



Technical Note

Simultaneous reduction of nitrate and perchlorate by sulfide-based denitrification process

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ABSTRACT

In this study, sulfide-based denitrification was used for the first time for perchlorate reduction. Perchlorates (ClO₄⁻) are the salts obtained from perchloric acid and are widely used in missile and rocket systems as well as in the automotive industry, fireworks production and some pharmaceutical industries (in the treatment of hyperthyroidism). Perchlorate prevents the iodine uptake of the thyroid gland and inhibits the production of thyroid hormones (triiodothyronine-T₃ and thyroxine-T₄). Autotrophic reduction methods are gaining popularity in the removal of perchlorate, which is often found together with nitrate in water. In this study, sulfide produced in an ethanol-based sulfidogenic reactor was used for simultaneous reduction of nitrate, nitrite and perchlorate. The study was conducted with batch reactors in 196 hours with varying concentrations of nitrate, nitrite and perchlorate in the presence of 30 mg/L total sulfide. The study showed that the perchlorate removal rate was gradually reduced in reactors containing 10 mg/L nitrite, 10 mg/L nitrate and 20 mg/L nitrite and 20 mg/L nitrate, respectively. The control group reactors revealed that sulfide is used as an electron donor and the process is biological. It was observed that sulfide, a dissolved inorganic sulfur compound, can reduce perchlorate in the presence of nitrate and/or nitrite, and the reduction rate is largely dependent on the presence of nitrate and nitrite.

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INTRODUCTION

Perchlorate is a chemical that can form in natural and anthropogenic way and can be found in rocket fuel, missiles, fireworks and flares [1]. Perchlorate can also

be found in certain bleaches and fertilizers [2]. Its high solubility (200 g/L for NH₄ClO₄) and its low adsorption properties make it easy to interact with groundwater [3].

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Perchlorate can also be produced naturally by the reaction of chlorine with ozone gas in the atmosphere and can accumulate in the earth like Atacama desert [4]. It can also be found in some minerals such as playa shell, hanksite, silvit and potash ore [5,6]. When taken into the body, it reduces the metabolism rate by reducing the thyroid hormones (triiodothyronine-T3 and thyroxine-T4). Low metabolic rate causes many diseases, especially heart diseases, in adult and adversely affected growth and development in children. These hormones are responsible for the development of the central nervous system, brain and skeletal structure [7]. However, perchlorate is not yet included in the World Health Organization (WHO), European Union, TS266 (Regulations on water intended for human consumption) and EPA drinking water standards. EPA reported a temporary limit of 24.5 µg/L, while some states in the United States set their own standards (these values ranged from 2 to 18 µg/L depending on the standard and the state) [8].

Another pollutant that can be found together with perchlorate in drinking waters is nitrate. Most important sources of nitrate in groundwater are the nitrogen containing fertilizer and sand from the release of improperly treated wastewater from industrial and domestic sources. Nitrate can be converted to nitrite in gastrointestinal and produced nitrite may react with hemoglobin in blood therefore blocks oxygen transfer. This condition is known as blue baby syndrome (methemoglobinemia). It is estimated that the mortality rate of methemoglobinemia, which occurred in infants is 8-10%. Furthermore, the metabolites of nitrosamines and nitrates can be carcinogenic [9]. Limit values of nitrate and nitrite in the WHO regulation are 50 and 3 mg/L, respectively [10].

A heterotrophic process is required for high efficient denitrification. This is also an alkalinity producing process. However, organic matter that can remain in the effluent without oxidation can be converted into disinfection by-products, or it can cause bacterial growth in distribution networks. Autotrophic denitrification methods are applied to reduce this risk [11,12]. Among several inorganic electron donor, sulfur derivatives are preferred electron sources because of reasons such as easy supply, low cost, non-explosive nature [13,14]. Elemental sulfur or thiosulfate are often used in column type autotrophic processes due to their high reducing potential and low cost.

Sulfide, on the other hand, can be produced as a result of sulfate reduction and can be present in the aqueous solutions either as free sulfide (S_2^{2-}), or as combined forms depending on solution pH. Oxidation of sulfide can result in elemental sulfur or sulfate production depending on oxygen amount. Complete oxidation of sulfide to sulfate requires eight electrons per S atom which is one of the most energetic reactions for chemoautotrophs [15]. In a study, autotrophic denitrifiers become dominant when sulfide rich anoxic sediments were treated by denitrification [16].

It has been reported that the production of intermediates such as N_2O also decreases with the use of sulfur [17,18].

Anions such as nitrate and perchlorate, which can be found in groundwater, are often associated with sulfate. Sulfide, which can be produced by sulfate reduction, can be used to remove other anions in water. For this purpose, sulfide-based nitrate, nitrite and perchlorate reduction should be investigated. In addition, there is no previous perchlorate reduction study in the literature with sulfide. When sulfate is present in the water together with nitrate, sulfate can be reduced to sulfide and then be used as electron donor in the denitrification processes [19]. However, it is not yet known that how perchlorate could affect this process and how perchlorate will be reduced when nitrate is present in the water by sulfide-based reduction. Therefore, in this study, the simultaneous reduction of nitrate and perchlorate with a sulfide-based denitrification process was investigated. Sulfide produced in a sulfidogenic reactor was extracted by the gas wash method and used as electron source in batch reactors. The reductions of nitrate, nitrite and perchlorate were monitored at different nitrate, nitrite and perchlorate levels to identify simultaneous reduction mechanism.

MATERIAL AND METHODS

Upflow Column Reactor

A column reactor with a 7 cm inner diameter and 40 cm (500 ml working volume) height was used in the study. Aquarium sand (1-2 mm) was used as the reactor filling material for microorganisms to grow. The reactor was covered with aluminum foil to prevent phototrophic growth and operated in a temperature-controlled room at 30°C. In order to collect the sulfide produced in the reactor, effluent container with nitrogen atmosphere was placed at the reactor outlet. An adjustable peristaltic pump (Ismatec™ ISM795C) was used to feed the reactor. The feed was kept at 4°C to prevent any biological degradation before the reactor.

OPERATING CONDITIONS

The sulfidogenic reactor was inoculated with sludge from an anaerobic reactor that produces biogas from pulp and paper industry wastewater and it was operated for 40 days to obtain the required amount of sulfide. During the operation, the hydraulic retention time (HRT) was set to 1 day. Ethanol was supplied to the reactor as the sole electron and carbon source. Reactor feed was prepared by adding 2567 mg/L $MgSO_4 \cdot 7H_2O$; 1479 mg/L $NaSO_4$; 56 mg/L KH_2PO_4 ; 110 mg/L NH_4Cl ; 11 mg/L ascorbic acid and 50 mg/L yeast extract to tap water. Sulfate, COD, alkalinity and sulfide analyzes were carried out from the reactor three times a week. Additionally, sulfate, COD and alkalinity analyzes were performed once a week from the feed.

Table 1. Operational conditions of reactors

	Batch Reactors					Control Group	
	1	2	3	4	5	K1	K2
NO ₃ ⁻ -N (mg/L)	20	0	10	10	0	20	20
NO ₂ ⁻ -N (mg/L)	0	20	10	0	10	20	20
ClO ₄ ⁻ (μg/L)	1000	1000	1000	1000	1000	1000	1000
Inoculum (ml)	5	5	5	5	5	-	5
S ²⁻ (mg/L)	30	30	30	30	30	30	-

Sulfur Extraction Setup

For sulfur-based nitrate and perchlorate experiments, the column reactor outlet fed with ethanol cannot be used directly since it contains unoxidized ethanol. Instead, a scrubbing process was carried out in order to extract only the sulfide content of the reactor effluent. For this process, sulfide in the reactor effluent was transported with a carrier gas (nitrogen) to distilled water. For this purpose, nitrogen gas tube, 2 gas washing bottles (Hazer glass) with a 500 ml volume were used. Gas wash bottles (Bottle 1: Sulfur donor, sulfidogenic reactor effluent; Bottle 2: Sulfur acceptor-pure water) were connected with silicone pipes. During the extraction process, pH and sulfur were monitored at 5 minutes intervals.

Batch Reactors

Batch reactors were set up for sulfide-based nitrate and perchlorate reduction. Seven sets of batch reactors were used with five different nitrate and perchlorate concentrations and two separate control groups. 50 ml falcon tubes were used as batch reactors and placed in an orbital shaker (BIOSAN OS-10, Orbital Shaker) to ensure complete mixing. Anion samples were taken from the reactors regularly and NO₃⁻-N, NO₂⁻-N, SO₄²⁻ and ClO₄⁻ analyzes were performed. Operating conditions in the reactors are presented in Table 1.

Analytical Methods

Prior to all analyzes, samples were centrifuged for 10 minutes at 3000 RPM (3024 RFC) with a Rotanta 460S centrifuge. For the pH measurement, a Hanna Instruments pH 211 pH meter was used. Alkalinity and chemical oxygen demand were measured according to Standard Methods, method no 2320-B and 5220-A (Closed Reflux, Titrimetric Method), respectively [20]. Sulfate was measured at 420 nm using a turbidimetric method in a spectrophotometer (Merck, Spectroquant Pharo 100) [21]. Total sulfide was measured as spectrophotometrically at 480 nm by the method described by CordRuwisch [22]. All anion analyzes were analyzed by ion chromatography system (Shimadzu HIC-SP) equipped with suppressed conductivity detector. DIONEX AS9 column (4×250 mm) was used for nitrate,

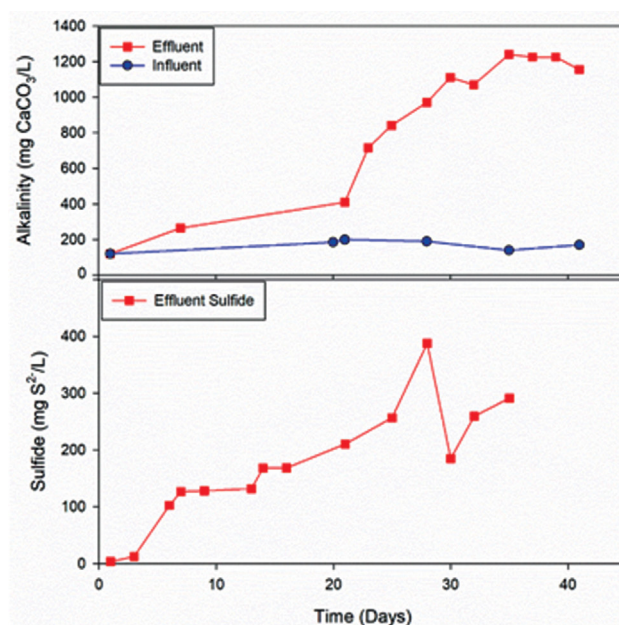


Figure 1. Alkalinity and sulfur values of the upflow sulfidogenic reactor.

nitrite, and sulfate analysis. AS20 (2×250 mm) column was used for perchlorate measurements. Detection limits for nitrate and perchlorate were 0.1 mg/L and 2 μg/L, respectively.

RESULTS

Performance of Column Reactor and Sulfur Production

The reactor was operated for 40 days in a temperature-controlled room by supplementing 1660 mg/L COD (with 743 mg/L ethanol and 122 mg/acetate) and 2000 mg/L SO₄²⁻. Sulfate and COD concentrations were decreased gradually starting from the first days of reactor operation. During the first 20 days of operation, COD was decreased from 1730 mg/L to 512 mg/L. During the study, the average effluent COD was measured as 473±117 mg/L, corresponding to a 76% COD oxidation.

Sulfate, on the other hand, showed a decreasing trend in parallel with COD oxidation. On the 20th day of the study, inlet and effluent sulfate concentrations were 2307 ± 42 and 1519 ± 45 mg/L, respectively, corresponding to a 34% sulfate reduction efficiency. At the end of the study, sulfate reduction rate was increased to 45%.

Alkalinity and sulfur followed an upward trend similar to COD and sulfate concentrations. Influent average alkalinity was 177 ± 23 mg/L CaCO_3 throughout the study. In the initial days of reactor operation, influent alkalinity was gradually increased from 120 mg/L to about 1150 mg/L. Sulfide was also gradually increased to 300 mg/L. The change in alkalinity and sulfur concentrations is presented in Figure 1.

Batch Reactors

Batch reactors containing different concentrations of nitrate, nitrite and perchlorate were examined in batch reactors for 192 hours (Figure 2). In the reactor set containing 20 mg/L NO_3^- -N total nitrogen, the influent nitrate was completely consumed within 144 hours. Additionally, nitrite formation was observed during the reduction process, but the nitrite was completely consumed at the end of the 168th hour. Perchlorate for this set showed a steady decrease trend, but at the end of the 192-hour experiment, its concentration dropped to 152 $\mu\text{g/L}$, corresponding to 85% removal efficiency.

In the second set, the influent total nitrogen concentration was 20 mg/L NO_2^- -N, and in this set, the conversion

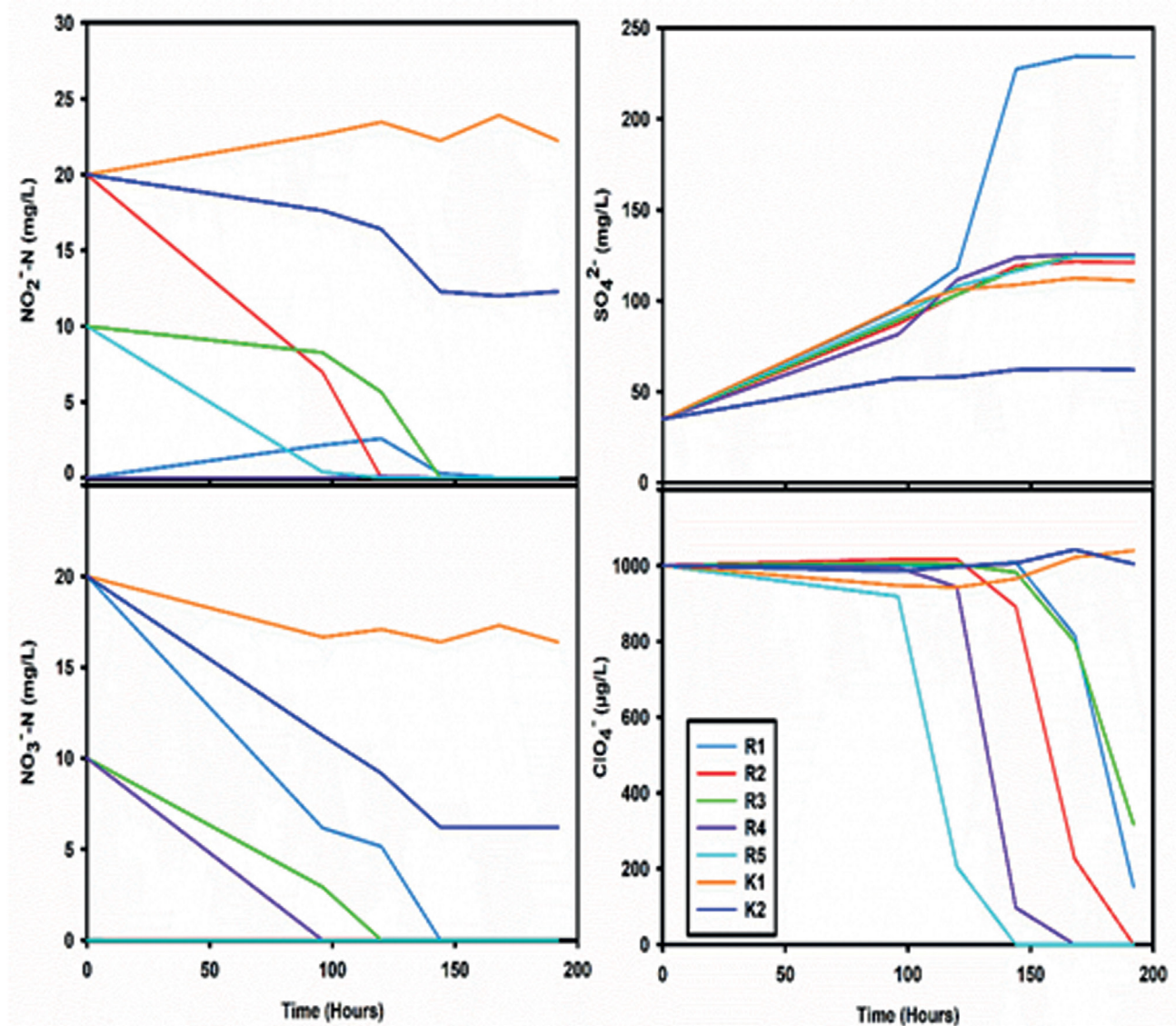


Figure 2. Nitrate, nitrite, sulfate, and perchlorate concentrations versus time in the batch reactors.

of influent nitrogen from nitrate to nitrite resulted in faster nitrogen removal. This is likely because less electrons are needed to reduce nitrite to nitrogen gas. Nitrite decreased to 6.97 mg/L at the end of 96th hours and further decreased to below the detection level in the 120th hour. Nitrate was measured at negligible levels in this set throughout the study. A similar rapid trend was observed for perchlorate reduction, and its concentration decreased from 1000 µg/L to 226 µg/L at the end of the 168th hour. At the 192nd hour, perchlorate was completely removed, and its concentration was below the detection limit.

Similar results to the first set were observed in the third set, where the total nitrogen load was given as 10 mg/L NO₃⁻ - N and 10 mg/L NO₂⁻ - N. Although nitrate and nitrite show a faster removal, perchlorate decreased from 1000 µg/L to 317 mg/L at the end of 192 hours, corresponding to a removal efficiency of 68%. It seems like the presence of nitrate has a negative effect on perchlorate reduction similar to many other simultaneous nitrate and perchlorate reduction [23,24].

In the other two sets, the influent nitrogen load was reduced to 10 mg/L, and it was given as nitrate nitrogen only in the 4th set. While effective nitrate removal efficiency was observed, perchlorate decreased from 1000 mg/L to 97 mg/L at the 144th hour measurement. Perchlorate was observed below the measurable level in the analysis performed at 168th hour. It seems like, the decrease in nitrate level had a positive effect on perchlorate removal. It is observed that perchlorate is reduced much faster compared to the first set of reactors containing 20 mg/L NO₃⁻ - N.

DISCUSSION

In the last set, the inlet nitrogen load was given as 10 mg/L NO₂⁻ - N, and 100% nitrate and nitrite removal was observed in the first 96 hours. Also 80% of the perchlorate was consumed within the first 120 hours, and complete perchlorate reduction was observed in 144th hours.

Perchlorate was detected in high concentrations in nitrate deposits in the Atacama Desert/Chili. This deposits were used as fertilizer over a century and led to nitrate and perchlorate contamination in related underground water [8]. Ammonium perchlorate is widely utilized in missile and rocket systems. When NH₄ClO₄ to water environment, ammonium could be nitrified and therefore nitrate, and perchlorate is found together in water sources. A study conducted in Hatay region proved that nitrate and perchlorate is present in water and food sources together [25].

To the best of the authors knowledge, in this study, sulfide was used for the first time in perchlorate reduction. Although there are various studies on the use of sulfide for nitrate reduction in the literature, it was determined that perchlorate can also be reduced by sulfur-based reduction process. However, it is also observed that the

process is greatly affected by the presence of nitrite and nitrate.

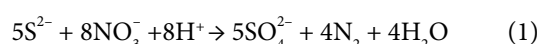
Although study shows that sulfide could be used for nitrate and perchlorate reduction, sulfide is toxic and has a rotten egg odor. Therefore, after its utilization in the reduction reactions, remaining sulfide should be removed by partial oxidation to elemental sulfur. Bekmezci et al. (2011) studied the elemental sulfur production as a result of partial oxidation of sulfide. They indicated that ORP is the key parameter that can be used to control partial oxidation [26]. In a similar way, partial oxidation could be applied to the effluent of sulfide-based reduction process.

The main advantages of sulfide-based denitrification to other forms of inorganic electron donors are the soluble nature of sulfide. As indicated in the literature, soluble forms of reduced sulfur sources have much higher reducing potential than that of insoluble forms of electron donors such as elemental sulfur.

Perchlorate reduction rate decreases in the presence of nitrate. For example, when there is 20 mg/L NO₃⁻ - N in the feed, the perchlorate reduction rate was 105.9 µg/(L.d). When the total nitrogen was given to reactor as nitrite, the perchlorate reduction rate increases to 125 µg/(L.d). Similarly, when feed was contained 10 mg/L NO₃⁻ - N, perchlorate reduction rate was 150 µg/(L.d) and it was further increased to 159 µg/(L.d) when the feed was contained 10 mg/L NO₂⁻ - N. This situation can be explained by the need for less electrons for the conversion of nitrite to nitrogen gas. Therefore, it means less competition for the electron donor in the environment.

Nitrate nitrite or perchlorate removal in the reactors containing no inoculum was negligible. Similarly, when inoculum was added without sulfide, approximately 50% nitrate and nitrite were observed with no perchlorate reduction. This indicates that sulfide was the main electron donor in the experimental sets. The reduction in the control group containing only inoculum was most probably because the organic substances (biodegradable organic substances originating from the dead microorganisms according to the Dead Regeneration Model) may have occurred in the inoculum [27].

Sulfate is generated as a result of sulfide-based reduction process. Reaction 1 below is the approximate sulfide based denitrification reaction [28].



Based on the Turkish Standard for Water Intended for Human Consumption (TS266), limit level for SO₄²⁻ is 250 mg/L. Hence, 58 mg/L NO₃⁻ - N can be theoretically reduced with sulfide-based reduction. In case of higher concentrations in the drinking water a mixotrophic process could be applied.

When the studies in the literature are examined, it is obvious that there is a competition between nitrate and

perchlorate. In a study by Coates and Achenbach, the order of priority for electron acceptors was defined as oxygen, nitrate and perchlorate [29]. Ghosh et al. (2011) stated in their study that perchlorate reduction starts in the presence of nitrate, but the complete removal of perchlorate completes after complete nitrate reduction [30]. Perchlorate reduction in sulfide-based batch studies also occurred after nitrate was completely consumed [24,31–33]. The close similarity in the reduction potential of the NO_3^-/N_2 pair with the $\text{ClO}_4^-/\text{Cl}^-$ pair ($E^\circ = 1.28 \text{ V}$) makes nitrate an excellent competitor of perchlorate [23]. Similar situations have been observed for sulfide-based perchlorate reduction, and the results are consistent with other electron sources. The study shows that sulfide could be used in simultaneous nitrate, nitrite, and perchlorate reduction. In real scale applications, sulfide can be provided in the form of Na_2S or can be produced on site as a result of sulfate reduction.

CONCLUSIONS

Complete oxidation of sulfide to sulfate requires eight electrons per S atom which is one of the most energetic reactions for chemoautotrophs. Because most groundwater contains sulfate, sulfide can be produced on site as a result of sulfate reduction. Biogenic sulfide produced by a sulfidogenic reactor was used for simultaneous denitrification and perchlorate reduction in the first time. Perchlorate reduction was greatly affected by the presence of nitrate and nitrite. In the three set reactors containing 10 mg/L $\text{NO}_2^- - \text{N}$, 10 mg/L $\text{NO}_3^- - \text{N}$ and 20 mg/L $\text{NO}_3^- - \text{N}$, perchlorate reduction rates were 159, 150 and 105.9 $\mu\text{g}/(\text{L}\cdot\text{d})$, respectively. Control group studies showed proved the sulfide based biological reduction was occurred.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

REFERENCES

- [1] Davenas A. Solid Rocket Propulsion Technology. 1st ed. New York: Pergamon Press Inc; 1993.
- [2] Collette TW, Williams TL, Urbansky ET, Magnuson ML, Hebert GN, Strauss SH. Analysis of hydroponic fertilizer matrixes for perchlorate: comparison of analytical techniques. *Analyst* 2003;128:88–97. [\[CrossRef\]](#)
- [3] Tikkanen MW. Development of a drinking water regulation for perchlorate in California. *Anal Chim Acta* 2006;567:20–25.
- [4] Catling DC, Claire MW, Zahnle KJ, Quinn RC, Clark BC, Hecht MH, et al. Atmospheric origins of perchlorate on mars and in the atacama. *J Geophys Res Planets* 2010;115:1–15.
- [5] Charnley G. Perchlorate: overview of risks and regulation. *Food Chem Toxicol* 2008;46:2307–2315. [\[CrossRef\]](#)
- [6] Orris GJ, Harvey GJ, Tsui DT, Eldrige JE. Preliminary analyses for perchlorate in selected natural materials and their derivative products. Washington. D. C.: US Government Printing Office; 2003. [\[CrossRef\]](#)
- [7] Chen HX, Shao YP, Wu FH, Li YP, Peng KL. Health survey of plant workers for an occupational exposure to ammonium perchlorate. *Chin J Ind Hyg Occup Dis* 2013;31:45–47.
- [8] Srinivasan R, Sorial GA. Treatment of perchlorate in drinking water: A critical review. *Sep Purif Technol* 2009;69:7–21. [\[CrossRef\]](#)
- [9] Ucar D, Cokgor EU, Sahinkaya E. Heterotrophic–autotrophic sequential system for reductive nitrate and perchlorate removal. *Environ Technol* 2016;37:183–191. [\[CrossRef\]](#)
- [10] Della Rocca C, Belgiorno V, Meric S. Overview of in-situ applicable nitrate removal processes. *Desalination* 2007;204:46–62. [\[CrossRef\]](#)
- [11] Sahinkaya E, Yurtsever A, Aktas O, Ucar D, Wang Z. Sulfur-based autotrophic denitrification of drinking water using a membrane bioreactor. *J Chem Eng* 2015;268:180–186. [\[CrossRef\]](#)
- [12] Ucar D, Yilmaz T, Di Capua F, Esposito G, Sahinkaya E. Comparison of biogenic and chemical sulfur as electron donors for autotrophic denitrification in sulfur-fed membrane bioreactor (SMBR). *Bioresour Technol* 2020;299:122574. [\[CrossRef\]](#)
- [13] Sahinkaya E, Yurtsever A, Ucar D. A novel elemental sulfur-based mixotrophic denitrifying membrane bioreactor for simultaneous Cr(VI) and nitrate reduction. *J Hazard Mater* 2016;324:15–21. [\[CrossRef\]](#)
- [14] Demirel S, Uyanik I, Yurtsever A, Celikten H, Ucar D. Simultaneous bromate and nitrate reduction in water using sulfur-utilizing autotrophic and mixotrophic denitrification processes in a fixed bed column reactor. *Clean Soil, Air, Water* 2014;42:1185–1189. [\[CrossRef\]](#)

- [15] Sorokin DY, Tourova TP, Galinski EA, Muyzer G, Kuenen JG. *Thiohalorhabdus denitrificans* gen. nov., sp. nov., an extremely halophilic, sulfur-oxidizing, deep-lineage gammaproteobacterium from hypersaline habitats. *Int J Syst Evol Microbiol* 2008;58:2890–2897. [CrossRef]
- [16] Shao M, Zhang T, Fang HHP. Autotrophic denitrification and its effect on metal speciation during marine sediment remediation. *Water Res* 2009;43:2961–2968. [CrossRef]
- [17] Tallec G, Garnier J, Billen G, Gossailles M. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: effect of oxygenation level. *Water Res* 2006;40:2972–2980. [CrossRef]
- [18] Capua FD. Sulfur-based denitrification of organic-deficient, acidic, low temperature and heavy metal contaminated waters in fluidized-bed reactors. Doctoral Thesis. Paris: Universite Paris-Est; 2016.
- [19] Qin Y, Wu C, Chen B, Ren J, Chen L. Short term performance and microbial community of a sulfide-based denitrification and Anammox coupling system at different N/S ratios. *Bioresour Technol* 2019;294:122130. [CrossRef]
- [20] Federation WE, Discussion G, Water A, Association W, Environment W, Discussion G. *Standard Methods for the Examination of Water and Wastewater*. 1st ed. Washington: American Public Health Association; 1999.
- [21] US EPA. Method 9038-sulfate (turbidimetric). (US Environ Prot Agency) Washington, DC, USA 1986. <https://www.epa.gov/sites/production/files/2015-12/documents/9038.pdf> (accessed January 21, 2018).
- [22] Cord-ruwisch R. A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* 1985;4:33–36. [CrossRef]
- [23] Bardiya N, Bae JH. Dissimilatory perchlorate reduction: A review. *Microbiol Res* 2011;166:237–254.
- [24] Ucar D, Cokgor EU, Sahinkaya E. Evaluation of nitrate and perchlorate reduction using sulfur-based autotrophic and mixotrophic denitrifying processes. *Water Sci Technol Water Supply* 2016;16:208–218.
- [25] Sungur S, Atan MM. Determination of nitrate, nitrite and perchlorate anions in meat, milk and their products consumed in Hatay region in Turkey. *Food Addit Contam Part B Surveill* 2013;6:6–10. [CrossRef]
- [26] Bekmezci OK, Ucar D, Kaksonen AH, Sahinkaya E. Sulfidogenic biotreatment of synthetic acid mine drainage and sulfide oxidation in an anaerobic baffled reactor. *J Hazard Mater* 2011;189:670–676. [CrossRef]
- [27] Orhon D. *Modeling of Activated Sludge Systems*. 1st ed. Pennsylvania: CRC Press; 1997.
- [28] Ghosh A, Pakshirajan K, Ghosh PK, Sahoo NK. Perchlorate degradation using an indigenous microbial consortium predominantly *Burkholderia* sp. *J Hazard Mater* 2011;187:133–139. [CrossRef]
- [29] Ucar D, Cokgor EU, Sahinkaya E, Cetin U, Bereketoglu C, Calimlioglu B, et al. Simultaneous nitrate and perchlorate removal from groundwater by heterotrophic-autotrophic sequential system. *Int Biodeterior Biodegradation* 2017;116:83–90. [CrossRef]
- [30] Ucar D, Cokgor EU, Sahinkaya E. Simultaneous nitrate and perchlorate reduction using sulfur-based autotrophic and heterotrophic denitrifying processes. *J Chem Technol Biotechnol* 2016;91:1471–1477. [CrossRef]
- [31] Ucar D, Sahinkaya E, Yilmaz T, Cakmak Y. Simultaneous nitrate and perchlorate reduction in an elemental sulfur-based denitrifying membrane bioreactor. *Int Biodeterior Biodegradation* 2019;144:104741. [CrossRef]