Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2014.1003606

57 (2016) 5350–5357
March

Taylor & Francis
Taylor & Francis Group

Correlations between the oxidation-reduction potential characteristics and microorganism activities in the subsurface wastewater infiltration system

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Received 2 April 2014; Accepted 27 December 2014

ABSTRACT

The removal abilities in subsurface wastewater infiltration system (SWIS) rely heavily on the metabolism of microorganisms. Although the role of microorganism is appreciated, a lack of effective indicators of microbial function in SWIS is apparent. This study explored the correlations between oxidation-reduction potential (ORP) and microorganism and enzyme activities involved in nitrogen removal processes. The results showed that SWIS exhibited a gradual change in ORP level, microbial activity, and abundance along the soil profile. The quantities of nitrifier and denitrifier were in positive correlations with potential nitrification activity and denitrification activity ($p < 0.05$). The correlation equations for ORP and nitrate reductase (NAR) activity were $N = 1250$ ORP—65.125 ($R^2 = 0.6165$, $p < 0.05$), $N = 333.3$ ORP—*21.27* ($R^2 = 0.7508$, $p < 0.05$) from the horizontal and longitudinal directions, respectively. The results suggested that ORP level could be used as an indicator of key functional microorganism and NAR activity involved in nitrogen cycling of SWIS. Furthermore, the distribution of ORP and aqueous rate behaved in the opposite way, confirming the running mechanism of SWIS theoretically.

Keywords: Subsurface wastewater infiltration system; Sewage treatment; Enzyme activity; Microorganism activity; Oxidation-reduction potential

1. Introduction

As one of the ecological treatment technologies, subsurface wastewater infiltration system (SWIS) is thought to be efficient in sewage treatment [1]. In SWIS, the polluted water percolates around the circumference through the capillary soakage and soil percolation actions [2]. The organic matters and nitrogen are decompounded and removed through the integrated actions of water, soil, microbe, and plant. SWIS has many advantages compared with the conventional activated sludge process, including excellent removal efficiency for organics and phosphorus, low construction, and operation cost and easy maintenance [3].

Similar to other eco-treatment technologies, the removal abilities in SWIS rely heavily on the metabolism of microorganisms contained within biofilm [4]. Although the role of biofilm in water treatment is appreciated, a lack of effective indicators of biofilm development and function in SWIS is apparent [5]. Lazarova and Manem [2] reviewed the technologies

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used by a variety of researchers to measure activity characteristics of biofilm and then concluded that simple and precise methods to measure microorganism's activities must be developed in order to take full advantages of biofilm processes.

The possible relationship between the oxidation-reduction potentials (ORP) of medium and the ability of microorganisms to initiate growth has been the research focus during the last decades [6]. The work has established the fact that the growth of certain anaerobes is greatly influenced by the ORP [6]. In the case of aerobic bacteria, however, conflicting results have been reported. Hand et al. [4] demonstrated that the potential of the medium was of considerable importance in determining whether or not small inocula of *Rhizobium*, an aerobic organism, could initiate growth. On the other hand, Oladoja and Ademoroti [5] concluded that the limiting factor in growth of *Bacillus megatherium* in vacuum is the oxygen content and not the ORP of the culture medium. As a whole, previous experiments provided little information, however, as to whether or not the potential of ORP affects the bacterial activity in SWIS from full-scale perspective. SWIS has been (and, to some extent, still are) frequently operated as "black boxes". In the past, much of the design of SWIS has been done with little knowledge of (or consideration for) the roles played by microorganisms and how their effects could be enhanced and optimized.

Therefore, the purposes of this study were as follows:

(1) To explore the ORP level characteristics in a field scale SWIS.
(2) To examine the microbial and enzyme activities involved in the nitrogen removal process.
(3) To discuss the correlations between the ORP level and microbial and enzyme activities.

2. Materials and methods

2.1. Raw wastewater characteristics

Field experiments were carried out at Shenyang University, China (East longitude 123°26′50.60″, North latitude 41°49′00.60″). The influent was combined wastewater, from toilets, restaurants, etc. The ranges of major water quality indices were pH 7.2–7.4, chemical oxygen demand (COD) 275–360 mg/L, biological oxygen demand (BOD_5) 155–220 mg/L, suspended solid (SS) 95–126 mg/L, total nitrogen (TN) 30–45 mg/L, total phosphorus (TP) 3–4 mg/L, ammonia nitrogen (NH_3-N) 20–30 mg/L, with an average ratio of 0.6 for BOD_5/COD.

2.2. Support matrix

In order to improve nitrogen removal efficiency, the matrix was composed of 5% activated sludge, 65% meadow brown soil, and 30% coal slag mixed evenly in volume ratio [7]. The soil used was meadow brown soil, sampled from the top 20 cm from Shenyang Ecological Station, with total organics 22.8 g/kg, TN 1.4 g/kg, and TP 0.85 g/kg. The activated sludge was obtained from the aeration tanks in Shenyang Northern Municipal Sewage Treatment Plant, China, air-dried after being centrifuged for 15 min at 1,500 rpm. Other materials (gravel and coal slag) were purchased from a local market (particle size: gravel 10–25 mm and coal slag 4–8 mm). The infiltration rate, porosity, and surface area of the matrix were 0.37 m^3/m^2 d, 59%, and 5.21 m^2/g, respectively.

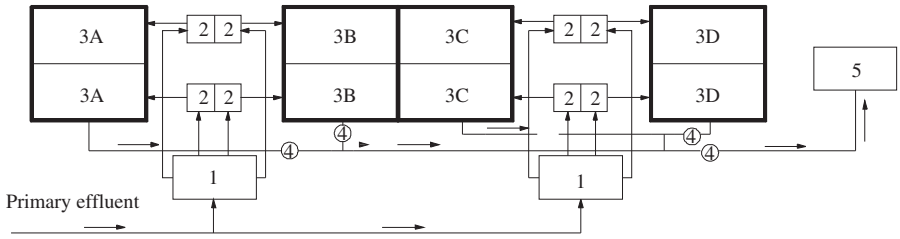
2.3. System description

The wastewater was firstly pretreated in a hydrolytic acidification tank. After the pretreatment, concentrations for COD, BOD_5, SS, and TP reduced to 200–290, 132–170, 20–30, and 2–3 mg/L, respectively. The concentrations for TN and NH_3-N changed little. The effluent flowed under gravity action through the SWIS system, which was composed of 8 subcells (Fig. 1). The dimension of each subcell was length × width = 20 m × 15 m, with effective depth of 1.5 m. Distributing pipes were 0.5 m underneath (100 mm in diameter with holes of 4 mm in diameter placed in the bottom every 60 mm). The collecting pipes were 1.5 m underneath (80 mm in diameter with 6 mm holes placed in the bottom side every 60 mm). The interval between two distributing pipes was 2.5 m (Fig. 2). The beds were planted with herbage, which was mainly for landscape planting.

2.4. Sampling and analytical method

Soil samples were sampled along each vertical profile which is in the middle of two adjacent distributing pipes. Five sample points were settled on the profile evenly, that is every 20 cm interval from 20 cm (up) to 100 cm (down) in depth (Fig. 2). Soil and water samples were collected twice a month. Sampling and measurements of ORP level were carried out at 9:00–9:30 am every three days, assuming the system was in a stable situation during monitoring and sampling. All results were repeated for three times.

The ammonifying, nitrifying, and denitrifying bacteria in the soil samples were counted using the most probable number calculation [7]. For the ammonifying bacteria, the medium contained per liter of distilled



1. Primary distributing tank 2. Secondary distributing tank 3A-D. SWIS 4. Sampling well for water quality analysis 5. Collecting tank

Fig. 1. Diagrammatic sketch of the SWIS.

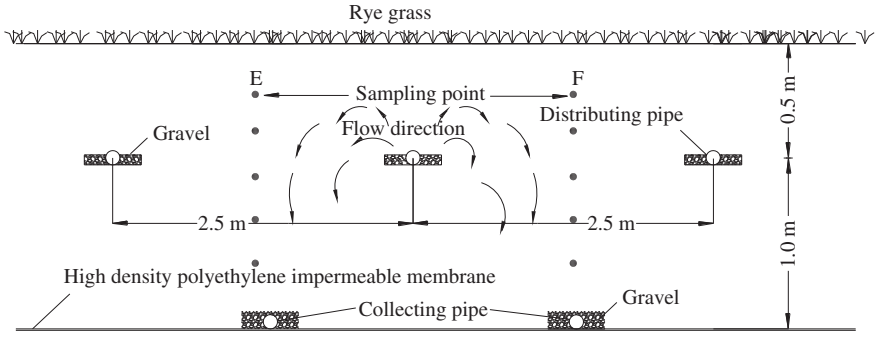


Fig. 2. Profile for the infiltration system and sampling position.

water: 5 g peptone, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, pH 7.2. The medium for the nitrifying bacteria contained per liter of distilled water: 13.5 g Na_2HPO_4, 0.7 g KH_2PO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.5 g $NaHCO_3$, 2.5 g $(NH_4)_2SO_4$, 14.4 mg $FeCl_3{\cdot}6H_2O$, and 18.4 mg $CaCl_2{\cdot}7H_2O$, pH 8.0. The medium for the denitrifying bacteria contained per liter distilled water: 1.0 g KNO_3, 0.1 g Na_2HPO_4, 2.0 g $Na_2S_2O_7$, 0.1 g $NaHCO_3$ and 0.1 g $MgCl_2$, pH 8.0. The soil samples were taken from 0.2, 0.4, 0.6, 0.8, and 1.0 m depths, respectively. Aliquot (1 mL) of serial 12-fold sterile distilled water dilutions of the soil samples was transferred to microtiter plates containing each type of medium, then incubated at 28℃ for 7 d (for the ammonifying bacteria), 14 d (for the nitrifying bacteria), and 15 d (for the denitrifying bacteria), respectively. Meanwhile, 10 g of soil samples were oven-dried at 105℃ for 12 h to produce a constant weight. The amounts of bacteria were analyzed every 3 d during the study.

Urease, nitrate reductase (NAR), and nitrite reductase activities were analyzed according to the method suggested by Guan [8] every 3 d.

The medium used for measuring the potential nitrification activity (PNA) contained per liter: 0.14 g K_2HPO_4, 0.027 g KH_2PO_4, 0.59 g $(NH_4)_2SO_4$, 1.20 g $NaHCO_3$, 0.30 g $CaCl_2{\cdot}2H_2O$, 0.20 g $MgSO_4$, 0.00625 g $FeSO_4$, 0.00625 g EDTA and 1.06 g $NaClO_3$, the pH was 7.5. Sodium chlorate was used to inhibit the oxidation of nitrite to nitrate. 20 g of soil samples were added to 100 mL of test medium and incubated at 25℃ on a horizontal shaker at 150 rpm. Subsamples were collected after 2, 6, 20, and 24 h of incubation. PNA was calculated by angular coefficient assessment of linear regression calculated for hours and the amount of nitrate produced. Results were normalized for volume loss during sampling and expressed as mg of nitrate per kilogram dry weight (DW) per hour. The medium for measuring the potential denitrification activity (PDA) contained per liter: 1.44 g KNO_3, 2.0 g glucose, 27.2 g KH_2PO_4 and 34.8 g K_2HPO_4, the pH was 7.2. 10 g of soil samples were added to 100 mL of test medium and incubated at 25℃ on a horizontal shaker at 150 rpm. Rubber plug was used during shaking to prevent gas from running out. Subsamples were collected after 48 h of incubation. PDA was calculated by angular coefficient assessment of linear regression and the amount of nitrite produced. Results were normalized for volume loss during sampling referred to by DW and expressed as mg of nitrite per kilogram dry matter per hour.

The measurement of ORP level was conducted by a platinum electrode coupled with calomel electrode. Electrode positions were arranged vertical to the distribution pipe with intervals 40 cm. The surfaces of electrode were cleaned and activated at regular intervals according to the method presented by Wang et al. [6] to ensure the accuracy of experimental results.

According to the method presented by Hand et al. [4], a certain quantity of matrix was weighed using electro-balance. Then, the samples were oven-dried for 2 h at 105℃ and reweighed. The aqueous rate was expressed by the following equation, where W (mg) refers to the matrix mass after dried, W_0 (mg) refers to the mass before dried.

$$\text{Aqueous rate} = (W_0 - W)/W_0 \quad (1)$$

2.5. Experimental operation

Sampling was conducted from June, 2012 to September, 2012 (room temperature 18.1–26.2℃). But, the systems were operated one month before sampling to allow grass establishment and microbial biomass maturation.

During the whole experimental period, intermittent operation mode was adopted as a passive method for oxygen transfer restoring. Each cycle of the intermittent operation included a continuous flow period of 24 h (between 9:00 and 9:00 am the next day) and a drying period of 24 h.

3. Results

3.1. ORP level characteristics

During the study period, ORP levels of different soil depth and distances to the distributing pipe were examined, as shown in Fig. 3(a). The distribution of ORP level presented different features across two dimensions. In the longitudinal direction, the level descended with the increase of depth layer. From 0.2 to 0.4 m depth, the ORP level was above 300 mV on average, suggesting the support matrix was in strong oxidizing property, whereas at the depth layer deeper than 0.8 m, the substrate was in a reducing environment with ORP below 100 mV was found. Horizontally, distance to the distributing pipe affected the ORP value significantly ($p < 0.05$). The nearer to the distributing pipe, the lower ORP level observed.

As shown in Fig. 3(b), the aqueous rate increased gradually from the top to bottom, from the area far from the distributing pipe to the nearer section, ranging from 3.5 to 44.3%. It was obvious that the distribution features of aqueous rate were opposite to

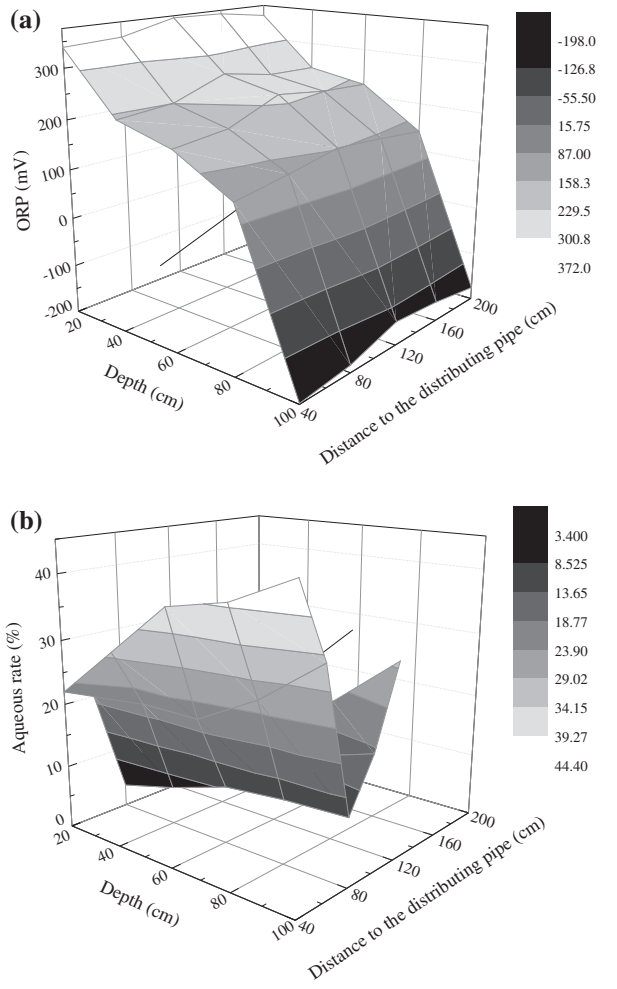


Fig. 3. Distribution characteristics of oxidation-reduction potential (a) and aqueous rate (b).

that of ORP. According to the previous studies [3,7], the flow path of wastewater is determined by both the actions of capillary and gravity in SWIS. Firstly, the wastewater flows up to a certain level under the force of capillary, then down to the bottom under the gravity. This flow characteristic determines the aqueous rate would be higher in the bottom and the area nearer the distributing pipe. Therefore, the experimental result confirmed the running mechanism of SWIS theoretically.

3.2. Microbial population characteristics

Purification processes in the SWIS were gradually established during the first three weeks. During stable purification period from the fourth week on (removal efficiency for COD was 81.8–86.1%, BOD_5 80.6–86.4%, SS 92.1–99.2%, TN 50.8–66.7%, TP 83.3–87.5%, NH_3-N

75.0–83.3%. The effluent quality met the standard of surface water quality in city landscape), soil samples were analyzed for the number of ammonifying, nitrifying, and denitrifying bacteria at different depths and positions. The amount of nitrifying bacteria declined with depths increase. At the same time, the nearer to the distributing pipe, the lower amount attained. The changes of denitrifying bacteria showed an opposite situation. In contrast, differences in the number of ammonifying bacteria were independent of both depths and positions.

Table 1 shows the PNA and PDA of the support matrix along the soil profile. Average PNA decreased with the increasing depth, while PDA increased with the increase of depth layer, which were consistent with the abundance of nitrifying and denitrifying bacteria ($p < 0.05$), respectively. The changes in PNA and PDA could be attributed to changes in organic carbon and soil texture, which in turn influenced the oxygen partial pressure and the activity of the nitrifiers and denitrifiers. A quantitative estimation of the nitrogen-cycling microbial communities and their corresponding PNA and PDA indicated higher numbers of nitrifying bacteria than denitrifying bacteria, and higher nitrification activity.

3.3. Enzyme activity characteristics

According to the previous studies [7], urease and NIR activities were in positive correlation with the TN removal efficiency and could be the biological indexes during the nitrogen removal process in SWIS. To test their correlations with ORP level, urease, NAR and NIR activity characteristics were examined as shown in Table 2.

The results suggested that soil depth and position had significant influence on the enzyme activity. For urease, the higher activity attained near the inlet, 0.6 m underneath, due to the high organic nitrogen concentration of the influent. The activity had no significant difference between sampling positions. For NAR, depth sequence for its activity from high to low was 0.2 m > 0.4 m > 0.6 m > 0.8 m > 1.0 m. From horizontal direction, activity sampled from the point with distance 120 cm from the distribution pipe was higher than that from the other positions. The result was in consistent with more quantity of nitrifying bacteria at that position. For NIR, the descending order for its activity of different layer was 0.4 m > 0.2 m ≈ 0.6 m > 0.8 m > 1.0 m.

According to the 80 sampling results for ORP and NAR, ORP had positive correlation with NAR distribution. The correlation equations were $N = 1250$ ORP —65.125 ($R^2 = 0.6165$, $p < 0.05$), $N = 333.3$ ORP—*21.27* ($R^2 = 0.7508$, $p < 0.05$) from the horizontal and longitudinal directions, respectively, where N refers to the NAR activity. No significant correlation was found between the ORP and urease and NIR activities. Therefore, ORP level can be used as an indicator for NAR activity.

Table 1
PNA and PDA in SWIS along the soil profile (mg/(kg h))

	Distance to the distributing pipe (cm)					
	40		80		120	
Depth (cm)	PNA	PDA	PNA	PDA	PNA	PDA
20	2.6 ± 0.1	1.6 ± 0.1	3.0 ± 0.4	1.3 ± 0.2	4.4 ± 0.2	1.0 ± 0.1
40	2.2 ± 0.1	1.9 ± 0.2	2.8 ± 0.2	1.6 ± 0.1	3.5 ± 0.3	1.4 ± 0.2
60	1.9 ± 0.1	2.4 ± 0.2	2.2 ± 0.2	2.2 ± 0.1	2.6 ± 0.2	1.9 ± 0.1
80	1.4 ± 0.2	2.9 ± 0.3	1.7 ± 0.2	2.7 ± 0.2	2.0 ± 0.1	2.4 ± 0.1
100	0.8 ± 0.2	3.8 ± 0.2	1.0 ± 0.1	3.5 ± 0.1	1.4 ± 0.1	3.3 ± 0.2
	160		200			
	PNA	PDA	PNA	PDA		
20	3.4 ± 0.5	1.4 ± 0.2	2.5 ± 0.3	1.5 ± 0.1		
40	2.7 ± 0.4	1.7 ± 0.2	2.4 ± 0.1	1.8 ± 0.1		
60	2.3 ± 0.1	2.1 ± 0.1	1.8 ± 0.2	2.3 ± 0.2		
80	1.6 ± 0.3	2.5 ± 0.3	1.3 ± 0.4	2.7 ± 0.2		
100	1.1 ± 0.1	3.6 ± 0.1	1.0 ± 0.2	3.6 ± 0.2		

Table 2
Enzyme activity characteristics in SWIS (mg/g d)

	Distance to the distributing pipe (cm)								
	40			80			120		
Depth (cm)	Urease	NAR	NIR	Urease	NAR	NIR	Urease	NAR	NIR
20	19.05 ± 0.05	0.90 ± 0.01	0.35 ± 0.03	20.03 ± 0.79	0.94 ± 0.20	0.37 ± 0.05	18.92 ± 2.17	1.18 ± 0.24	0.21 ± 0.01
40	20.35 ± 0.12	0.77 ± 0.01	0.41 ± 0.14	22.37 ± 3.25	0.79 ± 0.14	0.41 ± 0.03	18.30 ± 1.88	0.81 ± 0.07	0.26 ± 0.17
60	23.98 ± 1.23	0.52 ± 0.05	0.35 ± 0.01	22.27 ± 0.96	0.62 ± 0.03	0.36 ± 0.16	22.38 ± 1.09	0.77 ± 0.25	0.20 ± 0.02
80	19.77 ± 2.08	0.43 ± 0.11	0.29 ± 0.10	19.93 ± 3.00	0.52 ± 0.01	0.30 ± 0.11	19.89 ± 0.25	0.64 ± 0.41	0.15 ± 0.08
100	19.32 ± 2.37	0.42 ± 0.03	0.25 ± 0.02	20.98 ± 1.07	0.40 ± 0.08	0.22 ± 0.06	19.03 ± 1.11	0.45 ± 0.07	0.11 ± 0.01

	160			200		
	Urease	NAR	NIR	Urease	NAR	NIR
20	18.42 ± 1.78	0.91 ± 0.07	0.33 ± 0.08	15.55 ± 1.24	0.91 ± 0.07	0.30 ± 0.10
40	18.96 ± 0.55	0.75 ± 0.03	0.38 ± 0.14	18.56 ± 1.65	0.75 ± 0.03	0.35 ± 0.11
60	20.30 ± 2.23	0.53 ± 0.01	0.32 ± 0.03	23.09 ± 1.84	0.53 ± 0.01	0.29 ± 0.06
80	18.18 ± 3.37	0.40 ± 0.07	0.24 ± 0.06	20.37 ± 1.49	0.40 ± 0.17	0.26 ± 0.08
100	19.38 ± 3.07	0.28 ± 0.02	0.17 ± 0.02	15.76 ± 1.96	0.21 ± 0.04	0.23 ± 0.03

4. Discussion

The previous studies have presented several indicators of biofilm development and activity in eco-treatment systems, such as volatile suspended solid (VSS), respiration intensity, protein, polysaccharide, and viable cell number [9,10]. VSS relates to biomass growth, representing the non-ash part of the total suspended solids. So, VSS can express the quantity of biofilm to some extent. Respiration intensity represents the metabolizable blooming degree of biofilm. Protein has been used as a measure of cell biomass in biofilm, even though protein excreted into the extracellular matrix can account for almost 15% of its total mass [11]. Viable cells were quantified by the extraction of phospholipids and analysis of phosphate which has some correlations with the viable biomass [12].

An advantage of using these parameters to study biofilm is that the measurement for VSS, respiration intensity, the viable cell number, and protein can be carried out without having to remove the biofilm from the surface and without relying on cultivation of microorganisms [13]. However, they could not reflect exactly the transferring ability of hydrogen electrons from substrates to acceptors [14]. In addition, nitrogen-cycling microbial activity had not been isolated and quantified. Therefore, they cannot be considered as good measures of microbial activity involved in nitrogen removal process.

In SWIS, nitrification coupled with denitrification is generally thought to be the major method for nitrogen removal [7,15]. As a kind of autotrophic bacteria, nitrifiers have strict environmental requirements (temperature 20–30 °C, pH 7–8, DO concentration > 2 mg/L) and significantly lower growth rate than that of denitrifiers, so nitrification is a thought to be the limiting step for nitrogen removal process [16]. It was reported that for NH_3-N with a concentration of 1 mg/L, nitrification will not occur successfully unless the dissolved oxygen (DO) concentration reaches 4.6 mg/L [17,18]. The error of direct measurement of DO in the matrix layer is very big due to the low DO concentration in soil, especially in deeper layer. The absolute value of ORP is big as well as its positive correlation with DO concentration [19]. As a result, measurement of ORP was used for instead of DO. The ORP level generally reflects the amount and type of oxidative–reductive substrates contained in the liquid. Kadam et al. [20] reported the spatial distribution applying ORP parameter in constructed wetlands. According to Morkved et al. [21], ORP greater than 100 mV is commonly interpreted to indicate an aerobic environment. Herein, benefiting from the oxygen transferring availability of the surface soil, environment in the upper layer of SWIS (0–0.6 m) lay in aerobic state basically (average ORP 304 mV). In comparison, the deep layer (0.6–1.5 m) was in anoxic/anaerobic condition (average ORP 60 mV). The possible reason for ORP

distribution is the higher of BOD loadings, the more requirement of oxygen amount would be presented for the oxidation of the organic matters. When the oxygen demand was higher than the DO amount, the system was in an anoxic condition resulting in the low ORP value and nitrification efficiency. Accordingly, the zone at 0.2–0.6 m depth was the most effective nitrifying reaction region. The denitrifying bacteria were more active at depths of 0.6–1.5 m. Horizontally, obvious correlations also existed between ORP values and the quantities of nitrifiers and denitrifiers. Therefore, the ORP levels could be used as an indicator of the activities of nitrifying and denitrifying bacteria from both horizontal and longitudinal directions.

5. Conclusions

The results showed that the distribution of ORP level presenting different features across the horizontal and longitudinal dimensions could be used as an indicator of microorganism's activities and NAR involved in nitrogen removal process of SWIS. Soil depth and distance from the distributing pipe had significant effect on ORP distributions. The matrix was in strong oxidizing property from the 0.2–0.4 m depth with the ORP level above 300 mV, whereas at the depth layer deeper than 0.8 m, the substrate was in a reducing environment. The nearer to the distribution pipe, the lower ORP level observed. The distribution of aqueous rate showed an opposite trend compared with that of ORP. This conclusion confirmed the running mechanism of SWIS theoretically. Nitrifiers and denitrifiers exhibited gradual changes in quantities along the soil profile, which were in positive correlations with PNA and denitrification activity, respectively. For NAR, its activity was higher in the upper layer and the area near to the distribution pipe, positively associating with the ORP characteristics. The results would be helpful for further establishment of the operation mechanism of SWIS.

Acknowledgements

This work has been financially supported by the National Natural Science Foundation of China (no. 51108275), the Program for Liaoning Excellent Talents in Universities (LNET) (no. LJQ2012101), the Program for New Century Excellent Talents in Universities (no. NCET-11-1012), the Science and Technology Program of Liaoning Province (nos. 2011229002 and 2013229012), and Basic Science Research Fund in Northeastern University (nos. N130501001 and N140105003).

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