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Microbial communities associated with uranium in-situ recovery mining process are related to acid mine drainage assemblages

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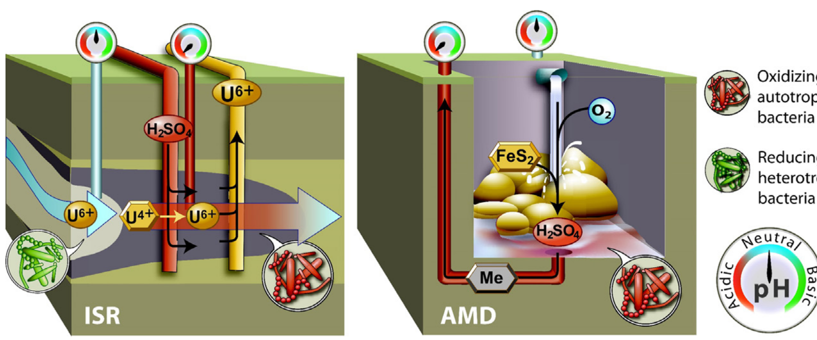
HIGHLIGHTS

- Redox gradient shaped community structures within the native aquifer zones.
- Acid injection favors acidophilic chemolithoautotrophic Bacteria and Archaea.
- This acidophilic community shared strong similarities with AMD-related communities.
- Up- and down-stream affected zones showed signs of resilience to ISR fluids.
- Assessing community structures is necessary for the setup of remediation strategies.

GRAPHICAL ABSTRACT



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ABSTRACT

A large fraction (47%) of the world's uranium is mined by a technique called "In Situ Recovery" (ISR). This mining technique involves the injection of a leaching fluid (acidic or alkaline) into a uranium-bearing aquifer and the pumping of the resulting solution through cation exchange columns for the recovery of dissolved uranium. The present study reports the in-depth alterations brought to autochthonous microbial communities during acidic ISR activities. Water samples were collected from a uranium roll-front deposit that is part of an ISR mine in operation (Tortkuduk, Kazakhstan). Water samples were obtained at a depth of *ca* 500 m below ground level from several zones of the Uyuk aquifer following the natural redox zonation inherited from the roll front deposit, including the native mineralized orebody and both upstream and downstream adjacent locations. Samples were collected equally from both the entrance and the exit of the uranium concentration plant. Next-generation sequencing data showed that the redox gradient shaped the community structures, within the anaerobic, reduced, and oligotrophic habitats of the native aquifer zones. Acid injection induced drastic changes in the structures of these communities, with a large decrease in both cell numbers and diversity. Communities present in the acidified (pH values < 2) mining areas exhibited similarities to those present in acid mine drainage, with the dominance of *Sulfobacillus* sp., *Leptospirillum* sp. and *Acidithiobacillus* sp., as well as the archaean *Ferroplasma* sp. Communities located up- and downstream of the mineralized zone under ISR and affected by acidic fluids were blended with additional facultative anaerobic and acidophilic microorganisms. These mixed biomes may

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be suitable communities for the natural attenuation of ISR mining-affected subsurface through the reduction of metals and sulfate. Assessing the effect of acidification on the microbial community is critical to evaluating the potential for natural attenuation or active bioremediation strategies.

1. Introduction

In Situ Recovery (ISR) is a mining strategy that accounts for almost half of the world's uranium production worldwide (World Nuclear Association, 2016). This alternative to conventional mining utilizes a series of injection and extraction wells to pump a leachate into a mineralized aquifer, resulting in ore dissolution, and to pump back the uranium bearing solution to the surface for further processing (Benes et al., 2001). The dissolved compound of interest (here uranium) is typically removed from solution using ion exchange columns. Further refining purifies the material into a commercial product (Morrell, 2013). ISR is considered advantageous over traditional mining techniques involving mechanical crushing and grinding because it requires lower operational costs and can be applied to relatively low-grade ores (Benes et al., 2001; Rawlings, 2002). ISR is deployed over a limited surface area and mill tailing or waste rock deposit are absent. Mining for uranium with ISR occurs in locations where the ore is deposited within a sedimentary rock or sediment layer with a relatively high hydraulic conductivity (e.g., sandstone) that lies between two relatively impermeable layers, and preferably below the water table (Benes et al., 2001). These deposits are formed when oxidized and mobile uranium precipitates within organic carbon-rich host unit, meeting reducing conditions. Alkaline ISR is used when the composition of the deposit contains typically more than 2% calcite, which corresponds to the vast majority of the cases. Else acid, a relatively dilute solution containing sulfuric acid, is used. Injection of sulfuric acid into the subsurface drastically alters the groundwater geochemistry (Saunders et al., 2016). The leachate, commonly at pH values < 2, dissolves minerals and mobilizes metals, thereby increasing the concentrations of dissolved solids in groundwater. The mobile metals have the potential to travel long distances and contaminate adjacent aquifer sections (Taylor et al., 2004; Belitz et al., 2015; Alrakabi et al., 2012). In addition to being radioactive and forming decay products, such as ^{226}radium, uranium itself exhibits toxicity. Recent examples of aquifer contamination demonstrate the urgent need to develop technologies for the removal of uranium released by mining activities (Watson et al., 2013; Williams et al., 2013; Romero-González et al., 2016).

The origin and the nature of the microbial communities associated with extremely acidic environments have been described in detail a few years ago (Johnson, 2012). These environments typically develop through the abiotic and microbial oxidation of metal sulfides in the presence of oxygen, producing sulfuric acid (Baker and Banfield, 2003; Kimura et al., 2011). Acid-mine drainage (AMD) is the highly acidic, metal-laden water produced from uncontrolled former mines (Johnson, 2012). Prokaryotes that are metabolically active in AMD have been reviewed in detail elsewhere (Johnson and Hallberg, 2003; Kimura et al., 2011; Dopson and Johnson, 2012; Volant et al., 2014). The main phyla present in these acidic habitats are *Proteobacteria*, *Nitrospira*, *Actinobacteria*, *Firmicutes*, and *Acidobacteria*, with the occasional presence of representatives of the phyla *Bacteroidetes* and TM7 (candidate phylum). Recent work on the ecology of prokaryotes involved in the oxidation of reduced sulfur compounds (such as *Sulfobacillus* sp. and *Acidithiobacillus* sp.) and metals (*Leptospirillum* sp., *Thiobacillus* sp., *Ferroplasma* sp.) in acidic environments has benefited from next-generation sequencing efforts and has shed light on community structure and biogeochemical cycling in these environments (Chen et al., 2016; Méndez-García et al., 2015; Kuang et al., 2013). Further research highlighted the ecological functioning of AMD-related communities, including aspects related to carbon cycle and molecular nitrogen fixation (Huang et al., 2016).

For ISR, acidification of the subsurface with sulfuric acid is hypothesized to contribute to the establishment of a microbial community with similarities to AMD communities. For either natural attenuation or active bioremediation to be successful post-ISR, it is critical to understand the effect of acidification on the microbial communities within the impacted groundwater. Remediation of uranium contamination typically relies on the establishment of reducing conditions in the subsurface and natural or engineered processes resulting in a reduced zone would allow the aquifer section affected by ISR to return to its original state. For instance, stimulation of anaerobic subsurface bacteria with organic matter has the potential to neutralize the acid and either create mineral species capable of uranium reduction or to directly (enzymatically) precipitate uranium species (Wall and Krumholz, 2006; Bernier-Latmani et al., 2010; Williams et al., 2013; Newsome et al., 2015). Furthermore, metals could be immobilized as sulfides precipitates (Watson et al., 2013). The goals of this study are i) to assess comprehensively the impact of ISR fluids on the autochthonous microbial communities present within an aquifer undergoing mining activities and ii) to compare these communities to those found commonly in AMD habitats.

2. Material and methods

2.1. Groundwater sampling

More than 50% of the uranium production in Kazakhstan comes from the Chu-Sarysu artesian basin (Dahlkamp, 2009). This region is a geological depression located between the Karataou and Chu-Illi mountains (Yazhikov, 1996). Samples (Table 1) were obtained from various redox compartments of the Uyuk aquifer within the Tortkuduk deposit (Eastern Chu-Sarysu), in agreement with the roll front deposit geometry.

With the exception of the “IN” and “OUT” samples, groundwater samples were taken from a depth of ca. 500 m below ground level (bgl) using a submerged pump. Sampling was carried out after stabilization of the on-line parameters measured in-situ (temperature, pH, redox, conductivity, dissolved oxygen) and the pumping out of a minimum of three well volumes. Samples were collected in 10 l plastic vessels treated beforehand with 5% hydrochloric acid for 24 h. Samples were kept in a cooled ice-box protected from light and processed within the next 4 h.

Table 1
List of samples collected from the Uyuk aquifer and the uranium collection factory. Both “upstream” and “downstream” are relative to the roll front area in which ISR is conducted.

Sample names	Sample origins	Details
UP	Uyuk aquifer	Upstream zone, native aquifer, pristine
MIN	Uyuk aquifer	Mineralized, roll-front uranium-rich, native aquifer, pristine
DWN	Uyuk aquifer	Downstream zone, native aquifer, pristine
UP-acid	Uyuk aquifer	Upstream zone, affected by ISR fluids
DWN1-acid	Uyuk aquifer	Downstream, affected by ISR fluids
DWN2-acid	Uyuk aquifer	Downstream, affected by ISR fluids
IN	Concentration factory	Inlet, before uranium concentration
OUT	Concentration factory	Outlet, after uranium concentration and before re-injection into the aquifer