







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  $2307 \pm 42$  and  $1519 \pm 45$  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  $177 \pm 23$  mg/L  $\text{CaCO}_3$  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  $\text{NO}_3^-$ -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 168<sup>th</sup> 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  $\text{NO}_2^-$ -N, and in this set, the conversion

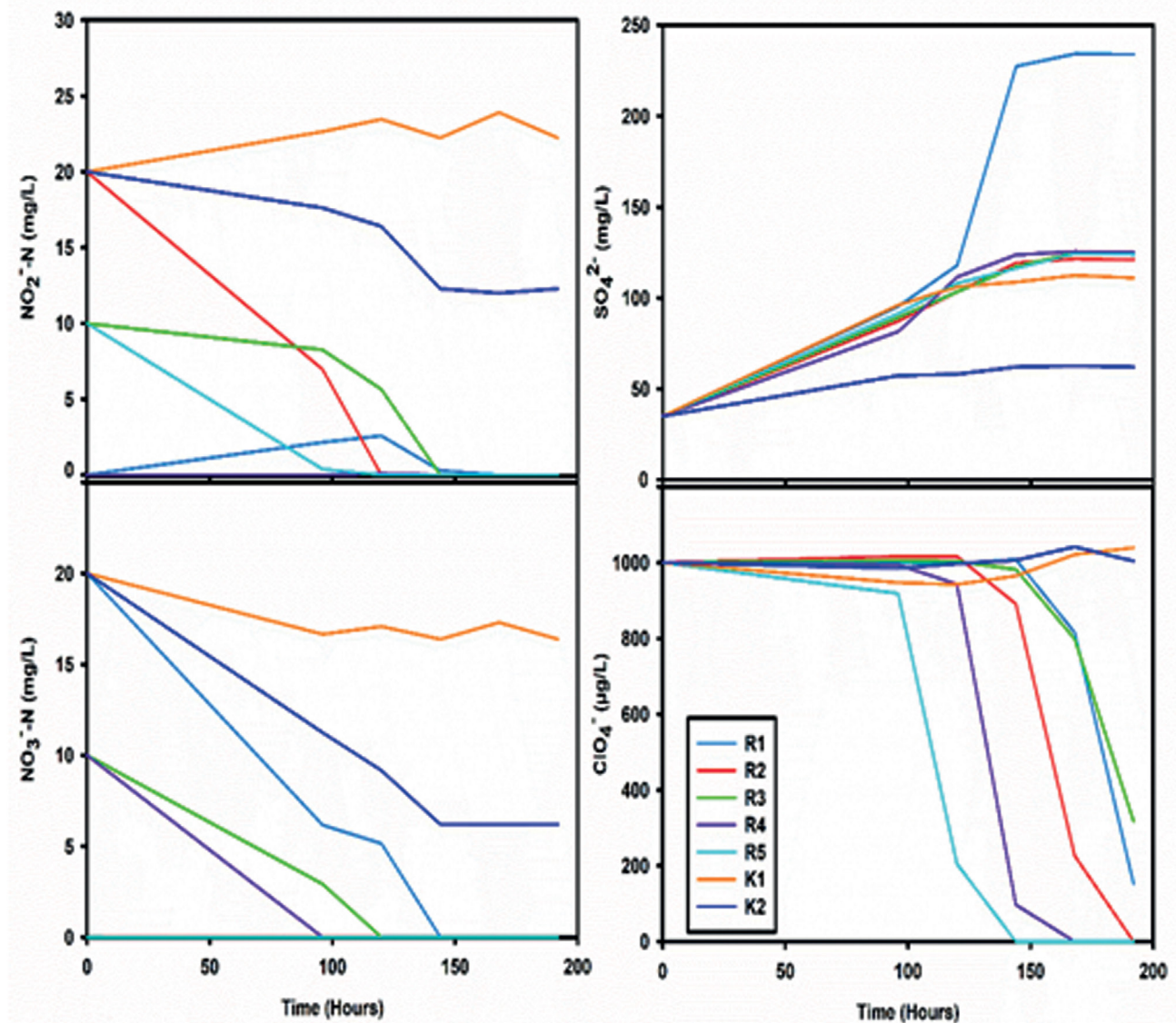


Figure 2. Nitrate, nitrite, sulfate, and perchlorate concentrations versus time in the batch reactors.

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 96<sup>th</sup> hours and further decreased to below the detection level in the 120<sup>th</sup> 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 168<sup>th</sup> hour. At the 192<sup>nd</sup> 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 NO<sub>3</sub><sup>-</sup> - N and 10 mg/L NO<sub>2</sub><sup>-</sup> - 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 4<sup>th</sup> set. While effective nitrate removal efficiency was observed, perchlorate decreased from 1000 mg/L to 97 mg/L at the 144<sup>th</sup> hour measurement. Perchlorate was observed below the measurable level in the analysis performed at 168<sup>th</sup> 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 NO<sub>3</sub><sup>-</sup> - N.

## DISCUSSION

In the last set, the inlet nitrogen load was given as 10 mg/L NO<sub>2</sub><sup>-</sup> - 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 144<sup>th</sup> 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 NH<sub>4</sub>ClO<sub>4</sub> 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.

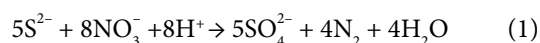
Although study shows that sulfide could be used for nitrate and perchlorate reduction, sulfide is toxic and has a rotten egg odor. Therefore, after its utilization in the reduction reactions, remaining sulfide should be removed by partial oxidation to elemental sulfur. Bekmezci et al. (2011) studied 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 NO<sub>3</sub><sup>-</sup> - 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 NO<sub>3</sub><sup>-</sup> - 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 NO<sub>2</sub><sup>-</sup> - 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 SO<sub>4</sub><sup>2-</sup> is 250 mg/L. Hence, 58 mg/L NO<sub>3</sub><sup>-</sup> - 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  $\text{NO}_3^-/\text{N}_2$  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  $\text{Na}_2\text{S}$  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.

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