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# Effect of amended soil and hydraulic load on enhanced biological nitrogen removal in lab-scale SWIS

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ABSTRACT

To characterize the effect of amended soil on nitrogen removal in subsurface wastewater infiltration system (SWIS), culture, grass carbon, and zeolite were mixed to produce microbial inoculums, and then the optimal microbial inoculums, nutrient substance, cinder, and original soil were mixed to produce the soils through bioaugmentation. Results indicate that the microbial inoculums (culture + 50% grass carbon + 50% zeolite) and the amended soil (12.5% microbial inoculums + 25% nutrient substrate + 12.5% cinder + 50% original soil) have the optimal biogenic stimulating properties, and the adsorption capacity of the amended soil are 1.216 mg-P $g^{-1}$ and 0.495 mg-N $g^{-1}$. The laboratory soil column experiment indicates that the efficient mode of nitrogen removal in lab-scale SWIS is adsorption–nitrification–denitrification and the nitrification/denitrification can be enhanced by the application of the amended soil. On average, the SWIS filled with amended soil converts 85% of ammonia nitrogen ($NH_4^+$-N) to $NO_x^-$-N and removes 49.8–60.6% of total nitrogen (TN), while the system filled with original soil removes 80% of $NH_4^+$-N and 31.3–43.2% of TN at 4–8 cm $day^{-1}$. Two systems are overloads at 10 cm $day^{-1}$. It is concluded that the microbial activities and nitrogen removal efficiencies are improved in SWIS after bioaugmentation.


## 1. Introduction

About half of the properties in onsite and small community of China discharge their mostly ordinary domestic sewage directly into sewer, watercourses, lakes, or sea. This discharge of poorly treated sewage is responsible for many watercourses and lakes not presently meeting their quality objectives. Therefore, national regulations have been adopted that define the permissible discharge of organic matter and nutrients from these properties. The regulations (Integrated Wastewater Discharge Standard<GB 8978–1996>) stipulate three treatment classes that have to be met in different areas depending on the quality objectives of the receiving water body. Removal of organic matter, measured as chemical oxygen demand (COD), is always required to a level of 80 or 90% removal.

Wastewater treatment for onsite and small community commonly relies on infiltration and percolation of primary effluent through soil to achieve purification, and subsurface wastewater infiltration system (SWIS) or other constructed wetland with various technological designs can meet the demands [1–7]. SWIS is an effective process for wastewater treatment according to the integrated mechanisms of chemical, physical, and biological reactions if the system is carefully designed and managed [8–10]. The efficiency of SWIS is affected by an inter-related series of factors, namely activities of microorganisms, temperature, characteristics of soil, hydraulic load, and characteristics of wastewater [11].

Biological nitrogen removal is of important concern throughout many part of the world, especially in densely populated areas, such as China, that rely primarily on SWIS for treatment and disposal of domestic wastewater. Recently, much research has been carried out to study nitrogen removal performance in SWIS [11–13]. The presence of nitrogen excess in the environment has caused serious alterations of the natural nutrient cycle between the living world and the soil, water, and atmosphere [14,15]. Ammonia nitrogen removal in SWIS may follow several pathways and the biological nitrogen removal (BNR) process is the most common methodology for removing it. Soluble ammonia can be adsorbed by the soil or removed by volatilization directly into the atmosphere as ammonia gas. The ammonia adsorbed in soil is available for uptake by microorganisms, or for conversion to nitrite nitrogen ($NO_2^-$-N) and nitrate nitrogen ($NO_3^-$-N) through nitrification. $NO_x^-$-N is not held by exchange reactions but remains in solution and is transported in the percolate if there is no denitrification. Denitrification in the

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**Table 1**
Count of bacteria of each scheme (cfu $g^{-1}$)

| Microbial inoculums (wt.%) | April (average) | | | May (average) | | |
|---|---|---|---|---|---|---|
| | AB[a] | NB[b] | DB[c] | AB | NB | DB |
| C[d] + 90% GC[e] + 10% Z[f] | $5.3 \times 10^8$ | $3.0 \times 10^6$ | $2.0 \times 10^{10}$ | $2.17 \times 10^{10}$ | $3.0 \times 10^6$ | $3.0 \times 10^{10}$ |
| C + 70% GC + 30% Z | $1.1 \times 10^{10}$ | $2.0 \times 10^6$ | $3.0 \times 10^9$ | $3.15 \times 10^{10}$ | $2.0 \times 10^7$ | $2.0 \times 10^{11}$ |
| C + 50% GC + 50% Z | $2.6 \times 10^{10}$ | $6.5 \times 10^6$ | $1.4 \times 10^{10}$ | $1.33 \times 10^{12}$ | $1.1 \times 10^8$ | $2.0 \times 10^{11}$ |
| C + 30% GC + 70% Z | $4.2 \times 10^8$ | $1.1 \times 10^6$ | $1.1 \times 10^{10}$ | $2.75 \times 10^{10}$ | $1.1 \times 10^8$ | $1.4 \times 10^{11}$ |
| C + 10% GC + 90% Z | $1.2 \times 10^9$ | $1.1 \times 10^6$ | $3.0 \times 10^9$ | $1.75 \times 10^{10}$ | $1.1 \times 10^8$ | $3.0 \times 10^{11}$ |

[a] AB: ammonifying bacteria.
[b] NB: nitrifying bacteria.
[c] DB: denitrifying bacteria.
[d] C: culture.
[e] GC: grass carbon.
[f] Z: zeolite.

conventional SWIS is usually affected by the denitrifying biomass, temperature, and the characteristic of the soil. Total nitrogen (TN) removal efficiencies can only reach 20–35% because the denitrifying biomass is usually too low in conventional SWIS. Ineffective treatment of nitrogen in conventional SWIS can eventually lead to eutrophication of water bodies and other contaminant-related problems that result from the discharge of partially treated wastewater into the surrounding environment.

The combination of the conventional SWIS with bioaugmentation for improving the removal efficiency of N compounds is an interesting concern. Bioaugmentation can be explained as a process in which the application of indigenous or wild type or genetically modified organism to the bioreactor or to the polluted sites in order to improve the performance of the on-going biological processes [16–20]. The microbial inoculums made with the mixtures of some specialized microorganisms (e.g. denitrifying bacteria) can be added to the conventional SWIS, and the addition point of bioaugmenting biomass can be also optimized to improve the TN removal efficiencies. The goal of this study is to determine if bioaugmentation can enhance nitrification/denitrification in SWIS.

The main purpose of the present work is as follows: (i) to determine the best scheme of microbial inoculums that is of optimal properties for the growth of ammonifying, nitrifying, and denitrifying biomass, and to prepare the soils by mixing microbial inoculums with nutrient substance, cinder, and original soil; (ii) to assess the phosphorus ($PO_4^{3-}$-P) and ammonia nitrogen ($NH_4^+$-N) absorption capacities of the soil; (iii) to determine whether microbial inoculums can act as a seed source to enhance biomass growth in the soil; (iv) to identify the contribution of the amended soil on the nitrogen removal at various hydraulic loads from 4 to 10 cm $day^{-1}$ in modified lab-scale SWIS by comparing with conventional lab-scale SWIS; and (v) to analyze the mechanisms of nitrogen removal in the two SWIS, as well as to establish effective parameters for evaluation.

## 2. Materials and methods

### 2.1. Preparation of microbial inoculums

The microbial inoculums were obtained from mixtures of culture, grass carbon, and natural zeolite (particle sizes below 0.17 mm) as shown in Table 1. The culture was isolated from the activated sludge collected from the sludge-dewatering unit of the Wen-chang Wastewater Treatment Plant located in Daowai, Harbin, China. The microbial diversity of the different groups of bacteria was done by employing selective agar plates under aseptic conditions by measuring CFU. Grass carbon consisted of partially decayed vegetal matter was used as initial substrate (35.6% organic carbon, 1.7% N, 0.3% P, 0.2% K) to provide nutrients for microorganisms growth. Zeolite is a well-known material for its ability to preferentially remove ammonium ions and it was used as a medium for maintaining a high amount of active biomass. Natural zeolite framework consists of symmetrically stacked alumina and silica tetrahedral, which results in an open and stable three dimensional honey comb structure with a negative charge [21,22]. Usually, natural zeolite can be used to remove ammonium ions from secondary effluent by selective ion-exchange, but it is rarely used as an additive for making microbial inoculums which could not only exert its higher selective ion-exchange capability for ammonium ion, but also save the chemical regeneration cost of the used zeolite.

The physiological–biochemical characteristics and activities of microorganisms were tested to obtain the optimal microbial inoculums scheme. It can be seen from Table 1 that the microbial inoculums (C + 50% GC + 50% Z) are the best scheme for growth of ammonifying, nitrifying, and denitrifying bacteria. Then the microbial inoculums are mixed with nutrient substance, cinder, and original soil for preparing the soil.

### 2.2. Preparation of soil

Cinder and original soil were ground and passed through a 100-mesh sieve (particle sizes below 0.17 mm) that are sufficiently fine to be mixed homogeneously. The components of zeolite, cinder, and original soil were analyzed using a Philips PW 4400 XR spectrometer (X-ray fluorescence-XRF, Japan) (Table 2). Nutrient substance solution contains the following nutrients per liter: 50 mg glucose, 5 mg peptone, 3.5 mg $(NH_4)_2SO_4$, 1.2 mg $KH_2PO_4$, 0.5 mg NaCl, 0.5 mg $NaHCO_3$, 1.4 mg $CaCl_2{\cdot}2H_2O$, 2 mg $MgSO_4{\cdot}7H_2O$, and trace elements 1 mL. Trace elements solution contains the following components per liter: 30 mg $FeCl_3{\cdot}6H_2O$, 1 mg $CuSO_4{\cdot}5H_2O$, 4 mg $MnCl_2{\cdot}4H_2O$, 2 mg $Na_2MoO_4{\cdot}2H_2O$, 3 mg $ZnSO_4{\cdot}7H_2O$, 5 mg $CoCl_2{\cdot}6H_2O$. The microbial inoculums, nutrient substance, cinder, and original soil were mixed to produce the soil, and the soil samples were kept in an incubator at 25 °C for 10 days.

**Table 2**
Components of zeolite, cinder, and original soil (wt.%)

| Components | Zeolite | Cinder | Original soil |
|---|---|---|---|
| $SiO_2$ | 54.50 | 61.65 | 56.31 |
| $Al_2O_3$ | 15.22 | 16.50 | 9.68 |
| CaO | 5.68 | 3.68 | 4.31 |
| $Fe_2O_3$ | 4.23 | 4.59 | 1.92 |
| MgO | 2.21 | 1.23 | 1.67 |
| $TiO_2$ | 0.92 | 0.65 | 1.37 |
| $K_2O$ | 0.83 | 2.32 | 0.98 |
| Others | <0.71 | <0.58 | <0.89 |
| Loss of ignition | <15.70 | <8.8 | <22.90 |

**Table 3**
Average count of bacteria in the soils (cfu $g^{-1}$)

| Scheme (wt.%) | AB[a] | NB[b] | DB[c] |
|---|---|---|---|
| 1# (2.5% MI[d] + 45% NS[e] + 2.5% C[f] + 50% OS[g]) | $1.04 \times 10^{11}$ | $1.40 \times 10^{7}$ | $1.40 \times 10^{12}$ |
| 2# (5% MI + 40% NS + 5% C + 50% OS) | $1.12 \times 10^{11}$ | $4.50 \times 10^{7}$ | $3.00 \times 10^{11}$ |
| 3# (7.5% MI + 35% NS + 7.5% C + 50% OS) | $3.16 \times 10^{10}$ | $1.50 \times 10^{7}$ | $3.00 \times 10^{11}$ |
| 4# (10% MI + 30% NS + 10% C + 50% OS) | $7.53 \times 10^{11}$ | $1.10 \times 10^{8}$ | $4.00 \times 10^{10}$ |
| 5# (12.5% MI + 25% NS + 12.5% C + 50% OS) | $1.02 \times 10^{12}$ | $1.40 \times 10^{8}$ | $1.40 \times 10^{12}$ |
| 6# (15% MI + 20% NS + 15% C + 50% OS) | $1.13 \times 10^{12}$ | $1.10 \times 10^{8}$ | $1.40 \times 10^{12}$ |
| 7# (17.5% MI + 15% NS + 17.5% C + 50% OS) | $8.40 \times 10^{11}$ | $1.10 \times 10^{8}$ | $1.10 \times 10^{12}$ |
| 8# (20% MI + 10% NS + 20% C + 50% OS) | $7.83 \times 10^{10}$ | $1.40 \times 10^{8}$ | $1.40 \times 10^{11}$ |
| 9# (22.5% MI + 5% NS + 22.5% C + 50% OS) | $3.17 \times 10^{10}$ | $2.00 \times 10^{7}$ | $1.40 \times 10^{11}$ |
| 10# (25% MI + 0% NS + 25% C + 50% OS) | $5.98 \times 10^{10}$ | $1.50 \times 10^{7}$ | $3.50 \times 10^{9}$ |
| 11# (100% OS) | $7.36 \times 10^{6}$ | $2.50 \times 10^{4}$ | $4.00 \times 10^{6}$ |

[a] AB: ammonifying bacteria.
[b] NB: nitrifying bacteria.
[c] DB: denitrifying bacteria.
[d] MI: microbial inoculums.
[e] NS: nutrient substance.
[f] C: cinder.
[g] OS: original soil.

The count of bacteria in each kind of soil samples was conducted for three times with interval of 3 days and the results were the average values of the three tests. The bacteria from the soils was sampled and quantified for CFU. The average count of ammonifying, nitrifying, and denitrifying bacteria in the soil was given in Table 3. It can be seen from Table 3 that soil 5# (12.5% MI + 25% NS + 12.5% C + 50% OS) is the best scheme for growth of ammonifying, nitrifying, and denitrifying bacteria. It should be noted that the average count of ammonifying bacteria in soil 6# (15% MI + 20% NS + 15% C + 50% OS) is greater than that in soil 5#. The reason for selecting soil 5# as the best scheme is that the incubation of ammonifying bacteria is easier than that of nitrifying bacteria and that the nitrification is a more important process for biological nitrogen removal.

The specific surface area (SSA) of the soils (1#–11#) was evaluated using the Brunauer–Emmett–Teller (BET) nitrogen adsorption technique at 77 K, using an automated manometric gas adsorption apparatus (Autosorb-1, Quantachrome Instruments, U.S.) and ultrahigh-purity gaseous nitrogen (99.9%). Soil samples were outgassed at 50 °C and equilibrated under vacuum for 12 h before measuring the $N_2$ adsorption isotherm. The details of the method and uncertainties associated with the measurement have been published elsewhere [23]. The adsorption test (Section 3.1) is performed with the soils to determine which one can be used in the lab-scale SWIS for treating wastewater.

### 2.3. Lab-scale SWIS

Two lab-scale subsurface wastewater infiltration systems with a diameter of 220 mm and a height of 800 mm were constructed in the lab as shown in Fig. 1. The sequential layers of soil in system 1 from bottom to top are gravel (100 mm), sand (60 mm), amended soil (500 mm), and original soil (40 mm). The sequential layers of soil in system 2 from bottom to top are gravel (100 mm), sand (60 mm), and original soil (540 mm).

The influent wastewater is filled into a network of distribution pipes at the top of infiltration system, and the treated effluent is collected through collecting pipes at the bottom of infiltration system. The wastewater is evenly distributed over the surface of the bed by a network of distribution pipes. The distribution pipes have a diameter of 8.2 mm and 3–5 mm holes placed in the bottom of the pipes approximately every 30–35 mm. The wastewater in the tank is intermittently aerated by air pump to improve oxygen transfer to wastewater, which can provide the necessary oxygen for the growth of microorganisms in the soil [24]. One-third of the effluent containing $NO_x^-$-N from the system is recirculated to the wastewater tank to enhance denitrification and to stabilize the treatment performance of the systems. The initial concentration of organic pollutant, pH, and operation conditions are identical in the two systems. Average hydraulic load is calculated by dividing the volume of water applied by the time from application until no water is visible above the surface of the soil [25].

### 2.4. Analysis methods for water quality

Wastewater used in the study was taken from one of the main sewers located in east of the campus (Heilongjiang Institute of Technology, Harbin, China), mainly carrying the wastewater of the dormitories, kitchen, plunge bath, etc. Characteristics of the unsettled and settled wastewater (after 1 h of settling) are given in Table 4.

Water samples were collected from the inlet and outlet pipes, and all the samples were stored at 4 °C for less than 24 h before the water quality was measured. According to Chinese EPA standard methods [26], potassium dichromate method was used for chemical oxygen demand (COD) analysis, colorimetric method was used for $NH_4^+$-N, $NO_3^-$-N, $NO_2^-$-N, TN and total phosphorus (TP) analysis, and gravimetric method was used for SS analysis. Bacteria in samples were quantified and isolated following Chinese EPA standard methods [25]. Nitrification/denitrification potential was also intermittently measured by batch test to indicate the activities of nitrifying/denitrifying bacteria in the soil [26].

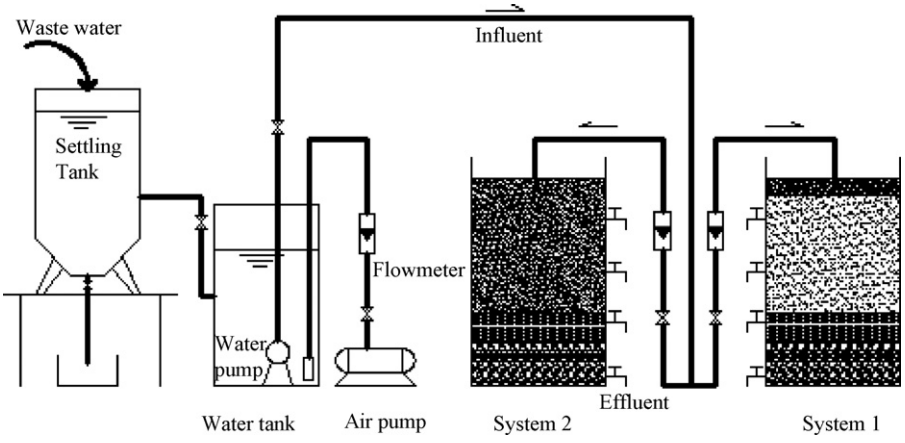


**Fig. 1.** Schematic diagram of the lab-scale systems.

**Table 4**
Characteristics of the wastewater

| Parameter | COD (mg $L^{-1}$) | TN (mg $L^{-1}$) | TP (mg $L^{-1}$) | $NH_4^+$-N (mg $L^{-1}$) | SS (mg $L^{-1}$) | $BOD_5$ (mg $L_{-1}$) | pH |
|---|---|---|---|---|---|---|---|
| Unsettled | 150–250 | 39–48 | 2.5–7.6 | 38–47 | 75–260 | 90–190 | 6.5–7.6 |
| Settled | 120–200 | 35–45 | 1.5–5.5 | 30–42 | 45–160 | 65–130 | 6.5–7.6 |

## 3. Results and discussion

### 3.1. Adsorption test of soil

Brunnaer–Emmett–Teller (BET) surface area analyses of the dried soils (1#–11#) were shown in Table 5. The phosphorus and ammonia nitrogen adsorption capacities of the soils were conducted by comparing the amended soil with that of the original soil (as shown in Table 5). Soil samples were dried at 105 °C until constant weight was achieved and then 5.0 g subsamples of the dried soil were placed into 250-mL conical flasks. The synthetic solution was prepared by dissolving 1.91 g $L^{-1}$ of $NH_4Cl$ and 3.68 g $L^{-1}$ of $K_2HPO_4{\cdot}3H_2O$ in distilled water. A given volume (100 or 50 mL) of solutions containing $NH_4^+$ and $PO_4^{3-}$ were separately added into each flask, and these flasks were shaken at 110 rpm at 25 °C for 24 h. The solution was filtered with a 0.45 μm membrane filter for analysis after adsorption equilibrium was achieved. The adsorption capacity of soil for N or P is calculated by the equation that follows: $q = (C_i - C_e)V/M$, where $q$ is the adsorption capacity of soil (mg $g^{-1}$), $C_i$ is the initial concentration of the target substance in the mixed soil solution (mg $L^{-1}$), $C_e$ is the equilibrium concentration of the target substance in the mixed soil solution (mg $L^{-1}$), $V$ is the volume of the solution (L) and $M$ is the weight of dried soil (g). The adsorption tests were conducted with each composition of soils for three times and the adsorption capacity results were the average values of the three tests.

The average data in Table 5 indicates that the soil 5# has a comparatively higher adsorption capacity for phosphorus ($PO_4^{3-}$-P) and ammonia nitrogen ($NH_4^+$-N) by comparing with the other soils (1#, 2#, 3#, 4#, and 11#), which is due to large surface area (5.16 $m^2$ $g^{-1}$) of the mixture 5#. Cinder are known to have a good adsorption capacity of both anion and cation so that the specific surface area and adsorption capacity of phosphorus ($PO_4^{3-}$-P) and ammonia nitrogen ($NH_4^+$-N) of the soils 6#–10# are improved by the increasing amount of cinder in the mixture. The soils 7#–10# are not good enough for the biomass growth (as shown in Table 3). The adsorption capacity of soil 5# (1.216 mg-P $g^{-1}$ and 0.495 mg-N $g^{-1}$) is approximate to that of soil 6# (1.224 mg-P $g^{-1}$ and 0.497 mg-N $g^{-1}$). Soil 5# is therefore selected as the best soil mixture to use in SWIS for treating wastewater because of its optimal physicochemical and biogenic stimulating properties.

### 3.2. Effect of hydraulic load on the nitrogen removal efficiencies

System 1 and system 2 are filled with the amended soil 5# and the original soil, respectively, to evaluate the impact of hydraulic load on the performance of the SWIS. Each cycle of the operation includes a flooding period of 12 h and a drying period of 12 h. Purification processes are established over time yielding comparatively stable purification efficiencies for key constituents (COD, TP, and TN) over 6 or 7 weeks at various hydraulic loads from 4 to 10 cm $day^{-1}$. During week 6 or week 7, there was likely continued build-up of biomass and establishment of bioprocesses important to purification as noted below. From that time on, greater than 80% of COD, 90% of TP and 75% of $NH_4^+$-N were removed from both of the systems. Pollutants removal in the columns correlated very well with total viable biomass in the biozone of the infiltrative layer, supporting the fact that the observed removal was based on biological processes and that the increased removal resulted in the increased soil biomass levels during the operation [27]. The effect of hydraulic load on the nitrogen removal efficiencies in the two systems was studied as shown in Figs. 2–9 and the average removal efficiencies of COD, TP, and SS at each hydraulic load were shown in Table 6. The behaviours observed in the two systems are different suggesting that the amended soil exerts a measurable effect on hydraulic and purification performance under the conditions studied, and further discussion of the potential reasons for this is given below.

It can be seen from Figs. 2 and 3 that system 1 and system 2 eliminate on average 17.65 and 11.89 mg $L^{-1}$ of nitrogen, accounting for 54.9 and 37.3% of TN, respectively. The mechanisms of nitrogen removal in SWIS include soil adsorption, ammonia volatilization and nitrification/denitrification. Volatilization losses of $NH_4^+$-N can be disregarded due to the amended soil and the original soil are acidic (pH about 6.1), and the adsorption of $NH_4^+$-N can be considered as the preparative process for nitrification due to most of the adsorbed $NH_4^+$-N is nitrified to $NO_x^-$-N by nitrifying bacteria. $NO_x^-$-N cannot be adsorbed by the soil due to its negative charge,

**Table 5**
Specific surface area (SSA–BET) measurements and adsorption tests of the soils (average)

| Scheme (wt.%) | SSA ($m^2$ $g^{-1}$) | $q_{avg}$ (mg-P $g^{-1}$) | $q_{avg}$ (mg-N $g^{-1}$) |
|---|---|---|---|
| 1# (2.5% MI[a] + 45% NS[b] + 2.5% C[c] + 50% OS[d]) | 4.16 | 0.914 | 0.162 |
| 2# (5% MI + 40% NS + 5% C + 50% OS) | 4.34 | 0.970 | 0.223 |
| 3# (7.5% MI + 35% NS + 7.5% C + 50% OS) | 4.58 | 0.996 | 0.306 |
| 4# (10% MI + 30% NS + 10% C + 50% OS) | 4.89 | 1.155 | 0.412 |
| 5# (12.5% MI + 25% NS + 12.5% C + 50% OS) | 5.16 | 1.216 | 0.495 |
| 6# (15% MI + 20% NS + 15% C + 50% OS) | 5.24 | 1.224 | 0.497 |
| 7# (17.5% MI + 15% NS + 17.5% C + 50% OS) | 5.32 | 1.226 | 0.501 |
| 8# (20% MI + 10% NS + 20% C + 50% OS) | 5.50 | 1.230 | 0.505 |
| 9# (22.5% MI + 5% NS + 22.5% C + 50% OS) | 5.71 | 1.229 | 0.509 |
| 10# (25% MI + 0% NS + 25% C + 50% OS) | 5.92 | 1.235 | 0.517 |
| 11# (100% OS) | 4.01 | 0.967 | 0.216 |

[a] MI: microbial inoculums.
[b] NS: nutrient substance.
[c] C: cinder.
[d] OS: original soil.

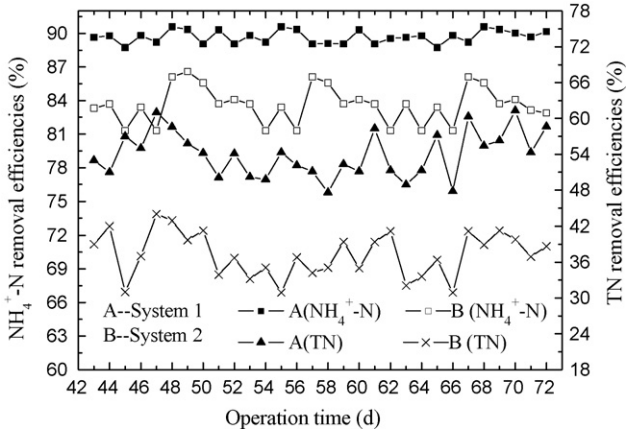


**Fig. 2.** $NH_4^+$-N and TN removal performances of the two systems at 4 cm day$^{-1}$.

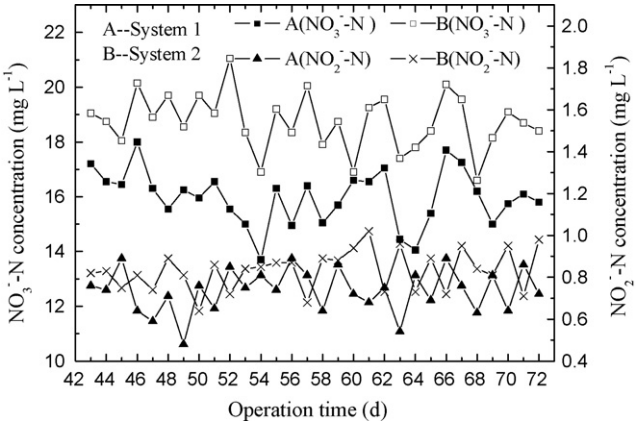


**Fig. 3.** Effluent concentration of $NO_3^-$-N and $NO_2^-$-N of the two systems at 4 cm day$^{-1}$.

so denitrification is the main way for $NO_x^-$-N removal in SWIS, and the removed nitrogen is in the form of $N_2$ or $N_2O$ [13].

It can be seen from Figs. 4 and 5 that nitrification efficiencies and TN removal efficiencies in system 1 are higher than those in system 2 at 6 cm day$^{-1}$ due to the higher microbial activities of ammonifying, nitrifying, and denitrifying bacteria in system 1. During the soil treatment of wastewater in the two systems, $NH_4^+$-N is rapidly absorbed onto the soil with negative charges and is subsequently nitrified to $NO_x^-$-N in the depth of 6–20 cm,

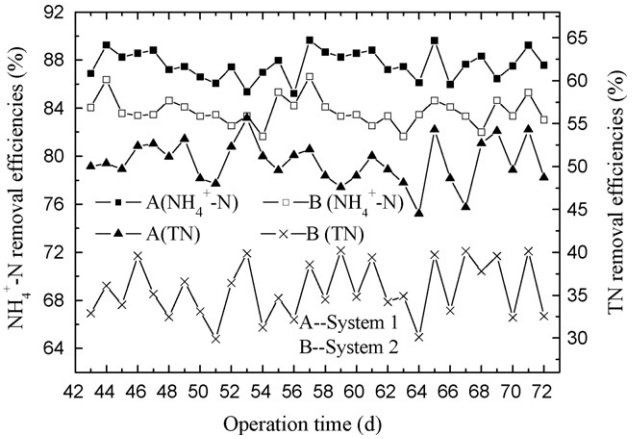


**Fig. 4.** $NH_4^+$-N and TN removal performances of the two systems at 6 cm day$^{-1}$.

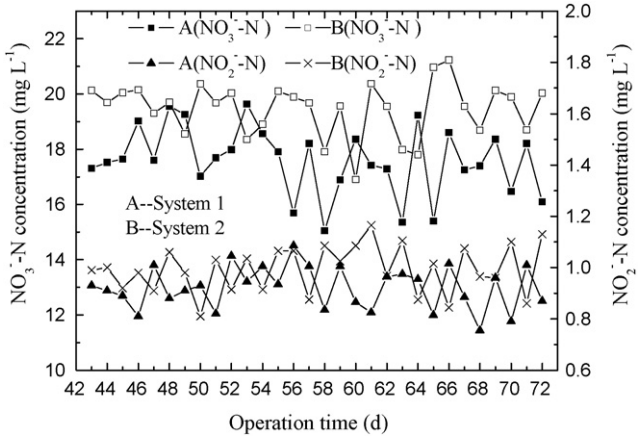


**Fig. 5.** Effluent concentration of $NO_3^-$-N and $NO_2^-$-N of the two systems at 6 cm day$^{-1}$.

and most $NO_x^-$-N is denitrified by denitrifying bacteria under anoxic condition in the depth of 15–50 cm. It is therefore concluded that nitrification/denitrification are the main and efficient way for nitrogen removal in SWIS, and the addition of bioaugmenting soil biomass (microbial inoculums) can enhance the nitrogen removal efficiency. Since purification with respect to some constituents such as $NH_4^+$-N, $NO_2^-$-N, and $NO_3^-$-N continue to improve until stabilizing at week 7 or later, it is speculated that there is a continued wastewater-induced clogging and a further establishment of purification processes within an operative infiltration zone, rather than an increased expansion of the infiltration area being utilized. Further detailed analysis of the hydraulic load and volumetric utilization efficiency data, and their effect on the purification performance is in progress.

It can be seen from Fig. 6 that the average removal efficiencies of TN in system 1 and system 2 are 48.0 and 34.1% at 8 cm day$^{-1}$, respectively. Nitrification was well established in the two systems and the higher denitrification rate was obtained in system 1 by week 7 (as shown in Figs. 6 and 7). TN removal efficiencies was initially low, possibly due to the main way for nitrogen removal is sorption and bio-uptake before week 5, but after the week 6 it stabilized at a TN removal of 36–55% in system 1 and a TN removal of 25–37% in system 2. The results observed in the two systems are different due to the biomass in system 2 is less than that of system 1. It is therefore speculated that the better performance of TN removal in system 1 at 8 cm day$^{-1}$ is due to a sufficient degree of soil clog-

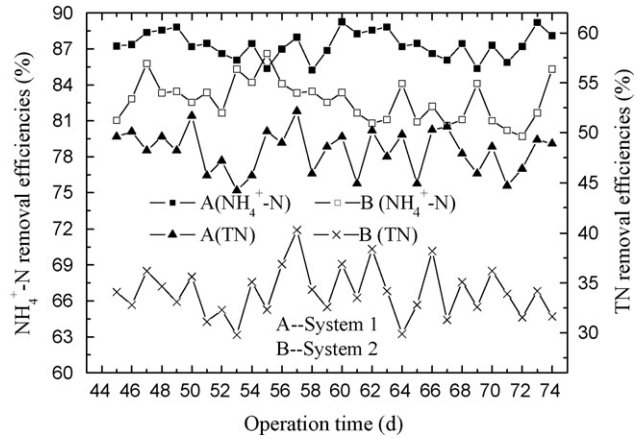


**Fig. 6.** $NH_4^+$-N and TN removal performances of the two systems at 8 cm day$^{-1}$.

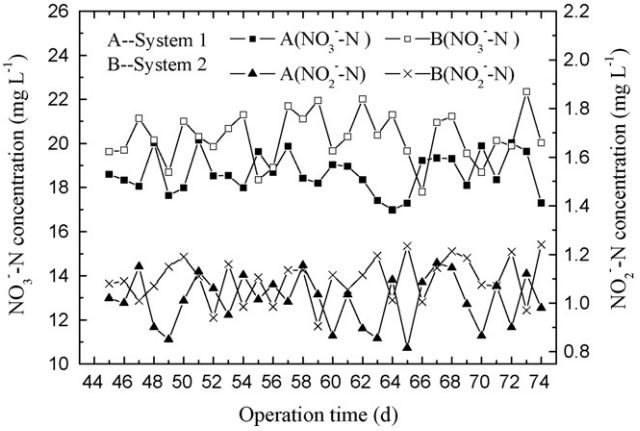


**Fig. 7.** Effluent concentration of $NO_3^-$-N and $NO_2^-$-N of the two systems at 8 cm day$^{-1}$.

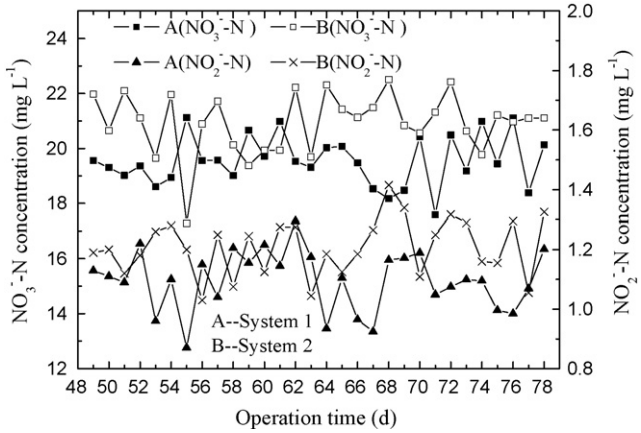


**Fig. 9.** Effluent concentration of $NO_3^-$-N and $NO_2^-$-N of the two systems at 10 cm day$^{-1}$.

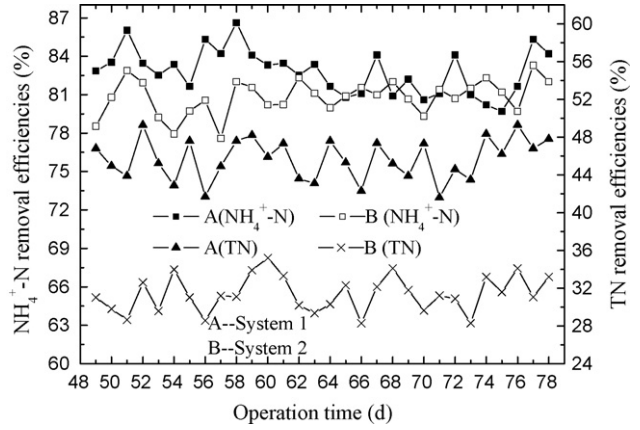


**Fig. 8.** $NH_4^+$-N and TN removal performances of the two systems at 10 cm day$^{-1}$.

ging genesis coupled with bioprocesses that effectively purified the wastewater influent given the adequate retention times and high volumetric utilization of the amended soil [2,28].

In both of the systems, the removal efficiencies of $NH_4^+$-N and TN and the rate of converting $NH_4^+$-N to $NO_x^-$-N at 10 cm day$^{-1}$ decrease because the influent has shorter residence time in the soil (as shown in Figs. 8 and 9). The removal efficiencies of TN in system 1 and system 2 are 44.7 and 29.6%, respectively. It is to be expected that higher hydraulic load lead to faster transport of pollutants into deeper soil depths, resulting in lower purification efficiency and higher pollutants concentration treated by lower soil biomass in deeper depths [27]. Some microbes were transported to the regions below the surface of the soil due to the hydraulic overload, causing a weaker nitrification/denitrification process in the two systems. During soil treatment of wastewater, organic nitrogen is easily converted to $NH_4^+$-N by ammonifying bacteria in SWIS and then the $NH_4^+$-N can be adsorbed onto the soil because the zeta potential of the soil particles are negative, and the adsorption capacity of the soil can be quickly recovered to the initial state by nitrifying $NH_4^+$-N to $NO_x^-$-N, which is subsequently denitrified to $N_2$ or $N_2O$ by the denitrifying biomass under the anoxic condition. So, this is the circulation mode of nitrogen removal in SWIS.

It can be deduced from the results as shown in Figs. 2–9 that the interaction of hydraulic and purification processes during soil treatment of wastewater changes the microbial activities, the retention times, and the volumetric utilization efficiency (VUE) of the soil beneath the infiltrative surface. These interactions can dramatically affect treatment efficiencies as they determine the transport and fate of wastewater constituents and the extent of reactions that occur in the aqueous or adsorptive phases during infiltration of the influent. The results of effluent analyses for N species and nitrification rates in the two systems suggest that nitrification mainly occurs within the depth of 6–20 cm and denitrification mainly occurs within the depth of 15–50 cm below the infiltrative surface.

**Table 6**
Concentration and removal efficiencies of key constituents (average)

| Parameter | 4 cm day$^{-1}$ | 6 cm day$^{-1}$ | 8 cm day$^{-1}$ | 10 cm day$^{-1}$ |
|---|---|---|---|---|
| COD (mg L$^{-1}$) | 20.3 | 25.2 | 31.5 | 37.7 |
| COD (%) | 88.6 | 86.1 | 83.8 | 81.2 |
| TP (mg L$^{-1}$) | 0.26 | 0.32 | 0.29 | 0.41 |
| TP (%) | 94.3 | 93.2 | 93.4 | 91.1 |
| SS (mg L$^{-1}$) | 20.1 | 25.6 | 27.9 | 29.9 |
| SS (%) | 85.7 | 83.1 | 80.6 | 79.3 |

## 4. Conclusions

This study finds that the bioaugmentation and hydraulic load have a significant influence on enhanced biological nitrogen removal in lab-scale SWIS. The most important conclusions are as follows: (1) The microbial inoculums (wt.%) made of culture, 50% grass carbon, and 50% zeolite are the best scheme for growth of ammonifying, nitrifying, and denitrifying biomass. (2) Due to its optimal physicochemical and biogenic stimulating properties, the soil (wt.%) made of 12.5% microbial inoculums, 25% nutrient substance, 12.5% cinder, and 50% original soil is best to use in SWIS to treat wastewater. (3) Purification processes in modified SWIS were gradually established over 6 weeks or longer, after which there were high removal efficiencies for organic matter (>80%) and $NH_4^+$-N (>75%); the microbial activities, impact load, and nitrogen removal efficiencies in modified SWIS are improved by filling with the amended soil, and the approximate sequence of TN removal efficiencies in modified SWIS is 4 cm day$^{-1}$ > 6 cm day$^{-1}$ > 8 cm day$^{-1}$ > 10 cm day$^{-1}$. The nitrogen removal efficiencies is improved by using the amended soil, emphasizing the fact that soil properties are of high importance in determining the microbial activity and nitrogen removal efficiency.

Only limited removal of TN (<61%) was obtained in the two systems due to the carbon substrate was deficient at the denitrification phase. Practically, it is crucial to determine the carbon substrate feeding limits at the beginning of denitrification phase

and to change the intervals between feeding and resting periods. The study of this point is under progress in our laboratory.

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