Filamentous organism bulking in nutrient removal activated sludge systems

Paper 8: The effect of nitrate and nitrite

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Abstract

The presence of nitrate (NO_3^-) and nitrite (NO_2^-) has a significant effect on the proliferation of low F/M filamentous organisms in nitrogen (N) and nutrient (N and phosphorus-P) removal activated sludge systems. In experiments in which the concentrations of NO_3^- and NO_2^- were manipulated in the 2nd anoxic reactor of a modified University of Cape Town (MUCT) nutrient removal system, either by addition of ammonium to the influent or by dosing NO_3^- or NO_2^- to the 2nd anoxic reactor, the extent of low F/M filamentous organism proliferation could be controlled, as measured by the diluted sludge volume index (DSVI). With a sufficiently high TKN/COD ratio (> 0.10 mgN/mgCOD) in the influent, or by dosing NO_3^- and NO_2^- at a level at which the denitrification potential of the anoxic zone was exceeded by the NO_3^- or NO_2^- load, the DSVI increased from values less than 100 mℓ/g to values greater than 150 mℓ/g in periods of between 3 and 5 sludge ages. It could not be determined which of NO_3^- or NO_2^- had the most significant effect on filament proliferation. While the stimulatory effect of the NO_3^- or NO_2^- passing into the aerobic zone on low F/M filamentous organism proliferation was positively identified, the mechanism by which this effect operated could not be established.

List of symbols

AA	=	anoxic-aerobic filament classification group
AVSS	=	active volatile suspended solids
COD	=	chemical oxygen demand
DSVI	=	diluted sludge volume index
F/M	=	food to micro-organism ratio
$f_{av,OHO}$)	=	the theoretical fraction of the VSS that is ordinary heterotrophic organisms (OHO)
$f_{S,up}$	=	wastewater unbiodegradable particulate COD fraction
IAND	=	intermittently aerated nitrification-denitrification
K_2 , K'_2	=	second (slow) rate of denitrification, in mgNO_3^--N/(mgAVSS·d) in the primary anoxic reactor utilising SBCOD in ND and NDBEPR systems respectively
MLSS	=	mixed liquor suspended solids
MLVSS	=	mixed liquor volatile suspended solids
MUCT	=	modified University of Cape Town
ND	=	nitrification-denitrification
NDBEPR	=	nitrification-denitrification biological excess phosphorus removal
RBCOD	=	readily biodegradable COD
SBCOD	=	slowly biodegradable COD
TKN	=	total Kjeldahl nitrogen
VSS	=	volatile suspended solids
MLE	=	modified Ludzack-Ettinger

Introduction

From observations on the experimental investigation into various factors proposed as possibly being associated with low F/M filamentous organism bulking (Lakay et al., 1999), it was concluded that the exposure of sludge to alternating anoxic and aerobic conditions, combined with the presence of NO_3^- and/or NO_2^-, played a significant role in proliferation of these organisms. This conclusion was drawn from observations that experimental changes which resulted in significant increases and decreases in sludge settleability were accompanied by significant increases and decreases in the concentration of NO_3^- and NO_2^-, either in the anoxic zone or in the effluent. The reasons for this association were not clear. To determine more precisely the relationship between low F/M filament proliferation and the NO_3^-/NO_2^- concentration, experiments were conducted in which the NO_3^-/NO_2^- concentration in the system was manipulated by increasing the influent TKN/COD ratio by addition of ammonium (NH_4^+) to the influent (in the form of an ammonium chloride solution); and direct addition of NO_3^- and/or NO_2^- to the anoxic reactor of the system by drip feeding a concentrated solution of sodium nitrate or sodium nitrite directly to the anoxic reactor.

Preliminary tests

Effect of changes in influent TKN/COD ratio on low F/M filament proliferation

The effect on low F/M filament proliferation of changes in the TKN/COD ratio through the addition of ammonium to the influent was examined at laboratory-scale in two MUCT systems (referred to as MUCT1 and MUCT2), the design and operating parameters of which are given in Table 1.

Initially, the two systems had the same operating conditions, including the same anaerobic, anoxic and aerobic mass fractions, i.e. anaerobic:anoxic:aerobic; 3:10½: 6½ [*Note: The anoxic mass fractions (50 to 65%) employed in the N&P removal (MUCT) systems in these experiments are considerably larger than would be employed in practice. This is to ensure complete anoxic denitrification even for high TKN/COD ratios (0.12 mgN/mgCOD) encountered from time to time with the sewage batches fed to the*

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Received 11 November 1998; accepted in revised form 20 April 1999.

TABLE 1
OPERATING PARAMETERS AND CONDITIONS OF MODIFIED UNIVERSITY OF CAPE TOWN NUTRIENT (N&P) REMOVAL SYSTEMS MUCT1 AND MUCT2

System	MUCT1	MUCT2
Operating conditions	Continuously fed, completely mixed multi-reactor systems	
DO in aerobic zone (mgO/ℓ)	2.0 - 4.0	2.0 - 4.0
Feed	Continuous (24 h)	
Sewage source	Mitchell's Plain raw	
Sludge source	Laboratory MUCT systems	
Mass of COD fed (mg/d)	10000	10000
Volume of feed (ℓ/d)	10	10
Target concentration (mgCOD/ℓ)	1000	1000
Influent TKN (mgN/ℓ)		
Days 1 to 39	103.9	104.3
Days 40 to 169	101.5	134.3
Days 170 to 198	138.2	107.5
Sludge age (d)	20	20
Temperature (°C)	20	20
Reactor volumes (ℓ); Mass fractions (%)		
Anaerobic	6 ; 15	6 ; 15
1st anoxic	4 ; 20	4 ; 20
2nd anoxic	6.5 ; 32.5	6.5 ; 32.5
Aerobic	6.5 ; 32.5	6.5 ; 32.5
MLVSS concentration (mg/ℓ)	2 435	2 436
MLSS concentration (mg/ℓ)	2 934	2 900
F/M [mgCOD applied/(gVSS·d)]	205	205
Hydraulic retention time (h)	48	48
Recycle ratios		
Aerobic (a)	4:1	4:1
Sludge (s)	1:1	1:1
Return (r)	1:1	1:1
pH of mixed liquor	7.2 - 8.2	7.2 - 8.2

laboratory systems. This resulted in very small aerobic mass fractions (20 to 33%) which may inhibit nitrification and P uptake. The anaerobic reactor mass fraction was retained at 15% to ensure that all the influent RBCOD would be utilised in the anaerobic zone and not affect the kinetics of denitrification (Clayton et al., 1989, 1991) and filament proliferation (Ekama et al., 1996)]. The changes in DSVI with time and the changes in operating conditions for MUCT1 and MUCT2 are illustrated in Fig. 1. During the first 40 d, the two systems were operated without ammonium addition to the influent (TKN/COD = 0.08 to 0.10 mgN/mgCOD) and the DSVIs of both systems were between 100 and 150 mℓ/g. On Day 40, ammonium was added to the influent of MUCT1 which increased the influent TKN/COD to 0.12 to 0.14 mgN/mgCOD and over a period of 5 sludge ages (Days 40 to 169), the DSVI increased from ≈110 to ≈ 250 mℓ/g.

During the same period for MUCT2, to which no ammonium was added (TKN/COD = 0.08 to 0.10 mgN/mgCOD), the DSVI remained between 100 and 150 mℓ/g. It should be noted that during the period Day 121 to Day 151, the sewage contained a high concentration of sulphide. On Day 170, addition of ammonium to the influent of MUCT1 was stopped and was transferred to MUCT2. This reduced the influent TKN/COD of MUCT1 to 0.08 to 0.10 mgN/mgCOD and the DSVI decreased from 250 mℓ/g to 160 mℓ/g in less than two sludge ages (Days 170 to 198). With ammonium addition to the influent of MUCT2, the influent TKN/COD increased to 0.12 to 0.14 mgN/mgCOD and the DSVI increased from ≈ 100 to 220 mℓ/g over the same two sludge age period (Days 170 to 198). Regular filament identifications (see Fig. 1) indicated that these changes in DSVI were mainly attributable to changes in low F/M filament proliferation, in particular type 0092. During the period in which the concentration of sulphide in the sewage was high (Days 121to151) filament type 021N became dominant in MUCT1 and the DSVI was very high (> 400 mℓ/g). Jenkins et al. (1984, 1993) list type 021N both as a low F/M filament and as a septic wastewater/high sulphide filament and in these experimental systems it would appear that its proliferation is a consequence of sulphide in the sewage.

Figures 2(a) to 2(c) illustrate for MUCT1 the variation with time of DSVI, VSS and concentration of nitrogen oxides (NO_x^-) (i.e. NO_3^- + NO_2^-) in the second anoxic reactor. Figures 3(a) to 3(c) illustrate the same parameters for MUCT2. The concentration of NO_x^- was measured in each of the reactors of each system and from an examination of the results (not shown in this paper) it appears that the strongest relationship between the NO_x^- concentration and low F/M filament proliferation (measured as DSVI) is for the NO_x^- concentration measured in the 2nd anoxic reactor. With increase in TKN/COD ratio and corresponding increase in DSVI, the concentration of NO_x^- in the 2nd anoxic reactors of both MUCT1 and MUCT2 increased from ≈5 to ≈20 mgN/ℓ. An additional finding in these experiments was that as the DSVI increased, the VSS concentration in the system decreased, and conversely, as the DSVI decreased, so the VSS concentration increased. Hypotheses, based on microbiological kinetics and biochemical mechanisms, are proposed in Paper 11 of this series (Casey et al., 1999a) in order to explain the associations between the parameters DSVI, VSS and NO_x^- in the 2nd anoxic reactor.

From the results described above, it can be concluded that high concentrations of NO_3^- and NO_2^- in the MUCT system in general, and in the 2nd anoxic reactor in particular, are associated with low F/M filamentous organism proliferation. The focus of the investigation from this point was to examine this association more closely with particular emphasis on the roles of nitrate and nitrite.

Experimental investigation

Research direction

Accepting that the presence of NO_3^- or NO_2^- is the cause of low F/M filament proliferation, the first task was to determine, firstly, which of the two, NO_3^- or NO_2^- is responsible, and secondly, in

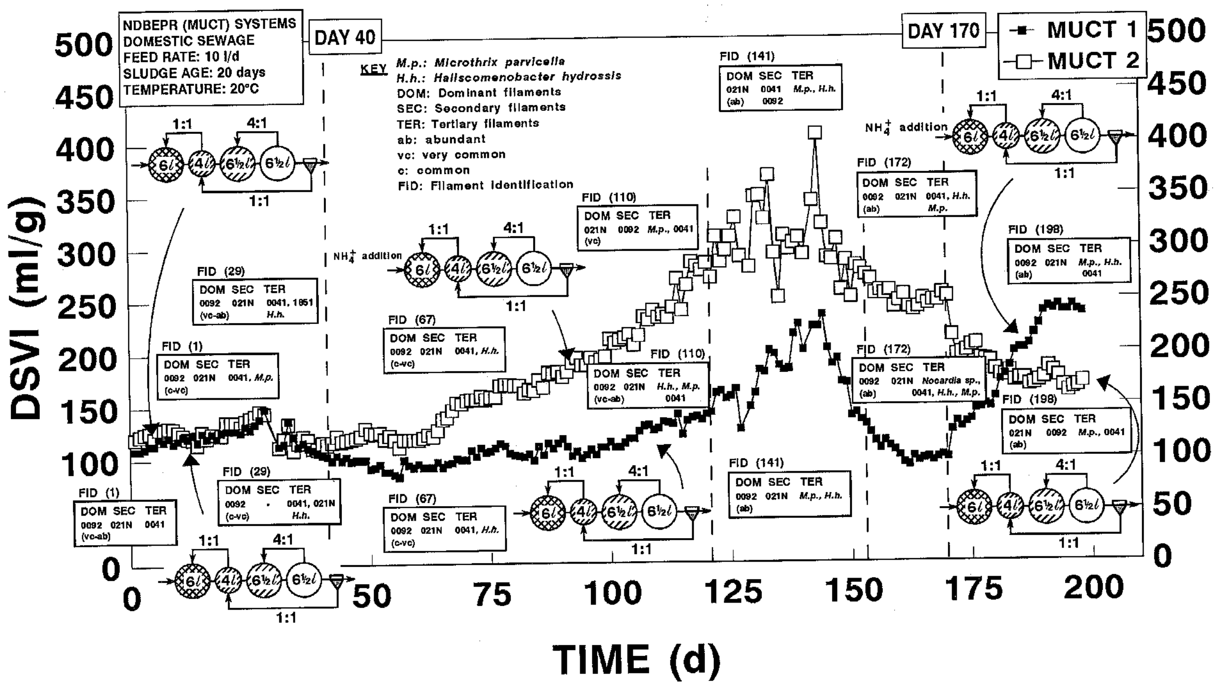

Figure 1

Sludge settleability as DSVI (mℓ/g) and changes in system operation with time for MUCT Systems 1 and 2

which zone, anoxic or aerobic, do the filaments proliferate? This is a complex problem since in anoxic-aerobic systems, each of NO_3^- and NO_2^- is produced as a result of microbiological action on the other, either NO_2^- to NO_3^- by nitrification under aerobic conditions (i.e. $NO_2^- \rightarrow NO_3^-$), or NO_3^- to NO_2^- by reduction of NO_3^- under anoxic conditions (i.e. $NO_3^- \rightarrow NO_2^-$).

The requirement for the presence of either NO_3^- or NO_2^- for low F/M filament proliferation was investigated in two MUCT configurations (MUCT3 and MUCT4) to which either NO_3^- or NO_2^- were dosed or not. Complete details of the operation and results of these experiments are given by Musvoto et al. (1992, 1994).

Effect of NO_3^- dosing on low F/M filament proliferation in an MUCT configuration

In investigating the role of NO_3^-, MUCT3 was operated with the design and operating parameters given in Table 2. The variation in DSVI with time for MUCT3 is shown in Fig. 4. The system was operated for more than 6 sludge ages under these conditions and the DSVI decreased from ≈ 160 mℓ/g to ≈ 70 mℓ/g by Day 128. During this period the TKN/COD ratio was ≈ 0.08 mgN/mgCOD and the average NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor were 0.5 and 0.2 mgN/ℓ respectively. During this period, the NO_3^- load on the anoxic zone was 50% of its denitrification potential, which was calculated from anoxic batch tests conducted on sludge harvested from the system. From Day 129, NO_3^- in the form of a solution of $NaNO_3$ was continuously dosed to the 2nd anoxic reactor to ensure the presence of NO_3^- at all times. As a means of ensuring that sufficient NO_3^- was dosed to exceed the denitrification potential of the anoxic zone, the denitrification rate of the sludge in the system was calculated from denitrification rates measured in two anoxic denitrification batch tests, conducted on sludge from the system on Days 104 and 113. From the denitrification rates, a denitrification potential of 720 $mgNO_3^-$-N/d was calculated and dosing at a rate somewhat greater than this (960 $mgNO_3^-$-N/d) was started on Day 129 to ensure an excess of nitrate in the system. During the following 110 d until Day 239 when NO_3^- dosing was terminated, the DSVI increased from ≈ 65 mℓ/g to 170 mℓ/g. During this period, average NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor were 6.8 and 2.1 mgN/ℓ respectively, concentrations considerably higher than the concentrations of 0.5 and 0.2 mgN/ℓ respectively measured during the period when NO_3^- was not dosed.

During the dosing period (Days 129 to 239), three filament identifications were conducted (Days 181, 202 and 237). Filament type 0092 was dominant or secondary on all three occasions, and other filaments present were types 0914 and 0041 and *Microthrix parvicella, Haliscomenobacter hydrossis* and *Flexibacter*. All dominant and secondary filaments were low F/M types.

An interesting result associated with the period during which NO_3^- was dosed and during which the DSVI increased, was a decrease in the amount of sludge generated in the system per unit mass of COD fed. Between Day 1 and Day 128, when the system had a decreasing DSVI, the MLSS and MLVSS of MUCT3 were high (average values of 3 600 and 2 900 mg/ℓ respectively). From Day 129 to Day 239, when the DSVI increased from ≈ 65 mℓ/g to 170 mℓ/g, the average values of MLSS and MLVSS decreased from 3 900 and 3 116 mg/ℓ respectively to 2 873 and 2 350 mg/ℓ respectively by Day 239. This is a similar result to that noted for MUCT1 and MUCT2 systems operated in the preliminary tests described above.

The NO_3^- dose was stopped on Day 239 and the system was operated in the same mode as between Days 1 and 128. The DSVI immediately began to decrease and reached a value of 91 mℓ/g on Day 309. The NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor decreased to averages of 0.7 and 0.14 mgN/ℓ respectively. From Days 310 to 340, the DSVI increased slightly to 105 mℓ/g, apparently a consequence of an increased influent TKN/COD ratio, from 0.09 to 0.10 mgN/mgCOD which caused the average NO_2^- concentration in the 2nd anoxic reactor to increase to a value of 0.3 from 0.14 mgN/ℓ, the NO_3^- concentration remaining constant at 0.7 mgN/ℓ. In a result similar to that noted earlier in this system, the amount of sludge generated in the system per unit mass of COD fed,

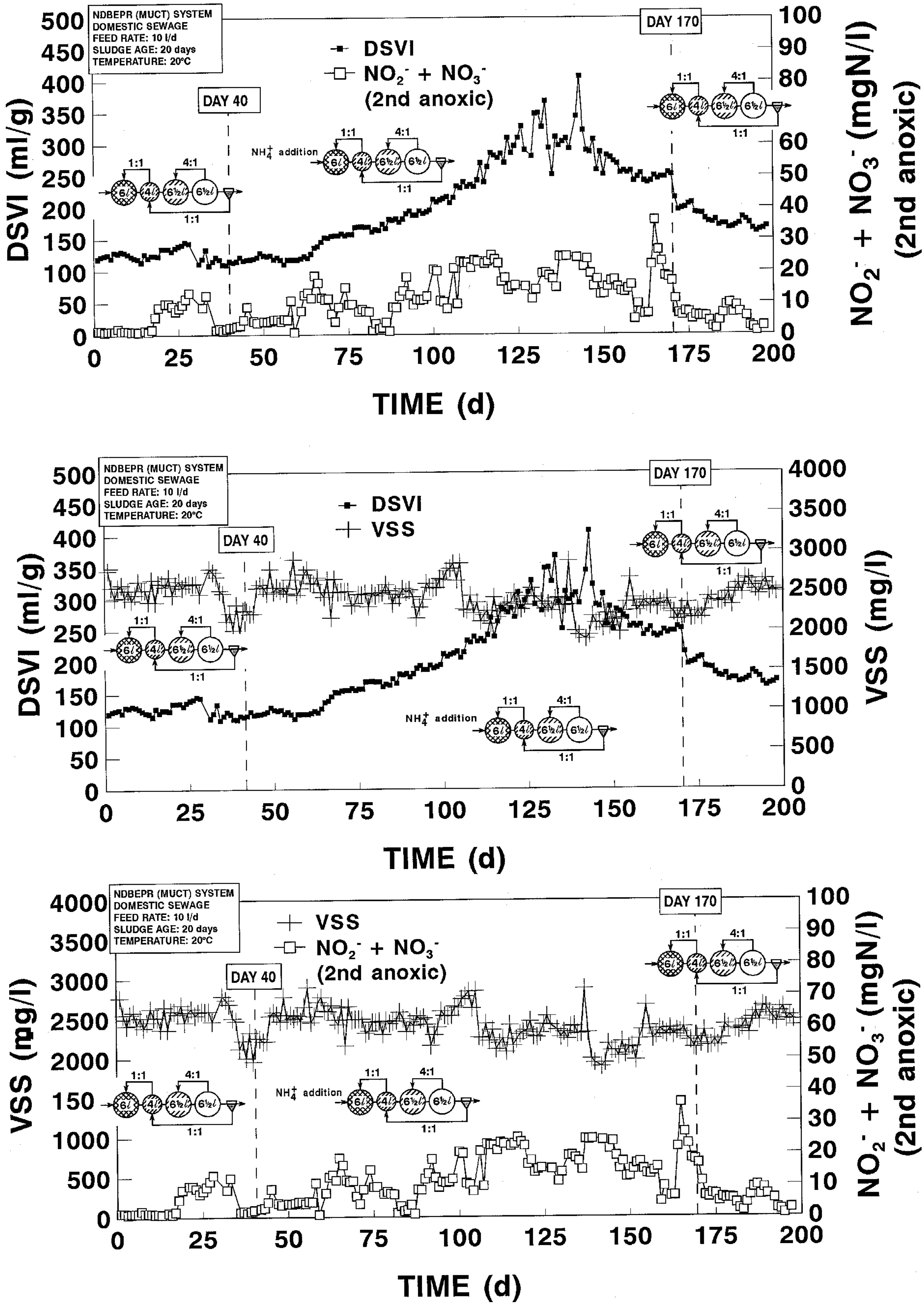

Figure 2

MUCT System 1, with and without ammonia additon to the influent, illustrating the effect on (a) DSVI and VSS with time, (b) DSVI and 2nd anoxic reactor nitrate + nitrite (NO_3^- + NO_2^-) concentration with time, and (c) VSS and 2nd anoxic reactor nitrate + nitrite (NO_3^- + NO_2^-) concentration with time

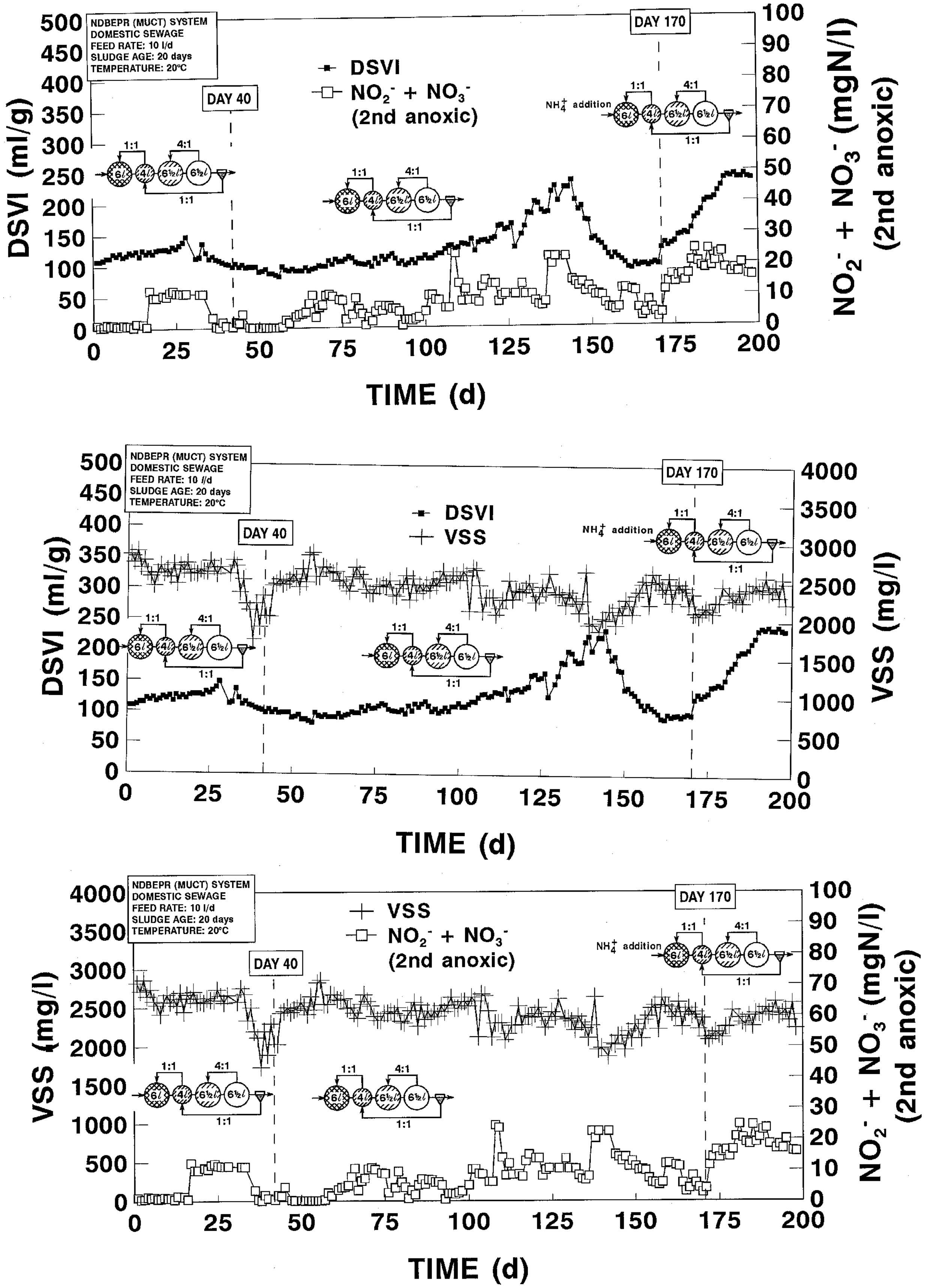

Figure 3

MUCT System 2, with and without ammonia additon to the influent, illustrating the effect on (a) DSVI and VSS with time, (b) DSVI and 2nd anoxic reactor nitrate + nitrite (NO_3^- + NO_2^-) concentration with time, and (c) VSS and 2nd anoxic reactor nitrate + nitrite (NO_3^- + NO_2^-) concentration with time

Table 2 **Operating Parameters and Conditions of Modified University of Cape Town Nutrient (N&P) Removal Systems MUCT3 and MUCT4**		
System	**MUCT3**	**MUCT4**
Operating conditions	Continuously fed, completely mixed multi-reactor systems	
DO in aerobic zone (mgO/ℓ)	2.5 - 4.0	2.5 - 4.0
Feed Sewage source Sludge source	Continuous (24 h) Mitchell's Plain raw Laboratory MUCT systems	
Mass of COD fed (mg/d) Volume of feed (ℓ/d) Target concentration (mgCOD/ℓ)	10 000 10 1 000	10 000 10 1 000
Influent TKN (mgN/ℓ)	60 - 100	60 - 100
Nitrate/Nitrite to 2nd anoxic (mgN/d) NO_3^- NO_2^-	 720 (Days 129 to 239) Nil	 Nil 900 (Days 291 to 340)
Sludge age (d) Temperature (°C)	20 20	20 20
Reactor volumes (ℓ); Mass fractions (%) Anaerobic 1st anoxic 2nd anoxic Aerobic	 6 ; 15 4 ; 20 9 ; 45 4 ; 20	 6 ; 15 4 ; 20 9 ; 45 4 ; 20
MLVSS concentration (mg/ℓ) MLSS concentration (mg/ℓ)	2 986 3 555	3 035 3 613
F/M [mgCOD applied/(gVSS·d)]	167	167
Hydraulic retention time (h)	48	48
Recycle ratios Aerobic (a) Day 1 to Day 27 Day 28 to Day 340 Sludge (s) Return (r)	 2:1 3:1 1:1 1:1	 3:1 3:1 1:1 1:1
pH of mixed liquor	7.2 - 8.2	

changed with the change in DSVI. After the nitrate dose was stopped on Day 239, the sludge mass generated per COD mass load on the system increased, as the DSVI decreased. The average MLSS and MLVSS concentrations increased from values of 2 873 and 2 350 mg/ℓ respectively, just before NO_3^- dosing was terminated (when the DSVI was high, ≈170 mℓ/g), to values of 3 748 and 3 083 mg/ℓ respectively after NO_3^- dosing was terminated (when the DSVI was lower, ≈140 mℓ/g and decreasing). A similar interrelationship between sludge production, DSVI and the NO_3^- concentration in the 2nd anoxic reactor was also noted earlier in the change between the period when nitrate was not dosed (Day 1 to Day 128) and when nitrate was dosed (Day 129 to Day 239). In that relationship, when nitrate was dosed, the DSVI increased and the MLSS and MLVSS decreased.

Effect of NO_2^- dosing on low F/M filament proliferation in an MUCT configuration

To examine more closely the effect of NO_2^- on low F/M filament proliferation, a second MUCT configuration (MUCT4) was started up, with operating conditions the same as for MUCT3, as described in Table 2. The changes in DSVI and NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor with time for MUCT4 are shown in Fig. 5.

The system was started on Day 171 with sludge from other MUCT systems operated in the laboratory and had a starting DSVI of 130 mℓ/g caused by low F/M filaments typical of nutrient removal systems. Neither NO_3^- nor NO_2^- were dosed between Days 171 and 290 and the DSVI decreased to 90 mℓ/g; the concentrations of NO_3^- and NO_2^- in the 2nd anoxic reactor were 0.2 to 0.7 mgNO_3^--N/ℓ and 0.1 to 0.2 mgNO_2^--N/ℓ respectively.

From a NO_2^- denitrification batch test conducted on Day 258, a NO_2^- denitrification rate was calculated and a NO_2^- dosing rate determined for MUCT4 such that the dosed NO_2^- and the NO_2^- and NO_3^- formed from nitrification of the influent TKN, would load the 2nd anoxic reactor to more than its denitrification potential.

On Day 291, 900 mg NO_2^--N/d was dosed to the 2nd anoxic reactor (equivalent to 90 mg NO_2^--N/ℓ influent), raising the effective influent TKN/COD ratio from 0.08 to 0.10 mgN/mgCOD. The DSVI increased rapidly over 50 d, from 90 mℓ/g on Day 290 to 174 mℓ/g on Day 340. During this period, the NO_2^- concentration in the 2nd anoxic reactor increased from between 0.1 and 0.2 mgN/ℓ to between 10 and 17 mgN/ℓ, and the NO_3^- concentration increased from between 0.2 and 0.7 mgN/ℓ to between 1.0 and 3.0 mgN/ℓ. In a filament identification conducted on Day 308, the dominant filament was type 0092 and types 021N and 0675 were secondary.

In comparing the influences of NO_3^- and NO_2^- on low F/M filament proliferation, it is interesting to examine the comparative rates of increase of DSVI with NO_3^- and NO_2^- dosing. With NO_3^-

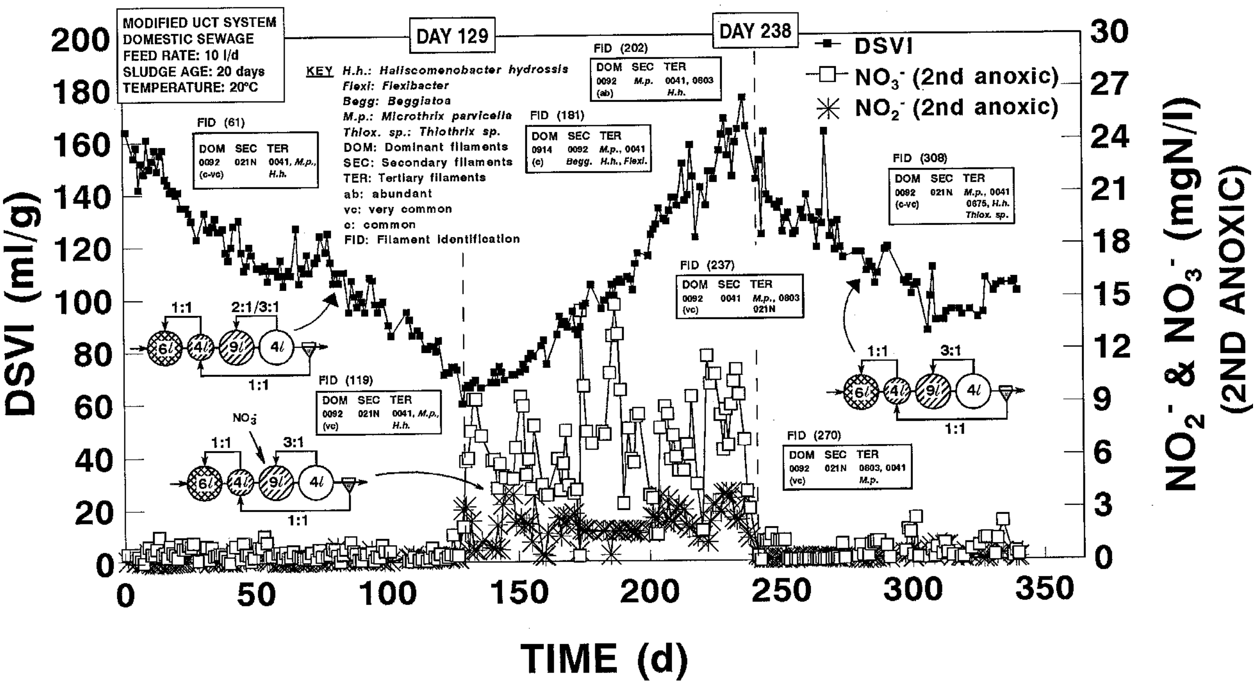

Figure 4

Sludge settleability as DSVI (mℓ/g) and concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) in the 2nd anoxic reactor of MUCT System 3 with time as a consequence of the addition and removal of NO_3^- to the 2nd anoxic reactor

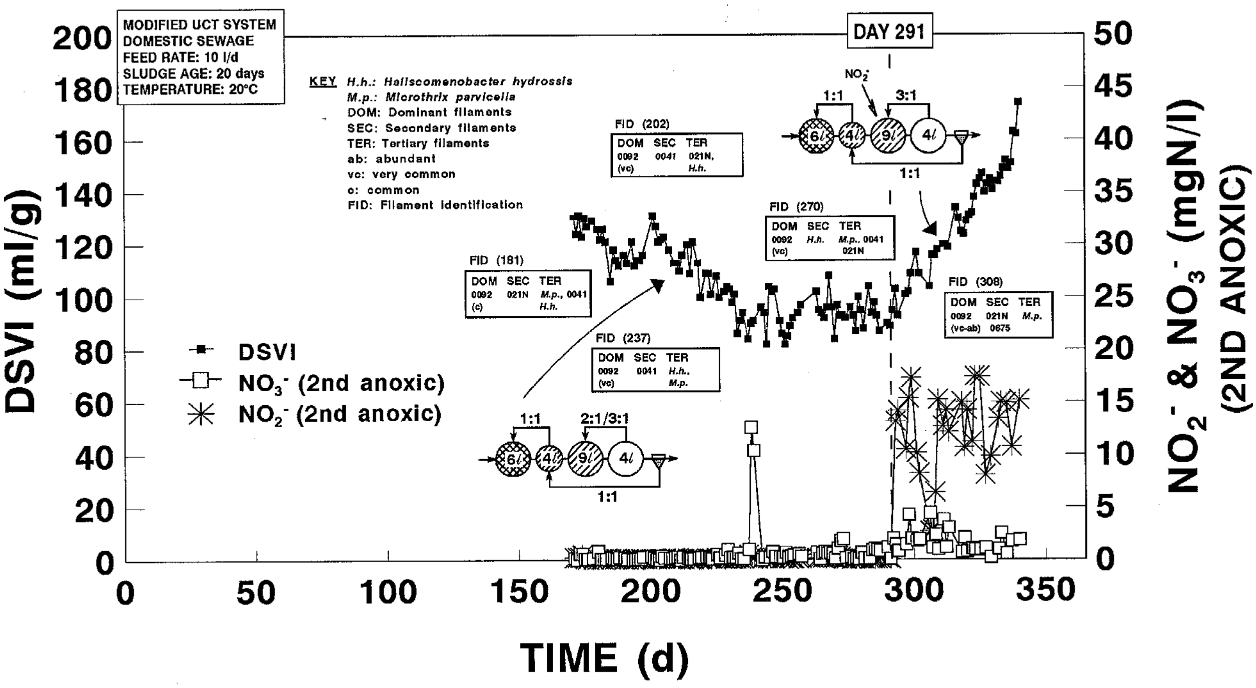

Figure 5

Sludge settleability as DSVI (mℓ/g) and concentrations of nitrate (NO_3^-) and nitrite (NO_2^-) in the 2nd anoxic reactor of MUCT System 4 with time as a consequence of the addition and removal of NO_2^- to the 2nd anoxic reactor dosing to MUCT3, and with NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor of 6.8 and 2.1 mgN/ℓ respectively, the DSVI increased from 66 to 176 mℓ/g in 111 d (i.e. 1.0 mℓ/g per d). Comparatively, with NO_2^- dosing to MUCT4, and with NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor of 1.7 and 11.3 mgN/ℓ respectively, the DSVI increased from 90 to 174 mℓ/g in 55 d (i.e. 1.5 mℓ/g per d).

From these results it is apparent that both NO_3^- and NO_2^- have an effect on low F/M filament proliferation. However, it is not possible to conclude as to which has the greater effect since, although the DSVI increased 1½ times more quickly with NO_2^- than with NO_3^- dosing, the concentration of NO_2^- present in the 2nd anoxic reactor with NO_2^- dosing (11.3 mgN/ℓ) was also about 1½ times greater than the concentration of NO_2^- present in the 2nd anoxic reactor with NO_3^- dosing (6.8 mgN/ℓ). Additionally, in the MUCT4 system with NO_2^- dosing, in which the DSVI increased 1½ times more quickly, the sum of the average NO_3^- and average NO_2^- concentrations in the 2nd anoxic reactor was about 1½ times the average in the MUCT3 system with NO_3^- dosing in which the DSVI increased 1½ times more slowly.

Comparison of effect of NO_3^- and NO_2^- dosing on the denitrification rates of NO_3^- and NO_2^- and on low F/M filament proliferation

An unusual feature of the experiments in which NO_3^- and NO_2^- were dosed to the 2nd anoxic reactors of MUCT3 and MUCT4 respectively, concerns the effect of NO_3^- and NO_2^- concentrations in the 2nd anoxic reactor on the K'_2 denitrification rate i.e. the NO_3^- and NO_2^- denitrification rate on particulate slowly biodegradable COD (SBCOD). For MUCT3, the denitrification rate of NO_3^- decreased from 0.429 $mgNO_3^-$-N/(mgAVSS·d) (average of 2 batch tests) before NO_3^- dosing (when the DSVI was 86 mℓ/g) to 0.202 $mgNO_3^-$-N/(mgAVSS·d) (one batch test) at the end of NO_3^- dosing when the DSVI was 165 mℓ/g. Upon termination of dosing, the rate increased to 0.249 $mgNO_3^-$-N/(mgAVSS·d) when the DSVI was 95 mℓ/g.

The nitrite denitrification rate $K'_{NO2,2}$ was measured at the end of the investigation when the nitrate dosing had terminated and an average of 0.237 $mgNO_2^-$-N/(mgAVSS·d) was obtained from three batch tests conducted between Days 290 and 317.

For MUCT4, the denitrification rate of NO_3^- decreased from 0.420 $mgNO_3^-$-N/(mgAVSS·d) (one batch test) before NO_2^- dosing to 0.224 mg NO_3^--N/(mgAVSS·d) (average of two batch tests) about 30 d after NO_2^- dosing commenced.

The nitrite denitrification rate decreased from 0.372 mg NO_2^--N/(mgAVSS·d) before nitrite dosing to 0.249 $mgNO_2^-$-N/(mgAVSS·d) about 30 d after nitrite dosing was started. Nitrite dosing to MUCT4 continued to Day 340, when operation of the system was terminated, so unlike MUCT3, a follow-on non-nitrite dosing period was not evaluated on MUCT4.

From these results it can be concluded that high NO_3^- concentrations in the 2nd anoxic reactor of an MUCT system result in a low NO_3^- denitrification rate and low NO_3^- concentrations result in a high rate. Similarly, high NO_2^- concentrations in the 2nd anoxic reactor of an MUCT system result in a low NO_2^- denitrification rate and low NO_2^- concentrations result in a high rate.

In investigations into the denitrification kinetics of nutrient (N&P) removal systems, Clayton et al. (1989, 1991) found that the K'_2 rate of denitrification of NO_3^- for an MUCT system was 0.224 $mgNO_3^-$-N/(mgAVSS·d), a value considerably higher than the K'_2 rate of denitrification for NO_3^- of 0.101 $mgNO_3^-$-N/(mgAVSS·d) measured by Marais and co-workers (see Van Haandel et al., 1981 and Warburton et al., 1991) for MLE and IAND nitrogen (N) removal systems respectively. A significant difference between N&P and N removal systems is the absence of an anaerobic zone in N removal systems, i.e. for an N removal system (low NO_3^- denitrification rate) with its anoxic reactor loaded with NO_3^- in excess of the denitrification potential, there is no zone in which NO_3^- is absent or at a low concentration. Clayton et al. (1989, 1991) concluded that the higher K'_2 denitrification rate in N&P removal systems is a consequence of an increase in the adsorbed SBCOD hydrolysis/utilisation rate for the ordinary heterotrophic (non-polyP) organisms, apparently induced by anaerobic-anoxic-aerobic sequencing in N & P removal systems. Comparatively, in MUCT3 and MUCT4 before the addition of NO_3^- and NO_2^- respectively (sum of the concentrations of NO_3^- and NO_2^- [i.e. NO_x^-] in the 2nd anoxic reactor less than 1.0 mgN/ℓ), the K'_2 denitrification rates for NO_3^- and NO_2^- were higher than after the addition of NO_3^- and NO_2^- (NO_x^- in the 2nd anoxic reactor between 3.5 and 13.5 mgN/ℓ for MUCT3 and between 17 and 35 mgN/ℓ for MUCT4). Similarly, for an IAND system (System 5) described by Lakay et al. (1999), before ammonium addition to the influent (<0.4 $mgNO_2^-$-N/ℓ present at the end of the anoxic period), the K'_2 denitrification rate of NO_3^- during the anoxic period on Day 189 of 0.103 $mgNO_3^-$-N/(mgAVSS·d) (see Fig. 9 in Lakay et al., 1999) was considerably higher than the value of 0.066 $mgNO_3^-$-N/(mgAVSS·d) measured during the anoxic period on Day 209 when the system had ammonium addition to the influent (>1 $mgNO_2^-$-N/ℓ present at the end of the anoxic period). [*A low concentration of nitrite implies also a low concentration of nitrate because significant nitrite denitrification does not take place until the nitrate concentration is low (< 1 mgNO$_2^-$-N/ℓ) - see Fig. 9 in Lakay et al. (1999) and Ekama and Wentzel (1999)*].

Factors that influence the K_2 and K'_2 denitrification rates in ND and NDBEPR systems respectively, are reviewed by Ekama and Wentzel (1997). Determination of these K rates requires calculation of the active ordinary heterotrophic organism (OHO) fraction of the VSS ($f_{av,OHO}$) in the system via the steady state ND (WRC, 1984) and NDBEPR (Wentzel et al., 1990) models. For the ND systems, because reasonably consistent results are obtained for the wastewater unbiodegradable COD fraction ($f_{S,up}$) and hence also for the $f_{av,OHO}$ fraction, variation in the calculated K_2 rates substantially reflects real variation in this rate. However, for the NDBEPR systems, the K'_2 rate varies significantly, not only between different NDBEPR systems fed the same wastewater but also in the same system as noted above in the MUCT3 and MUCT4 systems of this investigation. This variation in K'_2 rate is in part a consequence of significant variation in the calculated wastewater $f_{S,up}$ fraction, on which the $f_{av,OHO}$ fraction depends, for the same wastewater source. The variation in the calculated $f_{S,up}$ fraction in turn, results from varying specific VSS sludge production rates (mass VSS in system per mass COD load per day) in the NDBEPR systems which appeared to be associated with the sludge settleability (DSVI) and hence low F/M filament bulking (Musvoto et al., 1994; Casey et al., 1994). The two MUCT systems MUCT 3 and MUCT4, produced between 15 and 30% more VSS than expected from the steady state WRC (1984) ND model, the VSS mass increasing as the DSVI decreased in the absence of nitrate or nitrite dosing, then decreased as the DSVI increased with dosing and then increased again as the DSVI decreased after cessation of dosing (Figs. 4 and 5). Although not so strongly linked as in this investigation, Clayton et al. (1989, 1991) also observed significantly increased specific VSS sludge production rates in their MUCT system compared with ND systems at the same sludge age and receiving the same wastewater (Warburton et al., 1991). While NDBEPR systems are expected to have greater specific VSS sludge production rates for the ND and NDBEPR models, no explanation for the variation of this rate in

TABLE 3 **OPERATING PARAMETERS AND CONDITIONS OF MODIFIED UNIVERSITY OF CAPE TOWN NUTRIENT (N&P) REMOVAL SYSTEMS MUCT5 AND MUCT6**		
System	**MUCT5**	**MUCT6**
Operating conditions	Continuously fed, completely mixed multi-reactor systems	
DO in aerobic zone (mgO/ℓ)	2.0 - 4.0	2.0 - 4.0
Feed Sewage source Sludge source	Continuous (24 h) Mitchell's Plain raw Laboratory MUCT systems	
Mass of COD fed (mg/d) Volume of feed (ℓ/d) Target concentration (mgCOD/ℓ)	10000 10 1000	10000 10 1000
Influent TKN (mgN/ℓ)	140	100
Nitrite addition to 2nd anoxic (mgN/d) (Day 62 to Day 97)	-	250
Sludge age (d) Temperature (°C)	20 20	20 20
Reactor volumes (ℓ) ; Mass fractions (%) Anaerobic 1st anoxic 2nd anoxic Aerobic	 6 ; 15 4 ; 20 6.5 ; 32.5 6.5 ; 32.5	 6 ; 15 4 ; 20 6.5 ; 32.5 6.5 ; 32.5
MLVSS concentration (mg/ℓ) MLSS concentration (mg/ℓ) F/M [mgCOD applied/(gVSS·d)] Hydraulic retention time (h)	2 610 3 110 192 48	2 812 3 348 178 48
Recycle ratios Aerobic (a) Sludge (s) Return (r)	 4:1 1:1 1:1	 2:1 2:1 2:1
pH of mixed liquor	7.6	7.6

NDBEPR systems with the DSVI can be advanced.

In summary, three examples have been presented in which the denitrification rate is affected either by the presence or absence of an anaerobic zone or by the concentration of NO_3^- and NO_2^- in the anoxic period, i.e. N&P removal systems (which have an anaerobic zone) have higher K'_2 denitrification rates than N removal systems (which do not an anaerobic zone), and N removal systems with low concentrations of NO_3^- and NO_2^- (< 1 mgN/ℓ) have higher denitrification rates than N removal systems with high concentrations of NO_3^- and NO_2^- (>1 mgN/ℓ).

Given that systems with high concentrations of NO_3^- and NO_2^- in the anoxic zone in N removal systems and in the 2nd anoxic zone of N&P removal systems have higher DSVI values than systems with low concentrations of NO_3^- and NO_2^-, then the finding that high concentrations of NO_3^- and NO_2^- result in (or result from) lower denitrification rates, implies that a relationship exists between high DSVIs, lower denitrification rates and high concentrations of NO_3^- and NO_2^- in the anoxic zone prior to the aerobic zone.

With regard to the relationship between denitrification rate and low F/M filament proliferation, the finding that high concentrations of NO_3^- and NO_2^- result in lower denitrification rates may be linked to the finding that systems with high concentrations of NO_3^- and NO_2^- in the anoxic zone in N removal systems and in the 2nd anoxic zone of N&P removal systems have higher DSVIs than systems with low concentrations of NO_3^- and NO_2^- in the same reactors. That is, lower denitrification rates result in higher concentrations of NO_3^- and NO_2^- (and *vice versa*) which results in higher DSVIs.

It is unclear as to the relationship between DSVI, denitrification rate and higher concentrations of NO_3^- and NO_2^- in the 2nd anoxic zone, as to which of the parameters are the cause and which are the effect. However at this point it is sufficient to say that lower denitrification rates, higher concentrations of NO_3^- and NO_2^- and high DSVI values are interconnected, but the mechanisms of this interconnection are not understood.

Comparison of effect of ammonium and NO_3^- /NO_2^- on low F/M filament proliferation

In the experimental programme thus far, the role of the concentration of NO_3^- and NO_2^- on low F/M filament proliferation has been examined through two approaches: by addition of ammonium to the influent, resulting in an increase in concentration of NO_3^- and NO_2^- in the 2nd anoxic reactor of an MUCT system through nitrification of the ammonium in the aerobic reactor; and by direct addition of NO_3^- or NO_2^- to the 2nd anoxic reactor of an MUCT system. Although these different approaches had the same end result, i.e. an increase in low F/M filament proliferation, the experiments were conducted in the laboratory at different times and therefore received different batches of municipal sewage. From earlier experiments it had been noted that different batches of sewage can lead to differences in the extent of low F/M proliferation and DSVI. From the experiments conducted so far, it was suspected that these differences arose from different influent TKN/COD ratios of the sewage batches, but a direct comparison of the effects on low F/M proliferation of ammonium addition to the influent and NO_3^- or NO_2^- dosing to the 2nd anoxic reactor of parallel MUCT systems had not yet been conducted.

To examine this aspect directly, two MUCT systems (MUCT5 and MUCT6) were operated with the characteristics outlined in Table 3. MUCT5 was operated with a TKN/COD ratio ≈ 0.13 mgN/mgCOD (NH_4^+ in the form of NH_4Cl added to the influent) and MUCT6 was operated with a TKN/COD ratio ≈ 0.09 mgN/mgCOD (no NH_4^+ added to the influent). The change in DSVI with time for the two systems is illustrated in Fig. 6. For the 162 d of operation of MUCT5, the NO_2^- concentration in the 2nd anoxic reactor was between 0.5 and 1.5 mgN/ℓ and the DSVI between 200 and 250 mℓ/g. For MUCT6 between Days 1

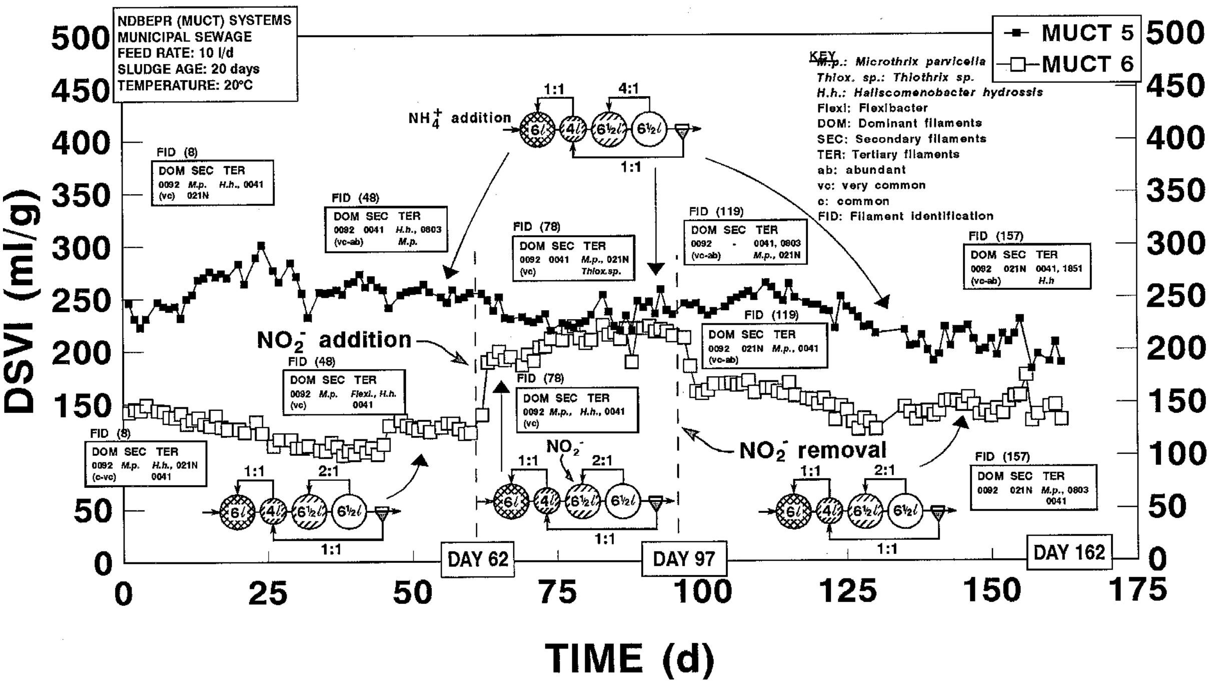

Figure 6

*Sludge settleability as DSVI (m*ℓ*/g) with time for MUCT Systems 5 and 6 with addition of ammonium to the influent (MUCT 5), and for addition and removal of NO_2^- to 2nd anoxic reactor (MUCT 6)*

and 62, the NO_2^- concentration in the 2nd anoxic reactor was less than 0.4 mgN/ℓ, and the DSVI was between 100 and 150 mℓ/g. From Day 62, NO_2^- (in the form of a concentrated $NaNO_2$ solution) was dosed to the 2nd anoxic reactor of MUCT6. The NO_2^- concentration in that reactor increased to between 1 and 10 mgN/ℓ and the DSVI increased rapidly to between 200 and 230 mℓ/g. After 35 d, on Day 97, NO_2^- addition was terminated, the NO_2^- in the 2nd anoxic reactor thereafter decreased to ≈0.3 mgN/ℓ and the DSVI declined within 3 d from 220 mℓ/g to 170 mℓ/g and within 25 d to 150 mℓ/g. In both MUCT5 and MUCT6, the dominant filament throughout the experimental period was type 0092, and the secondary filaments were usually *M. parvicella* or type 0041, all of which are low F/M types.

From the results it can be concluded that addition of ammonium to the influent and addition of NO_2^- directly to the 2nd anoxic reactor of MUCT systems produce similar measurements in DSVI for similar concentrations of NO_2^- in the 2nd anoxic reactor. A similar result was noted for MUCT4, the system described above, in which nitrite was dosed to the 2nd anoxic reactor. An interesting aspect in comparing the results of MUCT6 and MUCT4 is the considerably more rapid increase in DSVI in MUCT6 than in MUCT4 following NO_2^- dosing to each system. The only significant difference between the systems is the somewhat larger aerobic mass fraction in MUCT6 (32½%) than in MUCT4 (20%). This result is an agreement with the results illustrated in Fig. 5 of Paper 7 of this series (Lakay et al., 1999) in which IAND systems with 30 to 40% aerobic mass fraction developed higher DSVIs than similar IAND systems with lower percentage aerobic mass fractions and achieved the high DSVIs more rapidly with additions of similar concentrations of nitrite.

Conclusions

In the previous paper of this series (Lakay et al., 1999), it became clear that an association exists between the mass fractions of the anoxic and aerobic zones, the concentrations NO_3^- and NO_2^- in the anoxic zone prior to the aerobic zone and the proliferation of low F/M filaments. In Lakay et al. (1999), the roles of the aerobic and anoxic zones in producing low F/M filament proliferation were examined. To summarise, the results of those experiments were:

- In IAND systems, anoxic mass fractions in the range 60 to 70% resulted in the highest DSVI values. As the anoxic mass fraction increased from 70 to 100% (i.e. fully anoxic conditions) the DSVI decreased, and as the anoxic mass fraction decreased from 60% to 0% (i.e. fully aerobic conditions), the DSVI decreased.
- Completely aerobic systems did not bulk (DSVI < 150 mℓ/g).
- Single reactor completely anoxic systems with continuous NO_3^- addition, and fed either the SBCOD fraction of sewage, or complete municipal sewage, did not bulk (DSVI < 150 mℓ/g).

The role of NO_2^- (as opposed to NO_3^-) under completely anoxic conditions was investigated in the same series of experiments.

- A completely anoxic system in which continuous NO_3^- addition was replaced with continuous NO_2^- addition demonstrated a decreased DSVI, from 120 mℓ/g to 75 mℓ/g over 20 d.

These results with single-reactor systems are supported by the experimental work conducted with the multi-reactor N&P removal MUCT systems described in this paper, i.e. low F/M filamentous organisms proliferate in N and N&P removal systems when the sludge is subjected to sequential anoxic-aerobic conditions in which either, or both of NO_3^- or NO_2^- are present in the anoxic zone immediately preceding the aerobic zone, at concentrations greater than about 2.0 $mgNO_3^-$-N/ℓ or 1.0 $mgNO_2^-$-N/ℓ.

- Filaments proliferate in multi-reactor (MUCT) systems with the addition of ammonium to the influent (apparently

as a consequence of the concomitant production of NO_3^- and NO_2^-), or as a consequence of the direct addition of NO_3^- or NO_2^- via continuous dosing to the 2nd anoxic reactor.
- In agreement with results from N removal systems, the size of the aerobic mass fraction of an N&P removal system affects the development of the filamentous organism population; for a system with excess NO_2^- passing from the anoxic to the aerobic reactor, an aerobic mass fraction of 30 to 40% results in a rapid increase in DSVI whereas an aerobic mass fraction of 20% results in a considerably lower rate of increase in DSVI.

From the results, the primary factor implicated in promoting low F/M filament proliferation is alternating anoxic-aerobic conditions with NO_3^- and NO_2^- present during the anoxic conditions which immediately precede the start of aerobic conditions. Assuming that the organism population which mediates denitrification is predominantly facultative heterotrophic organisms, the alternating anoxic-aerobic conditions would force these organisms to alternatingly switch between NO_3^-/NO_2^- and oxygen respectively as terminal electron acceptor. From the body of experimental work presented in this series of papers, it is hypothesised that the cause for bulking lies in the requirement for the facultative organisms (irrespective of whether filament or floc-former), to frequently switch between aerobic and anoxic respiration as the environmental conditions change, this switching either providing an advantage for the filaments or acting as a disadvantage to the floc-formers. Testing of such a hypothesis requires investigation of the respiratory processes of facultative organisms at a level more fundamental than the externally measured manifestations of activated sludge processes such as, COD removal, and oxygen and NO_3^- utilisation rates. Such a fundamental investigation would entail an examination of the biochemical mechanisms of transport of protons, electrons, and the electron acceptors, oxygen, and NO_3^- and NO_2^-, and the synthesis of enzymes mediating these mechanisms under aerobic, anoxic, and alternating anoxic-aerobic conditions. Investigations into these aspects were prompted also by necessity - all areas of experimental research considered to be influential in low F/M filamentous organism bulking were apparently exhausted. As a first step in this investigation, an in-depth review was conducted of the biochemical aspects of respiration of obligate aerobic and facultative heterotrophic organisms and this is presented in the next paper of this series (Casey et al., 1999b).

From the results of the experiments described in this paper it is apparent that the so-called low F/M filaments do not proliferate under all low F/M conditions; in particular, under continuous aerobic and continuous anoxic conditions at long sludge ages (i.e. > 10 d) which constitute low F/M conditions, low F/M filaments do not develop. As a consequence of the finding that this group of filaments **do not** necessarily proliferate under all low F/M conditions, but invariably proliferate under alternating anoxic-aerobic conditions, it was concluded that the filaments should be renamed anoxic-aerobic (AA) filaments as a name more descriptive of the conditions under which they apparently proliferate. This name is used throughout the remainder of the papers in this series in place of the term **low F/M**, the name assigned to the filament group which includes the following filamentous organisms; types 0092 and 0041, *M. parvicella* and types 1851, 0675 and 0914.

Acknowledgements

This research was financially supported by the Water Research Commission, Foundation for Research Development and University of Cape Town and is published with their permission.

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