# Groundwater treatment study by a sequencing batch bioreactor amended with molecular nitrogen.

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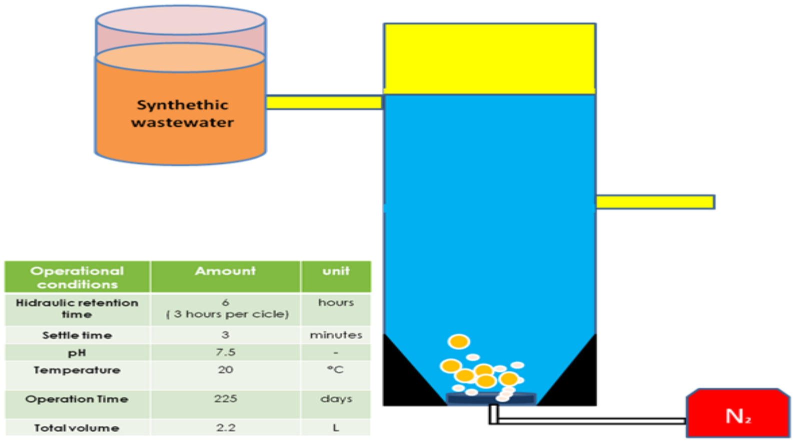
It has been observed that human activity over land use has deteriorated the quality of water bodies all around the world. It is of special significance the deterioration of groundwater, which is a concern because it is used in many human settlements as a primary source of drinking water (SheikhyNarany et al., 2018). One of the primary contaminants of groundwater in human settlements is nitrogen, which is extensively utilized as fertilizer in agricultural applications. Nitrogen in fertilizers passes to groundwater dissolved in irrigation water and stays retained in the aquifer (Logsdon & Cole, 2018). One of the most promising technologies for the treatment of wastewater is the aerobic granular sludge technology. In this technology, the sequencing batch operational conditions in the bioreactor favor the spontaneous generation of granular biomass, attachments of microorganisms and extracellular polymeric substance that achieve great density and settle ability (Gonzalez-Martinez et al., 2018b). Aerobic granular sludge systems have been proven for removal of nitrogen via denitrification. In this project, a bioreactor was built and configured as granular sludge technology, and was started-up and operated for the treatment of nitrate-contaminated synthetic groundwater.

The system showed that granular biomass could be formed under anaerobic conditions and agitation with $N_2$ gas. The bioreactor used in this experiment was a cylinder of 2.2 L volume (**Figure 1**).



| Operational conditions | Amount | unit |
|---|---|---|
| Hidraulic retention time | 6<br>( 3 hours per cicle) | hours |
| Settle time | 3 | minutes |
| pH | 7.5 | - |
| Temperature | 20 | °C |
| Operation Time | 225 | days |
| Total volume | 2.2 | L |

**Figure 1** – Schematic of the bioreactor used in the study

The bioreactor was operated in sequencing batch reactor mode, with a hydraulic retention time of 6 hours. The bioreactor operated in cycles as follows: 3 hours of agitation with $N_2$ gas; 3 minutes of settling; discard of 50% of upper volume of the bioreactor; filling of 50% of water volume with synthetic groundwater. The pH was controlled at 7.5, and the bioreactor operated for 225 days. The bioreactor was amended with a synthetic wastewater simulating a real groundwater composition with 100mg-N $L^{-1}$ of Nitrate. However, the carbon source concentration was modified during the operation time. In this sense, the modification was related to a decrease of influent organic carbon (NaAc) concentration: 1 g $L^{-1}$ from start-up to operational day 90; 0.5 g $L^{-1}$ from day 91 to 135; 0.25 g $L^{-1}$ from day 136 to day 180; 0.20 g $L^{-1}$ from day 181 to day 225. All other components of the synthetic wastewater remained equal during the operation time.

**Table 1** – Physicochemical performance in the SBR

| Day | 90 | 135 | 180 | 225 |
|---|---|---|---|---|
| **Nitrite mg-N /L** | 0 | 0 | 12 | 15 |
| **Nitrate mg-N /L** | 17 | 26 | 46 | 52 |
| **Nitrogen removal (%)** | 83 | 74 | 42 | 33 |

The performance of the bioreactor in terms of nitrogen removal during the whole operational time is shown in Table1. The system experienced an increase in the nitrogen removal capacity during the granular biomass formation stage at 1 g and 0.5 g of $L^{-1}$NaAc, respectively. The effluent concentrations of nitrite were negligible and the effluent nitrate concentration were correlated to effluent total nitrogen, showing that the system eliminated nitrite rapidly and that nitrate reduction was the limiting step in the nitrogen removal bioprocess. Nevertheless, since the carbon source concentration was decreased to 0.25 mg $L^{-1}$or lower, nitrite started to accumulate in the bioreactor, leading to decrease in total nitrogen removalthat achieved a final value in the range of 30-40%.

The microbial communities of *Bacteria*, *Archaea* and *Fungi* domain were analyzed by the means of high throughput sequencing, revealing their structure and their dynamics during the operation of the system at different influent organic matter concentrations. In this way, for the collection of biological samples, an amount of 100 mL of biomass was collected from the bioreactor when it was under complete mixing conditions. These were kept at -20 °C and sent to RTLGenomics Laboratory (Lubbock, TX, USA) for subsequent Illumina MiSeq high throughput sequencing protocol.

In this way, the ordination of microbial communities during the experimentation time suggested that there was more similarity among all samples for the *Archaea* domain than for the *Bacteria* or *Fungi* domains (**Figure 2**).

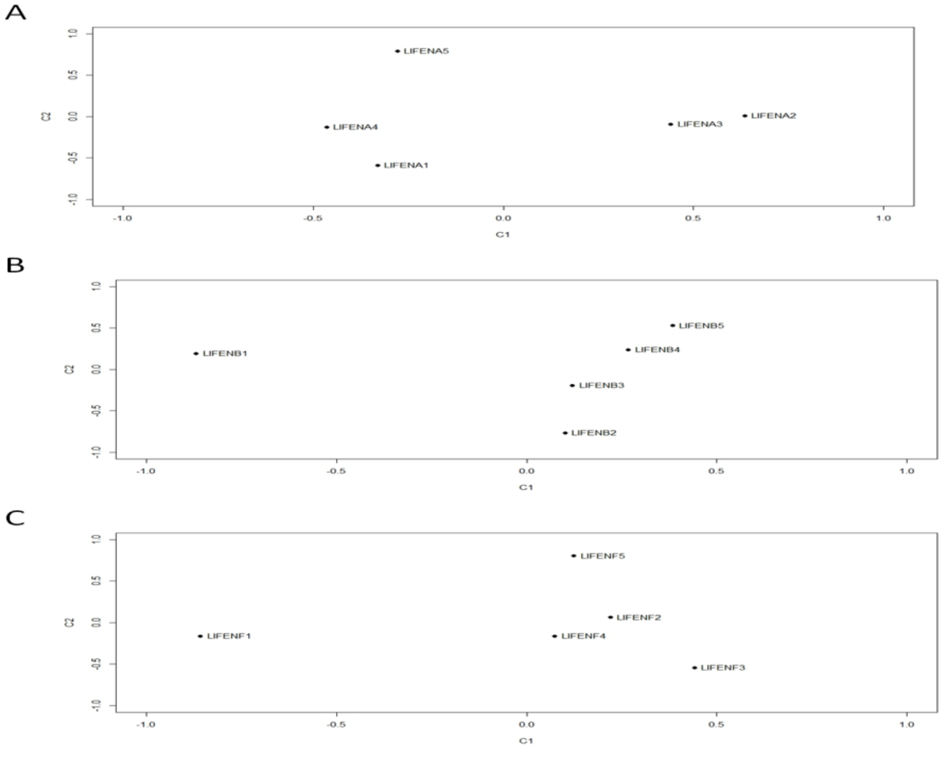


**Figure 2** – Singular value decomposition for the *Archaea* (A), *Bacteria* (B) and *Fungi* (C) domains

For *Bacteria* and *Fungi*, the initial sample was greatly different than all others, suggesting that there was an adaptation of the bacterial and fungal biomass during the operation time of the bioreactor. On the other hand, the singular value decomposition of *Archaea* domain suggested that the communities of *Archaea* did not suffered a great change over the operation time.

The members of *Archaea* identified were all identified as *Euryarchaeaota* phylum. The importance of *Archaea* was not relevant in the sample N1, since only 1 high-quality read was found in that sample. Dominant archaeal phylotypes were shared in samples N2, N3, N4 and N5, and archaeal community structure shifts consisted on differences in importance of other minor OTUs, as seen in the singular value decomposition analyses.

Bacterial phyla identified in the samples were Actinobacteria, Bacteriodetes, Firmicutes, Planctomycetes, Proteobacteria and Verrucomicrobia. Among them, Proteobacteriawas the dominant. Among the Proteobacteria in the system, potential denitrifiers within the *Burkholderiaceae* family were found. *Burkholderiaeae*members has been identified as potential denitrifiers in paddy rice soil and natural watersheds (Yoshida et al., 2012; Tomasek et al., 2017). The reduction of influent organic carbon concentration from 0.5 to 0.25 mg L$^{-1}$ shifted the dominant bacterial OTU, which suggested that dominant denitrifiers in the system had different affinities for organic matter.

All dominant OTUs found in the *Fungi* domain were classified as Basidiomycotaphylum. The predominance of the Basidiomycotaphylum, in granular biomass and other wastewater treatment technologies operating under low temperature conditions has been reported (Gonzalez-Martinez et al., 2018a; 2018b), and so their domination in this experiment could be explained by their adaptation to these conditions.

In conclusion, until now,the results obtained pointed out that the granular sludge technology is a feasible solution for the treatment of nitrogen-contaminated groundwater. The preliminary analyses developed showed the efficiency of the system to remove nitrogen at NaAc concentrations higher than 0.5 g L$^{-1}$ and the inefficiency to operate at lower influent organic matter concentrations, which was coupled to a turnover in dominant OTUs.However more studies are needed for a better understanding of this technology.

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