Influence of shear on nitrification rates in a membrane bioreactor

M. A. Lou, P. Larsen, P. H. Nielsen and S. N. Bak

ABSTRACT

Grundfos BioBooster (GBB) installed and operated a membrane bioreactor (MBR) test plant in 2012. During the period it became evident that the nitrification rate was lower than expected and a study was carried out to investigate the possible reasons for the observed low-nitrification rate. Tests were conducted at a pilot plant and the effect of shear from the BioBooster membrane system and the pressure reduction component on the nitrification rate was investigated. The possible effect of selection of microbial communities caused by the filtration unit was also investigated. The results revealed an unchanged nitrification rate when exposed to shear from the filtration unit and the pressure reduction component. When testing the effect of selection, the nitrification rate was also unchanged.

Key words | maximum potential nitrification rate, MBR, selection, shear

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INTRODUCTION

The world is currently faced with serious issues regarding water availability. A solution can be found in reliable decentralized wastewater treatment plants (WWTPs), which are designed as a cost-effective investment and ensure cheap operation costs and also deliver a superior water quality. Furthermore, in order to ensure low operating costs and reliability it is essential that the WWTP is operating with a high degree of robustness in order to withstand changes in incoming waste water.

Membrane bioreactors (MBRs) have, during the recent decade, gained more and more popularity due to the advantages of compactness, disinfection, improved effluent quality and robustness. However, in many MBR plants the microbial population is also exposed to significantly more shear originating from membrane cleaning than in a conventional activated sludge (CAS) plant.

From a process perspective the design of MBRs is based upon process knowledge from CAS plants. However, the literature is inconclusive as to whether elements such as the effect of increased shear can be scaled up from CAS without influencing the biological processes in the system. The most sensitive process in CAS and MBR is typically associated with the nitrification process due to the relatively slow growth rate of the nitrifying populations.

This paper is reporting experimental work carried out to determine the impact of shear and selection of microbial communities due to membrane separation on the nitrification capacity of a MBR under different operational conditions, and comparing this to the CAS plant from which the sludge originated.

Grundfos BioBooster (GBB) has, since May 2012, operated a full-scale MBR test plant at Bjerringbro WWTP, Jutland, Denmark with a capacity of 800 PE. The GBB system has a unique Membrane Filtration Unit (MFU) of rotating membranes with ceramic discs. During the test period it became evident that nitrification rates were lower than originally expected (<1 gNH_4-N/(VSS*h)). The effect of MBR operation on the nitrification rate compared to CAS operation is not well known. Different studies have found lower (Witzig *et al.* 2002; Parco *et al.* 2006; Monti & Hall 2008), higher (Gao *et al.* 2004; Munz *et al.* 2008; Fenu *et al.* 2010) or unchanged (Manser *et al.* 2004, 2005; du Toit *et al.* 2010) nitrification rates when comparing MBR and CAS operation.

The main difference between the CAS and GBB systems is the shear induced by the rotating discs (120 rpm at nominal operation) and the pressure reduction component (needle valve), and also the changed selection pressure on microbial communities introduced by MBR reactors in general. Introducing a membrane instead of settling tanks causes almost complete retention of suspended solids and among this, inert/dead material, which will dilute the nitrifying bacteria in the sludge. Furthermore, species that would normally be washed out due to poor settling/adhesion characteristics

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will be retained and grow in the MBR system, causing further dilution of nitrifiers in the system. In order to determine whether nitrification rates are affected by the GBB MBR system, an investigation of the main differences between the GBB MBR and CAS was executed.

MATERIALS AND METHODS

A pilot plant was constructed for experimental purposes. The pilot plant is located at the GBB test plant at Bjerringbro WWTP, Jutland, Denmark. The pilot plant has a process volume of 10.8 m^3 and a bottom aeration disc diffusion system. The pilot plant has two types of sludge separation, decanting equipment and GBB MFU. Settings of the plant when operating were a mixed liquor suspended solids (MLSS) of 5 kg/m^3, with 2 mg/l of dissolved oxygen (DO), pH 7, food to microorganism (F/M) ratio of 0.12 kgCOD/(kgSS*d), hydraulic retention time of 13 h, and an aerobic sludge age of 20 days.

Execution of the nitrification test was based on the idea of operating the pilot plant as a CAS plant and introducing the component which could potentially influence the nitrification rate. The three potential components/mechanisms prioritized from theoretical considerations were as follows:

(a) A test to expose activated sludge to shear from GBB MFU rotating discs. The pilot plant was operated as a Sequencing Batch Reactor (SBR) with an integrated filtration unit (GBB MFU) in order to eliminate the influence of microbial selection by the MFU. This setup made it possible to test the shear effect of the GBB MFU as the main component that could affect the nitrification process compared to CAS operation. An SBR cycle consists of an aeration period of 2 h and a settling period of 1 h.

(b) A test to expose activated sludge to shear from the pressure reduction component. Executed as described in (a). Furthermore, the pressure reduction component (needle valve) was introduced to the system.

Figure 1 shows the setup made to evaluate test a and test b.

(c) A test to introduce a selection from GBB MFU to the system. The setup is operated as a MBR plant with filtration by the GBB MFU, see Figure 2.

The pilot plant was seeded with sludge from Bjerringbro WWTP at the beginning of each of the described tests.

The test period was 1 month for test a and test b and 3 months for test c. Samples were taken twice a week to be

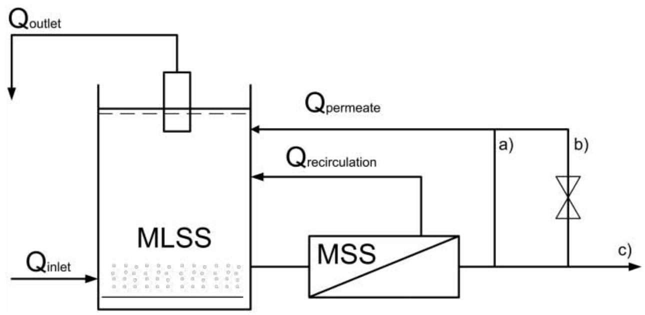

Figure 1 | Overview of test setup to expose activated sludge to shear from GBB membranes (test a) and the pressure reduction component (test b). MSS: membrane suspended solids.

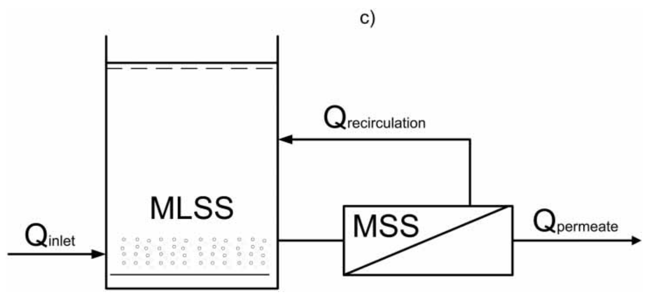

Figure 2 | Overview of test set up to expose activated sludge to selection from GBB membranes (test c).

analysed for standard wastewater parameters (chemical oxygen demand (COD), total nitrogen (TN), NH_4-N, NO_3-N), standard sludge parameters (sludge volume index (SVI), suspended solids (SS), volatile suspended solids (VSS), microscopy) and maximum potential nitrification rate (MPNR). At the start and end of the test period, nitrifiers were quantified with fluorescence *in situ* hybridization (FISH) for the biovolume of ammonium and nitrite oxidizing bacteria (AOB and NOB, respectively) with probe Nso190 specific for betaproteobacterial ammonium oxidizing bacteria, probe Cluster6a-192 specific for *Nitrosomonas oligotropha* lineage and probe Ntspa662 specific for the nitrite oxidizing *Nitrospira*. For quantitative FISH, random fields were chosen and images acquired with a specific probe-defined population (Cy3) and the general EUBMIX (FLUOS). The biovolume of probe defined-populations was determined by image analysis software (ImageJ, http://rsbweb.nih.gov/ij/) and custom-made macros were used for post-processing and data acquisition of all images. Further details on the probes can be found in Loy *et al.* (2007) and the hybridization was performed according to Morgan-Sagastume *et al.* (2008).

Measurements of MPNR took place in a container with 5 l of fresh activated sludge from the pilot plant with continuous measurement of DO and pH. The pH was stabilized by the addition of bicarbonate. Ammonium was added to a concentration of ~25 mg NH_4-N/l and the decrease of ammonium was measured over a period of 2 h.

All data were corrected to a temperature of 15 °C using a temperature correction constant of 1.12. To verify the correction constant, identical tests were executed at different temperatures, and the results showed that the correction constant was valid in the temperature interval from 10–25 °C.

RESULTS

Two different levels of shear from rotating discs were tested (test a): Low shear with a disc rotation of 110 rpm and 20 kg MSS/m^3 representing a shear stress of approximately 1,300 s-1 and high shear with a disc rotation of 180 rpm and 40 kg MSS/m^3 representing a shear stress of approximately 2,200 s-1, see Figure 3.

A decrease in MPNR was observed during the first 2 weeks with low shear and a poor N removal, most likely due to a low F/M ratio. After a minor modification it was possible to maintain the F/M ratio at 0.12 kgCOD/(kgSS*d) and MPNR increased to the original level. Overall, there were no significant changes in MPNR during the test phase for the two tested shear levels.

During the test period, light microscopy was conducted weekly to visually observe the floc size. Typical images are shown below, see Figure 4.

The results at the start of the tests showed a floc size of 50–500 μm as expected of sludge from a CAS plant. After 1 month of operation the floc size decreased to approximately 20–100 μm. The number of single cells in the bulk liquid increased during the test. These results were seen both for high and low shear.

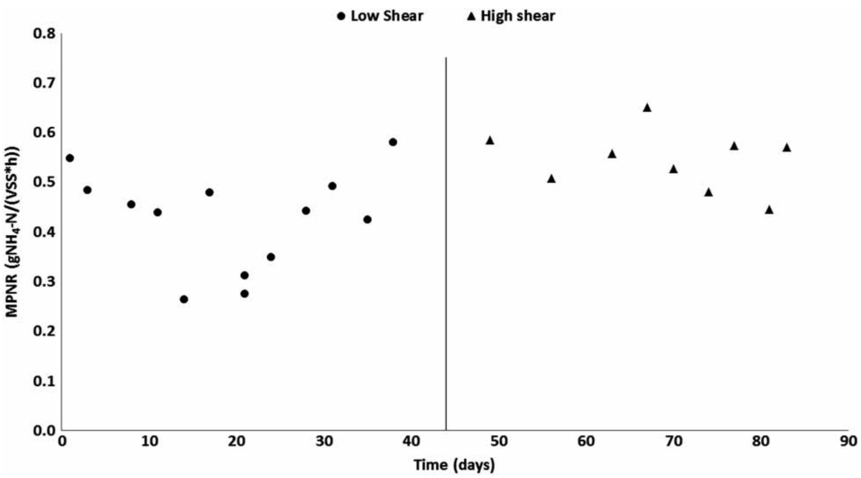

Figure 3 | MPNR when testing shear from GBB MFU (test a). MPNR is normalized to 15 °C.

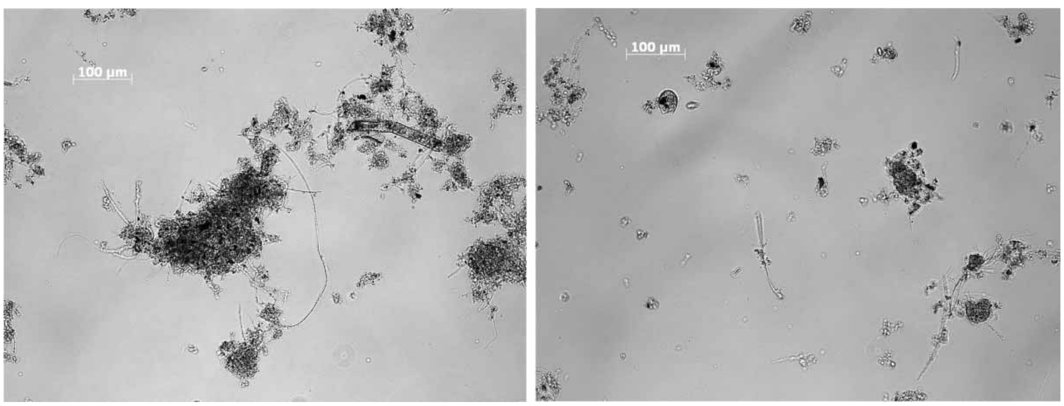

Figure 4 | Examples from microscopy from start (left) and end (right) of test a.

To investigate whether the nitrifying microbial community changed with shear, FISH analyses were carried out at the start and end of each test phase. Initially, a screening with many probes was performed and the probes, representing more than 0.5% of all *Bacteria*, were selected for quantification. The results are shown in Table 1. Taking the standard deviations into account, there was a slight tendency to a decreased fraction, especially of AOB and *N. oligotropha* and also NOB when exposed to high shear. Compared to CAS sludge, the nitrifier fractions were within the range identified in Danish Enhanced Biological Phosphorus Removal (EBPR) plants.

Compared to CAS systems, the FISH analyses showed an increased tendency to grow as single cells or relatively small micro colonies, which are atypical for nitrifying bacteria (see images below, Figure 5). This tendency was found in both start and end samples on both tests.

Results from testing the pressure reduction component (test b) are shown in Figure 6.

During the test there were errors in the measurements of MPNR from days 25–33. When introducing the pressure reduction component to the system, no significant change of MPNR was identified. The fluctuations observed are within the expected uncertainty when measuring MPNR.

A tendency for decreased abundance of AOB was observed using the broad probe, but using the more specific probe for *N. oligotropha*, an increase was observed (see Table 2). This suggests that subpopulations of AOB were affected differently during the test period. Taking the standard deviation into account, NOB was not affected by introducing the pressure reduction component. For comparison, the same groups were quantified in a parallel full-scale CAS system receiving the same wastewater. In this system all groups increased relatively more than in the test system over the same period. However, a decrease in MPNR was also observed during the period, which could perhaps originate from modification of the CAS plant taking place in the same period.

Results from testing MPNR when introducing selection to the system from the GBB MFU are shown in Figure 7.

The test period was set to 3 months (>3 aerobic sludge ages) to ensure a possible change of bacterial composition to occur due to the total retention of all biomass.

The first measurement of MPNR was low due to stressed seeding sludge from Bjerringbro WWTP caused by fundamental rebuilding of the plant. The sludge adapted to the test conditions during the first 3 days where the MPNR

Table 1 | Results from FISH analysis (% of all bacteria and standard deviation). The results from CAS systems are from Nielsen *et al.* (2010)

	Low shear		**High shear**		**CAS (EBPR)**	
	Start	**End**	**Start**	**End**	**Average**	**Range**
AOB	7.9 (1.3)	7.4 (1.3)	4.3 (1.2)	3.5 (1.0)	3.8	1.2–8.2
N. oligotropha	4.3 (1.7)	1.9 (0.04)	3.8 (2.4)	1.9 (0.5)	1.1	0.2–2.3
(NOB) *Nitrospira*	2.3 (0.5)	2.0 (0.3)	2.6 (0.8)	1.3 (0.5)	3.1	0.5–5.2

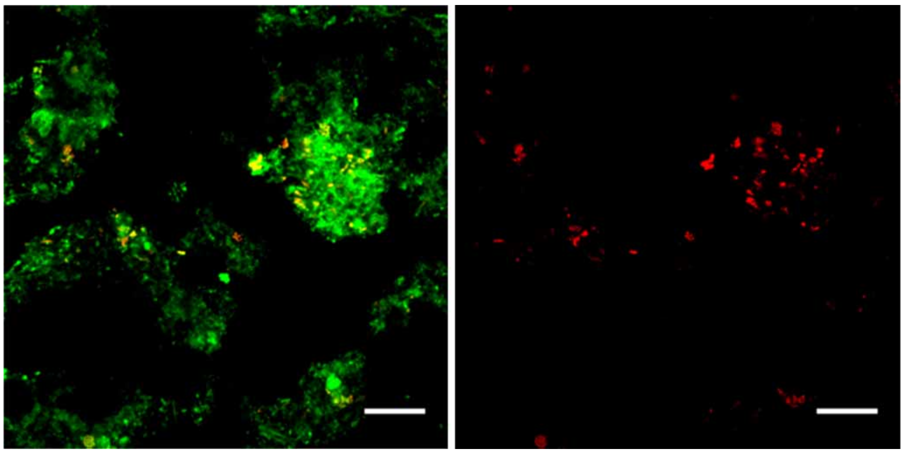

Figure 5 | FISH images of same field of view. Left is an overlay image of EUB MIX (green + yellow) and AOB (yellow), right is the single cells of AOB (red). The scale bar represents 20 μm. The full colour version of this figure is available online at http://www.iwaponline.com/wst/toc.htm.

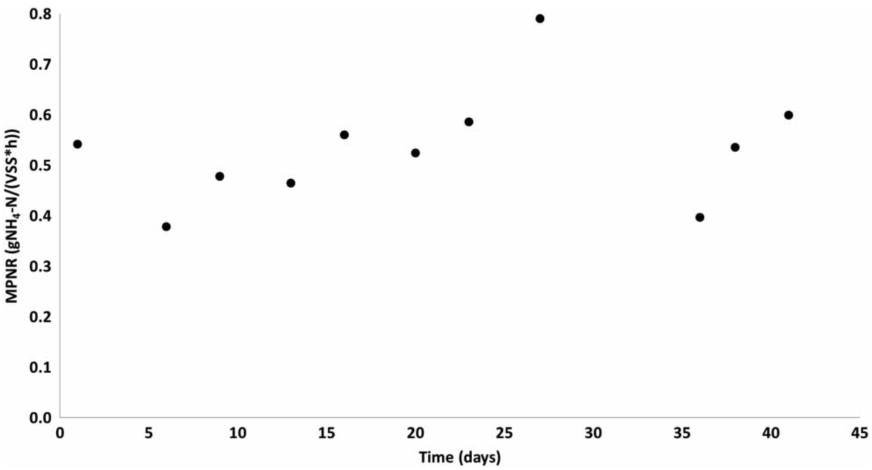

Figure 6 | MPNR when testing shear from the pressure reduction component (test b). MPNR is normalized at 15 °C.

Table 2 | Results from FISH analysis (% of total bacteria and standard deviation). The results from CAS systems are from Nielsen *et al*. (2010). CAS WWTP is a sample from a parallel CAS system receiving the same wastewater

	Seeding	Pressure reduction	CAS WWTP	CAS	
	Start	End	End	Average (%)	Range (%)
AOB	2.1 (0.1)	1.5 (0.1)	2.4 (0.3)	3.8	1.2–8.2
N. oligotropha	< 0.5	1.0 (0.2)	2.6 (0.6)	1.1	0.2–2.3
Nitrospira	1.5 (0.7)	1.0 (0.1)	2.0 (0.4)	3.1	0.2–5.2

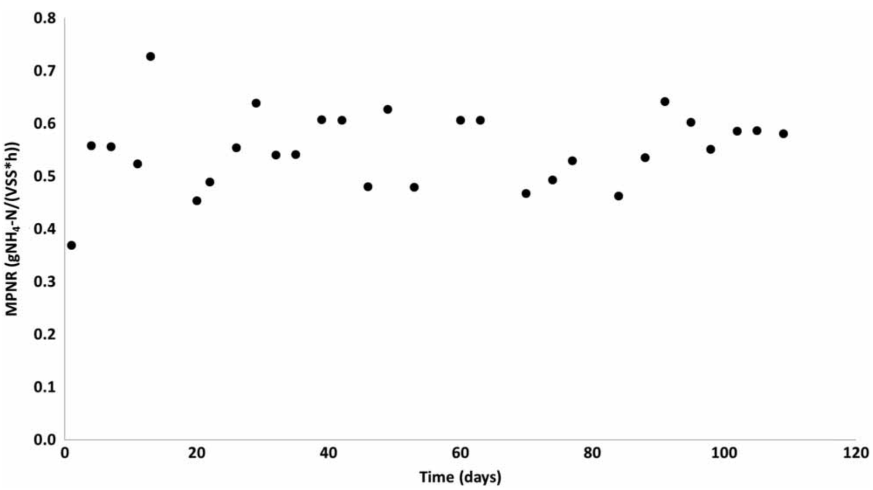

Figure 7 | MPNR when testing selection from the GBB MFU (test c). MPNR is normalized at 15 °C.

was increased to the level seen in earlier tests. The first measurement of MPNR was discarded and the test start was defined at day 4 of the test period.

During the test period, acceptable fluctuation was seen in the measurement of MPNR. Over the test period, there was no significant reduction of MPNR compared to the starting point. It was not possible to document a negative effect on MPNR caused by selection due to MBR filtration of the sludge.

In comparing the biovolume of AOB and NOB at the start and end of the selection test, the results showed a trend similar to the test of the effect of the pressure

Table 3 | Results from FISH analysis (% of total bacteria and standard deviation). The results from CAS systems are from Nielsen *et al.* (2010)

	Selection test		CAS	
	Start	End	Average (%)	Range (%)
AOB	3.3 (0.4)	1.8 (0.2)	3.8	1.2–8.2
N. oligotropha	1.2 (0.2)	1.8 (0.3)	1.1	0.2–2.3
Nitrospira	1.5 (0.4)	1.7 (0.19)	3.1	0.2–5.2

reduction component, as the fraction of AOB decreased while *N. oligotropha* and *Nitrospira* increased slightly (see Table 3).

DISCUSSION

During operation of the GBB MBR full-scale plants, low nitrification rates were observed. Hence, a test program was established to record the potential effects of specific components/effects introduced by the GBB system on nitrification rates. For all tests an MPNR decrease of more than 20% during the test period was considered significant.

Increased shear levels

When testing increased shear levels, three different parameters were used to determine the effect: MPNR, light microscopy and FISH. The results from MPNR showed no change during testing. At the same time, the microscopy clearly showed a diminishing floc size during the test period of 1 month and a larger number of single cells in the bulk liquid. FISH results revealed a tendency for decreased content of AOB and NOB at the end of the test period and more nitrifying bacteria growing as single cells. AOB and NOB are known mostly to grow as strong shear resistant mono-species microcolonies when tested in sludge from CAS-plants (Larsen *et al.* 2008). Overall, this indicates that a change from traditional CAS operation to a pilot plant with higher shear levels can cause population dynamics for nitrifiers without affecting the MPNR. Hence, this example indicates that different groups of nitrifiers pose different nitrification capacity to the system, and that fewer nitrifiers do not necessarily cause changes in system performance. Furthermore, it is possible that nitrifiers not targeted by the three gene probes were selected during the pilot test. This has not been tested in this study. Future studies on this subject could benefit from application of a combination of high throughput amplicon sequencing and FISH. This combination would ensure a screening independent of prior knowledge to species composition, which is necessary to select gene probes and then confirm with FISH probes to get data on growth as micro-colonies or single cells.

The actual shear level introduced in test a was estimated and b was not measured. The decision to test these specific shear compounds was based on theoretical calculations as to where the highest levels of shear were introduced in the GBB system. The results from test a and test b did not reveal the full-scale effect of a GBB system. Verification is being performed as a long-term full-scale performance test during 2013/2014.

Changed selection pressure

Three different parameters were used to determine the effect from changed selection pressure: MPNR, microscopy and FISH. The results are as described when testing shear levels. See discussion above.

The quality of FISH was poorer for the selection tests, a relatively high background noise was identified which causes a low signal to noise ratio. It is, however, considered not to influence the conclusion.

CONCLUSION

During operation of a GBB full-scale MBR plant, suspicion arose that shear from GBB MFU or changed selection pressure was causing low nitrification rates.

Measurement of MPNR was not affected when introduced to:

- shear from GBB MFU disc rotation;
- shear from pressure reduction component;
- selection from GBB MFU.

FISH analysis of the nitrifying populations indicated that abundance decreased slightly without causing changes in the system performance.

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