Distinctive denitrifying capabilities leads to different N_2O production in dPAO and dGAO cultures

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INTRODUCTION

Denitrifying polyphosphate accumulating organisms (dPAOs) are a group of bacteria competent to simultaneously remove nitrogen and phosphorus. This process is highly interesting for wastewater treatment plants due to its energy savings and the reduction of the carbon needs for nutrient removal. However, the presence of glycogen accumulating organisms (GAOs) may cause instability on enhanced biological phosphorus removal due to competition with PAOs for carbon uptake without performing phosphorus removal. Nitrous oxide (N_2O) is a potent green house gas that has been reported to accumulate in systems where denitrification is conducted using storage polymers (PHA) such as dPAO or dGAO reactors (Zeng, 2003). This study aims to investigate the different denitrification kinetics and the N_2O potential in two separated enriched cultures of dPAO and dGAO performing denitrification with PHA as their only carbon source. Experiments under anoxic conditions with a single or multiple electrons acceptors present simultaneously were conducted to assess the potential effect of electron competition in the different denitrification kinetics from the two cultures.

MATERIALS AND METHODS

Two lab-scale SBRs were operated to enrich a dPAO and a dGAO culture. Both reactors were operated in a 6h cycle consisting in: 5 min feed-1 (COD and in the case of the dPAO reactor also P); 1.7h anaerobic phase, 4 min feed-2 (NO_3^-), 1.9h anoxic phase, 1.5h aerobic phase and 45 min of settling, purge and decant. The pH was controlled at 7.5 ± 0.1 with 0.1M HCl. The sludge retention time (SRT) was 10 days. 7 different batch tests with different combinations of electron acceptors (A (NO_3^-), B (NO_2^-), C (N_2O), D(NO_3^-, N_2O), E (NO_2^-, N_2O), F (NO_3^-,NO_2^-) and G(NO_3^-,NO_2^-, N_2O)) were carried out with enriched dPAO or dGAO sludge withdrawn from the end of the anaerobic phase of the parent SBR in a sealed batch reactor with no head-space. In each batch, a concentration of 20 mg N-NOx/L was initially added as a pulse. Dissolved N_2O was monitored with an online N_2O microsensor (Unisense A/S) and samples for the analysis of nitrate, nitrite, phosphate, ammonia, VFAs, glycogen and PHA were taken along the experiment. Biomass concentration (MLVSS) was also analyzed at the end of each test to calculate the specific reduction rates. Fluorescence in situ hybridization (FISH) was also performed to quantify the microbial communities in each of the reactors.

RESULTS AND DISCUSSION

Figure 1a shows a representative example of the batch tests conducted with a single electron acceptor (tests A- C) with dPAOs (fig.1a, top graphs) and dGAOs (fig.1a, bottom graphs). In all tests, N_2O accumulated to a certain degree, indicating that N_2O reduction was always slower than the nitrate and/or nitrite reduction rates in both cultures. Also, in the case of the dGAO tests, nitrite accumulated in batch test A when nitrate was added as the sole electron acceptor indicating that dGAO had a preference to consume nitrate against nitrite. Therefore the highest denitrification rate for dGAOs was found with nitrate.

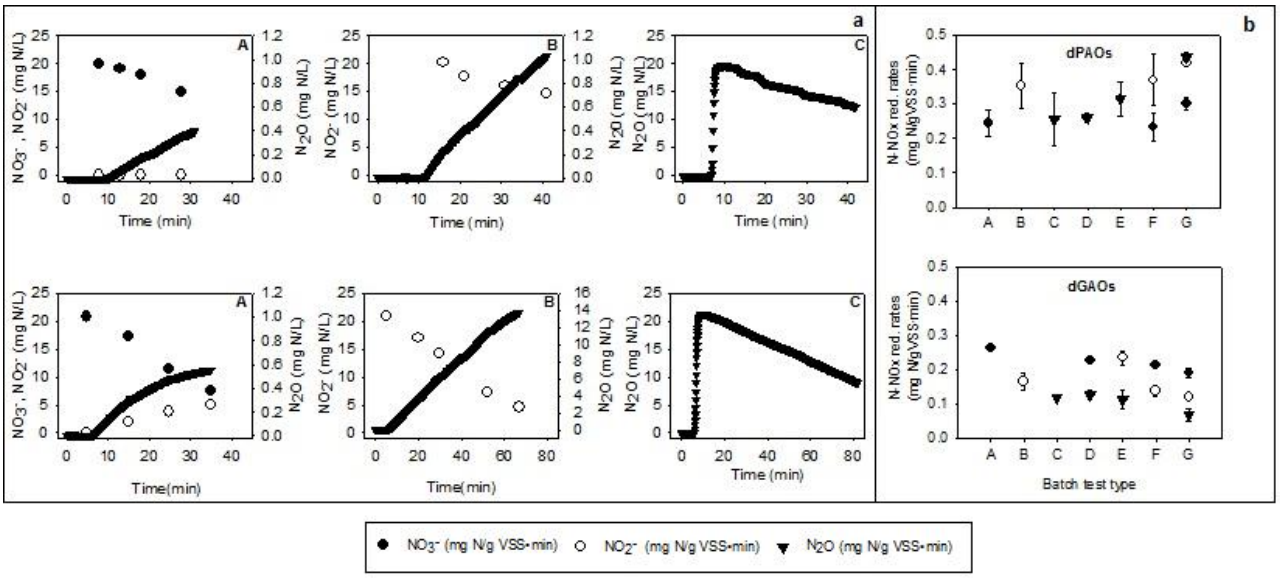

Figure 1 $NO3^-$, $NO2^-$ and N_2O profiles for batch tests A- C for the dPAO and the dGAO reactors (a), nitrogen oxides reduction rates for dPAO and dGAO cultures, respectively (b).

In the tests conducted with dPAOs, the highest denitrification rate was obtained when nitrite was used as electron acceptor. This could suggest the presence of two different dPAO groups as it has been reported previously by Oehmen, (2010), one able to denitrify from nitrate and another one only from nitrite and would imply that the denitrification rates are lower in the case of nitrate. Microbial characterization revealed that 42% of the microbial community belonged to the Accumulibacter group, with 26% being type PAO I (able to denitrify from nitrate and nitrite) and 15% being type PAO II (able only to denitrify from nitrite). Therefore there were more dPAOs able to denitrify nitrite than to denitrify nitrate. For batch tests D-G (fig 1b), different combinations of electron acceptors were added in order to see if a competition for electrons was occurring when PHA was used as carbon source for denitrification. Pan, (2013) and Ribera-Guardia, (2014) found a reduction on the denitrification kinetics of the different electron acceptors when these ones were added in combination, compared to the tests done with a single electron acceptor, in denitrifying cultures with external carbon supply. In our study, no electron competition was found in any of the two cultures, suggesting a different response when an internal carbon source such as PHA is used for denitrification.

CONCLUSIONS

The main conclusions from this investigation are:

- dPAOs have a higher denitrifying capacity than dGAOs as their nitrogen oxides reduction rates are higher.
- No electron competition was detected when using PHA as the carbon source for denitrification in any of the cultures.
- dGAOs accumulate more nitrous oxide than dPAOs under all the electron acceptor scenarios tested.

REFERENCES

Oehmen, A., Carvalho, G. et al. (2010) Incorporating microbial ecology into the metabolic modelling of polyphosphate accumulating organisms and glycogen accumulating organisms. *Water research*, **44**(17), 4992–5004.

Pan, Y., Ni, B.-J.,et al.(2013) Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water research*, **47**(10), 3273–81.

Ribera-Guardia, A., Kassotaki, E., et al. (2014) Effect of carbon source and competition for electrons on nitrous oxide reduction in a mixed denitrifying microbial community. *Process Biochemistry*, **49**(12), 2228–2234.

Zeng, R. J., Yuan, Z., and Keller, J. (2003) Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system. *Biotechnology and Bioengineering*, **81**(4), 397–404.