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Simultaneous nitrate and perchlorate removal from groundwater by heterotrophic-autotrophic sequential system

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ABSTRACT

Perchlorate is a naturally occurring and manufactured chemical anion and can be present in water sources together with nitrate. This study aims at (1) determining the nitrate and perchlorate contamination in a semi-arid plain (Harran Plain) and (2) evaluating the performance of a heterotrophic-autotrophic sequential denitrification process for nitrate and perchlorate removal from the groundwater of this plain. The nitrate in the groundwater samples varied between 4.07 and 83.22 mg l^{-1} NO_3^--N. Perchlorate was added to groundwater samples externally and its concentration was increased from 100 to 1500 μg l^{-1}. The total nitrogen concentrations in the sequential system effluent throughout the study were always below 0.5 mg l^{-1}. C/N ratio was 2.44 which was slightly lower than the theoretical level of 2.47. Therefore the average NO_3^--N in the heterotrophic reactor effluent was 19 ± 3.7 mg l^{-1} corresponding to an efficiency of 75% reduction. The remaining nitrate and nitrite were almost completely reduced in the autotrophic process. The system's perchlorate removal efficiency was above 98%, except during the last period (82%), at which influent perchlorate was 1500 μg l^{-1}. The maximum perchlorate reduction rate throughout the study was around 15 mg/(L.d). Both perchlorate and nitrate reduction were partial in the heterotrophic reactor, but completed in the following autotrophic process.

1. Introduction

Nitrate and perchlorate can be present together in water sources, because nitrate is a common co-contaminant in surface and ground waters. Excess fertilizer utilization and discharge of wastewaters without proper treatment are some of the reasons for nitrate contamination. Nitrate in drinking water may be considered hazardous because it causes methemoglobinemia, a disorder characterized by the presence of higher than normal levels of methemoglobin (metHb, i.e., ferric [Fe^{3+}] rather than ferrous [Fe^{2+}] hemoglobin) in the blood (Idi et al., 2015). When methemoglobin forms in an infant's blood, less oxygen is carried, leading to oxygen deficiency in the tissues. This disorder is called blue-baby syndrome, due to the observable bruising and tarnishing in the skin of these infants. TS266 (Turkish Standards for Water Intended for Human Consumption) and the US-EPA recommends maximum contaminant levels of 10 mg l^{-1} NO_3^--N and 1.0 mg l^{-1} NO_2^--N for drinking water (Keskin, 2010).

Perchlorate, on the other hand, is an anion that consists of a chlorine atom at the center, surrounded by four oxygen atoms in a tetrahedral array. It has been used in rocket propellants, highway safety flares, air-bag inflators, fireworks and matches (Motzer, 2001). It naturally forms from the chemical reaction between chlorine gas and ozone and can be found in some natural materials such as Chilean saltpeter, kelp, fishmeal, hanksite, potash ore (sylvinite), and playa crust (Charnley, 2008).

Perchlorate may block iodine uptake by the thyroid. It competitively inhibits iodide from entering the thyroid by affecting the Na^+/I^- symporter. Hence, the synthesis of thyroid hormones (known as triiodothyronine-T3 and thyroxine-T4) is prevented.

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These hormones regulate basal metabolism in adults, and aid in the proper development of children. The US-EPA and some states in the US have determined the standard levels for perchlorate. In 2009, 15 μg l^{-1} was suggested as the interim health advisory level by EPA. The standard levels in some states in the US also vary between 1 and 18 μg l^{-1} depending on the state and the type of standard (Srinivasan and Sorial, 2009).

Detection of perchlorate in such low concentrations became possible with the development of new chromatographic methods (Motzer, 2001). Current technology allows perchlorate detection to 55 ng l^{-1} (Wagner et al., 2007). This technology has revealed that many drinking water sources are actually contaminated and require a wide range of monitoring studies. It is also necessary to monitor nitrate levels in the groundwater as nitrate concentrations could increase in summer and decrease in winter due to dilution.

Nitrate deposits in the Atacama Desert/Chile also contain high concentrations of perchlorate (~8000 mg kg^{-1}) (Catling et al., 2010). With the utilization of these deposits as fertilizer, perchlorate might be more commonly found in the groundwater together with nitrate. Harran plain is known for its nitrate contamination (Yesilnacar and Gulluoglu, 2008). This contamination is due to excess fertilizer utilization which affects groundwater; the fertilizer used might also contain perchlorate. Monitoring this pollution and determining the treatment approaches are, therefore, of great importance.

The extent of nitrate pollution in Harran Plain has been investigated in several studies (Yesilnacar and Gulluoglu, 2008; Yesilnacar et al., 2008). In a study conducted in 2008 some wells in Harran Plain was found to contain nitrate as high as 180 mg l^{-1} NO_3^--N and the average concentration of the whole plain was 35 mg l^{-1} NO_3^--N (Yesilnacar and Gulluoglu, 2008). In another study conducted on deep aquifers, the highest nitrate concentration was 163 mg l^{-1} NO_3^--N with an average value of 9 mg l^{-1} NO_3^--N (Yesilnacar and Yenigun, 2011). The presence of nitrate also suggests potential perchlorate pollution as reported in other studies (Ziv-El and Rittmann, 2009). However, perchlorate pollution in the Harran plain has not been studied so far.

Biological nitrate and perchlorate reduction was studied using both heterotrophic and autotrophic processes (Chiu and Chung, 2003; Karanasios et al., 2016; Sahinkaya et al., 2015). Although each process has its own advantages, the autotrophic process may have the disadvantages of sulfate and acid generation. The main disadvantage of the heterotrophic process is the contamination of the treated effluent with unused organic matter. Mixotrophic denitrification combines the advantages and eliminates the disadvantages of both processes (Uçar et al., 2015b). Single and sequential reactor systems have been reported as being used for the successful removal of both contaminants from drinking water (Ucar et al., 2016; Uçar et al., 2015b). In the previous study, the reduction rates were 1.2 g NO_3^--N/(L.d) and 12 mg ClO_4^-/(L.d) for nitrate and perchlorate, respectively (Ucar et al., 2016). Combining autotrophic and heterotrophic processes, hence, seems to be a promising approach to solve the nitrate pollution problem in Harran Plain.

Therefore the aims of this study are (1) to determine the nitrate and perchlorate contamination level of the groundwater in Harran Plain with regard to its suitability for human consumption, (2) designing and operating a biological reduction process for the successful removal of nitrate and perchlorate from the groundwater collected from Harran Plain. Nitrate, nitrite and perchlorate were monitored in 22 observation wells and the water obtained from the highest nitrate-containing well was treated in a heterotrophic-autotrophic sequential process. Additionally, PCR-DGGE and real time PCR analysis were performed to identify microbial community structure in the reactors.

2. Materials and method

2.1. The region

Turkey is currently running a project to develop water resources in the semi-arid southeastern region of the country. This project's Turkish acronym is GAP. GAP includes 22 dams in the upper Euphrates-Tigris Basin and aims to provide irrigation for 1.7 million ha of land by 2015 (Yesilnacar and Yenigun, 2011). The Harran Plain is situated in the south focal part of the GAP Project inside the Sanliurfa-Harran irrigation area. The plain is 30 by 50 km in area, and is located amongst rolling hills and expansive flatlands that spread south towards Syria. The Harran plain is the largest in the region with 141,500 ha of irrigable land, 3700 km^2 of drainage area and 1500 km^2 of plain area. The plain is approximately located at latitudes 36°43′–37°10′ North and longitudes 38°47′–39°10′ East. In order to monitor the pollution in the Harran Plain, groundwater samples from 22 observation wells were taken. These samples were subjected to NO_3^--N, NO_2^--N, SO_4^{2-}, ClO_4^- and Cl^- analysis.

2.2. Heterotrophic - autotrophic sequential process

Two up-flow column bioreactors were operated in series as shown in Fig. 1. Firstly groundwater was fed to the heterotrophic bioreactor and the effluent was then fed to the autotrophic bioreactor. Methanol and elemental sulfur were used as electron sources in the heterotrophic and autotrophic bioreactors, respectively. The total and working volumes of both bioreactors were 550 and 400 ml, respectively.

Sand particles were used as packing material for the heterotrophic reactor. In the autotrophic bioreactor, elemental sulfur was used both as an energy source and packing material. The bioreactors were inoculated with the sludge obtained from denitrifying autotrophic and heterotrophic reactors, which had been operated for more than 100 days using stimulated nitrate polluted groundwater. Dissolved oxygen in the feed was removed by bubbling the feed with N_2 gas. Feed containers were kept at 4 °C to prevent possible microbial growth. The reactors were covered with aluminum foil to prevent phototrophic growth. The reactors were fed with groundwater taken from well no 12 which contained 74.4 ± 4.10 mg l^{-1} NO_3^--N; 227.5 ± 1.25 mgl^{-1} SO_4^{2-}. Methanol and K_2HPO_4 were added to groundwater externally as a source of carbon and phosphorous for heterotrophic growth. The ground water was also supplemented with $NaClO_4^- \cdot H_2O$, since the groundwater contained no existing perchlorate. Total hydraulic retention time of the sequential process was 2 h throughout the study (1 h for each).

2.3. Operation of the reactors

The study was composed of four operational periods. In these periods perchlorate was added to groundwater samples and its concentration was increased gradually from 100 to 1500 μg l^{-1}. Groundwater sulfate concentration averaged 227.5 ± 1.25 mg l^{-1}, hence we aimed to reduce most of the nitrate using heterotrophic denitrification, not to increase sulfate concentrations higher than the standard values, as autotrophic denitrification generates sulfate. Methanol concentration, therefore, was 182 ± 3.5 mg l^{-1} throughout the study, corresponding to 2.44 mg CH_3OH/NO_3^--N. This ratio was slightly lower than the theoretical ratio of 2.47. The reactors were sampled every 2.5–3 h for the measurement of NO_3^--N, NO_2^--N, SO_4^{2-}, pH, dissolved organic carbon, alkalinity, HS^-, ClO_4^-, ClO_3^-, ClO_2^- and Cl^-.

2.4. Real-time PCR analyses

Quantitative evaluation of the bacteria responsible for the