical oxygen demand (COD)) based on the results of the batch tests. The CCD results suggested that the optimal values of variables were initial Oedogonium sp. density of 399.2 mg/L and DPW diluted by 16.3, while the predicted value of NH₄⁺-N removal efficiency obtained was 97.0%.

Key words | ammonium nitrogen removal, central composite design, digested piggery wastewater, microalgae

INTRODUCTION

The pig industry is one of the main industries in the suburban economy of China (McOrist et al. 2011). Effluents from pig farming contain high concentrations of nitrogen, phosphorus, and organic matter in both soluble and particulate forms, the composition mainly depending on animal nutrition and farming practices. Anaerobic digestion is often attractive as the first treatment process for piggery wastewater due to the recovery of renewable energy (biogas) and the reduction of organic matter, waste volume and odors, through the fermentative degradation of organic constituents. However, levels of nutrients such as ammonia are not reduced during anaerobic digestion because the microorganisms employed generally lack sufficient autotrophic metabolism of inorganic nitrogen (Noike et al. 2004; Uludag-Demirer et al. 2008) Thus, it is necessary to treat digested piggery effluent effectively prior to disposal in order to avoid causing severe environmental problems such as eutrophication of water bodies which can lead to highly undesirable changes in the ecosystem structure and function (Novotny 1999), groundwater contamination, air pollution by ammonia gas volatilization, and soil degradation due to over-fertilization and also risks to human health (Godos et al. 2010).

Traditional bacterial nitrification–denitrification can be used to remove ammonia, but it requires the assimilation of extra organic carbon. Another important feature of anaerobic digestion is the high concentration of alkalinity involved. High alkalinity makes it difficult to apply advanced oxidation processes using ozone or peroxides because bicarbonate acts as a radical scavenger (Ma & Graham 2000; Currie et al. 2005). Additionally, the high complexity and energy inputs associated with these technologies have not promoted their widespread implementation in rural areas. Agricultural land disposal methods have traditionally been used to solve swine manure management, however, the recent intensive farming context has overtaxed the natural
capacity of the farm surrounding lands to cope with piggery wastewaters. In this context, the development of cost-effective technologies, which support a simultaneous carbon oxidation and nutrient recovery, is crucial in the establishment of sustainable farming.

Microalgae such as green microalgae and blue-green algae have a high ability to assimilate nutrients (N and P) and inorganic carbon into their biomass from wastewater for photosynthesis. Digested piggery effluent could be used as an alternative nutrient source for growing microalgae especially when a substantial amount of nitrogen remains following the reduction of organic matter by the first anaerobic digestion (Noike et al. 2004; Park et al. 2010). The use of a wide range of microalgae such as Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas and Spirulina for treating domestic wastewater has been reported and the efficacy of this method is promising (Tam & Wong 1990; Zhang et al. 2008; Magro et al. 2012; Yonezawa et al. 2012). At the same time, microalgae can be used as a functional food such as Spirulina platensis which presents antioxidant and antimutagenic properties as an animal feed additive (Peiretti & Meineri 2008). Due to the high amount of lipids or oils contained in microalgal biomass, biodiesel and other biofuel can also be produced from microalgae, which has been considered an important renewable and cost-effective source of energy for the future (Rusten & Sahu 2011; Larkum et al. 2012). Thus, wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling.

However, there is little information on the use of microalgae for treating digested piggery effluents and the comparison of treatment efficiency of the different microalgal species. Easily cultured and high-performance microalgae are desirable for removing the nutrients (N, P, C), especially NH\textsubscript{4}\textsuperscript{+}-N, from digested piggery wastewater (DPW). A microalgal system was employed as an alternative secondary or post-secondary treatment process to remove nutrients from digested piggery effluent in our study. The nutrient removal performance of four green microalgae (Hydrodictyaceae reticulatum Lag, Scenedesmus obliquus, Oedogonium sp. and Chlorella pyrenoidosa) and three blue-green algae (Anabaena flos-aquae, Oscillatoria amoena Gom and Spirulina platensis) from DPW were studied in batch tests. The microalgal species that exhibited a high-performance NH\textsubscript{4}\textsuperscript{+}-N removal efficiency was then selected for further optimization of the variables to improve NH\textsubscript{4}\textsuperscript{+}-N removal efficiency using a central composite design (CCD).

**METHODS**

**Algal strains**

Algal strains of green eukaryotic microalgae (Hydrodictyaceae reticulatum Lag, Scenedesmus obliquus, Oedogonium sp. and Chlorella pyrenoidosa) and blue-green algae (Anabaena flos-aquae, Oscillatoria amoena Gom and Spirulina platensis) were obtained from the Freshwater Microalgae Culture Collection of the Institute of Hydrobiology (FACHB-Collection), the Chinese Academy of Sciences, China. Cells were grown in culture media (FACHB-Collection) under room temperature and a 12-h photoperiod. Once the microalgae were acclimatized to the culture conditions, algal cells were harvested by centrifugation at 4,000 rpm for 10 min and then were used for the initial algal inoculations in the following tests.

**Digested piggery wastewater**

The raw piggery effluent was collected from a local pig farm (Wuhan, China) and digested by anaerobic digester inoculated with granular sludge. Before use as culture media, DPW (total phosphorus (TP) 93 mg/L; NH\textsubscript{4}\textsuperscript{+}-N 891 mg/L; chemical oxygen demand (COD) 1,360 mg/L; total nitrogen (TN) 983 mg/L; total solids (TS) 4.1%) was diluted with tap-water to obtain initial nutrient concentrations and reduce the ammonia toxic influence on algae growth.

**Nutrient removal batch tests**

In order to compare the nutrient removal and growth of seven different microalgae, a series of batch tests were carried out. Batch cultures were conducted in 2 L flasks with a total working volume of 1 L under 3,500 Lux cool-white fluorescent tube illumination with a photoperiod of 12 h light:12 h dark. The DPW was diluted with tap-water (dilution factor = 15) to obtain initial nutrient concentrations (TP 6.2 mg/L; NH\textsubscript{4}\textsuperscript{+}-N 59.4 mg/L; COD 90.7 mg/L). The initial algal inoculum density was 300 mg/L dry weight (DW) for all treatments. Cultures were continuously agitated by atmospheric air bubbling (0.5 L/min) that also provided CO\textsubscript{2}. Once the best alga for nutrient removal from DPW was established, it was chosen to be used in the following tests.
Central composite design tests

In order to evaluate the effect of initial cell density (mg/L) and wastewater dilution factor on NH$_4^+$-N removal efficiency and further improve the NH$_4^+$-N removal efficiency from DPW during the Oedogonium sp. growth, CCD, which is an effective statistical and mathematical tool, was used to optimize the two independent variables of initial cell density (mg/L) ($X_1$) and wastewater dilution factor ($X_2$) in this study. The extreme values of the independent variables were selected based on values obtained in preliminary experiments. Initial cell density ($X_1$) was varied between 17 and 582 mg/L and DPW dilution factor ($X_2$) between 0.86 and 29.14. Other running conditions were the same as the batch tests. The experimental ranges and the levels of the variables for NH$_4^+$-N removal efficiency are given in Table 2. Experiments 1–8 were performed at different combinations of the two variables while those from 9 to 15 were under the same conditions as center points. The repeated center points were used to evaluate the confidence level of the method. Experimental data were analyzed using response surface regression software (Design-Expert). The following second-order polynomial equation was then fitted to the data by multiple regression:

$$Y = b_0 + \sum b_iX_i + \sum b_{ii}X_i^2 + \sum b_{ij}X_iX_j$$

where, $Y =$ the predicted response; $b_0 =$ constant; $b_i =$ the linear coefficients; $b_{ii} =$ squared coefficients; $b_{ij} =$ cross-product coefficients; and $X_i$ and $X_j$ are variables.

Analytical procedures

NH$_4^+$-N, TN, TP and COD and TS analyses were performed following the standard methods (APHA 1998). The algal DW per litre (DW) of culture suspension was used to evaluate the algal density. The DW was evaluated by drying cells at 105°C to constant weight after centrifugation at 4,000 rpm for 10 min to separate microalgae.

RESULTS AND DISCUSSION

Piggy wastewater characteristics before and after digestion

The anaerobic treatment was conducted in a covered 10 L digester with a granular sludge bed. The characteristics of the piggy wastewater before and after digestion (data not shown) show a good reduction of the various parameters after anaerobic digestion. The COD of raw wastewater was 7,300 mg/L and decreased to 1,360 mg/L, accounting for 81.3% reduction. TS was also reduced from 7.5% down to 4.1%. TP was reduced by anaerobic digestion from 176 to 93 mg/L (47.2% reduction). In contrast, the NH$_4^+$-N as the main component of TN increased slightly due to the anaerobic bioconversion of proteins contained in piggery wastewater into amino acids and then to ammonia. The 8.9% increase of TN for a final value of 983 mg/L was such that an N/P ratio of 21:2 was obtained in the decanted DPW. Therefore, it is essential to further treat the DPW before discharge since critical nutrients were still retained in the piggery wastewater after anaerobic digestion.

Nutrient removal efficiencies under batch culture conditions

For all eukaryotic microalgae, the only forms of inorganic nitrogen that are directly assimilable are nitrate (NO$_3^-$), nitrite (NO$_2^-$), and NH$_4^+$-N (Barsanti & Gualtieri 2006). The form of NH$_4^+$-N took up more than 80% of the TN in the DPW and therefore was readily available to microalgae. Efficient and continuous nutrient removal from wastewater could be provided by algae growth in the log-phase due to the rapid nutrient uptake and productivity (Aslan & Kapdan 2006; Park et al. 2010). Results from this experiment (Table 1) showed that Oedogonium sp. and Scenedesmus obliquus lowered the NH$_4^+$-N concentration of the medium to near zero after 7 days with the NH$_4^+$-N removal rate of 95.9 and 93.2%, respectively. There might be an ammonium disappearance through gas stripping.
due to active photosynthesis. However, ammonia volatilization was negligible compared with total \( \text{NH}_4^+ - \text{N} \) removal if \( \text{CO}_2 \) was supplied during the algae culture (Woertz et al. 2009). A significant reduction (92.9–64.1%) of TP was also found for all of the seven samples with the initial phosphorus concentration of 6.2 mg/L, which indicated complete exhaustion of phosphate may need a longer time than 7 days for all microalgae. *Oedogonium* sp. and *Chlorella pyrenoidosa* presented the highest TP removal of 92.9 and 91.7%, respectively. *Scenedesmus obliquus* also showed a good performance with TP removal of 90.5%. This was also observed by Ruiz-Marin who achieved 83.3% reduction of TP when treating urban wastewater with *Scenedesmus obliquus* but starting at a lower concentration (2.5 mg PO4/L) and a shorter cultivation time (2 days) (Ruiz-Marin et al. 2010). The results from the elemental analyses suggest that the N/P ratios in DPW varied from 16.0 to 4.4 after being consumed by algal growth.

COD in DPW was utilized by algae to some extent (67.3–38.4%) but not as efficiently as ammonium and phosphorus. Since algae used the organic carbon in the DPW only as part of their carbon source, while \( \text{CO}_2 \) was also assimilated through the process of autotrophic photosynthesis (Magro et al. 2012). *Spirulina platensis* gave the smallest COD reduction of 38.4%, whereas *Scenedesmus obliquus* performed the best with a COD reduction of more than 67.3% from the DPW, following the *Chlorella pyrenoidosa* and *Oedogonium* sp. with a COD reduction of 63.3 and 62.5%, respectively. Uptake rate of COD followed a pattern consistent with COD reduction. *Spirulina platensis* was clearly less performatory than the other species, when the highest COD uptake rate of 1.21 mg/L/h was observed by *Scenedesmus obliquus*.

### CCD model and optimization of variables

Baced on the results of the batch tests, *Oedogonium* sp. showed the best performance of nutrient absorption, especially the removal efficiency of 95.9% \( \text{NH}_4^+ - \text{N} \) and 92.9% TP. Thus, the species *Oedogonium* sp. was chosen for the CCD tests for optimizing the variables. The CCD matrix of two independent variables and experimental results obtained in the batch tests with *Oedogonium* sp. are presented in Table 2. Based on these results, an empirical relationship between the response and independent variables was attained and expressed by the following second-order polynomial equation:

\[
Y = 95.58 + 5.02X_1 + 2.81X_2 - 2.63X_1X_2 - 4.71X_1^2 - 5.68X_2^2
\]

\( \text{NH}_4^+ - \text{N} \) removal efficiencies have been predicted by the equation and are presented in Table 2.

The correlation coefficient \( R^2 \) quantitatively evaluates the correlation between the experimental data and the predicted responses obtained from the model. It was found that the predicted values matched the experimental values reasonably well with \( R^2 = 0.9464 \), suggesting good

### Table 2 | Central composite design matrix and the value of response function

<table>
<thead>
<tr>
<th>No. of test</th>
<th>Ranges</th>
<th>Levels</th>
<th>NH(_4)(^+) - N removal efficiency (%)</th>
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</thead>
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<tr>
<td></td>
<td>( X_1 )</td>
<td>( X_2 )</td>
<td>Experimental</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>5</td>
<td>–1</td>
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<tr>
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<td>5</td>
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<td>3</td>
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agreements between the experimental and predicted values of NH$_4^+$-N removal efficiency.

In addition to correlation coefficient, the adequacy of the models was evaluated by the residuals (difference between the observed and the predicted response value). Residuals are thought of as elements of variation unexplained by the fitted model and then it is expected that they occur according to a normal distribution. Normal probability plots are a suitable graphical method for judging residuals normality (Khataee et al. 2010). The observed residuals are plotted against the expected values, given by a normal distribution (data not shown). Based on the residual plot (data not shown), the residuals appear to be randomly scattered. The trends reveal reasonably well-behaved residuals.

The $p$-values were used as a tool to check the significance of each of the coefficients, which in turn, are necessary to understand the pattern of the mutual interactions between the test variables. The smaller the $p$-value, the more significant the corresponding coefficient (Liu & Chiou 2005; Zarei et al. 2010). The results indicate that the linear term of initial Oedogonium sp. density ($X_1$) and DPW dilution factor ($X_2$) and their square term had a significant effect on the NH$_4^+$-N removal efficiency with low $p$-values of less than 0.05. While the square term of $X_2$ had the strongest effect on the NH$_4^+$-N removal efficiency ratio with the lowest $p$-values of 0.0003. The interactive term of the two variables $X_1 X_2$ had no significant impact on correlation of coefficients because its $p$-value was bigger than 0.05 (data not shown). $F$-value is the ratio between the mean square of the model and the residual error. The computed $F$-value obtained of 35.23 was clearly higher than the tabulated $F$ at 5% level (2.352 at 95% significance), which indicates the adequacy of the model.

**Optimization of variables as response surface and counter plots**

CCDs have been successfully used for optimizing culture conditions for mass production of algae and hazardous materials removal by other microorganisms (Rigas et al. 2005; Hasan et al. 2009; Kim et al. 2012). Figure 1 shows the response surface and contour plots of the NH$_4^+$-N removal efficiency as a function of initial Oedogonium sp. density and DPW diluted time. The response surfaces of the NH$_4^+$-N removal efficiency show a clear peak, increasing or decreasing of variables to deviate the peak value would reduce the NH$_4^+$-N removal efficiency. The results indicate that the low level of NH$_4^+$-N was the limiting factor for Oedogonium sp. productivity. A higher NH$_4^+$-N removal efficiency was obtained as an accelerated Oedogonium sp. productivity following increased NH$_4^+$-N levels. But the growth of Oedogonium sp. is inhibited at higher ammonia concentrations due to the toxic influence (Tam & Wong 1990) which evidences that it is essential to dilute the DPW before using for microalgae growth. Too low a Oedogonium sp. seeding concentration failed to favor
ammonia removal during cell cultivation. The higher ammonium removal was obtained at the moderate *Oedogonium* sp. density. However, there was no further increase of ammonium removal at the higher cell concentrations. The reason could be the low light availability arising from self-shading at high algal density (Li et al. 2011). This is in agreement with literature reports of the initial nutrient concentration and the alga seeding concentration effect on the NH$_4^+$-N removal efficiency (Lau et al. 1995; Godos et al. 2010).

By solving the regression equation, the optimal values of $X_1$ and $X_2$ were initial *Oedogonium* sp. density of 399.2 mg/L and DPW dilution factor of 16.3, respectively, while the predicted value of the ratio of NH$_4^+$-N removal efficiency obtained was 97.0%.

**CONCLUSIONS**

A microalgal *Oedogonium* sp. system with optimized operating parameters can be proposed as an effective way to assimilate the nutrient from DPW, especially the high concentration of NH$_4^+$-N, and to reduce contamination to the environment. The analytic results of the CCD experiment indicates the adequacy of the model for optimizing the values of the variables of the initial *Oedogonium* sp. density and the DPW dilution factor to improve the NH$_4^+$-N removal efficiency. The NH$_4^+$-N removal efficiency of DPW using *Oedogonium* sp. in continuous tests with optimal factors will be further studied in the future.

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**REFERENCES**


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