



# In-situ Control of Typical Taste & Odor Matters in River Sediments and Identification of Functional Bacteria Species

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## ABSTRACT

This paper conducted an in-situ control project in Shenzhen River located along the boundary of Hong Kong and Shenzhen in China. Main odor matters included acid volatile sulfide (AVS), geosmin (GEM) and 2- methylisoborneol (2-MIB). This project demonstrated that appropriate injection at 1.68g-N/g-AVS can obtain 95% removal of AVS within two weeks. In addition, the heavy metal ions did not release to overlying water body, the microbiological toxicity reduced substantially. Although injection of calcium nitrate can also remove both GEM and 2-MIB, its efficiency was not as good as on AVS. Microbial communities varied distinctly on 7th day during remediation with nitrate. *Proteobacteria* increased distinctly in sediments, major bacteria from the taxa of  $\beta$ -,  $\epsilon$ -, and  $\gamma$ -*proteobacteria*. *Thiobacillus* was the most abundant species for AVS removal followed by *Sulfurimona* belonging to  $\epsilon$ -*proteobacteria* and *Rhodanobacter* from  $\beta$ -*proteobacteria*. The results on bacterial community structure and its function provide the scientific principles for engineering control of odor in sediment of Shenzhen River.

## INTRODUCTION

Shenzhen River, 37 km long with watershed area of 3125 km<sup>2</sup>, is located in the south of China (Fig.1). A pilot-scale in situ remediation operation with nitrate injection was conducted within two representative regions of Shenzhen River, which were severely contaminated by AVS (acid volatile sulfide). The two regions were located on the Luohuqiao (Region 1) and Lingang community (Region 2). There were two neighboring sections in each region, including one treated section (A) and one control section (C). The area of each section in Region 1 (1A and 1C) was 400 m<sup>2</sup> and the area in Region 2 (2A and 2C) was 2500 m<sup>2</sup>. Sediment properties of experimental regions were presented in Table 1. Calcium nitrate was evenly injected into sediments of the sections of regions 1 and 2 with a treatment depth of around 60 cm (Figure 2). The nitrate dosage in the sections of 1A and 2A were 3600 and 4053 mg N/kg dry sediment, respectively, calculated by the AVS content in the sediments. No calcium nitrate was injected into the sections of 1C and 2C, which was used as the control areas to compare with the treated areas. Core sediments were collected from each section before nitrate injection, on the 7<sup>th</sup> and 14<sup>th</sup> after nitrate injection. There was no sediment collection for 1C and 2C on 7<sup>th</sup> d.



Figure 1 Location of experimental regions

Items	Region 1	Region 2
Density (g/cm <sup>3</sup> )	1.31 ± 0.10	1.38 ± 0.11
Water content (%)	63.38 ± 1.9	55.31 ± 4.4
AVS (mg N/kg dry sediment)	3056.63 ± 222.2	3060.63 ± 301.3
Nitrate (mg N/kg dry sediment)	641 ± 55.8	313 ± 6.1
Total organic carbon (S w/w)	3.86 ± 0.06	3.80 ± 0.52
Sediment oxygen demand (mg O <sub>2</sub> /kg dry sediment)	9206 ± 773.2	8194 ± 1273.3
Particle size distribution (%)		
<0.063 mm	89.96 ± 0.76	84.62 ± 0.59
0.063-0.125 mm	3.90 ± 0.01	4.77 ± 0.58
0.125-0.25 mm	1.68 ± 0.06	2.19 ± 0.43
0.25-0.5 mm	1.36 ± 0.11	2.22 ± 0.45
0.5-1 mm	1.15 ± 0.06	1.51 ± 0.43
1.06-2.5 mm	1.19 ± 0.04	2.11 ± 0.09
2.43-4.0 mm	0.65 ± 0.03	1.39 ± 0.35
>4 mm	0.11 ± 0.04	1.18 ± 0.02

Figure 2 Sediments properties of experimental regions



Figure 3 Injection vessel in demonstration spot

## RESULTS & DISCUSSION

The monitoring result of sediment properties after dosing nitrate, including pH, ORP, AVS, TOC and nitrate content were shown in Table 2. During 14 d since remediation began, most of the nitrates, 79.7% for 1A and 93.3% for 2A, respectively, were consumed. The removal ratio of AVS for 1A and 2A were 92.4% and 80.6%, respectively. This means that remediation of river sediment for the AVS control can be quickly completely in 14 d. During this period of time, increase in ORP and decrease in pH values were observed. There was a slight decrease of TOC content with removal rate of 4.4% and 12.7% for 1A and 2A, respectively.

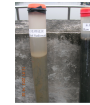


Figure 4 Remediation effect on sediment from treated section (left) compared with control (right)

Table 1 Variation of sediment properties after dosing nitrate calcium

Samples	ORP (mV)	pH	AVS (mg S/kg dry weight)	TOC (%)	Nitrate (mg N/kg dry weight)
1A0	-2440 ± 6.2	7.47 ± 0.07	3202.25 ± 193.7	4.10 ± 0.21	3816.79 ± 127.3
1A7	-1905 ± 12.6	7.31 ± 0.05	1731.15 ± 112.7	4.05 ± 0.48	1543.18 ± 118.4
1A14	-1535 ± 7.4	7.11 ± 0.04	244.00 ± 192.7	3.92 ± 0.79	77460 ± 124.8
2A0	-2342 ± 4.6	7.34 ± 0.08	2241.25 ± 219.8	3.63 ± 0.56	4106.28 ± 138.8
2A7	-2231 ± 11.3	7.01 ± 0.06	1520.82 ± 160.9	3.45 ± 0.82	135416 ± 102.7
2A14	-1453 ± 9.5	7.03 ± 0.02	627.50 ± 114.7	3.17 ± 0.23	27300 ± 86.9
1C0	-2480 ± 5.8	7.40 ± 0.03	2911.00 ± 145.4	3.63 ± 0.16	8240 ± 10.6
1C14	-2587 ± 16.2	7.35 ± 0.09	3065.53 ± 155.6	3.82 ± 0.25	7497 ± 15.5
2C0	-2280 ± 14.7	7.36 ± 0.01	2880.00 ± 136.3	4.17 ± 0.43	2895 ± 6.7
2C14	-2395 ± 8.9	7.23 ± 0.08	2895.02 ± 236.4	4.07 ± 0.66	3130 ± 6.2

The first number of samples name represent experiment region; "A" represent nitrate injection; "C" represent no nitrate addition; the number 0, 7, and 14 represent before nitrate injection, 7 days after nitrate injection, and 14 days after nitrate injection, respectively.

Bacterial sequences affiliated with *Proteobacteria* (44.09% on average) were the most abundant followed by the sequences affiliated with *Firmicutes* (7.22% on average) and *Chloroflexi* (6.70% on average) (Figure 5). Microbial community composition varied distinctly on the 7<sup>th</sup> d during remediation, when *proteobacteria* increased distinctly as the most abundant in sediments, mainly the taxa of  $\beta$ -,  $\epsilon$ -, and  $\gamma$ -*proteobacteria* (Figure 6). The species belonging to seven phyla (*Planctomycetes*, *Chloroflexi*, *BCR1*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, and *Chlamydiae*) and three classes (*Acidobacteria*, *Thermomicrobia*, and *Delta-proteobacteria*) were significantly abundant in sediments before nitrate injection (Figure 7). *Thiobacillus* was the most abundant species for AVS removal followed by *Sulfurimona* belonging to  $\epsilon$ -*proteobacteria* and *Rhodanobacter* from  $\beta$ -*proteobacteria*. The microbial community richness and diversity decreased on the 7<sup>th</sup> d after nitrate injection, but revived on the 14<sup>th</sup> d when the remediation process tended to be finished.

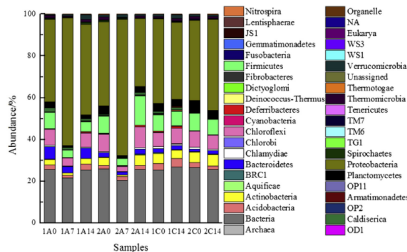


Figure 5 Phylogenetic distribution of sequences assigned on phylum

## RESULTS & DISCUSSION

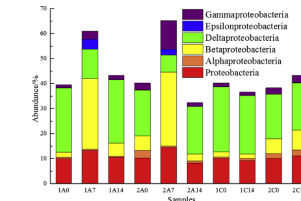


Figure 6 Phylogenetic distribution of sequences assigned on classes of *Proteobacteria*

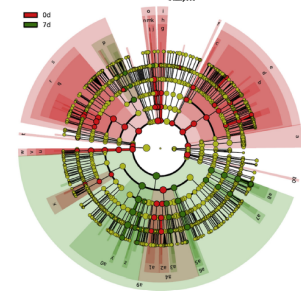


Figure 7 LefSe cladogram of comparison result between samples before nitrate injection and samples on the 7<sup>th</sup> d after nitrate injection (The black circles from inner to outer stand for phylum, class, family, genus, and species. Red circles stand for taxa which were abundant at 0<sup>th</sup> d, and green circles stand for taxa which were abundant at 7<sup>th</sup> d)

## CONCLUSIONS

Injection of nitrate into contaminated sediments in Shenzhen river in this study has been demonstrated to be effective in controlling AVS as odor matters in a biological way. About 86.5% AVS can be removed with the consumption of 86.5% nitrate on average within 14 days. The most abundant phylum obtained by the sequences affiliated to bacterial domain is *Proteobacteria* in both treated and untreated sediment samples. Microbial community composition varied distinctly on the 7<sup>th</sup> day during remediation, when *proteobacteria* increased distinctly as the most abundant in sediments, mainly the taxa of  $\beta$ -,  $\epsilon$ -, and  $\gamma$ -*proteobacteria*. The species belonging to seven phyla (*Planctomycetes*, *Chloroflexi*, *BCR1*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, and *Chlamydiae*) and three classes (*Acidobacteria*, *Thermomicrobia*, and *Delta-proteobacteria*) were significantly abundant in sediments before nitrate injection. The microbial community richness and diversity decreased on the 7<sup>th</sup> day after nitrate injection, but revived on the 14<sup>th</sup> day when the remediation process tended to be finished. Nitrate and ORP resulted in variation of microbial community structure and were strongly related with dominance of *NRB*.

References: Chen L, Wang L Y, Liu S J, et al. Profiling of microbial community during in situ remediation of volatile sulfide compounds in river sediment with nitrate by high throughput sequencing. *International Biodegradation & Biodegradation*, 2013, 429-437.

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