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Microarray-based analysis of survival of soil microbial community during ozonation

Jian Wang[1, 2, 3], Joy D. Van Nostrand[3, 4], Zhili He[3, 4], Liyou Wu[3, 4], Ye Deng[3, 4], Xu Zhang[1, 2], Jizhong Zhou[3, 4], Guanghe Li[1, 2]

[1] Department of Environmental Science and Engineering, Tsinghua University, Beijing 10084, China [2] State Key Joint Laboratory of Environmental Simulation and Pollution Control, Tsinghua University, Beijing 100084, China
[3]Institue for Environmental Genomics, University of Oklahoma, Norman, OK 73019 [4] Virtual Institute for Microbial Stress and Survival, http://vimss.lbl.gov

ABSTRACT

A 15 h ozonation was performed on bioremediated soil to remove recalcitrant residual oil. To monitor the survival of indigenous microorganisms in the soil during in-situ chemical oxidation (ISCO) culturing and a functional gene array, GeoChip, was used to examine the functional genes and structure of the microbial community during ozonation (0 h, 2 h, 4 h, 6 h, 10 h and 15 h). Breakthrough ozonation decreased the population of cultivable heterotrophic bacteria by about 3 orders of magnitude. The total functional gene abundance and diversity decreased during ozonation, as the number of functional genes was reduced by 48% after 15 h. However, functional genes were evenly distributed during ozonation as judged by the Shannon-Weaver Evenness index. A sharp decrease in gene number was observed in the first 6 h of ozonation followed by a slower decrease in the next 9 h, which was consistent with microbial populations measured by a culture based method. Functional genes involved in carbon, nitrogen, phosphors and sulfur cycling, metal resistance and organic remediation were detected in all samples. Though the pattern of gene categories detected was similar for all time points, hierarchical cluster of all functional genes and major functional categories all showed a time-serial pattern. Bacteria, archaea and fungi decreased by 96.1%, 95.1% and 91.3%, respectively, after 15 h ozonation. *Deltaproteobacteria*, which were reduced by 94.3%, showed the highest resistance to ozonation while *Actinobacteria*, reduced by 96.3%, showed the lowest resistance. Microorganisms similar to *Rhodothermus*, *Obesumbacterium*, *Staphylothermus*, *Gluconobacter* and *Enterococcus* were dominant at all time points. Functional genes related to petroleum degradation decreased 1~2 orders of magnitude. Most of the key functional genes were still detected after ozonation, allowing a rapid recovery of the microbial community after ozonation. While ozone had a large impact on the indigenous soil microorganisms, a fraction of the key functional gene-containing microorganisms survived during ozonation and kept the community functional.

BACKGROUND

■ ***In situ* ozonation** has been proved to be an effective method to remediate organic pollutants in soil, such as PAHs, diesels. Also it was shown to be feasible to remove absorbed hydrophobic organic compounds (HOCs) and high molecular weight residual oil.

■ Ozone can rapidly remove contaminants through direct or radical pathways and produce more biodegradable intermediates. Therefore, **integrated chemical–biological treatment** has commonly been used.

■ **Sterilization of soil microorganisms** is one of major concerns for the integrated treatment. As a wide used disinfection reagent, ozone inactivates microorganisms by reacting with intracellular enzymes, nucleic acids, and components of the cell envelope, spore coats, or viral capsids.

■ **Understanding of survival of indigenous microorganisms during ozonation** is necessary to better develop a combined remediation strategy.

METHODS

■. **Soil**

- Contaminated by crude oil, sampled from Daqing oil field, China
- Bioremediated for more than 600 d
- Total solvent extractable matters (TSEM) was 27 mg/g

■ **Ozonation**

- 100 g soil, passed 1.25 mm sieve
- Soil column experiments, ozone was injected upwards
- O_3 flow rate-500 mL/min, inlet concentration-44 ± 2.6 mg/L
- Ozonatioin time- 0 h, 2 h, 4 h, 6 h, 10 h, 15 h

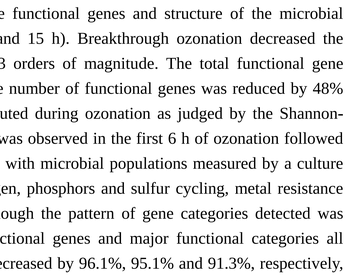

Fig. 1. Ozone breakthrough curve (BTC) and TSEM removal during ozonation

■. **Microarray-based analysis**

- Soil DNA was extracted by freeze-grinding method (Zhou et al., 1996). Purified DNA was amplified using a whole community genome amplification (WCGA) method (Wu et al., 2006). The amplification products were labeled with Cy-5 using random priming method.
- **Geochip 3.0**, a comprehensive functional gene array (FGA), containing more > 28 000 probes covering > 24 000 genes in >290 groups, including functional genes involved in cycling of C, N, S and P, metal reduction and resistance, and in organic remediation, was used to examine the functional genes of soil microbial community.
- After overnight hybridization at 42 ºC, microarrays were scanned on a ScanArray 5000 Microarray Analysis System (Perkin-Elmer, Massachusetts, USA) at 90% laser power and 80% PMT. Signal intensity of each spot was determined by ImaGene 6.1 (Biodiscovery Inc., CA, USA). Spots with signal-to-noise ratio [SNR = (signal intensity-background intensity)/background standard deviation] <2.0 were removed.
- A common oligo reference standard (CORS) was used to normalized the data (Liang et al., 2010). Then Signal intensity of each gene was normalized by raw DNA yield so that normalized signal had a unit of signal intensity per gram of soil.

RESULTS

● **Overall functional gene diversity**

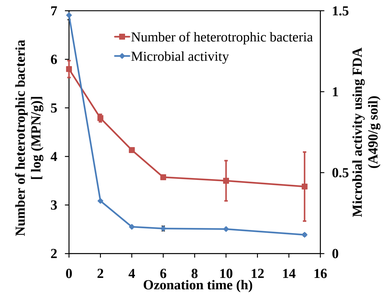

Fig. 2. Change of population and activity of soil indigenous microorganisms during ozonation. Culture-based method showed population of microorganisms decreased by > 2 orders of magnitude.

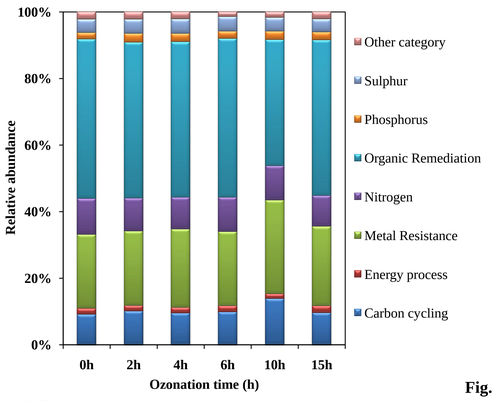

Fig. 3. Relative abundance of all functional gene categories detected. The total number of genes detected at each time point was used to calculate the relative abundance of each gene group.

Table 2. Functional genes detected, overlap (italicized), unique (bold), diversity indices

	0 h	2 h	4 h	6 h	10 h	15 h
0 h	**24.2%**	*46.3%*	*39.0%*	*36.7%*	*32.9%*	*33.8%*
2 h		**16.9%**	*41.6%*	*38.0%*	*36.7%*	*36.2%*
4 h			**13.5%**	*37.3%*	*39.0%*	*38.7%*
6 h				**11.2%**	*36.6%*	*39.7%*
10 h					**13.9%**	*40.0%*
15 h						**11.5%**
No. gene detected	2814	2344	1818	1544	1471	1459
Shannon-Weaver (H')	7.94	7.75	7.50	7.33	7.29	7.28
Shannon-Weaver Evenness	0.9997	0.9996	0.9996	0.9997	0.9998	0.9997

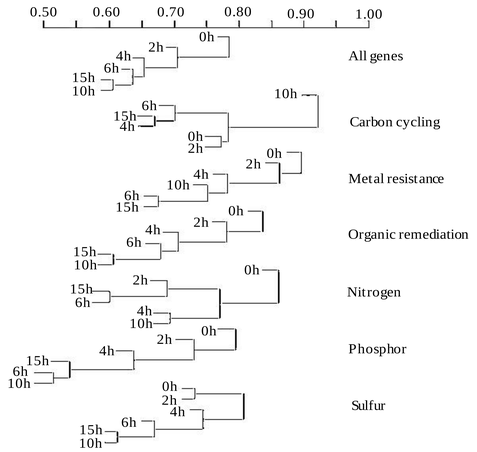

Fig. 4. Hierarchical cluster analysis of all functional genes and major functional categories. Though gene category structure showed similar pattern for all time points, hierarchical cluster analysis of all functional genes and major functional categories all showed time-serial pattern. Functional genes of 4 h 6 h, 10 h and 15 h samples showed more similarity than those of 0 h and 2 h, and functional genes of 6 h, 10 h and 15 h clustered together for most of categories.

Microarray results showed that the number of functional genes was reduced by 48% after 15 h. A sharp decrease of gene number was observed in the first 6 h ozonation followed by a slower decrease in the next 9 h, which was consistent with culture-based method. Microbial diversity decreased during 15 h ozonation as indicated by Shannon-Weaver index, while Shannon-Weaver Evenness indices for all the samples were close to 1, indicating functional genes were evenly distributed during ozonation.

● **Change of functional genes involved in oil remediation during ozonation**

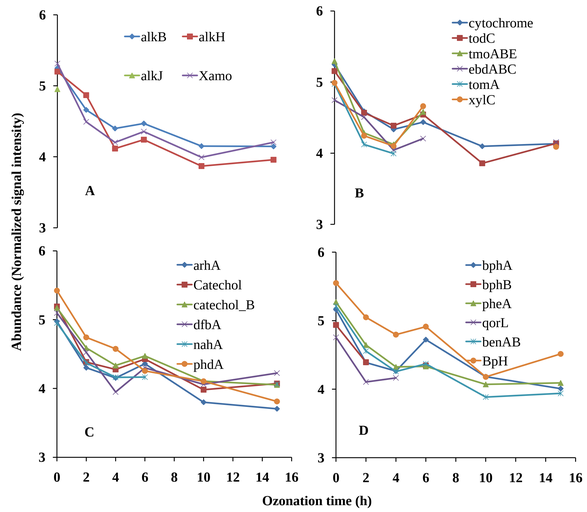

Fig. 6. Abundance of oil remediation related genes in ozoned soils. (A) Chain hydrocarbon degradation genes, (B) BETX related degradation genes, (C) PAH degradation genes, (D) Other aromatics degradation genes. 17 out of 22 petroleum degrading genes remained detected during 15 h ozonation process. Signal intensity of these genes decreased by 1~2 orders of magnitude with different sensitivity to ozone.

● **Phylogenetic diversity**

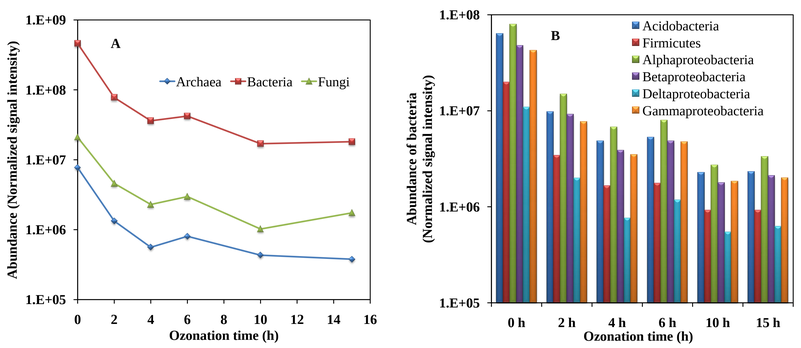

Fig. 5. Abundance of bacteria, archaea and fungi (A) and classes of bacteria (*Actinobacteria*, *Firmicute*, *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria*) during ozonation based on total signal intensity of detected by Geochip hybridization. Bacteria, archaea and fungi decreased by 96.1%, 95.1% and 91.3% after 15 h ozonation. Fungi were the most resistant to ozone while bacteria were the least. Abundance of all classes of bacteria decreased and significant negative relationships were observed between abundances and ozonation time (P = 0.02~0.04). *Deltaproteobacteria* which were reduced by 94.3% showed highest resistance while *Actinobacteria* which both were reduced by 96.3% showed lowest resistance to ozonation. Microorganisms similar to *Rhodothermus*, *Obesumbacterium*, *Staphylothermus*, *Gluconobacter* and *Enterococcus* were dominant at all the time points.

SUMMARY & CONCLUSION

■ Ozone had a big impact on indigenous microorganisms in soil. It will decrease microbial diversity and change the community structure.

■ Different microorganisms and functional genes showed different tolerances to ozone. No microorganism was found to be completely resistant to ozone.

■ Soil might provided a shelter preventing microorganism from thorough dying off.

■ Most of the key functional genes were still detected after ozonation, allowing a rapid recovery of the microbial community after ozonation.

■ While ozone had a large impact on the indigenous soil microorganisms, a fraction of the key functional gene-containing microorganisms survived during ozonation and kept the community functional.

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Contact information: wang-jian02@mails.tsinghua.edu.cn; jzhou@ou.edu; ligh@tsinghua.edu.cn