

## Nitrogen Removal in Duckweed-Based Ponds with Effluent Recirculation

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### ABSTRACT

A pilot-scale set of duckweed-based ponds (DWBPs) were set up with effluent recirculation added into the influent line to see what effect this had on solving system failure and enhancing nitrogen removal. The experimental results showed that the optimum performance in terms of nitrogen removal was obtained when the system was operated at a hydraulic retention time (HRT) of 16 days with 100% effluent recirculation. Average removal efficiencies were 72% for total nitrogen (TN), 72% for total kjeldahl nitrogen (TKN) and 73% for ammonia-nitrogen (NH<sub>4</sub>-N). During the six-month study period, it was found that all the experimental systems had good stability with no die-off of the duckweed. A nitrogen mass balance study indicated that the three main mechanisms for nitrogen removal in the DWBPs with effluent recirculation were; duckweed uptake, nitrification-denitrification and sedimentation, respectively. The first order constant rates (h<sup>-1</sup>) for TN removal and for NH<sub>4</sub>-N were in the range of 0.048-0.074 and 0.047-0.78 per day, respectively.

**Key words:** effluent recirculation, duckweed, municipal wastewater, nitrogen removal

### INTRODUCTION

The discharge of nitrogen-rich wastewater effluents into receiving rivers can have several adverse impacts such as algal bloom, a proliferation of nuisance plants and eutrophication, that in turn have severe environmental impacts and lead to detrimental conditions for aquatic life. In addition, the leaching of nitrogen into the groundwater can pose potential human-health hazards, as high levels of nitrates have been associated with blue-baby syndrome in infants. To maintain water quality, the regulations governing the discharge of treated wastewater increasingly require the significant removal of nutrients.

In recent decades, there have been many studies on various types of wastewater treatment systems available for treating nutrients and some nitrogen-oriented treatment technologies, such as the bio-ceramic sequencing batch reactor and biological nutrient removal (BNR) processes are available for practical implementation (Henz, 1991). But these technologies are energy intensive and depend on mechanical equipment, electrical energy and the availability of skilled personnel for their ongoing operation. They are relatively expensive and none seems to be applicable for small or rural communities. Considering the socio-economic conditions of the developing countries, an appropriate technology should: be user friendly;

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have low installation and maintenance costs; require low energy usage; and also implement reuse-recovery processes. Duckweed-based ponds (DWBPs) have all of these characteristics and so may be a technology applicable to developing countries. Several research studies have been carried out on duckweed-based ponds for wastewater treatment (Alaerts *et al.*, 1996; Korner and Vermaat, 1998; Zimmo, 2003 and Nhapi *et al.*, 2003), with the treatment efficiencies differing considerably depending on regional conditions and in particular on: the retention time; water depth; initial nutrient concentration; duckweed density; duckweed genera; and harvesting regimes. (Nhapi, 2004). Some of the research related to DWBPs reported poor and uncertain effluent qualities mainly due to the operational constraints, such as duckweed die-off resulting in system instability (Shome and Neogi, 2001; Nhapi, 2004). Other factors such as the enrichment of ammonia nitrogen were also reported to have toxic effects on duckweed (Clement and Merlin, 1995; Caicedo *et al.*, 2000). Consequently, there were some recommendations that appropriate modifications, such as effluent recirculation, may help the system to function properly (Nhapi *et al.*, 2003). This study was conducted through field trials using conventional DWBPs with the addition of effluent recirculation. The main objectives were to: (a) investigate the effect of HRT and effluent recirculation rates on the system performance; (b) determine the nitrogen mass balance; and (c) determine the first-order nitrogen removal rate constants.

## MATERIALS AND METHODS

### System set-up

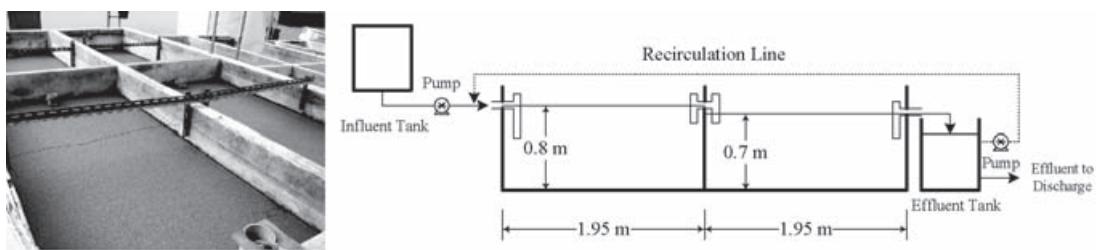
A set of pilot-scale DWBPs was installed at the Asian Institute of Technology (AIT), Thailand. Operations were conducted under ambient conditions with a temperature range of 15-38 °C and a mean of 28 °C. The system consisted of two rectangular concrete tanks, with dimensions for width × length × water depth of 0.90 × 1.95 × 0.80 m and 0.90 × 1.95 × 0.70 m, respectively (Figure 1). The total volume and surface area of the system were 2.6 m<sup>3</sup> and 3.5 m<sup>2</sup> respectively. The Duckweed species *Spirodella polyrrhiza* was used for the study with specimens taken from the surrounding area and allowed to acclimatize before use. The duckweed stock density at the start of the study was 600 g/m<sup>2</sup> (wet weight).

### Experimental conditions

The DWBPs were continuously fed with AIT campus wastewater under the operating conditions shown in Table 1. Experimental conditions were determined based on pre-test results. Harvesting of the duckweed was undertaken every four days on 50% of the total surface area, which was based on the typical reported doubling time of duckweed of 2.3-7.3 days (Oron *et al.*, 1986).

### Sample collection and parameter analysis

The DWBPs system was run with AIT wastewater until a steady state was reached based



**Figure 1** The study set of pilot-scale DWBPs and its schematic diagram.

on N removal and then sampling and duckweed harvesting including *in situ* measurement were carried out on a regular basis. Major water quality parameters such as pH, electrical conductivity (EC), dissolved oxygen (DO), suspended solids (SS), chemical oxygen demand (COD), TKN,  $\text{NH}_4^+$ -N, nitrite-nitrogen ( $\text{NO}_2$ -N), nitrate-nitrogen ( $\text{NO}_3$ -N) and ortho-phosphate ( $\text{PO}_4^{3-}$ ) were periodically monitored and the weight of duckweed was measured to determine biomass production and the N content. All the analysis was based on the standard methods (APHA, 1998). DO, temperature and pH were measured by meters.

### Calculation and statistical analysis

The nitrogen mass balance was computed according to the mass flow rate. Statistical analysis for the comparison of different treatment conditions was done using a two-sample paired t-test. Performance differences were considered significant for  $P < 0.05$ .

### Nitrogen mass balance

Nitrogen mass balance was calculated according to equation 1.

$Q_{\text{inf}}(N)_{\text{inf}} = Q_{\text{eff}}(N)_{\text{eff}} + N_{\text{dw}} + N_{\text{unacc}}$  (1)  
where:  $Q_{\text{inf}}$  &  $Q_{\text{eff}}$  = inflows and outflows (L/d).

$N_{\text{dw}}$  = nitrogen uptake by duckweed (mg/d).

$N_{\text{unacc}}$  = unaccounted-for balance of nitrogen (mg/d).

### Tracer study

A tracer study was conducted prior to the operation of the DWBPs. Sodium chloride (NaCl) was used as a tracer in the impulse feeding starting point. This was done to characterize the actual hydraulic retention time ( $\text{HRT}_{\text{actual}}$ ) and dispersion numbers ( $d^*$ ) of the designed pilot-scale DWBPs.

## RESULTS AND DISCUSSION

The dispersion numbers ( $d^*$ ) of the DWBPs under all operating conditions were in the range of 0.15-0.16, corresponding to moderate dispersion according to Metcalf and Eddy, 1991. The  $\text{HRT}_{\text{actual}}$  was calculated from the tracer results from the 50 and 100% effluent recirculation rates, resulting in values of 8.3 and 8.5 days at the theoretical hydraulic retention time ( $\text{HRT}_{\text{th}}$ ) of 8 days. The  $\text{HRT}_{\text{actual}}$  values for the 50 and 100% effluent recirculation rates were 15.9 and 16.6 days at the  $\text{HRT}_{\text{th}}$  of 16 days (Table 2). In theory, the  $\text{HRT}_{\text{actual}}$  should have been less than the  $\text{HRT}_{\text{th}}$  due to inactive pond volume or the fraction of dead-

**Table 1** Experimental conditions in the study.

No.	Effluent recirculation rate (%)	$\text{HRT}_{\text{th}}(\text{d})$	TN loading(g/m <sup>2</sup> .d)
1	50	8	2.5
2	100	8	2.3
3	50	16	1.1
4	100	16	0.9

**Table 2** Actual HRT and dispersion numbers of pilot-scale DWBPs.

$\text{HRT}_{\text{th}}(\text{d})$	Effluent recirculation rate (%)	$\text{HRT}_{\text{actual}}(\text{d})$	Dispersion No.( $d^*$ )
8	50	8.3	0.15
	100	8.5	0.15
16	50	15.9	0.15
	100	16.6	0.16

space present in the ponds. The contradictory results for the study were possibly caused by the effluent recirculation carrying back some of the tracer into the influent.

### Performance of the recirculated DWBPs

Measurements were taken for a six month period. The Effluent Guideline of EU Directive 91/271/EEC, required TN concentrations to be less than 15 mg/L for 10,000–100,000 p.e. (CEC, 1991). The results of this study showed that the effluent quality of the DWBPs operated at HRT 16 days under conditions of 50 and 100% effluent recirculation rates met the criteria for discharge from urban wastewater treatment plants to areas that are sensitive to eutrophication. However, the TN concentrations of the DWBPs operated at HRT 8 days in this study were slightly higher than the maximum allowable limit of the EU Directive 91/271/EEC. The average values for the removal efficiency are shown in Table 3.

### N removal

The DWBPs received influent wastewater with an average of 28 mg/L of TN and

23 mg/L of NH<sub>4</sub>-N. At HRT 16 days, the average TN removals were 72 and 61% under conditions of 50 and 100% effluent recirculation rates, respectively. At HRT 8 days, the average TN removals were 32 and 36% under conditions of 50 and 100% effluent recirculation rates, respectively. A comparison of the TN and NH<sub>4</sub>-N removal efficiencies between the four treatments is shown in Figure 2. The results showed that the DWBPs at the longer HRT could provide better treatment efficiencies and there were no significant differences ( $P > 0.05$ ) in terms of TN removal between the two effluent recirculation rates applied (50 and 100%) at the same HRT.

### N uptake by plant

A portion of the total nutrients could be attributed to aquatic plant assimilation which was then removed from the system via plant harvesting. In this study, the average value of the harvested biomass in all treatments ranged from 79.0–86.6 g/m<sup>2</sup>.d (wet weight), which contained 93.1–96.0% water. The nitrogen content in the biomass was 60.6–65.4 gN/kg (dry weight). The TN removal via duckweed harvesting was calculated to be 0.26–

**Table 3** Treatment efficiencies in the DWBPs with effluent recirculation.

Parameters	Influent (mg/L)	Removal efficiency (%)			
		HRT (d); Recirculation rate (%)			
		8d; 50%	8d; 100%	16d; 50%	16d; 100%
pH	7.5 (0.1)	-	-	-	-
EC(μS/cm)	602 (55)	-	-	-	-
TN	28 (6)	32 (9)	36 (10)	61 (21)	72 (9)
TKN	27 (6)	32 (10)	36 (10)	61 (21)	72 (9)
Org-N	4 (2)	48 (19)	44 (22)	50 (29)	66 (17)
NH <sub>4</sub> -N	23 (5)	30 (10)	35 (12)	64 (22)	73 (11)
NO <sub>2</sub> -N	0.2 (0.1)	-	-	-	-
NO <sub>3</sub> -N	0.3 (0.1)	-	-	-	-
COD	78 (15)	50 (12)	52 (12)	50 (10)	58 (12)
SS	29 (14)	69 (16)	76 (10)	66 (14)	72 (14)
PO <sub>4</sub> <sup>3-</sup>	6.5 (1)	33 (15)	45 (18)	60 (4)	61 (5)
Alkalinity	155 (14)	-	-	-	-

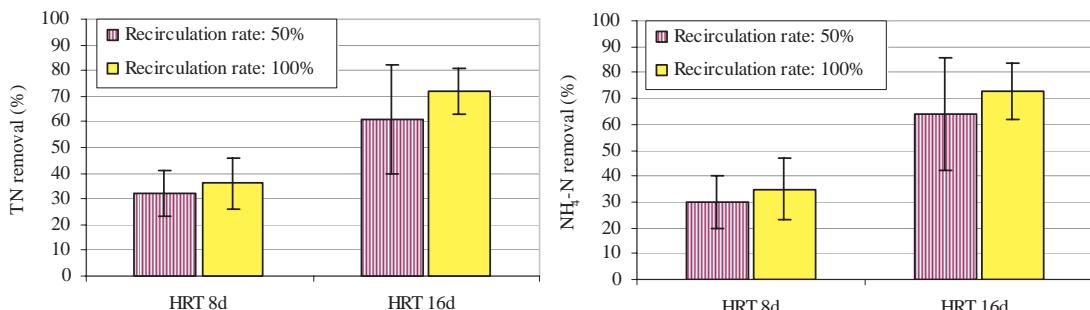
*Note:*  $\bar{X}$  ( $\pm SD$ ), n = 24

0.30 gN/m<sup>2</sup>.d. These values corresponded well with the amount of 0.26 gN/m<sup>2</sup>.d reported by Alaerts *et al.* (1996). Similar biomass production and TN content were observed for the TN loadings of 2.4 g/m<sup>2</sup>.d (HRT 8 days) and 1.0 g/m<sup>2</sup>.d (HRT 16 days) applied in this study. This suggested that this level of TN loading had no inhibition on the duckweed growth, but rather promoted growth rates. In addition, the relative growth rate (RGR) was 0.4 per day and the doubling time was 1.73 days. However, this result did not match with previous studies, where some researchers had observed a higher duckweed production rate in a more concentrated medium (Hammouda *et al.*, 1995; Van der steen *et al.*, 1998), while some reported a decrease in the growth rate at a higher medium concentration (Al-Nozaily *et al.*, 2000; Bergmann *et al.*, 2000). Different types of media and duckweed species used at different operational conditions could have been the main reasons for this inconsistency. It was apparent that the duckweed had a limited capability for N assimilation up to a certain level. To enhance

nitrogen removal via plant uptake, it would be better to increase the pond surface area per volume ratio of the duckweed rather than increase the medium concentration. Biomass production and the moisture and nitrogen contents of the treatments are summarized in Table 4.

### Nitrogen mass balance

N input and output in the duckweed ponds system were estimated using mass-balance analysis assuming that the water inflow was equal to the water outflow. The objective of this analysis was to investigate the major N-removal pathway in the DWBPs with applied effluent recirculation. Based on the calculation, it was found that some loss of N would be unaccounted for. Mechanisms that would mainly contribute to this unaccounted part were: denitrification; ammonia volatilization; and sedimentation (Alaerts *et al.*, 1996; Zimmo *et al.*, 2004; El-shafai *et al.*, 2007). Based on the experimental operation, ammonia volatilization was predicted to have little impact on the system. This was due to the neutral pH condition present



**Figure 2** Comparison of TN and NH<sub>4</sub>-N removal efficiencies in the DWBPs with effluent recirculation.

**Table 4** Biomass production, nitrogen and moisture content in the duckweed.

	HRT 8 days		HRT 16 days	
	Effluent recirculation rates (%)		Effluent recirculation rates (%)	
	50%	100%	50%	100%
Harvested biomass (gN/m <sup>2</sup> .d (wet weight))	79.0	86.6	83.3	85.2
Moisture contents (%)	95.0	94.7	95.1	95.2
N-contents (gN/kg; (dry weight))	65.4	65.3	61.0	60.6

in the pond, whereas volatilization was reported to play an important role if the pH were higher than 8 (Nalbur *et al.*, 2003). From the pond-situ measurements, the pH value was in the range of 7.2-7.9 while the DO and ORP values were in the ranges of 0.1-2.5 mg/L and 70-180 mV, respectively, which indicated a nitrification-denitrification process was occurring in the DWBPs. The values in Table 5 represent the

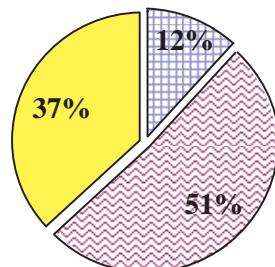
overall nitrogen mass flow in the system during the operating period.

Figures 3 and 4 summarize the breakdown of the major causes of nitrogen removal. Based on the nitrogen mass balance, the main mechanisms that were considered likely to take part in nitrogen removal in the DWBPs with effluent recirculation were nitrification-denitrification and duckweed uptake. At a TN

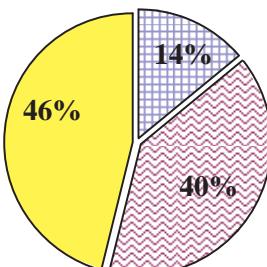
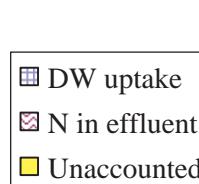
**Table 5** Overall nitrogen removal rates in the DWBPs with effluent recirculation during the experimental period.

	Operating condition			
	8d; 50%	8d; 100%	16d; 50%	16d; 100%
<b>Overall N removal (mgN/m<sup>2</sup>.d)</b>	1165	1324	686	647
(mgN/m <sup>2</sup> .d)	2393	2214	1046	854
(mgN/m <sup>2</sup> .d)	1228	890	360	207
(mgN/m <sup>2</sup> .d)	270	300	260	260
(mgN/m <sup>2</sup> .d)	895	1024	426	387

*Note:* n=24, operating period 73 days.

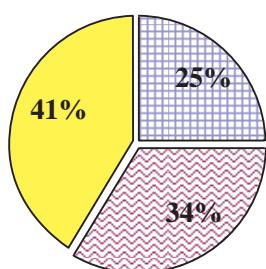


a) HRT 8d, Effluent recirculation: 50%

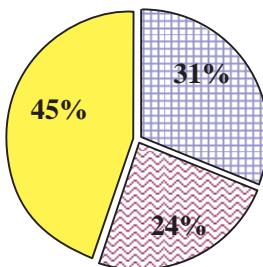
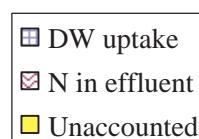


b) HRT 8d, Effluent recirculation: 100%

**Figure 3** N-mass balances of the pilot-scale DWBPs at HRT 8 days.



a) HRT 16d, Effluent recirculation: 50%



b) HRT 16d, Effluent recirculation: 100%

**Figure 4** N-mass balance of the pilot-scale DWBPs at HRT 16 days.

loading of 2.4 g/m<sup>2</sup>.d (HRT 8 days), the high amount of total nitrogen mass found in the effluent and the unaccounted part indicated a high TN concentration left in the effluents. The results also showed the mechanisms for TN removal were the same based on either the TN loading rate or HRT, which was probably related to their similar TN mass. In the study, though all mechanisms could not be identified by measurement, the nitrogen mass balance indicated that the major mechanisms responsible for N removal in the DWBPs with effluent recirculation were; duckweed uptake, nitrification-denitrification and sedimentation, respectively.

#### TN and NH<sub>4</sub>-N constant rate of removal

A first-order plug-flow model was used to describe the TN and NH<sub>4</sub>-N reduction in the DWBPs with the first-order rate constants being calculated from a linear regression using equation 2 (Polprasert, 1996):

$$\frac{C_e}{C_o} = e^{-k_T \cdot t} \quad (2)$$

where:  $C_e$  = Effluent concentration, mg/L

$C_o$  = Influent concentration, mg/L

$k_T$  = Removal rate coefficient at T °C, per day

$t$  = HRT, day

Based on this calculation, the first order rate constants of TN and NH<sub>4</sub>-N removal are shown in Table 6.

As N removal was the focus of this study, the TN and NH<sub>4</sub>-N efficiencies according to the HRT were calculated using the constants, with

these parameters being known to follow a first-order decay model. The values for TN were in the range of 0.048–0.074 per day whereas the values for NH<sub>4</sub>-N were 0.047–0.078° per day. These values were in the same range as the first-order rate constants reported for the reduction of nutrients using floating aquatic macrophytes (Sookhah and Wilkie, 2004). These kinetic coefficients can be useful in forecasting the removal rate or system efficiencies in system designs.

#### CONCLUSIONS

DWBPs with effluent recirculation were suited to treating nitrogen in municipal wastewater and the effluent TN standard of 15 mg/L (CEC, 1991) was achievable providing the system had a retention time of not less than 16 days. HRT and the TN loading rate should be used as important parameters in the design of DWBPs to achieve a good removal of nitrogen. Although duckweed ponds are more subject to instability than a waste stabilization pond system, the DWBPs with effluent recirculation enhanced the system stability as there was no duckweed die-off during the operating period. Based on the nitrogen mass balance in this study, major mechanisms responsible for N removal were; nitrification-denitrification, duckweed uptake and sedimentation, respectively. For the range of TN loading rates applied, the nitrogen uptake rates of duckweed were in the range of 260–300 mgN/m<sup>2</sup>.d. The plant had limited nitrogen assimilation up to a certain level and the frequency of harvesting

**Table 6** First-order rate constants of TN and NH<sub>4</sub>-N removal.

HRT (d)	Treatment		TN loading (gN/m <sup>2</sup> .d)	$k_{TN}$ (/d)	$k_{NH_4-N}$ (/d)
		Effluent recirculation rate (%)			
8		50	2.5	0.048	0.047
8		100	2.3	0.055	0.055
16		50	1.1	0.054	0.060
16		100	0.9	0.074	0.078

*Note:*  $k$  = removal rate coefficient at mean water temperature 26 °C.

could enhance nitrogen removal. The plant uptake rate would also increase with an increased area per volume ratio for the duckweed.

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