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Enhancing bio-remediation of nitrate-contaminated drinking water source using a newly prepared solid-phase carbon source and evaluating risks of disinfection by-product formation and biostability

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ABSTRACT

Nitrate concentrations exceed standards in many drinking water sources. Solid carbon source (SCS) is usually used to increase the biological denitrification in waters with a low carbon-nitrogen ratio. However, there is a lack of research on the enhanced denitrification in drinking water sources with nitrate exceeding limits by the introduction of SCS. In this study, a SCS composite was prepared using agricultural waste corncob and polybutylene succinate and the SCS carbon release performance, denitrification effect and potential chemical and biological risks were investigated via a series of batch and column experiments. The SCS composite presented a long-term, stable carbon release performance and good microbial utilization capability for denitrification: SCS composite has a sustained release capacity for over 28 days in dynamic waters. Short-chain fatty acids accounted for over 60 % of the released carbon, and predominant fluorescence compounds were protein organic compounds and soluble microbial metabolites. The introduction of SCS composite evidently improved bio-rope biomass and its denitrification effect. The removal percentage of NO_3^- by SCS reactor pretreatment reached 90.9 %, remaining stable within 28 days. The SCS pretreatment increased formation potentials of trihalomethanes, but formation potentials of haloacetonitriles with higher toxicity were effectively reduced, so the comprehensive toxicity risk of disinfection by-products was reduced. The SCS pretreatment has slight negative impact on drinking water biological stability only during the first 10 days, which needs attention. This study demonstrated that the prepared SCS composite has excellent carbon release performance and denitrification effect, and its chemical and biological risks were controllable.

1. Introduction

Social and economic development have resulted in significant environmental problems and damage to aquatic ecosystems. In China, one major water quality issue is the high concentration of nitrate (NO_3^-) in surface waters [1]. Studies have shown the concentrations in the river waters of the Liaohe River, Haihe River and Yellow River Basin were higher than 45 mg/L. Annual NO_3^- concentrations ranging from 103.6 to 155.9 mg/L were detected at the estuary of the Yangtze River in Shanghai [2]. As a result, many drinking water sources are affected by high NO_3^- concentrations [3]. NO_3^- accounts for a significant proportion of total nitrogen (TN) in Chinese drinking water sources [4], causing the TN concentration frequently exceeds 1 mg/L, the surface water standard of Class III, which is also the standard for drinking water sources (GB 3838-2002). Since 1940, studies have proven that NO_3^- can cause blue baby syndrome or methemoglobinemia and cyanosis syndrome [5]. Drinking water is one of the most important routes for daily exposure to NO_3^- for humans. Usually, NO_3^- removal from drinking water sources and during drinking water treatment are two options and the later one has been studied previously [6]. NO_3^- removal from drinking water sources is the key to solve the problem of nitrogen exceeding the standard and reduce the risks to human health. Biological denitrification has

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Table 1
Basic water quality of experimental water.

	TN (mg/L)	TP (mg/L)	COD mn (mg/L)	NO_3^-N (mg/L)	NH_4^+-N (mg/L)	pH	DO (mg/L)
Micro-polluted river water	4.8–7.5	>0.3	6.0–10.0	2.07–4.13	1.9–2.5	6.0–9.0	3.5–4.5

been proven to be an economically and environmentally friendly method for nitrate removal [1,7]. Bio-carriers have been proven to be a promising alternative for nitrate removal from water. In many micro-polluted drinking water sources, artificial medium bio-rope as a carrier of biofilm growth is often used to remove nitrate by denitrification. During the denitrification process, nitrate acts as electron acceptor when the inner biofilm is anoxic and the carbon acts as electron donor in heterotrophic denitrification. However, the bio-rope existing in micro-polluted drinking water sources currently has achieved unsatisfactory denitrification effects [8], as a result of insufficient carbon source and low ratio of carbon to nitrogen [9]. Therefore, the addition of an external carbon source to the current bio-rope is crucial to improve its denitrification efficiency and achieve the required nitrate removal.

Previous studies showed that multiple substances, classified into liquid carbon source and solid carbon source (SCS) based on their physical form, can be used as external carbon sources to enhance denitrification processes [10]. In practice, low molecular weight organic compounds and saccharides are often used as liquid carbon source, such as methanol, ethanol, glucose and fructose [11]. In previous studies, nitrate removal over 90 % has been achieved by adding methanol and ethanol to wastewater as external carbon sources [12]. Liquid carbon sources have the advantage of high utilization efficiency, but they are prone to leaking into water, and the carbon pollution could lead to excessive microbial growth during drinking water treatment processes and water distribution through pipelines, which limits their application in enhancing denitrification in water sources. In contrast, SCS can slowly and steadily release organic matter so that the carbon leakage can be controlled well [13]. Naturally derived cellulose carbon sources, such as agricultural wastes, and artificially synthesized biodegradable polymer carbon sources have been extensively studied as single solid-phase carbon sources both in China and elsewhere [14,15]. Research findings indicate that the addition of agricultural waste and artificially synthesized biodegradable polymer materials exhibit favorable denitrification effects. However, challenges such as high leachate coloration caused by nitrogen and phosphorus leaching, significant organic matter release and unstable release from agricultural wastes, and the prohibitively high cost of artificially synthesized biodegradable polymers restrict their engineering application. Therefore, the development of a SCS with appropriate DOC release, high denitrification effect and low cost is a major challenge.

Currently, there is a lack of knowledge regarding SCS preparation and application in enhancing denitrification effect in micro-polluted water sources. The authors speculate that low-cost agricultural wastes and artificially synthesized biodegradable polymers with stable carbon release capacity can be combined into a SCS composite with good carbon release performance and high denitrification effect. It is noteworthy that adding SCS in drinking water sources could potentially bring chemical and biological risks caused by carbon leakage into the micro-polluted water sources. For chemical risks, disinfection by-products (DBPs), such as trihalomethanes (THMs) and haloacetonitriles (HANs) with high detection frequencies in waters, are a class of harmful substances with cytotoxic, genotoxic, carcinogenic, teratogenic and mutagenic characteristics, which can be formed under the presence of their precursors, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON), during chlorine disinfection process [16,17]. For biological risks, excessive carbon source leakage can reduce the biological stability of the treated water in the drinking water pipe network and adversely affect the water distribution system and human health.

In this study, a new SCS composite based on agriculture waste and artificially synthesized biodegradable polymer was prepared, and its nitrate removal effect in drinking water sources was assessed. The specific objectives were 1) to prepare a SCS composite with a stable carbon release performance and good carbon-bioavailability, 2) to explore the effect of the prepared SCS composite on denitrification effect with bio-rope as biomass carrier in drinking water sources, and with a SCS reactor as a pretreatment process for raw water, and 3) to assess the chemical risks of DBP FPs and the biological stability of drinking water due to the introduction of SCS composite.

2. Material and methods

2.1. Materials

Corncob (particle size of 100 mesh) and polybutylene succinate (molecular weight of 1,000,000-2,000,000 g/mol) were purchased from Lianfeng Agricultural Product Processing Factory (Jiangsu, China) and Solvay PPA (Solvay China Group), respectively. Before use, the corncob was dried in an oven at 50 °C for 12 h until constant weight.

The standard products of DBPs including THMs (trichloromethane (TCM), bromodichloromethane (BDCM), chlorodibromomethane (DBCM) and tribromomethane (TBM)); HANs (trichloroacetonitrile (TCAN), dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN) and dibromoacetonitrile (DBAN)) chromatographic Methyl tert-butyl ether (MtBE, high performance liquid chromatography grade) were all purchased from Shanghai Bailingwei Technology Co., LTD. Other chemicals and reagents including Bac Titer-Glo Microbial Cell Viability™ Assay Kit, Adenosine Triphosphate (ATP) Standard (Beijing Promag Biotechnology Co., Ltd.), sodium hypochlorite, phosphate buffered saline, anhydrous sodium sulfate, hydrochloric acid, sulfuric acid, anhydrous calcium chloride and boric acid, were all of at least analytical grade (Shanghai Sinopharm Reagent Co., Ltd., Shanghai); Milli-Q ultrapure water was used to prepare standard solutions (Millipore, USA).

The micro-polluted water used in the batch and the column experiments was collected from a lake located at the campus of Shanghai University. The water quality of the lake water was similar to drinking water sources, so it was used to simulate drinking water sources. The water quality indicators are shown in Table 1. In order to stabilize the concentration of NO_3^--N in the influent at approximately 4 mg/L, an appropriate amount of potassium nitrate was added. The activated sludge with MLSS of 5000 mg/L was taken from the anaerobic-anoxic-oxic process of a municipal wastewater treatment plant in Shanghai for inoculation during batch and column experiments. To build the simulated sand filtration and biological activated carbon (BAC) filtration columns, quartz sand and BAC were collected from a drinking water treatment plant (DWTP) in Wuxi, Jiangsu Province. The quartz sand was taken from a sand filter with stable operation for >5 years, and the BAC with a particle size of 0.88 mm was collected from a BAC filter with stable operation for 1 year.

2.2. Experimental design

2.2.1. Preparation of SCS composite

Biodegradable Polyvinyl alcohol and sodium alginate are widely used and therefore were decided as skeleton materials of the prepared SCS composite in this study. The contribution of polyvinyl alcohol and sodium alginate to the SCS composite were similar as in a previous study [18] and the contribution of corncob and polybutylene succinate to the SCS composite were as similar as in a previous study [19]. The ratio of the four materials, polyvinyl alcohol, sodium alginate, polybutylene

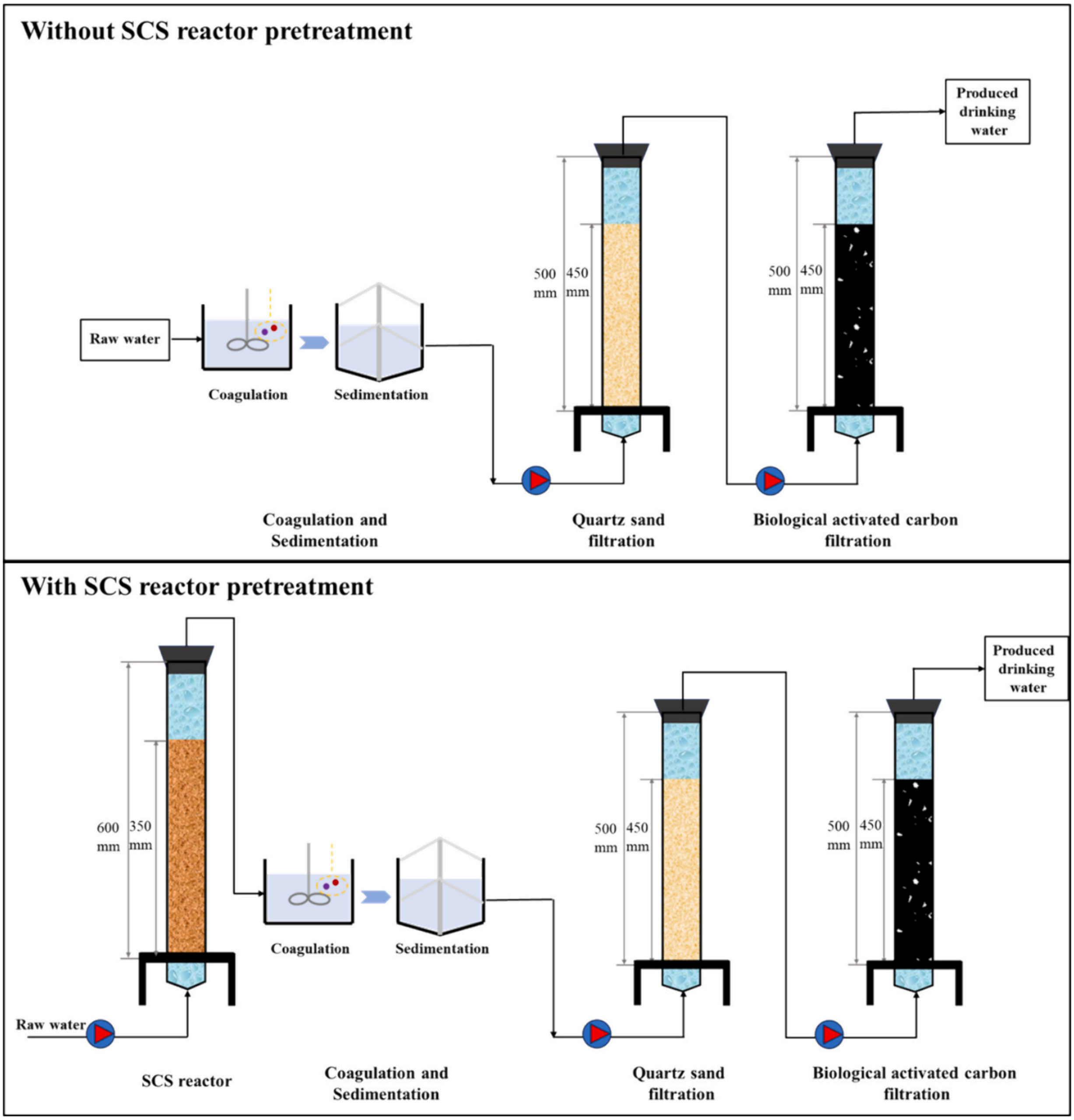


Fig. 1. Column experiment design for estimating chemical and biological risks of SCS reactor pretreatment on produced drinking water.

succinate and corncob, was 10/2/5/5. Meanwhile, 5 % calcium chloride saturated boric acid solution was added to further increase the stability of polyvinyl alcohol and sodium alginate [20]. The detailed preparation process is shown in Fig. S1, and the information of morphology, crystal structure and chemical composite of the SCS composite is shown in Fig. S2.

2.2.2. *Carbon release performance of prepared SCS composite*

The carbon release performance was assessed by measuring the concentration of DOC and short-chain fatty acids (SCFs) in glass bottles with fixed volume of water and amount of SCS composite. SCFs are easy to be utilized by microorganisms, so they can characterize the microbial availability of carbon released by the prepared SCS composite. Furthermore, the organic matter was characterized with fluorescence excitation emission matrix (EEM) experiments. All the experiments were conducted in triplicate and all glass bottles filled with water and SCS composite were shaken (oscillation frequency 100 rpm/min) at a constant temperature oscillation incubator of 25 ± 1 °C,

For the DOC release experiments, 6 g SCS composite was added to sealed glass bottles with 900 mL lake water. On day 0.5, 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28, 5 mL samples were taken from the bottles for DOC analysis using a three-dimensional FEEM (F-7100 analyzer) to further evaluate the carbon release performance of SCS composite.

The SCFs, acetic acid, propionic acid, butyric acid and isobutyric acid were analyzed in a similar procedure [21]. Here 4 g SCS composite was added to 200 mL sterilized water and samples were analyzed after 24, 48 and 72 h, using gas chromatography (GC-2010-plus).

2.2.3. *Denitrification experiments*

2.2.3.1. Effect of SCS composite on denitrification with bio-rope. Polypropylene rope as biofilm carrier is widely used to remove nitrate by denitrification in Chinese drinking water sources [8,22]. Anoxic conditions in the deep biofilm will enable denitrification which can be enhanced by carbon released from SCS composite. The effect of SCS addition on bio-rope denitrification was explored through batch experiment, which was divided into two phases: the first phase (length 14 days) was the biofilm development on the bio-ropes, followed by the second phase (day 14 to 35) where the denitrification was measured. To provide new nutrients for biofilm growth, the water, namely lake water, in the bottle was replaced every 7 days without disturbing the bio-rope biofilm. In the first phase, activated sludge was added into the water in a 1:9 ratio to artificially inoculate the bio-rope when refreshing the water. From day 14 onwards activated sludge suspension was no longer added to the bottle. Microbial activity (represented by ATP) on bio-rope was measured at 0 d, 7 d, 14 d, 21 d, 28 d, and 35 d, and NH_4^+-N, NO_3^--N, and TN were measured at 14 d, 18 d, 21 d, 25 d, 28 d, 32 d, and 35 d.

2.2.3.2. Denitrification effect of SCS reactor in drinking water pretreatment. In China, the raw water treatment plant is primarily responsible for extracting source water and conducting pretreatment, followed by distributing the source water to several DWTPs. To address the issue of nitrate exceeding the standard in source waters, one approach is to introduce SCS to enhance existing bio-rope denitrification in water sources, while another approach involves installing SCS denitrification reactors in the raw water treatment plant. In order to estimate the nitrate removal in SCS denitrification reactors, cylindrical organic resin glass packed columns with an inner diameter of 40 mm and a height of 600 mm, filled with 145 g of the prepared SCS composite, were used as SCS denitrification reactors. The prepared SCS composite was added as the carbon source and biofilm carrier, reaching a height of 350 mm. A hydraulic retention time of 3 h (flow rate: 2.44 mL/min) was set and the column was run in up-flow. This column experiment was conducted for 28 days. The first 3 days concerned inoculation with activated sludge, during the subsequent 25 days denitrification was measured. The influent and the effluent water samples were collected on day 7, 14, 21 and 28 for the analysis of NO_3^--N, TN, DON and DOC.

2.2.4. Chemical and biological risks of SCS composite

To assess the chemical and biological risks in the subsequent drinking water production, concerning DBP FPs and biostability, as a result of the introduction of the SCS denitrification reactor in source water pretreatment, two sets of simulation devices were used in this study: one set was with and the other set was without a SCS reactor on front of the coagulation-sedimentation-filtration process (Fig. 1). It is well known that coagulation-sedimentation-filtration-chlorination as traditional treatment processes have been widely used in many DWTPs. In this study, coagulation and sedimentation processes were simulated in a 30 L automated stirrer (SP-MDC60). Filtration was simulated in columns. Both sand and BAC filtration columns had an inner diameter of 20 mm and a height of 500 mm, with a filling height of 450 mm, which were operated in series. Hydraulic retention times for both columns were 30 min. This column experiment was divided into two phases: a start-up phase without connection of the SCS reactor to the coagulation/sedimentation/filtration process in the first 45 days, and a risk estimation phase with connection of the SCS reactor in the following 28 days. Figs. S3 and S4 show the influent and effluent DOC, NH_4^+-N and NO_3^--N concentrations of the two sets without SCS pretreatment during the start-up phase, indicating that the internal stable microbial environment had successfully formed and exhibited good organic matter removal performance. Afterwards, the SCS pretreatment was connected to the following traditional drinking water treatment processes for one set, while no SCS reactor was used in the other set. During the risk estimation phase, the produced water samples were collected from the two sets with and without SCS pretreatment on day 7, 14, 21 and 28 for the subsequent DBP FP test as well as biodegradable dissolved organic carbon (BDOC) as an indicator for regrowth potential and colony counts as indicator for actual regrowth.

2.3. Analysis

2.3.1. DOC, ATP, TN, NH_4^+-N and NO_3^--N measurement and DOC release kinetics

DOC concentrations were determined using a total organic carbon analyzer (Jena multi C/N 2100, German) after prefiltration through a 0.45 μm cellulose membrane [23]. ATP plays a key role in metabolic processes in the cells and can therefore be used as a measure for living biomass. Total ATP concentration was measured using Bac Titer-Glo Microbial Cell Viability™ Assay Kit and ATP Standard (Beijing Promag Biotechnology Co., Ltd.) [24].

TN was determined using the alkaline potassium persulfate oxidation spectrophotometric method. NH_4^+-N was determined using the Nessler's reagent spectrophotometric method. NO_3^--N was determined by Ultraviolet spectrophotometry.

The carbon release mechanism was determined by Ritger-Peppas equation (Eq. (1)) [25]:

$$\frac{M_t}{M_\infty} = kt^N \tag{1}$$

2.3.2. DBP FP test, DBPs measurement and toxicity

A test of DBP formation potentials (DBP FPs) were performed in 40 mL amber glass volumetric bottles under headspace-free conditions in a dark incubator [26]. A NaClO stock solution with a concentration of 85 g/L was prepared as chlorine disinfectant. In the DBP FPs tests, the chlorine demand was calculated according to Eq. (2) [27]. At the end of the predetermined reaction time, THMs and HANs were immediately extracted by adding 2 mL MtBE to a 10 mL sample. The samples were shaked for 5 min using a multi-tube vortex mixer (DMT-2500, Shanghai, China) at 2300 rpm. Finally, samples were kept in the refrigerator for <24 h before measurement.

$$\text{Chlorine } (Cl_2) \text{ dosage} = 3 \times \text{DOC mg/L} + 7.6 \times NH_4{}^+\text{-Nmg/L} + 10\text{mg/L} \tag{2}$$

THM4 and HAN4 were decided to be the target DBPs in this study because they were frequently detected in drinking water [28]. Detailed information on DBP analytical methods is described in Table S1 and the authors' previous studies [29]. They were measured by gas chromatography equipped with an electron capture detector (GC/ECD) (Clarus 680, PerkinElmer, USA) based on the United States Environmental Protection Agency method 551.1 and 552.3.2.

A comprehensive toxicity analysis method was employed, which included the DBP cytotoxicity and genotoxicity determined by the Plewa [30], as well as the composite toxicity reported in previous literature [31]. The toxicity of DBPs was calculated using the formula shown in Eq. (3).

$$ITRV_X = \sum [CTV_X \times FP_X] \tag{3}$$

2.3.3. EEM and other fluorescence parameters

The sources, optical properties, structures and chemical behaviors of the DOC in water sources and produced water were analyzed by Fluorescence spectrophotometer (HITACHI F-7000, Japan) with xenon lamp as excitation source [32]. EEM spectra were divided into five regions representing specific components of organic matters: Region I (EX200-250/EM280-330, Aromatic I), Region II (EX200-250/EM330-380, Aromatic II), Region III (EX200-250/EM380-550, fluvic acid-like), Region IV (EX250-450/EM280-380, soluble microbial products-like) and Region V (EX250-450/EM380-550, humic acid-like).

2.3.4. BDOC and bacterial measurement

BDOC was determined using the dynamic cultivation method with biofilm on sand as stated in a previous study [33]. The biofilm was collected from the sand filter of a DWTP in Wuxi, Jiangsu Province, China. Prior to use, the biofilm was activated for at least three days in

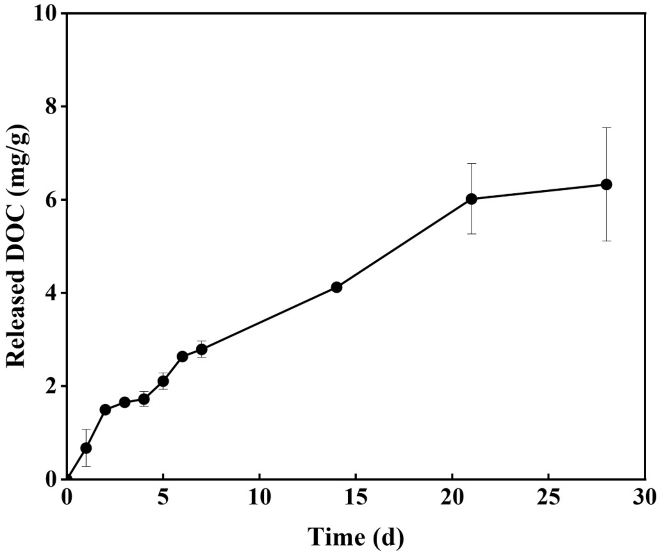


Fig. 2. The DOC released from SCS composite in batch reactors with lake water ($n = 3$).

water from a campus lake (the test water). Colony counts were measured using culture plate counting method as described in a previous study [34].

3. Results and discussion

3.1. Carbon release performance of prepared SCS composite

The DOC released from the prepared SCS composite in the bottles with lake water during 28 days is depicted in Fig. 2. The carbon release rate from SCS composite was 0.40, 0.19, 0.27 and 0.04 mg DOC/g SCS composite/week in the four weeks, respectively. It was observed that SCS underwent a rapid carbon release phase in the first 7 days, followed by a slower and stable release phase. On day 28, the released DOC content was 6.33 ± 1.32 mg/g SCS composite, which was between artificial synthetic biodegradable materials and natural cellulose materials reported in previous literature [19]. Furthermore, the N value in the Ritger-Peppas equation can characterize the carbon release mechanism, which is widely used to assess the stability of SCS [35]. When N value is <0.45, the carbon release is dominated by Fick diffusion, otherwise the carbon release mechanism includes the dissolution of the skeleton material [36]. In this study, the N value of SCS composite was 0.26 (Table S2), confirming stable carbon release mechanism and ability of SCS composite. Till day 28, the carbon release still had not reached a state of equilibrium, indicating that the carbon release of SCS was not exhausted even on day 28. In dynamic waters such as lakes and rivers, SCS composite may have a more sustained release capacity as the carbon concentration difference between SCS composite and water is larger in dynamic waters than in the static laboratory experiment.

The short-chain fatty acids (SCFs) released from SCS composite are shown in Fig. 3. SCFs can be easily utilized by microorganism and promote the occurrence of biological denitrification. The total concentration of SCFs decreased with time, probably because the released SCFs, especially acetic acid, were consumed by microorganism as a result of unsealed bottle and bacterial contamination. It can be observed that the maximum amount of SCFs released from SCS composite was at least 1.5 mg/g on day 1, and acetic acid was the predominant component, up to 1.02 mg/g. Combined with the results of DOC concentrations, preliminary calculations showed that the SCFs released from SCS composite accounted for at least 60 % of the total released carbon, indicating that most carbon released from SCS can be easily utilized by microorganism. Additionally, in SCFs, acetic acid shows a higher denitrification rate because of its simpler biodegradation pathway [37], which further indicates that the carbon source released from SCS will have better denitrification effects.

Fig. 4 shows the DOM fluorescence components released from the

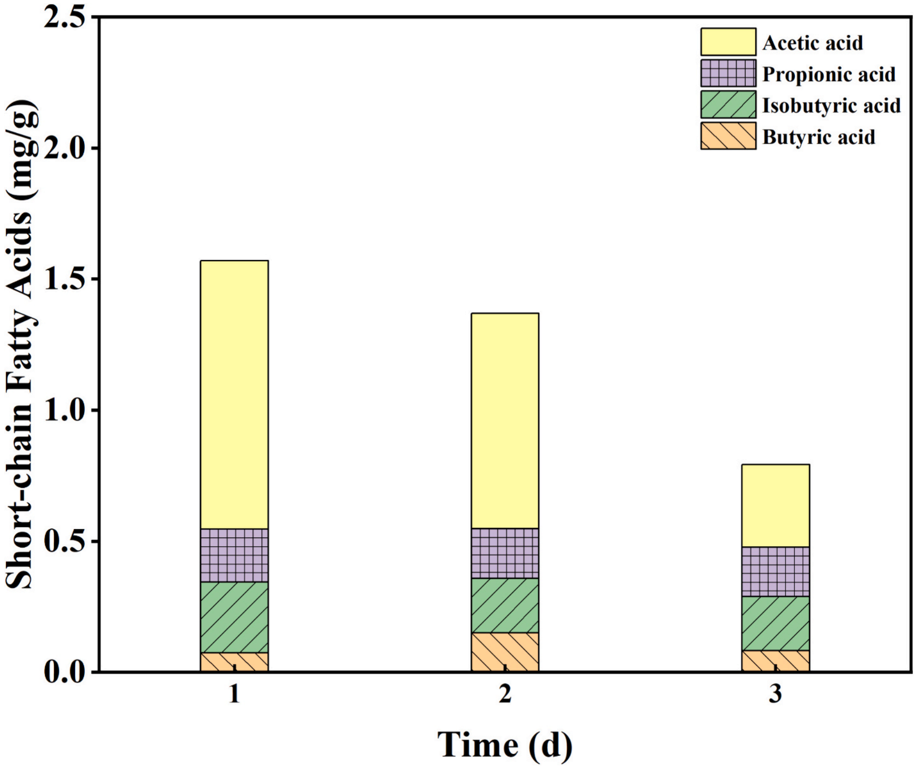


Fig. 3. Short-chain fatty acids released from SCS in batch reactors with ultrapure water.

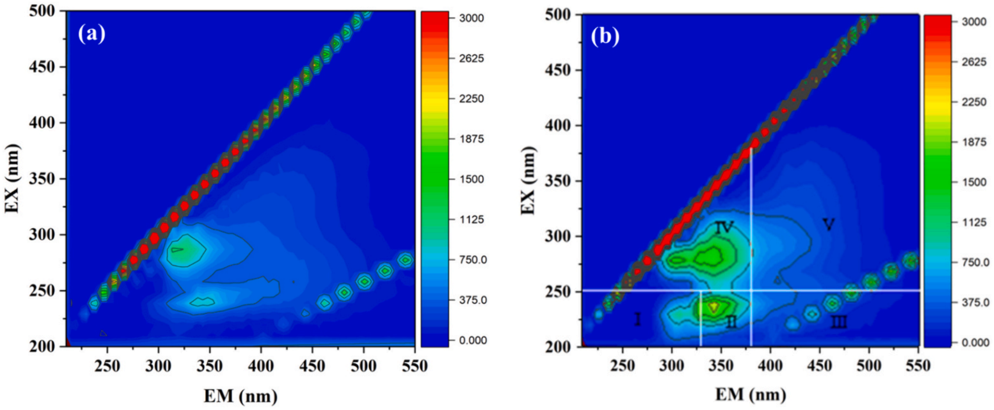


Fig. 4. The DOM fluorescence components in raw water without (a) and with SCS composite (b).

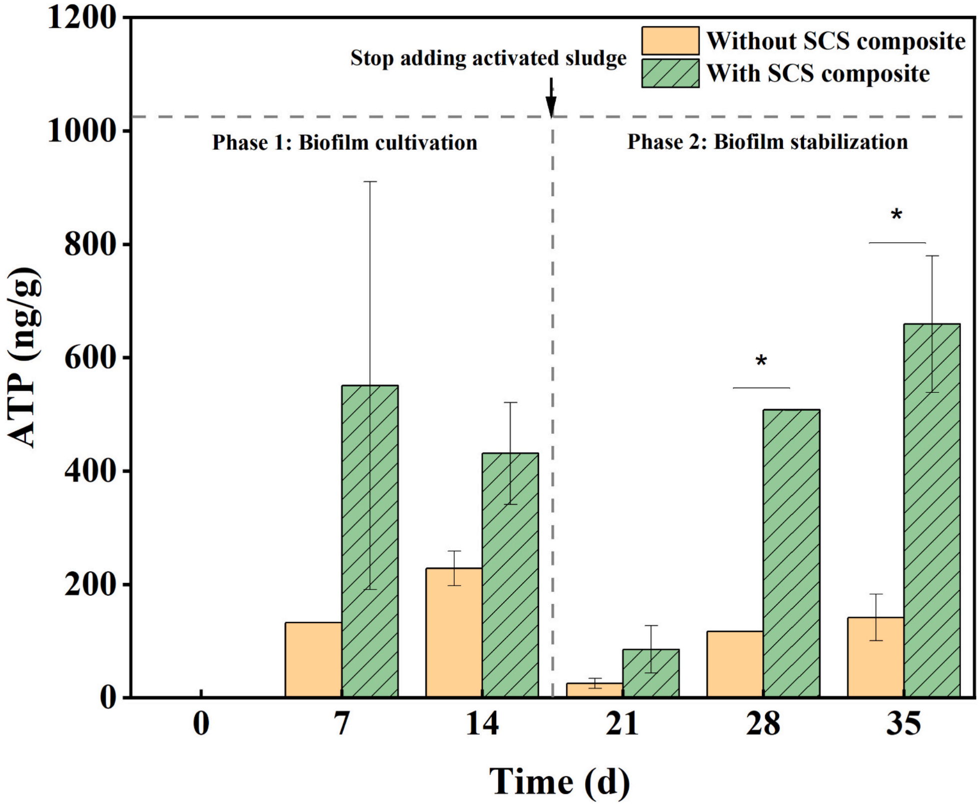


Fig. 5. Effects of adding SCS composite on the biomass on bio-rope in batch reactors with lake water ($n = 3$). * represents significant differences between the groups without and with SCS composite ($p < 0.05$).

prepared SCS composite within 72h. Compared to the raw water (Fig. 4a), the DOM released by SCS composite has obvious fluorescence peaks at aromatic protein substances (Region II) and soluble microbial metabolites (Region IV) (Fig. 4b). Aromatic protein organic compounds and soluble microbial metabolites can be used as carbon sources by microorganisms in the absence of carbon sources [38]. Consequently, it further confirmed that the carbon released from SCS composite is available for microorganism and can stimulate denitrification in water source with low C/N ratio water and high nitrate content.

Fig. 5 shows the effect of adding SCS composite on the biomass, represented by ATP, on the bio-rope. At the initial stage, the biomass in both groups with and without SCS composite was 0 ng ATP/g bio-rope as the bio-rope was new. It can be observed that the biomass in the SCS group was significantly increased and noticeably higher than that in the control group, in both phases with and without activated sludge inoculation, although the ATP was lower on day 21 than day 14 as the addition of activated sludge was stopped. For example, the bio-rope biomass on the 35th day reached 480.12 ng ATP/g for the SCS group, while it was only 129.54 ng ATP/g for the control group. These results indicate that the carbon released by SCS composite can be used by microorganisms to promote the rapid formation of biofilms as it increased the biomass on the bio-rope, and therefore may improve the denitrification effect of micro-polluted water sources. It is consistent with SCF result in Fig. 3.

3.2. *Effect of SCS addition on nitrogen removal by bio-rope*

Fig. 6 shows the denitrification effect of bio-rope with and without SCS addition in batch reactors. As is shown in Fig. 6a, the addition of SCS has no remarkable impact on the removal of NH_4^+-N. In Fig. 6b and 6c,

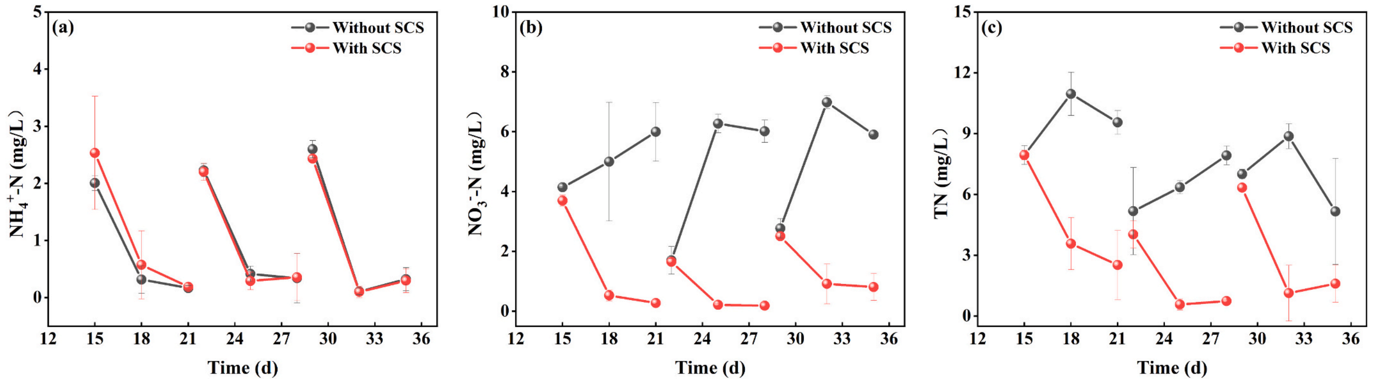


Fig. 6. Effects of SCS composite addition on nitrogen removal by bio-rope in batch reactors with lake water (n = 3).

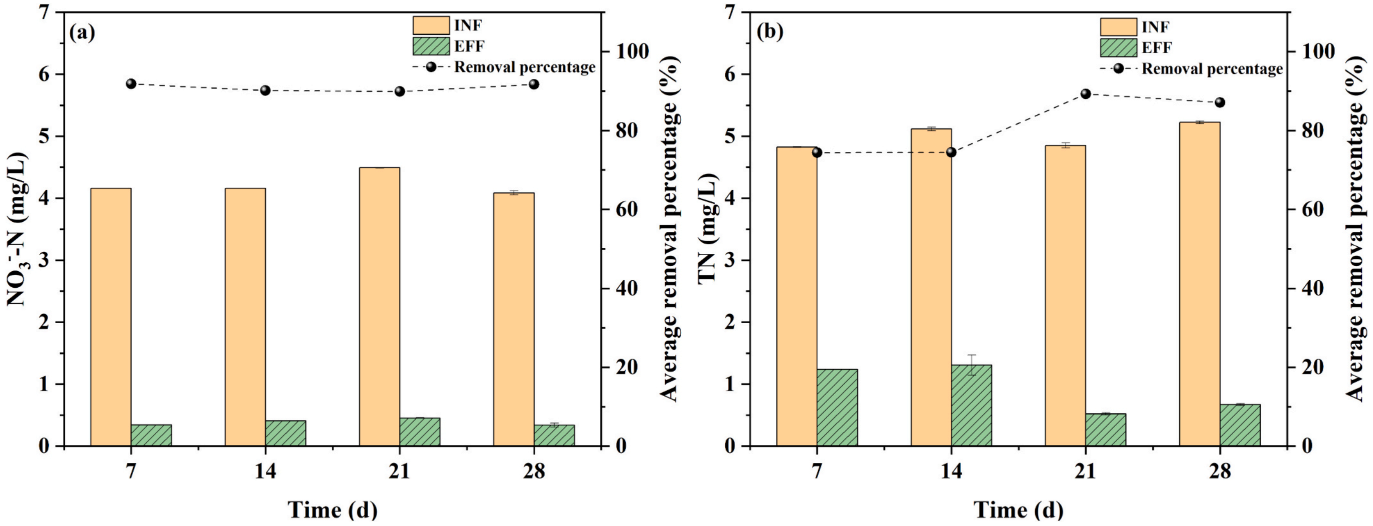


Fig. 7. The NO_3^--N (a) and the TN (b) removal by SCS column.

NO_3^--N and TN accumulated initially and then underwent a slight removal in the control group, which may be attributed to microbial death and decomposition due to the lack of carbon sources for microbial activity. In the group with SCS addition, NO_3^--N decreased from an initial average concentration of 2.62 mg/L to 0.42 mg/L, with a removal percentage of 83.1 ± 13.5 %, and TN decreased from 6.10 mg/L to 1.61 mg/L, with a removal percentage of 75.0 ± 6.7 %, within 7 days. Studies reported that bio-rope can form an aerobic-facultative-anaerobic dynamic microbial environment from the outside to the inside, facilitating and promoting the processes of nitrification and denitrification [39,40]. The results in Fig. 6 further proved that the biofilm on the surface of the bio-rope removed NO_3^--N by denitrification and therefore reduced the TN. In summary, the addition of SCS composite remarkably improved the TN removal of bio-rope by denitrification.

To assess the nitrogen removal effect of SCS reactor pretreatment after the microbial inoculation (3 d) has been completed, Fig. 7 presents NO_3^--N and TN concentrations in the influent and the effluent of SCS column. In Fig. 7a, the NO_3^--N concentration in the influent of the SCS reactor was 4.20 ± 0.18 mg/L, while in the effluent it was <0.5 mg/L during the whole experimental period. The average removal percentage of NO_3^--N by SCS pretreatment reached 90.9 ± 1.01 %, indicating that the SCS pretreatment remarkably removed NO_3^--N by denitrification. Fig. 7b shows that the removal percentage of TN by SCS pretreatment reached 81.3 ± 8.0 %, as a result of denitrification. The NO_3^--N removal percentage of several agriculture wastes reported in the literature was <80 % and the TN removal rate was <70 %, and there was a trend of decreasing percentage with the increase of running time [41]. In contrast, these results of this study show that the prepared SCS composite has not only an excellent denitrification effect but also a stable denitrification ability.

3.3. *Chemical risk of SCS reactor pretreatment*

3.3.1. *Effect of SCS reactor pretreatment on DOC and DON*

DOC and DON are usually considered as the precursors of C-DBPs and N-DBPs respectively [42], posing potential health risks to human. To assess the potential chemical risk of SCS reactor pretreatment, Fig. 8 shows the DOC and DON concentrations after drinking water traditional treatment processes, with and without SCS reactor pretreatment. It can be observed that DOC was removed in some degree by each treatment process and finally reached a concentration below 2 mg/L in the control group (Fig. 8a), while SCS pretreatment led to a dramatic increase of DOC concentrations (Fig. 8b), because microorganisms did not fully utilize the carbon released from SCS composite in the pretreatment. With the prolongation of operation time, the leaked carbon from SCS reactor pretreatment rapidly decreased, and DOC concentration in the produced water of the whole set decreased to below 4 mg/L on day 28 (Fig. 8c), which is comparable to the effluent of traditional drinking water treatment process reported in the literature (3–5 mg/L) [43,44]. This indicates that the SCS reactor pretreatment has almost no effect on the final DOC concentration and further confirmed that it has almost no risk of carbon leakage compared to traditional drinking water pretreatment processes. However, it does not mean that the effect of SCS composite on DOC can be ignored, especially in the first 21 days,

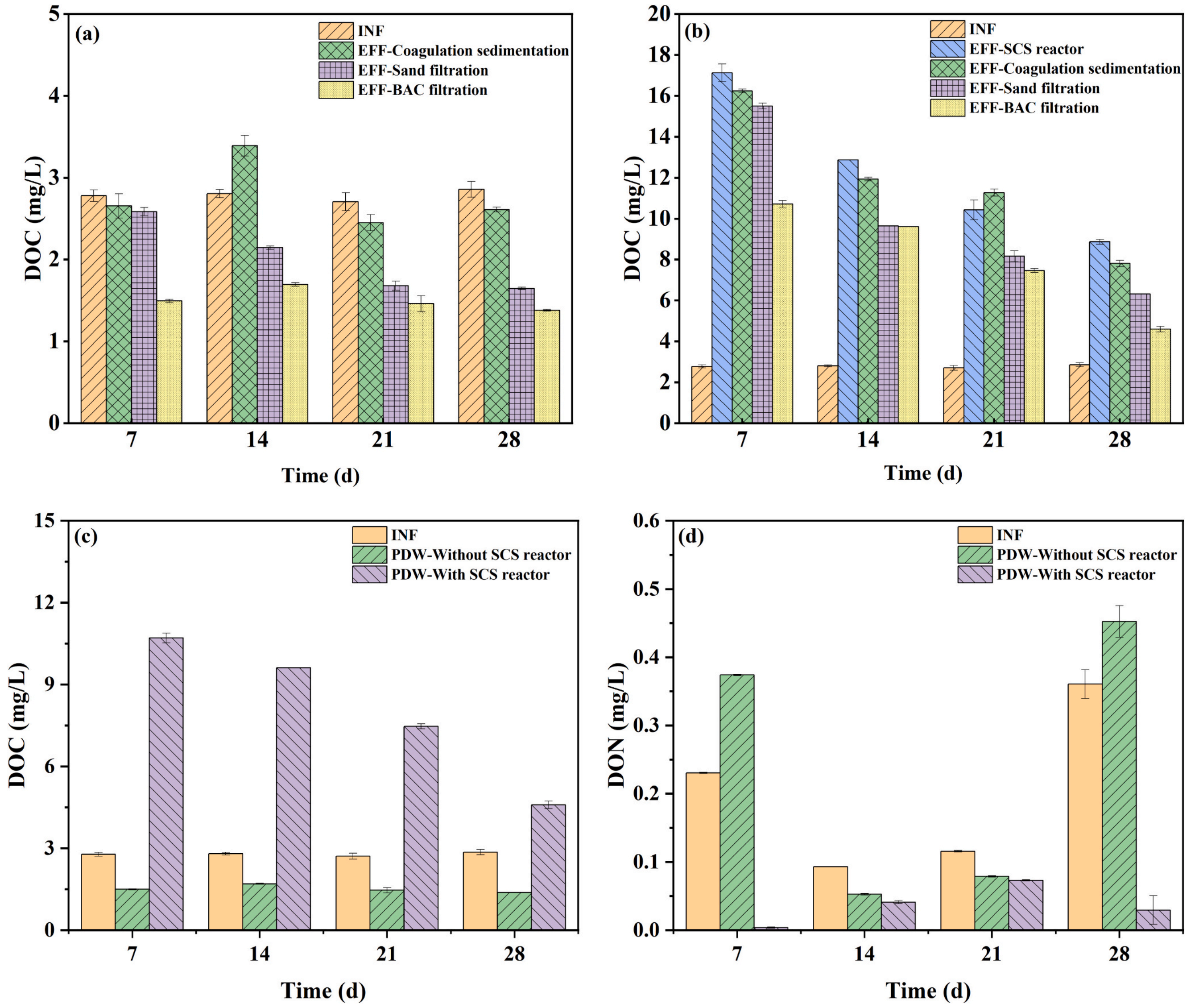


Fig. 8. The effect of SCS reactor pretreatment on the concentrations of DOC and DON in produced drinking water: DOC concentrations in the effluent of each treatment process without (a) and with (b) SCS column pretreatment, and DOC (c) and DON (d) concentrations in the produced drinking water of the whole set without and with SCS column pretreatment. PDW in the legend represents the produced drinking water.

because DOC is one of the important precursors of C-DBPs [45]. The DOC leakage problem could be controlled by increasing the water flow rate of SCS column and decreasing SCS composite dosage.

Differently from DOC concentrations, SCS reactor pretreatment remarkably decreased the concentration of DON in the produced water of the whole set, with an average concentration of <0.1 mg/L (Fig. 8d). However, without SCS pretreatment, the whole set of traditional drinking water treatment processes did not remove DON apparently. 0.2 μg/L HANs were detected in waters with a DON concentration of 0.4 mg/L [46]. HANs, formed by the reaction of DON with chlorine-containing disinfectants, are believed to have higher carcinogenicity compared to C-DBPs [47]. Therefore, attention should be paid on the concentration of DON, as it nearly reached 0.4 mg/L in influent sometimes. Notably, sometimes DON concentration increased after drinking water treatment processes without SCS pretreatment. This could be explained by the lack of a carbon source, leading to endogenous respiration by microorganisms within the BAC column [48] and the accumulated soluble microbial products by microorganisms during endogenous respiration released into the effluent [49]. These results indicate that probably the SCS composite could reduce N-DBP FPs but increase the C-DBP FPs in drinking water.

3.3.2. *Effect of SCS reactor pretreatment on THM FPs and HAN FPs*

Fig. 9 shows the effect of SCS pretreatment on the formation potentials of C-DBPs (THMs) and N-DBPs (HANs). Both the DBP FPs in the produced drinking water after the whole treatment train, with and without SCS reactor pretreatment, are shown bromine-DBPs were formed in the both classes of DBPs, which can be explained by the presence of bromide in the source water, resulting in the substitution reaction of bromine during the reaction of these precursors with chlorine [50]. It can be observed in Fig. 9a–d that SCS pretreatment decreased the FPs of DBCM and TBM, while it first increased (0–21 days) and then decreased (28 days) the FPs of BDCM and TCM. In general, the total THMs concentration increased due to the introduction of SCS column, which is consistent with the DOC results in Fig. 8c. However, considering the toxicity of BDCM and TCM is much lower than TBM and DBCM [51], the total toxicity of THMs could be decreased by SCS column pretreatment. It can be observed in Fig. 9e–h that the FPs of the four detected HANs in the produced drinking water were all reduced due to the introduction of SCS reactor pretreatment, which is consistent with the lowered DON concentration as shown in Fig. 8d. To comprehensively assess the chemical risk of DBPs in the produced drinking water, it is necessary to further analyze the comprehensive toxicity of DBPs,

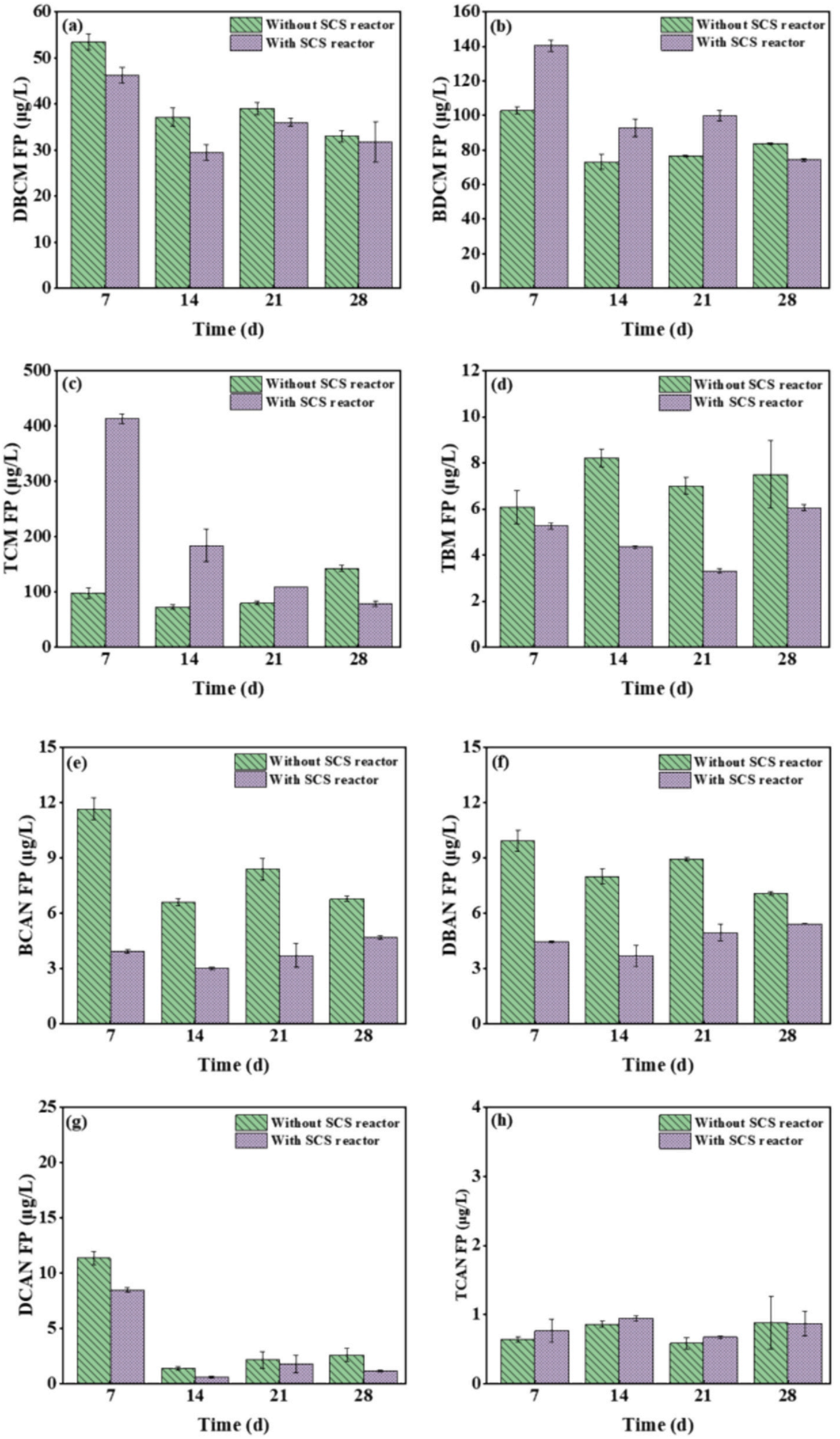
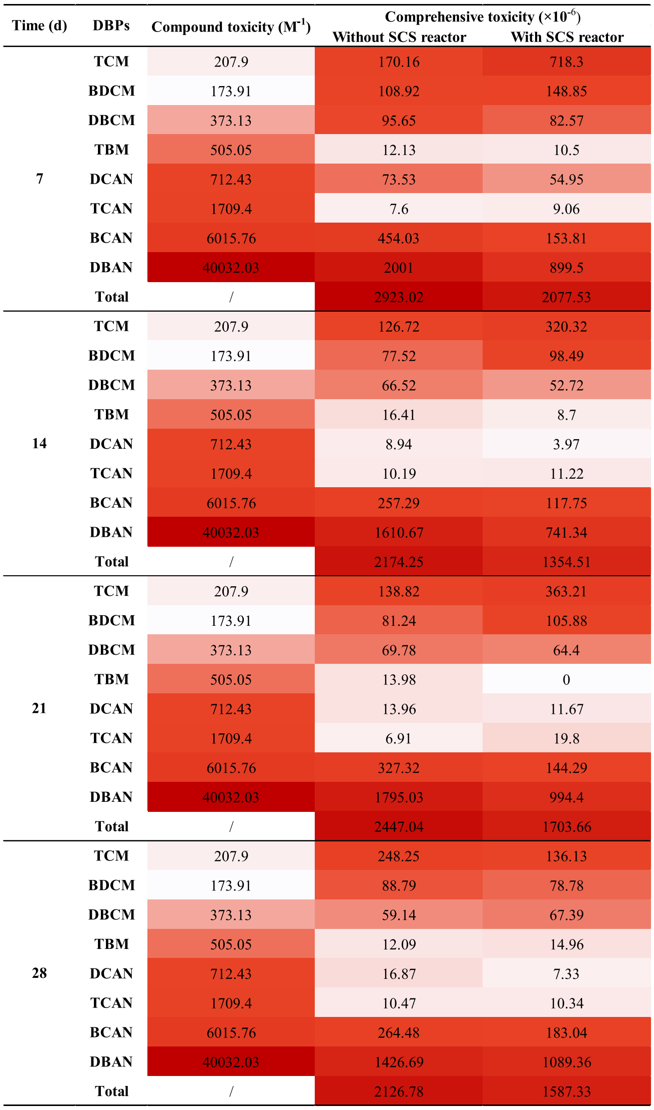


Fig. 9. Effects of SCS column pretreatment on the THM FPs (a-d) and HAN FPs (e-h) in produced drinking water ($n = 3$).

which is done in the next section.

3.3.3. *Comprehensive toxicity analysis of DBPs by SCS pretreatment*

Fig. 10 shows the comprehensive toxicity of THMs and HANs in the produced water with and without SCS column pretreatment. It can be seen that the comprehensive toxicity of THMs and HANs on the 7th, 14th, 21st and 28th days were lower in the group with SCS pretreatment than the group without SCS pretreatment, mainly due to the contribution of the decrease of N-DBP FPs. These results indicate that SCS pretreatment could effectively reduce the DBP toxicity of produced drinking water. The introduction of SCS column pretreatment in drinking water treatment has important prospects as it could limit the



Time (d)	DBPs	Compound toxicity (M^{-1})	Comprehensive toxicity (×10^{-6}) Without SCS reactor	Comprehensive toxicity (×10^{-6}) With SCS reactor
7	TCM	207.9	170.16	718.3
	BDCM	173.91	108.92	148.85
	DBCM	373.13	95.65	82.57
	TBM	505.05	12.13	10.5
	DCAN	712.43	73.53	54.95
	TCAN	1709.4	7.6	9.06
	BCAN	6015.76	454.03	153.81
	DBAN	40032.03	2001	899.5
	Total	/	2923.02	2077.53
14	TCM	207.9	126.72	320.32
	BDCM	173.91	77.52	98.49
	DBCM	373.13	66.52	52.72
	TBM	505.05	16.41	8.7
	DCAN	712.43	8.94	3.97
	TCAN	1709.4	10.19	11.22
	BCAN	6015.76	257.29	117.75
	DBAN	40032.03	1610.67	741.34
	Total	/	2174.25	1354.51
21	TCM	207.9	138.82	363.21
	BDCM	173.91	81.24	105.88
	DBCM	373.13	69.78	64.4
	TBM	505.05	13.98	0
	DCAN	712.43	13.96	11.67
	TCAN	1709.4	6.91	19.8
	BCAN	6015.76	327.32	144.29
	DBAN	40032.03	1795.03	994.4
	Total	/	2447.04	1703.66
28	TCM	207.9	248.25	136.13
	BDCM	173.91	88.79	78.78
	DBCM	373.13	59.14	67.39
	TBM	505.05	12.09	14.96
	DCAN	712.43	16.87	7.33
	TCAN	1709.4	10.47	10.34
	BCAN	6015.76	264.48	183.04
	DBAN	40032.03	1426.69	1089.36
	Total	/	2126.78	1587.33

Fig. 10. Effects of SCS column pretreatment on the DBPs comprehensive toxicity of produced drinking water.

generation of N-DBPs, which are usually more toxic than C-DBPs [52,53].

3.4. Effect of SCS pretreatment on biological stability

3.4.1. Effect of SCS pretreatment on EEM components of organic matter

Fig. 11 shows EEM components of organic matter in the produced drinking water with and without SCS pretreatment. By comparing the results of the two groups, it can be found that the carbon released from SCS reactor pretreatment was mostly removed by the existing drinking water treatment processes. The remaining substances in the produced drinking water mainly consisted of tryptophan-like substances (Region II) and microbial metabolite analogues (Region V), which is in line with previous studies [29,32]. Due to the introduction of SCS reactor pretreatment, the microbial metabolite analogues (Region V) in the produced drinking water increased slightly in the first 7 days, which corresponds to the DOC results (Fig. 8). With the increase of running time, the organic components in the produced drinking water with SCS reactor pretreatment were not significantly different from those of the control group, which indicated that the carbon released by the SCS reactor pretreatment could be effectively removed by subsequent drinking water treatment processes.

3.4.2. Effects of SCS pretreatment on BDOC and colony counts

BDOC is an important indicator for microbial growth potential in drinking water distribution systems. Fig. 12 shows the concentration of BDOC and colony counts in the influent and the produced drinking water with and without SCS reactor pretreatment. As is shown in Fig. 12a, BDOC concentrations in the influent ranged from 0.67 to 0.86 mg/L, which is in line with a previous study [54]. The introduction of SCS reactor pretreatment resulted in higher concentration of BDOC in the produced drinking water in the first 20 days, which is in line with the DOC results shown in Fig. 8. While, from day 20 onwards, BDOC in the produced drinking water without and with SCS reactor pretreatment were close. EEM results showed that organic matter was mainly composed of hydrophobic tryptophan-like substances which are not components of BDOC [55]. This could explain why SCS reactor pretreatment increased DOC concentrations but decreased BDOC concentrations in the produced drinking water after a certain running time. The produced drinking water with SCS reactor pretreatment might contain more aromatic substances and large molecular substances. It has been reported that an increase in aromatic substances and molecular weight in DOC correspond to a lower biodegradability [56]. In conclusion, the issue of the release of a small amount of low molecular weight substances in the first 20 days of SCS reactor pretreatment led to a slight increase in BDOC in the produced drinking water, which needs attention. However, with operation time, SCS reactor pretreatment could not impact the biostability. As is shown in Fig. 12b, the colony counts in the influent ranged from 1.44×10^5 to 1.82×10^5 CFU /mL, and in the produced drinking water with and without SCS reactor pretreatment it was 0.70×10^5–1.30×10^5 CFU /mL, which is comparable to the concentrations reported in DWTPs [57]. With operation time, the colony counts in the produced drinking water with SCS reactor pretreatment gradually decreased. On day 30, colony counts became lower due to the introduction of SCS reactor pretreatment. This result is consistent with BDOC results shown in Fig. 12a. Overall, the impact of SCS reactor pretreatment on the biological stability of the produced water was minimal, but still colony counts in the effluent during the first 10 days needs to be closely monitored.

4. Conclusions

In this study, the effects of SCS addition to raw water, or use in a pretreatment reactor, for nitrate removal was evaluated. Also, its potential chemical and biological risks in drinking water treatment were assessed. The prepared SCS composite presented a long-term and stable carbon release performance. The carbon release of SCS composite was not exhausted in batch bottles with lake water even after 28 days, indicating SCS composite may have more sustained release capacity in dynamic waters such as lakes and rivers. The SCFs accounted for at least 60 % of the released carbon, and the predominant fluorescence compounds were protein organic compounds and soluble microbial metabolites, indicating that the carbon released from SCS composite has good microbial utilization capability for denitrifying bacteria.

The introduction of SCS composite significantly improved bio-rope biomass and its denitrification effect: the removal percentages of NO_3^--N and TN were 83.1 % and 75.0 %. The removal percentages of NO_3^--N and TN by SCS reactor pretreatment was 90.9 % and 81.3 %, and the denitrification effect of SCS pretreatment maintained at a relatively stable level within 28 days.

SCS reactor pretreatment increased THM FPs, but the FPs of HANs with higher toxicity were effectively removed, so the comprehensive toxicity risk of DBPs was reduced. However, it should be noted that the SCS reactor pretreatment increased BDOC and colony counts during the first 10 days, which had a slight negative impact on biological stability. However, the negative impact disappeared afterwards.

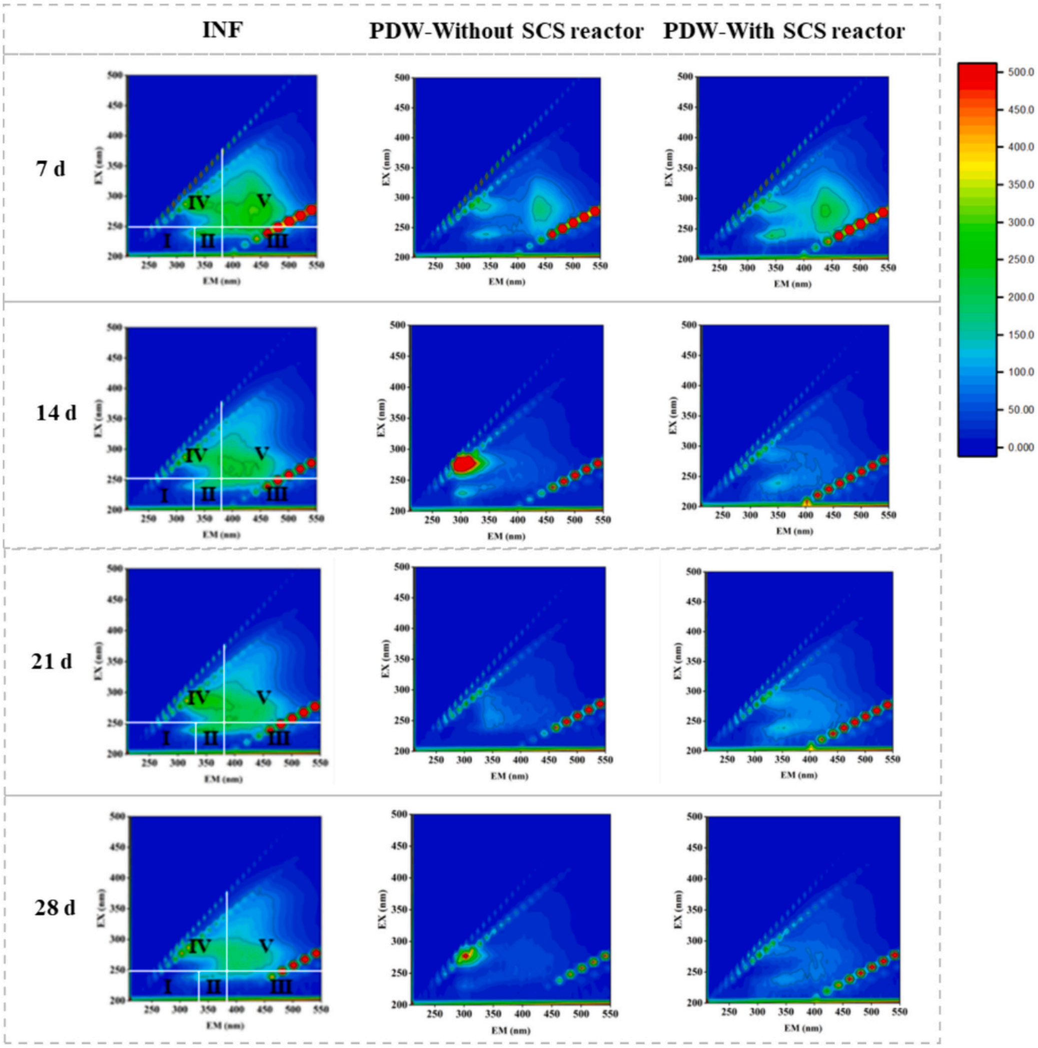


Fig. 11. The effect of SCS pretreatment on EEM components of organic matter in produced drinking water. PDW in the legend means the produced drinking water.

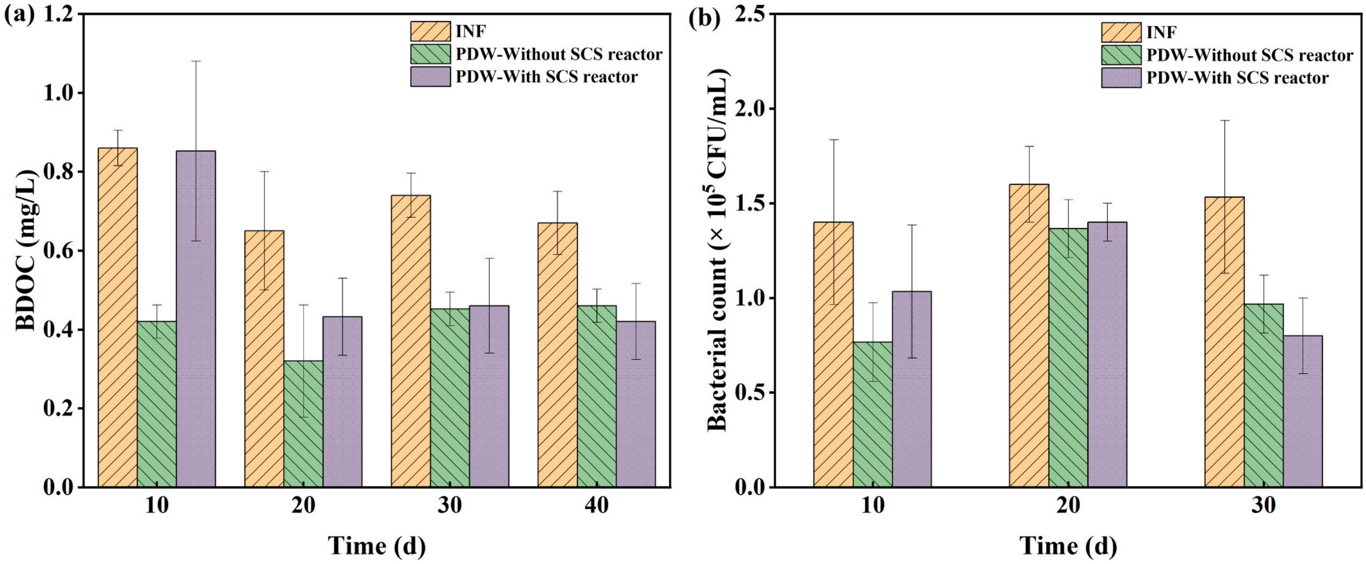


Fig. 12. The effect of SCS pretreatment on BDOC (a) and colony counts (b) in produced drinking water ($n = 3$). PDW in the legend means the produced drinking water. No significant difference was observed between the two groups with and without SCS reactor ($p>0.05$).

CRediT authorship contribution statement

Feifei Wang: Writing – review & editing, Project administration, Methodology, Conceptualization. **Jie Zhou:** Writing – original draft, Validation, Investigation, Formal analysis. **Jiazheng Pan:** Writing – review & editing, Investigation. **Haiting Zhang:** Writing – review & editing, Investigation. **Chiquan He:** Writing – review & editing, Conceptualization. **Xiaodi Duan:** Writing – review & editing, Conceptualization. **Jan Hofman:** Writing – review & editing, Supervision. **Jan Peter van der Hoek:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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