Nitrogen removal in recirculated duckweed ponds system

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Abstract Duckweed-based ponds (DWBPs) have the potential for nitrogen (N) removal from wastewater; however, operational problems such as duckweed die-off regularly occur. In this study, effluent recirculation was applied to the DWBPs to solve the above problem as well as to investigate N removal mechanisms. Two pilot scale recirculated DWBPs were employed to treat municipal wastewater. The average removal efficiencies for TN, TKN and NH4-N were 75%, 89% and 92%, respectively at TN loading of 1.3 g/m².d and were 73%, 74% and 76%, respectively at TN loading of 3.3 g/m².d. The effluent of the system under both operational conditions had stable quality and met the effluent standard. Duckweed die-off was not observed during the study, which proves the system stability and effluent recirculation which is thought to be a reason. N-mass balance revealed that nitrification–denitrification and duckweed uptake play major roles in these recirculated DWBPs. The rates of nitrification–denitrification were increased as TN loading was higher, which might be an influence from an abundance of N and a suitable condition. The rates of N uptake by duckweed were found similar and did not depend on the higher TN loading applied, as the duckweed has limited capacity to assimilate it.

Keywords Effluent recirculation; municipal wastewater; N-mass balance; nitrogen removal; recirculated duckweed-based ponds

Introduction
Nitrogen (N) removal from municipal wastewater has been set as a priority concern in recent decades because of its potential harm to the aquatic environment. Though many N-treatment technologies are available such as activated sludge, biological nutrient removal (BNR) (Henze, 1991), these technologies are energy intensive and also depend on mechanical equipment, electrical energy and the availability of skilled personnel. They are relatively expensive and none of them seem applicable for small or rural communities.

In developing countries where resource and skilled manpower are limited, a new effective technology with high treatment efficiencies and low construction and operation costs is required to tackle the increasing wastewater problems. The conventional treatment system known to date for the majority of applications is waste stabilization ponds (WSP). Drawbacks associated with these include land requirement, odour, high Suspended Solids (SS) (Polprasert, 1996; Senzia et al., 2003) and low N removal. In these circumstances, introducing an aquatic plant such as duckweed might provide a more convenient alternative that can be used to supplement WSP and could treat nitrogenous wastewater in a cost-effective manner (FAO, 1999). Many research projects dealing with DWBPs have been carried out (Alaerts et al., 1996; Korner and Vermaat, 1998; Nhapi et al., 2003; Zimmo, 2003). Some of these researches reported poor and uncertain effluent qualities. Mainly, it was due to operational constraints such as duckweed die-off resulting in system instability. Other factors related to wastewater strength such as ammonia toxicity were also reported to have toxic effects on duckweed (Wang, 1991; Clement and Merlin,
Thus, there were some recommendations that appropriate modifications such as effluent recirculation may help the system to function properly (Nhapi et al., 2003).

To address these problems, this study was conducted through field trials on recirculated DWBPs with emphasis on N-removal efficiency and its removal mechanisms. The main objectives were:

(a) to determine the hydraulic characteristics of the recirculated DWBPs and their treatment performance operating at HRT of 16 days with 100% recirculation rate;
(b) to determine N removal mechanisms and
(c) to determine N-mass balance of the recirculated DWBPs.

Materials and methods

Experimental set-up

Pilot-scale DWBPs were set-up at the Environmental Research Station, Asian Institute of Technology (AIT), Thailand. These operated under an ambient temperature of 16 to 39°C (average 28°C). The pilot-scale system comprised two rectangular concrete tanks, illustrated in Figure 1, each of which has dimensions of 0.90 × 1.95 × 0.80 m and 0.90 × 1.95 × 0.70 m (width × length × water depth). The total volume and surface area of the tanks were 2.6 m³ and 3.5 m² respectively. The system was carried out under a transparent plastic roof to avoid rainfall interference. Duckweeds of mixed species between *Lemna minor* and *Wolffia arrhiza* from the surrounding ponds were used based on its abundance and suitability. They were acclimatized before use and started at a stock density of 600 g wet-wt/m².

Experimental conditions

The experiments were set-up under two different TN loadings applied to the recirculated DWBPs. The influents were AIT municipal wastewater, which had background TN concentration of average 20 mg/L. Details of the operational conditions for this study are summarized in Table 1.

Sample collection and parameter analysis

The DWBPs were acclimatized with AIT wastewater until they reached steady state based on N-removal. Samplings were carried out regularly including *in situ* measurement of pH, temperature and Oxidation–reduction potential (ORP). Duckweed biomasses were harvested by 60% of total surface area every three days. Water samples were analysed for pH, Electrical Conductivity (EC), Suspended Solids (SS), Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), Ammonium (NH₄-N), Nitrite (NO₂-N), Nitrate (NO₃-N) and Ortho-Phosphate (PO₃⁴⁻). Duckweeds were analysed for biomass production and N-content. These analyses were performed according to the *Standard Methods*.
Quantification for N removal mechanisms

The measurements of individual N-removal mechanisms were carried out during the period of 12 months. N uptake rate was calculated from N-contents in the duckweed biomass, which was harvested regularly every three days. N-removal rate via sedimentation was calculated from N-content present in the sediments. Measurements were performed using plastic screenings of an area 0.2 m² to trap the sediments at the pond bottom, and these sediments were drawn at every month accumulation. Ammonia volatilization rate was measured by covering the pond surface area of 0.1 m² with an acrylic box for 24 hours and using 2% boric acid solution to trap the gases and then analysing for ammonia concentration. The details of the collected samples for ammonia volatilization and sedimentation are illustrated in Figure 2.

The nitrification–denitrification rate was measured by using a nitrate reduction technique (Andersen, 1977; Zimmo, 2003). Water samples were collected at the top (0–0.10 m) and the bottom (0.70–0.80 m) from each pond. The samples were divided into two types of treatment N being with nitrification inhibitor (1-allyl-2 thiourea (ATU) of 20 mg/L) and without inhibitor. Then, water samples were incubated for 24 hrs under in situ pond temperature (12 hrs in light and another 12 hrs in darkness). The measurements were considered due to loss and increase of NO₂-N and NO₃-N concentrations at two different times – before and after incubation.

Tracer study

A tracer study was conducted before the recirculated DWBPs were operated to determine the hydraulic characteristics in terms of actual HRT and dispersion number (d°). Sodium chloride (NaCl) was used as the tracer for the impulse feeding in this study.

![Figure 2 Experimental set-up for ammonia volatilization and sedimentation measurements](https://iwaponline.com/wst/article-pdf/55/11/103/439209/103.pdf)
Statistical analysis and N-mass balance

Statistical analysis for the comparison of different treatment conditions was done using the two sample paired \( t \)-test. Performance differences were deemed significant if \( p < 0.05 \). The N-mass balance was computed according to the N-mass flows.

Results and discussion

Tracer study

Based on the experimental results, chloride tracer studies showed that the operating condition of theoretical HRT of 16 days with 100% effluent recirculation had a dispersion number \( (d_p) \) of 0.23 and the actual HRT was 19 days. This indicated moderate dispersion of wastewater as categorized by Metcalf and Eddy, 1995 and the hydraulic nature of this system was dispersed-plug flow. These correlated with the tracer study in the pilot-scale duckweed based ponds series of Zimmo (2003); the study also stated that duckweed cover significantly improves the hydraulic behaviour in the pond system.

Overall system performances

Extensive monitoring of the treatment efficiency N revealed that during 12 months (Apr. 2005 – Apr. 2006), the averaged influent TN was 21 mg/L to DWBPs No.1 and 52 mg/L to DWBPs No.2. This resulted in TN loadings of 1.3 g/m².d and 3.3 g/m².d respectively. Influent was primarily composed of Inorganic-N. The overall efficiency of the system with the lower TN loading rate had the removal of TKN, NH₄-N, COD and SS higher than the system with the higher loading rate \( (p < 0.05) \). However, the efficiency of TN removal was not significantly different \( (p > 0.05) \) between the two conditions, which were 75% and 73% in DWBPs No. 1 and No. 2 respectively. The overall efficiencies of these recirculated DWBPs are presented in Table 2.

The system had high removal efficiencies of Inorganic-N (91% and 75%) rather than Organic-N (75% and 54%). The values in parentheses represent DWBPs No.1 and DWBPs No.2 respectively. This might result from the abundance of NH₄-N in the influent, which duckweed had more preference to uptake than other forms. COD removal was 71% and 64% in DWBPs No.1 and No.2 respectively. Oron et al. (1987) also observed COD removal at 63% for Lemna gibba covered mini-ponds operated at HRT 20 days using settled sewage. This indicated that DWBPs were not suited for treating high

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DWBPs No.1 (TN loading 1.3 g/m².d)</th>
<th>DWBPs No.2 (TN loading 3.3 g/m².d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent (mg/L)</td>
<td>Effluent (mg/L)</td>
</tr>
<tr>
<td>pH</td>
<td>7.7 ± 0.3</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>EC(µS/cm)</td>
<td>616 ± 83</td>
<td>523 ± 55</td>
</tr>
<tr>
<td>TN</td>
<td>21 ± 4</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>TKN</td>
<td>20 ± 4</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Org-N</td>
<td>4 ± 2</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>Inorg-N</td>
<td>17 ± 3</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>16 ± 3</td>
<td>1.3 ± 1</td>
</tr>
<tr>
<td>NO₂-N</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>0.9 ± 0.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>COD</td>
<td>91 ± 27</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>SS</td>
<td>29 ± 10</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>8 ± 2</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>180 ± 34</td>
<td>143 ± 18</td>
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</tbody>
</table>

Note: Mean ± SD; n = 93.
organic wastewater due to less organic removal capacity. Moreover, intensive harvesting (if applied) could affect organic removal due to loss of some microbes attached to the duckweed roots. The averaged efficiencies of suspended solids (SS) removal in DWBPs No.1 and No.2 were 91% and 82% respectively. The SS in the effluent have been reported to be less than 10 mg/L in both treatments. This high SS removal was considered due to the duckweed mat that promoted quiescent conditions and also inhibited algal growth. During the studied period, the TN, COD and SS concentration levels in the effluent were below the Thailand effluent standard (TKN = 35 mg/L; MONRE, 2005) and also below the stringent standard of the EU (TN = 15 mg/L; CEC, 1991).

**N removal mechanisms**

The calculated rates of each N-removal mechanism from each specific measurement are summarized in Table 3.

Rates of N-removal categorized in the individual mechanisms are listed in Table 3. The results showed that nitrification–denitrification and plant uptake rates were 0.31 gN/m².d and 0.11 gN/m².d in DWBPs No.1 while the rates in DWBPs No.2 were 0.35 gN/m².d and 0.77 gN/m².d respectively. There was only a small fraction of N attributed to ammonia volatilization and sedimentation. The negligible amount of ammonia volatilization could be supported by the neutral pH (7.2–7.9) and water temperature (24–29.5 °C) present in the pond water. Ammonia volatilization is reported, which mainly depends on pH, temperature and TN input (Gomez et al., 1995). Dissolved oxygen (DO) was observed in the range of 0.1–3.4 mg/L, and oxidation–reduction potential (ORP) in the range of 70–180 mV. These values indicated aerobic conditions in which nitrification is likely to have taken place. Denitrification is likely to occur in the pond bottom where anoxic conditions are present. The duckweed consumed N of average 0.31 gN/m².d and 0.35 gN/m².d in DWBPs No.1 and 2 respectively. Alearts et al. (1996) recorded that the duckweed consumed N of 0.26 gN/m².d, while Korner and Vermaat (1998) reported N uptake by *Lemma gibba* was about 0.59 gN/m².d. Al-Nozaily et al. (2000) reported 0.46 gN/m².d and El-Shafai et al. (2007) reported 0.26 gN/m².d. These rates were found to be similar mainly due to a limited capability for nutrient uptake and/or a limitation of plant assimilation.

**N-mass balance**

N inputs and outputs throughout the study were estimated using the mass-balance process. The calculation assumed water inflow equal to water outflow. This is due to less water loss by evapo-transpiration (7%) and rainfall being prevented by a transparent plastic roof.

The results revealed that there were two dominant effective pathways for N-removal in the recirculated DWBPs, one through the nitrification–denitrification sequential reaction and the second through duckweed assimilation or plant uptake. At the lower TN

<table>
<thead>
<tr>
<th>N removal mechanism</th>
<th>DWBPs No.1 (TN loading 1.3 g/m².d)</th>
<th>DWBPs No.2 (TN loading 3.3 g/m².d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant uptake (gN/m².d)</td>
<td>0.31 ± 0.07</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>Ammonia volatilization (gN/m².d)</td>
<td>0.001 ± 0.001</td>
<td>0.007 ± 0.003</td>
</tr>
<tr>
<td>Sediment accumulation (gN/m².d)</td>
<td>0.05 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Nitrification–denitrification (gN/m².d)</td>
<td>0.11 ± 0.02</td>
<td>0.77 ± 0.15</td>
</tr>
</tbody>
</table>

Note: Mean ± SD; n = 109 for plant uptake; n = 47 for ammonia volatilization; n = 32 for sedimentation; and n = 28 for nitrification–denitrification.
loading (1.3 g/m².d), plant uptake contributed 45% and nitrification–denitrification 16% of N removal. At the higher TN loading (3.3 g/m².d) 47% and 21% of N-removal comes from nitrification–denitrification and plant uptake respectively (Figure 3). From the N-mass balance, though plant uptake showed different percentages between two conditions, the amounts of N-mass throughout the study were about the same (396 gN and 352 gN in TN loading of 1.3 g/m².d and 3.3 g/m².d respectively). This indicated a limitation of the plant assimilation capacity.

Minor pathways contributing to N-removal in this system were sedimentation and ammonia volatilization. Approximately 21% and 22% of N-mass were discharged as effluents. This left approximately 9.7% and 5.3% unaccounted for in DWBPs No. 1 and No. 2 respectively. This data indicates that the N-mass in the system was well explained with the N fluxes measured. The unaccounted parts could probably be due to unmeasured algal and microbial uptake, incomplete nitrification–denitrification and other minor losses. In the literature cited, there were also many studies that report the percentage of N attributed to each mechanism via N-mass balance in the applied duckweed system. These are summarized in Table 4.

Most of these studies found that the major N-removal mechanisms were plant uptake and the nitrification–denitrification process. Other pathways were negligible or played a less important role. However, there were some differences between the N-removal rates via plant uptake and nitrification–denitrification in these studies (Table 4). This could be attributed to the following reasons: (a) differences in duckweed biomass per water volume ratio, by which higher the better for N-removal via plant uptake; and (b) differences for operating conditions such as initial TN loading, HRT, water temperatures, DO, etc.

Figure 3  N-mass balance in the recirculated DWBPs (gN was based on N-mass along the study period)

<table>
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</thead>
<tbody>
<tr>
<td>Duckweed uptake</td>
<td>44%</td>
<td>18%</td>
<td>34%</td>
<td>30%</td>
<td>80%</td>
<td>45%, 21%</td>
<td></td>
</tr>
<tr>
<td>Nitrification</td>
<td>–</td>
<td>–</td>
<td>15–25%</td>
<td>–</td>
<td>–</td>
<td>16%, 47%</td>
<td></td>
</tr>
<tr>
<td>Sedimentation</td>
<td>10%</td>
<td>6%</td>
<td>–</td>
<td>–</td>
<td>5%</td>
<td>8%, 4.2%</td>
<td></td>
</tr>
<tr>
<td>Volatilization</td>
<td>73%</td>
<td>6%</td>
<td>–</td>
<td>1.1%</td>
<td>negligible</td>
<td>0.3%, 0.5%</td>
<td></td>
</tr>
<tr>
<td>Unaccounted-for</td>
<td>9%</td>
<td>–</td>
<td>50%</td>
<td>–</td>
<td>15%</td>
<td>9.7%, 5.3%</td>
<td></td>
</tr>
<tr>
<td>Outflow/effluent</td>
<td>9%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>21%, 22%</td>
<td></td>
</tr>
<tr>
<td>Seepage</td>
<td>28%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

Note: a,b represent the treatment conditions for TN loading of 1.3 g/m².d and 3.3 g/m².d respectively; c represents nitrification only; d represents nitrification–denitrification.
However, percentages from the N-mass balances might not be good for making comparisons between the studies. This is because the calculations were based on the N-removal mechanisms within the system studied. Though the removal amount/rates may be found to be the same, the contribution into percentage would not appear to be the same for the different systems.

Conclusions
Based on the above findings and observations, the conclusions are summarized as follows.

- The recirculated DWBPs showed relatively higher removal efficiencies (89% TN and 93% NH₄-N) at the lower TN loading than at the higher TN loading (78% TN and 75% NH₄-N). The TN, COD and SS in the effluents of these recirculated DWBPs were found to meet the stringent EU standard (CEC, 1991). The quality of effluents was stable. One reason for this stability was that when effluent recirculation was applied it caused influent dilution, oxygen addition, and provided a suitable environment for duckweed and microbial growth.
- Two dominant effective pathways were found in removing N from the recirculated DWBPs. The first was through nitrification–denitrification, and the second was through duckweed uptake.
- TN was removed via duckweed at relatively the same rate under different TN concentrations applied to the system. This indicated that the N-removal rate via this route was limited by the capability of N assimilation. The proper harvesting regime in consideration of doubling time could help to promote N-removal via duckweed uptake.
- The recirculated DWBPs showed a promising alternative for treating N-rich wastewater where an ammonium (NH₄-N) was a major species. The effluent recirculation could be a solution for duckweed die-off and unstable effluent qualities, which occasionally occurs in conventional DWBPs especially at high influent TN concentrations. The recirculation seems to enhance efficient nitrification–denitrification due to indirect adding of oxygen into the system.

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References


