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Variations of dominant microbial populations in groundwater in response to the leachate from Laogang Landfill

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Abstract: Temporal changes of dominant microbial populations in groundwater in response to the leachate from Shanghai Laogang Landfill were investigated. Concentrations of dissolved redox-relevant species in groundwater suggested that the dominating redox process had changed from denitrification to methane-production/sulfate-reduction due to landfilling. Dominant microbial populations were determined using restriction fragment length polymorphism(RFLP) analyses of 16S rRNA gene libraries, which were further studied by sequencing and phylogenetic analyses. The results indicated that obvious shifts of dominant microbial populations had occurred in groundwater in response to the pollution of leachate. The closest relatives of some dominant clones are accordant with the dominating redox processes determined by hydrochemical analyses, based on the GenBank's indications on the ability to perform redox reactions.

Keywords: groundwater; landfill; 16S rRNA; microbe; redox process

Introduction

In some coastal cities of China, refuse was ultimately landfilled at seashore zones, due to cheaper land-value and less public disturbance. Shanghai Laogang Landfill(120.97° E, 31°N) is a primary example of seashore landfills. It was constructed along the shore of the East China Sea and began operation at the end of 1989. However, this landfill was constructed without the presence of appropriate liners, accordingly dramatic hydrochemical changes in underlying aquifers have been detected as a result of the high organic load of leachate.

Laogang Landfill has been monitored for a long time, and many mathematic models (Zhao, 2000; 2002) were established based on data collected. However, the impact of leachate on subsurface environments was seldom investigated, and such work is significant for a better understanding of ecological processes in leachate-polluted aquifers.

Limitations of the traditional cultivation-based techniques have restricted our knowledge of the microbiology in various ecosystems. Recently, several studies on microbial communities using 16S rRNA gene analysis have been reported(Xie, 2003). They have provided unprecedentedly detailed insights into the microbial communities. Spatial differences among microbial communities in leachate-polluted aquifers have been described using molecular approaches (Röling, 2001). However, to our knowledge, there has been no comparable investigation about variations of dominant microbial populations in groundwater subject to a landfill with time.

This paper describes temporal variations of hydrochemical conditions and dominant microbial populations in response to the pollution of leachate. Dominant microbial populations were determined using restriction fragment length polymorphism(RFLP) analyses of 16S rRNA gene libraries.

1 Materials and methods

1.1 Sampling

The Laogang Landfill was separated into many compartments by clay dams, with 10 hectares for each compartment. A spare compartment was scheduled to operate at the end of 2002, accordingly, a monitor-well which is 10 *m* downstream from the border of this compartment was chosen for our investigation. Before this compartment was put to operation, groundwater was sampled in November 2002; after the compartment began operation, groundwater was sampled twice more in January and May 2003. Samples of 3 different dates were named as A, B and C by time sequence.

Sampling procedures of groundwater: after 3 volumes of standing water of the monitor-well was removed with a special device, groundwater was collected into 500 ml pre-sterilized and nitrogen flushed PVC bottles, which were filled to capacity and tightly capped with screw-cap. Samples were transferred to our laboratory on ice and stored at 4℃ for less than 12 h. Fresh groundwater samples were filtrated with 150 mm diameter, 0.22 μm pore-size filters by air-compressing, and filters were frozen at −80℃ until DNA extraction.

1.2 Chemical analysis

At the sampling location, the temperature, pH, and dissolved oxygen were measured *in situ*, and the concentrations of H_2S and CH_4 in the headspace of the monitor-well also were measured by a portable gas analyzer.

Other hydrochemical analyses were performed following procedures described by "Chinese national measurement standards for oceanic environments (GB17378-1998)". Parameters measured included total dissolved solid, total organic carbon, SO_4^{2-}, S^{2-}, chemical oxygen demand, Cl^-, NH_4^+-N, NO_3^--N, F^-, hardness, and metals.

1.3 DNA extraction and 16S rRNA gene amplification

Filters containing microbes were cut in small pieces,

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and DNA was extracted by using Röling's method (Röling, 2000). After purifying by using the 3S DNA column purification kit (Biocolor, China), the DNA was used as a template to amplify 16S rRNA gene using the universal primers 27f and 1492r. PCR reaction mixtures as well as thermal cycling were performed under the conditions described by Martin-Laurent (Martin-Laurent, 2001) with the GeneAmp PCR System 2400 (Applied Biosystems, USA).

1.4 Construction of 16S rRNA gene libraries

The PCR-amplified 16S rRNA gene products were purified by using the 3S PCR product purification kit (Biocolor) prior to ligation. Clone libraries were constructed using the T-Vector PCR product cloning kit (Biocolor). Clones were screened with PCR for the presence of the inserts using primers (27f/1492r) in separate PCR reactions. If its length were approximate 1550 bp, the PCR product of the clone would be further analyzed by restriction fragment length polymorphism (RFLP).

1.5 RFLP analyses and sequencing

The PCR products were separately digested with restriction enzymes *Msp*I and *Rsa*I (Sibenzyme) for 2 h at 37℃. The above digests were resolved on 2% agarose gels, and a molecular weight marker was included in each gel. The gel was stained with ethidium bromide and the pattern was documented using a gel documentation system (GelDoc2000, Bio-Rad, USA). Gel images were converted, normalized, and analyzed with the Diversity Database 2.2.0 software (Bio-Rad).

Purified PCR products of the selected clones from RFLP analyses were used as the sequencing template, and DNA sequences were determined with automated fluorescent *Taq* cycle sequencing using the ABI 377 Sequencer (Applied Biosystems).

2 Results and discussion

The microbes in groundwater was investigated rather than sediment-associated microbes, due to the fact that a large portion of the sediment-bound microbes could be physically (e.g., in pores) or biologically (e.g., in biofilms) protected from the influence of environmental perturbations. Research on groundwater could generate more specific data on how variations in environmental conditions are reflected in changes of the dominant microbial populations.

2.1 Hydrochemical changes of groundwater

The values of hydrochemical parameters of groundwater sample A, B and C are shown in Table1. Leachate is rich in dissolved organic matter, inorganic species and heavy metals, which may be reflected by parameters such as COD (chemical oxygen demand), NH_4^+-N, TOC (total organic carbon), TDS (total dissolved solid), Cl^-, hardness, Pb, Ni and Cr. There were obvious increases in values of these parameters from A to C. These increases indicated that the leachate from that compartment had made a impact on the downstream groundwater.

Table 1 Hydrochemical data of groundwater sample A, B and C

Parameters	Value (in mg/L unless noted)			Parameters	Value (in mg/L unless noted)		
	A*	B*	C*		A*	B*	C*
T**	16.6℃	16℃	17℃	pH	7.1	7.0	7.0
COD_{Cr}	5.1	65.2	67.3	NH_4^+-N	0.6	15.3	16.9
TOC**	6.2	14.3	15.8	Cl^-	3723	4116	4123
Hardness	2012	2354	2524	TDS**	7546	7936	8066
F^-	0.33	0.39	0.03	Pb	0.03	0.052	0.054
Cr	< 0.004	< 0.004	< 0.004	Ni	0.011	0.019	0.01
DO**	< 0.1	< 0.1	< 0.1	NO_3^--N	33.2	1.1	0.76
Fe	5.5	7.1	6.3	Mn	0.1	0.2	0.4
SO_4^{2-}	< 8.0	15.0	15.0	H_2S***	0	10 ppm	8 ppm
S^{2-}	0	0.5	0.5	CH_4***	0	0.4 ppm	0.4 ppm

Notes: *: Sampling date of A, B and C: November 2002, January 2003 and May 2003; **: *T*: temperature; TOC: total organic carbon; TDS: total dissolved solid; DO: dissolved oxygen; ***: actual concentration in headspace of the monitor-well

Depending on the impact of the leachate and the redox-buffering capacity of aquifers, a sequence of anaerobic redox zones (e.g. methane production, sulfate, iron, manganese, and nitrate reduction) may develop in aquifers, and this sequence strongly affects the behavior of the pollutants leaching from the landfill.

Determination of the dominating redox process by composition of dissolved redox-relevant species in groundwater has been proved to be effective (Ludvigsen, 1998). After analyses of redox-relevant species (DO, NO_3^--N, Fe, Mn, SO_4^{2-}, S^{2-}, H_2S, CH_4), the dominating redox process have been roughly determined. It was estimated that nitrate (33.2 mg/L) was likely to be the dominant electron acceptor and accordingly denitrification would be a major redox process for the groundwater represented by the sample A, because the contents of DO, Fe, Mn were very low, and H_2S and CH_4 were not detected. Likewise, methane-production and sulfate-reduction were deduced to be simultaneous major redox processes for the groudwater represented by both B and C, since CH_4, H_2S were detected and the concentration of

other dissolved redox-relevant ions were low. It is apparent that landfilling has produced a profound effect on redox conditions of the underlying aquifer.

It is noticeable that the values of TDS, Cl^- and hardness are remarkably high, even before that compartment was landfilled (sample A). Leachate alone is not likely to raise these values to such a high level as referred to data from non-seashore landfills. Seawater was deduced to be responsible for this situation, because seashore aquifers should have been inevitably infiltrated by seawater with the flowing tide. It is worthy of further investigation since combined pollution by leachate and seawater have turned these aquifers into environments of special physicochemical condition.

2.2 Dominant microbial populations in groundwater samples

In the process of construction of 16S rRNA gene libraries, blue-white selection was used for screening, and 120 white colonies were randomly picked for each sample. A total of 113, 115 and 108 clones with correct inserts were selected for samples A, B and C.

A large variety of different RFLP patterns was found when the clones were analyzed by *Msp*I and *Rsa*I digestions. The combined genotype was defined as a group of sequences that have indistinguishable *Msp*I and *Rsa*I restriction patterns. Accordingly, there were 45, 49 and 48 combined genotypes to be grouped for sample A, B and C, respectively. From a statistical standpoint, each combined genotype was generally regarded as a unique population (Yu, 2001).

From 45, 49 and 48 combined genotypes in A, B and C libraries, there were 7, 8 and 7 including 4 clones or more, which been regarded as the representatives of the dominant microbial populations. Inserts of one clone from each dominant combined genotype were successfully partially sequenced ($\approx$ 600 bp). Sequences were assigned to major microbial groups and the closest known relatives were chosen by searching in GenBank databases based on BLAST similarities. Summary of phylogenetic affiliation of the dominant microbial populations are shown in Table 2.

There is strong dominance by bacteria belonging to β-Proteobacteria in the A library, while no clones showed affiliation to β-Proteobacteria, and relatively dominant microbial groups are gram-positive bacteria (e.g. *Firmicutes*, Fusobacteria) in the libraries B and C. The results indicated that there is a complete shift in the composition of the dominant populations after landfilling. On the other hand, there is no clear transition of dominant microbial populations found between the sample B and C.

Table 2 Summary of phylogenetic affiliation of the dominant microbial populations in sample A, B and C, as determined by partial 16S rDNA sequencing

Clones			Closest known relative in GenBank database		
Source	No.	Similarity, %	Name*	Accession No.	Affiliation
A	2NL-11	96	*Azoarcus tolulyticus*[a]	AF229861	β-Proteobacteria
A	2NL-16	94	*Dechlorosoma* sp. PCC	AY126453.1	β-Proteobacteria
A	2NL-24	95	*Paenibacillus azotofixans*	AJ251195	*Firmicutes*
A	2NL-49	91	*Cytophaga* sp. Dex80-64	AJ431235	*Bacteroidetes*
A	2NL-77	94	*Methylobacillus flagellatus*	gi:175379	β-Proteobacteria
A	2NL-96	92	*Pelobacter* sp. A3b3	AJ271656	δ-Proteobacteria
A	2NL-116	92	*Nitrosomonas* sp. JL21	AB000700	β-Proteobacteria
B	3JL-26	96	*Methylosarcina fibrata*[c]	AF177296	γ-Proteobacteria
B	3JL-65	98	*Propionigenium modestum*	gi:45626	*Fusobacteria*
B	3JL-66	97	*Flavobacterium limicola*	AB075232	*Bacteroidetes*
B	3JL-73	91	*Polyangium cellulosum*	AY039304	δ-Proteobacteria
B	3JL-88	93	*Aminobacterium colombiense*	AF069287	*Firmicutes*
B	3JL-93	96	*Ilyobacter polytropus*	AJ307981	*Fusobacteria*
B	3JL-102	95	*Dethiosulfovibrio marinus*[b]	AY005466	*Firmicutes*
C	3ML-8	94	*Methylocaldum szegediense*[c]	gi:2911788	γ-Proteobacteria
C	3ML-19	96	*Desulfuromonas thiophila*[b]	gi:2326420	δ-Proteobacteria
C	3ML-33	95	*Propionigenium maris*	gi:8574387	*Fusobacteria*
C	3ML-45	96	*Methylosarcina quisquiliarum*[c]	AF177297	γ-Proteobacteria
C	3ML-89	90	*Anaerobaculum mobile*	AJ243189	*Firmicutes*
C	3ML-92	94	*Aminobacterium colombiense*	AF069287	*Firmicutes*
C	3ML-109	94	*Cellulophaga fucicola*	AJ005973	*Bacteroidetes*
C	3ML-119	94	*Dethiosulfovibrio peptidovorans*[b]	gi:1777783	*Firmicutes*

Notes: * : According to the GenBank's indications, the bacteria with superscript a, b, c have the ability to perform reactions of denitrification, sulfate reduction and methane oxidation, respectively

Based on the GenBank's indications of the closest relative of a retrieved sequence, a possible biochemical role of the bacterium represented by the sequence could be surmised. It should be noted that 1 sequence related to

genera capable of denitrification (*Azoarcus* related) were retrieved. Remarkably, 3 sequences are closely related to sulfate reducers. Three sequences are associated with the methanotrophic bacteria though no sequence affiliated with methanogenic bacterium was encountered. This situation is in accordance with major redox processes in these environments implied by hydrochemical analyses. It was found that several redox processes could take place simultaneously in many cases (Ludvigsen, 1998), which is consistent with coexistence of sulfate-reducing bacteria and methanotrophic bacteria in groundwater samples B and C.

A phylogenetic description of dominant microbial populations is an important step in understanding of ecological processes in aquifers beneath a landfill. However, phylogenetic affiliation alone is not sufficient to firmly connect certain microbial taxa with a biochemical reaction, therefore we can only hypothesize about its possible biochemical role. On the other hand, evidences from other aspects can be used to support the validity of our speculations. It is reasonable that more sequences are related to complex-compound-degrading fermentative bacteria in the samples B and C. For example, 2 sequences (3JL-88 and 3ML-92) are affiliated with *aminobacterium colombiense*, an aminoacid-degrading obligate anaerobe. A sequence (3JL-93) is affiliated with *Ilyobacter polytropus*, a fermentative Fusobacterium which specialized in the degradation of hydroaromatic compounds. In this study, it is significative that phylogenetic information showing variations of dominant microbial populations is in accordance with hydrochemical changes of groundwater on the whole.

3 Conclusions

The results indicated that obvious changes of dominant microbial populations had occurred in groundwater in response to leachate from Laogang Landfill. Concentration variations of dissolved redox-relevant species suggested that the dominating redox process had changed from denitrification to methane-production/sulfate-reduction. The dominating redox process determined is accordant with the closest relatives of some dominant clones, based on their indications on the ability to perform redox reactions in the GenBank database.

References:

Ludvigsen L, Albrechtsen H J, Heron G *et al*., 1998. Anaerobic microbial redox processes in a landfill leachate contaminated aquifer (Grindsted, Denmark) [J]. J Contam Hydrol, 33: 273—291.

Martin-Laurent F, Philippot L, Hallet S *et al*., 2001. DNA extraction from soils: old bias for new microbial diversity analysis methods[J]. Appl Environ Microbiol, 67: 2354—2359.

Röling W F M, Breukelen B M *et al*., 2000. Analysis of microbial communities in a landfill leachate polluted aquifer using a new method for anaerobic physiological profiling and 16S rDNA based fingerprinting[J]. Microb Ecol, 40: 177—188.

Röling W F M, Breukelen B M, Braster M *et al*., 2001. Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer[J]. Appl Environ Microbiol, 67: 4619—4629.

Xie B, Kang K, 2003. Uptake of copper ion by activated sludge and its bacterial community variation analyzed by 16S rDNA[J]. Journal of Environmental Sciences, 15(3): 328—333.

Yu Z, Mohn W W, 2001. Bacterial diversity and community structure in an aerated lagoon revealed ribosomal intergenic spacer analysis and 16S ribosomal DNA sequencing[J]. Appl Environ Microbiol, 67: 1565—1574.

Zhao Y, Liu J, Huang R, 2000. Long-term monitoring and prediction for leachate concentrations in Shanghai refuse landfill[J]. Water Air and Soil Pollution (The Netherlands), 122(3—4): 281—297.

Zhao Y, 2002. Excavation and characterization of refuse in closed landfill[J]. Journal of Environmental Sciences, 14(3): 303—308.

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