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Influence of bicarbonate on the abundance of microbial communities capable of reducing U(VI) in groundwater

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In order to investigate the influence of different concentrations of bicarbonate on the abundance of microbial communities capable of reducing U(VI) in groundwater, 7 groups of experiments incubated with lactate and amended with 7 initial concentrations of bicarbonate (0, 5, 10, 15, 20, 25, and 30 mM), respectively, were conducted. It was found that U(VI) concentration failed to decrease below the Chinese uranium wastewater discharge standard (0.05 mg L^{-1}) when the initial bicarbonate concentration was higher than 10 mM. During the nitrate reduction, the abundance of nitrate reduction bacteria (*NRB*) was 22% in B0, while the abundance of *NRB* was 3.4% in B10 and B15. The main functional microbial community in B0 was *NRB Brevundimonas*, whose abundance was over 19%. During the U(VI), sulfate, and Fe(III) reduction, the abundance of sulfate and Fe reduction bacteria (*SRB* and *FRB*) was 44% in B0, while the abundance of *SRB* and *FRB* were 26% and 27% in B10 and B15, respectively. The main functional microbial communities capable of reducing U(VI) in B0 were *Cellulomonas*, *Desulfosporosinus*, and *Desulfovibrio*, whose abundance was as high as 36%. The main microbial community capable of reducing U(VI) in B10 was *Desulfitobacterium*, and its abundance was higher than 14%. The main functional microbial communities capable of reducing U(VI) in B15 were *Desulfovibrio* and *Geobacter*, and their abundance was over 13%. The overall experimental results indicate that the higher initial bicarbonate concentration leads to the lower abundance of microbial communities capable of reducing U(VI) in groundwater.

Introduction

Uranium mining and processing in the nuclear industry have resulted in contamination of groundwater which was due to the release of uranium into the groundwater.[1,2] Uranium commonly appears as soluble U(VI) or sparingly soluble U(IV) phases and exists in the anaerobic conditions of groundwater.[3,4] Soluble U(VI) can migrate with the flowing groundwater. Once it accumulates in the human's body, it will be seriously harmful for people's health.[5]

In the alkaline *in situ* leaching mining operation, bicarbonate is widely used as an agent to extract uranium from the uranium-bearing ores.[6] CO_3^{2-} or HCO_3^- in groundwater can help to form U–carbonate complexes, such as UO_2CO_3, $UO_2(CO_3)_2^{2-}$, and $UO_2(CO_3)_3^{4-}$, which resulted in the high mobility of U(VI).[7] Van Engelen *et al.* demonstrated that high concentration of bicarbonate in solution promoted the formation of negatively charged uranyl–carbonate complexes and it inhibited the toxicity of U(VI) to bacteria, but high concentration of bicarbonate reduced the abundance of microbial communities.[8] Bicarbonate which presents in the groundwater with neutral pH could decrease the absorption of U(VI) in sediments.[9]

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Previous studies demonstrated that low concentration of bicarbonate was beneficial for U(VI) bioreduction in sediment, while high concentration of bicarbonate and sulfate could inhibit the reduction.[10] Philip E. Long *et al.* found that when low concentration of bicarbonate existed in groundwater, the abundance of Ca–uranyl–carbonate complexes increased, and acetate–bicarbonate treatments could increase the rate of U(VI) reduction in the bicarbonate-impacted sediment.[11] Sheng Ling *et al.* showed that increasing the concentration of sodium bicarbonate in the system would lead to the slower kinetics for U(VI) reduction and it would have profound effects on the speciation of U(VI) in the bacterial cell envelope.[12] Obviously, these studies made it clear that high concentration of bicarbonate and sulfate would inhibit the U(VI) bioreduction, acetate–bicarbonate treatments would promote the formation of Ca–uranyl–carbonate complexes. What's more, increasing the concentration of bicarbonate would influence the reduction kinetics and the speciation of U(VI) on the bacterial cell envelope. High concentration of bicarbonate may be able to inhibit U(VI) reduction by influencing the abundance of microbial communities capable of reducing U(VI) in groundwater. However, it is still not clear what concentrations of bicarbonate affect the abundance of microbial communities capable of reducing U(VI) in groundwater. Nitrate can inhibit U(VI) bioreduction.[13] Some *SRB*, including *Desulfosporosinus* and

Desulfovibrio, have the ability of reducing U(vi),[14,15] and some *FRB*, such as *Geobacter*, can also reduce U(vi).[16] U(vi) bioreduction is often after nitrate reduction, and it usually accompanies with sulfate and iron reduction.[17] Therefore, in studying what concentrations of bicarbonate affect the abundance of microbial communities capable of reducing U(vi), the impact of *NRB*, *SRB* and *FRB* should also be taken into consideration.

Our previous study demonstrated that high concentration of bicarbonate can inhibit uranium bioreduction, but we did not know what concentrations of bicarbonate in groundwater would affect the bioreduction of uranium and influence the abundance of microbial communities capable of reducing U(vi).[18] In this paper, each microcosm was prepared with 50 g sediment and 1000 mL uranium contaminated groundwater. The sediment was taken from a monitoring well near a uranium tailings repository situated in South China, and the groundwater was also taken from the monitoring well. 7 groups of experiments, which were incubated with lactate and amended with 0, 5, 10, 15, 20, 25, and 30 mM initial concentrations of bicarbonate, respectively, were designed. Indigenous microbial communities were stimulated by sodium lactate in the anaerobic microcosms. Functional genes were quantized by qPCR. The abundance of microbial communities was analyzed using Miseq sequencing method.

Materials and methods

Sample site and sample collection

Samples were taken from a monitoring well near a uranium mill tailings repository situated in South China. The repository has a subtropical continental climate with an annual average temperature of 17.9 °C, an annual average rainfall of 1452.0 mm, an annual average evaporation capacity of 1324.5 mm, and the altitude from 210.5 m to 307.0 m above sea level. It covers an area of approximately 1.70 km², and contains approximately 188 million *t* of uranium mill tailings produced by a nearby uranium mill where the uranium ore was processed by the acid leaching from 1960s to 1990s.[19] Large amounts of waste residue and waste water, which contain nitrate, sulfate, (bi)carbonate, Ca^{2+}, Mg^{2+}, U and other heavy metal ions, and uranium tailings, were discharged into the tailings repository.[2] These discharged wastes have heavily polluted the surface environment and underground water.

Uranium contaminated groundwater was taken from the monitoring well described above, whose water level was at the depth of 10 m below the surface. The dissolved oxygen (DO) concentration of the contaminated groundwater was below 0.3 mg L^{-1}. The sediment samples were collected from the monitoring well, either. All the samples were immediately taken to the laboratory with ice bags under anaerobic conditions, and they were preserved in an anaerobic glove box at the temperature which was lower than 4 °C until use.[20] Geochemical compositions of the sediment and groundwater samples were detected before incubation, and the results were shown in Table 1.

Microcosm experiment

Experimental groups were described above. Each group of the experiment was conducted in triplicate.[12] For each of 21 sterilized microcosms, 50 g sediment and 1000 mL contaminated groundwater were both added into each of them, respectively,[21] and they were divided into 7 groups as previously described, with each group having three microcosms. 0, 0.42, 0.84, 1.26, 1.68, 2.10 and 2.52 g of sodium bicarbonate (AR) were dissolved into each group of microcosms, and each group was named as B0, B10, B15, B20, B25, B30, respectively. To make the microcosms reach balance, they were kept motionless for 24 h. The pH of solutions in all the microcosms was then adjusted to 7.0 with diluted HCl or NaOH.[12,22] After that, 2 mL sterile sodium lactate was added into each microcosm, and all microcosms were bubbled with the mixed gas of 95% N_2 and 5% H_2 for half an hour to remove DO in them. Finally, all microcosms were incubated at 20 °C in dark in the anaerobic glove box.[2]

The interval for taking solution samples was set at 3 days. All the solution samples were collected through the indwelling sampling pump of each microcosm, and they were passed through a 2 μm diameter filter for geochemical analysis. Sediment samples were collected at day 10, day 25, and day 64 after incubation for microbiological analysis.

Analytical techniques

The total U concentration was determined by ICP-MS (Agilent Technologies 7700 Series. USA), and its measurement error was ±2 ng L^{-1}. The total Fe concentration was determined by atomic absorption spectrophotometer (PerkinElmer, PinAAcle900F, USA), and its measurement error was ±0.03 mg L^{-1}. The NO_3^-, SO_4^{2-}, and CO_3^{2-} concentrations were determined by ion chromatography (ICS-900. USA), and their measurement errors were ±0.05 mg L^{-1}, ±0.1 mg L^{-1} and ±0.1 mg L^{-1}, respectively. The COD concentration was determined by COD rapid determination instrument (AWM. France), and its measurement error was ±0.1 mg L^{-1}. The metal cation

Table 1 Geochemical characteristics of sediment and groundwater samples used for microcosm experiment

Physical and chemical characteristics	Sediment (g kg^{-1})	Groundwater (mg L^{-1})
Total Fe	7.6	0.1
Mg	4.5	10.2
Ca	1.7	106.5
Mn	0.5	5.3
K	2.2	26.2
Na	0.6	47.1
Zn	1.0	0.3
Cr	0.02	0.5
U	0.1	6.5
SO$_4^{2-}$	—	2790.5
HCO$_3^-$	—	0.6
DO	—	0.3
COD	—	11.0
pH	6.0	6.5

concentrations were also determined by atomic absorption spectrophotometer.[18] The DO concentration was determined by dissolved oxygen meters (INESA JPSJ-605, China). pH was determined by pH-meter (INESA JPSJ-3F, China).

qPCR analyses

The $TM1389$, $dsrB$ and $gltA$, qPCR analyses were performed using SYBR green-based detection chemistry, and their primer pairs were BACT1369/PROK1492R, DSRq1F/DSRq1R and CS375F/CS598R, respectively.[23,24] 25 μL qPCR mixtures contained 12.5 μL of SybrGreen qPCR Master Mix, 0.5 μL of 10 μM concentrations of primer F, 0.5 μL of 10 μM concentrations of primer R, 9.5 μL of ddH$_2$O, and 2 μL cDNA templates. All qPCR experiments were conducted in triplicate. PCR amplification was performed with ABI7500 real-time PCR system.[25] The thermal cycling for activation included the initial heating at 95 °C for 10 min, and the 40 cycles with each one including melting at 95 °C for 15 s and annealing or extending at 60 °C for 1 min. Standard curves were drawn using a 10-fold dilution series of quantified plasmid $TM1389$, $dsrB$, and $gltA$ gene, respectively.

16S rRNA gene amplicon pyrosequencing

The triplicate of 16S rRNA genes was amplified from each DNA extract. Two primers (338F [ACTCCTACGGGAGGCAGCA] and 806R [GGACTACHVGGGTWTCTAAT]) were designed for PCR amplification.[26] Cycling was performed using a TransGenAP221-02 (TransStart Fastpfu DNA polymerase, 20 μL). PCRs were performed in a final volume of 20 μL mixture, which was obtained by mixing 4 μL of 5×FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forward Primer (5 μM), 0.8 μL of reverse Primer (5 μM), 0.4 μL of FastPfu Polymerase, and 2 μL of template DNA together, and diluting the mixture with sterile ddH$_2$O to 20 μL. The amplicon process consists of three steps: (a) the initial denaturation at 95 °C for 3 min; (b) 27 cycles with each one including heating at 95 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 45 s; and (c) a final extension at 72 °C for 10 min, and at 10 °C.[27] Amplifications were run by PCR instrument (ABI GeneAmp® 9700).

Sequences were clustered into OTUs (Operational Taxonomic Units) using Usearch software (version 7.1 http://drive5.com/uparse/). In order to obtain the taxonomic information of each OTU cluster, 97% of similar levels of the OTU were subjected to taxonomic-based analysis using the pipeline initial process of the RDP pyrosequencing pipeline (release 11.3 http://rdp.cme.msu.edu/).[28] The community composition of each sample was classified as phylum and genus levels by statistical analysis. Based on the statistical analysis results, diversity and abundance of microbial communities were determined for each sample. Furthermore, the heat-map was drawn to show the consequences.

Results and discussion

Geochemical characteristics of sediment and groundwater samples

To illustrate the geochemical composition of microcosms, the previous sediment and groundwater samples were detected. The measured data were shown in Table 1. Iron was very abundant in sediments, while it was not detected in groundwater. Therefore, microbial communities would reduce ferric ions in sediment to ferrous ones, and ferrous and ferric cations would migrate to groundwater, accompanied by uranium reduction.[29] Sediment and groundwater were enriched with calcium, and this resulted in the Ca–uranyl–carbonate complexes with lactate consumption. Thereby, those complexes increased the uranium mobility in groundwater (Table 2).[30] As buffer, the low concentration of bicarbonate in groundwater could decrease the toxicity of heavy metals to microbial communities.[31] The carbonate of previous groundwater was in very low concentration. However, after 64 days of incubation, the concentration of carbonate was raised to a high level due to the consumption of lactate. The higher concentration of bicarbonate previously added, the higher the carbonate concentration would be generated (Table 2). The variation of nitrate, uranium, sulfate, pH, and COD will be discussed in the text below.

Variation of nitrate, uranium, sulfate, iron, pH and COD in solution

Fig. 1 shows that nitrate concentrations began to decrease after the electron donor was added. In the first 10 days, nitrate concentrations of all groups decreased fast, and the nitrate reduction speed decreased fastest in the group without bicarbonate (B0). This phenomenon indicates that low concentration of bicarbonate is in favor of nitrate reduction.[32] Nitrate concentrations declined to the detection limit at day 22 in all groups, and they kept unchanged after that.

Uranium concentrations of all experimental groups declined from 6.5 mg L^{-1} to 4.2–5.4 mg L^{-1} after all the microcosms were kept motionless for 24 h. The uranium concentration increased with the increase of the initial bicarbonate concentration, but it does not increase proportionally with the bicarbonate concentration. The variation of uranium concentrations in the microcosms is shown in Fig. 1B. It can be observed that U(VI) was reduced slowly in the first 12 days due to nitrate inhibition,[33] and since then the concentration of U(VI) decreased fast. For these groups, the uranium concentration decreased faster in the control group (B0) than in the other groups. The final concentrations of U(VI) decreased bellow the maximum contaminant limit for uranium wastewater discharge (0.05 mg L^{-1}) by Chinese Environmental Protection Ministry (GB 23727-2009) in B0, B5, B10, and they remained stable. However, uranium concentrations

Table 2 Carbonate concentrations before and after incubation

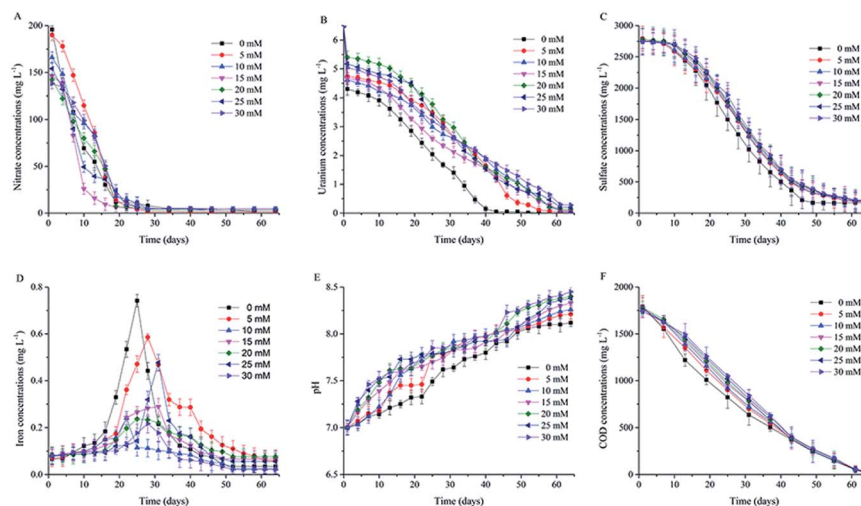| Group	Carbonate concentration (mg L^{-1})
Previous groundwater	14.7
B0	1152.2
B5	1564.6
B10	1865.9
B15	2131.7
B20	2334.2
B25	2443.4
B30	2532.5

Fig. 1 Variation of nitrate, uranium, sulfate, iron, pH and COD in solution during incubation.

remained at 0.1 to 0.28 mg L^{-1} in other groups depending on the bicarbonate concentration. This phenomenon indicates that U(VI) concentrations decreased more quickly in the microcosms amended with low concentrations of bicarbonate than in those amended with high concentrations of bicarbonate; what's more, the U(IV) was more stable in the microcosms amended with low concentrations of bicarbonate. These results obviously illustrated that bicarbonate had the inhibitory effect on U(VI) bio-reduction.[12,18] The reason why the final concentration of U(VI) was lower in B0 than in other groups was that microbial communities consumed the electron donors and the metabolic CO_3^{2-} was produced by them. The metabolic CO_3^{2-} desorbed the U(VI) that was previously adsorbed by sediments, which was also reduced by microbial communities.[11] However, bicarbonate concentrations were too high for microbial communities capable of reducing U(VI) in groups. Their reduction ability was inhibited by high bicarbonate concentrations, and they could not decrease the desorbed U(VI) concentrations to the lower level.

Fig. 1C shows that sulfate concentrations in the microcosms decreased slowly in the first 12 days, and then, they decreased faster. The concentration of sulfate in B0 stopped declining and kept stable at day 49. However, the sulfate concentrations in other groups continued declining until day 58. Fig. 1B and C show that U(VI) reduction accompanied with sulfate reduction. The reason for this was that some kinds of *SRB* could reduce U(VI) while they were reducing sulfate.[34] At the same time, it is clear that the activities of the indigenous microbial communities were inhibited significantly by the high concentration of bicarbonate.[35]

Total iron concentrations were very low before incubation in all groups. After incubation, they increased to peak values ranging from 0.21 to 0.73 mg L^{-1} between day 22 and day 31. Then, they began to decline (Fig. 1D). The reason for the increase was that iron was reduced by *FRB* in sediment; and the reduction resulted in the increase of total iron concentration.[36] The subsequent decrease was due to the precipitation of biogenic Fe(II).[37] Total iron concentrations increased faster in the low bicarbonate

concentration groups (0 mM and 5 mM) than in other groups, and their peak values were higher. This can be attributed to the growth promotion of the low bicarbonate concentration on the indigenous microbial communities.[12]

It can be seen that the pH of all the microcosms increased to 8.12–8.45 after 64 days of incubation in Fig. 1E. The reason for this was that the microbial communities continuously consumed electron donors and produced carbonate in the solutions (Table 2).[11] Significantly, the higher the previous bicarbonate concentrations, the higher the pH would be.

Fig. 1F shows the variation of COD concentrations in the microcosms. COD concentrations decreased quickly as soon as incubation began. The COD was consumed faster in B0 than in other groups since the microbial communities were more active in B0 before the sulfate reduction. After the sulfate was completely reduced, COD was only used to maintain the activities of the microbial communities.[38]

Based on the above results, we chose the control group (B0), the group whose U(VI) concentration could decrease to 0.05 mg L^{-1} (B10), and the group whose U(VI) concentration could not decrease to 0.05 mg L^{-1} (B15) for, qPCR, and PCR amplification analyses.

qPCR results

TM1389, *dsrB*, and *gltA* are the gene of *Eubacteria*, *SRB*, and *FRB*, respectively. The variations of the numbers of *TM1389*, *dsrB*, and *gltA* can reflect their growth.[25,39] In order to quantify *TM1389*, *dsrB* and *gltA*, qPCR was used to analyze sediment samples at day 0, 10, 25, 37, and 64, respectively. Fig. 2 shows that the growth of *Eubacteria*, *SRB* and *gltA* was detected at the five key time points. During uranium, iron and sulfate reducing process, the number of *TM1389* increased over time, which indicated that microbial communities were growing during this process.[40] Afterwards, it began to decrease (Fig. 2A), and the activities of the microbial communities also decreased. The number of *dsrB* was detected to be the highest at day 25 in B0, and it reached 5×10^3 copies per μL. However, the numbers of
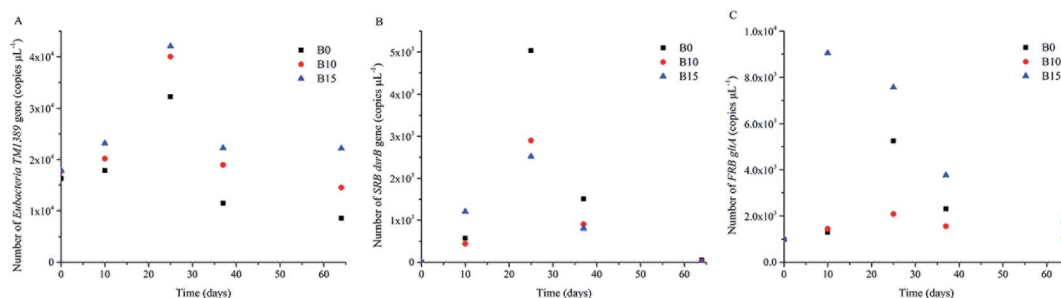
Fig. 2 Number of *Eubacteria TM1389*, *SRB dsrB* and *FRB gltA* mRNA gene copies per μL total DNA.

dsrB in B10 and B15 were just half of that in B0, which indicated that the lactate stimulated *SRB* were more active in B0 than in B10 and B15. The transcriptional peak of *dsrB* was agreeable with the result of 16S rRNA gene pyrosequencing, and they showed that the dominant genus of *SRB*, including *Desulfitobacterium*, *Desulfobulbus*, *Desulfosporosinus*, *Desulfovibrio*, and *Desulfurispora*, were detected.[26,41,42] The number of *gltA* was detected to be very high at day 10 in B15, while it was tested to be very low in B0 and B10. The transcriptional peak of *gltA* was in agreement with the result of the 16S rRNA gene pyrosequencing, and they showed that *Geobacter* and *Geothrix* were detected at day 10 in B15.[25,43] At day 25, the number of *gltA* increased to 5.2×10^3 copies per μL in B0, while the number of *gltA* decreased a little in B15. The main function microbial community was *Geobacter* in B0 and B15. This phenomenon indicated that the lactate stimulated *FRB* were more active in B15 than in B0 and B10.

Abundance of the functionalized indigenous microbial communities during bioreduction

In order to study the abundance of the functionalized indigenous microbial communities in the microcosms amended with lactate, the amplification microarray was used to analyze the preliminary sediment and the sediments of B0, B10 and B15 which were sampled at day 10 and 25, respectively. Fig. 3 illustrates that the compositions of microbial communities whose abundance is more than 1%.[44] Microbial communities, including *Bacillus*, *Brevundimonas*, *Carnobacterium*, *Enterococcus*, *Lactococcus*, *Lysinibacillus*, *Solibacillus*, *Spirochaeta*, and *Sporosarcina*, were detected in the preliminary sediment. Among these microbial communities, only *Brevundimonas* was reported to have the ability of reducing nitrate,[45] which indicated that functionalized indigenous microbial communities cannot be stimulated without adding electron donor.

At day 10, microbial communities, including *Bradyrhizobium*, *Enterococcus*, *Sphingomonas*, and *Xanthobacter*, were simultaneously found in the three groups. Among these microbial communities, *Sphingomonas* is able to bioprecipitate uranium from alkaline solutions,[46] and *Bradyrhizobium* can precipitate metallic cations.[47] Microbial communities, including *Algoriphagus*, *Anaerolinea*, *Azospirillum*, *Brevundimonas*, *Devosia*, *Flavobacterium*, *Luteimonas*, *Pseudoxanthobacter*, *Reyranella*, *Sphingopyxis*, and *Rhizobium*, were also detected in B0. Of which,

Azospirillum, *Brevundimonas*, and *Rhizobium* are nitrate reducing bacteria.[45,48,49] Some microbial communities were detected in B10, including *Bacillus*, *Bacteroides*, *Brevundimonas*, *Burkholderia*, *Dialister*, *Lactococcus*, *Lysinibacillus*, *Nevskia*, *Novosphingobium*, *Prevotella*, *Solibacillus*, and *Sporosarcina*. Among aforementioned microbial communities, *Brevundimonas* is a kind of nitrate reducing bacteria which previously mentioned, so is *Novosphingobium*.[50] *Bacteroides* is a kind of enzyme capable of reducing sulfate,[51] and *Burkholderia* can reduce the toxicity of nickel and uranium.[52] And the microbial communities were detected in B15, including *Acidovorax*, *Bacteroides*, *Burkholderia*, *Dechloromonas*, *Desulfovibrio*, *Dialister*, *Faecalibacterium*, *Geobacter*, *Geothrix*, *Lactococcus*, *Methyloversatilis*, *Nevskia*, *Prevotella*, and *Solibacillus*. Thereinto, *Acidovorax* has the ability to remove uranium from solution,[53] *Bacteroides* is a kind of enzyme capable of reducing
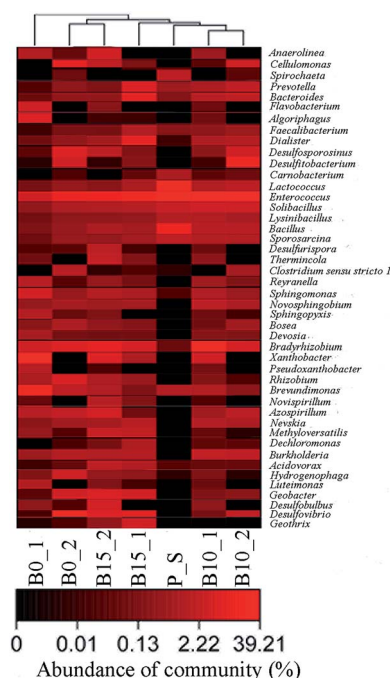

Fig. 3 Abundance of microbial communities in response to lactate and bicarbonate treatments. P_S is the preliminary sediment. B0_1, B10_1, and B15_1 indicate the functionalized indigenous microbial communities in B0, B10 and B15, respectively, at day 10; and B0_2, B10_2, and B15_2 indicate the functionalized indigenous microbial communities in B0, B10 and B15, respectively, at day 25.

sulfate which previously mentioned, *Burkholderia* can reduce nickel and uranium toxicity which is previously stated, *Dechloromonas* can reduce nitrate and oxidize Fe(II),[54] *Desulfovibrio* is a sulfate reducing bacteria which can reduce sulfate and U(VI),[15] *Geobacter* is an Fe(III)- and U(VI)-reducing bacterium,[16] *Geothrix* is a kind of iron reducing bacteria,[55] and *Methyloversatilis* can reduce nitrate to nitrite.[56] During this period, nitrate reduction proceeded faster in B0 than in the other two groups (Fig. 1A). The reason for this was that the abundance of the nitrate reducing bacteria was over 20% in B0, while the abundance of the nitrate reducing bacteria was only 3.4% in B10 and B15. The main functional microbial community was the *NRB Brevundimonas* in B0, and its abundance was up to 19%. Although *SRB* and *FRB* were detected in B15, the nitrate inhibited the reduction of U(VI), sulfate, and Fe(III) (Fig. 1B–D).[17]

At day 25, many microbial communities, including *Azospirillum*, *Bradyrhizobium*, *Burkholderia*, *Cellulomonas*, *Desulfosporosinus*, *Desulfovibrio*, *Enterococcus*, and *Solibacillus*, were simultaneously detected in the three groups. The functional microbial communities including *Azospirillum*, *Bradyrhizobium*, and *Desulfovibrio* have been described above. *Cellulomonas* is a kind of dissimilatory reduction bacteria capable of reducing Cr(VI), Fe(III), and U(VI),[57] and *Desulfosporosinus* is a kind of sulfate reduction bacteria capable of reducing sulfate and U(VI).[14] Some other microbial communities were detected in B0, including *Bosea*, *Brevundimonas*, *Clostridium_sensu_stricto_1*, *Desulfitobacterium*, *Geobacter*, *Hydrogenophaga*, and *Rhizobium*. The functions of *Brevundimonas*, *Geobacter*, and *Rhizobium*, have been described above. *Clostridium_sensu_stricto_1* can reduce nitrate and nitrite,[58] and *Desulfitobacterium* can reduce sulfate and U(VI).[59] The abundance of *SRB* and *FRB* was 44% in B0. The main functional microbial communities capable of reducing U(VI) were *Cellulomonas*, *Desulfosporosinus*, and *Desulfovibrio*, whose abundance was up to 36%. Many other microbial communities, including *Bacillus*, *Bacteroides*, *Desulfitobacterium*, *Dialister*, *Lactococcus*, *Nevskia*, *Novosphingobium*, *Prevotella*, and *Sporosarcina*, were found in B10. All the functional microbial communities including *Bacteroides*, *Novosphingobium*, and *Desulfitobacterium*, have been described before. The abundance of *SRB* and *FRB* was 27% in B10. The main functional microbial community capable of reducing U(VI) was *Desulfitobacterium*, and its abundance was up to 14%. The microbial communities including *Acidovorax*, *Anaerolinea*, *Bacillus*, *Brevundimonas*, *Desulfobulbus*, *Desulfurispora*, *Geobacter*, *Methyloversatilis*, *Novispirillum*, *Rhizobium*, *Sphingomonas*, *Thermincola*, and *Xanthobacter* were detected in B15. Among these microbial communities, the functional microbial communities, including *Acidovorax*, *Brevundimonas*, *Geobacter*, *Methyloversatilis*, *Sphingomonas*, and *Rhizobium*, have been described previously. *Desulfobulbus* and *Desulfurispora* are sulfate reduction bacteria.[42,60] The abundance of *SRB* and *FRB* was 44% in B15. The main functional microbial communities capable of reducing U(VI) were *Desulfovibrio* and *Geobacter*, and their abundance was up to 13%. During this period, U(VI) reduction proceeded faster in B0 than in B10 and B15 (Fig. 1B). The reason for this was that the abundance of *SRB* and *FRB* capable of reducing U(VI) was higher in B0 than in B10 and B15.

Sulfate reduction speed was also higher in B0 than in B10 and B15 (Fig. 1C), which was due to the fact that the abundance of *SRB* was higher in B0 than in the other two groups. Since the activity of *FRB* was the highest in B0, the concentration of the total iron in the solution was also the highest in it. These results were agreeable with the whole reduction process (Fig. 1).

Conclusions

7 groups of experiments with 7 initial concentrations of bicarbonate (0, 5, 10, 15, 20, 25, and 30 mM) were conducted, and the results showed that the U(VI) concentration could not be decreased below the Chinese uranium wastewater discharge standard (0.05 mg L^{-1}) when the initial bicarbonate concentration was higher than 10 mM. Miseq sequencing results showed that while the nitrate was being reduced, the main functional microbial community was a kind of *NRB Brevundimonas* in B0, and its abundance was 19%. Moreover, while U(VI), sulfate and Fe(III) were being reduced, it was found that the main functional microbial communities capable of reducing U(VI) were *Cellulomonas*, *Desulfosporosinus*, and *Desulfovibrio* in B0 and their abundance was higher than 36%, that the main functional microbial communities capable of reducing U(VI) was *Desulfitobacterium* in B10 and its abundance was 14%, and that the main function microbial communities capable of reducing U(VI) were *Desulfovibrio* and *Geobacter* in B15 and their abundance was over 13%. The experimental results indicate that the higher initial bicarbonate concentration leads to the lower abundance of microbial communities capable of reducing U(VI) in groundwater.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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