A novel PSB-EDI system for high ammonia wastewater treatment, biomass production and nitrogen resource recovery: PSB system
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ABSTRACT

A novel process coupling photosynthetic bacteria (PSB) with electrodeionization (EDI) treatment was proposed to treat high ammonia wastewater and recover bio-resources and nitrogen. The first stage (PSB treatment) was used to degrade organic pollutants and accumulate biomass, while the second stage (EDI) was for nitrogen removal and recovery. The first stage was the focus in this study. The results showed that using PSB to transform organic pollutants in wastewater into biomass was practical. PSB could acclimatize to wastewater with a chemical oxygen demand (COD) of 2,300 mg/L and an ammonia nitrogen (NH$_4^+$-N) concentration of 288–4,600 mg/L. The suitable pH was 6.0–9.0, the average COD removal reached 80%, and the biomass increased by an average of 9.16 times. The wastewater COD removal was independent of the NH$_4^+$-N concentration. Moreover, the PSB functioned effectively when the inoculum size was only 10 mg/L. The PSB-treated wastewater was then further handled in an EDI system. More than 90% of the NH$_4^+$-N was removed from the wastewater and condensed in the concentrate, which could be used to produce nitrogen fertilizer. In the whole system, the average NH$_4^+$-N removal was 94%, and the average NH$_4^+$-N condensing ratio was 10.0.

Key words | biomass production, EDI, nitrogen condensing, NH$_4^+$-N treatment, photosynthetic bacteria

INTRODUCTION

Rapid development of fertilizer, food and chemical industries has led to a great deal of ammonia nitrogen in wastewater. Discharging this type of wastewater without proper treatment would contribute to eutrophication and poison the aquatic biological life (Kurniawan et al. 2006; Escudero et al. 2014). In general, traditional physical or chemical process methods combined with biological nitrification and denitrification are used to treat high ammonia nitrogen wastewater with transformation from ammonia nitrogen to N$_2$ (Ozturk et al. 2005). Although effective, these processes are long and complicated (Lin et al. 2015). In addition, large amounts of excess sludge are generated. The excess sludge is difficult to handle and often causes secondary pollution.

To solve the difficulties in handling industrial wastewater containing high concentrations of ammonia nitrogen, a novel process, photosynthetic bacteria (PSB) treatment coupled with an electrodeionization (EDI) system, was proposed to effectively treat wastewater and recover useful biomass and nitrogen resources. The wastewater was initially treated by PSB to degrade the organic pollutants and culture useful biomass and then treated by an EDI system to remove the residual ammonia nitrogen and produce a useful N-rich concentrate. The emphasis in this study was on the first stage of the novel process: PSB treatment.

PSB wastewater treatment technology has attracted much attention because this bio-technology possesses many advantages. PSB can tolerate harsh conditions and remove pollutants satisfactorily without many tanks. The treatment was used to treat high organic wastewater (Kim et al. 2004; Lu et al. 2010) and some toxic wastewater, such as pharmaceutical wastewater (Madukasi et al. 2010).
PSB could effectively address toxic substances, such as 4-chlorophenol, and were tolerant of heavy metal ions, such as Cr$^{6+}$ (Kong et al. 2014; Kis et al. 2015). In addition, the biomass of PSB is rich in valuable substances, such as single-cell proteins and biopolymers (Azad et al. 2004). Hence, the biomass can be recycled as useful raw material in the food, medical and agriculture industries (Nakajima et al. 1997). There are also some disadvantages in PSB wastewater treatment. For example, illumination is needed, and pre-treatment is necessary sometimes with extra expense. However, in general, using PSB to treat high ammonia nitrogen wastewater can achieve organic pollutant removal and useful biomass production, thus avoiding excess sludge production and treatment compared to traditional wastewater treatment technologies.

The EDI system is composed of electrodialysis and ion-exchange and is widely studied in water purification and water treatment (Alvarado & Chen 2014). This technology allows for the removal of ions from the influent and recovery of concentrated solutions (Mahmoud et al. 2003). It was used to reduce ammonia ions in previous studies with successful results (Spiegel et al. 1999; Goffin & Calay 2000). Thus, ammonia nitrogen can be removed and concentrated in an EDI system. The concentrated effluent, rich in ammonia nitrogen and relatively pure, has the potential to be used to produce fertilizer. In general, on the premise of treating the wastewater, resource recycling can be realized in the PSB-EDI process.

In this study, the feasibility of PSB to reduce organic pollutants together with biomass recovery in high ammonia nitrogen wastewater was investigated. In view of efficiency and economy, the suitable operative parameters were determined by an L$_{25}(5^3)$ orthogonal test. The practice of treating the PSB effluent with an EDI system was also explored.

**MATERIALS AND METHODS**

**Materials and experimental set-up**

Synthetic ammonia nitrogen wastewater was used in this study. The wastewater was prepared with glucose, ammonium sulphate and dipotassium phosphate. The chemical oxygen demand (COD) was 2,500 mg/L, and total phosphorus (TP) was 45 mg/L. The NH$_4^+$-N concentration ranged between 288 and 4,600 mg/L. The pH was adjusted by adding 1 mol/L NaOH or HCl (5.0–9.0).

The PSB used in this study was a wild strain that was isolated from a fish farm. It was identified as *Rhodopseudomonas palustris* by 16S rDNA and was named Z13. Z13 was cultured using RCVBN medium in a thermostatic shaker (120 rpm, 26–30 °C) (Wu et al. 2015). RCVBN medium is one of the most important PSB culture media. It was prepared with DL-malate, MgSO$_4$, (NH$_4$)$_2$SO$_4$, CaCl$_2$, KH$_2$PO$_4$, Na$_2$EDTA, VB$_1$, nicotinic acid, biotin, and trace element solution (which contained ZnSO$_4$·7H$_2$O, MnCl$_2$·4H$_2$O, H$_3$BO$_3$, CoCl$_2$·6H$_2$O, CuCl$_2$·2H$_2$O, and Na$_2$MoO$_4$). After 48 h, Z13 was at the logarithmic growth phase and was used for wastewater treatment.

The EDI system consisted of ion exchange membranes (HeCEM Grion 7321C and HeAEM Grion 7171C), ion exchange resins (D001 and D201, Zhengguang Industrial, Hangzhou, China), anode and cathode plates (ruthenium-coated titanium alloy and stainless steel, respectively), and peristaltic pumps (BT 100-2J and BT 300-2J, Longer Pump, Baoding, China). The working volume of the system was 0.3 L. The internal structure is shown in Figure S1 (available with the online version of this paper).

**METHODS**

**PSB wastewater treatment process**

The bioreactors were 500 mL glass flasks. These flasks were sterilized at 121 °C for 30 min before use. A 400 mL volume of wastewater was added into the bioreactor each time. These bioreactors were shaken at 120 rpm at 30 °C with a shaker and illuminated by fluorescent lamps with a wavelength of 400 to 750 nm and an illumination intensity of 2,000 lux. Oxygen-permeable membranes were used to seal the bioreactors, and the dissolved oxygen (DO) was kept at approximately 0.5–1.5 mg/L by shaking. These light-oxygen conditions were chosen following our previous study and have been used by other researchers (Kim et al. 2004; Tao et al. 2008). In large-scale applications, the studied light condition could be realized by sunlight during the day and supplementary artificial light at night, and the studied oxygen condition could be realized by stirring.

In the feasibility study, the feasibility of PSB to reduce organic pollutants together with biomass recovery in high ammonia nitrogen wastewater was investigated. The NH$_4^+$-N concentration, COD, and TP of the wastewater was 2,300, 2,300 and 45 mg/L, respectively. The PSB inoculum size was 20 mg/L (dry weight). The initial pH was 7.0.

In the operative parameters study, an L$_{25}(5^3)$ orthogonal test was designed with NH$_4^+$-N concentration levels of 288, 575, 1,150, 2,300 and 4,600 mg/L, initial pH levels of 5.0, and light conditions between 0.5 and 1.5 mg/L by shaking.
6.0, 7.0, 8.0 and 9.0, and inoculum size levels of 10, 20, 40, 60 and 90 mg/L (Table 1). There were 25 experimental groups in total.

After the PSB treatment, the biomass was separated by centrifugation. The PSB biomass recycled after the first stage could be used as raw material to produce protein and high value substances or could be used as fish feed after some processing. The supernatant was then treated by the EDI system.

**EDI system**

The effluent from the first stage was treated in an EDI system, where the NH\textsubscript{4}\textsuperscript{+}N was removed from the wastewater and concentrated into the EDI concentrated effluent. The purified water compartment (the central one) of the EDI was filled with mixed anion and cation exchange resins (1:1, v/v). The EDI system was set up by feeding different compartments with three different liquids under 30 voltages. The liquids for the five compartments were driven by five peristaltic pumps from the bottom up through the system. The liquids were separated by anionic and cationic exchange membranes (AEM and CEM) without interference. The purified water compartment was fed with PSB-treated effluent, and the flow was 5 mL/min. The concentrate compartments were fed with circulated deionized water, and the flow was 20 mL/min. The electrode compartments were fed with 1 mol/L circulated sodium sulphate solution, and the flow was 20 mL/min. The duration of the treatment in the EDI was 80 min. Ammonia nitrogen was transferred from the central compartment to concentrate compartment 2 in the electric field. The H\textsubscript{2}O was split between the anion and cation exchange resins, anion exchange resins and CEM, and cation exchange resins and AEM. The resins absorbed and saturated by NH\textsubscript{4}\textsuperscript{+}-N were regenerated in situ by H\textsuperscript{+} and OH\textsuperscript{-}, and the absorption capacity for NH\textsubscript{4}\textsuperscript{+}-N was recovered. NH\textsubscript{4}\textsuperscript{+}-N was removed from the feed water (PSB-treated wastewater) and was enriched in concentrate compartment 2. Purified effluent and concentrated effluent were obtained in the end.

**Analysis methods**

For the PSB wastewater treatment section, samples were collected from bioreactors and were centrifuged at 9,000 rpm for 10 min. The supernatant was used to test the COD and NH\textsubscript{4}\textsuperscript{+}-N concentrations using APHA standard methods (closed reflux, colorimetric method and titrimetric method), and the collected PSB were used to measure the biomass (dry weight) (Wu et al., 2015). The pH was measured using a pH tester (PHS-3C, Inesa Instrument Inc., Shanghai, China), and the DO level was controlled using a DO meter (JPB-607A, Inesa Instrument Inc., Shanghai, China).

For the EDI wastewater treatment section, the samples were collected from the purified water compartment and concentrate compartment 2. NH\textsubscript{4}\textsuperscript{+}-N removal and the condensing ratio in this section were calculated as follows:

\[
\text{NH}_4^+ \text{-N removal} = \frac{C_1 - C_2}{C_1} \times 100\% \quad (1)
\]

\[
\text{NH}_4^+ \text{-N condensing ratio} = \frac{C_3}{C_1} \quad (2)
\]

where \(C_1\) is the NH\textsubscript{4}\textsuperscript{+}-N concentration of the PSB-treated wastewater, \(C_2\) is the NH\textsubscript{4}\textsuperscript{+}-N concentration of the purified
The feasibility of using PSB to degrade organic pollutants and accumulate biomass in high NH₄⁺-N wastewater was investigated first, and the results are shown in Figure 1. It can be observed from Figure 1 that PSB could tolerate high NH₄⁺-N concentrations with very low COD/N (1.0) and could transform organic compounds in the wastewater into valuable biomass.

COD represents the organic pollutants in the wastewater, and biomass proliferation represents how many times the PSB biomass increases after 96 h. As Figure 1 shows, the COD decreased effectively and the biomass increased synchronously in high NH₄⁺-N wastewater. The COD removal was 75.1% at 96 h and the PSB biomass increased by 10.25 times. In previous studies, the PSB cells showed an increase of 3–28 times in wastewater treatment or hydrogen production (Takabatake et al. 2004; Wuwansaard et al. 2009; Lu et al. 2011). Hence, the PSB biomass accumulation in this study was within the normal range, which was excellent considering the extremely low C/N ratio.

**RESULTS AND DISCUSSION**

**COD removal and biomass proliferation using PSB to treat high ammonia wastewater**

The NH₄⁺-N concentration ranges from 200 to 5,000 mg/L in different ammonia nitrogen wastewater (Carrera et al. 2006). Different wastewaters usually present different pH values, and pH has a huge influence on microorganisms. In addition, the inoculum size is an important parameter for the microorganism culture because it significantly influences the microorganism growth. This is because bacteria...
regulate their performance through quorum-sensing when the number of individuals changes (Bassler & Losick 2006). Therefore, the NH₄⁺-N concentration, initial wastewater pH, and the inoculum size of the PSB are three important factors that need to be controlled to obtain satisfactory performance from PSB treatment of high NH₄⁺-N wastewater.

The detailed results are summarized in Table S1 (available with the online version of this paper). In wastewater treatment, COD removal is the main aim, and biomass production is a useful by-product; therefore, the performances were evaluated mainly according to the COD removal. In general, COD removals were higher than 60% in 88% of the experimental groups, and the biomass showed at least a 100% increase in 72% of the experimental groups (Table S1). These results indicated that the high NH₄⁺-N wastewaters were effectively treated by PSB in most cases. The average COD removals and average biomass proliferation under different conditions are summarized in Figure 2.

As Figure 2(a) shows, when the NH₄⁺-N concentration ranged from 288 to 4,600 mg/L, the average COD removals among the different experimental runs were close, and PSB grew normally. These results suggested that the appropriate range of NH₄⁺-N concentration for PSB growth was broad. Previous studies reported that PSB thrived in wastewaters with 5–1,000 mg/L NH₄⁺-N (Idi et al. 2015; Zhang et al. 2015). These reports meant PSB could handle very high NH₄⁺-N loads. Generally, microbe growth is inhibited by NH₄⁺-N at concentrations beyond a few hundred milligrams per litre. According to the literature, the inhibition of nitrite-oxidation might occur when the NH₄⁺-N concentration ranged from 16 to 500 mg/L (Philips et al. 2002). In addition, the proper COD/N for activated sludge is approximately 10–20. In contrast, PSB grew well with NH₄⁺-N from 288 to 4,600 mg/L and low COD/N ratios of 0.5–8.0. The results showed that the PSB method was much more flexible than the activated sludge process in treating high NH₄⁺-N wastewater.

As Figure 2(b) shows, there was no significant difference in COD removal and the biomass proliferation with an initial wastewater pH of 6.0–9.0. This result suggested that the appropriate range of initial pH values for PSB was relatively broad: weakly acidic, neutral and slightly alkaline conditions were all acceptable. However, the effect was much worse at an initial wastewater pH of 5.0. Acidic conditions did harm the PSB. Similar phenomena regarding the adaptability to a wide pH range were observed for hydrogen production by PSB. An initial pH of 6.0–9.0 did not affect the cumulative and maximum rates of the evolved hydrogen too much with a strain of *Rhodobacter sphaeroides* (Tao et al. 2008). Combining the results in this study and literature, PSB were not significantly influenced by and worked effectively in the pH range of 6.0–9.0, which was the same as conventional biological processes.

Figure 2(c) shows that the inoculum size affected COD removal. The optimal inoculum size was 90 mg/L. However, the results of all of the experimental runs were acceptable. Thus, the appropriate range for the inoculum size for PSB was quite broad, which provided high flexibility. It was notable that the average COD removal under an inoculum size of 10 mg/L was 70.4%. Satisfactory effectiveness was obtained under such a low inoculum size, which meant easy process start-up. Some previously reported inoculum sizes were much higher (approximately 100–1,000 mg/L) (Azad et al. 2004; Kim et al. 2004), which might be because of using different PSB genres and wastewaters.

Please note that under the same NH₄⁺-N concentration (or pH or inoculum size), all other operational parameters were different, so the deviation was high. For example, in Figure 2(a), when the NH₄⁺-N concentration was 288 mg/L, the deviation of the average COD removal was calculated by the data in Table S1, Nos 21–25. For these five experimental runs, the initial pH and inoculum size were all different from each other.

**Quantitative influences of the NH₄⁺-N concentration, initial pH and inoculum size on the treatment efficiency**

As mentioned above, the NH₄⁺-N concentration, initial pH and inoculum size are crucial factors for PSB treating the high NH₄⁺-N wastewater. Hence, the quantitative influences of these three factors on COD removal and biomass production were further analysed.

Variance analysis is usually used to judge whether there is a difference among the observed values in different experimental groups. Linear regression analysis is usually used to obtain a functional model and conclude how the dependent variable would be influenced by independent variables. Thus, based on the data in Table S1, these two methods were used to figure out the quantitative influences of the NH₄⁺-N concentration, initial pH, and inoculum size on COD removal and biomass production.

**Quantitative influences of the NH₄⁺-N concentration, initial pH and inoculum size on COD removal**

Through variance analysis, the NH₄⁺-N concentration and inoculum size had no significant influence on COD removal (*P* > 0.05), and initial pH was the opposite (*P* < 0.05).
Figure 2 | Average COD removal and biomass proliferation at 96 h under different (a) NH$_4$-N concentrations, (b) initial pH, and (c) inoculum sizes.
ally, the initial pH had no significant influence on COD removal. Actu-
pered the ability of PSB to degrade organics, so this index
above. As for the initial pH, acidic conditions severely ham-

of the PSB. These results were consistent with the
findings above. The expla-
nation for the initial pH was the same as that for the
quantitative influences of the initial pH on COD removal.

**Further NH$_4^+$-N removal from wastewater and recovery as concentrate by the EDI system**

The results showed that in the PSB stage, the average COD removal reached 80.0%, and the biomass increased by an average of 9.16 times when the COD was 2,300 mg/L, NH$_4^+$-N was 288–4,600 mg/L, and pH was 6.0–9.0. However, the NH$_4^+$-N removal in this stage was very low (approximately 20%), and the concentration was still 230–3,900 mg/L depending on the initial NH$_4^+$-N concentration. The PSB-treated effluent was rich in NH$_4^+$-N,

The results of the linear regression analysis shown in Table 3. According to Table 3, the regression model-fitting analysis of biomass production was as follows (the model was significant, $P < 0.05$):

\[
\text{Biomass production} = -107.134 - 0.012 \times \text{NH}_4^+ - N + 45.400 \times \text{pH} - 0.615 \times \text{Inoculum size} \quad (5)
\]

The units were mg/L for biomass production, NH$_4^+$-N and the inoculum size.

As Equation (5) shows, the regression coefficients of NH$_4^+$-N, the pH and the inoculum size were $-0.012$, $45.400$ and $-0.615$. Obviously, there were slight negative correlations between biomass production and the NH$_4^+$-N concentration and inoculum size, but there was a strong positive correlation between biomass production and the initial pH. These results suggested that large amounts of biomass could be recovered effectively irrespective of different NH$_4^+$-N concentrations and inoculum sizes. These results were also consistent with the findings above. The explanation for the initial pH was the same as that for the quantitative influences of the initial pH on COD removal.

**Table 3 | The results of linear regression analysis on biomass production**

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized coefficient</th>
<th>Standard error</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(constant)</td>
<td>-107.134</td>
<td>91.462</td>
<td>-1.171</td>
<td>0.255</td>
</tr>
<tr>
<td>NH$_4^+$-N concentration</td>
<td>-0.012</td>
<td>0.011</td>
<td>-1.144</td>
<td>0.266</td>
</tr>
<tr>
<td>Initial pH</td>
<td>45.400</td>
<td>11.980</td>
<td>3.789</td>
<td>0.001</td>
</tr>
<tr>
<td>Inoculum size</td>
<td>-0.615</td>
<td>0.590</td>
<td>-1.041</td>
<td>0.310</td>
</tr>
</tbody>
</table>

Dependent variable: biomass production.
B: regression coefficient; t: result of t-test for the regression coefficient. Sig.: significance of the t-test.

**Quantitative influences of the NH$_4^+$-N concentration, initial pH and inoculum size on biomass production**

Through variance analysis, the NH$_4^+$-N concentration and inoculum size had no significant influence on biomass production ($P > 0.05$), and pH was the opposite ($P < 0.05$).
which was then treated by EDI. The N load to the PSB stage and the EDI stage was 0.058–0.92 kg/(m³·d) and 5.52–93.6 kg/(m³·d), respectively.

After EDI treatment, more than 90% of the ammonia nitrogen was removed from the wastewater (90.3–95.2% depending on the initial NH₄⁺-N concentration). At the same time, NH₄⁺-N was concentrated in concentrate compartment 2, and the highest NH₄⁺-N concentration was 43,400 mg/L with a condensing ratio of 11.1. This concentrated NH₄⁺-N might be used to produce fertilizer.

In total, the PSB-EDI process provides an ideal solution for high NH₄⁺-N wastewater. Through this process, wastewater was effectively purified, and a useful biomass and a nitrogen fertilizer ingredient were obtained. Furthermore, there was no residual sludge production, and the pollution caused by residual sludge could be avoided. This novel process is worth being promoted to pilot or full scale for its multiple benefits (effective wastewater treatment, PSB biomass for feed production and NH₄⁺-N for nitrogen fertilizer production, and minimization of excess sludge problem). Furthermore, the process is simple and can be easily operated. The EDI operation processing will be further optimized in a future study. Continuous operation and natural light conditions will be adopted.

CONCLUSIONS

PSB-EDI as a novel process to treat high NH₄⁺-N wastewater was proposed. It could produce useful biomass, obtain a nitrogen fertilizer ingredient and remove pollutants. In the first stage (PSB treatment), organic pollutants in the wastewater were converted to PSB biomass. The operation was quite flexible: an NH₄⁺-N concentration from 288 to 4,600 mg/L and an initial pH from 6.0 to 9.0 did not hinder the PSB efficiency, and the inoculum size could be minimized to 10 mg/L. After the second stage (EDI system), NH₄⁺-N removal was up to 90%, and the condensed concentrate might be used to produce fertilizer. The average COD and NH₄⁺-N removals from the whole system were 80.0% (±9.7) and 94.0% (±3.0), respectively, the average biomass proliferation was 9.16 (±8.7), and the average NH₄⁺-N condensing ratio was 10.0 (±1.6).

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