

Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture

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ADDITIONAL INFORMATION

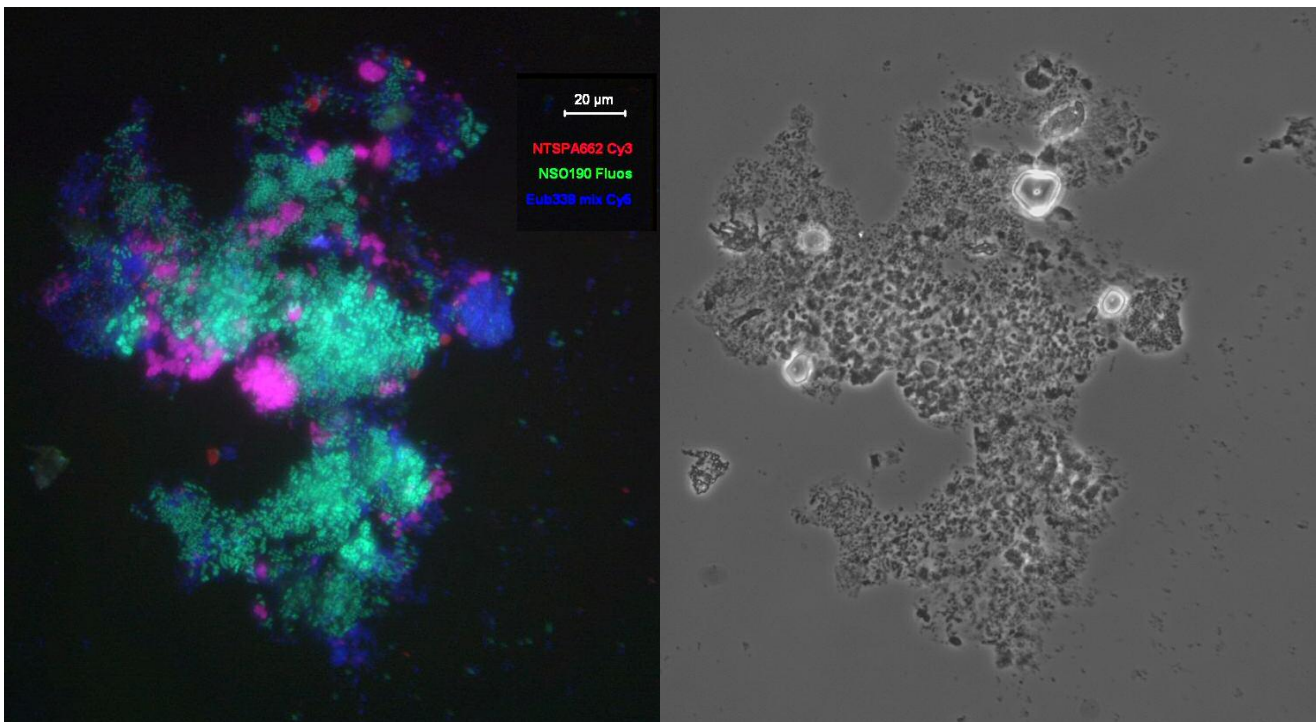


Figure I FISH image of a nitrifying sludge granule from reactor A. The following probes were used: NSO 190 green (ammonium oxidising Beta proteobacteria) NTSPA 662 red (Nitrospira-like organism) EUB 338 blue (Eubacteria).

Analysis of the biomass composition

Fluorescence In Situ Hybridization (FISH) was used to get an impression of the microbial composition of the nitrifying community (Figure I gives an example). It was estimated that more than 90% of the biomass consisted of ammonium-oxidizing and nitrite-oxidizing bacteria. The probe binding indicated that the AOB are most likely ammonium-oxidizing *beta-proteobacteria* comprising, *Nitrospira*, *Nitrosococcus* and *Nitrosomonas* species (covered by NSO_190 probe), but do not belong to the subgroup of *Nitrosomonas europaea*, *N. eutropha* and *N. halophila* (because the NSE_1472 probe did not give a signal). The NOB most likely belong to the cluster of *Nitrospira*-like organisms (covered by NTSPA_662 probe), and not to *Nitrobacter* species (negative response with NIT_1035 probe). The

micro-organisms that did not hybridise with the NSO and NTSPA probes could either be heterotrophic micro-organisms or nitrifying micro-organisms that do not have the probe-binding site.

In a nitrifying mixed cultures like those described in this paper, the presence of heterotrophic microorganisms cannot be excluded based solely on the use autotrophic growth medium. The organic carbon necessary for heterotrophic bacteria may originate from decaying biomass, influent impurities or the yeast extract in the influent. The estimated maximum amount of biomass that may originate from growth on yeast extract is limited to 0.0032 g dw/L, representing no more 1.5% of the total biomass in the system. The contribution of heterotrophic biomass, grown on decaying biomass or organic compounds excreted by nitrifying micro-organisms, is largely unknown but may amount to the approximately 10% non-nitrifying cells found by FISH analysis.

Estimation of kinetic parameters of nitrifiers

Values for the biomass specific maximum substrate uptake rates (q^{\max} values) for AOB and NOB were estimated. From the FISH analysis the nitrifying populations was roughly estimated to consist of 75% ammonium oxidizers and 25% nitrite oxidizers. This composition corresponds to the number of electrons involved in both nitrification steps (6 and 2 electrons per mole nitrogen converted for ammonium and nitrite oxidation respectively) as well as the values reported in literature. FISH analysis furthermore indicated the presence of approximately 10% non-nitrifying biomass, resulting in an estimated overall biomass composition of 68% AOB, 22% NOB, and 10% other eubacteria. Relating this biomass composition to the actual measured overall biomass concentrations and the maximum volumetric ammonium and nitrite uptake rates allows for identification of the following maximum specific activity values: $q_{AOB}^{\max} = 120 \text{ mgN} \cdot g_{dw} \text{AOB}^{-1} \cdot h^{-1}$ and $q_{NOB}^{\max} = 247 \text{ mgN} \cdot g_{dw} \text{NOB}^{-1} \cdot h^{-1}$. The overall biomass yield was estimated from the measured biomass concentration in the reactor and the

measured SRT-value: $Y_{X/N} = 0.10 g_{dw} \cdot gN^{-1}$. Using the previously described ratios of AOB and NOB the individual yields can be estimated: $Y_{AOB/N} = 0.07 gX_{dw} \cdot gN^{-1}$, and $Y_{NOB/N} = 0.02 gX_{dw} \cdot gN^{-1}$.

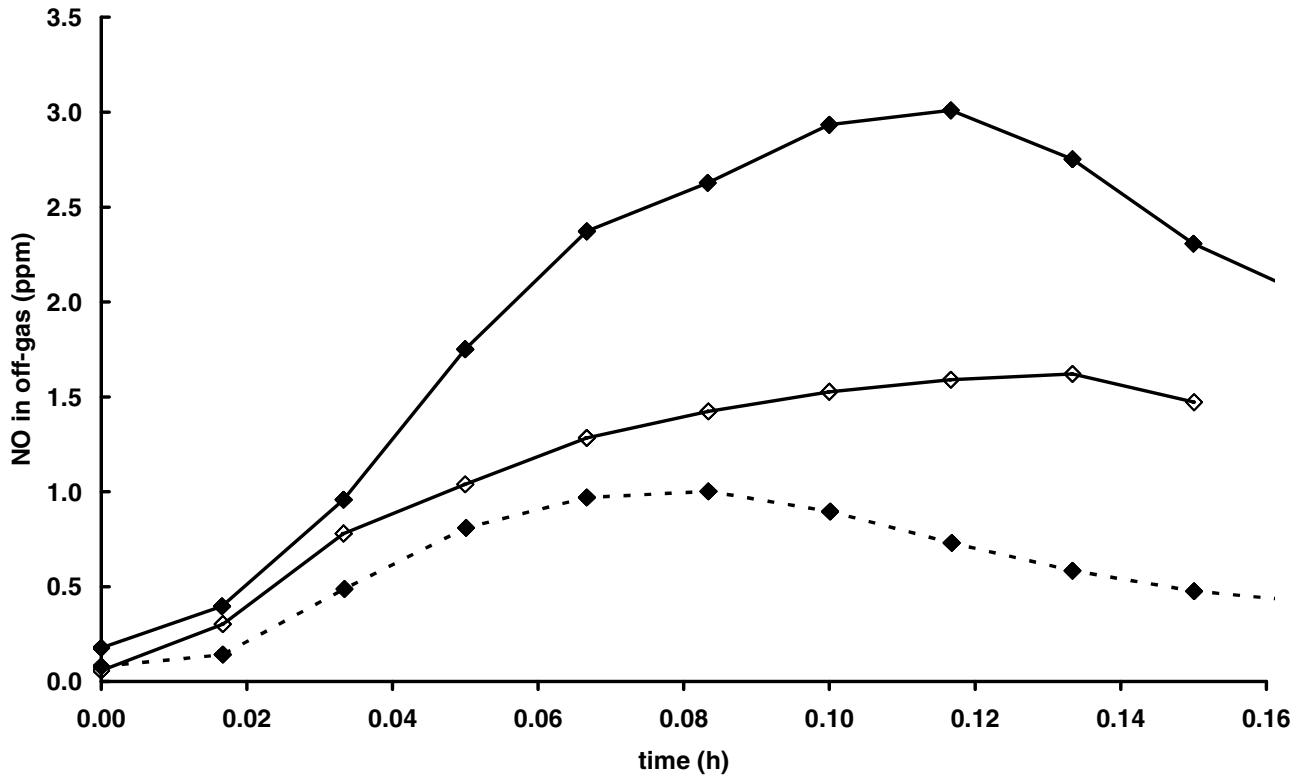


Figure II Ammonium pulses ((\blacklozenge) 45 mgN-NH₄⁺/L (\diamond) 26 mgN-NH₄⁺/L ($-\blacklozenge-$) 15 mgN-NH₄⁺/L) at the start of an SBR cycle lead to increased NO emission. The nitrite concentration was approximately 10 mg N-NO₂⁻/L. NO concentrations are measured in the gas phase.

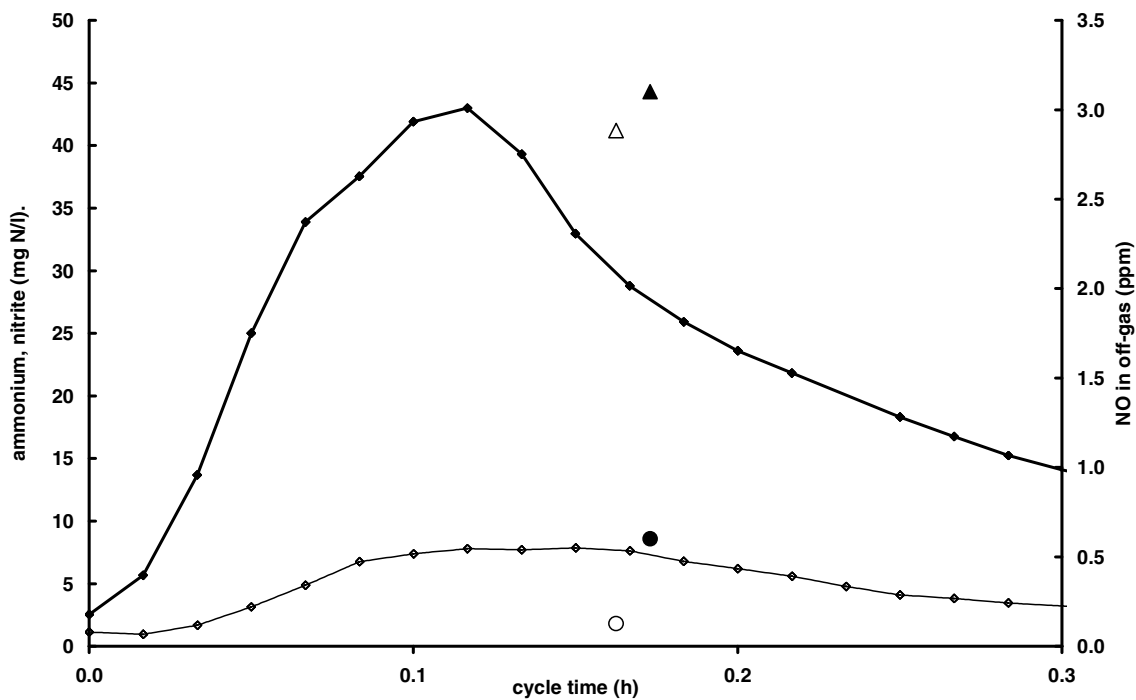
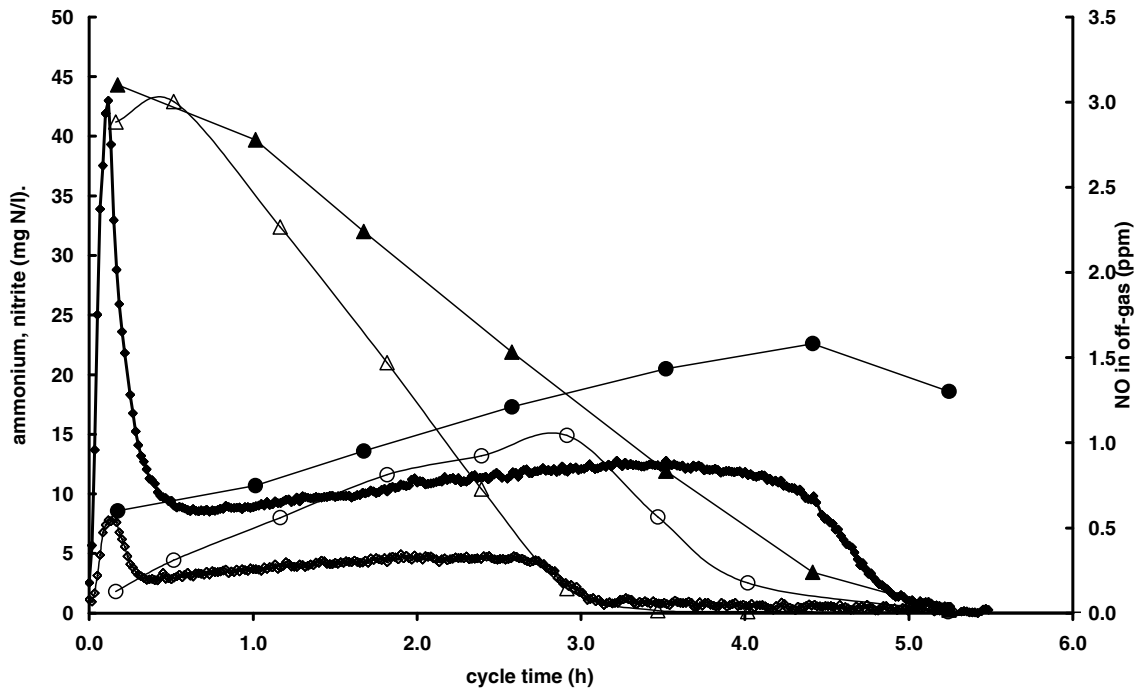


Figure IIIA. Increased nitrite concentrations lead to quicker and higher NO emission. Concentrations of ammonium (Δ), nitrite (\circ) and NO in the gas (\diamond) during one SBR cycle without residual nitrite (open symbols) and with residual nitrite in the reactor (filled symbols).

Figure IIIB First 0.3 hours of figure IIIA are depicted.