The Impact of High Mixed Liquor Concentration (3-13 gVSS/ℓ) on the Kinetic Rates of the N and P Removal Bioprocesses in Membrane Biological Nutrient Removal Activated Sludge Systems

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Abstract. The impact of including membranes for solid liquid separation and high volatile suspended solids (VSS) concentration (3-12 gVSS/ℓ) on the kinetics of biological nitrogen and phosphorus removal (BNR) was investigated. To achieve this, a membrane bioreactor (MBR) biological nutrient removal (BNR) activated sludge (AS) system was operated for 450 days in parallel with a conventional BNR system with a settling tank (CAS). The influence of high VSS concentration (up to 12 gVSS/ℓ) in the MBR system on the system performance and the nitrification, denitrification and phosphorus release and uptake kinetic rates were measured with aerobic, anoxic and anaerobic batch tests on mixed liquor (ML) harvested from the MBR system, diluted to different VSS concentrations, and from the CAS system. Also, the limitation of ammonia, oxygen, nitrate and acetate on the kinetic rates was investigated with batch tests. The results show that the BNRAS steady state and kinetic models developed for low VSS concentration BNRAS systems with secondary settling tanks can be applied with reasonable confidence to predict the performance of high VSS concentration BNRAS systems with membranes, except for the maximum specific growth rate of the nitrifiers, which was observed to be significantly lower in the MBR system.

Keywords: Membrane · Settling tanks · Nitrification · Denitrification · Biological phosphorus removal · Kinetics

1 Introduction

For conventional (with settling tanks) activated sludge (CAS) systems for biological nutrient removal (BNR), considerable knowledge has been accumulated on their performance, design and operation. Design procedures and performance simulation models have been developed based on well structured and researched stoichiometric and kinetic principles of the underlying fundamental biologically mediated processes. It is not certain whether this knowledge developed for CAS BNR systems can be applied directly to membrane bioreactor (MBR) BNR systems, given the significant differences

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that may arise when membranes are included such as (i) floc structure (Zhang et al. 1997; Cicek et al. 1999; Huang et al. 2001; Yamamoto 2002; Gao et al. 2004; Manser et al. 2005), (ii) bacterial communities (Ghyoot et al. 1999; Luxmy et al. 2000; Liebig et al. 2001; Smith et al. 2002; Manser et al. 2005), (iii) metabolic activities (Lee et al. 2003; Han et al. 2005; Sperandio et al. 2005; Li et al. 2005) and (iv) sludge production (Cicek et al. 1999; Smith et al. 2002; Holbrook et al. 2005; Monti et al. 2005).

Ramphao et al. (2005) concluded that incorporating membranes in BNR AS systems makes a profound difference not only to the design of the BNR system itself, but also to the approach to design of the whole wastewater treatment plant. This paper presents research that investigates whether the steady state and kinetic models developed for CAS BNR systems can be applied also with reasonable accuracy to model MBR BNR systems.

Accordingly, the kinetic rates of nitrification, denitrification, anaerobic acetate uptake and P release, anoxic P release/uptake and aerobic P uptake were measured in batch tests over a range of volatile suspended solids (VSS) concentrations (3-12 gVSS/ℓ) on sludge harvested from an MBR-BNR system and compared with the corresponding rates measured in a parallel CAS BNR system at 3 gVSS/ℓ. Also, the influence of the limitation of substrate (ammonia, oxygen, nitrate, phosphorus, acetic acid) concentrations on the kinetic rates was investigated in the batch tests. To provide additional information on the anoxic behaviour of phosphate accumulating organisms (PAO), the ability of the AS in MBR BNR systems to denitrify under anoxic conditions with simultaneous phosphate uptake was investigated and quantified.

2 Material and Methods

Two parallel lab-scale membrane (MBR) and conventional (CAS) activated sludge systems were operated for 450 days at 20°C allowing their behaviour to be monitored and their performance compared. Both systems were UCT configurations (Figs. 1 and 2, Table 1) so that denitrification and biological excess phosphorus removal (BEPR) could function independently, provided the recycles do not overload the anoxic reactor with nitrate. System design and operational parameters such as zone mass fractions, inter-reactor recycles and sludge ages were kept the same in both systems (Table 1). Five A4 size Kubota® membrane panels submerged in the aerobic reactor of the MBR system replaced the function of the SST.

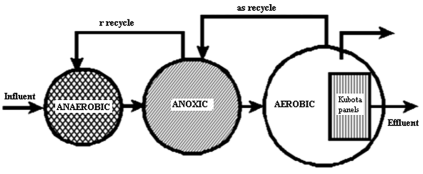

Fig. 1. Schematic layout of MBR UCT system

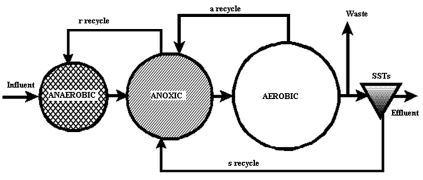

Fig. 2. Schematic layout of CAS UCT system

Table 1. MBR and CAS UCT systems' design and operating parameters

System parameters	MBR UCT	CAS UCT
Sludge age (d)	20	20
Anaerobic (R1) mass fraction (%), Volume (ℓ)	12.6[a]/19	12.6[a]/5.6
Anoxic (R2) mass fraction (%), Volume (ℓ)	27.9[a]/21	27.9[a]/6.2
Aerobic (R3) mass fraction (%), Volume (ℓ)	59.5[a]/35	59.5[a]/13.2
a-recycle (R3 to R2)	3:1	2:1
r-recycle (R2 to R1)	1:1	1:1
s-sludge Return Recycle (SST to R2)	-	1:1
Hydraulic retention time (d)	0.53	1.67
MLVSS concentration (mg/ℓ)	12 500	3 600
MLTSS concentration (mg/ℓ)	18 000	5 000
Influent flow (ℓ/d)	140	15
Feed COD concentration (mg/ℓ)	1000	1000
Membrane flux ($m^3/m^2/d$)	0.239	-

[a] For the given a- and r-recycle ratios.

The systems were fed screened (1 mm mesh) raw unsettled municipal wastewater from the Mitchell's Plain Wastewater Treatment Plant (Cape Town, South Africa), augmented with sodium acetate (200 mgCOD/ℓ to accentuate BEPR), ammonia (20 mgN/ℓ to increase TKN/COD), phosphorus (to ensure > 5 mgP/ℓ in effluent) and sodium bicarbonate (to provide some alkalinity for pH buffering). The wastewater was collected in 2 m^3 batches, macerated and stored in stainless steel tanks at 4°C and served as feed for both systems for 15 to 20d. Daily, after thorough mixing, the required volume of wastewater was withdrawn from the stainless tanks and diluted with tap water to the target COD concentration (800 mgCOD/ℓ). After adding the supplements, a sample is taken and the required volume for 1 days feed transferred into the systems' refrigerated (8°C) feed drums. The feed drums were gently stirred (1–2 rpm) to keep settleable solids in suspension and covered with a floating lid to minimize oxygen entrainment. The influent was pumped into each system with a multi-channel peristaltic pump, which also pumped the recycle flows. The influent tube was passed through a water bath at 20°C to avoid temperature decrease in the anaerobic reactor, in particular the MBR system with the very short hydraulic retention time.

The two systems were monitored daily via the parameters listed in Table 2. Additionally, recycle flow rates and trans-membrane pressure (TMP, constant flux) were monitored daily. Once monthly mixed liquor samples were analysed by a microbiologist for filament identification and floc morphology. Also mixed liquor samples were sent fortnightly for FISH analysis (Maharaj et al. 2007). The influent readily biodegradable organics (RBO) COD) concentration (before supplement addition) was measured daily in a fully aerobic square wave fed (12 h feed on, 12 h feed off) AS system at 2.5 days sludge age according to Ekama et al. (1986).

For each wastewater batch (which was accepted to represent a steady-state period), the daily results were averaged (after analysis for outliers). These steady-state averages were used to assess the performance of the systems and the following process

Table 2. Sampling position and parameter measurement

Test	COD	TKN	FSA	NO_3	NO_2	T-P	TSS	VSS	OUR	DSVI	pH
Influent	F; UF	UF	F			UF					
Anaerobic				F	F	F	UF	UF			
Anoxic				F	F	F	UF	UF			
Aerobic	UF	UF		F	F	F	UF	UF	D	D[a]	D
Final effluent	F; UF	F; UF	F	F	F	F; UF					

F = 0.45 μm filtered; UF = Unfiltered samples; D = Direct measurement taken. COD; TKN; FSA (Free and Saline Ammonia); T-P (Total Phosphorus); TSS; VSS (Standard Methods 1985). DSVI = Dilute Sludge Volume Index; (Ekama and Marais 1984); OUR = Oxygen Utilization Rate (Randall et al. 1991).

[a] For the MBR system, the unfiltered COD was measured at the 800 mℓ mark of the 1000 mℓ measuring cylinder after 30 min settling.

characteristics were calculated: System COD and N mass balances; influent unbiodegradable soluble and particulate COD fractions ($f_{S'us}$ and $f_{S'up}$ respectively, Ekama and Wentzel 1999); mixed liquor VSS/TSS, COD/VSS and TKN/VSS ratios; nitrate and P mass changes across each reactor, sludge production and the influent readily biodegradable (RB) COD from the OUR measured in the square-wave fed 2.5d sludge age AS system (du Toit et al. 2007).

To determine the kinetics rates, aerobic, anoxic-aerobic and anaerobic-aerobic and anaerobic-anoxic-aerobic batch tests on the mixed liquor harvested from the different reactors of the two BNR systems were conducted (Parco 2006; Parco et al. 2006, 2007). Particularly on the MBR system the influence of the VSS concentration and of the limitation of ammonia, oxygen, nitrate and acetate concentrations on the kinetic rates was examined. Moreover, to provide additional information on the anoxic behaviour of phosphate accumulating organisms (PAO), the ability of AS in MBR and CAS systems to denitrify under anoxic conditions with simultaneous phosphate uptake was investigated and quantified to check the extent of anoxic P uptake BEPR in the systems. This is important to accurately separate OHO and PAO denitrification behaviour. Detailed results of the whole investigation summarised here are given by Parco (2006) or du Toit et al. (2010).

3 Batch Test Inventory

Three groups of aerobic nitrification batch tests (37 in all) were conducted to evaluate the effect of VSS, ammonia and dissolved oxygen (DO) concentration on the nitrification kinetics in the MBR system: 29 Group (1), i.e. 10 with 10-20 mgN-NH_4/ℓ, 12 with 30-40 mgN-NH_4/ℓ and 7 with 50 mgN-NH_4/ℓ on MBR system ML diluted (with effluent) to different VSS concentrations between 2 and 14 gVSS/ℓ, i.e. 8 with 2-3 gVSS/ℓ, 2 with ∼4 gVSS/ℓ, 6 with ∼5-6 gVSS/ℓ, 5 with 7-10 gVSS/ℓ, 2 with 10-11 gVSS/ℓ and 5 with 12-14 gVSS/ℓ on MBR system ML, 2 Group (2), i.e. 2 on MBR system ML at the same VSS concentration (∼9 gVSS/ℓ) but at different DO concentrations 2-5 and 10-15 mgO/ℓ) and 6 Group (3), i.e. in parallel, 3 on each of MBR

and CAS system ML with MBR ML diluted to the same low VSS concentration as that from the CAS system (2-3 gVSS/ℓ) to determine the effect of the membranes.

Five groups of anoxic batch tests for denitrification (33 in all) were conducted, viz. Group (1): On MBR system ML at different VSS concentrations between 2.5 and 12 gVSS/ℓ with ML from the anaerobic and aerobic reactors mixed in proportion to the recycles entering the anoxic reactor; Group (2): like Group (1) but at different nitrate concentrations; Group (3): like Groups (1) and (2) but with different proportions of anaerobic and aerobic ML (Set I - 50/50 by VSS mass, Set II - 100% anaerobic and Set III - 100% aerobic); Group (4) on MBR and CAS system ML in parallel with the MBR ML diluted to the same low VSS concentration as that from the CAS system (2-3 gVSS/ℓ) and with ML from the anaerobic and aerobic reactors mixed in proportion to the recycles entering the anoxic reactor and Group (5): like Group (4) but with wastewater added.

Altogether fifteen anaerobic batch tests were conducted, 13 (BTs 1 to 13) with low to moderate acetate dosages varying from 0.009 to 0.043 mgHAcCOD/mgVSS and VSS concentrations ranging from 2.7 to 11.2 gVSS/ℓ, one (BT14) with excess acetate addition at 0.166 gHAcCOD/gVSS at 6.37 gVSS/ℓ and one (BT15) with wastewater addition at 5.52 gVSS/ℓ.

4 Calculating the Bioprocess Specific Kinetic Rates

In the steady-state design procedures and dynamic models, the increased sludge production in MBR systems can be accommodated by increasing the influent unbiodegradable particulate COD fraction ($f_{S'up}$). This was done in this investigation. Fixing the unbiodegradable soluble COD fraction ($f_{S'us}$) for the MBR and CAS systems at the values found above, i.e. 0.045 and 0.066 respectively, the $f_{S'up}$ fraction for the MBR and CAS systems were calculated to be 0.241 and 0.084 mgCOD/mgCOD respectively to match the measured average mass of VSS in the systems (Ekama and Wentzel 1999). Noting that the model takes account of the different masses of PAOs in the two systems, it is a concern that for two systems with the same design and operating parameters fed the same wastewater, different $f_{S'up}$ fractions are obtained. If $f_{S'up}$ is really a wastewater characteristic, $f_{S'up}$ should be the same for both systems. The problem of obtaining different $f_{S'up}$ fractions for the MBR and CAS systems, is that they result in different OHO (f_{avOHO}) and PAO (f_{avPAO}) biomass fractions of the VSS in the systems, where $f_{avOHO} = X_{BH}/X_v$ and $f_{avPAO} = X_{BG}/X_v$ and X_{BH}, X_{BG} and X_v are the OHO, PAO and total VSS concentrations respectively. However, the method of calculating $f_{S'up}$ by matching the calculated mass of VSS in the system with that measured has always has been applied in the past to determine the f_{avOHO} and f_{avPAO} active fractions and the OHO and PAO specific kinetic rates (van Haandel et al. 1981; Wentzel et al. 1990; Clayton et al. 1991; Ekama and Wentzel 1999) and these specific rates have been adopted as default values in the ASM1 and ASM2 kinetic models. So because there is no other way of determining biomass specific kinetic rates from experimental systems fed real wastewater, the uncertainty that different $f_{S'up}$ fractions will have on the kinetic rates, while not ideal, has to be accepted as it has been in the past (Ekama and Wentzel 1999) because expressing kinetic rates in terms of VSS

makes the rates incomparable between different BNR systems. In the end, steady state models aligned with and based on the same but simplified principles as kinetic models are the only interface between experimental systems and the kinetic models.

Because the kinetic rates determined from the batch tests results were assigned to the biomass population mediating the particular bioprocess, and the steady state NDBEPR model (Wentzel et al. 1990) was used to determine the OHO (f_{avOHO}) and PAO (f_{avPAO}) active fractions from the measured data on the MBR and CAS systems, it was important for the OHO specific denitrification rate and the PAO specific P release and P uptake rates that the observed and predicted P removal of the systems matched well. This ensured that the OHO and PAO specific kinetic rates were consistent with estimates of the OHO (f_{avOHO}) and PAO (f_{avOHO}) active fractions determined in the past. The wastewater batch average calculated P removal of the MBR system based on the known system operating parameters, dosed acetate (200 mg/ℓ) and measured wastewater RBO concentration was > 2 mgP/l below that measure P removal but thereafter matched well. The nitrification batch tests, for which a close correlation between predicted and measured P removal was not important, were conducted at the beginning of the investigation when the predicted and measured P removal did not match well. The denitrification (anoxic) and P release and P uptake (anaerobic-anoxic/aerobic) batch tests were conducted during wastewater batches 10 to 25, when the predicted and measured P removal did match well. The measured kinetic rates in the MBR and CAS systems can therefore be legitimately compared with rates measured in previous investigations.

5 Conclusions

To assess the impact of high VSS concentration in membrane bioreactor biological nutrient removal (BNR) activated sludge (AS) systems on the bioprocess kinetic rates that mediate biological N and P removal, two identical (except for the hydraulic retention time) parallel laboratory scale University of Cape Town (UCT) nitrification denitrification (ND) biological excess phosphorus removal (BEPR) systems fed the same real wastewater were operated for 450 days, one at a low VSS concentration (3 gVSS/ℓ) and solid liquid separation with a secondary settling tank (CAS system), the other at a high VSS concentration (13 gVSS/ℓ) and solid liquid separation with submerged panel membranes (MBR system). From the BNR performance of these two systems and from aerobic, anoxic-aerobic and anaerobic-anoxic-aerobic batch tests on sludge harvested from the two systems the following conclusions were drawn.

The MBR system achieved a higher COD removal (effluent COD 41 mgCOD/ℓ) compared with the CAS system (unfiltered 74 mgCOD/ℓ, 0.45 μm filtered 51 mgCOD/ℓ) due to the complete retention of particulate organics and some colloidal organics considered soluble in CAS systems. However, the "unfiltered effluent" COD concentration from the MBR system (measured at the 800 ml mark in the 1000 ml measuring cylinder after 30 min settling in the diluted sludge volume index test) was much higher (139 mgCOD/ℓ) than the unfiltered COD from the CAS system (73 mgCOD/ℓ). Both systems achieved similar in N removals (MBR 83%, CAS 81%). Nitrification was complete in both systems - effluent free and saline ammonia

(FSA) concentration from the MBR system was 0.7 mgFSA-N/ℓ and from the CAS system 0.9 mgFSA-N/ℓ. Denitrification was better in the MBR system (effluent nitrate MBR 18.0 mgNO_3-N/ℓ and CAS 20.0 mgNO_3-N/ℓ) due to the negligible impact of the dissolved oxygen in the recycle to the anoxic reactor at the high VSS concentration of the MBR system. The P removal in the MBR system (22.5 mgP/ℓ) was higher than that in the CAS system (17.4 mgP/ℓ). This was due to the recycle of nitrate from the anoxic reactor to the anaerobic reactor and greater anoxic P uptake in the CAS system due to the non-zero nitrate concentration in the anoxic reactor. This made the kinetic rates associated with BEPR measured in the batch tests incomparable between the two systems. Due to the higher sludge production by the MBR system [0.31 (gVSS/d)/(gCOD/d)] than by the CAS system [0.20 (gVSS/d)/(gCOD/d)], the influent unbiodegradable particulate COD fraction ($f_{S',up}$) of the MBR system was higher (0.241) than that of the CAS system (0.084). This affected the fractionation of the VSS into the ordinary heterotrophic organism (OHO) and phosphate accumulating organism (PAO) active fractions in the two systems with the steady state BNR models, which also affected the observed OHO and PAO VSS specific kinetic rates calculated from the results of the batch tests on sludge harvested from two systems. This affect was unavoidable because kinetic rates expressed in terms of VSS are not comparable between different BNR systems. This effect was unaviodable because steady state models aligned with and based on the same but simplified principles as kinetic models are the only interface between experimental systems and the kinetic models.

From the aerobic nitrification batch tests: (1) At the same low VSS concentration, the MBR system exhibited lower VSS specific ammonia utilization rate (SAUR) and autotrophic nitrifier organism (ANO) maximum specific growth rates (μ_A) than the parallel CAS system, apparently due to different selection pressures imposed by membranes and SSTs. (2) For the MBR system, as the VSS concentration increased, the SAUR and μ_A decreased, apparently due to ammonia and/or oxygen transfer limitations. (3) For the MBR system at the VSS concentration, as the initial ammonia concentration increased, the SAUR and μ_A increased, indicating possible ammonia transport limitation at increasing VSS concentration.

From the above, it was evident that the ANOs in the MBR and CAS systems exhibited different behaviour, apparently induced by different environments under which the ANOs develop. The reasons for this possibly are: (1) In CAS systems with SSTs, organism loss via the effluent occurs including ANOs. Therefore CAS system may select ANOs with higher maximum specific growth rates (μ_A) than MBR systems. In the MBR system all the ANOs are retained, including slow growing ones. (2) At the high VSS concentrations in the MBR system, oxygen and ammonia transport limitations decrease the observed SAUR and μ_A.

From the anoxic-aerobic batch tests, the OHOVSS specific denitrification rate by OHOs (K_{2OHO}) utilizing slowly biodegradable organics (SBO) obtained at different MBR system VSS concentrations (2.5-12 gVSS/ℓ) and different initial nitrate concentrations ranging from 30 to 90 mgN/ℓ showed no effect to initial nitrate concentration, in agreement with past work (van Haandel et al. 1981, Clayton et al. 1991; Ekama and Wentzel 1999) and no effect to VSS concentration. From all the anoxic batch tests, the average K_{2OHO} was 0.264 mgNO_3-N/(mgOHOVSS.d), which is very

close to the average K_{2OHO} rate reported in the literature for conventional (low VSS) BNR systems with SSTs, i.e. 0.255 from Ekama and Wentzel (1999).

From the anaerobic-anoxic-aerobic batch tests, the specific VSS and specific PAOVSS anaerobic acetate (as COD) uptake and P release rates showed no effect of VSS or initial acetate concentration. Also, the results obtained with different concentrations of acetate added showed the acetate uptake rate to be zero order with respect to acetate concentration, which is in agreement with literature studies (Wentzel et al. 1985, 1989). The P release to acetate uptake ratio also showed no effect with acetate dose and VSS concentration. The specific VSS and specific PAOVSS aerobic and anoxic P uptake rates also showed no effect of VSS concentration. The average PAOVSS specific anaerobic acetate uptake and P release rates and the aerobic P uptake rate obtained over the VSS concentration range were within the range of literature rates observed on enhanced PAO culture systems, confirming that within experimental variation, high VSS concentration does not affect the rates.

In the anaerobic-anoxic/aerobic batch tests with acetate uptake, the PAOs showed significantly higher anoxic P uptake and denitrification rates than in the MBR system itself, where high acetate and excess nitrate did not occur. In the former the PAOs denitrified 22% of the nitrate whereas in the MBR system only 11%. The OHOVSS specific denitrification rates were within the same 0.2 to 0.3 $mgNO_3$-N/(mgOHOVSS.d) range in all the batch with an anoxic phase. While the PAOVSS specific denitrification rate in the anaerobic-anoxic/aerobic batch tests was about half of the OHOVSS specific denitrification rate, in the MBR system, the PAOVSS specific denitrification rate was only $1/14^{th}$ of the OHOVSS specific denitrification rate because the conditions in the anaerobic-anoxic/aerobic batch tests (high acetate and nitrate) were not prevalent in continuous flow BNR systems fed real wastewater. The large reduction in P removal resulting from significant anoxic P uptake BEPR seems counter-productive for the very small PAO contribution to denitrification.

The results from this investigation show that the BNRAS steady state and kinetic models developed for low VSS concentration BNRAS systems with secondary settling tanks can be applied with reasonable confidence to predict the performance of high VSS concentration BNRAS systems with membranes, except for the maximum specific growth rate of the nitrifiers, which was observed to be significantly lower in the MBR system.

Specific denitrification rates are zero order with respect to nitrate concentration and HAc consumption rates are zero order respect to HAc concentration in agreement with previous observations on conventional BNR systems. Anoxic P uptake has been consistently observed and the existence of 2 groups of PAO bacteria has been demonstrated. Anoxic P uptake is detrimental to the BEPR performance in a BNR system. However, quantitative links between design and operational parameters and the extent of anoxic P uptake have not been established. This has hindered incorporation of anoxic P uptake in the design and simulation models for BNR systems, with or without membranes, and requires resolution. The specific denitrification rates of OHOs are significantly higher than those of PAOs, to confirm the greater affinity of OHOs than PAOs for nitrate.

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