Treatment Performance and Bacterial Populations in Subsurface Horizontal Flow Constructed Wetland System Treating Young and Stabilized Waste Leachate

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Abstract This research was carried out to determine organic and nitrogen removal efficiency of subsurface horizontal flow wetland system (HSF) in treating leachate with different degree of stabilization. Hydraulic loading rate (HLR) to the system was varied at 10, 28 and 56 mm d^{-1}. Average BOD removals in the system were 98% and 71% when applied to young and stabilized waste leachate at HLR of 10 mm d^{-1}. In term of TKN, average removal efficiency was about 43% and 46%. High nitrogen characteristics in stabilized leachate adversely affected treatment performance and plant in the system. Bacterial population targeting nitrifying bacteria in soil and surrounding plant root was studied by using fluorescent in-situ hybridization (FISH) technique. Bacterial population ratios between ammonium oxidizing bacteria (AOB) to nitrite oxidizing bacteria (NOB) and ANAMOX bacteria was found varied along the treatment system and they were affected by hydraulic loading rate and leachate characteristics. AOB was found at higher percentage in soil surrounding plant root whereas NOB and ANNAMOX bacteria were found in other soil area.

Keywords: Cattail; Constructed wetland; FISH; leachate; nitrifying bacteria; nitrogen removal

Introduction

One of the difficulties in dealing with solid waste landfill leachate is its wide variation in both quantity and characteristics terms. Various treatment methods have been applied to purify this problematic wastewater ranging from natural to mechanical intensive treatment systems. When considering leachate characteristics, organic substances and nitrogen are the major pollutants which need to be removed. However, high attention has been paid on nitrogen removal recently (Pelkonen et al., 1999; Kalyzhnyi and Gladchenko, 2004) especially when dealing with leachate from old landfill sites. Advanced leachate treatment systems using biological and chemical treatment methods are recently adopted in developed countries but high investment and operating costs limited their application in most of the developing countries. Natural based treatment systems such as constructed wetland would to be more appropriate and practical for their treatment as the systems have significant merits of low cost and versatile removal mechanisms (Lee et al., 2004). In most of the cases, they are used as post-treatment or polishing systems. Direct application of constructed wetland to high strength wastewater especially leachate is very limited.

This study deals with direct application of sub-surface horizontal flow constructed wetland to young and stabilized municipal solid waste leachate. The organic and nitrogen removal efficiencies were evaluated at different hydraulic loading rates. Effect of leachate on plant growth was also studied. Microbiological study targeting nitrifying bacteria in the systems was conducted using fluorescent in-situ hybridization (FISH) technique. This molecular biology methodology is recently applied to study microbial population in constructed wetland system (Baptista et al., 2003; Shipin et al., 2005). The target microorganisms locating in soil and surrounding plant root along the treatment system are determined to correlate microbial populations with the treatment mechanisms in the system.

Materials and methods

The schematic diagram of subsurface horizontal flow (HSF) constructed wetland system used in this study is shown in **Figure 1**. Four concrete ponds of 1 m wide 3 m long and 1 m deep were used. The inlet and outlet zones were filled with 30-60 mm gravel of 0.8 m depth and 1-2 mm sand was used in plantation zone. The operating water depth was 0.7 m. The wastewater was fed into the system by a centrifugal pump through an inlet pipe (10 mm size) with valve control. The wastewater flew downward, moved horizontally through treatment (plantation) zone and discharged from the outlet zone through an outlet pipe (50 mm size). Sampling pipes (2 inches size, 0.5 m. into the sand layer) were provided at three different locations. Cattail (*Typha augustifolia*) was used as emergent in the system at initial planting density of 40 rhizomes m^{-2}.

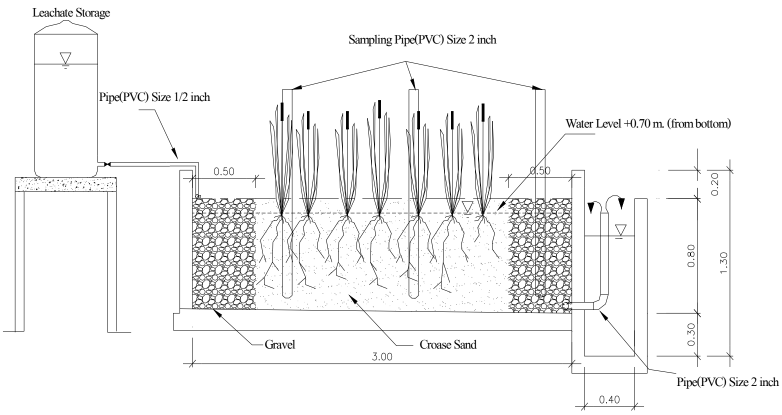


Figure 1. Schematic of subsurface horizontal flow (HSF) constructed wetland system

Two different types of wastewater were used, i.e. representing young and stabilized waste leachate. Their chemical characteristics are shown in **Table 1**. Fresh leachate was collected from a solid waste transfer station in Bangkok. It was diluted with tap water to obtain final COD of 5,000-10,000 mgL^{-1}. For stabilized waste leachate, collected wastewater from leachate storage pond in a closed landfill was used. Influent COD was controlled in range of 3,000-5,000 mgL^{-1} in the first experiment (Run I). It was diluted by rainwater in subsequent experiments (Run II and Run III) in order to control nitrogen loading into the system.

Treatment efficiencies of the system were examined at different hydraulic loading rates (HLR) of 10 mm d^{-1} (Run I), 28 mm d^{-1} (Run II) and 56 (Run III) mmd^{-1}. They were equivalent to the feeding rate of 20, 56 and 112 Ld^{-1} and hydraulic retention time (HRT) of 28, 10 and 5 d respectively. Water quality analyses included temperature, pH, BOD, COD, SS, NH_3-N, TKN, NO_2^-, NO_3^-, PO_4^3, Cl^- and EC. They were performed according to standard methods for the examination of water and wastewater (APHA, 1989)

Table 1. Chemical characteristics of young and stabilized waste leachate

Parameter	Unit	Young leachate (Run I-III)	Stabilized leachate (Run I)	Stabilized leachate (Run II-III)
pH	-	4.3-6.5	8.2-8.5	7.9-9.2
BOD	mgL^{-1}	3,150-7,400	209-278	15-68
COD	mgL^{-1}	5,850-12,820	1,613-4,506	414-2,184
SS	mgL^{-1}	320-825	124-223	10-158
NH_3-N	mgL^{-1}	43-108	711-967	88-441
TKN	mgL^{-1}	144-366	846-1,454	107-305
NO_2-N	mgL^{-1}	ND-3.2	3.0-3.3	1.0-2.7
NO_3-N	mgL^{-1}	0.3-3.8	1.6-2.9	1.0-2.5
PO_4^{3-}	mgL^{-1}	4.3-23.4	7.3 – 8.8	1.4-5.4
Cl^-	mgL^{-1}	125-1,000	2,699-3,199	400-875
EC	mS cm^{-1}	1.5-6.7	17.4-21.3	1.4-13.1

Fluorescent in-situ hybridization (FISH) technique was used to study nitrogen transforming microorganisms, i.e. nitrifying and ANNAMOX bacteria. Specific 16S rRNA-targeted oligonucleotide probes, i.e. Nsm156 (*Nitrosomonas* spp.) representing ammonium oxidizing bacteria (AOB), NIT3 (*Nitrobacter* spp.) representing nitrite oxidizing bacteria (NOB) and Amx820 (ANNAMOX bacteria) were used. The probes were labeled with FITC flourophors. Relative comparison of these bacterial populations in soil and surrounding plant root along the pathway of wastewater was determined as fraction in total microorganisms (DAPI).

Results and discussion

Organic and nitrogen removal efficiencies in the system

Table 2 shows the effluent qualities from the experimental system treating young and stabilized leachate at different hydraulic loading rates (HLR). In case of young leachate treatment, high organic removal was achieved at 98% and 94% in terms of BOD and COD during steady state conditions when the system was operated at HLR of 10 mmd^{-1}. Subsequent increase in HLR to 28 and 56 mmd^{-1} did not deteriorate organic removal efficiencies. They were in range between 94-99% resulting in average effluent BOD and COD concentrations of 32-136 and 364-757 mgL^{-1} respectively. High BOD and COD removal occurred in concurrent with moderate suspended solid removal efficiencies (71-88%) as major treatment mechanisms in the system were sedimentation and filtration of suspended solids in gravel bed, plant uptake and biological degradation of organic substances by attached growth microorganisms under aerobic, facultative and anaerobic conditions in the top (rhizosphere), middle and bottom zones respectively (Stottmeister et al., 2003). Organic concentration profiles along constructed wetland unit (Figure 2a) showed that most of the organic substances were removed within the first 1 m distance from the inlet.

In stabilized leachate treatment case, average BOD and COD removal efficiencies were 71% and 58% at HLR of 10 mmd^{-1}, significantly lower than young leachate case. When HLR was increased to 28 and 56 mmd^{-1}, BOD and COD removal efficiencies were reduced to 55%, 44% and 42%, 63% respectively despite the influent was diluted by rainwater. Lower treatment efficiencies obtained in this case was mainly due to the presence of hardly biodegradable organic in stabilized leachate (BOD/COD <0.1) as compared to young leachate (BOD/COD of 0.6-0.7). Although major fraction of organic substances was removed at the inlet zone, considerable removal of remaining COD along the system was observed especially in case of 10 mmd^{-1} HLR (Figure 2b).

Table 2. Effluent characteristics (mgL^{-1}, except pH) and steady state removal efficiencies (%)

Leachate	Parameters	HLR 10 mmd^{-1}		HLR 28 mmd^{-1}		HLR 56 mmd^{-1}	
		avg. (SD)	%	avg. (SD)	%	avg. (SD)	%
Young	pH	7.6 (0.3)		8.1 (0.1)		7.6 (0.3)	
Leachate	BOD	136 (171)	98	32 (31)	99	115 (36)	97
	COD	757 (692)	94	438 (294)	97	364 (169)	96
	SS	30 (25)	88	113 (128)	71	87 (19)	88
	NH_3-N	44 (20)		72 (40)		98 (18)	
	TKN	86 (40)	43	114 (37)	36	182 (24)	8
	NO_2-N	3.9 (6.6)		1.9 (2.3)		0.3 (0.1)	
	NO_3-N	0.7 (0.2)		4.2 (4.8)		3.0 (0.3)	
	PO_4^{3-}	0.1 (0.1)	99	0.5 (0.1)	95	0.9 (0.3)	95
	Cl^-	532 (127)	25	400 (178)	19	1,125 (105)	0
Stabilized	pH	8.4 (0.3)		8.6 (0.1)		7.9 (0.1)	
Leachate	BOD	47 (31)	71	27 (10)	55	11 (10)	44
	COD	1,489 (529)	58	1,037 (175)	42	185 (115)	63
	SS	40 (15)	56	51(10)	66	28 (4)	66
	NH_3-N	281 (191)		133 (62)		55 (6)	
	TKN	358 (191)	46	224 (50)	41	154 (14)	20
	NO_2-N	54 (47)		4.9 (3.4)		17.1 (3.1)	
	NO_3-N	4.2 (5.7)		1.6 (1.2)		6.8 (1.3)	
	PO_4^{3-}	2.2 (2.1)	21	2.1 (0.7)	48	1.2 (0.7)	25
	Cl^-	1,075 (1,035)	70	625 (210)	0	500 (85)	0

In term of nitrogen removal, average TKN removals in HSF were 43% and 46% at HLR of 10 mmd^{-1} in young and stabilized leachate application case. An increase in HLR to 28 mmd^{-1} did not significantly deteriorated TKN removal efficiencies. However, they were diminished as the loading rate was increased to 56 mmd^{-1}. Unlike organic substances, TKN removal took place gradually along the treatment unit. Organic nitrogen in the leachate was transformed by ammonification and nitrification reactions to oxidized forms (NO_2^- and NO_3^-) and subsequently removed by either denitrification or plant uptake. These mechanisms were evidenced by the observation of temporary accumulation of oxidized nitrogen in the middle zone of treatment unit. Though highest removal efficiencies was achieved at HLR of 10 mmd^{-1} in case of stabilized leachate treatment, some death of plant was observed at the inlet zone possibly caused by excessive NH_3-N loading (De Feo et al., 2005). Therefore, subsequent experiment at higher HLR was conducted with diluted leachate to control incoming TKN at the same level as young leachate.

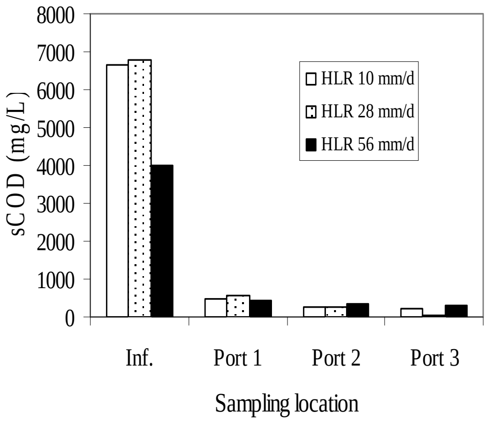
Considering COD and TKN profile along the treatment system under steady-state condition, their concentration changes along treatment system can be described by first-order kinetic as described by the follow equation.

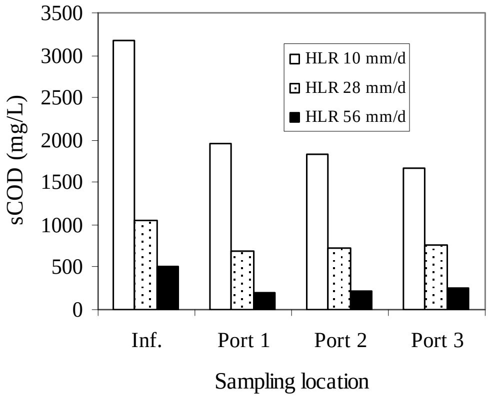
$$dC/dL = -kL \qquad 1)$$

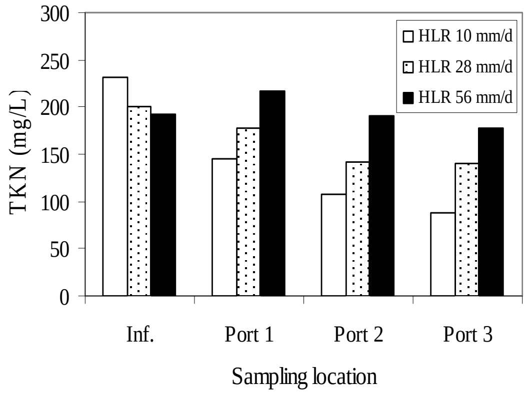
where
- C = COD or TKN concentration at any distance L (mg/l)
- C_0 = initial COD or TKN concentration (mg/l)
- k = first-order rate constant (m^{-1})
- L = treatment distance (m)

By using the above equation, first-order rate constants (k) for COD removal during young leachate treatment were determined as 1.11, 1.47 and 0.77 m^{-1} at HLR of 10, 28 and 56 mmd^{-1} respectively. These are considered much higher than stabilized leachate treatment cases where k values were found in range between 0.09-0.2 m^{-1}. The organic removal rates were also found much higher than those of TKN (0.32, 0.13, 0.04 m^{-1} at HLR of 10, 28 and 56 mmd^{-1}). For TKN removal from stabilized leachate, the reaction rate constant was highest at 0.1 m^{-1} at HLR of 28 mmd^{-1}.

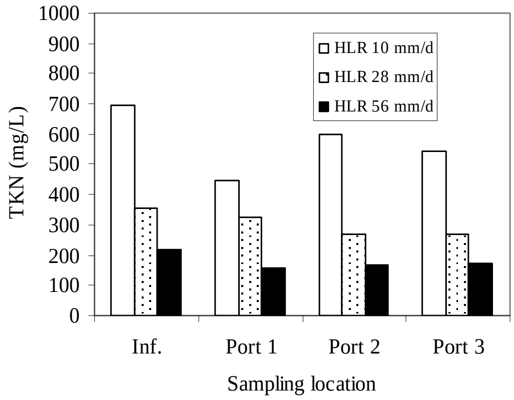
It can be concluded from previous described results that appropriate HLR of subsurface horizontal flow constructed wetland treating young leachate was 10 mmd^{-1}. Nevertheless, moderate nitrogen removal efficiencies was still achieved at HLR of 28 mmd^{-1} for stabilized leachate where influent TKN concentration was controlled at about 100-300 mgL^{-1}. It was also found that organic and nitrogen removal efficiencies obtained in this study are agreeable to the values reported in the literatures (Bulc et al., 1997; De Feo et al., 2005; Lee et al., 2004).







a)



b)

Figure 2. Steady state sCOD and TKN profile along constructed wetland unit in cases of a) young waste leachate b) stabilized waste leachate

Nitrogen transforming bacterial populations in the system

Fluorescent in-situ hybridization (FISH) technique was used to quantity nitrogen transforming bacterial population in the system targeting at nitrifying bacteria, i.e. ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), and ANNAMOX (AMX) bacteria. The results are expressed in terms of bacterial population percentage and ratio among target organisms, i.e. AOB/NOB, AOB/AMX and NOB/AMX in soil samples taken from mid-depth of the experimental unit (40 cm) and surround plant root at the inlet, middle and outlet of the experimental system when applied to the treatment of young and stabilized leachate. They are shown in **Figure 3** and **Figure 4**.

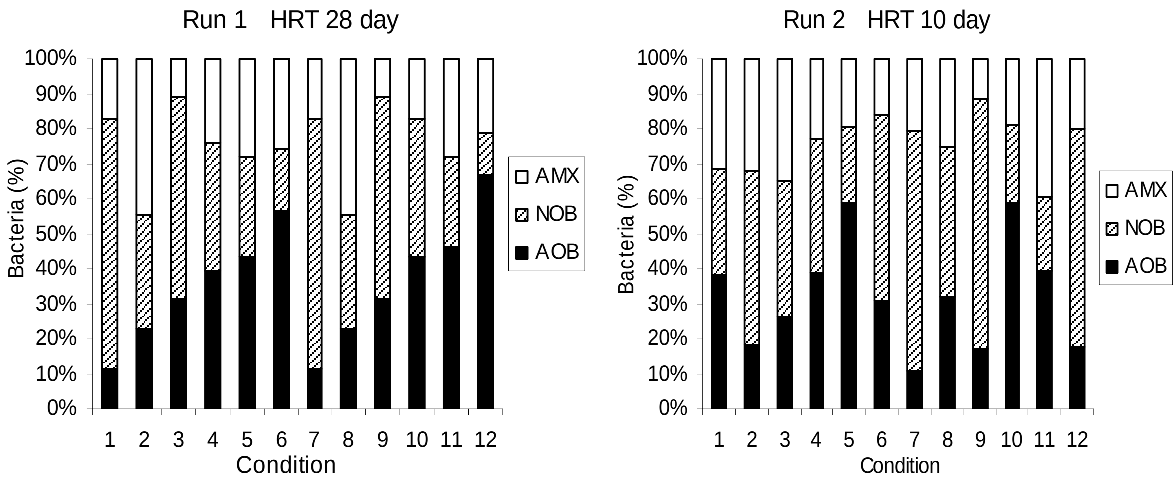


Figure 3. Percentage of AOB, NOB and ANNAMOX bacteria under different conditions

1) Young leachate, soil, port1	7) Stabilized leachate, soil, port1
2) Young leachate, soil, port2	8) Stabilized leachate, soil, port2
3) Young leachate, soil, port3	9) Stabilized leachate, soil, port3
4) Young leachate, plant root, port1	10) Stabilized leachate, plant root, port1
5) Young leachate, plant root, port2	11) Stabilized leachate, plant root, port2
6) Young leachate, plant root, port3	12) Stabilized leachate, plant root, port3

It was found that nitrogen transforming bacterial population varied along the treatment system and there are significant differences between soil samples taken at mid-depth (40 cm) and soil surrounding plant root. AOB population was found at higher percentage in the area surrounding plant root (condition 4-6 and 10-12) as compared to other area (condition 1-3 and 7-9). This observation can be explained by the fact that oxygen was supplied though plant root system (Stottmeister et al., 2003) and AOB utilized them to convert ammonium nitrogen to nitrite. Subsequent reaction took place in the outer zone in which nitrite was further oxidized to nitrate by NOB or reacted with incoming ammonium in anaerobic ammonium oxidation and converted to N_2 by ANNAMOX bacteria. This observation suggested that nitrogen transformation in this system could proceed through nitrification and anaerobic ammonium oxidation pathways. The presence of ANNAMOX bacteria in constructed wetland could be expected if the system was operated near optimum conditions for their growth, i.e. pH of 7.5, DO of 1.5 mgL^{-1} and temperature of 30^oC (Jianlong and Ning, 2004).

As shown in Figure 4, AOB/NOB ratio was relatively constant in soil samples along the treatment system. On the other hand, NOB/AMX ratio was found fluctuating but higher ratio was detected towards the outlet zone. The ratio was also affected by hydraulic loading rate and leachate characteristics (young or stabilized leachate). As major fraction of organic substances was removed at the inlet zone, oxygen was more available to nitrifying bacteria at the outlet zone and NOB could become predominant over ANNAMOX bacteria. Higher AOB/NOB and AOB/AMX ratios were detected in surrounding plant root as compared to other soil areas. Similar pattern of NOB/AMX ratio along the treatment system was also observed surrounding plant root.

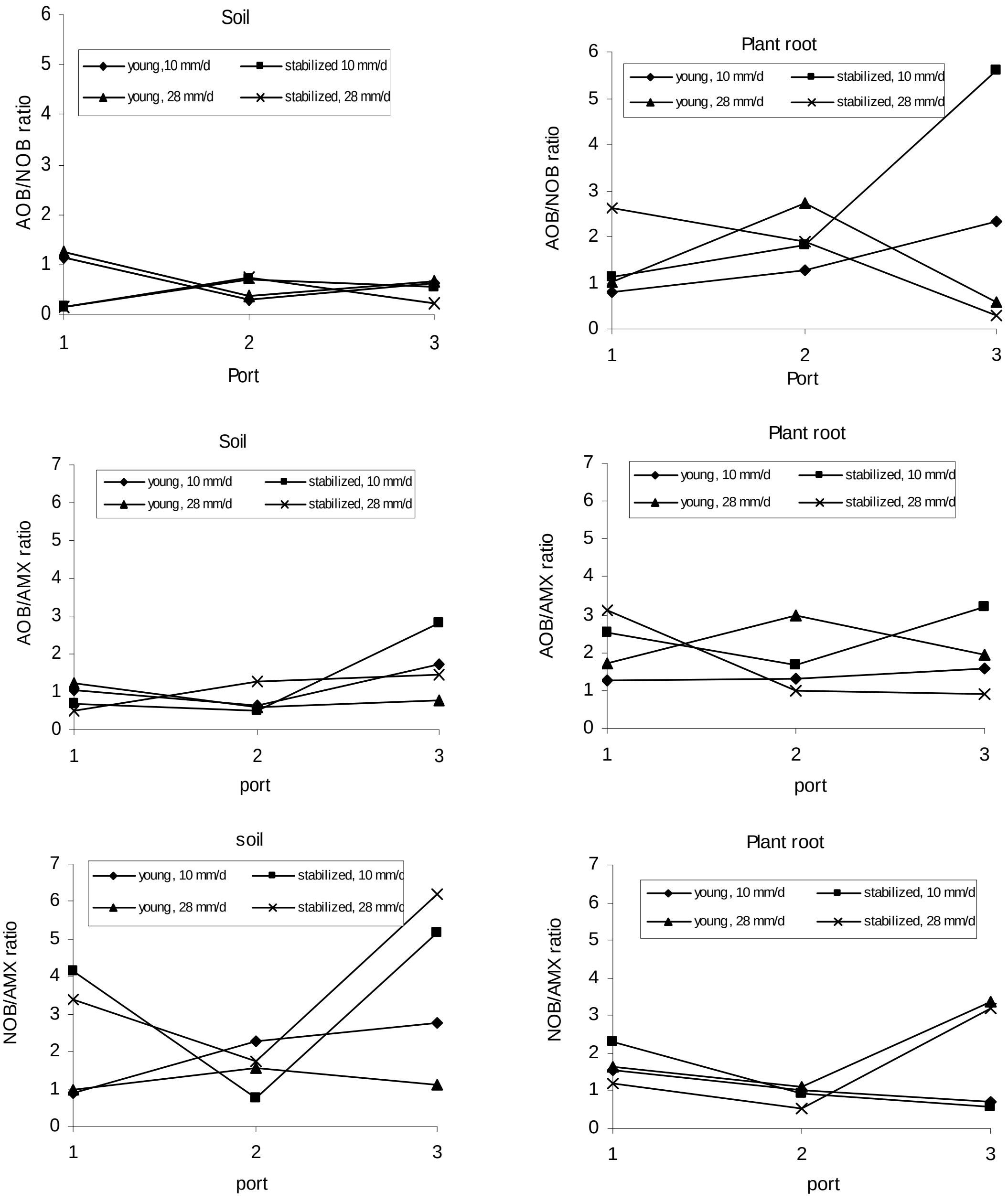


Figure 4. Ratio of AOB/NOB, AOB/AMX and NOB/AMX in soil and surrounding plant root

Conclusions

In this study, subsurface horizontal flow constructed wetland was applied to the treatment of young and stabilized leachate. According to our experimental results, the following conclusions can be drawn.

1. High organic removal efficiencies (97-99% BOD and 94-97%COD) was achieved in subsurface horizontal flow constructed wetland system treating young leachate (3150-7400 mgBODL^{-1}, BOD/COD of 0.6-0.7) at hydraulic loading rate (HLR) of 10 to 56 mmd^{-1}. Majority of COD was removed in the first 1 m distance from the inlet. Moderate TKN removal (36-43%) was achieved at HLR of 10 and 28 mmd^{-1}.

2. Lower BOD removal efficiencies (41-77%) were observed when the system was applied to the treatment of stabilized leachate (BOD/COD < 0.1). Moderate TKN removal (41%) was achieved at HLR of 28 mmd^{-1} when influent TKN was controlled at about 100-300 mgL^{-1}.

3. Nitrifying and ANNAMOX bacteria populations were found varied along the treatment system. AOB population was detected at higher percentage in the area surrounding plant root as oxygen was supplied through plant root system. Detection of both NOB and ANNAMOX bacteria suggested that nitrogen transformation in the system could proceed through nitrification and anaerobic ammonium oxidation pathways. Bacterial population ratio was also affected by hydraulic loading rate and leachate characteristics.

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