Water Qual Expo Health (2010) 2: 31–46
DOI 10.1007/s12403-010-0022-7

Nitrification and Denitrification in a Passive On-site Wastewater Treatment System with a Recirculation Filtration Tank

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Received: 12 August 2009 / Revised: 2 January 2010 / Accepted: 7 January 2010 / Published online: 30 January 2010

Abstract Groundwater contamination due to the failure of septic tank systems is a vital concern in environmental health. Active on-site wastewater treatment counts on the use of pumps to sustain the aerobic condition in the process and promote the nitrification which might not be sustainable in terms of energy saving. In current practice, passive on-site wastewater treatment processing is deemed a cost-effective option to improve the nutrient removal. The recirculation filtration tank (RFT) is an intermediate process installed to trigger or promote the proper nitrification/denitrification process between the septic tank and the drain field. However quantification of the nitrification remains difficult. To explore the structure and function of the microbiological community in the RFT, two types of sands—fine and coarse—were used in two consecutive phases for elucidating the nitrification and denitrification effects. With the aid of real-time PCR, the growth of nitrifiers and denitrifiers in sand was monitored in the RFT without adding any external carbon source to the sand. Further, phosphorus removal from the wastewater and the ability of limestone for phosphorus removal were also confirmed in the RFT. Fine sand with limestone mixture performed better in nutrient removal if clogging was overcome by using a grinder pump for dosing. On average, removal efficiencies of 60.54% ammonium, 49.48% total Kjeldahl nitrogen (TKN), 42.57% total nitrogen (TN), 92.06% soluble reactive phosphorus (SRP) and 87.16% total phosphorus (TP) were achieved by the RFT with fine sand. The E. Coli removal efficiency by the RFT was 99.9% in both phases.

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Keywords Wastewater treatment · Nutrient removal · Real-time PCR · Recirculation filtration tank · Environmental health

1 Introduction

Removal of the nutrients from septic tank wastewater is of crucial importance in maintaining the sustainability of the aquatic ecosystem and human health. According to USEPA, unionized ammonia (NH_3) is very toxic for salmonid and non-salmonid fish species (USEPA 1993). Fish mortality, health and reproduction can be hampered by the presence of 0.100 mg/L to 10.00 mg/L of ammonia (USEPA 1993). Nitrite is more toxic than nitrate and can cause human health problems such as liver damage and even cancers (Gabel et al. 1982; Huang et al. 1998). Nitrate/nitrite can bind with hemoglobin causing oxygen deficiency known as methemoglobinemia (MHB) by forming methemoglobin in infant's bodies. If the methemoglobin concentration rises to >10%, it can result in cyanosis ("blue-baby" syndrome). MHB affects infants under 6 months of age. The most characteristic symptom is an ashen, bluish (cyanotic) hue to the skin and nails (WHO 2003; WEF 2005). Nitrate is also responsible for a wide range of tumors in the human body (Mirvish 1991; Aslan and Türkman 2003). Nitrate is also responsible for formation of N-nitroso compounds in the digestive system (WHO 2003; Rocca et al. 2005). These compounds are considered carcinogens. Nitrite can react with amines chemically or enzymatically to form nitrosamines that are very potent carcinogens (Sawyer et al. 2003). Nitrate can inhibit iodine uptake and harmfully affect the thyroid gland (WHO 2003). Wastewater also has different phosphorus species which have a significant harmful impact on water

bodies. Both nitrogen and phosphorus can trigger eutrophication in water bodies through excessive growth of algae.

According to USEPA, about 25% of US homes depend on OWTS due to the unavailability of a centralized wastewater treatment system; this number is increasing over time (USEPA 2002, 2003). Among these, about one third do not reach their expected life or fail to treat the wastewater as they should (Smith et al. 2008). But all these systems are operated by an active process that consumes a large amount of energy for the aeration pumps. Untreated or improperly treated septic tank wastewater is a major source of groundwater contamination. Due to widespread septic tank failure, scientists, engineers, and manufacturers in the wastewater treatment industry have developed a wide range of alternative passive technologies designed to address increasing hydraulic loads, energy saving requirements, and water contamination by nutrients and pathogens in on-site wastewater treatment. These alternative systems with new materials and methods require increased focus on system performance, pollutant transport and fate, resultant environmental impacts, and an integration of the planning, design, siting, installation, maintenance, and management functions. Passive on-site wastewater treatment is defined by the Florida Department of Health (FDOH) as a type of on-site sewage treatment and disposal system that excludes the use of aerator pumps and includes no more than one effluent dosing pump with mechanical and moving parts and uses reactive media to assist in nitrogen removal. Nutrient removal, including both nitrogen and phosphorus species, is the main focus of our study in performance-based passive on-site wastewater treatment systems (OWTSs). Under "performance-based" we understand the efficiency of nutrient removal, the longevity of sorption media, etc.

The Florida Keys On-site Wastewater Nutrient Reduction Systems (OWNRS) Demonstration Project was initiated in 1995 to demonstrate the use of OSTS to reduce the concentrations of nutrients discharged to the coastal region of the Keys (Anderson et al. 1998). Five treatment trains had been adopted for testing in that study, including: (1) a septic tank followed by a recirculating sand filter (RSF) and an anoxic bio-filter (ABF) with effluent discharged to an unlined drip irrigation field, (2) a septic tank with effluent discharged to a lined drip irrigation field, (3) a fixed-film activated sludge (FAS) treatment known as the Bio-Microbics FAST^{TM} aerobic treatment unit (ATU) and an anoxic bio-filter (ABF) with effluent discharged to an unlined drip irrigation field, (4) a suspended growth biological treatment system operating a continuous feed cyclic reactor (CFCR), which is known as the AES BESTEP-IDEA^{TM} system similar to a sequencing batch reactor (SBR), with effluent discharged to an unlined drip irrigation field, and (5) a rotating biological contactor (RBC) and an anoxic bio-filter (ABF) with effluent discharged to an unlined drip irrigation field. Additional unit operations, such as chemical precipitation, supplemental carbon addition for denitrification, and additional phosphorus adsorption media, were available. Twenty-four-hour flow composite samples were collected from the influent mix tank and from each of the three treatment process effluents. Samples were analyzed according to Standard Methods (APHA 1992) for biochemical oxygen demand (BOD_5), carbonaceous biochemical oxygen demand ($CBOD_5$), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), nitrate–nitrogen (NO_3–N), nitrite–nitrogen (NO_2–N), and total phosphorus (TP). Total nitrogen (TN) was obtained by summation. However, without chemical precipitation, the quality of the effluents did not meet the Florida advanced wastewater treatment (AWT) standards of 5 mg/L for CBOD and TSS, 3 mg/L for TN, and 1 mg/L for TP.

Septic tanks followed by lined and/or unlined subsurface wetlands in sequence had also been used for on-site wastewater treatment in the last decade (Mankin and Powell 1998; Thom et al. 1998; Sun et al. 1998). The University of West Florida installed a constructed wetland in 1994 to treat 1.89 m^3/day (500 gpd) residential wastewater. The system utilizes a hybrid approach which combines subsurface and free-water surface flow designs. It consists of one 3.78 m^3 (1000 gallon) primary treatment septic tank and a 0.76 m × 0.76 m (30′ × 30′) cell of wetland subdivided into three compartments. The removal efficiency of TSS, total phosphates (TPO_4), NH_3, BOD_5, TKN, and fecal-coliform were 98%, 88%, 60%, 94%, 77%, and 97%. These tests proved the potential of using wetland as a means to polish the septic tank effluents without involving the use of complicated aerobic/anaerobic wastewater treatment technologies, such as AES BESTEP-IDEA^{TM} and Bio-Microbics FAST^{TM}. However, Florida's current septic tank regulations require subsurface flow of wastewater effluents.

If the RFT cannot fulfill full nitrification, disposal facilities in an OWTS may act as supplemental installations, which include many options such as absorption trenches, absorption beds, elevated mounds, and even injection wells. Drain field modifications also provide a good channel for denitrification. Many other commercial units were developed such as the Waterloo Biofilter® combined with a leaching trench and NITREX^{TM} and a drain field as a whole. The former uses a trickling filter for aeration with foam media; the latter is used to separately perform the nitrification and denitrification in two units in sequence. This configuration was arrived at after testing fifteen technologies in Oregon. An anoxic attached growth reactor was used to foster the denitrification. It is also known that the use of sulfur and limestone may create a similar anoxic environment for denitrification in the drain field (Shan and Zhang 1998). In terms of nutrient removal Chang et al. (2009a) compared

astatula sand with builders' washed sand in two traditional drain fields. It was found that both sands had similar performance in terms of nitrification and denitrification. Chang et al. (2009b) further tested an innovative underground drain field filled with a mixture of sorption media for nutrient removal to improve the nitrification and denitrification in the drain field.

A septic tank generally creates an anoxic/anaerobic environment with time due to accumulation of oil/scum layer on the top portion of the tank (Bounds 1997). As a consequence, a septic tank can support the denitrification process but it may not support the nitrification process due to the lack of oxygen diffusion in the scum layer. A 24-hour hydraulic retention time is generally assumed for the sludge accumulation and scum forming process (USEPA 1980). An RFT may be used to support the nitrification process before the treated wastewater goes to the drain field. A combination of septic tank and RFT is chosen for this study as a passive treatment system. The process was slow in winter due to slow bacterial activity at low temperature. Up to now, little has been reported about the nitrification and denitrification performance of the RFT in passive OWTS. Hence this kind of studies is expected to have a great impact on decentralized wastewater treatment systems. In the early stage, the overall passive OWTS can remove 96.52% TSS, 95.46% TKN, 47.58% TN and 92.84% TP (Anderson et al. 1997). Healy et al. (2004) found removal efficiencies of 83.2% TN, 100% NH_4–N, 43.3% P and 100% SS from dairy parlor washing with 6.6 days hydraulic retention time (HRT) and recirculation ratio of 3.0 associated with the RFT. If properly operated, an RFT can remove 87% of NH_3–N, 96% of BOD, 96% of TSS, and 50% of TP (IDNR 2007). Urynowicz et al. (2007) tried to evaluate the performance of the RFT in terms of nitrogen removal from septic tank wastewater and found 72.0% nitrogen removal with a recirculation ratio of 5.0 and 63.0% nitrogen removal with a recirculation ratio of 3.7 (Urynowicz et al. 2007). There is a potential problem of clogging in the sand filter due to physical (i.e. solid accumulation), chemical (i.e. precipitation reaction) and biological (i.e. biofilm growth or slow decomposition of organic matter) activities occurring in the filter (Venhuizen 1998; Hurst 2006). An RFT may be a chamber for simultaneous nitrification and denitrification if properly designed. But the denitrification process could be slow in an RFT (USEPA 1980). The objective of this paper is to present an exhaustive examination of the RFT functionality in a passive on-site wastewater treatment system for nutrient removal with the emphasis on an intercomparison between fine and coarse sand. The analysis via using the real-time Polymerase Chain Reaction (PCR) will deepen understanding of the nitrification and denitrification effects in a septic tank system. Phosphorus and pathogen removal was not the focus although it is briefly discussed in the paper.

2 Materials and Methods

2.1 Rationale of Nitrification in the RFT

Although different physical–chemical–biological processes are available for removal of nitrogen species from wastewater, biological processes are the most common due to their environmentally sound nature and cost effectiveness. Nitrification and denitrification are the two most common components of the biological wastewater treatment process. In the nitrification process, ammonium is converted to nitrite, and nitrite to nitrate. In the denitrification process, nitrate/nitrite is converted to nitrogen gas in a stepwise manner by heterotrophic bacteria in an anoxic environment with the presence of an organic carbon source. Nitrite is an intermediate product in both the nitrification and denitrification processes. For this reason, maintaining an anoxic environment is very important in the denitrification process to ensure the complete removal of nitrate. A typical septic system diagram is shown in Fig. 1 in which the nitrification can be promoted in the RSF while denitrification mainly occurs in

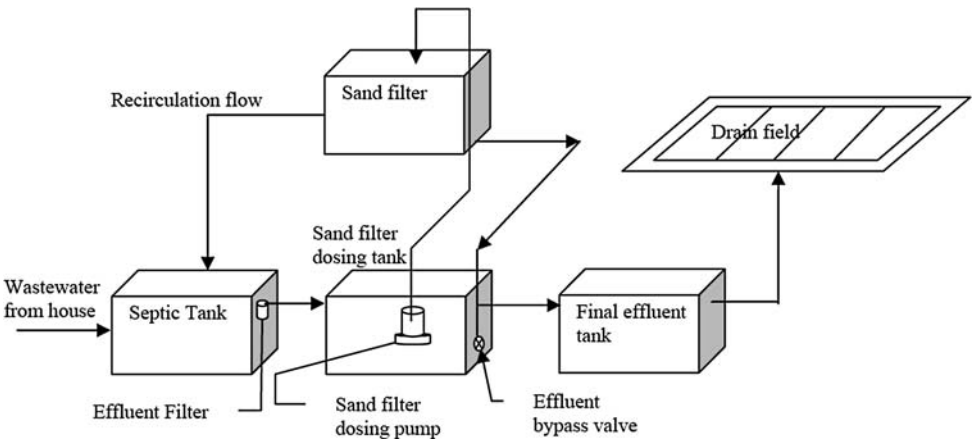


Fig. 1 The septic system including the RFT (Venhuizen 1998; Chang et al. 2007, 2009a)

the septic tank and drain field. The nitrification and denitrification mechanisms (i.e. (1)–(4)) can be expressed as below. For simplification, (3) is just the combination of (1) and (2):

- Nitrification:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

- Denitrification:

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad (4)$$

2.2 System Description of the UCF Pilot Plant

A septic tank with a capacity of 5.110 m^3 (1350 gallons) received sewage from the 15-person BPW scholarship house on campus at University of Central Florida (UCF), USA. An HRT of 24 h was applied in the septic tank. The septic tank then discharges the sewage to a dosing tank. The capacity of the RFT was about 3.975 m^3 (1050 gallons). It has been reported that multiple dosing is very effective in increasing removal efficiency (USEPA 1980). Multiple dosing means that wastewater will be discharged to the RFT intermittently. The multiple dosing concept is also rational with the wastewater generation pattern in a household. The recycled flow from the RFT went to the septic tank and mixed with the fresh sewage flow, promoting further denitrification. This RFT was basically designed to support nitrification. The RFT received hourly dosing from the dosing tank. The dosing tank sent the flow into two directions: one part went to the distribution tank as feed-forward flow and the other three parts went to the RFT as recycled flow (see Fig. 1). Thus, the recycling ratio was about 3:1. The RFT has a depth of about 1.2 m (4 feet) and it was filled with filter medium up to 0.914 m (3.0 feet) in total. It is configured as a three-layer medium: the top layer is a gravel layer, the middle layer is a fine or coarse sand mixture layer with depth of 0.6 m (2 feet), and the bottom layer is a gravel layer with depth of 0.152 m (0.5 foot). The fine sand mixture layer includes a mixture of 80% fine sand and 20% limestone in Phase I. The system was initially operated for two months to ensure equilibrium. In Phase I, the performance of the RFT was evaluated for about three months from September to November, 2008. Then, in Phase II, the middle layer was replaced with course sand to compare the performance against its previous counterpart. In Phase II, samples were collected for additional three months (February to April, 2009) for comparison.

From the distribution tank, the wastewater went to the two drain fields in equal volumes so that each drain field filled with either astatula sand or builders' washed sand received about 50% of the total flow from the dosing tank. The size of each drain field was 6.09 × 4.57 × 1.22 m (20 × 15 × 4 feet). Each drain field was filled with native soil on the top, to a depth of 0.61 m, and the remaining 0.61 m was filled with astatula sand or builders' washed sand. In this paper the performance of builders' washed sand drain field was evaluated as an integral part of the system assessment.

To sample the infiltrate and proceed with water quality monitoring in the vadose zone, three lysimeters (e.g., Soil Moisture Equipment Corporation) were installed in each of the two standard drain fields. The lysimeter equipment collects water from the unsaturated or vadose zone by a porcelain cup. The water from a lysimeter was collected using a vacuum pump. The lysimeters were placed at three different depths: 20.3 cm (8 inches), 40.6 cm (16 inches), and 61 cm (24 inches) after the top 61 cm. In this way the position of the lysimeters covered the entire depth of washed builders' sand. A schematic diagram of the system layout at the UCF Test Center is shown in Fig. 2. Samples were taken at 10 sampling points (Fig. 2, denoted S1–S10). Samples from points S5–S10 were collected by the lysimeters directly. Sampling points S5, S6, and S7 were situated in the drain field filled with astatula sand; sample points S8, S9, and S10 in drain fields filled with washed building sand. For this study, a composite sampling method was applied for sample collection. Samples were collected biweekly in the morning (from 6:00 to 8:00 am), at mid-day (from 11:00 am to 1:00 pm), and in the evening (from 5:00 to 7:00 pm). Major nutrients of concern included NH_3–N, NO_x–N (the sum of nitrate and nitrite), NO_2^-–N, organic nitrogen, TN, SRP, organic phosphorus, TP, fecal coliforms, and *E. coli*. All samples were analyzed by a certified laboratory (e.g., Environmental Research & Design Inc., ERD) in Orlando, Florida.

The pH, temperature, conductivity, dissolved oxygen (DO) were measured on-site using a HACH HQ40d multiparameter instrument. Dissolved organic carbon (DOC) was determined using a Phoenix 8000 UV-per sulfate TOC analyzer at UCF. The data was analyzed by using TOC Talk 3.0 software. The sample was filtered by 0.45 micron membrane filter before performing the DOC analysis. It was very difficult to get absolutely organic carbon free distilled (DI) water. The standard curve and the dilution for DOC analysis were carried out using ultrapure DI water (DOC 0.3 mg/L). This concentration was subtracted from the result for the standard curve and sample.

2.3 Real-Time PCR for Microorganism Identification and Quantification

The nature of the microorganism population can affect the effluent water quality in a biological wastewater treatment

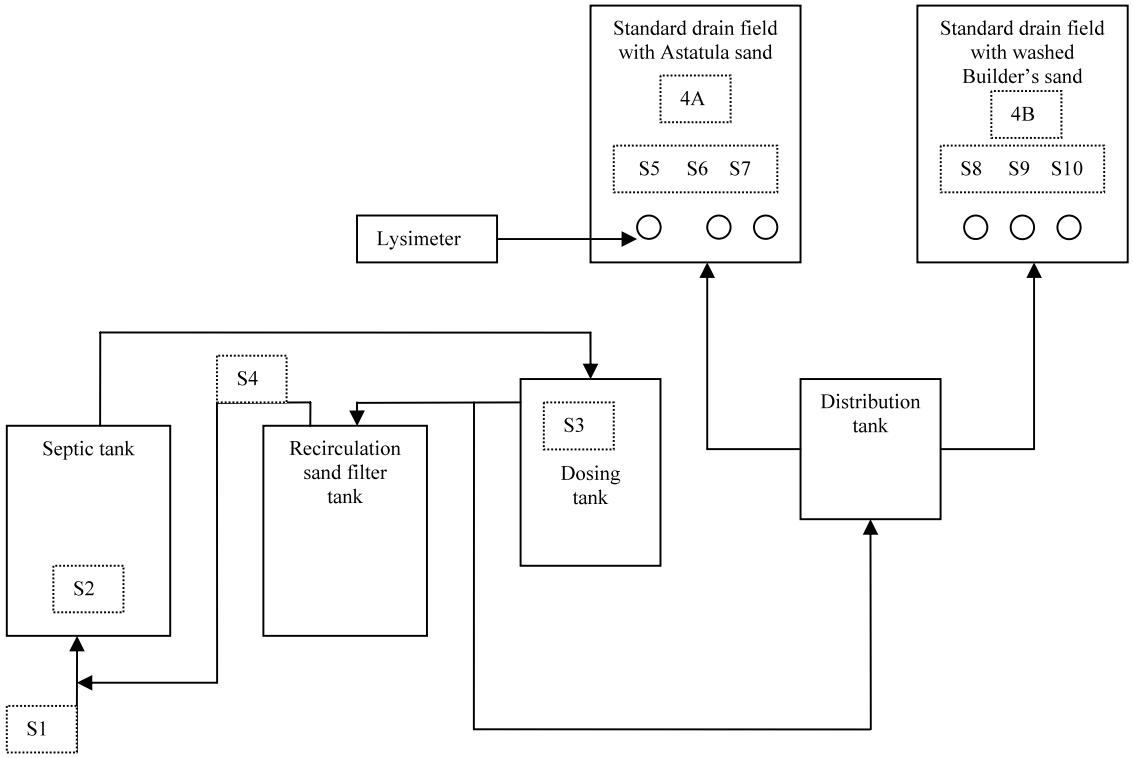


Fig. 2 Schematic diagram of the septic tank system at UCF

Table 1 The oligonucleotide sequences of the primers

Ammonium monooxygenase (*amoA*)	amoA-1F GGGGTTTCTACTGGTGGT amoA-2R CCCCTCKGSAAAGCCTTCTTC	Rotthauwe et al. (1997)
16S rRNA Nitrospira sp. (*NSR*)	NSR 1113F CCTGCTTTCAGTTGCTACCG NSR 1264R GTTTGCAGCGCTTTGTACCG	Dionisi et al. (2002)
Nitrite reductase (*nirK*)	nirK 876 ATYGGCGGVAYGGCGA	Braker et al. (1998)

system (Hurst 2006). To confirm the presence of nitrifiers and denitrifiers in the system, Real-time PCR (i.e., Applied Biosystem, Step One real-time PCR system) was used to determine the presence of nitrifiers and denitrifiers. The samples for PCR analysis were collected in 1.5 mL microcentrifuge tubes from three locations in the RSF: (1) from the top of the middle layer, (2) from the half way point of the middle layer, and (3) from the bottom of the middle layer. The samples were kept at −20°C for a short period before extracting the DNA. The DNA of the microorganisms was extracted from the sand sample by following the procedure described in the SoilMaster^{TM} DNA extraction kit (e.g., EPICENTER Biotechnologies). It was possible to get about 300 µL of DNA sample by following this procedure. Subsequently the samples were analyzed in the PCR by a using specific primer; the remaining samples are kept frozen at −20°C for future use. Real-time PCR quantification was done to amplify amoA gene (ammonia monooxygenase gene) from ammonia oxidizing bacteria (AOB), NSR gene (nitrite reductase gene) from nitrite oxidizing bacteria (NOB) in nitrification and nirK gene (nitrite reductase gene) from nitrite reductase denitrifiers. The oligonucleotide sequences of the primers are shown in Table 1.

Table 2 shows the PCR mixture for amplification of a different gene. The PCR protocol for AOB was as follows: first stage—2 min at 50°C, 10 min at 95°C; second stage—with 40 cycles of 45 s at 95°C, 60 s at 55°C and 45 s at 72°C (Okano et al. 2004). The PCR protocol for NSR was as follows: first stage—2 min at 50°C, 10 min at 95°C; second stage—with 35 cycles of 15 s at 95°C and 30 s at 63°C (Harms et al. 2003). The PCR protocol for nirK was as follows: first stage—120 s at 50°C, 600 s at 95°C; second stage—with 6 touchdown cycles consisting of 15 s at 95°C for denaturation, 30 s at 63°C for annealing, 30 s at 72°C for extension and 15 s at 80°C for the final data capture step. The annealing temperature was decreased by 1°C from the second cycle up to 58°C, the last cycle with a annealing temperature of 58°C was repeated 40 times (Henry

Table 2 The composition of PCR mixture for amplification of different gene

	amoA (Ammonia oxidizing bacteria)	NSR (Nitrite reductase nitrifiers)	nirK (Nitrite reductase denitrifiers)
SYBR Green	12.5	12.5	12.5
Primer	1.5	1.5	1.0
Standard DNA or template DNA	2.0	2.0	1.0
DEPC water	9.0	9.0	10.5
Total	25.0	25.0	25.0

et al. 2004). Step One v2.1 software developed by Applied Biosystem was used for the PCR data analysis. Since the standard curve was developed based on the gene copy number per μL, the copy number of amoA, NSR and nirK can be calculated using the standard curve.

3 Results and Discussion

3.1 Phase I Testing Results

The overall performance of the nutrient removal system wide is shown in Tables 3 and 4. RFT had a significant role in the removal of nitrogen species. The removal efficiencies of NH_3–N, TKN and TN in RFT were 60.54%, 49.48% and 42.57%, respectively. As fine sand was used in the RFT, an increase in the HRT was expected since the nitrifiers had more time to perform the nitrification process. Generally, the optimal condition for nitrification may occur at pH 7.5 to 8.0 with the DO concentration $>$ 0.5 mg/L (Metcalf & Eddy, Inc et al. 2003). In our case, the pH was about 7.49 and DO concentration was about 0.67 mg/L in the RFT sample. In addition, the alkalinity all over the system was $>$119.0 mg/l (i.e., 322 mg/l in the RFT influent and 236 mg/L in the RFT effluent), which supports the nitrification functionally. A decreasing trend in the alkalinity further confirmed the presence of nitrification process in the system. To some extent, increasing ammonium concentrations (i.e. about 14.22%) in the septic tank effluent were observed. It may be due to accumulation of ammonium or decomposition of organic nitrogen or microorganism cells in the septic tank. The drain field showed some potential for nutrient removal. The overall removal efficiencies of NH_3–N, TKN and TN by the system were about 97.74%, 86.90% and −10.20%, respectively. The negative value of TN implies an increase rather than a decrease of these complements in the effluent mainly due to the increase of nitrate concentration via the nitrification process. The alkalinity concentration was about 164.5 mg/L in the drain field. The DO and pH range in the drain field was about 3.49–4.87 mg/L and 7.49–8.29, respectively. So alkalinity and DO should not be the limiting factors in the drain field for nitrification in such a favorable pH range. The high DO level might act as an inhibitor for a nitrate reductive pathway in denitrification process. The septic tank alone removed about 99.22% of E. Coli, so decomposition of E. Coli cells might also contribute some ammonium. According to real-time PCR analysis, as shown in Table 5, the AOB population decreased with depth. A significant number of AOB was detected at the bottom of the middle layer. It was observed that the ammonium concentration was 14.8 mg/L, DO was 0.67 mg/L, alkalinity was 236 mg/L and the pH was 7.49 in the effluent of the RFT in Phase I. Given these conditions, the presence of some nitrifiers at the bottom is possible.

The nitrite concentration had increased due to ammonium conversion to nitrite. Growth of NOB might interact with AOB. The AOB might also outcompete the NOB in terms of substrate consumption (i.e. CBOD) in the sense that the NOB population was not sufficient to produce the nitrate. As nitrite transformed from ammonium at the top of RFT could not reach the middle layer due to the lower permeability of the fine sand, the NOB at the top layer of the RFT had time to consume nitrite and increase its population in that portion. The NOB population decreased with the availability of nitrite in the middle layer. The TKN removal was therefore satisfactory (i.e. 86.89%) due to the initial ammonification in the system, but this was not the case in TN removal (i.e. −10.2%) due to the increase of nitrate and nitrite concentrations in the system. The temperature during the sampling program varied from 25.3°C to 29.05°C, which should not be the key limiting factor in both nitrification and denitrification.

There was little or no denitrification in the system due to the lack of organic carbon in the fine sand. Thus sand alone was not capable of supporting full denitrification and organic carbon available in this wastewater was not sufficient for fostering denitrification. The average DOC concentration in the wastewater was about 59.9 mg/l. During denitrification, the pH can be generally elevated due to alkalinity production and a pH range of 7.0 and 8.0 is sufficient to start the denitrification process (Metcalf & Eddy, Inc et al. 2003). In this system, the pH range was 6.88 to 7.95 but there was no increasing trend of pH in the system. Although the DO concentration in the influent was about 0.25 mg/L, no denitrification was observed in the septic tank with an HRT of 24 hours.

Table 3 (a) Nitrogen removal (μg/L) in Phase I. (b) Phosphorus removal (μg/L) in Phase I

(a)

Location	NH_3–N	NO_x–N	NO_2^-–N	NO_3^-–N	TKN	TN
S1 (i.e. wastewater influent)	32 864	11	8	3	46 259	46 270
S3 (i.e. septic tank effluent)	37 538	41	32	9	42 704	42 745
S4 (i.e. RFT effluent)	14 813	2978	1635	1343	21 571	24 549
S8 (Sample from drain field)	2193	32 579	935	31 644	9516	42 095
S9 (Sample from drain field)	15	60 200	33	60 167	4870	65 070
S10 (Sample from drain field)	20	41 947	9	41 938	3803	45 750
Average of S8, S9 & S10	742.7	44 909	325.7	44 583	6063	50 972

(b)

Location	SRP (μg/L)	Dis. Org. P (μg/L)	Par. Org. P (μg/L)	TP (μg/L)
S1 (i.e. wastewater influent)	4928	32	2240	7200
S3 (i.e. septic tank effluent)	4271	1254	915	6440
S4 (i.e. RFT effluent)	339	92	396	827
S8 (Sample from drain field)	3411	69	55	3535
S9 (Sample from drain field)	2332	33	65	2430
S10 (Sample from drain field)	4972	93	95	5160
Average of S8, S9 & S10	3572	65	71.7	3708

NO_x–N = NO_2^-N + NO_3^-–N

Table 4 (a) Nitrogen removal (%) in Phase I. (b) Phosphorus removal (%) in Phase I

(a)

Location	NH_3–N (%)	NO_x–N (%)	NO_2^-–N (%)	NO_3^-–N (%)	TKN (%)	TN (%)
[a]Removal from influent to RFT (%)	54.93	−26 973	−20 338	−44 666.70	53.40	46.90
[b]Removal by only RFT (%)	60.54	−7163.40	−5009	−14 822.20	49.50	42.60
[c]Removal from influent to washed builders' sand drain field (%)	97.74	−408 161	−3971	−1 486 000	86.90	−10.20

NO_x–N = NO_2^-N + NO_3^-–N

[a]Removal from influent to RFT i.e. S1 to S4

[b]Removal by RFT alone i.e. S3 to S4

[c]Removal from influent to builders' wash sand drain field (here average data of S8, S9, S10 is used)

(b)

Location	SRP (%)	Dis. Org. P (%)	Par. Org. P (%)	TP (%)
[a]Removal from influent to RFT (%)	93.10	−19	82.30	88.50
[b]Removal by only RFT (%)	92.10	92.70	56.70	87.20
[c]Removal from influent to washed builders' sand drain field (%)	27.50	−103.10	96.80	48.50

Table 5 The population of microorganism in the middle layer of the RFT in Phase I (Chang et al. 2010)

Target gene	Sample location	Gene copy number/g sample	Standard Curves		
			Slope	Y-Intercept	R^2
NSR (Nitrite reductase denitrifiers)	Top of the middle layer	9.8×10^4	−3.25	32.65	0.99
	Middle of the middle layer	1.6×10^4			
	Bottom of the middle layer	1.5×10^4			
amoA (Ammonia oxidizing bacteria)	Top of the middle layer	4.1×10^9	−3.45	49.15	0.99
	Middle of the middle layer	3.3×10^8			
	Bottom of the middle layer	1.8×10^9			

Nevertheless, phosphorus removal was much better than nitrogen removal. In the RFT, SRP and TP removal efficiencies were about 92.06% and 87.16%, respectively. This might be due to the presence of limestone in the RFT. Limestone undergoes a precipitation reaction with phosphorus and also has the capability to adsorb it. So it can remove phosphorus in both physical and chemical processes. Obviously, the phosphorus removal was not good at all before and after the RFT. Besides, the RFT pH (i.e. 7.49) favored the phosphorus removal by adsorption or chemical precipitation. Yet this was not the case in the drain field. One possible reason for the lower phosphorus removal in the drain field was that phosphorus might be accumulated in the drain field over time.

E. Coli removal was about 100.00% through the whole system. Obviously, fine sand was good enough for pathogens removal too. But it may cause clogging very quickly if not appropriately handled. In our system, clogging became noticeable after six months of running the RFT with fine sand. Clogging might happen in the system due to solid accumulation or precipitation of hydroxylapatite (end product of phosphorus and calcium precipitation reaction) as indicated by the fact that about 64.28% of the total suspended solids (TSS) were removed by RFT alone during Phase I. This caused the replacement of fine sand with coarse sand to prevent clogging in Phase II testing. Appendix summarizes the mass balance of the overall OWTS system in Phase I. It is obvious from this appendix that the total N and the total P concentrations are not being conserved. This is probably due to the accumulation occurring within certain system units being hard to quantify. For this reason, this appendix provides only an indication of the conditions during the treatment process.

4 Phase II Testing Results

The nutrient removal data in Phase II were presented in Tables 6 and 7. When the middle layer was replaced with course sand in the RFT, the overall nutrient removal efficiency deteriorated. Ammonium removal efficiencies in the RFT and the whole system were about 26.60% and 99.73%, respectively. As coarse sand was used, the HRT might be decreased due to the higher permeability. Consequently, microorganisms had less time to consume ammonium and form the biofilm on the surface of the coarse sand. The TNK removal efficiency was also decreased due to poor removal of ammonium in the RFT. In the RFT, there was a decreasing trend of alkalinity (i.e. 264 to 200 mg/L) and the suitable pH (i.e. 6.8–6.9) was maintained. The overall performance of the system for NH_3–N, TKN and TN removal was about 99.73%, 31.21% and 95.12%, respectively. So if it is possible to increase the HRT, the system may be able to increase the nitrification in the RFT. Some nitrification also appeared in the septic tank. Table 8 presents the microorganism population in the RFT. Growth of AOB in the RFT was opposite to the previous observation. Generally it is believed that AOB may grow better in upper portion of the system due

Table 6 (a) Nitrogen removal (μg/L) in Phase II.
(b) Phosphorus removal (μg/L) in concentration in Phase II

(a)

Sampling location	NH_3–N	NO_x–N	NO_2^-–N	NO_3^-–N	Org. N	TKN	TN
S1 (i.e. wastewater influent)	41 884	48	31	17	3016	44 900	44 948
S3 (i.e. septic tank effluent)	30 316	15	12	3	9380	39 696	39 711
S4 (i.e. RFT effluent)	22 253	3570	1147	2423	2626	24 879	28 449
S8 (Sample from drain field)	197	30 364	18	30 346	990	1187	31 551
S9 (Sample from drain field)	73	34 983	11	34 972	3708	3781	38 764
S10 (Sample from drain field)	69	35 596	13	35 583	1526	1595	37 191
Average of S8, S9 & S10	113	33 647.7	14	33 633.7	2074.67	2187.67	35 835.3

(b)

Sampling location	SRP	Org. P	TP
S1 (i.e. wastewater influent)	3164	3694	6858
S3 (i.e. septic tank effluent)	5577	639	6216
S4 (i.e. RFT effluent)	6354	909	7263
S8 (Sample from drain field)	7086	216	7302
S9 (Sample from drain field)	7196	773	7969
S10 (Sample from drain field)	6852	500	7352
Average of S8, S9 & S10	7044.67	496.333	7541

NO_x–N = NO_2^-N + NO_3^-–N

Table 7 (a) Nutrient removal (%) in Phase II. (b) Data of the nutrient removal (%) in Phase II

(a)

Location	NH_3–N	NO_x–N	NO_2^-–N	NO_3^-–N	TKN	TN
[a]Removal from influent to RFT (%)	46.90	−7337.50	−3600	−14 153	12.90	44.60
[b]Removal by only RFT (%)	26.60	−23 700	−9458.30	−80 667	72	37.30
[c]Removal from influent to Builder's wash sand drain field (%)	99.73	−69 999	54.84	−197 745	31.21	95.13

(b)

Location	SRP	Dis. Org. P	Par. Org. P	TP
[a]Removal from influent to RFT (%)	36.70	−100.80	75.39	−5.90
[b]Removal by only RFT (%)	28.40	−13.90	−42.30	−16.80
[c]Removal from influent to washed builders' sand drain field (%)	20.27	−122.65	86.56	−9.96

Table 8 Microorganism population in the middle layer of the RSF in Phase II

Target gene	Sample location	Gene copy number/gram sample	Slope	Y-Intercept	R-square
nirK (Nitrite reductase denitrifiers)	Top of the middle layer	10 932.3	−3.31	39.12	0.99
	Middle of the middle layer	66 392.84			
	Bottom of the middle layer	21 233.5			
NSR (Nitrite reductase nitrifiers)	Top of the middle layer	UD	−3.75	35.06	0.98
	Middle of the middle layer	65 761.4			
	Bottom of the middle layer	60 714.3			
amoA (Ammonia oxidizing bacteria)	Top of the middle layer	8.68E+10	−3.04	55.21	0.99
	Middle of the middle layer	9.44E+10			
	Bottom of the middle layer	1.20E+11			

to the presence of oxygen. However, during Phase II, the AOB population increased with depth in the RFT (i.e., 8% increase in the middle layer and 27% increase in the bottom layer). The ammonium concentration was 22.25 mg/L, DO was 1.96 mg/L, alkalinity was 116 mg/L, and pH was 6.85 in the RFT effluent. The DO, alkalinity and pH range in the drain field was 2.83–4.48 mg/L, 95–128 mg/L and 6.98–7.44, respectively. So the drain field provided a favorable environment to support the nitrification process. All the possible conditions were present for the growth of nitrifiers in the bottom of the RFT to support the possible outgrowth of nitrifiers at the bottom of the RFT. The NOB population at the top of the RFT was very low because in the top layer it had almost no/little nitrite to survive.

Denitrification was also hampered by the lack of organic carbon in both the RFT and the drain field. The only OC source was wastewater itself. The high porous area of coarse sand in the RFT could contain a higher concentration of DO (i.e. 1.96 mg/L). Although the presence of denitrifiers was shown in real-time PCR, those denitrifiers were using organic carbon from wastewater and consuming oxygen rather than nitrate due to their facultative nature. This is one of the reasons why the top portion still contained some denitrifiers. But it was observed that some denitrification was in progress in the septic tank with a DO concentration of 0.47 mg/L and a pH of 7.75. This result was consistent with other literature (Pochana and Keller 1999; Metcalf & Eddy, Inc et al. 2003). Hence, if a suitable environment is present in the septic tank, then that itself can start the simultaneous nitrification and denitrification processes.

Figure 3 shows a comparison of the microorganism population in the RFT over two phases. Since we are not certain of the trend between the data points over the depth of the RFT, the dashed and solid lines shown provide only an indication. The AOB growth in Phase II was higher than in Phase I due to high mass flow of ammonium in Phase II (i.e. 41.88 mg/L > 32.86 mg/L). As Phase II used coarse sand, this sand has a higher porosity for a high mass flow of ammonium such that the AOB population was probably greater than the other two population groups. Because ammonium

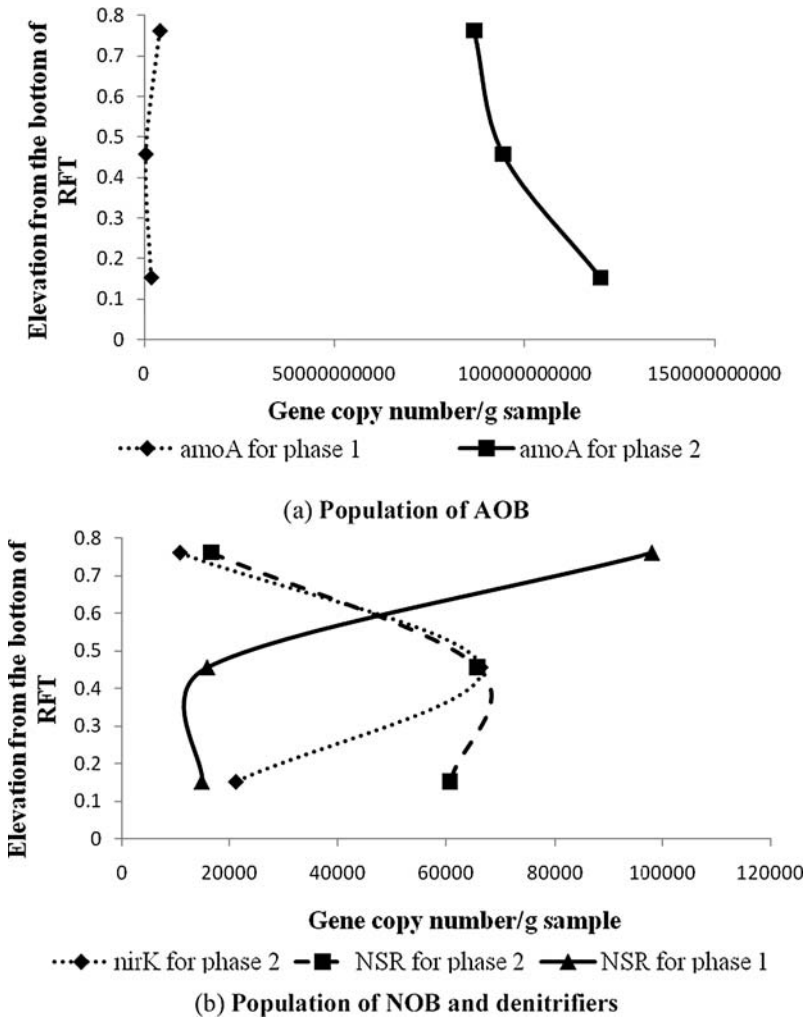
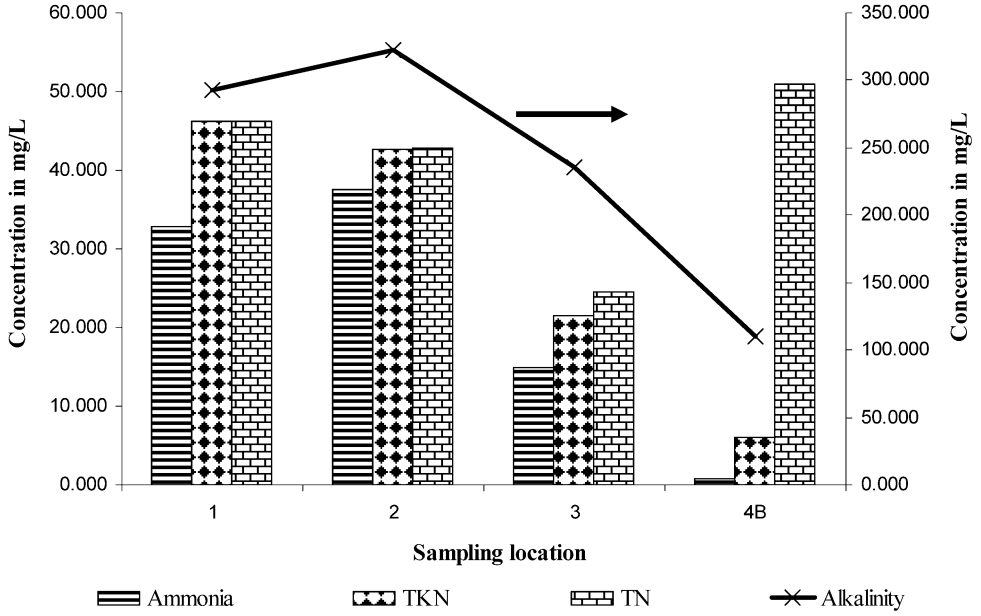


Fig. 3 Comparison of population of microorganisms in the RFT

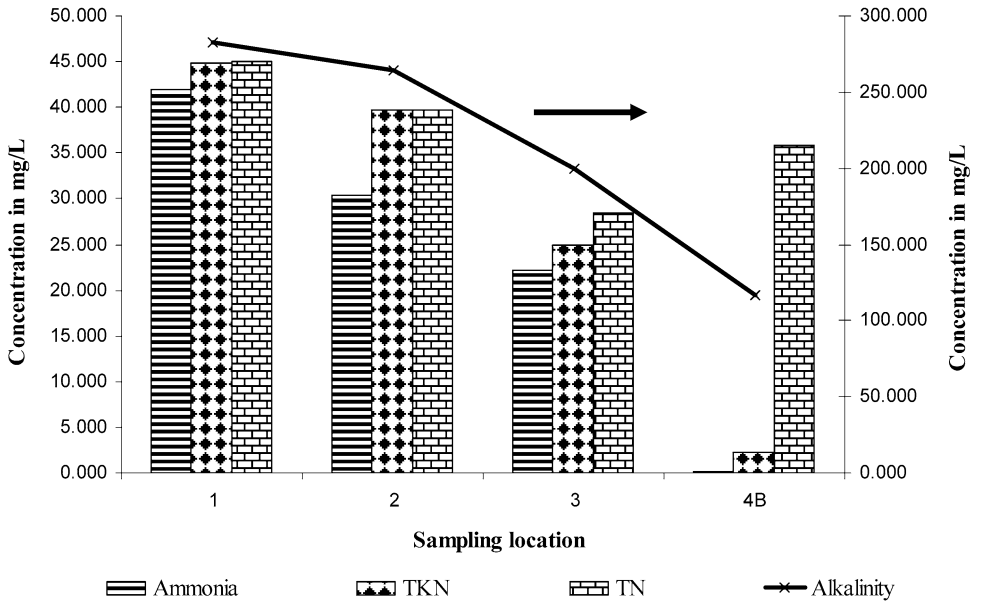
is the first nitrogen species to be involved in the nitrification process, AOB has the first opportunity to grow in the RFT. As a consequence, other microorganisms had less space to grow their colony in a specific volume. But Phase II had a lower ammonium removal efficiency with its higher AOB population due to the smaller HRT encountered in the RFT. During Phase II, no significant clogging problem was observed but the decrease in nutrient removal was substantial. Figure 4 shows comparative delineation of population dynamics over depth across both phases.

The removal of phosphorus deteriorated significantly due to the absence of limestone in the RFT. The coarse sand used in the RFT probably had no other minerals like Fe, Al or Ca that could support the phosphorus removal in RFT. The system was leaching noticeable amounts of SRP and TP. This might happen due to phosphorus accumulation with time or microorganism/pathogens decay in the system. Moreover, the conversion of organic P (i.e. about 73.26% organic P removal in the whole system) into an inorganic form also raised the inorganic P concentration in the system. The E. Coli removal in the system was about 100%. Although no clogging was observed, the RFT removed 21.88% TSS during Phase II.

Figure 4a and b illustrates the removal of nitrogen species relative to the alkalinity changes over five sampling locations (i.e., S1, S2, S3, S4, and 4B as shown in Fig. 2) in the treatment process. It clearly indicates that nitrification did occur from S1, to S2, to S3, to S4, and to 4B as evidenced by the generally decreasing trend of alkalinity. Both phases follow a very similar trend in nitrogen removal. If denitrification is substantial, the alkalinity level at 4B should gradually increase. This reverse trend did not happen due to the lower level of denitrifiers present in the RFT. One of the reasons is the lack of a carbon source as electron donor to trigger the denitrification. Extended research focusing on the use of different treatment media as electron donors, such as sawdust, zeolites, tire crumbs, oyster shell, and spodosols, for improving nutrient removal from alternative on-site wastewater treatment technologies will become the focus in the future. Figure 5a and b summarizes the removal of phosphorus species over the same sampling locations. Obviously, the performance of phosphorus removal in Phase I is much bet-



(a)



(b)

Fig. 4 Comparative nitrogen removal over two phases: **a** removal of nitrogen species in Phase I; **b** removal of nitrogen species in Phase II

ter than in Phase II due to the limited adsorption capacity in the RFT that may be already exhausted in Phase I.

5 Conclusions

This study confirms that the removal of nutrients by the RFT occurs by a combination of physicochemical and microbiological processes. An RFT can be judged cost-effective in terms of nitrification/denitrification processes if an appropriate design is developed. The lack of organic carbon in the effluent is responsible for the failure of denitrifiers to grow in the RFT since the sand itself cannot fully support the growth of denitrifiers without a source of organic carbon. Using either fine or coarse sand brings about advantages/disadvantages in terms of nutrient removal and clogging potential. When limestone was used in the RFT, phosphorus removal was significant. But no phosphorus removal

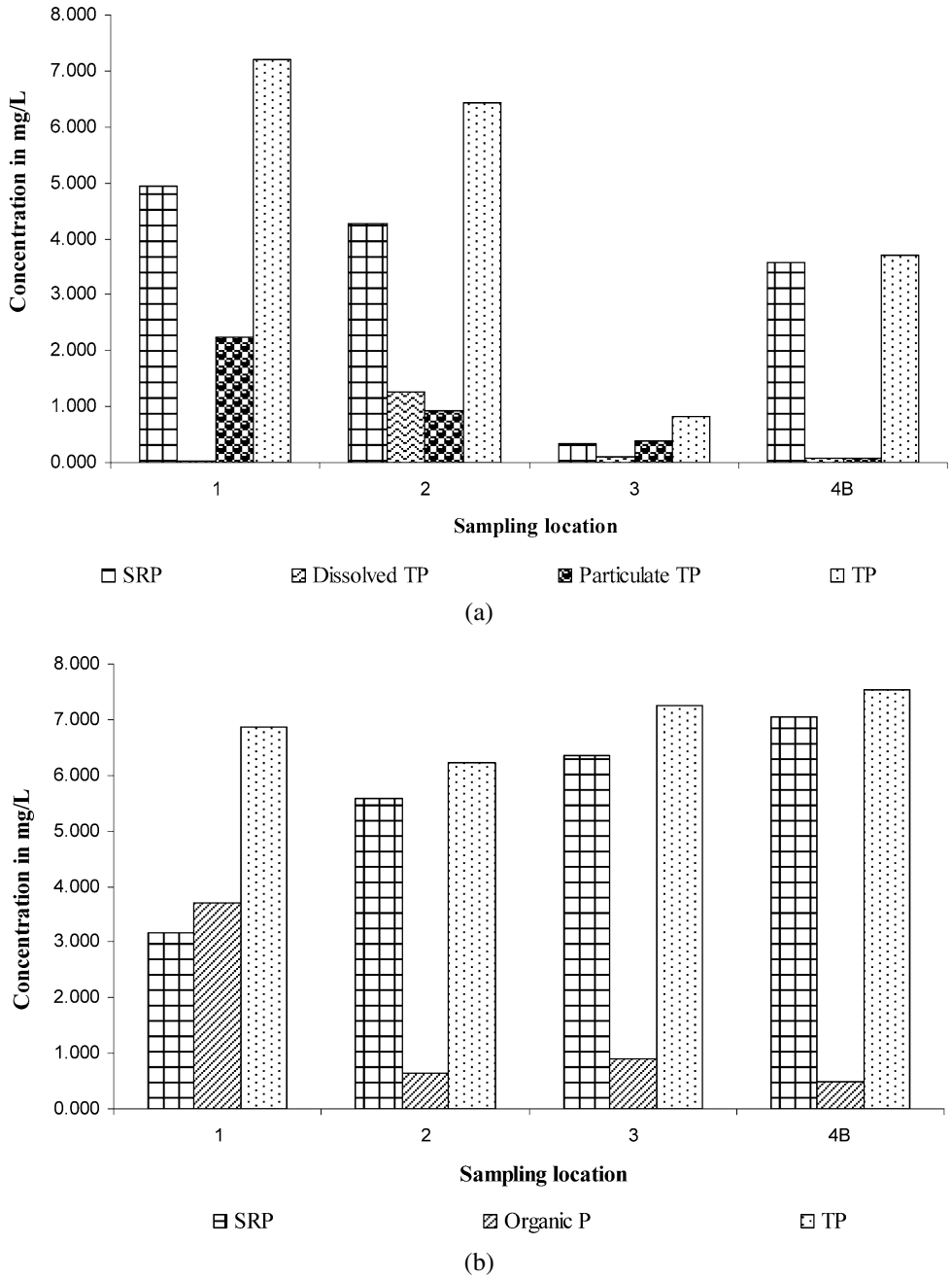


Fig. 5 Comparative phosphorus removal over two phases: **a** removal of phosphorus species in Phase I and **b** removal of phosphorus species in Phase II

was observed without limestone in the second phase. In these two phases, a septic tank might act as a potential denitrification chamber. However, the fresh wastewater had only a very low NO_x–N concentration. It was observed that the major portion of ammonia started to convert to nitrate after treatment in the septic tank as the remaining nitrification was not excessive in the RFT.

At the end of this study, we can establish that the passive OWT with the inclusion of the RFT presents a higher removal efficiency of nutrient species. Overall, both the RFT and drain field were considered as possible nitrification zones in these two phases if the NOB population is not high enough in the RFT. Nevertheless, it is possible to further modify the RFT as a reactor to implement simultaneous nitrification and denitrification if a suitable organic carbon source can be added. For this reason, any special media mixture to be introduced in the RFT as a sustainable source of organic carbon to trigger denitrification, should be placed at

the bottom of the tank as long as this portion can remain permanently submerged.

Acknowledgements The authors are grateful for financial support from the Florida Department of Environmental Protection (FDEP), provided through the Watershed Restoration Program's Urban BMP Research and Development Grant.

Appendix

Figures 6, 7, 8, 9 and 10 show free body diagrams of mass balance in the septic tank system in Phase I. Mass balance of Phase II can be carried out similarly. Evaporation of water and physical or chemical changes in the dosing and distribution tanks are considered negligible. The flow is measured by a flow meter placed in the inlet of the tank. These dia-

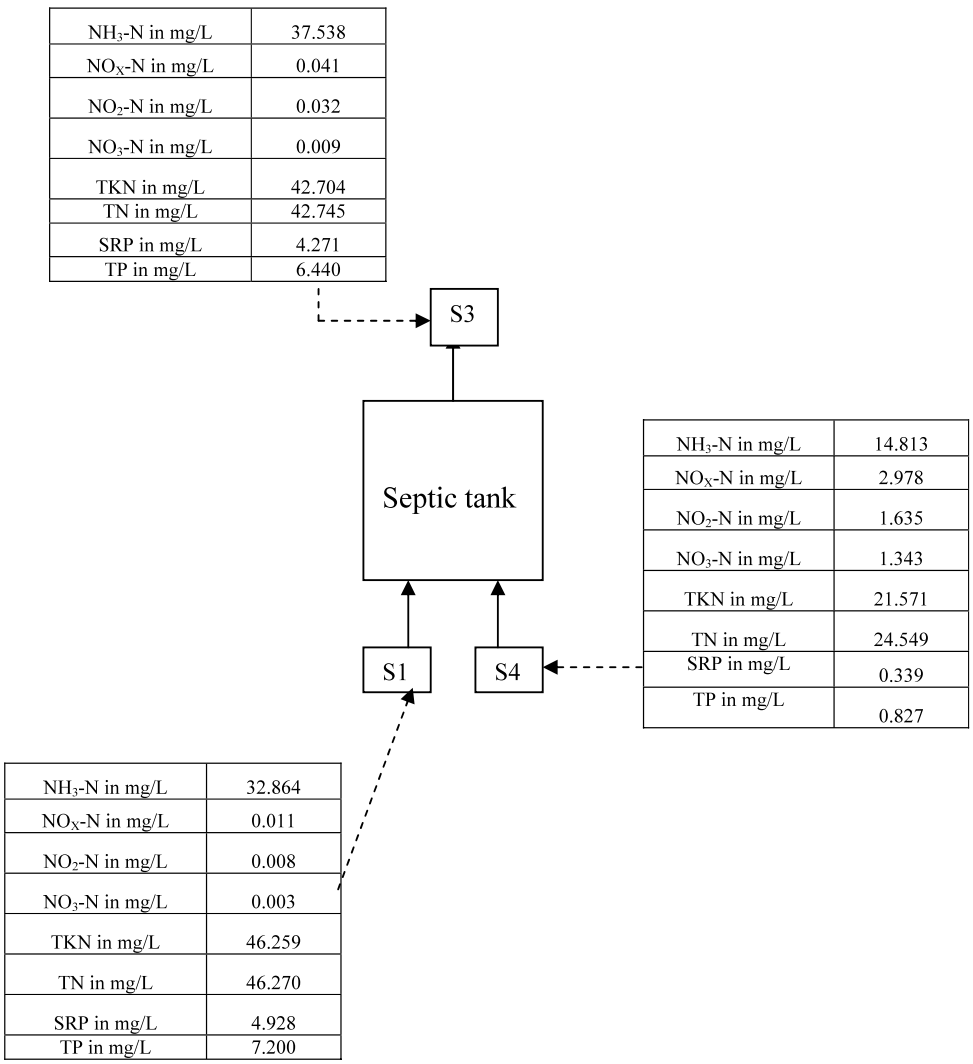


Fig. 6 Free body diagram for septic tank mass balance

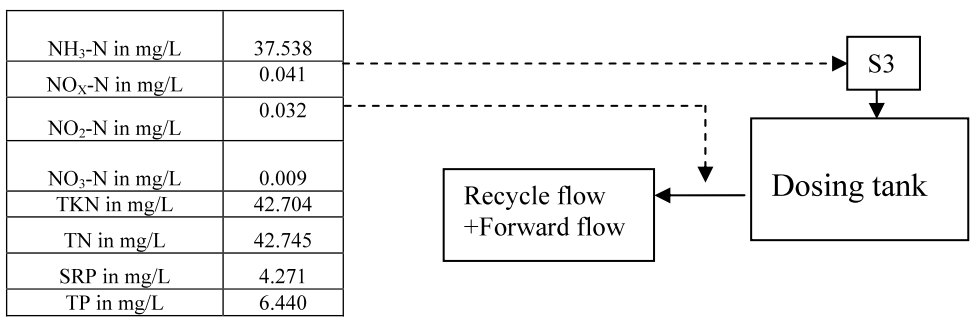
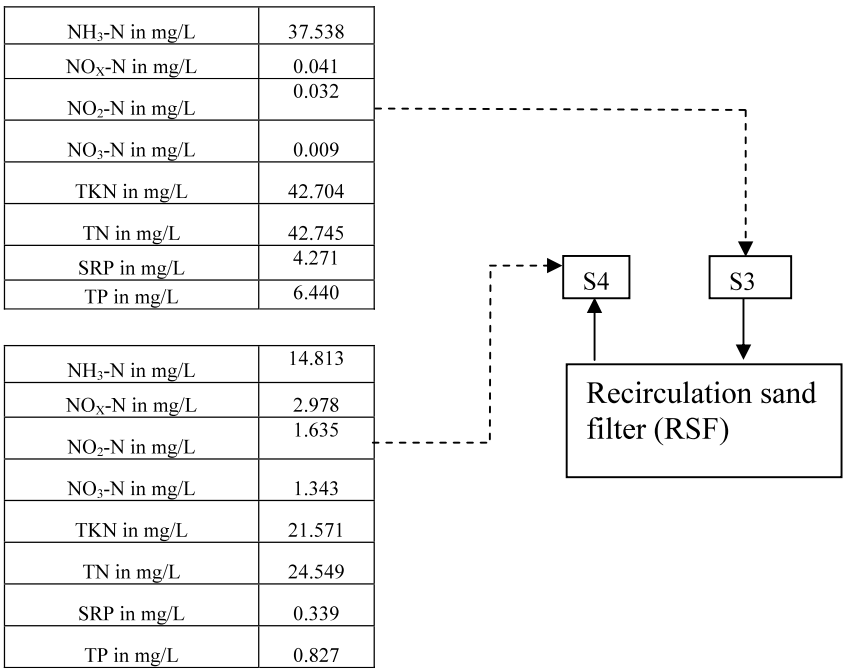


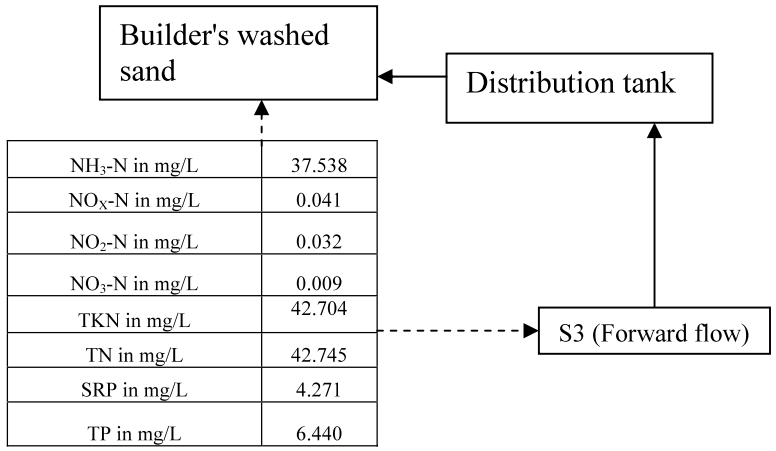
Fig. 7 Free body diagram for dosing tank mass balance



NH_3-N in mg/L	37.538
NO_X-N in mg/L	0.041
NO_2-N in mg/L	0.032
NO_3-N in mg/L	0.009
TKN in mg/L	42.704
TN in mg/L	42.745
SRP in mg/L	4.271
TP in mg/L	6.440

NH_3-N in mg/L	14.813
NO_X-N in mg/L	2.978
NO_2-N in mg/L	1.635
NO_3-N in mg/L	1.343
TKN in mg/L	21.571
TN in mg/L	24.549
SRP in mg/L	0.339
TP in mg/L	0.827

Fig. 8 Free body diagram for RSF mass balance



NH_3-N in mg/L	37.538
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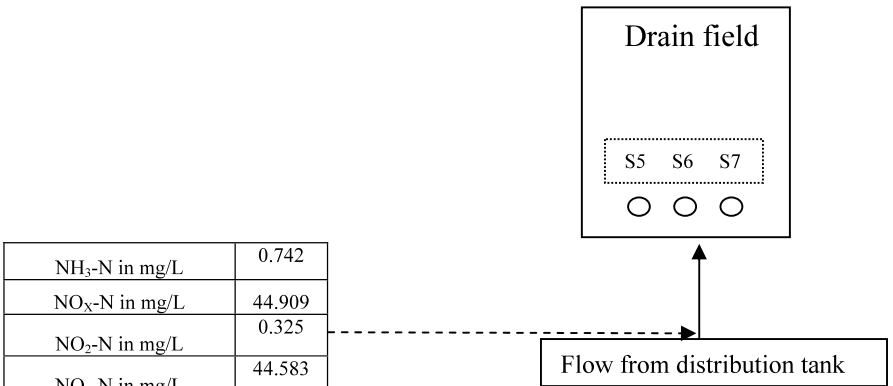
NO_3-N in mg/L	0.009
TKN in mg/L	42.704
TN in mg/L	42.745
SRP in mg/L	4.271
TP in mg/L	6.440

Fig. 9 Free body diagram for distribution tank mass balance



NH_3-N in mg/L	0.742
NO_X-N in mg/L	44.909
NO_2-N in mg/L	0.325
NO_3-N in mg/L	44.583
TKN in mg/L	6.063
TN in mg/L	50.972
SRP in mg/L	3.572
TP in mg/L	3.708

Fig. 10 Free body diagram for drain field mass balance

grams can help clarify average changes of nutrient concentrations in inflow and outflow of each unit.

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