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How does the operation time affect the performance and metabolomics of the subsurface wastewater infiltration bed?

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ABSTRACT

To reveal the effect of operation time on the wastewater treatment performance and microbial metabolites of subsurface wastewater infiltration systems (SWISs), the removal of typical pollutants (COD, NH_4^+–N, NO_3^-–N and TP) (COD – chemical oxygen demand; TP – total phosphorus) was evaluated and ultra-performance liquid chromatography-mass spectrometry was used to qualitatively identify the microbial metabolites under variable operation stages. Redundancy analysis (RDA) was carried out to analyze the relationship between the metabolites and influencing factors. The results showed that the pollutant removal performance of SWIS was highly operation dependent. During the early operation time (stage I), lower pollutant removal efficiency (COD: 75.2% ± 2.3%, NH_4^+–N: 28.5% ± 1.7% and NO_3^-–N: 22.7% ± 1.5%) was obtained. As the operation increased to stage II and III, the removal efficiency of pollutants was significantly improved (stage II: COD: 82.2% ± 3.5%, NH_4^+–N: 63.5% ± 2.6% and NO_3^-–N: 60.7% ± 2.1%; stage III: COD: 85.6% ± 3.9%, NH_4^+–N: 75.2% ± 2.7% and NO_3^-–N: 71.6% ± 2.0%). Meanwhile, there were more single peaks (represent the relative content of metabolites) collected in 40–60 min and the higher resolution was obtained during stages II and III. The RDA results further confirmed that the appearance of metabolites was negatively correlated with the concentration of COD, NH_4^+–N and NO_3^-–N. Since the removal of TP is mainly dominated by physical and chemical processes, its relationship with microbial metabolites was insignificant. The results indicated that it is feasible to qualitatively indicate the pollutant removal efficiency of SWIS at different stages of operation by identifying the representative microbial metabolites.

Keywords: Subsurface wastewater infiltration system; Operation time; Metabolomics; Pollutant removal; Redundant analysis

1. Introduction

In the context of extensive academic research focusing on water shortages and wastewater reuse, subsurface wastewater infiltration system (SWIS), an ecological treatment technology integrating physical, chemical, and biological reactions, is increasingly becoming a research hotspot [1–5]. Unlike aquifer recharge systems, trickling filters or treatment wetlands, SWIS is constructed below the ground and there is a U-shape groove under the distribution pipe, where the pollutants can precipitate and hydrolyze (Fig. 1). Then the wastewater passes through the infiltration layer under the action of capillary force and gravity, and is collected through the bottom collection pipe. Partially attributed to the purification ability of soil, SWIS has the advantages of low construction and operation costs, no aeration, low energy consumption, etc [6,7]. SWIS has been widely used to treat domestic wastewater in rural areas without perfect sewer system.

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The performance of SWIS is closely related to the activities of soil microorganisms [8]. The functions of these microorganisms include three ways: (1) they purify wastewater through the conversion of nutrients (especially nitrogen) resulting in microbial protein, (2) microbial protein consequently serve as a source of nutrition for protozoa in soil and (3) they suppress the growth of pathogens through the competition to ensure water quality. With the extension of running time, the growth of microorganisms goes through four stages [3,7]: lag phase, logarithmic phase, stable phase and decay phase. In the early stage of SWIS operation, microorganisms need to adapt to the new soil environment for a period of time, at which time the microbial metabolism is less strong. In the logarithmic growth period, the microbial cell activity is the strongest. The bacteria reproduce regularly at a high speed, and the generation time is the shortest. As the running time extends, the growth of microorganisms becomes stable, the reproduction rate and mortality rate tend to be balanced and the metabolic activity begins to decrease. After the stable period, the reproductive capacity of the microorganism and metabolic activity decline significantly, and products such as amino acids, enzymes, and antibiotics are released. It can be seen that the metabolic activities and products of microorganisms are operation time-dependent. Microbial metabolomics mainly studies all low-molecular-weight metabolites inside and outside the cell at a certain time in the cell growth or growth cycle. It is an important expansion of the research on the microbial distribution and functional gene diversity. Previous studies, however, mostly focused on the treatment performance of wastewater and several key groups of microorganisms and enzymes at a certain sampling time [4,7,9–11]. Although at different operation stages, significant differences in the removal efficiency of chemical oxygen demand (COD), nitrogen and phosphorus of SWIS have been reported [2], the microbial population and functional gene abundances have also been revealed [7], few reports presented in-depth discussion of microbial metabolomics. So far, only the changes of microbial metabolomics with loading rates of SWIS have been reported [12], while the relationship between metabolomics and operation time has not been revealed yet. Moreover, since extending the service life of SWIS is a major concern, it is important to clarify how microbial metabolomics and pollutant removal performance change over time.

2. Materials and methods

2.1. System description

As is shown in Fig. 2, three sets of SWISs were run in parallel. The size of the column was 180 cm × 30 cm (height × diameter). There are six groups of sampling holes at 25, 40, 65, 95, 145 and 170 cm. The wastewater was pumped through a peristaltic pump and dispersed through a "cross" perforated distribution pipe. The filling materials were mixed matrix, fine sand and gravel from top to bottom. Among them, the mixed matrix consisted of 10% sand, 25% slag and 65% farmland soil in volume ratio. Its main characteristics were: pH 6.8 ± 0.3, organic matter content 3.9% ± 1.1%, porosity 52.5% ± 0.9% and permeability (8.9 ± 0.5) × 10^{-5} cm/s. Vertically, 5 ORP (oxidation-reduction potential) probes were equipped at 25, 40, 65, 95 and 145 cm, respectively.

2.2. Wastewater

The influent of SWISs was combined wastewater taken from the outlet of the hydrolysis acidification tank. The quality was analyzed according to the American Public Health Association [13]: COD 250 ± 20 mg/L, the ratio of biochemical oxygen demand (BOD)/COD 0.55 ± 0.08, ammonia nitrogen (NH_4^+–N) 15 ± 4 mg/L, nitrate (NO_3^-–N) 2 ± 1 mg/L, total nitrogen 22.5 ± 6 mg/L and total phosphorus (TP) 6 ± 1 mg/L.

2.3. Experimental operation

During the experimental period, the temperature was 22°C ± 2°C and the hydraulic loading rate was controlled to be 0.14 m^3/m^2 d. The SWIS simulators were operated in a wet-dry alternation manner. In other words, each SWIS was continuously fed with water for 12 h (8 a.m.–8 p.m.), and then "rested" for 12 h (8 p.m.–8 a.m. the next day). To evaluate the effect of operation time on the performance and metabolomics of SWIS, the operation was divided

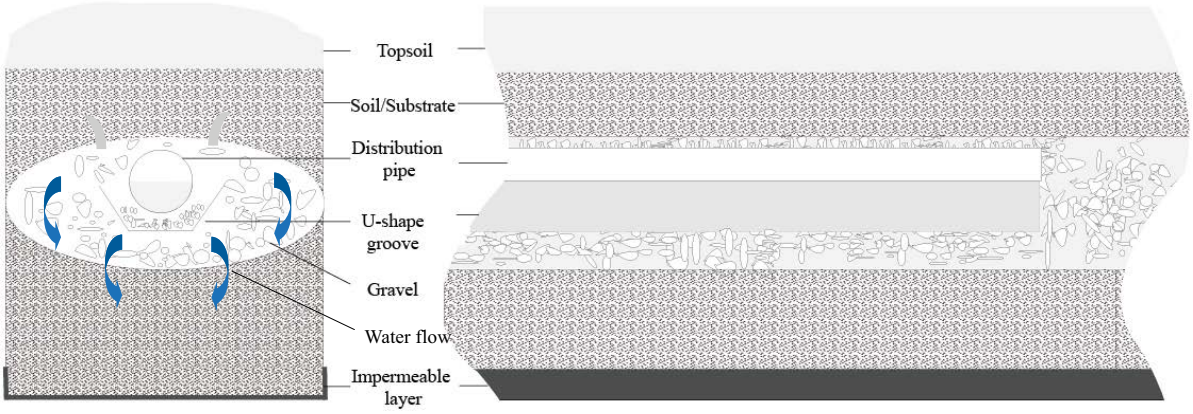

Fig. 1. Schematic diagram of subsurface wastewater infiltration system.

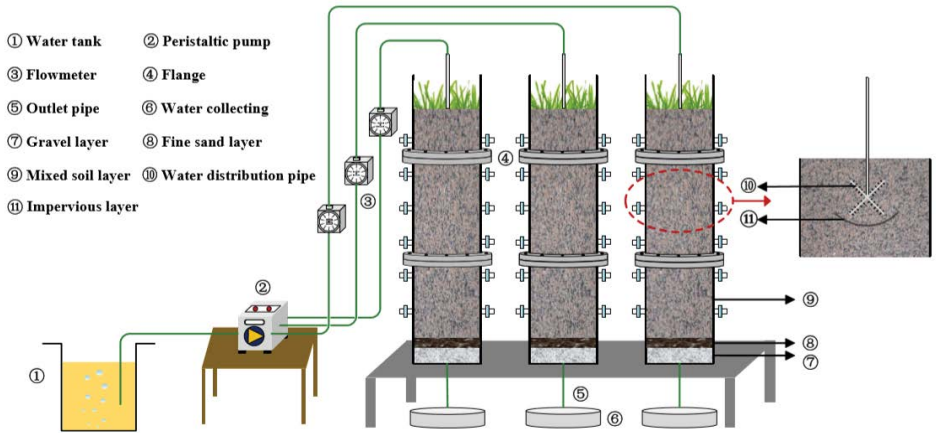

Fig. 2. SWIS simulation devices.

into three stages: stage I (0–10 d), stage II (10–20 d) and stage III (20–30 d).

2.4. Sample preparation

Soil samples were collected from the sampling points of each column at 9 a.m. every 2 d. At each sampling time, 2 g soil samples were taken from each sampling point. After blended, the samples were quickly inactivated with liquid nitrogen and extracted three times with 10 mL pure methanol. The extract was combined and dried under a vacuum. Subsequently, the supernatant was absorbed in 1 mL of methanol and centrifuged at 13,000 rpm for 10 min. It was then filled into a 1.5 ml sampling vial and stored at 4°C for testing. While collecting the soil samples, influent and effluent samples were sampled and analyzed [13].

2.5. Analytical method of metabolites

To qualitatively identify the metabolites, soil samples were determined by Agilent 1260 Infinity-6420 LC-triple quadrupole mass spectrometry (United States). The measurement conditions were: the mobile phase consists of phase A (formic acid: water = 1:1,000) and B (acetonitrile), flow rate 0.2 mL/min, column temperature 30°C gradient elution [10,12]. The conditions were: 0 min, 15% B; 10 min, 65% B; 15 min, 80% B; 30 min, 95% B; 38 min, 99% B; 55 min, 99% B; 56 min, 15% B; 69 min, 15% B. The mass spectrum used the ESI ion source and the number of acquisitions was in positive ion mode. The ion collection range was 50–1,800 m/z. The software tool MZ Convert (ProteoWizard, available at proteowizard.sourceforge) converted the initial data file into m/z format and performed peak integration and peak alignment processing. Multivariate statistical analysis of data was performed by SIMCA-P software (version 14.1, UMETRICS, Ume, Sweden).

2.6. Data processing

The obtained data file was subjected to AMDIS analysis in a simple model, and then the total ion chromatogram of the metabolites was identified and peak matched. By identifying the characteristic values of accurate mass-to-charge ratio (m/z), strength, and detection time, it was compared with METLIN (https://metlin.scripps.edu) metabolic database.

2.7. Quality control/assurance

To ensure the accuracy of the data, the Agilent 1260 Infinity-6420 LC-triple quadrupole mass spectrometry (United States) was regularly corrected before sampling. Each sample was provided with three replicates. While analyzing, blank matrix samples (operation time = 0) were prepared and the same test steps were repeated. The sample test results were deducted by the background value. One sample out of every five samples was randomly selected for repeated testing. If the deviation of the repeated testing was within 5%, it proves the effectiveness of the results.

3. Results

3.1. Pollutant removal performance

Fig. 3 shows the concentrations of COD, NH_4^+–N, NO_3^-–N and TP in effluent and removal efficiency during the whole experimental periods. The removals of COD, NH_4^+–N and NO_3^-–N in stage I were significantly lower than those in stage II and III. For COD, NH_4^+–N and NO_3^-–N, the removal efficiency increased from 75.2% ± 2.3%, 28.5% ± 1.7% and 22.7% ± 1.5% (stage I) to 82.2% ± 3.5%, 63.5% ± 2.6%, 60.7% ± 2.1% (stage II) and 85.6% ± 3.9%, 75.2% ± 2.7% and 71.6% ± 2.0% (stage III). The effluent quality of stage III met the Class I (A) requirement of the Cities Sewage Treatment Plant Pollutant Discharge Standard in China (GB18918–2016). In comparison, the TP removal efficiency didn't change significantly in the three stages, which were 85.0% ± 1.1% (stage I), 85.1% ± 1.6% (stage II) and 85.2% ± 1.3% (stage III), respectively.

3.2. Ultra-performance liquid chromatography-mass spectrometry analysis

Using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), the total ion chromatograms

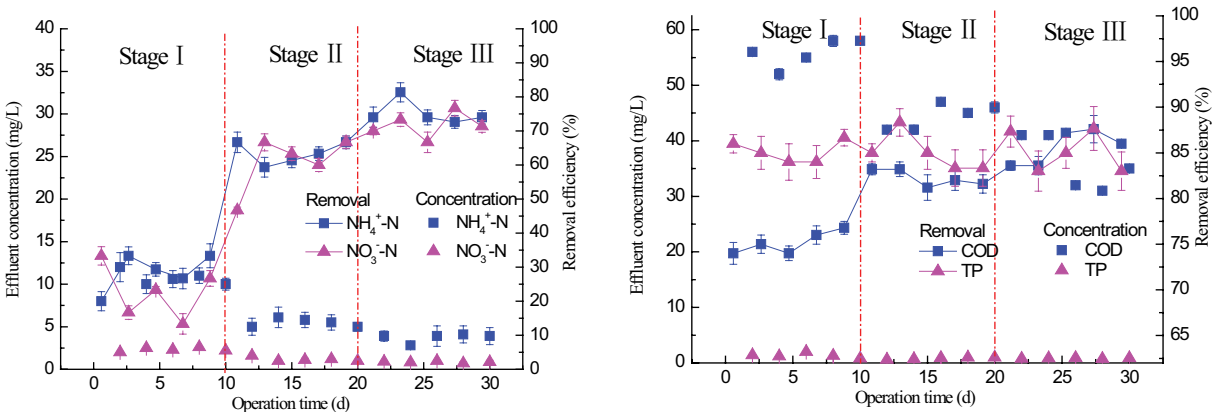

Fig. 3. Removal efficiency and concentration of pollutants at different stages.

of soil samples obtained at different operation time (0, stage I, stage II and stage III) is shown in Fig. 4. Positive and negative ion modes were simultaneously carried out to analyze the soil extracts. After removing duplicate samples, a total of 1,130 peaks, 180 clusters, and 698 singlet peaks were detected at stage II and III UPLC-MS analysis showed that the soil extracts yielded complex spectra dominated by spectral features of lipids, terpenes and sugars. More single peaks and higher resolution was obtained in stage II and III.

3.3. Screening and identification of differential metabolites

The screening and identification of metabolites include the following three steps: (1) the variable importance in the projection (VIP) score trend was obtained by partial least squares-discriminant analysis (PLS-DA), and (2) significant differences in different groups metabolites was identified by ANOVA analysis (analysis of variance, *t*-test, $P < 0.05$) and (3) METLIN database was used to match the metabolites [14]. In this way, a total of 198 metabolites were screened out. Then, *P* values of the metabolites were calculated by ANOVA and the 17 metabolites with $P < 0.05$ were matched using the METLIN metabolomics database. The identification results are shown in Table 1.

3.4. ORP variations

The ORP at different depths was tested (Fig. 5). It can be seen that the ORP at 25, 40, 95 and 145 cm of the bed did

Table 1
Qualitative analysis of soil microbial metabolites

N	RH(s)	m/z	METLIN ID	Metabolites	Formula	ppm	Adduct	VIP
1	1,362.2	355.0691	443756	L-Glutamic acid	$C_{15}H_{12}ClNO_6$	1	$[M+NH_4]+$	2.0923
2	2,070.5	413.2662	263643	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	1	[M+Na]+	2.02617
3	1,463.8	371.1941	978039	N-Octanoyl-L-phenylalanylglycine	$C_{19}H_{28}N_2O_4$	1	[M+Na]+	1.94596
4	1,198.4	579.2904	872356	Chloride	$C_{24}H_{40}ClN_5O_8$	4	$[M+NH_4]+$	1.9446
5	1,357.4	429.0904	806410	Hexopyranosiduronate	$C_{18}H_{14}NNaO_9$	6	$[M+NH_4]+$	1.89573
6	1,616	371.1026	509630	Didemethylasterriquinone D	$C_{22}H_{14}N_2O_4$	1	[M+H]+	1.85134
7	1,367.8	297.0821	693185	N-(Dimethylphosphorothioyl)-L-tryptophan	$C_{13}H_{17}N_2O_2PS$	0	[M+H]+	1.82845
8	1,199.7	319.2227	403895	Caproyl glutamic acid	$C_{15}H_{27}NO_5$	3	$[M+NH_4]+$	1.82619
9	1,615.4	355.0691	443756	N-(3-chloro-1,4-dihydro-1,4-dioxo-2-naphthalenyl)	$C_{15}H_{12}ClNO_6$	0	$[M+NH_4]+$	1.78684
10	1,292.2	429.3088	761640	Oleoyl glutamic acid	$C_{24}H_{42}N_2O_3$	25	[M+Na]+	1.72538
11	1,607.1	519.1407	501954	Phenylthiodiphenylpenicillin	$C_{28}H_{26}N_2O_4S_2$	7	[M+H]+	1.71393
12	1,560.1	457.3401	46716	PE(O-16:0/0:0)	$C_{21}H_{46}NO_6P$	21	$[M+NH_4]+$	1.64673
13	1,896.3	355.0723	403129	Alpha-2,3-Methylenepenicillin G	$C_{16}H_{16}N_2O_4S$	8	[M+Na]+	1.64513
14	1,468.4	683.5398	64003	2-Octaprenyl-6-methoxy-1, 4-benzoquinone	$C_{47}H_{70}O_3$	1	[M+H]+	1.6386
15	1,362.1	501.0795	63903	Coenzyme M-HTP heterodisulfide	$C_{13}H_{26}NO_{10}PS_3$	13	$[M+NH_4]+$	1.60194
16	970.9	293.2071	45741	Serratamic acid	$C_{13}H_{25}NO_5$	2	$[M+NH_4]+$	1.55569
17	76.3	444.068	452086	Tubercidin-5′-diphosphate	$C_{11}H_{16}N_4O_{10}P_2$	3	$[M+NH_4]+$	1.51336

not change significantly, which remained at 660 ± 11 mV, 790 ± 26 mV, –220 ± 17 mV and –260 ± 9 mV, respectively. While the ORP at 65 cm periodically changed significantly, increased from –100 ± 9 mV during the wetting period to 100 ± 4 mV during the drying period [12]. During the drying period, the water content of the bed decreases, O_2 diffuses through the bed, and the dissolved oxygen content increases, which can better stimulate the ability of microorganisms to aerobically oxidize and degrade organic matter.

4. Discussion

4.1. Pollutant removal pathway of SWIS

Take glucose as an example, in SWISs, organic matters (termed as COD and BOD) were firstly adsorbed by the soil particles and then be degraded by microorganisms, especially aerobic microorganisms through fermentation and respiration [15], as shown in formula (1–3).

$$C_6H_{12}O_6 \rightarrow 2C_3H_4O_3 + 4[H] + \text{Energy} \quad (1)$$

$$C_3H_4O_3 + 3H_2O \xrightarrow{\text{Aerobic}} 3CO_2 + 10[H] + \text{Energy} \quad (2)$$

$$4[H] + O_2 \rightarrow 2H_2O + \text{Energy} \quad (3)$$

As shown in Fig. 6, nitrogen in domestic sewage mainly exists in the form of organic nitrogen, while the nitrogen required for soil microbial activities and plant growth mainly comes from inorganic nitrogen, so most of the organic nitrogen is converted into inorganic nitrogen such as NH_4^+–N through microbial mineralization (①); Then, autotrophic nitrification and heterotrophic nitrification microorganisms oxidize NH_4^+–N or organic nitrogen into NO_2^-–N and NO_3^-–N (② and ③); Finally, under anaerobic or micro-oxygen supply conditions, the microorganisms use NO_2^-–N and NO_3^-–N as the electron acceptor and reduce them to N_2O and N_2 (④ and ⑤) [2,16,17].

The degradation of organic carbon and nitrogen is closely related to the metabolic process of microorganisms. SWIS has a strong ability to degrade organic matter. The soil has a huge specific surface area. As the operation time

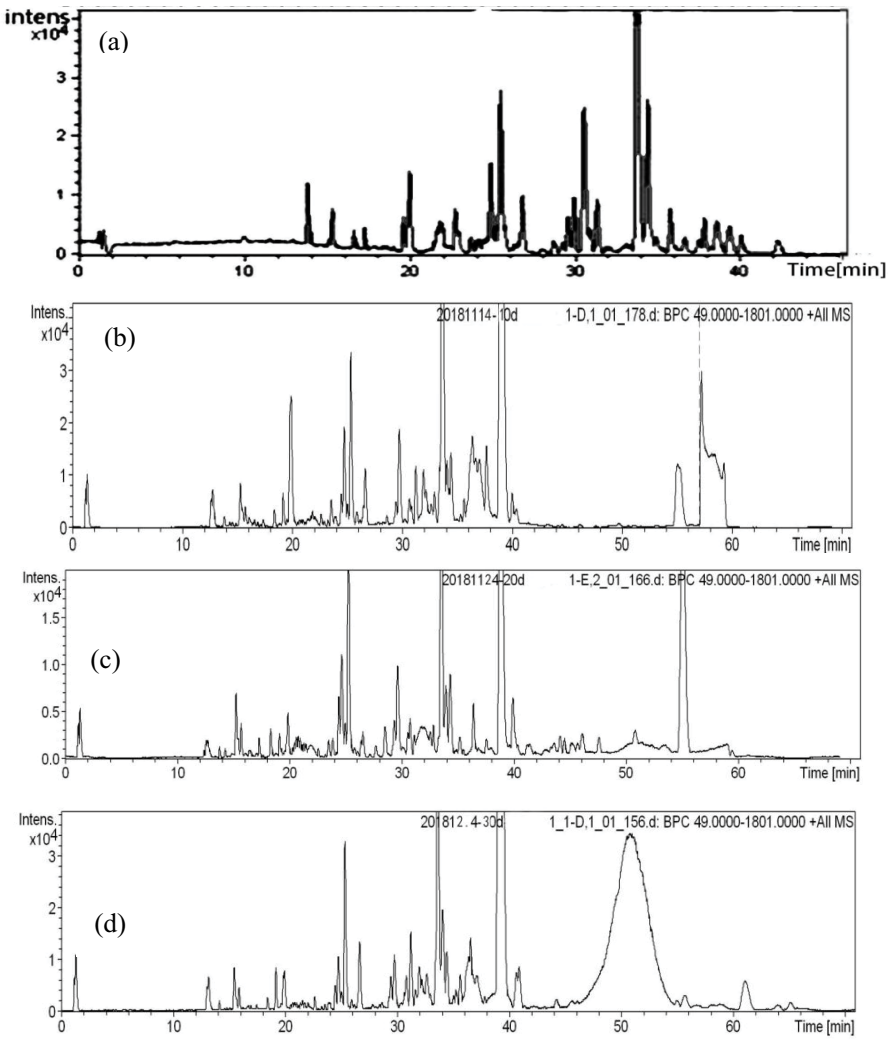

Fig. 4. Total ion chromatogram at different operation stages (a) operation time = 0, (b) stage I, (c) stage II, (d) stage III.

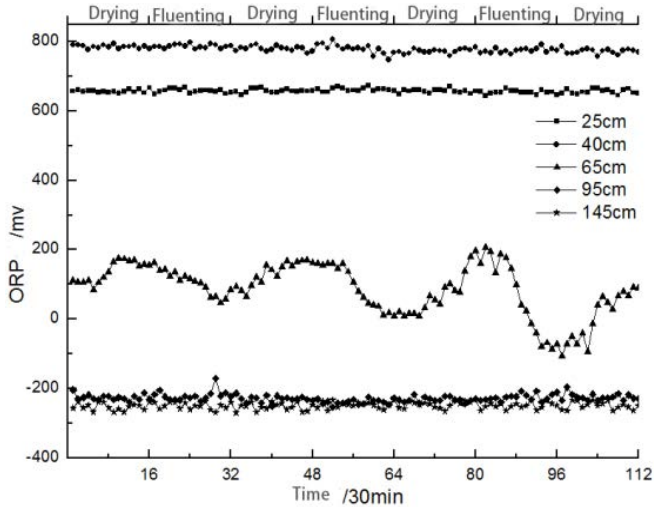

Fig. 5. ORP variations with time.

is extended, a layer of biofilm is gradually formed on the surface of soil particles [15–17]. When the wastewater flows through the surface of the particles, insoluble organic matter is precipitated, filtered, adsorbed and then used by tiny organisms [3,15]. Soluble organic matter is decomposed and removed through the adsorption of biofilms and the metabolism of microorganisms [4,17]. Since the operation time is associated with an increase in the microbial population, in stage I, the degradation rate of COD, NH_4^+–N and NO_3^-–N was significantly lower than that in stage II and III. Correspondingly, it can be seen from Fig. 4 that there were more metabolites in stages II and III. On the contrary, physical sedimentation and chemical adsorption are reported to be the dominant ways for phosphorus removal in the SWIS process [18]. During the 30 d period of this study, the TP removal efficiency did not change significantly, which further confirmed the insignificant association between TP removal and microbial metabolism. Since the adsorption capacity of the substrate is finite, it can be speculated that the ability to remove phosphorus will decrease over a longer operation time [18–20]. Previous reports showed a dramatic decrease of 24.0% of TP removal for a 7-year-old SWIS, which means that it has lower phosphorus adsorption and storage ability over a long operation time [2].

4.2. Correlation between metabolites and influencing factors

The essence of redundancy analysis (RDA) is the combination of regression analysis and principal component analysis, coupling multiple environmental factors and metabolomics data. RDA displays the relationship between metabolomics with environmental factors in a sorted axis manner [8,10,12]. According to the results of RDA, the interpretation rate of horizontal axis was 75.14%, and the ordinate RDA interpretation rate was 22.53%, which totally explained 97.67% of the variance (Fig. 7), revealing that the correlation coefficients between metabolites and environmental factors (NH_4^+, COD, ORP, NO_3^- and NO_2^-) were high. The angle between the metabolites and environmental factors suggested that NH_4^+, COD, ORP, NO_3^- had significantly negative correlation with most metabolites, while NO_2^- had positive correlation. Take Coenzyme M as an example (Number 15 in the microbial metabolites list of Table 1). It is a specific coenzyme of methanogens and acts as a carrier for methyl groups in the final reaction step of methane formation. Therefore, as shown in Fig. 7, there is a significant correlation between Coenzyme M and COD. It is revealed from the angle between the environmental factors that there was a positive correlation between NH_4^+, NO_3^-, ORP, COD and NO_2^-. This means that the degradation processes of pollutants are related to each other. For example, NH_4^+ will be sequentially oxidized into NO_2^- and NO_3^- under the action of ammonia monooxygenase, hydroxylamine oxidoreductase and nitrite oxidase (Fig. 6), and the concentration of substrate (NH_4^+) is the main influencing factors of nitrification (positive correlation). While in the denitrification process, nitrate reductase uses NO_3^- as the electron acceptor to complete respiration and obtain energy, reducing NO_3^- to NO_2^-. The concentration of the substrate (NO_3^-) is the main influencing factor (positive correlation) of this denitrification reaction. As the denitrification process is anaerobic, the refractory organic matter can be anaerobic decomposed with the assistance of Coenzyme M. In the RDA results, the longer the blue lines, the stronger the

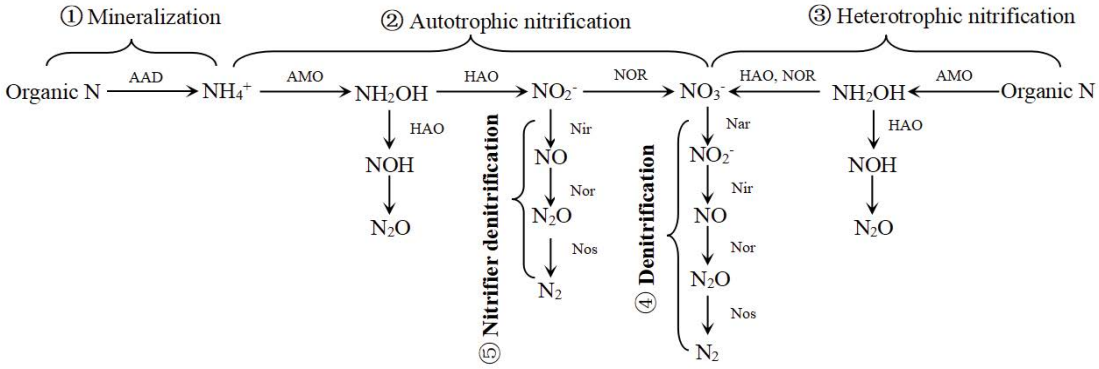

Fig. 6. Nitrogen removal pathway in the SWIS process (AAD: amino decarboxylase; AMO: ammonia monooxygenase; HAO: hydroxylamine oxidoreductase; NOR: nitrite oxidase; Nar: nitrate reductase; NiR: nitrite reductase; Nor: nitric oxide reductase; Nos: nitrous oxide reductase).

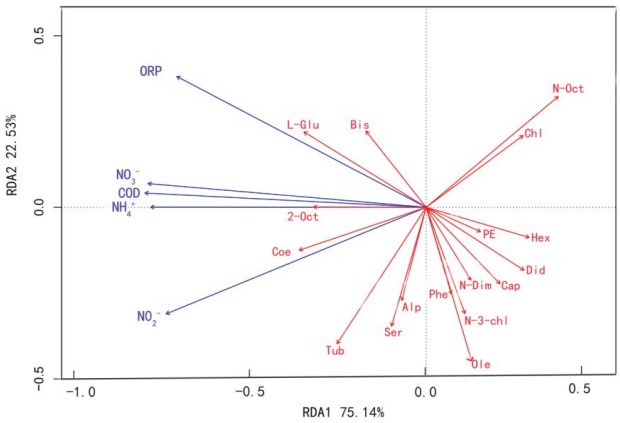

Fig. 7. RDA results of metabolites (Red arrows) and influencing factors (Blue arrows). (17 metabolites are abbreviated).

influence on the production of metabolites [12]. From the results in Fig. 7, NH_4^+, NO_3^-, ORP, COD and NO_2^- had almost the same strong influence. The RDA results showed that in SWIS, with the extension of the running time, the metabolites were greatly affected by the environmental factors, and had negative correlations with NO_2^-, NH_4^+, and COD. The results suggested that the 17 metabolites can be used to indicate the operation stage and performance of SWIS to remove pollutants, such as NO_2^-, NH_4^+, and COD. Fewer small-molecule metabolites detected means lower removal efficiency of pollutants, so that the bed configuration and operational conditions can be adjusted accordingly.

5. Conclusion

This study tested the pollutant removal performance and metabolic characteristics of SWIS under variable operation stages. The results indicated that the ability of SWIS to remove pollutants (COD, NH_4^+–N and NO_3^-–N) increased with the operation time. The removal efficiency of COD increased from 75.2% ± 2.3% (stage I) to 82.2% ± 3.5% (stage II) and 85.6% ± 3.9% (stage III). For NH_4^+–N, the removals were 28.5% ± 1.7% (stage I), 63.5% ± 2.6% (stage II) and 75.2% ± 2.7% (stage III) and for NO_3^-–N, the removals increased from 22.7% ± 1.5% (stage I) to 60.7% ± 2.1% (stage II) and 71.6% ± 2.0% (stage III). To recognize different operation stages, metabolites were screened out and 17 potential bio-markers were qualitatively obtained. Their appearance was negatively correlated with the concentrations of COD, NH_4^+–N and NO_3^-–N. The relationship between TP content and metabolites was insignificant, further confirming that the removal of phosphorus is not dominated by microbial processes. In addition, this study confirms the feasibility of using qualitative analysis of small molecule metabolites to characterize the ability of SWIS to remove organic carbon and inorganic nitrogen.

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References

[1] G.N. Magesan, J.C. Williamson, G.W. Yeates, A.R. Lloyd-Jones, Wastewater C:N ratio effects on soil hydraulic conductivity and potential mechanisms for recovery, Bioresour. Technol., 71 (2000) 21–27.
[2] Y.H. Li, H.B. Li, J. Pan, X. Wang, Performance evaluation of subsurface wastewater infiltration system in treating domestic sewage, Water Sci. Technol., 65 (2012) 713–722.
[3] L.M. Wang, F.H. Guo, Z. Zheng, X.Z. Luo, J.B. Zhang, Enhancement of rural domestic sewage treatment performance, and assessment of microbial community diversity and structure using tower vermifiltration, Bioresour. Technol., 102 (2011) 9462–9470.
[4] Y.F. Sun, S. Qi, F. Zheng, L. Huang, J. Pan, Y. Jiang, W. Hou, L. Xiao, Organics removal, nitrogen removal and N_2O emission in subsurface wastewater infiltration systems amended with/without biochar and sludge, Bioresour. Technol., 249 (2018) 57–61.
[5] C. Liang, Y. Li, B. Chai, H. Wu, Evaluating the effects of intermittent aeration and biochar addition on enhancing removal performance of subsurface wastewater infiltration systems with loess soil, Bioresour. Technol., 5 (2019) 12–19.
[6] A. Kadam, G. Oza, P. Nemade, S. Dutta, H. Shankar, Municipal wastewater treatment using novel constructed soil filter system, Chemosphere, 71 (2008) 975–981.
[7] J. Pan, S.Y. Qi, Y.F. Sun, Y.Y Jiang, N. Zhao, L.L. Huang, Y.N. Sun, Nitrogen removal and nitrogen functional gene abundances in three subsurface wastewater infiltration systems under different modes of aeration and influent C/N ratios, Bioresour. Technol., 241 (2017) 1162–1167.
[8] Y.Q. Wang, W.J. Zhang, C.X. Nie, C. Chen, X.Y. Zhang, J.H. Hu, Evaluation of sample extracting methods of FCSM by Lactobacillus acidophilus based on a UPLC-Q-TOF-MS global metabolomics analysis, Braz. J. Microbiol., 49 (2018) 392–400.
[9] J. Zhang, X. Huang, C.X. Liu, H.C. Shi, H.Y. Hu, C.F. Shao, Z.Q. Liu, Nitrogen removal enhanced by intermittent operation in a subsurface wastewater infiltration system, Ecol. Eng., 25 (2005) 419–428.
[10] M.L. Zhang, Y. Wang, Q. Zhan, C.S. Wang, D.W. Zhang, J.B. Wan, C.Y. Yan, UPLC/Q-TOF-MS-based metabolomics study of the anti-osteoporosis effects of *Achyranthes bidentata* polysaccharides in ovariectomized rats, Int. J. Biol. Macromol., 112 (2018) 433–441.
[11] M. Bali, H. Tlili, Removal of heavy metals from wastewater using infiltration-percolation process and adsorption on activated carbon, Int. J. Environ. Sci. Technol., 16 (2019) 249–258.
[12] L. Yang, Y.H. Li, F. Su, H.B. Li, Metabolomics study of subsurface wastewater infiltration system under fluctuation of organic load, Curr. Microbiol., 77 (2020) 261–272.
[13] American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Waterworks Association and Water Pollution Control Federation, Washington, 2003.
[14] C. Willers, P.J. Jansen van Rensburg, S. Claassens, Can a metabolomics-based approach be used as alternative to analyse fatty acid methyl esters from soil microbial communities?, Soil Biol. Biochem., 103 (2016) 417–428.
[15] C.J. Liu, J.Z. Xie, M.L. Sun, Z.L. Gao, D.X. Zheng, X. Liu, G.H. Ning, X. Cheng, H. Bruning, Nitrogen removal performance and microbial community changes in subsurface wastewater infiltration systems (SWISs) at low temperature with different bioaugmentation strategies, Bioresour. Technol., 250 (2018) 603–610.
[16] L.Y. Zhang, Y.B. Ye, L.J. Wang, B.D. Xi, H.Q. Wang, Y. Li, Nitrogen removal processes in deep subsurface wastewater infiltration systems, Ecol. Eng., 77 (2015) 275–283.

[17] L.B. Zhang, J. Yang, The influence of hydraulic loading on biofilm properties in a subsurface wastewater infiltration system, Arch. Environ. Prot., 42 (2016) 70–79.
[18] D. Eveborn, D.G. Kong, J.P. Gustafsson, Wastewater treatment by soil infiltration: long-term phosphorus removal, J. Contam. Hydrol., 140 (2012) 24–33.
[19] H.P. Yuan, J.Y. Nie, N.W. Zhu, C. Miao, N. Lu, Effect of temperature on the wastewater treatment of a novel anti-clogging soil infiltration system, Ecol. Eng., 57 (2013) 375–379.
[20] J.L. Pang, J. Pan, D.L. Tong, X. Fu, B. Sun, M. Yang, H.B. Li, How do hydraulic load and intermittent aeration affect pollutants removal and greenhouse gases emission in wastewater ecological soil infiltration system?, Ecol. Eng., 1 (2020) 146–149.