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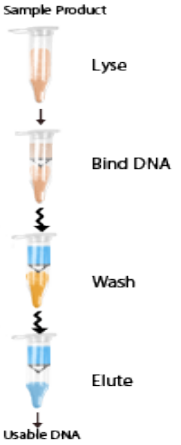
water | onderzoek | advies

# Quantification of Nitrifying and Manganese oxidizing bacteria with real-time qPCR to evaluate purification efficiency of sand filters in drinking water production

## Introduction

Dutch drinking water is the safest and cleanest drinking water of the world. Drinking water can be produced out of groundwater or out of surface water after extensive purification. Groundwater once felt down as rain/snow and trickled into the earth. The earth is like a huge filter. On its way, groundwater takes lots of natural compounds from the earth. Often groundwater has manganese (Mn), iron (Fe) and ammonia concentrations above the WHO guidelines. In an aerated slow sand filter, biomass develops easy, which can decrease chemicals by biological metabolism. Biological treatment of drinking water is more and more common. Manganese oxidizing by Leptothrix and Nitrification by Nitrosomonas for instance. These microorganisms growth very slow, that's why we developed two qPCR's to get a closer look on the biological effect on removal of Mn and NH4+.

## Method




Water samples were taken before and during sand filtration, also a few sand samples were measured. 100mL water samples were filtered on a 0.45µm membrane filter. The filter was used in the 'powerbiofilm DNA extraction kit' from MoBio. DNA extraction is based on mechanical and chemical lysis, DNA binds to a silica membrane followed by wash steps, DNA is eluted in 100µl elution buffer from the MoBio kit.

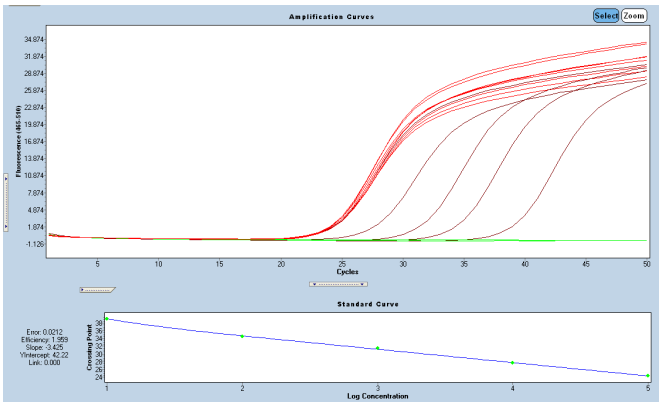
Primers and probe for *Nitrosomonas sp.* were heterogeneous for the ammonium mono-oxidizing gene. Primers and probe for *Leptothrix sp.* were heterogeneous for its 16S rRNA. Two upstream primers for *Leptothirx* are required to get all relevant species.

| | |
|---|---|
| amoA-F1 | 5' GGGGTTTCTACTGGTGGT 3' |
| amoA-R2 | 5' GAAGAAGGGTTTCCGGAGGGG 3' |
| amoA-pr2 | 5' TGTATGTGCGTACAGGTACACCGG 3' *FAM |
| | |
| Forward primer1 PS-1: | 5' ACGGTAGAGGAGCAATC 3' |
| Forward primer2 PSP-6: | 5' CAGTAGTGGGGGATAGCC 3' |
| Reverse primer DSP-6: | 5' GCTTTTGTCAGGGAAGAAATC 3' |
| Lepto-pr6 forward: | 5' CACGCGGCATGGCT 3' *Cy5 |

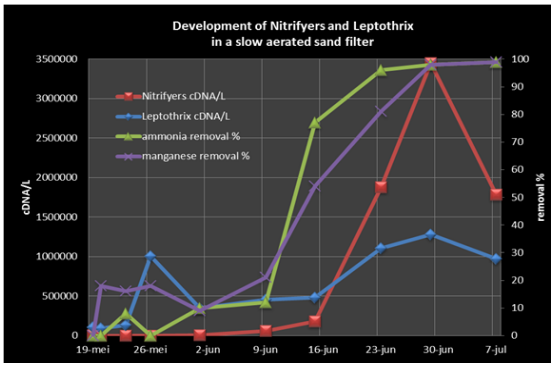
PCR program was as followed: 10min. Roomtemp. (uracil glycosylase), 5 min. 95°C (Denaturation), 50x 30sec. 95°C; 1min. 55°C; 10sec. 72°C.

For quantifying the amount of bacteria in the sand filter we developed a WLN-III plasmid with both target genes. The begin concentration of this plasmid is referenced to the mip- gene of the standardized Minerva plasmid, and is about 160.000 cDNA/L for both nitrifying bacteria and *leptothrix sp*.

## Results




Standard curve for *Nitrosomonas sp.* and *Leptothrix sp.* with a PCR efficiency of 95.9% and a slope of -3.425.




Removal of chemical compounds by biology

## Conclusion

The results showed us that biological treatment of groundwater is a good way to remove unwanted compounds. The start of natural biological removal takes long time, it would be interesting to see if we can fasten biological removal by adding *Leptothrix* and *Nitrosomonas* to an aerated slow sand filter.