Supplementary information

Acceleration of sand filtration start-up for manganesecontaining groundwater treatment: microbial-mediated autocatalytic oxidation of manganese oxides

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1. Schematic diagram of sand filtration system



Fig. S1 Schematic diagram of sand filtration system

2. DNA extraction and 16S rRNA analysis

Each sample consisted of approximately 5 g of sand and was stored at -80°C before further analysis. All extraction tools were sterilized in an autoclave for 30 min before use. According to the manufacturer's instructions, samples were subjected to total DNA extraction using Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA). The extracted DNA of bacteria was PCR amplified with primer 338F (5'-ACTCCTACGGGagGGCAGCAG-3') in the V3-V5 region by 16S rDNA gene via a thermocycling PCR system (Gene Amp® 9700, ABI, USA). Samples were pooled in equimolar, sequenced by Majorbio Bio-Pharm Technologies Ltd. (Shanghai, China) on the Illumina MiSeq platform (Illumina, San Diego, USA), and clustered into operational taxonomic units (OTUs) using 97% similarity threshold of the sequence results. The relative abundance of populations characterized the microbial community of samples.

3. Surface morphology of raw seeding sand grain







4. Mn removal efficiency under different start-up strategies

Fig. S3 Mn removal efficiencies in four sand filters with different start-up strategies

5. MnOB amount after PG injection



Fig. S4 Amount of MnOB in the suspension and sand media for 9 days after PG injection. Data of day 0 is the MnOB content of the ripened sand filter at day 120.

6. Raman spectra of four BioMnOx samples



Fig. S5 Raman spectra of four BioMnOx samples.

7. Table S1 Characteristics of raw groundwater and standard water

| Properties | Unit | Groundwater after aeration | Water quality limit |
|----------------|-------------------------|----------------------------|------------------------|
| Manganese | mg/L | 1.23-1.88 | 0.10 |
| Iron | mg/L | 1.16-3.52 | 0.30 |
| Ammonia | mg/L | 0.38-0.85 | 0.50 |
| Alkalinity | mg CaCO ₃ /L | 250-260 | |
| Total hardness | mg CaCO ₃ /L | 244-263 | 450 |
| TDS | mg/L | 265-432 | 1000 |
| Turbidity | NTU | 0.42-10.2 | 1 |
| DO | mg/L | 4.98-8.51 | |
| pН | | 7.0-7.4 | 6.5-8.5 |
| Temperature | °C | 3.6-8.9 | |

quality limit

Remark: "---" represented undetected or unlimited.

| Species | Percentage (%) | | | | |
|------------|----------------|-------|-------|-------|--|
| | SF0 | SF1 | SF2 | SF3 | |
| Mn(II) | 20.27 | 62.16 | 32.96 | 29.85 | |
| Mn(III) | 63.23 | 13.31 | 42.39 | 21.40 | |
| Mn(IV) | 16.50 | 24.53 | 24.65 | 48.75 | |
| Mn average | 2.962 | 2.624 | 2.917 | 3.189 | |

8. Table S2 Proportions of Mn species in the BioMnOx of the four

sand filters

9. Table S3 OTUs, Chao1, ACE, Shannon, Simpson and Good's coverage indexes obtained at the different start-up methods in the sand filters at the threshold of 97%

| Sand filter | OTU | Chao1 | ACE | Shannon | Simpson | Good's coverage |
|-------------|-----|---------|---------|---------|---------|-----------------|
| SF0 | 308 | 1212.35 | 1259.78 | 3.49 | 0.82 | 0.99 |
| SF1 | 264 | 1180.47 | 1224.69 | 2.83 | 0.84 | 0.99 |
| SF2 | 529 | 1273.42 | 1330.18 | 4.68 | 0.93 | 0.99 |
| SF3 | 416 | 1228.58 | 1264.90 | 3.57 | 0.88 | 0.99 |

The Good's coverages for all samples were 0.99, indicating that the sequence analysis reflected almost the entire bacterial population. All the indexes were generally lower in SF1 than in the other filters, suggesting that the diversity of the microbial communities and the bacterial homogeneity were significantly affected by PG addition. Moreover, the higher Shannon and Simpson indices of SF2 and SF3 implied that inoculation with MnOB and BioMnOx could be beneficial to increasing the diversity of microbial communities.