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Influence of septic tank attached growth media on total nitrogen removal in a recirculating vertical flow constructed wetland for treatment of domestic wastewater

Iyad Al-Zreiqat[a], Bassim Abbassi[b,*], Tom Headley[c], Jaime Nivala[d], Manfred van Afferden[d], Roland A. Müller[d]

[a] Department of Urban Water Management, Faculty of Civil Engineering, Technical University of Berlin, Germany
[b] School of Engineering, University of Guelph, Ontario, Canada
[c] Wetland & Ecological Treatment Systems, Bolwarra Heights, NSW, Australia
[d] Helmholtz Center for Environmental Research – UFZ, Leipzig, Germany

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A B S T R A C T

Among different decentralized wastewater treatment systems, constructed wetlands are particularly robust, reliable and cost-effective technologies. However, traditional treatment wetland designs have a limited capacity to remove total nitrogen. The recirculating vertical-flow constructed wetland (RVFCW) system is a novel modification of the vertical flow wetland (VFW), allowing for increased denitrification by circulating the nitrified effluent back into a recirculation tank, where it is mixed with primary treated wastewater. Microcosm experiments were conducted to investigate the effects on nitrogen removal of mixing recirculated VFW effluent with raw wastewater after different degrees of primary treatment, with and without attached growth media. The results show that the inclusion of attached growth media in the first chamber of the recirculation tank resulted in enhanced total nitrogen removal. The microcosms that contained a mixture of raw wastewater and VFW effluent showed denitrification efficiency of 83% after 48 h of contact time. An increase in the denitrification efficiency (up to 99.5%) was observed in microcosms that also contained attached growth media. The majority of nitrate-N (NO_3-N) removal was achieved in the first 24 h. Inclusion of media increased the denitrification efficiency after 48 h contact time from 36 to 93% and from 31 to 88% in microcosms containing VFW effluent mixed at a ratio of 3:1 with wastewater after 1 and 2 days residence time in a septic tank respectively. It was inferred that the lower the degree of pre-treatment of wastewater into which the recirculated VFW effluent was mixed, the greater is the denitrification rate and thereby the lower TN concentration in the effluent.

1. Introduction

Water scarcity in arid and semi-arid countries has led to increased interest in recycling of various wastewater streams, especially in decentralized systems. Nitrogen needs to be managed appropriately to minimize eutrophication of waterways and contamination of groundwater with nitrate. With respect to total nitrogen removal in onsite wastewater systems, the conventional approach to providing advanced treatment is by using supplementary aeration, which enhances nitrification but does not accomplish denitrification (Charles et al., 2005; Peterson, 2006; Moelants et al., 2008; Levett et al., 2010; Oakley et al., 2010). Constructed wetlands are engineered systems designed to treat wastewater using the same processes that occur in a natural wetland, but within a more controlled environment (Vymazal, 2007; Lee et al., 2009; Vymazal, 2010; Almuktar et al., 2015). Among the different types of constructed wetlands, the vertical flow wetland (VFW) has been commonly used as a decentralized wastewater treatment system. VFWs are recognized as a robust treatment technology that can withstand disturbances and variable influent quality (e.g. high ammonium concentrations) and temperature changes. The system is characterized by a high oxygen transfer rate, and consequently a high level of organic matter removal and nitrification, although its denitrification efficiency is considered poor (Sklarz et al., 2009; Garcia et al., 2010). This is largely because a conventional VFW cannot simultaneously maintain the required aerobic and anaerobic conditions for total nitrogen removal (Arias et al., 2005).

The recirculating vertical flow constructed wetland (RVFCW) is a novel modification of the VFW that is designed to achieve higher

* Corresponding author.
E-mail address: babbassi@uoguelph.ca (B. Abbassi).

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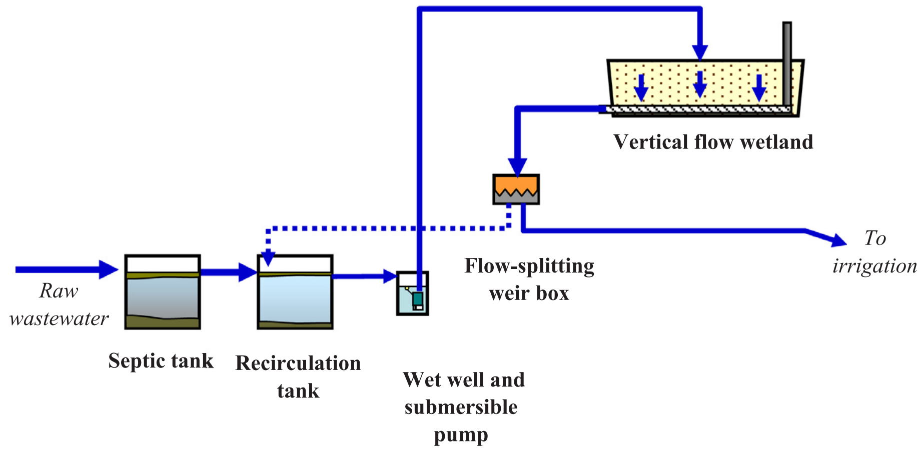

Fig. 1. Components of the recirculating vertical flow constructed wetland system.

nitrogen removal rates (García-Pérez et al., 2006; Gross et al., 2007; Tanner et al., 2012). Recirculation plays an important role in improving the performance of the VFW, because it enhances the interactions and contact between wastewater pollutants and the microorganisms responsible for treatment (Lian-sheng et al., 2006; Zapter et al., 2011). Part of the nitrified VFW effluent is recirculated back to the septic tank, where the presence of anoxic conditions and organic carbon favour denitrification (Vymazal, 2007; Kadlec and Wallace, 2009). This gives rise to the question of how much pre-treatment of the influent is required prior to mixing with the recirculated VFW effluent. The right balance needs to be struck between providing anoxic, carbon-rich conditions for denitrification in the recirculation tank and maintaining sustainable solids and organic loading rates on the VFW bed which will avoid clogging. Sundaravadivel and Vigneswaran (2001) reported that while recycling within a RVFCW system may not change the overall system hydraulic retention time (HRT), it may increase local flow velocities. Accordingly, the system design should be optimized to avoid disturbance in septic tank function.

The portion of the VFW effluent that is recirculated is normally characterized by high dissolved oxygen concentration which varies with the recirculation ratio. Arias et al. (2005) found that as recycling rates increased, the oxygen saturation varied from zero with no recycling to 12, 30, and 60% as recirculation rates increased to 100, 200, and 300%, respectively. While this is favourable for nitrification and aerobic degradation of organic matter, elevated levels of dissolved oxygen in the recirculation tank may inhibit denitrification. Accordingly, the RVFCW has proven to be an effective alternative to remove organic matter, suspended solids, and ammonium in domestic wastewater (Sundaravadivel and Vigneswaran, 2001; Gross et al., 2007; Arias et al., 2005; Li and Tao, 2017), but the design and operation needs to be optimized for total nitrogen removal.

The focus of this study is optimization of nitrogen removal from domestic wastewater in RVFCWs. Experiments were conducted in Jordan on a full-scale RVFCW system as well as lab-scale microcosms, using real municipal wastewater. To assess the effect of different levels of wastewater pre-treatment and the inclusion of attached-growth media on total nitrogen removal, microcosms were established comparing different combinations of VFW effluent and untreated wastewater collected from various locations within a septic tank, both with and without attached-growth media.

2. Materials and methods

2.1. Full-scale vertical flow constructed wetland

A RVFCW was constructed at a demonstration facility in Fuhais, Jordan as one example of a potential decentralized system for onsite treatment of domestic wastewater. The constructed wetland system consists of a septic tank, a recirculation tank, splitter-box, wet well with a submersible pump (pump well), and a recirculating VFW (Fig. 1). The system was designed to treat domestic wastewater with a daily flow of 2160 L/d at a hydraulic loading rate of 108 L/m^2 d. The septic tank is fed with raw wastewater from the adjacent central wastewater treatment plant (25,000 Person Equivalents) of the city of Fuhais in Jordan. The septic tank design allows the assumption of plug flow pattern through the tank. The VFW is intermittently loaded with effluent from the recirculation tank at a rate of 90 L/h, divided into three episodes of feeding per hour. Each individual feed (every 20 min) discharges 30 L of wastewater within a few minutes. The inlet flow volume to the RVFCW system is measured with an electromagnetic flow meter (ELPIS) and the intermittent loading is controlled using a programmable logic control (PLC) SIEMENS-SIMATIC S7-200.

The wastewater receives primary treatment in a septic tank with a working volume of 4.6 m^3, ensuring an average residence time of two days. The effluent from the septic tank flows into the recirculation tank with a working volume of 6.2 m^3, where it is mixed with 75% of the effluent from the VFW (i.e. a recirculation rate of 300%). After a residence time of approximately one day, the effluent from the recirculation tank flows to the pump well, from where the submersible pump distributes the water over the surface of the VFW via a network of pipes. After each pass through the wetland, 25% of the VFW outflow leaves the system as final effluent.

The VFW has a surface area of 20 m^2 (4 m × 5 m) and a depth of one meter. A bedding layer of fine sand was used under the liner across the bottom, while the side-walls were built with concrete bricks. The bottom and the sides were lined with a polyethylene (PE) liner (1-mm thick) to prevent leakage. Crushed volcanic rock, known as zeotuff, was used as filling material, because it is locally available in Jordan. Zeotuff was quarried and processed in the eastern part of Jordan by the Green Technology company. The main filter media of 2–4 mm zeotuff comprises approximately 80 cm of the total depth. This filter media is underlain by a drainage zone, in which treated wastewater is collected in three parallel perforated lateral pipes and then drains from the system by gravity. The drainage pipes are connected to a common pipe downstream of the wetland bed. The other ends of the perforated lateral pipes are connected to ventilation risers, which passively provide the wetland filter bed with oxygen. The drainage pipes are made of polyvinyl chloride (PVC) with an inside diameter of 110 mm. The perforated lateral pipes are covered by a layer of 20 cm of Zeotuff with a size range of 10–25 mm, to serve as a drainage zone overlain by the layer of 80 cm of 2–4 mm Zeotuff, as shown in Fig. 2.

Wastewater is intermittently pumped and distributed over the surface of the bed through a network of eight lateral pipes located directly at the top of the main filter material and designed to achieve uniform

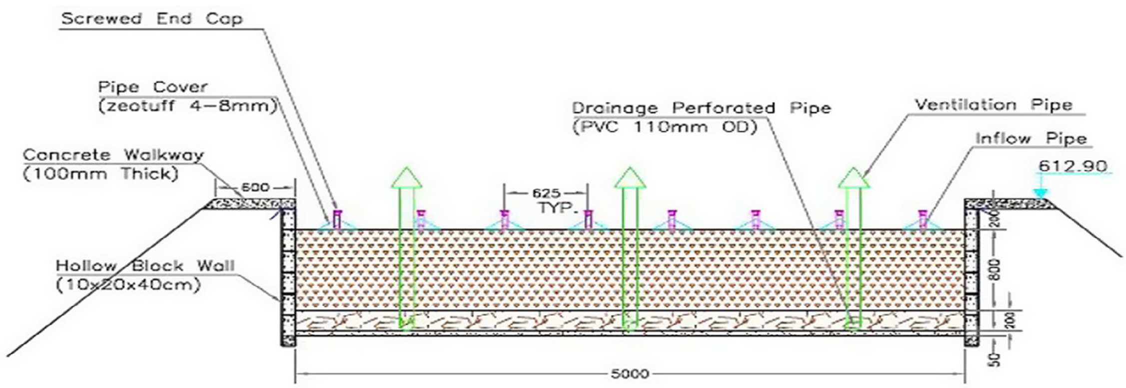

Fig. 2. Schematic diagram of the recirculating vertical flow wetland.

Table 1
Experimental setup of the microcosm reactors.

	No.	Microcosm setup	Level of primary treatment	Attached media	Amount of VFW effluent (%)
Control 1	1	Raw wastewater	0 (raw WW)	No	0
Pair 1	2	Raw wastewater, No media	0 (raw WW)	No	75
	3	Raw wastewater + media	0 (raw WW)	Yes	75
Pair 2	4	1-day HRT Septic tank, No media	Halfway point of septic tank	No	75
	5	1-day HRT Septic tank + media	Halfway point of septic tank	Yes	75
Pair 3	6	2-day HRT Septic tank, No media	Septic tank outlet	No	75
	7	2-day HRT Septic tank + media	Septic tank outlet	Yes	75
Control 2	8	VFW effluent	–	No	100

distribution of influent. Orifices of 6 mm diameter are spaced every 60 cm along each distribution pipe. The distribution pipes are covered by 4–8 mm zeotuff. The ends of distribution pipes have screw cap at each end that can be removed to flush the pipes in case of required maintenance.

The system has been designed to mix a portion of the VFW effluent with the septic tank effluent in a recirculation tank, allowing the denitrification process to occur under anoxic conditions. Water samples were taken at different locations representing the different system components and analyzed for various wastewater parameters according to both Standard Methods for Examination of Water and Wastewater (APHA, 1995) and HACH-Lange test kits.

2.2. Microcosm experiment

A series of bench-scale experiments were conducted to investigate the effect of different degrees of primary treatment as well as the effect of attached-growth media in the recirculation tank on denitrification. Eight microcosm batch-reactors representing different treatments were prepared. Each microcosm was constructed using a plastic bucket with a working volume of 10 L. In order to simulate the full-scale RVFCW system, the mixing ratio of the VFW effluent to wastewater was identical to that in the full-scale system (3:1). The microcosms were loaded with a mix of 75% VFW effluent and 25% wastewater that had received various degrees of primary treatment (Table 1). The different qualities of primary treated wastewater were collected from different locations throughout the full-scale septic tank, representing different hydraulic retention times (HRT) of 0, 1, and 2 days, corresponding to influent (raw) wastewater, the middle of the tank, and effluent from the septic tank.

Two microcosms were constructed as controls. One received only raw wastewater (i.e., no VFW effluent) to provide a reference for background total nitrogen removal in the microcosms. A second control received only VFW effluent to provide a reference of the background level of denitrification that might occur without mixing with primary treated effluent (i.e., no recirculation). To study the role of attached growth (biofilm) in combination with different levels of primary treatment, three microcosms containing attached-growth media were tested; one for each level of primary treatment. Attached-growth media were made of plastic conduit tubing, which is normally used for household electric wiring, cut into cylindrical pieces of 1.9-cm diameter and 1-cm length. Each microcosm with attached-growth media received a textile net filled with 900 pieces of conduit tubing. A total of 8 microcosms (6 test and 2 control) were used in this phase of the investigation (Table 1).

Each run in the experiment was repeated four times. The attached-growth media were pre-conditioned by storing them in the full-scale recirculation tank for a period of three weeks prior to the start of the experiment. This measure was taken to allow the denitrifying biofilm to grow and enrich on the surface of the plastic media. Each pair of microcosms (suspended and attached growth) with similar degrees of primary treatment (0, 1, or 2 HRT), were filled with the relevant wastewater mixture after being prepared in a separate container. All experiments were conducted at ambient room temperature inside the onsite laboratory building.

2.3. Sampling and analysis

During each batch experiment, water samples were collected after 0, 3, 6, 9, 12, 24, 36 and 48 h since the start of the batch. Samples of 100 mL were syringe-drawn and emptied into a clean glass bottle for subsequent analysis. Each water sample was analyzed onsite for dissolved oxygen, pH, redox, and temperature using a HACH-Lange multimeter HQ40D. Samples were analyzed in the lab for chemical oxygen demand (COD), total nitrogen (TN), nitrate-N (NO_3-N), and ammonium-N (NH_4-N) according to Standard Methods for Examination of Water and Wastewater (APHA, 1995) and HACH-Lange test kit instructions. A HACH-Lange DR 2800 spectrophotometer was used for test-kit analyses.

2.4. Statistical analysis

A regression analysis was conducted using SAS software version 9.3, where General Liner Model (GLM) analysis was carried out to investigate whether the relationships between concentration and contact time were significant for each of the treatments (different primary treatment (0, 1, and 2 day HRT in septic tank) and presence or absence of media). The coefficient of determination (r^2) of the liner regression of each treatment was also generated in order to evaluate the strength of the relationship between concentration and contact time for each treatment.

3. Results and discussion

3.1. Full-scale RVFCW results

The RVFCW was operated under a hydraulic loading of 108 L/m^2 d. Weekly sampling was performed for a period of 11 months. Fig. 3 summarizes the VFW loading rates for the monitored parameters, calculated from the septic tank effluent samples.

The loading rates indicate that most of the influent nitrogen is in the form of ammonium (loading rate of 4.7 g/m^2 d). NO_3-N was observed in the VFW influent wastewater, with a loading rate of 2.4 g/m^2 d. This nitrate was derived from the nitrified effluent being recirculated from the VFW and is evidence that the denitrification process in the recirculation tank was incomplete. An organic nitrogen loading rate of 3.3 g/m^2 d was calculated by subtracting inorganic N from TN.

In order to investigate the performance of each component of the RVFCW system, samples were taken at different locations representing the different components, as described in Section 2.1. Table 2 summarizes the average concentrations of the monitored parameters in each component. The ratio of the average chemical oxygen demand (COD) to biological oxygen demand (BOD_5) of the feed wastewater was found to be approximately 1.8, similar to domestic wastewater.

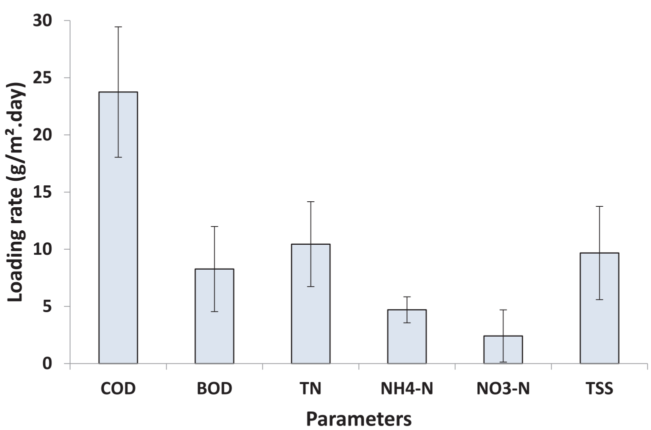

Fig. 3. The pollutant loading rates for the VFW. The error bars denote +/− one standard error of the mean.

Table 2
Mean concentration (+/− one standard deviation) of the main parameters monitored at different sampling points.

Parameter	Inlet (mg/L)	Septic-out (mg/L)	Recirculation Tank-in# (mg/L)	VFW-in (mg/L)	VFW-out (mg/L)
COD	934 ± 288	627 ± 159	188 ± 44	126 ± 26	42 ± 9
BOD_5	513 ± 98	193 ± 40	72 ± 17	44 ± 16	32 ± 14
TSS	409 ± 228	202 ± 67	62 ± 19	36 ± 18	16 ± 11
TN	107 ± 27	101 ± 21	62 ± 20	55 ± 17	52 ± 22
NH_4-N	69 ± 13	84 ± 15	22 ± 4	25 ± 5	1 ± 1
NO_3-N	0.5 ± 0.1	0.6 ± 0.1	27 ± 12	13 ± 10	38 ± 14

Concentrations for the inlet of the recirculation tank are calculated values, assuming that the septic tank effluent and the recirculated portion of VFW effluent were uniformly mixed at a ratio of 3:1 corresponding to the recirculation rate.

Table 3
Percentage removal of parameters at different stages of the RVFCW system.

Removal efficiency				
Parameter	Septic tank (%)	Recirculation tank (%)	VFW (%)	Overall (%)
TSS	50.6	41.7	56.1	96.1
COD	32.9	33.3	66.4	95.5
BOD_5	62.4	39.7	26.4	93.7
TN	5.9	10.9	6.7	51.9
NH_4-N	−21.8	−13.1	95.1	98.2

Table 2 shows that raw wastewater (inlet) has a fairly high total nitrogen concentration of 107 mg/L, which is expected in locations with limited water supply (Metcalf and Eddy, 2003). The difference in TN concentrations between Septic-out and VFW-out (i.e. the final effluent) indicates an overall denitrification of approximately 49 mg/L throughout the recirculating part of the system, apparently due to nitrification in the VFW and subsequent denitrification in the recirculation tank. Most of the TN entering the system is in the form of ammonium, with a mean concentration of 69 mg/L. All but a small fraction of the remainder is in the form of organic nitrogen, with a mean concentration of 37 mg/L. When wastewater is pumped to the RVFCW system, it receives primary treatment in the septic tank. Some of the organic N is transformed to NH_4-N, which explains the increase in the ammonium concentration to 84 mg/L at the septic tank outlet.

Table 3 shows the overall recirculating system performance; with mean concentration removal efficiencies for BOD_5, TSS, and TN of 93.8, 96.1, and 51.4%, respectively. The total removal efficiencies of BOD_5 and TN are in high agreement with the results obtained by Põldvere et al. (2009), who also concluded that higher recirculation rates of treated water can result in increased purification efficiency for most water quality indicators. The calculated removal efficiencies of BOD_5, NH_4-N, and TSS within the VFW component were 26.4, 95.1, and 68.7%, respectively. The high ammonium removal clearly indicates the nitrification capacity of the VFW system; which is attributed to the activity of the biofilm attached to the filter material, and to the abundant oxygen due to the intermittent feeding mode (Tanner et al., 2012; Ye et al., 2012). In contrast, the RVFCW system exhibited only a moderate total nitrogen removal (51.4%), indicating a limited denitrification process, possibly due to the high oxygen level in the VFW effluent that is mixed with insufficient organic carbon in the effluent from the septic tank and insufficient contact time between wastewater and denitrifying bacteria within the recirculation tank. Table 3 also shows that a major part of the BOD_5 (62.4%) was removed in the septic tank before coming in contact with the nitrified effluent from the VFW. This BOD_5 removal is primarily due to sedimentation of organic solids and the anaerobic degradation processes (Vymazal, 2007). Under the predominantly quiescent conditions, it was also found that 50.6% of the TSS content was retained in the septic tank.

Analytical results for nitrogen compounds TN, NO_x-N (NO_3-N & NO_2-N), and NH_4-N for all RVFCW system stages are summarized in Fig. 4, which shows that total nitrogen was reduced by 51.4% throughout the entire treatment system. This is consistent with works conducted by Põldvere et al. (2009) and Tanner et al. (2012), who reported TN removal of 51 and 58%, respectively. The removal of TN in the RVFCW system is mainly attributed to the nitrification of ammonium in the VFW and subsequent denitrification of nitrate in the recirculation tank. However, Filali et al. (2017) showed that in recirculating wetland systems with intermittent flow regime, both incomplete nitrification and denitrification processes can lead to a

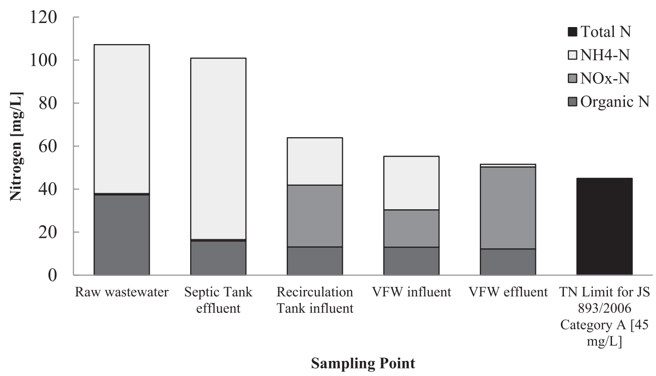

Fig. 4. Nitrogen transformation throughout the RVFCW system.

production of soluble and gaseous nitrous oxide (N_2O). Therefore, the emissions of greenhouse gases (GHG) including the N_2O compound that has a high global warming characterization factor should be considered within a context of life cycle analysis of the wetland systems (Mander et al., 2014).

Alternative microbial processes of nitrogen transformation are the dissimilatory reduction of nitrate to ammonium (DNRA) and the anaerobic ammonium oxidation (ANAMMOX). However, DNRA in this investigation is most likely negligible compared to denitrification due to the short HRT in the anoxic zone (Tournebize et al., 2015). Li and Tao (2017) reported that the intermittent feeding regime, which resulted in an unsteady “labile” supply of organic carbon for denitrification, could result in a suppression of ANAMMOX by competing for nitrite. They also concluded that ANAMMOX activity in constructed wetlands could be limited by the low nitrite concentrations. Accordingly, ANAMMOX is also most likely negligible in this investigation, so that denitrification is the main transformation process to reduce nitrate.

Fig. 4 also shows that the treated wastewater quality does not quite comply with the Jordanian Standard (JS: 893/2006), which sets the allowable discharge limit of total nitrogen and nitrate to be 45 and 30 mg/L, respectively, for irrigation of cooked vegetables and public areas (Category A). Although there is significant removal of total nitrogen and the effluent satisfies Category B of the standard (70 and 45 mg/L for TN and NO_3 respectively) for lower risk irrigation options, optimization of the RVFCW performance is needed to satisfy the more stringent Category A standard in Jordan for total nitrogen and nitrate.

3.2. Microcosm experimental results

The change in mean COD concentration over contact time in the microcosms are shown in Fig. 5. In all microcosms, except for control 2 (VFW effluent only), the COD concentration decreased between 45 and 60% over the 48-h batch period. This is consistent with Arias et al. (2005), who found that as the recycling ratio increases, COD removal also increases. As shown in Fig. 5, most COD reduction occurred within the first 24 h, indicating that a HRT of 1 day should be sufficient in the recirculation tank. This reduction in COD is primarily attributed to the breakdown of organic material and the utilization of the organic matter in the denitrification process. No significant effect of attached growth media on organic matter degradation was detected throughout the entire experiment.

Similarly, nitrate-N concentrations were determined throughout the 48-h batch periods; results are shown in Fig. 6. Nitrate-N concentration remained unchanged for the control 1 and 2. Despite the high NO_3-N concentration in VFW effluent (28.3 mg/l), no denitrification was observed in control 2 (VFW effluent only) through the full contact time of 48 h (29.2 mg/l). This is due to the limited organic carbon in the VFW effluent and undeveloped anoxic conditions; the average redox potentials at the beginning and the end of the experiment were found to be −90 and 87 mV, respectively. Accordingly, the existing RVFCW system

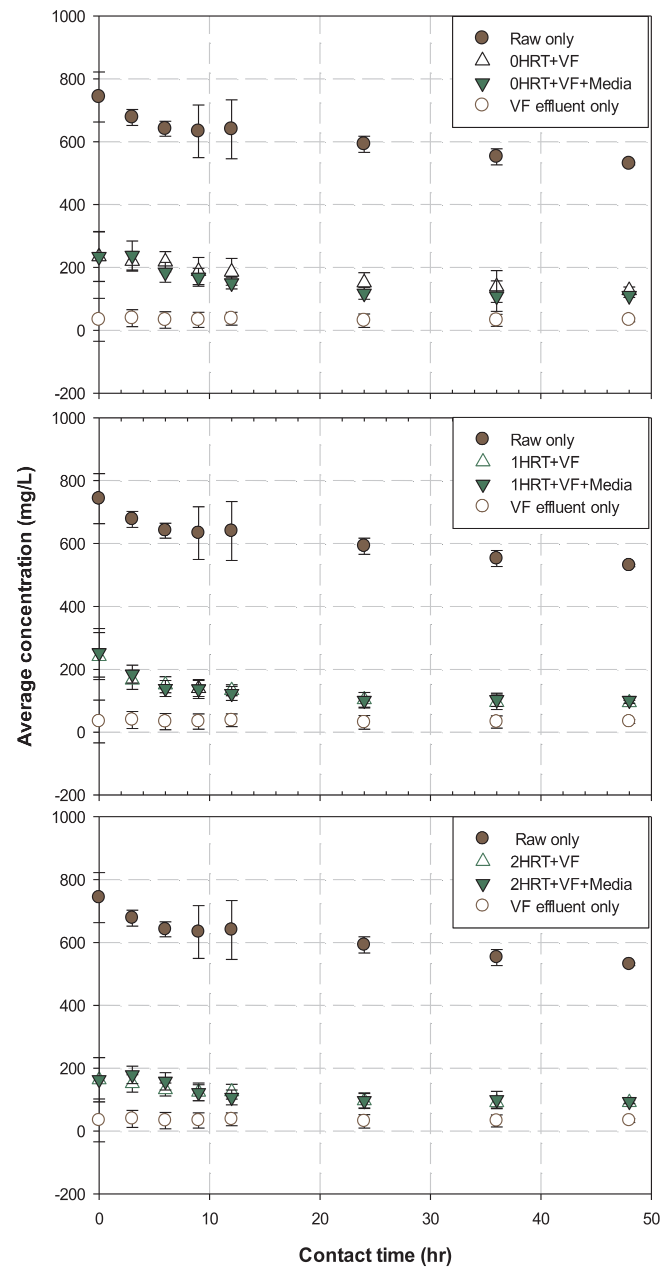

Fig. 5. Average COD concentration over contact time in the microcosms. The error bars represent +/− one standard error of the mean.

under the current operating conditions will not be able to obtain further reduction of nitrogen, even at feasible retention times in the effluent collection tank; further nitrogen reduction requires innovative modification of the RVFCW system. Considering the redox potential analysis for the other microcosms (i.e. not the controls), the values were fluctuating between −150 and 120 mV indicating both nitrification and carbonaceous BOD degradation. Similarly, average redox potential in control 1 (raw wastewater) was found to be −234 mV, indicating a complete anaerobic condition (Faulwetter et al., 2009).

The concentration of nitrate-N decreased as the retention time increased in those microcosms containing a mixture of nitrified VFW effluent and influent wastewater after various degrees of pre-treatment. In general, the lower the amount of pre-treatment (higher organic matter content), the greater the rate of nitrate reduction. Furthermore, the inclusion of media in the microcosms has greatly increased the rate of nitrate removal. Comparison of Figs. 5 and 6 shows that denitrification was clearly enhanced by the higher COD concentrations that were present when wastewater with lower levels of primary treatment were mixed with the nitrified VFW effluent. The suspended growth microcosms containing raw wastewater (HRT = 0) showed higher nitrate removal than that those microcosms with septic tank effluent at HRT of 1-day and 2-day. Nitrate-N reduction after 48 h in suspended growth microcosms containing wastewater with different levels of septic tank pre-treatment (0, 1, and 2 HRT) was found to be 83, 36, and 31%, respectively, indicating that the organic carbon removal achieved by primary treatment before a recirculation tank has a detrimental effect on the level of denitrification.

The inclusion of media in the microcosms improved the rate of nitrate removal, due to the presence of a fixed surface area within the reactors for attached growth, denitrifying biofilms to develop. In the microcosms receiving VFW effluent mixed with raw wastewater (no pre-treatment), average nitrate-N removal after 48 h increased from 83% in the suspended-growth microcosm to 99.5% in the attached-growth microcosm. Almost complete nitrate-N removal was achieved after 24 h in the microcosm containing media which received VFW effluent mixed with raw wastewater, whereas without media approximately 12 mg/L of nitrate-N remained after 24 h. This demonstrates that, for the wastewater under investigation, the organic carbon in the raw sewage was sufficient to achieve completely denitrify all of the nitrate present in the VFW effluent when a residence time of at least 24 h and attached-growth media is provided in the recirculation tank. A similar improvement in denitrification rate by the inclusion of attached-growth media was observed for the microcosms containing VFW effluent mixed with wastewater after 1 and 2 days of primary treatment in a septic tank. In these microcosms, denitrification after 48 h increased from 36 to 93% (1 day primary treatment) and from 31 to 88% (2 days primary treatment) respectively, with the inclusion of media. This is attributed to the enriched denitrifying population in the media-attached biofilm. Moreover, the denitrifying microorganisms tend to be more active inside the biofilm, where oxygen concentration is usually very limited (Matsumoto et al., 2007). Due to low oxygen concentration in the bulk solution of the microcosms, mass transfer of oxygen inside the biofilm is also low, leaving most of the biofilm under anoxic conditions. Moreover, due to the high concentration of nitrate in the bulk solution, penetration of nitrate in the biofilm is rather high. Regions in the biofilm containing high nitrate-N and low dissolved oxygen concentrations would therefore be ideal sites for denitrification (Matsumoto et al., 2007).

The effect of the contact time in the microcosms on nitrogen removal was also apparent, especially in the first few hours of each run. Within these periods, the COD concentration is high enough to provide denitrifying microorganisms with the required source of carbon. This is seen in the attached-growth microcosms containing mixtures with raw wastewater (0-day HRT), where more than 84% of nitrate reduction was achieved within the first 12 h. In all treatments, contact times longer than 12 h have revealed a slowing in the rates of denitrification, corresponding to a reduction in COD concentrations below approximately 150 mg/L.

The ammonia-N concentration did not change over the entire contact time in all treatments. Thus, any change in nitrate-N concentration was clearly reflected in the total nitrogen concentration (Fig. 7). However, the percentage reductions of total nitrogen were less than the percentage reductions of nitrate-N. For example, in the microcosms receiving VFW effluent with untreated raw wastewater and attached-growth media, the nitrate-N and Total N reductions after 48 h contact time were calculated to be 99 and 67%, respectively. This difference is attributed to the content of ammonium in the primary treated wastewater (ranging from 10 to 15 mg/L additional N in the final mixtures) that elevated the total nitrogen concentrations. This ammonium was

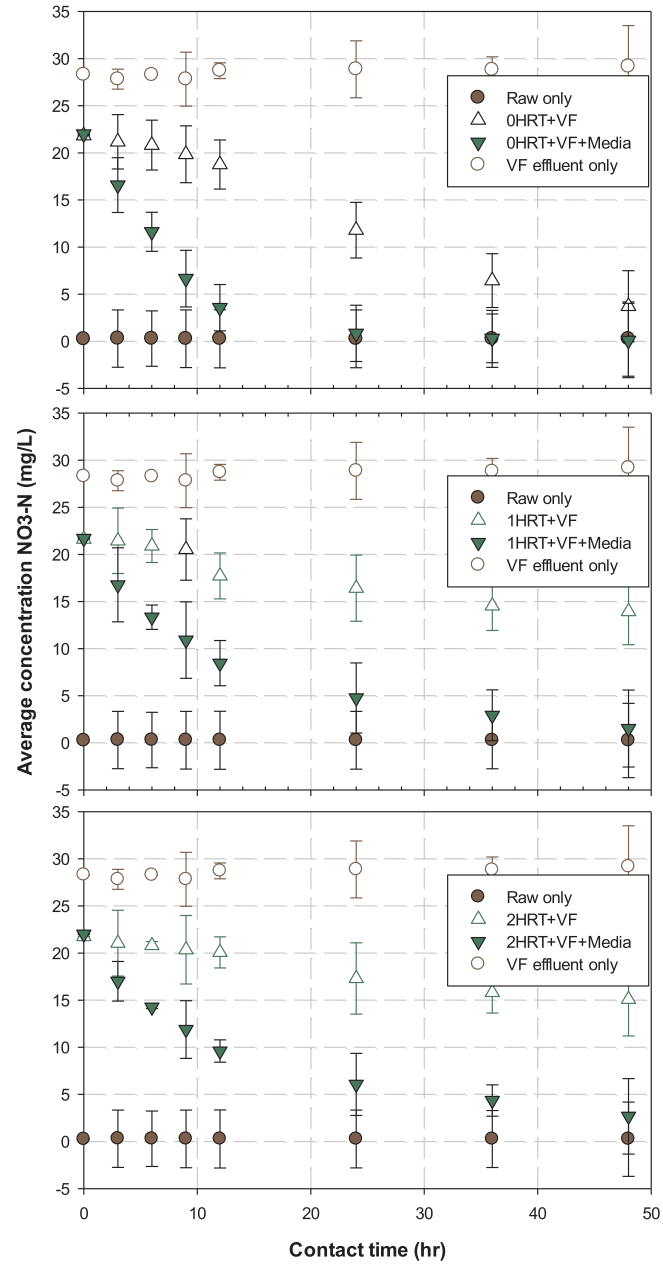

Fig. 6. Average nitrate-N concentration over contact time in the microcosms. The error bars represent +/− one standard error of the mean.

not removed within the anoxic microcosms. In the context of a full-scale RVFCW, this residual ammonium in the outlet of the recirculation tank would be subsequently nitrified in the VFW and remain in the effluent as nitrate. Therefore, a residual amount of Total N will remain in the RVFCW effluent, which will be proportional to the recirculation rate employed, even if complete denitrification is achieved in the recirculation tank.

As for nitrate, the effect of media on TN removal was clearly seen, in particular for the microcosms receiving VFW effluent mixed with raw wastewater, where the removal increased from 35% (suspended growth) to 68% (attached growth) as shown in Fig. 7.

ANOVA regression analysis was conducted to compare the significance of the different treatments for nitrate-N reduction over

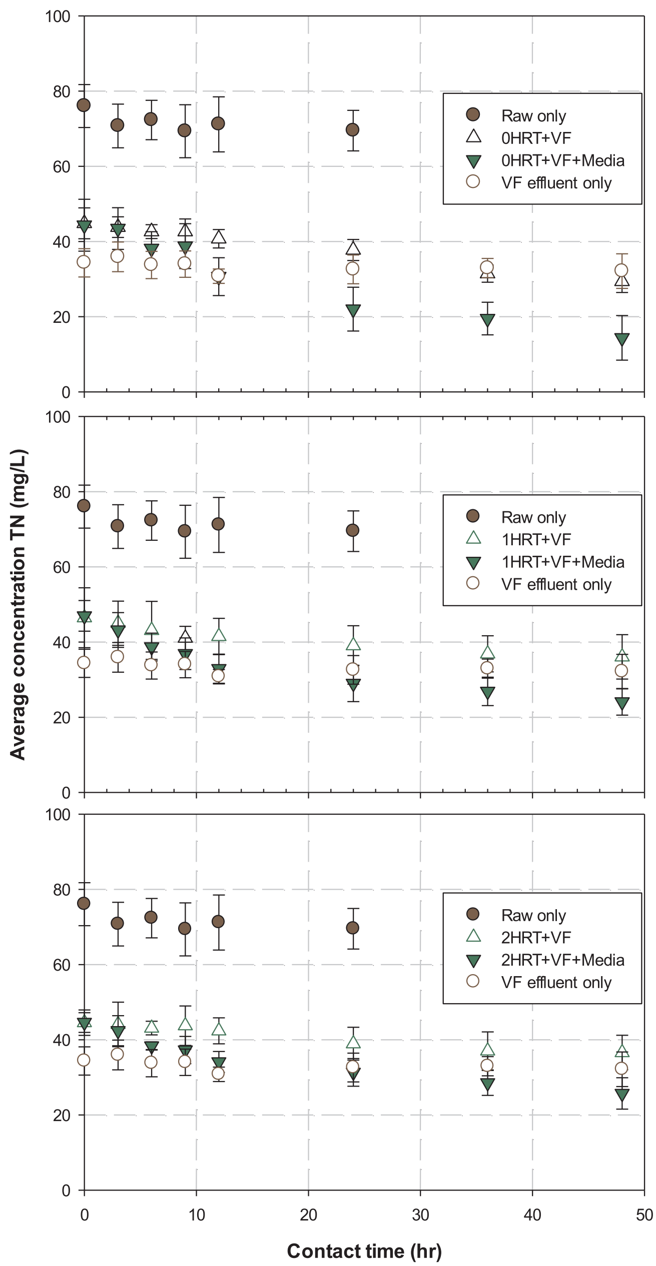

Fig. 7. Average total nitrogen concentration over contact time in the microcosms. The error bars represent +/− one standard error of the mean.

contact time as shown in Table 4.

The ANOVA table shows that the regression equations for all treatments were highly significant with $P < 0.0001$. In most cases, r^2 for the attached growth treatments were showing a stronger correlation than the ones with suspended growth. The regression analysis revealed that r^2 for nitrate concentration in the attached growth treatments with primary treated wastewater after 0, 1, and 2 days residence time in the septic tank were 0.62, 0.71 and 0.68 respectively. This is also seen in Fig. 6. The reduction in TN over contact time was found to be significant for all treatments, where P values were less than 0.0001. The treatment of (0 HRT + Media) showed the highest r^2 among all treatments.

4. Conclusion

The performance of the full-scale RVFCW system for ammonia removal was substantial; 98% of the ammonia in the raw wastewater was removed. This indicates the effective aerobic character of the VFW, as well as the presence of an enriched nitrifying population in the biofilm of the wetland media. At the studied recirculation rate of 300%, denitrification in the recirculation tank of the high nitrate-N concentration in the VFW effluent was, however, limited with a reduction of only 51%. Under the current operating conditions, the RVFCW system produced an effluent with TN and nitrate concentrations compliant with the Jordanian regulations (JS 893/2006) for lower-risk Category B irrigation reuse, but not with the more stringent Category A. The microcosm experiment, comparing several combinations of wastewater primary treatment, VFW effluent recycling, and presence/absence of attached-growth media within the recirculation tank, has revealed new findings. Increasing organic matter in the primary treated wastewater (less pre-treatment) increases the denitrification rate. The denitrification rate was especially enhanced by the inclusion of attached-growth media in the recirculation tank microcosms. The maximum nitrate-N removal efficiency of 99.5% was seen after 48 h of contact time in the microcosms containing attached-growth media and receiving the nitrified VFW effluent mixed with raw wastewater (no primary treatment) at a ratio of 3:1. The microcosm receiving the same wastewater mixture, but without attached-growth media, achieved 83% removal of nitrate-N after 48 h, showing that, in addition to the rich carbon source available in the raw wastewater (0 d HRT), the effect of attached-growth media on the denitrification rate was significant. The results also revealed that the microcosm containing media achieved 96% denitrification after a contact time of 24 h when receiving VFW effluent mixed with raw wastewater at a ratio of 3:1. From the perspective of designing full-scale RVFCW systems, these results indicate that, under the loading conditions studied, recirculating the nitrified VFW effluent back to the inlet of a septic tank with a residence time of at least 1 day (considering both the forward and recirculated flow rates) at a recirculation rate of 300% will achieve essentially complete removal of nitrate. However, some Total N will remain in the treated effluent due to the ammonium in the influent raw wastewater which is not removed in the recirculation tank and will subsequently leave the RVFCW system as nitrate after being nitrified in the VFW. In this regard, the ultimate removal of Total N achievable by the RVFCW system is proportional to the recirculation rate employed (e.g. 75% removal at the 3:1 recirculation rate studied here), with a higher recirculation rate required to achieve higher levels of Total N removal. Further research is needed to study the effect of higher recirculation ratio on denitrification efficiency under the conditions used in this experiment.

Table 4
ANOVA table of the regression analysis.

Treatment	COD		TN		NO_3-N	
	r^2	P value	r^2	P value	r^2	P value
0d HRT + VFW	0.65	< 0.0001	0.77	< 0.0001	0.86	< 0.0001
0d HRT + VFW + Media	0.62	< 0.0001	0.81	< 0.0001	0.62	< 0.0001
1d HRT + VFW	0.51	< 0.0001	0.35	< 0.0005	0.55	< 0.0001
1d HRT + VFW + Media	0.40	< 0.0001	0.62	< 0.0001	0.71	< 0.0001
2d HRT + VFW	0.70	< 0.0001	0.56	< 0.0001	0.56	< 0.0001
2d HRT + VFW + Media	0.51	< 0.0001	0.71	< 0.0001	0.68	< 0.0001

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