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Reduction of Nitrate by Biogenic Sulfide in Denitrifying Membrane – Sulfidogenic Up Flow Column Reactor System

Yeraltı sularından nitrat ve sülfatın denitrifikasyon membran-sülfidojenik yukarı akışlı kolon sıralı reaktör sisteminde giderimi

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Abstract

Sulfur- or thiosulfate- based autotrophic denitrification presents an effective and economical solution for nitrate removal from wastewater or groundwater with low concentrations of organic matter. However, the substantial production of sulfate can limit its wider application, particularly in groundwater that already exhibits high sulfate concentrations. This study evaluated the performance of a sequential system engineered for the effective removal of both nitrate and sulfate from groundwater. The system integrates autotrophic denitrification, which occurs within a membrane bioreactor (MBR), with ethanol-based sulfate reduction. A key design feature of this system is the utilization of sulfide, generated in a sulfidogenic column bioreactor, as the primary electron source for the denitrification process. The system was operated using synthetic groundwater containing nitrate at concentrations of 25 and 100 mg N/L in successive phases. While the system achieved near-complete nitrate removal across both influent nitrate concentrations, it demonstrated poor sulfate reduction performance. The original design intended for the sulfide produced from sulfate reduction in the column bioreactor to be used solely as an electron source for nitrate removal in the MBR. However, the observed low sulfate removal efficiency resulted in the carryover of organic matter from the sulfidogenic column bioreactor to the MBR, thereby fostering mixotrophic denitrification conditions. The study underscores that optimizing sulfur-based combined systems is crucial for achieving the simultaneous and efficient removal of both nitrate and sulfate.

Keywords: Sulfide-based denitrification, Sulfate reduction, Sequential system, Membrane bioreactor

Öz

Kükürt ya da tiyosülfat bazlı ototrofik denitrifikasyon prosesleri, düşük organik madde konsantrasyonlarına sahip atıksu veya yeraltı suyundan nitrat giderimi için etkili ve ekonomik bir çözüm sunmaktadır. Ancak, yüksek sülfat üretimleri, özellikle halihazırda yüksek sülfat konsantrasyonlarına sahip atıksu veya yeraltı sularında, bu yöntemin yaygınlaşmasını sınırlayabilmektedir. Bu çalışmada, yeraltı suyundan hem nitratın hem de sülfatın giderimi için tasarlanmış ardışık iki farklı prosesten oluşan kombine bir sistemin performansı değerlendirilmiştir. Sistemde, bir membran biyoreaktörde (MBR) gerçekleşen ototrofik denitrifikasyon, yukarı akışlı kolon bir reaktörde gerçekleşen etanol bazlı sülfat indirgeme prosesiyle birleştirilmiştir. Sistemde, yukarı akışlı sülfidojenik kolon biyoreaktörde üretilen sülfürün denitrifikasyon işlemi için birincil elektron kaynağı olarak kullanılması amaçlanmıştır. Sistem, 25 ve 100 mg N/L nitrat içeren sentetik yeraltı suyu kullanılarak ardışık iki farklı işletme koşulu altında işletilmiştir. Sistem, her iki giriş nitrat konsantrasyonunda da neredeyse tam nitrat giderimi sağlamasına rağmen, düşük sülfat indirgeme performansı göstermiştir. Kolon biyoreaktöründe sülfat indirgemesiyle üretilen sülfürün yalnızca MBR'de nitrat giderimi için bir elektron kaynağı olarak kullanılması amaçlanmış olmasına rağmen, düşük sülfat giderim verimliliği, organik maddenin sülfidojenik kolon biyoreaktörden MBR'ye taşınmasına ve miksotrofik denitrifikasyon koşullarının oluşmasına neden olmuştur. Çalışma, kükürt bazlı kombine sistemlerin optimizasyonunun hem nitratın hem de sülfatın eş zamanlı ve etkin giderimi için kritik önem taşıdığını ortaya çıkarmıştır.

Anahtar kelimeler: Sülfür-bazlı denitrifikasyon, Sülfat indirgeme, Sıralı sistem, Membran biyoreaktör

1 Introduction

Nitrate contamination in groundwater poses a global challenge due to deficiencies in sewage infrastructure, animal husbandry, septic systems, uncontrolled discharge of industrial waste and wastewater, and excessive use of fertilizers [1]. Biological denitrification, which includes both heterotrophic and autotrophic processes, is a common method to remove nitrate from water source. Heterotrophic denitrification process requires organic carbon as an electron source, whereas autotrophic denitrification process utilizes inorganic electron donors [2] to achieve complete nitrate reduction [3].

The autotrophic denitrification using inorganic electron donors has several advantages over heterotrophic denitrification. Notably, the effluent from autotrophic processes is less likely to be contaminated with organic substances, a common concern in heterotrophic denitrification. Moreover, hydrogen gas (H_2), zero-valent iron (Fe°), elemental sulfur (S°), hydrogen sulfide (H_2S), and thiosulfate ($S_2O_3^{2-}$) are generally more affordable inorganic electron donors than organic electron donors [2]. In particular, reduced sulfur compounds such as S°, H_2S and $S_2O_3^{2-}$ have advantages over other inorganic electron donors due to their non-explosive, non-toxic nature, low cost, and stability under normal conditions [4]. For example, sulfur-

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based denitrification processes have been preferred successfully to remove nitrate from groundwater [5], drinking water [3], and industrial wastewater [6] due to its low cost, easy handle and transport [7].

A major drawback of using reduced sulfur compounds is the production of sulfate and acidity as byproducts. Theoretically, for every milligram of nitrate nitrogen reduced, 7.54 milligrams of sulfate are expected to be produced. The US-EPA and TS266 drinking water sulfate standard of 250 mg/L [8],[9] limits the extent of nitrate reduction achievable using reduced sulfur compounds. Theoretically, the elemental sulfur-based denitrification process could reduce 33 mg/L of $N\text{-}NO_3^-$ in groundwater initially devoid of sulfate, while potentially remaining within permissible sulfate concentration limits. However, sulfate is another anion commonly present in groundwater. According to Ucar et al. (2017), sulfate concentrations in Harran Plain exhibited significant variation, ranging from 4.07±0.3 to a maximum of 425.7±36.1 mg/L. The average sulfate concentration throughout the plain was found to be 82±103.1 mg/L. Similarly, nitrate concentrations demonstrated a wide range, with values between 4.07±0.3 mg/L and 83.2±5.4 mg/L, averaging 19±20.8 mg/L [5]. The co-occurrence of nitrate and sulfate ions in groundwater limits the efficacy of denitrification processes based on reduced sulfur compounds for achieving regulatory water quality standards. Although advanced technologies such as reverse osmosis can effectively remove excess sulfate from treated water [3], they often incur high costs and operational complexities. To optimize sulfate production, a combined approach of sulfur-based denitrification and sulfate reduction can be applied. This strategy involves converting excess sulfate into hydrogen sulfide, which can then be consumed as an electron source in the denitrification. A basic stoichiometry of sulfide formation is presented in Reaction 1 below.

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \quad (1)$$

Sulfide in wastewater can exist in three forms: hydrogen sulfide gas (H_2S), the non-volatile ionic species hydrogen sulfide (HS^-), and sulfide (S^{2-}). The complete oxidation of S^{2-} to sulfate (SO_4^{2-}), involving the transfer of eight electrons per sulfur atom, represents a highly energy-yielding process for chemoautotrophic microorganisms [10],[11]. Simple form of the reaction is presented below [12].

$$5S^{2-} + 8NO_3^- + 8H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O \quad (2)$$

Several studies in the literature have successfully demonstrated the use of sulfide as an electron donor for effective nitrogen removal from wastewater [13]-[16]. For example, Andreides et al. (2021) investigated nitrogen removal efficiency in a packed bed reactors fed with two separate waste streams: nitrified and sulfide wastewater from an industrial plant. Nitrate removal performance exceeded 94% in the reactors operating at a maximum sulfide loading rate of 0.17 kg/(m³·d) and a nitrate-nitrogen loading rate of 0.25 kg/(m³·d) [16]. Although extensive research exists on using sulfur as an electron donor for nitrogen removal, studies investigating the conversion of sulfate, either produced during denitrification or present in the water, into sulfide through sulfate reduction, and the subsequent use of this generated sulfide as an electron donor for re-denitrification, remain relatively limited. For this purpose, this study aims to determine the performance of an innovative combined process combining sulfate reduction and denitrification processes for the simultaneous removal of nitrate and sulfate from groundwater. This process utilized sulfide-based autotrophic denitrification to reduce nitrate, while ethanol oxidation was used to reduce sulfate and produce the required sulfide. The efficiency of nitrate removal and sulfate reduction and sulfur utilization of the combined system at two different nitrate loading rates were comparatively investigated in detail.

2 Material and Method

2.1 Denitrifying membrane, up-flow anaerobic sulfidogenic sequential system

In this study, a fully mixed denitrifying membrane bioreactor was coupled in parallel with an up-flow sulfidogenic column reactor, as depicted in Figure 1. The feed was initially mixed with recycled effluent from the sulfidogenic reactor and then introduced into the membrane bioreactor. Subsequently, the membrane bioreactor effluent, supplemented with ethanol, was pumped to the up-flow sulfidogenic column reactor. Half of the effluent from the column reactor was recirculated to the membrane bioreactor, while the remaining half was left the bioreactor. Four peristaltic pumps (Ismatec Reglo-Z/1-3290 ml/min interval) were used. Two pumps were used for feeding of membrane and up flow sulfidogenic reactors, one pump for suctioning and one pump was used for recycling as shown in Figure 1.

2.2 Denitrifying membrane bioreactor

The denitrifying membrane bioreactor was made of plexiglass material. The dimensions were 15 cm x 15 cm x 30 cm corresponding to 6.75 L total volume. The active volume of the reactor was 3 L, and it was covered with aluminum foil to prevent phototrophic growth. The reactor was placed on a magnetic stirrer and mixed at 150 RPM to ensure complete mixing. Double sided membrane module (10 cm x 10 cm and total area 0.02 m2) was placed in the reactor and 0.04 µm pore size flat sheet polyether sulfone (PES) ultrafiltration membrane was used. Membrane fouling was monitored by a manometer on the suctioning line. To reduce membrane fouling, intermittent suction was applied (5 min suction and 1-min relaxing). Feeding was provided by chrome probes with liquid level role (Tense SSR05).

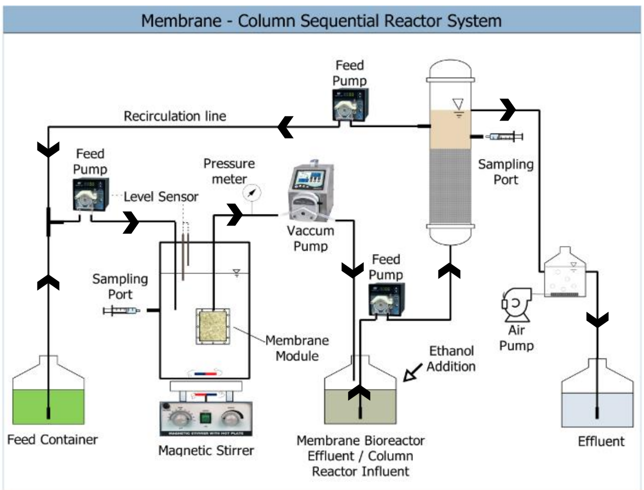

Figure 1. Denitrifying membrane – sulfidogenic up flow column reactor sequential system.

2.3 Up-flow anaerobic sulfidogenic sequential system

Laboratory scale anaerobic up-flow glass column reactor was used. The column bioreactor has a working volume of 500 ml and has an inner diameter of 7 cm and a height of 40 cm. Aquarium sand (1-2 mm) was used as filling material and covered with aluminum foil. A recycling from the sulfidogenic reactor to denitrifying reactor was provided with a peristaltic pump to transfer alkalinity and sulfide.

2.4 Operational Conditions

The system was operated at 35 °C in a temperature-controlled room for 227 days. Sludge taken from an anaerobic digester treating pulp and paper industry was used as inoculum. HRT and SRT were 1 day and infinite, respectively, during the study. In the first part of the study (days 1-154), the influent nitrate was 25 mg $N\text{-}NO_3^-$/L, while in the second part (days 155-227), nitrate was increased to 100 mg $N\text{-}NO_3^-$/L. The influent sulfate was approximately 500 mg/L and COD/SO_4^{2-} ratio was 0.67. Synthetic groundwater was prepared by adding the following chemicals to tap water: KNO_3 (181 and 722 mg/L for 25 and 100 mg $N\text{-}NO_3^-$/L, respectively), $NaSO_4$ (620 mg/L), KH_2PO_4 (50 mg/L), NH_4Cl (110 mg/L), ascorbic acid (11 mg/L), and $NaHCO_3$ (823 mg/L). To maintain a COD/SO_4^{2-} ratio of 0.67, ethanol was added at 335 mg/L COD (161 mg ethanol or 0.102 mL of 96% purity ethanol with a density of 789 kg/m^3 per liter) for an influent sulfate concentration of 500 mg/L. In order to remove sulfide from the effluent, an aeration unit was placed on the system effluent. Aeration unit was added to the effluent tank to control the effluent sulfide. The volume of aeration unit was one liter, and it was aerated with an air pump at a flow rate of 187.5±3.12 ml/min. The cleaning of the membranes was performed physically and/or chemically when the pressure increased to 200 mbar. Physical cleaning was performed by cleaning the membrane surface with a sponge. Chemical cleaning was done by holding the membranes in 3% sodium hypochlorite solution for 1 h, and then in acidic solution adjusted to pH 3 with H_2SO_4 for 1 h. Mass balances were calculated according to the Reaction 1 and 2. Sulfide-based denitrification produces 4.28 mg SO_4^{2-}, 3.57 mg $CaCO_3$ alkalinity; consumes 1.43 mg S^{2-} for each mg $N\text{-}NO_3^-$ reduced. The results were evaluated according to this stoichiometry in each relevant section.

3 Results and Discussion

3.1 Variations of sulfate and sulfide in the sulfidogenic bioreactor

The sulfidogenic column bioreactor was fed with MBR effluent by adding ethanol to maintain a constant $COD/SO4^{2-}$ ratio of 0.67. The average sulfate concentration in the influent of the sulfidogenic column bioreactor was 459±109 mg/L throughout the entire operation.

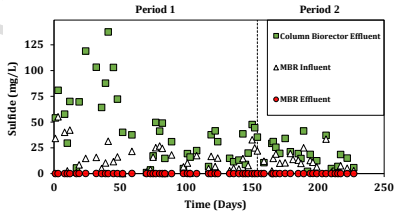

Figure 2. Variations of sulfide concentration in the denitrifying membrane – sulfidogenic up flow column reactor sequential system.

In the initial period, the sulfidogenic column bioreactor demonstrated a low sulfate reduction efficiency, with an approximate removal rate of 27%. The average sulfide concentration generated during this period was 43±33 mg/L. However, upon increasing the system feed nitrate to 100 mg N/L, sulfate reduction performance within the sulfidogenic column bioreactor began to decline and ultimately ceased. Consequently, sulfide production within the system was significantly reduced during the second period, with an average concentration of only 18±11 mg/L.

Numerous studies in the literature have demonstrated high sulfate removal efficiencies under similar operating conditions in the absence of nitrate. For example, Yildiz et al. (2019) achieved a significant sulfate reduction in an ethanol-fed sulfidogenic bioreactor, lowering the influent sulfate concentration of 2000 mg/L to a final level of 51 ± 7 mg/L at COD/SO_4^{2-} ratio of 0.67 [17]. The low sulfate removal efficiency observed in this study can be attributed to two primary factors. Firstly, the microbial community within the column bioreactor may not have shifted to favor the dominance of sulfate-reducing bacteria, hindering efficient sulfate reduction. Secondly, the presence of residual nitrate in the membrane bioreactor effluent, which was subsequently fed to the column bioreactor, likely inhibited the growth and activity of sulfate-reducing bacteria. Heterotrophic nitrate-reducing microorganisms and sulfate-reducing microorganisms may be potential competitors for the same electron donors. Especially, high nitrate concentrations can create an unfavorable environment for sulfate-reducing bacteria [18]. Furthermore, some studies indicate that some specific sulfate-reducing microorganisms may preferentially utilize nitrate or nitrite as electron acceptors over sulfate under certain conditions [19],[20].

3.2 Variations of nitrate in the denitrifying membrane – sulfidogenic up-flow column reactor system

The nitrate concentration in the system feed was 25±2.3 and 101±3.5 mg N/L in the Period 1 and 2, respectively. The MBR influent was generated by mixing the system feed with the effluent from the sulfidogenic column bioreactor. This facilitated the transfer of sulfide from the sulfidogenic reactor to the MBR, providing the essential electron donor for nitrate reduction. Throughout the study, nitrate concentrations at the sulfidogenic reactor effluent remained below detectable levels (Figure 2). Consequently, the feed nitrate concentration was diluted only upon entering the MBR. The average nitrate concentration at the MBR influent inlet was 13.8 ± 4.5 mg N/L and 40.3 ± 10.3 mg N/L for the first and second parts of the study, respectively. At the end of the first period, the nitrate concentration at the membrane effluent decreased to an average of 0.8±2.3 mg N/L. In contrast, nitrate removal performance decreased in the second period, leading to a significant increase in the average MBR effluent nitrate to 28.8 ± 29.02 mg N/L.

In the MBR, the aim was to establish a fully autotrophic nitrate reduction process utilizing sulfide derived from the column bioreactor. According to Reaction 1 and 2, sulfide-based denitrification produces 4.28 mg SO_4^{2-}, 3.57 mg $CaCO_3$ alkalinity; consumes 1.43 mg S^{2-} for each mg $N\text{-}NO_3^-$ reduced. During periods 1 and 2, an average of 15 ± 13 mg/L and 11 ± 8 mg/L of sulfur, respectively, was introduced into the MBR from the column bioreactor, by diluting with the system feed at the MBR inlet. Despite complete sulfide consumption within the MBR, the system was fed insufficient sulfide to facilitate the reduction of all incoming nitrate. Furthermore, while the

column bioreactor supplied sulfide for nitrate reduction to the MBR, residual ethanol, resulting from incomplete sulfate reduction in the column bioreactor, was also transported to the MBR. This led to the formation of mixotrophic denitrification conditions within the MBR. Detailed information about COD consumption within the MBR is presented in subsequent sections.

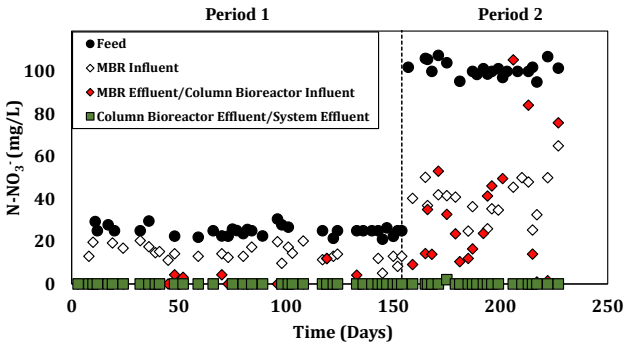

Figure 3. Variations of nitrate concentration in the denitrifying membrane – sulfidogenic up flow column reactor sequential system.

Nitrate concentrations at the system effluent (sulfidogenic column bioreactor effluent) were consistently below the detection limit (Figure 3), indicating complete nitrate removal by the sulfidogenic column bioreactor. Heterotrophic bacteria likely utilized ethanol to remove nitrate that could not be reduced autotrophically within the column bioreactor. This heterotrophic activity may have significantly contributed to the observed low sulfate and COD removal efficiencies within the column bioreactor.

Nitrite, an intermediate product, was monitored regularly throughout our study. Elevated nitrite concentrations of 4.6 ± 0.9 and 7.4 ± 6.1 mg/L were observed in two specific periods (days 179-181 and 203-215), which coincided with operational issues related to the membrane bioreactor effluent (Figure 4). In all other instances, nitrite levels remained below the detection limit.

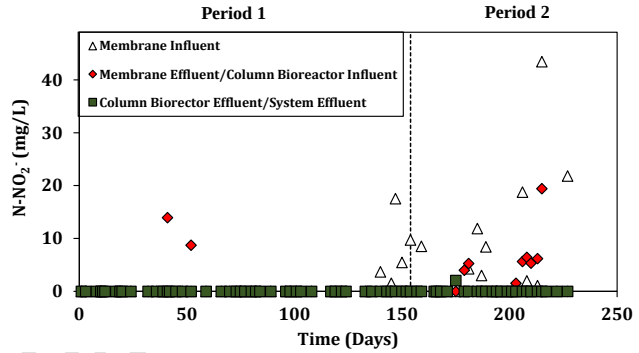

Figure 4. Variations of nitrite concentration in the denitrifying membrane – sulfidogenic up flow column reactor sequential system.

The complete oxidation of S^{2-} to SO_4^{2-}, is an energetically attractive reaction that transfers eight electrons per sulfur (S^0) atom [11]. It has been observed that autotrophic denitrification bacteria dominate the whole process when nitrate is mixed with sulfur-rich anoxic sediments [21],[22]. Sulfide-based denitrification could be used in the removal of sulfate and nitrate in groundwater. There are also studies in the literature on the treatment of sulfur rich sediment sludge. The two most commonly reported denitrification bacteria in the literature are *Thiobacillus Denitrificans* and *Thiomicrospira Denitrificans*. Studies indicate that autotrophic denitrification bacteria may become more dominant in the presence of nitrate than heterotrophic bacteria [22].

Although there are many studies in the literature that provide high nitrate removal with sulfide-based denitrification, only partial nitrate removal was achieved in this study. This limitation can be attributed primarily to insufficient sulfide delivery to the MBR, a consequence of the low sulfate reduction efficiency observed within the column bioreactor. For example, Yang et al. (2016) observed a direct correlation between nitrate removal efficiency and the sulfide/nitrogen (S/N) ratio. Their study demonstrated a four-fold increase in the biomass-specific nitrate reduction rate when the S/N ratio was elevated from 3 to 5 [15]. In the study by Liang et al. (2024), nitrate, sulfide and phosphate removal efficiencies were reported as 87.63±3.12, 99.61±1.02 and 85.38±4.07%, respectively, at influent nitrate, sulfide and phosphate concentrations of 80.55±2.98 mg N/L, 380.15±20.83 mg S/L and 47.70±4.35 mg P/L, respectively and at the HRT of 8.8 h [23]. In a study by Hove et al. (2020), optimal influent concentrations for effective nitrite removal via sulfide-based denitrification in municipal wastewater were reported as follows: TKN at 30 g N/m^3, hydrogen sulfide at 24 g S/m^3, and organic matter at 16 g COD/m^3 [13].

The removal of both nitrate and sulfate from aquatic environments is typically achieved using advanced filtration methods (i.e., nanofiltration or reverse osmosis [24] or ion exchange techniques [25]) all of which generate a concentrate that must be managed. In rural areas where groundwater use for drinking water is necessary, the management of this concentrate becomes even more challenging. A process in which both nitrate and sulfate are biologically removed, instead of being concentrated, can offer a significant advantage. To this end, a robust system providing high performance can be established by combining heterotrophic denitrification and heterotrophic sulfate reduction followed by partial oxidation. However, this approach may again result in high sludge production and also require a high organic substrate input, thereby increasing operational costs. The proposed system is important because it features low sludge production, low substrate requirement, reuse of the produced sulfur, and the final discharge of both pollutants from the system boundaries. Nevertheless, the fragile structure of the system may limit its application.

In the sulfidogenic column reactor, sulfide production depends on medium pH [26], the presence of surfaces where sulfur can adsorb, and ultimately on sulfate reduction performance. Any variation in this process, and consequently low sulfide production, directly affects nitrate removal in the sulfide-based autotrophic denitrification reactor. As a result, if nitrate reaches the sulfidogenic reactor, a nitrate/sulfate competition may arise that further reduces sulfide production [27] in the sulfidogenic column reactor. This chain reaction could result in complete performance loss of the entire system.

To prevent this, ensuring continuous denitrification even during sulfide deficiency is essential. Therefore, denitrification conditions can be maintained not only with sulfide, but also with elemental-sulfur or thiosulfate-based approaches. Elemental sulfur is a particularly attractive alternative electron donor, as it can be used as reactor filling material and dissolve when needed [28], eliminating the requirement for continuous dosing.

3.3 Variations of COD and alkalinity in the denitrifying membrane – sulfidogenic up-flow column reactor system

The column bioreactor was fed with ethanol added to the MBR effluent, and the average influent COD was 312±91 mg/L during the study. The COD concentrations in effluent of the sulfidogenic column bioreactor were averaged 197±103 and 132±43 in Period 1 and 2. COD removal efficiency in the column bioreactor exhibited an increase from 37% in the first period to 58% in the second period, despite a decrease in sulfate removal efficiency (Figure 5). This strongly suggests that heterotrophic processes are the dominant mechanism driving the high nitrate removal efficiency observed at the sulfidogenic column bioreactor effluent.

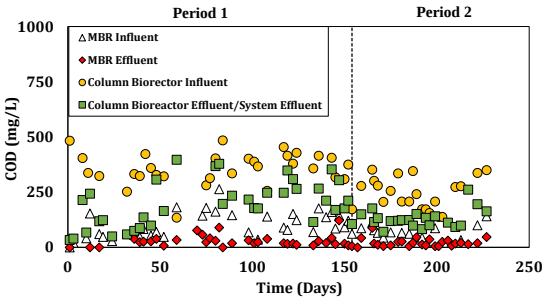

Figure 5. Variations of COD concentration in the denitrifying membrane – sulfidogenic up flow column reactor sequential system.

No external COD source was introduced into the MBR system feed. However, the recycle line transported unoxidized COD from the column reactor to the MBR. The average COD in the MBR influent was 101±59 and 76±30 mg/L in the Period 1 and 2, respectively, which was subsequently reduced to 28±26 and 22±19 mg/L in the MBR effluent. The reduction of nitrate to nitrogen gas by bacteria from the presence of organic matter is known as heterotrophic denitrification. The expected stoichiometry of an ethanol-based denitrification is presented below [29].

$$0.154NO_3^- + 0.154H^+ + 0.0833C_2H_5OH \rightarrow 0.292H_2O + 0.01C_5H_7NO_2 + 0.1167CO_2 + 0.072N_2 \quad (3)$$

According to Reaction 3, 1.77 mg ethanol is required in the ethanol-based denitrification of each gram $N\text{-}NO_3^-$. This corresponds to a requirement of approximately 3.7 mg COD for the reduction of 1 mg of $N\text{-}NO_3^-$ in the ethanol-based denitrification. Based on the measured COD concentrations in the MBR influent and effluent, an average of 19.8 and 14.6 mg $N\text{-}NO_3^-/L$ could be removed in the MBR via heterotrophic denitrification using COD carried over from the column reactor. This estimate assumes that all nitrate removal in the MBR can be attributed to this pathway.

According to Reactions 1 and 2, theoretical alkalinity production per a nitrate-nitrogen removed was estimated at 1.041 mg $CaCO_3$ for ethanol-based sulfate reduction and 3.56 mg $CaCO_3$ for sulfide-based denitrification, respectively. Alkalinity variations throughout the study are visually represented in Figure 6. No significant increase or decrease was observed in the influent and effluent alkalinity concentrations of the MBR and sulfidogenic column bioreactor.

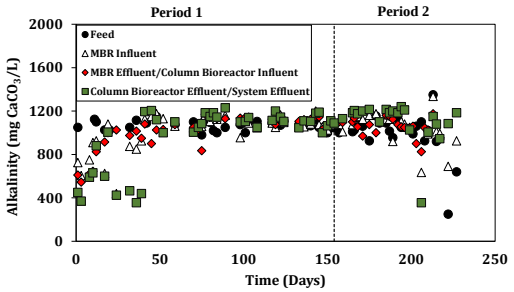

Figure 6. Variations of alkalinity concentration in the denitrifying membrane – sulfidogenic up flow column reactor sequential system.

4 Conclusion

In this study, the removal performance of high concentrations of sulfate and nitrate in groundwater by sequential autotrophic denitrification and sulfate reduction processes was investigated. Sulfate was first reduced to sulfide in an ethanol-based system and then produced sulfide was used as an electron mediator for nitrate reduction. Nitrate was almost completely removed at the system effluent but the sulfate removal efficiency of the sequential system was quite low. Ethanol-based denitrification conditions occurred in both reactors, and nitrate was reduced both partially autotrophically and heterotrophically. Operating conditions were insufficient to maintain a more dominant sulfate-reducing bacteria population in a sulfidogenic column bioreactor, and autotrophic nitrate-reducing bacteria in the MBR. Furthermore, to increase the reactor's resilience to sulfide fluctuations, an alternative electron donor should be available in the MBR in case of sulfide deficiency. Future studies could explore initially operating the denitrification reactor with elemental sulfur to promote a microbial community capable of utilizing both sulfur and sulfide, thereby maintaining nitrate removal performance during sulfide interruptions.

5 Author contribution statements

In this study, Author 1 conducted the collecting data and performing analyzes. Author 2 was responsible for the evaluation and validation of the obtained results, data visualization, and preparation of the manuscript. Author 3 contributed to the conceptualization of the research topic, supervision of the project, literature reviews, reviewing the manuscript.

6 Ethics committee approval and conflict of interest statement

"There is no need to obtain permission from the ethics committee for the article prepared"

"There is no conflict of interest with any person / institution in the article prepared"

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