DEVELOPMENT AND EVALUATION OF SPECIFIC CONTROL METHODS FOR AMELIORATING LOW F/M FILAMENT BULKING

UNIVERSITY OF CAPE TOWN
(Department of Civil Engineering)

WRC Report No. 286/1/94

UNIVERSITY OF CAPE TOWN
Department of Civil Engineering
(Water Research Group)

FINAL REPORT
on the
FOUR YEAR RESEARCH CONTRACT (1989–1992)
into
DEVELOPMENT AND EVALUATION OF SPECIFIC CONTROL METHODS FOR AMELIORATING LOW F/M FILAMENT BULKING
for the
WATER RESEARCH COMMISSION

by
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WRC Report 286/1/94
ISBN No. 1 86845 076 7
ISBN Set No. 1 86845 078 3

Research Report No. W 82
August, 1993

Final Report to the Water Research Commission on a 4–year research contract (1989–1992) into development and evaluation of specific control methods for ameliorating low F/M filament bulking

SYNOPSIS

Following the finding in the previous research contract that the selector effect did not control low F/M filament bulking[1] in N and N & P removal systems, new approaches for dealing with this common problem in N and N & P removal plants needed to be developed. In this research contract a number of directions were explored to try to identify the cause for the low F/M filament bulking; if the cause could be understood, then it becomes possible to devise strategies for the control of the low F/M filament proliferation.

From the results of an experimental investigation over 3 years in which various laboratory[2] N and N & P removal plants were operated (all at 20° C) to examine the effect of:

(i) readily biodegradable or slowly biodegradable COD only as feed,
(ii) the aerobic mass fraction,
(iii) frequency of exposure to anoxic and aerobic conditions,
(iv) nitrate and nitrite concentrations in the anoxic zone or during the anoxic period,
(v) DO concentration in the aerobic zone or during the aerobic period,
(vi) sludge age,
(vii) fully aerobic and fully anoxic conditions, and
(viii) differences in the anoxic–aerobic condition in intermittently aerated single reactor and 2 reactor anoxic aerobic systems,

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[1] Caused principally by filaments 0092, *Microthrix parvicella*, 0041, 0675, 0914 and 185l.

[2] In full–scale N and N & P removal systems, *M. Parvicella* is frequently associated with significant scum formation on the reactor surfaces, particularly when the reactors are designed with subsurface inter–reactor connections allowing the foam to accumulate on the surface. In the laboratory N and N & P removal systems, *M. Parvicella* was frequently identified in the mixed liquor and often found to be the dominant filament in particular in the N removal systems. However, even though the laboratory systems also had subsurface inter–reactor connections scum formation was not observed in the investigation. The reason for this difference in *M. Parvicella* behaviour between laboratory and full–scale N and N & P removal systems is not clear and not dealt with in this investigation.

it was concluded that the single most important factor influencing low F/M filament proliferation was alternating anoxic–aerobic conditions with significant concentrations of nitrate and/or nitrite (>5 $mgNO_3$–N/ℓ and >2 $mgNO_2$–N/ℓ) present at the commencement of aerobic conditions. Under these conditions the activated sludge is forced to switch between aerobic and anoxic metabolic pathways in which nitrate/nitrite and oxygen respectively serve as terminal electron acceptors, this switching conferring some competitive advantage onto the filaments or some disadvantage onto the floc–formers. As a consequence of the direct influence of anoxic–aerobic conditions on low F/M filament proliferation irrespective of sludge age, and a virtual absence of these filaments under fully aerobic or fully anoxic conditions, these filaments were renamed anoxic–aerobic (AA) filaments as a name more descriptive of the conditions under which they proliferate. From the observations and a review of the microbiological and biochemical literature on facultative heterotrophic denitrification pathways, a hypothesis for the cause of the AA filaments was developed, viz:

If denitrification is not complete upon commencement of aerobic conditions the facultative heterotrophic floc–formers, which denitrify nitrate completely to dinitrogen gas, are inhibited in the oxygen uptake oxidase cytochromes under the aerobic conditions by denitrification intermediates in particular nitric oxide (NO) accumulated under the previous anoxic conditions. In contrast, the AA filaments, which reduce nitrate only as far as nitrite, will not be inhibited in their oxygen uptake cytochromes because they do not accumulate the inhibiting NO intermediates from the nitrite to dinitrogen gas step.

Because the hypothesis is at a microbiological and biochemical level, it could not be directly experimentally verified in this investigation i.e. it could not be tested that floc–formers denitrify to dinitrogen gas whereas the AA filaments only as far as nitrite. This aspect will need to be taken up by microbiologists and biochemists in specialist pure culture work. However, convincing indirect evidence in support of the hypothesis was obtained in the investigation from

1) The literature survey which demonstrates that inhibition of oxidase cytochrome by denitrification intermediates is a recognized phenomenon by microbiologists and biochemists.

2) Specifically designed batch tests on sludges harvested from bulking and

non–bulking N and N & P removal activated sludge systems in demonstrating the existence of the inhibition.

3) In intermittent aeration single reactor and two–reactor anoxic–aerobic N removal systems, when nitrate and nitrite concentrations were low (< 2 $mgNO_3$–N/ℓ and <0,5 $mgNO_2$–N/ℓ) in the anoxic reactor upstream of the aerobic or at the time aerobic conditions commenced, the systems demonstrated a significantly reduced level of filaments and lower DSVIs compared with systems that had high nitrate and nitrite concentrations at commencement of aerobic conditions; this feature was particularly notable in Modified UCT N & P removal systems.

4) Most of the experimental results observed in the exploratory investigation.

The implications of the above conclusions regarding AA filament bulking on the design and operation of N and N & P removal plants still need to be examined, but it would appear that these plants need to be designed and operated such that the nitrate/nitrite recycled to the anoxic reactor(s) should be fully denitrified before re–entering to the aerobic zone or period.

Further investigations with the objective of (1) confirming the new framework for understanding AA filament bulking, and (2) examining its implications on design and operation are being conducted in a new three year (1993–1995) follow–up research contract with the Water Research Commission, the 4th in a series since 1983.

Final Report to the Water Research Commission
on a 4 year research contract (1989–1992) into
development and evaluation of specific control methods
for ameliorating low F/M filament bulking

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ACKNOWLEDGEMENTS

The writers wish to express their gratitude to the following persons for their contribution to the research work reported here:

- Mr Taliep Lakay – Laboratory Technical Assistant, for his invaluable help in running the experimental laboratory systems, analytical equipment, stores, and being the helping hands required at the right time and right place.
- Mrs Heather Bain – Clerical and Administrative Assistant, for so cheerfully and unquestioningly typing and re–typing the seemingly unending drafts, attending to the accounts and seeing to all the clerical details that we so easily overlook.

The contribution of these two persons is not that of support only – they are vital members of the research team.

- The staff of the Civil Engineering Workshop and Laboratory, Messrs E von Guerard, C Nicholas and D Botha, Principal, Senior and Senior Technical Officers respectively, and N Hassen, Technical Assistant, for construction and maintenance of the laboratory equipment.
- Mr Dougie Swartz, Departmental Assistant, for his help in the Water Research Laboratory.

All of the experimental work conducted under this research contract was done by the following post graduate students:

Mr Tim Casey, Research Officer, who conducted a large part of the experiments, and inspired the many others done by the MSc students listed below, for his PhD degree.

Ms Eustina Musvoto and Messrs Charles Warburton, Dave Ketley, Andrew Hulsman and Michael de Villiers who undertook the many experiments which examined the effect of the various system design and operating parameters on AA filament bulking for their MSc degrees.

It is the dedication and effort of these students that produced the large body of experimental data out of which flowed the new framework for understanding the AA filamentous bulking problem in nutrient removal plants.

A special word of gratitude and appreciation is expressed to Mrs Mara Segal and Mrs Lee Boyd, Principal and Senior Professional Officers respectively of the Johannesburg Scientific Services Department at Cydna Laboratory, for doing so willingly and capably all the filament identifications throughout the 4 year contract period.

Acknowledgement is due to the members of the Steering Committee of the project who guided the research work during the 4 year period:

Dr S A (Steve) Mitchell – Water Research Commission (Chairman)
Dr L H (Laurraine) Lötter – Johannesburg Scientific Services Department
Mr A R (Tony) Pitman – Johannesburg Wastewater Department
Mr G (Gerhard) Offringa – Water Research Commission
Dr J J (Koos) Barnard – Department of Water Affairs
Prof W A (At) Pretorius – Pretoria University
Mr H G J (Henk) Beekman – Cape Town City Engineer's Department
Mr P W (Piet) Weideman – Water Research Commission (Committee Secretary)
Mr F (Fanus) Venter – Division of Water Technology, CSIR

Gratitude is expressed to the Water Research Commission and Foundation for Research Development for financial support of the research.

Finally the writers express their appreciation to all their colleagues and associates in the field for their willingness to inform us of their experiences and observations on full-scale plant behaviour. We value this contact with practice, not only for the information it provides, but also for the sobering reminders of the magnitude and urgency of the bulking problem.

PAPERS, REPORTS AND OTHER CONTRIBUTIONS PUBLISHED DURING CONTRACT PERIOD (January 1989 to December 1992)

A. PAPERS PUBLISHED

A.1 Journals and Conference

1. Gabb DMD, Still DA, Ekama GA, Jenkins D and Marais GvR (1991). The selector effect on filamentous bulking in long sludge age activated sludge systems. Wat.Sci.Tech. 23(4/6–2), Kyoto, 867–877.

2. Casey TG, Wentzel MC, Loewenthal RE, Ekama GA and Marais GvR (1992). A hypothesis for the cause of low F/M filament bulking in nutrient removal activated sludge systems. Water Research, 26(6), 867–869.

3. Musvoto EV, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993). The effect of incomplete denitrification on anoxic–aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. Presented at first IAWQ ASPD Specialist Conference, Paris, Sept. 1993. (To appear in Wat.Sci.Tech.)

4. Casey TG, Wentzel MC, Ekama GA, Loewenthal RE and Marais GvR (1993). An hypothesis for the causes and control of anoxic–aerobic (AA) (low F/M) filament bulking in biological N and N & P removal activated sludge systems. Presented at first IAWQ ASPD Specialist Conference, Paris, Sept. 1993. (To appear in Wat.Sci.Tech.)

5. Gabb DMD, Ekama GA, Jenkins D and Marais GvR (1989). Evaluation of bulking control methods for long sludge age activated sludge systems. Procs. 1st biennial WISA conference, Cape Town, March 1989.

6. Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). Causes and control of low F/M filament bulking in nutrient removal activated sludge systems. Procs. Two–day Workshop on prevention and control of bulking activated sludge, Perugia, Italy, June 1992.

B. REPORTS PUBLISHED

1. W 65[3] Warburton CA, Lakay MT, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1991). The effect of sludge age and aerobic mass fraction on low F/M filament bulking in intermittent aeration nitrogen removal systems.

2. W 68[3] Ketley DA, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1991). The effect of fully anoxic conditions and frequency of exposure to aerobic and anoxic conditions on the growth of low F/M filaments in nitrogen removal systems.

3. W 73[3] Hulsman A, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). The effect of type, size, position and recycle ratio of the anoxic

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[3] Research Report Number, obtainable from Department of Civil Engineering, University of Cape Town, Rondebosch 7700, Cape, South Africa. Attention Prof G A Ekama. Fax 0927 21 650 2603.

zone on low F/M filament bulking in nitrogen removal activated sludge systems.

4. W 77[3] Musvoto EV, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). The effect of large anoxic fractions and concentration of nitrate and nitrite in the primary anoxic zone in low F/M filament sludge systems.

5. W 81[3] De Villiers ME, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993). The effect of nitrate and nitrite concentrations at the commencement of aerobic conditions on AA (low F/M) filament bulking in N removal activated sludge systems.

6. W 82[4] Casey TG, Ekama GA, Wentzel MC, Lakay TM and Marais GvR (1993). Development and evaluation of specific control methods for ameliorating low F/M filament bulking. Summary final report to Water Research Commission on 4 year (1989–1992) research contract K5/286. WRC 286/1/93, P O Box 824, Pretoria, 0001. (This report)

7. W 83[4] Casey TG, Wentzel MC, Ekama GA, Lakay MT and Marais GvR (1993). Causes and control of anoxic–aerobic (AA) filament bulking in biological N and N & P removal systems. Detailed final report to Water Research Commission on 4 year (1989–1992) research contract K5/286, WRC 286/2/93, P O Box 824, Pretoria 0001.

[4] Reports obtainable from Water Research Commission, P O Box 824, Pretoria, 0001, South Africa. Attention Dr S Mitchell. Fax 0927 12 331 2565.

Final Report to the Water Research Commission on a 4 year research contract (1989–1992) into development and evaluation of specific control methods for ameliorating low F/M filament bulking

SUMMARY REPORT

1. OBJECTIVES

This 4 year research contract from 1989 to 1992 is the third in a consecutive series which commenced in 1983. The first of these contracts (1983–84) was an exploratory study and survey of the extent and severity of the bulking problems in South African activated sludge plants and identification of the principal filamentous organisms causing the problems. In this work, which surveyed 110 activated sludge plants, the majority of which were long sludge nitrification plants incorporating denitrification either intentionally (in identified non–aerated zones) or unintentionally (in poorly mixed "dead" zone) and 26 of which were biological N & P removal plants, the principal dominant filamentous organisms were the so–called low F/M ones (i.e. 0092, 0041, *Microthrix parvicella*, 0675, 9014 and 1851) (Blackbeard *et al.*, 1985).

In the second contract, the promoted non–specific and specific control strategies for low F/M filaments were evaluated. The main non–specific strategy, chlorination, so named because it is effective for any filamentous organism type, was found to be successful without significant influence on nitrification, denitrification and biological P removal. However, the non–specific methods treat only the symptoms of bulking, i.e. kills the filaments, and does not eliminate the causes for the proliferation of the filaments. Consequently the promoted specific control method for low F/M filaments was evaluated. This method, called the selector effect, involves modifying the activated sludge reactor in such a way as to impose alternating feed starve conditions and a readily biodegradable COD concentration gradient on the sludge by introducing plug–flow conditions, or selector reactors, which are small reactors receiving the influent and underflow recycle ahead of the main reactor. It was found that when selector reactors, either aerobic or anoxic, were installed on intermittent aeration nitrification–denitrification activated sludge systems (mimicing Carousel or Orbal plants), the selector effect induced by the selector reactor did not control the low F/M filament proliferation. Although failure of the selector effect to control the low F/M filament proliferation was disappointing, it was reassuring because it brought consistency with regard to the effect of the anaerobic reactor. This reactor

in effect also functioned as a selector in that the RBCOD was taken up in it by floc–formers (in this case the polyP organisms that effect biological excess P removal) and it was noted in laboratory and full–scale N & P removal plants that the anaerobic reactor did not control low F/M filament proliferation because these systems seemed prone to low F/M filament bulking. Curiously, the major influence that appeared to ameliorate the low F/M filament proliferation was continuous aeration. This was effective in rapidly (in 2 to 3 weeks) reducing the low F/M filament population and improving sludge settleability, but it also eliminated the conditions required for biological N & P removal. The investigation concluded that alternative specific control methods for low F/M filament proliferation in N and N & P removal systems would need to be found (Gabb *et al.*, 1989).

The 4 year research contract results presented in this report commenced at the termination of the 2nd bulking contract described above. The finding that the selector effect did not control the low F/M filament proliferation placed the research back into an exploratory phase. As a consequence the central task of this research contract was to establish and pursue new directions of research that held promise of controlling the low F/M filament proliferation without compromising biological N & P removal. By considering the implications of the research conducted in the previous contract, *inter alia*:

i) the low F/M filaments appear not to require influent RBCOD for proliferation,
ii) anoxic–aerobic conditions appear to stimulate low F/M filament proliferation,

a wide–ranging experimental investigation was initiated to determine the influence of a range of factors on low F/M filament proliferation. The experimental results obtained in this investigation and conclusions gleaned from them forms the central activity of this research contract.

2. RESEARCH DIRECTIONS AND SYSTEMS OPERATED

In order to establish which conditions in N and N & P removal systems promote (or inhibit) low F/M filament proliferation, the following system factors were examined:

1. Readily biodegradable COD (RBCOD) or slowly biodegradable COD (SBCOD) only as influent. (Casey *et al.*, Report W 83)

2. Fully aerobic and fully anoxic conditions. (Ketley *et al.*, W 68)

3. Differences in alternating anoxic–aerobic conditions caused by (i) intermittent aeration in a single reactor, or (ii) separate anoxic and aerobic reactors in single sludge systems. (Hulsman *et al.*, W 73; de Villiers *et al.*, W 81)

4. The magnitude of the aerobic mass fraction, i.e. (i) the fraction of the day the sludge mass is under aerobic conditions, or (iii) the proportion of the mass of sludge in the system under aerobic conditions. (Warburton *et al.*, W 65; Ketley *et al.*, W 68; Musvoto *et al.*, W 77)

5. The magnitude of the sludge age. (Warburton *et al.*, W 65)

6. The nitrate and nitrite concentrations during the anoxic periods. (Warburton *et al.*, W 65; Musvoto *et al.*, W 77; de Villiers *et al.*, W 81; Casey *et al.*, W 83)

7. The frequency of alternation between anoxic and aerobic conditions. (Warburton *et al.*, W 65; Ketley *et al.*, W 68; Hulsman *et al.*, W 73)

8. The dissolved oxygen (DO) concentration in fully aerobic systems or during the aerobic period or zone of anoxic aerobic systems. (Casey *et al.*, W 83)

In the experiments to examine the above factors (except sludge age), three basic types of long sludge age (15–30 d) laboratory scale activated sludge systems were operated at 20° C:

(1) continuously fed single reactor systems fed either real sewage, artificial sewage or real sewage separated into its soluble and particulate fractions by 0,45μm membrane filtration, operated either fully aerobic (FA), fully anoxic (FX) or anoxic–aerobic by intermittent aeration (IA) with varying aerobic mass fractions and frequencies of alternation between anoxic and aerobic conditions,

(2) continuously fed two reactor (separate) anoxic–aerobic nitrification–denitrification (2RND) pre– and post–denitrification systems fed real or artificial sewage with varying aerobic mass fractions and frequencies of alternation between anoxic and aerobic conditions by adjustment of the mixed liquor recycle ratio,

(3) continuously fed modified UCT N & P removal systems fed real sewage with varying influent TKN/COD ratios and varying anoxic mass fractions between 20 and 65% (with constant anaerobic mass fraction of 15% giving unaerated mass fractions between 35 and 80%).

The effect of sludge age at 20, 15, 10, 8, 7, 6 and 5 days was examined in single reactor intermittently aerated ND systems fed real sewage with 30% aerobic mass fraction.

3. RESULTS FROM THE INVESTIGATION

From monitoring the behaviour of the above types of systems, the following significant observations were made (details given in references cited and summarized in Table 2 on pages 26 and 27):

3.1 In fully anoxic (FX) systems with effluent NO_3^- concentrations > 5 mgN/ℓ (i.e. by NO_3^- dosing) and in fully aerobic systems with high DO (>2 mgO/ℓ) and low DO (0,2<DO<0,5 mgO/ℓ), all fed real sewage, the low F/M filaments (0041, *H.hydrossis*, 0092, *M.parvicella*, 1851) were present but *did not* proliferate and low DSVIs (<80 mℓ/g) were obtained. When the effluent NO_3^- was <5 mgN/ℓ in the fully anoxic systems due to cessation or insufficient nitrate dosing, the sludge settleability became very poor by floc dispersion, not by filament proliferation, presumably due to the unavailability of sufficient external electron acceptors on a continuous basis. In contrast, in the intermittent aeration (IA) systems (30% aerobic fraction) with either high or low DO concentration during the aerobic period, the low F/M filaments *did* proliferate causing high DSVIs (> 200 mℓ/g). These observations indicated that (i) alternation between anoxic and aerobic conditions was necessary for low F/M filament proliferation and (ii) the low DO conditions in the IA system as it moves between anoxic and aerobic conditions did not appear to play a role in promoting low F/M filament proliferation (Casey *et al.*, 1993; Ketley *et al.*, 1991).

3.2 In the intermittent aeration (IA) systems fed artificial sewage, low F/M filaments, in particular *H.hydrossis* and 1851, but also 0092, 0675 and 0041, proliferated both with RBCOD alone, SBCOD alone and with mixtures of RBCOD and SBCOD as feed. With RBCOD alone, excessively high DSVIs (>1000 mℓ/g) were quickly reached (in days) whereas with SBCOD alone the

DSVIs increased but not to such very high values (DSVI ≈ 500 mℓ/g) and much more slowly (weeks). These effects could be interchanged on a pair of IA systems by interchanging the RBCOD only and SBCOD only feeds between them. In doing this a number of times with both systems receiving the same mass of COD daily and neither nitrate limited, it was observed that increases in DSVI (which occurred when switching to RBCOD only as feed) were accompanied by increases in effluent nitrate + nitrite concentrations. This seemed unusual because RBCOD is more rapidly degradable and therefore is able to induce a higher denitrification potential than SBCOD (Casey *et al.*, 1993; Ketley *et al.*, 1991).

These observations demonstrate that low F/M filaments can grow on SBCOD only and confirmed the earlier conclusion that the selector effect was unable to control low F/M proliferation; selectors stimulate the preferential uptake of RBCOD by floc–formers and the SBCOD passes through the selectors to the main intermittently aerated reactor. Further it was noted in these experiments that periods of high DSVI were accompanied by corresponding periods of increased effluent NO_3^- concentrations. This seemed to indicate that sludge with large populations of low F/M filaments did not denitrify to the same degree as the well settling sludges.

3.3 In the intermittent aeration (IA) systems fed real or artificial sewage, the aerobic fraction and the nitrate concentration during the anoxic period of the anoxic–aerobic cycle influenced the proliferation of the low F/M filaments (*M.parvicella*, 0092, 1851, 0041 and *H.hydrossis*). The aerobic fraction had a strong influence with proliferation reaching a maximum (highest DSVI 300 to 400 mℓ/g for real sewage) at aerobic mass fractions between 30 to 40%, decreasing as the aerobic mass fraction became greater and smaller to fully aerobic and fully anoxic conditions (DSVI ~80 mℓ/g). At aerobic mass fractions between 30 and 40%, (i) the nitrate + nitrite concentration during the anoxic period had only a mild influence, with proliferation generally increasing as the nitrate concentration increased above 5 mgN/ℓ and (ii) the frequency of alternation between anoxic and aerobic conditions from 72 times per day to once per day did *not* significantly influence the DSVI and therefore low F/M filament proliferation (Warburton *et al.*, 1991; Ketley *et al.*, 1991).

3.4 In the two–reactor anoxic–aerobic (2RND) pre– and post–denitrification

systems fed either real or artificial sewage with aerobic mass fractions from 45% to 30%, the size of the aerobic mass fraction had a significant effect on the level of filament proliferation. With an aerobic mass fraction of 30% and fed artificial substrate the DSVI was high (400 mℓ/g) but with a higher aerobic mass fraction of 45%, the DSVI was considerably lower (100 mℓ/g). For systems with an aerobic mass fraction of 30 or 45% and fed municipal sewage, the DSVI's were low (<150 mℓ/g), values being somewhat lower than expected given the IA system results cited above. With an aerobic mass fraction of 45%, manipulating the a-recycle ratio such that the frequency of exposure to anoxic and aerobic conditions changed from once per day to 15 times per day and dosing nitrate into the anoxic reactor resulting in high concentrations of nitrate in the outflow of the anoxic reactor (>5 mgN/ℓ) did not lead to low F/M filament proliferation (Hulsman *et al.*, 1992).

In repeating the experiments with a pair of the 2RND predenitrification systems fed real sewage, supplementing the influent with ammonium, instead of dosing nitrate into the anoxic reactor, *did* cause low F/M filament proliferation and high DSVIs (>200 mℓ/g); in the control system which did not receive dosed ammonium, the DSVI remained low (<100mℓ/g). These results were reproducible by switching the ammonium dose between the two systems (De Villiers *et al.*, 1993; Casey *et al.*, 1993). This second experiment with 2RND systems is difficult to reconcile with the first because insofar as nitrate loads on the anoxic reactor is concerned, it should make little difference whether the nitrate is dosed directly into the reactor or generated by nitrification provided the frequency of exposure remains unchanged. In both cases, i.e. with nitrate dosing and ammonium supplementation, high concentrations of nitrate + nitrite (> 5mgN/ℓ) were observed in the outflow of the anoxic reactor, yet in the former case low F/M filaments did not proliferate whereas in the latter case they did.

3.5 In the Modified UCT systems fed real sewage it was found possible to manipulate the sludge settleability (DSVI), and hence low F/M filament proliferation (generally 0092 dominant, 0041 and *M.parvicella* secondary and *Thiothrix*, 021N, *H.hydrossis* and 1851 incidental and occasional irrespective of filament abundance and DSVI), between high values (>200 mℓ/g) and low values (<120 mℓ/g) by:

(i) Manipulating the anoxic sludge mass fraction. In a pair of MUCT systems, one with a high anoxic mass fraction (15% anaerobic, 20% first anoxic and 32% second anoxic and 33% aerobic), the DSVI was high (200–250 mℓ/g); in the other, with a low anoxic mass fraction (15% anaerobic, 20% first anoxic and 32+33 = 65% aerobic) the DSVI tended to be low (100–150 mℓ/g). This experiment was prompted by the work on N removal systems mentioned above which indicated that the greater the aerobic mass fraction from 35%, the lower the DSVI and shows that this also applies to N & P removal systems (Casey *et al.*, 1993).

(ii) Manipulating the influent TKN concentration with ammonium dosing to the influent. At low TKN/COD ratio (no ammonium dosing) the concentration of nitrate generated to be denitrified by the anoxic reactors was such that the nitrate and nitrite concentrations leaving the anoxic reactors was very low (< 5mgN/ℓ). At high TKN/COD ratios (with ammonium dosing) complete denitrification in the anoxic reactors (mainly the second anoxic) was no longer possible leading to high concentrations of nitrate and nitrite leaving the anoxic reactor (> 10 mgN/ℓ). With ammonium dosing, the DSVI increased (from 100 to 280 mℓ/g) and without ammonium dosing, the DSVI decreased (from 250 to 170 mℓ/g) (Casey *et al.*, 1993).

(iii) Manipulating the nitrate concentration to be denitrified by dosing nitrate to the second anoxic reactor: In a pair of MUCT systems with very large anoxic mass fraction (15% anaerobic, 20% first anoxic, 45% second anoxic and 20% aerobic) to obtain a low DSVI [see (i) above], one receiving dosed nitrate, the other not, the DSVI increased (from 60 mℓ/g to 160 mℓ/g) from the time nitrate dosing commenced at a rate which caused each of the nitrate and nitrite concentrations in the outflow of the second anoxic reactor to increase from <0,1 mgN/ℓ to >5 mgNO_3^-–N/ℓ and >1 mgNO_2^-–N respectively. In the system with no nitrate dosing, the nitrate and nitrite concentrations in the outflow of the second anoxic reactor were each <0,1 mgN/ℓ and the DSVI remained low (~70 mℓ/g) (Musvoto *et al.*, 1992).

(iv) Manipulating the nitrite concentrations in the outflow of the secondary anoxic reactor: In a pair of MUCT systems with large anoxic sludge mass

fraction (15% anaerobic, 20% first anoxic, 33% second anoxic and 32% aerobic), one receiving ammonium supplemented to the influent, the other not, the DSVI in the former was high (250 mℓ/g) whereas that in the latter was low (<150 mℓ/g). To check whether or not the ammonium supplementation was an indirect way of increasing the nitrite concentration in the outflow of the second anoxic reactor, nitrite was dosed into the second anoxic reactor of the system not receiving ammonium supplementation. Upon commencement of dosing, the DSVI increased sharply from 130 to 220 mℓ/g and upon termination of dosing after 40 days (2 sludge ages), the DSVI sharply decreased to <150 mℓ/g. Without nitrite dosing the nitrite concentration in the second anoxic reactor was low (<1,0 mgN/ℓ) but during dosing the concentration increased to 8 mgN/ℓ while that in the ammonium supplemented system was between 1 and 3 mgN/ℓ (Casey *et al.*, 1993, Musvoto *et al.*, 1992).

In the discussion of the experimental results above, only those relating to the system operating parameters, sludge settleability and filament identification were presented. In the detailed reports cited a range of additional data is given such as COD and N mass balances over the systems, COD, nitrification, denitrification, N removal and biological P removal (where applicable) performances, as well as kinetic rates of nitrate and nitrite denitrification and oxygen utilization calculated from the system data or from ancillary batch tests on sludge harvested from the systems.

4. CONCLUSIONS FROM THE EXPLORATORY INVESTIGATION

From the above experiments on N and N & P removal systems throughout which filaments common to N and N & P removal plants were observed in greater or lesser quantities (i.e. 0092, *M.parvicella*, 0041, *Thiothrix*, 021N, *H.hydrossis* and 1851) it was concluded that a major factor influencing filament proliferation was intermittent aeration, causing the organisms to be alternately exposed to aerobic conditions (where oxygen serves as terminal electron acceptor) and anoxic conditions (where NO_3^- or NO_2^- serve as terminal electron acceptor), provided complete reduction of NO_3^- and denitrification of NO_2^- did not take place. From this two conclusions emerged: (i) That the name low F/M filaments was no longer appropriate and because the conditions for their proliferation appeared to be closely linked to anoxic–aerobic conditions, they were renamed anoxic–aerobic (AA) filaments; (ii) that the cause for the AA filament proliferation lay in the *requirement for the sludge mass to switch between aerobic and anoxic metabolic*

pathways, this switching providing some competitive advantage to the filamentous organisms or disadvantage to the floc-forming organisms. With these conclusions as a basis, attention was focused on facultative heterotrophic denitrification pathways.

5. LITERATURE REVIEW OF DENITRIFICATION PATHWAYS

Payne (1973) proposed the general denitrification pathway

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

nitrate → nitrite → nitric oxide → nitrous oxide → nitrogen gas

Initially denitrification was considered a strictly anoxic process, occurring only in the total absence of oxygen. However, subsequently it has been demonstrated quite convincingly in pure cultures that denitrification can continue under aerobic conditions, albeit at a lower rate [Pichinoty and d'Ornano (1961), Showe and De Moss (1968), Krul and Veeningen (1977), Robertson and Kuenen (1984)]. Pure culture studies have also demonstrated that one or more of the intermediates in the denitrification pathway have an inhibitory effect on the aerobic utilization of substrate with oxygen as terminal electron acceptor. Krul (1976) in pure culture studies on a denitrifying organism isolated from activated sludge, cultured under anoxic conditions and tested under aerobic conditions, concluded that the accumulation of the intermediate nitric oxide (NO) during denitrification caused a measurable and prolonged inhibition of oxygen utilization under subsequent aerobic conditions. Curiously, this inhibition could be demonstrated for a pure culture of an isolate from activated sludge but not for a mixed culture of activated sludge.

Some controversy arose as to whether the inhibitory effect was due to NO_2^- or NO, but recent work has concluded that the inhibitory effect is due to NO and not NO_2^-. However, the degree of inhibition is exacerbated by the presence of NO_2^- and NO_3^- (Kučera *et al.*, 1987; Carr and Ferguson, 1990).

6. HYPOTHESIS (EXPLANATION) FOR AA FILAMENT BULKING

The findings of the literature review together with the experimental results described earlier provided the basis for an explanation for the proliferation of AA filamentous organisms in N and N & P removal systems:

When denitrification is not complete under anoxic conditions, floc-formers are inhibited in their oxygen uptake cytochromes (oxidases) under subsequent

aerobic conditions by the remaining denitrification intermediates accumulated under the anoxic conditions; this inhibition of floc–formers places them at disadvantage for substrate uptake and utilization against the filamentous organisms. The denitrification intermediate causing the inhibition is nitric oxide (NO).

For this hypothesis to be valid requires the AA filaments to denitrify only as far as NO_2^- so that they do not accumulate NO, and the floc–formers to denitrify completely to N_2 gas and thereby accumulate NO under certain conditions. The literature review was regarded as providing sufficient evidence that NO inhibition of oxidases under aerobic conditions is a known phenomenon. Therefore to verify the hypothesis, it was required to be shown that (1) filaments denitrify only to NO_2^- and floc–formers to dinitrogen gas and (2) oxygen uptake inhibition is manifest in bulking activated sludge. Because the first proof requires specialized biochemical and microbiological techniques, this could not be conclusively tested in this project and is a question that would need to be taken up by microbiologists and biochemists in pure culture work. Consequently only indirect evidence for the hypothesis could be collected. This evidence is briefly presented below; for details see Casey *et al.* (1993) and Musvoto *et al.* (1992).

7. SOME EXPERIMENTAL EVIDENCE SUPPORTING THE HYPOTHESIS

7.1 Demonstration of Inhibition

To determine whether or not inhibition of oxygen utilization (and correspondingly substrate utilization) takes place in activated sludge subjected to alternating anoxic aerobic conditions, a series of batch tests was conducted on sludge harvested from the anoxic reactor of the 2RND system operated by de Villiers *et al.* (1993). In these tests the maximum specific OUR was measured (Ekama *et al.*, 1986, Randall *et al.*, 1991) upon sewage addition, with different anoxic or aerobic pretreatment conditions.

7.1.1 *Anoxic denitrification*

Figure 1 shows that inhibition of OUR was induced in the sludge after a 2 hr anoxic period with NO_2^- present during both anoxic and aerobic periods ($\approx$ 25,0 mgN/ℓ at the start of the aerobic period). Inhibition was less marked in a sludge subjected to the same conditions but with less NO_2^- present (5,5 mgN/ℓ). Almost no inhibition was measured in a sludge subjected to the same conditions with only 0,1 mgN/ℓ NO_2^- present. These batch tests show that (1) inhibition of OUR in the presence of

NO_2^- is observed and (2) the degree of inhibition is directly related to the concentration of NO_2^- at the commencement of aerobic conditions. However, it was not clear whether the inhibition results from the NO generated by NO_2^- denitrification under anoxic conditions or under aerobic conditions.

7.1.2 *Aerobic denitrification*

To check whether or not activated sludge from the 2RND system exhibited denitrification of NO_2^- under aerobic conditions, aerobic batch tests were conducted on specially prepared sludge samples. In the preparation, first, virtually all of the NO_3^- and NO_2^- were removed from the sludge by diluting with tap water, settling and decanting the supernatant 3 successive times. Then the sludge was held anoxic in the presence of about 120 mgCOD/ℓ sewage in order to denitrify any remaining NO that might be present in the organisms. After 2 h, during which thiourea was added (10 mg/ℓ) to inhibit NO_2^- formation by *Nitrosomonas*, aeration was commenced ($2{,}0 < DO < 4{,}0$ mgO/ℓ). After 1 h aeration, 20 mgNO_2^-–N/ℓ batch volume was added. After a further 1 h aeration, 360 mgCOD/ℓ (final batch volume) sewage was added and the OUR, NO_3^- and NO_2^- concentrations measured with time. Figure 2 shows that OUR inhibition is exhibited. In a similar test but with NO_3^- addition (20 mgN/ℓ) instead of NO_2^-, no inhibition is exhibited. These tests seem to indicate that NO inhibition under aerobic conditions does take place with NO_2^-, (the NO apparently produced by aerobic denitrification of NO_2^-) but not with NO_3^-. In a control batch test, in which no NO_2^- or NO_3^- was added, no inhibition was exhibited. The results of these batch tests were reproducible with sludges from intermittently aerated N and Modified UCT N & P removal systems.

In the batch tests presented so far, it appears that during the aerobic period after sewage addition the inhibition is relieved, reflected in a steadily increasing maximum specific OUR, in some cases leveling off at a constant value before the precipitous decrease in OUR when the RBCOD has been depleted. The relief of OUR inhibition possibly arises because the presence of significant quantities of RBCOD under aerobic conditions accelerates the $NO \rightarrow N_2O \rightarrow N_2$ part of the denitrification pathway so that the NO produced from NO_2^- denitrification does not accumulate.

7.1.3 *Effect of RBCOD on OUR inhibition by NO*

To check if OUR inhibition takes place in the presence of significant quantities of RBCOD, an aerobic batch test was conducted in which NO_2^- was added after the

sewage addition but while RBCOD was still present, rather than before sewage addition when only SBCOD (principally generated from organism death and lysis) is present as in the previous batch experiments. In this test no inhibition was noted, and it was concluded that the presence of RBCOD (in sufficient quantity) prevented or relieved the inhibition. From this it seemed reasonable to accept the suggestion above that the RBCOD accelerates the NO $\rightarrow$ N_2 steps of the pathway in such a way that NO no longer is accumulated, is reasonable.

7.2 Determination of the extent of NO_3^- reduction and denitrification under anoxic conditions by filaments and floc-formers

With the experiments above, it was demonstrated that OUR inhibition, hypothesized to be by NO, takes place in the presence of NO_2^- in switching from anoxic to aerobic conditions. For the proposed explanation to be acceptable, it needed to be shown even superficially that floc-formers denitrify from NO_3^- to N_2 gas, and so are susceptible to OUR inhibition by accumulated NO, whereas the low F/M filaments reduce NO_3^- to NO_2^- only, and therefore do not accumulate NO and so are not susceptible to this inhibition. Clearly this is an experiment that needs to be taken up by microbiologists and biochemists, but for the purposes of testing the hypothesis, sludge samples from a fully anoxic *(FX)* system (low DSVI) and the 2RND system on which the batch tests above were done (high DSVI), both fed real sewage, were subjected to a nitrate reduction test, a test which allows the generation of NO_2^- and/or N_2 gas to be determined. The sample with the high DSVI (many low F/M filaments) showed an accumulation of NO_2^- with no N_2 gas being detected in 8 out of 10 tests. The sample with the low DSVI (few low F/M filaments) accumulated N_2 gas, but no NO_2^- accumulated in 8 out of 10 tests. From this it is reasonable to accept that qualitatively, filaments tend to reduce NO_3^- to NO_2^- only, whereas floc-formers denitrify NO_3^- to N_2 gas. This observation lends credibility to the proposed hypothesis for low F/M filament proliferation. With a reasonable hypothesis for low F/M filament proliferation in N and N & P removal systems, attention was directed at devising strategies for the control of these filaments in the systems.

7.3 The effect of incomplete denitrification – an example in a biological N & P removal system (ex Musvoto *et al.*, 1992)

7.3.1 *Experimental Set-up*

Two identical Modified UCT systems (MUCT 1 and MUCT 2) for biological N and P removal were set up. Their anoxic mass fraction was very large at 65% (1st

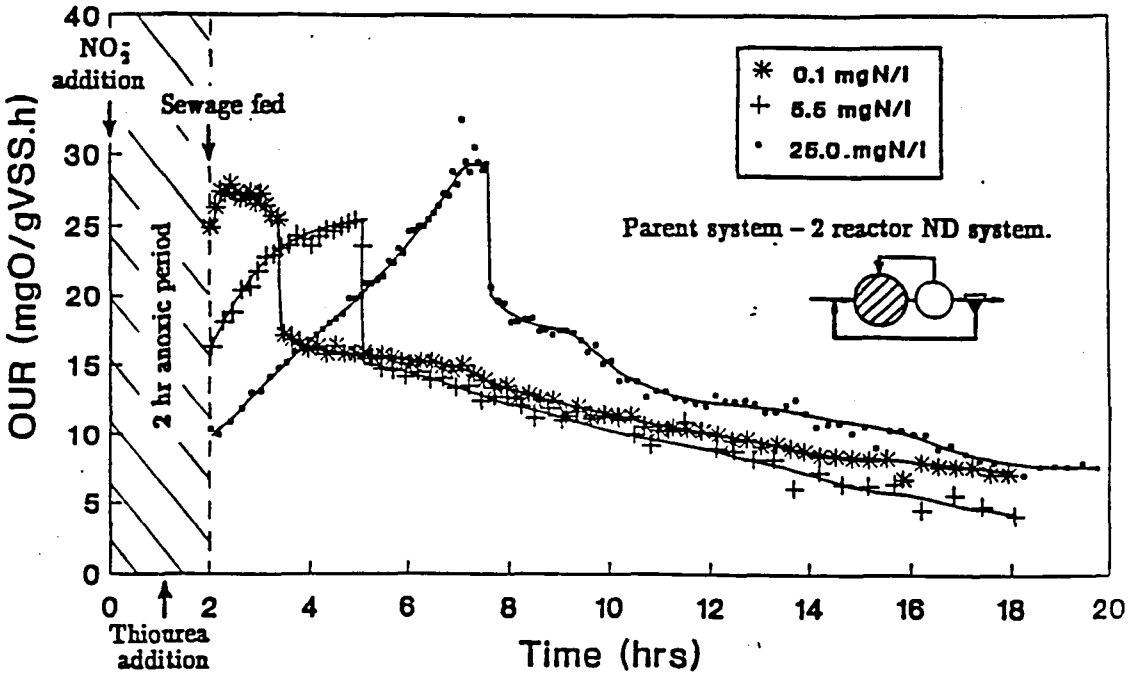


*Fig 1: Oxygen utilization rate [OUR, in mgO/(gVSS.h)] with time under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2 reactor ND system with a 2hr anoxic period prior to the aerobic test and with varying nitrite concentrations at the start of the aerobic test, demonstrating the initial but gradually declining inhibitory effect of NO_2^- on maximum specific OUR (*0,1, + 5,5 and · 25 NO_2^-–N/ℓ).*

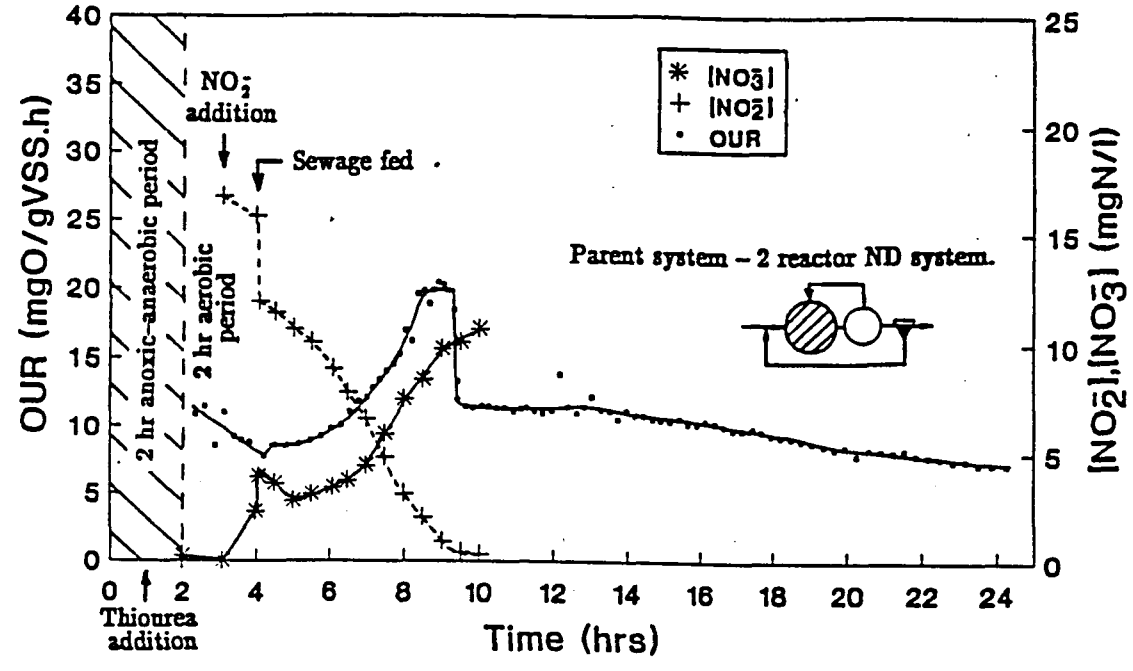


*Fig 2: Oxygen utilization rate [· OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (+NO_2^- and *NO_3^-, in mgN/ℓ) with time under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2 reactor ND system with a 2hr anoxic–anaerobic period prior to a 2hr aerobic period during which NO_2^- was added (20 mgN/ℓ), prior to the aerobic test.*

anoxic 20%, 2nd anoxic 45%) to ensure even at high TKN/COD ratios (up to 0,11 mgN/mgCOD) complete denitrification in the second anoxic reactor could be achieved at a fixed mixed liquor (aerobic to 2nd anoxic) recycle ratio of 3:1 and, as a result, produce a nitrate and nitrite free inflow to the aerobic reactor. Both systems were started up with an AA (low F/M) bulking sludge from other laboratory MUCT systems containing typical filaments for these systems, i.e. 0092, 0041, *M.parvicella* and some *H.hydrossis*. Both were operated identically at 20 days sludge age and each fed 10ℓ/d of the same Mitchell's Plain (Cape) unsettled real sewage diluted with tap water to a concentration of 1000 mgCOD/ℓ. The MUCT 1 system was operated for 340 days; for 111 days from day 129 to 240, *nitrate* was dosed into the 2nd anoxic reactor at a rate of 720 mgNO_3–N/d (72 mgN/ℓ influent). The MUCT 2 system was operated for 169 days from day 171 to day 340; for 50 days from day 290 to 340 *nitrite* was dosed into the second anoxic reactor at a rate of 900 mgNO_2–N/d (90 mgN/ℓ influent). The two systems were monitored almost daily for influent and effluent COD, TKN, nitrate, nitrite and total P concentrations, individual reactor nitrate, nitrite and total P concentrations, aerobic reactor MLSS and MLVSS concentrations, oxygen utilization rate and sludge settleability in terms of DSVI. Filament identifications were done every 3 to 4 weeks. Regular batch tests were conducted on sludge harvested from the systems to measure the nitrate and nitrite denitrification rates so that the nitrate load on the anoxic reactors could be compared with their denitrification potential.

7.3.2 *Experimental Results*

With nitrate dosing. *In the MUCT 1 system before nitrate dosing, the DSVI decreased from a start up value of 164 to 80 mℓ/g* in 128 days. The nitrate and nitrite concentrations in the outflow of the second anoxic reactor were very low i.e. <0,5 mgNO_3–N/ℓ and <0,2 mgNO_2–N/ℓ (Fig 3). The filaments were of the AA (low F/M) type i.e. 0092 and 0914 dominant with 0041, *M.parvicella*, *H.hydrossis* and 0803 present (Table 1). After commencement of nitrate dosing the DSVI increased slowly reaching 176 mℓ/g in 111 days. The nitrate and nitrite concentrations in the outflow of the second anoxic reactor increased to between 2 and 10 mgNO_3–N/ℓ and between 1,5 and 3 mgNO_2–N/ℓ respectively. The increase in nitrite concentration with nitrate dosing is acceptable because in this investigation as well as in those of Clayton *et al.* (1989) and Stern and Marais (1974), it was found that nitrite was formed at a slow rate of 0,060 mgNO_2–N/(mgAVSS.d) while nitrate was being denitrified, and that only when the nitrate concentration reaches low values (<1 mgNO_3–N/ℓ) did nett nitrite removal

commence. The same filaments as earlier were identified to be dominant and present. After cessation of nitrate dosing the DSVI declined from 176 to 91 mℓ/g in 69 days. A few days after cessation of nitrate dosing, the nitrate and nitrite concentrations in the outflow of the second anoxic reactor decreased to similarly low values (<0,5 mgNO_3–N/ℓ and <0,2 mgNO_2–N/ℓ respectively) as observed earlier before nitrate dosing. The same low F/M filaments as earlier were identified as dominant and present.

With nitrite dosing. In the MUCT 2 system before nitrite dosing, the DSVI decreased slowly from a start-up value of 131 to 90 mℓ/g in 118 days (Fig 4). The concentrations of nitrate and nitrite in the outflow of the second anoxic reactor were very low <0,2 mgNO_2–N/ℓ and <0,7 mgNO_3–N/ℓ. The dominant filaments were of the AA (low F/M) type i.e. 0092 dominant with *M.parvicella*, 0041 and *H.hydrossis* present (Table 2). From commencement of nitrite dosing the DSVI initially increased sharply from 90 to 116 mℓ/g in 11 days and thereafter more slowly, reaching 174 mℓ/g, 39 days later. The nitrite concentration in the outflow of the second anoxic reactor increased to between 10 and 20 mgNO_2–N/ℓ. The same filaments as earlier were identified to be dominant and present. The effect of withdrawing the nitrite dose was not investigated.

7.3.3 *Other Observations*

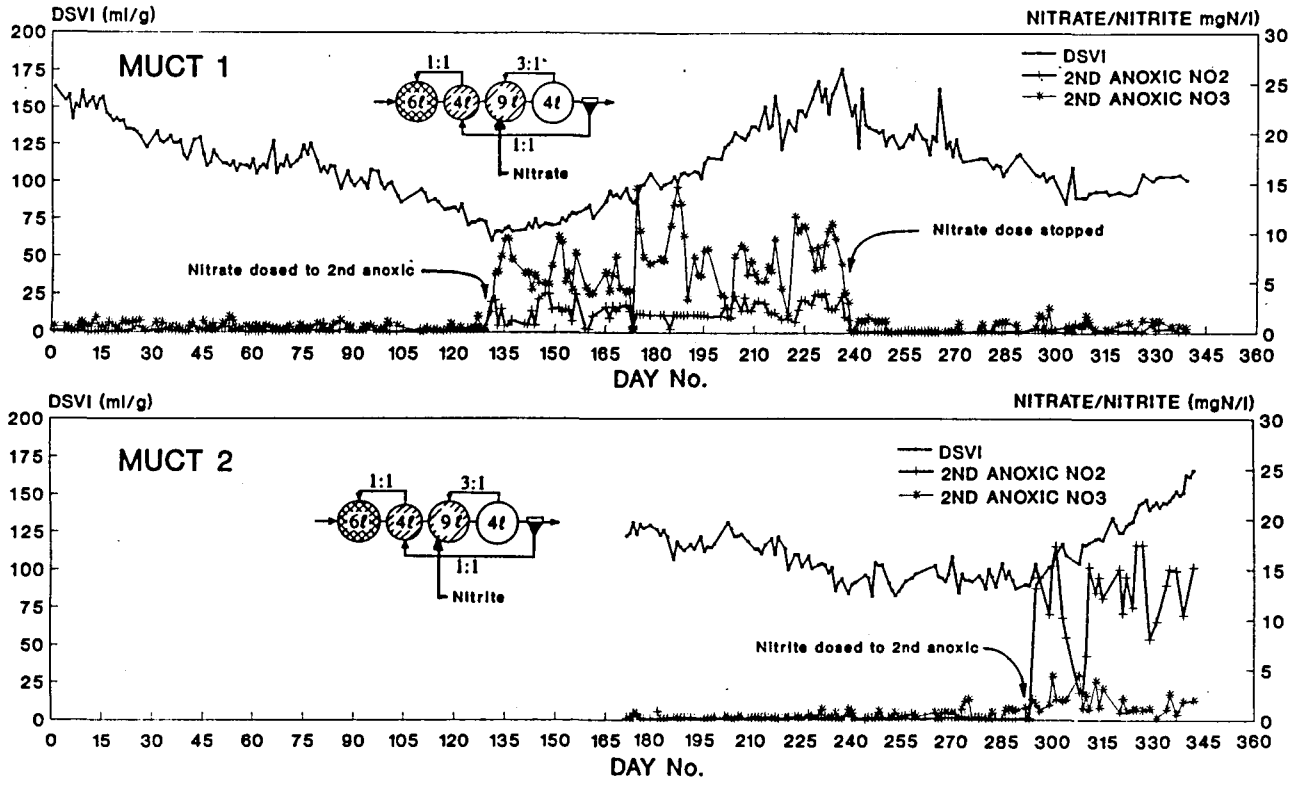
VSS mass. The two systems produced between 15 to 30% more VSS than expected in terms of the steady state design model of Wentzel *et al.* (1990) and WRC (1984). The VSS mass in the system was found to be dependent on the DSVI (or bulking); initially the VSS increased as the DSVI decreased in the absence of nitrate or nitrite dosing, then decreased as the DSVI increased with dosing and then increased again as the DSVI decreased upon withdrawal of the dosing. Although not so strongly connected to AA filament bulking as in this investigation, Clayton *et al.* (1989) also observed significantly increased sludge mass in MUCT systems compared to single or 2-reactor N removal systems at the same sludge age receiving the same wastewater and daily COD mass load (Warburton *et al.*, 1991).

Denitrification rates. The nitrate and nitrite denitrification rates observed were 0,296 mgNO_3–N/(mgAVSS.d) and 0,247 mgNO_2–N/(mgAVSS.d); the former is 24% higher than that observed by Clayton *et al.* (1989) and about 3 times higher than the second rate of denitrification (K_2) in N removal systems; due to an absence of earlier determinations, the nitrite rate could not be compared. In batch tests with

MUCT1 FILAMENT IDENTIFICATION					
DAY No.	DSVI	DOMINANT FILAMENT	SECONDARY FILAMENT	OTHER FILAMENTS PRESENT	RELATIVE AMOUNT OF FILAMENT
61	105	0092	021N	0041 *M.parvicella* *H.hydrossis*	Common to V.common
119	82	0092	021N	*H.hydrossis* *M.parvicella* 0041	V.common
181	96	0914	0092 Beggiatoa	*M.parvicella* 0041 *H.hydrossis* *Flexibacter*	Common
202	126	0092	*M.parvicella*	0803 0041 *H.hydrossis*	Abundant
237	165	0092	0041	*M.parvicella* 0803 O21N	Common
270	129	0092	021N	0803 0041 *M.parvicella*	V.common
308	91	0092	021N	*M.parvicella* 0041, 0675 *H.hydrossis* *Thiothrix* sp.	Common to v.common

Table 1: Filament identifications and DSVI for MUCT 1 and MUCT 2 systems during the investigation. Both systems operated at 20 days sludge age and 20° C.

MUCT2 FILAMENT IDENTIFICATION					
DAY No.	DSVI	DOMINANT FILAMENT	SECONDARY FILAMENT	OTHER FILAMENTS PRESENT	RELATIVE AMOUNT OF FILAMENT
181	122	0092	021N	*M.parvicella* 0041 *H.hydrossis*	Common
202	127	0092	0041	021N *H.hydrossis*	V.common
237	94	0092	0041	*H.hydrossis* *M.parvicella*	Common
270	84	0092	*H.hydrossis*	*M.parvicella* 0041 O21N	Common to V.common
308	116	0092	021N 0675	*M.parvicella*	V.common to abundant



Figs 3 and 4: Sludge settleability (in DSVI, mℓ/g) and nitrate and nitrite concentration in the 2nd anoxic reactor for MUCT 1 (Fig 1, top) and MUCT 2 (Fig 2, bottom). Note the increase in these parameters upon commencement of nitrate dosing to MUCT 1 on day 129 and nitrite dosing to MUCT 2 on day 291. Also the decrease in these parameters in MUCT 1 upon cessation of nitrate dosing to MUCT 1.

high nitrate and nitrite concentrations (>10 mgN/ℓ), nett nitrite denitrification commenced only when the nitrate concentration reached low concentrations (<1 mgNO_3–N/ℓ); at high nitrate concentrations, the nitrite concentration increased at a rate of about 0,060 mgNO_2–N/(mgAVSS.d).

8. A PROPOSED STRATEGY FOR LOW F/M FILAMENT BULKING CONTROL IN N AND N & P REMOVAL SYSTEMS

In the batch tests described above (Figs 1–2), not only was OUR inhibition demonstrated but also it appeared that, in the presence of RBCOD the inhibition was progressively relieved. In the parent N and N & P removal system from which the sludges were harvested, this relief probably does not take place because RBCOD is not available in sufficient quantity upon entry into the aerobic reactor. If a system modification could be devised in which RBCOD is available (in sufficient quantity) at the end of the anoxic reactor to reduce the accumulated NO in the floc–formers, then the floc–formers would enter the aerobic reactor in a more competitive condition against the low F/M filaments. This proposal was implemented by installing a small (containing 4% of system sludge mass) completely mixed reactor between the anoxic and aerobic zones of the MUCT and 2RND systems (De Villiers *et al.*, 1993; Casey *et al.*, 1993). These reactors, called Denox for convenience, received influent RBCOD by diverting 10% of the daily influent flow to them. Although from the batch tests it would appear logical to have made these reactors aerobic, it was decided to make them anoxic because it was thought that this would deplete the accumulated NO more rapidly.

With a pair of MUCT systems with 52,5% anoxic mass fraction (15% anaerobic) after installing a Denox reactor on one of the systems, the DSVI decreased from 150 mℓ/g to 100 mℓ/g in 30 days while the DSVI in the control MUCT system (which received supplemented NH_4^+) increased from 180 to 230 mℓ/g. Transferring the Denox reactor to the control system, caused the DSVI to decline from 230 to 120 mℓ/g in 25 days while that in the experimental system increased from 100 to 170 mℓ/g. Curiously, after this the DSVI of the control system (with the Denox reactor) began to increase slowly and after about 30 days the DSVI of both systems was around 140 to 180 mℓ/g. During this last period similarly high concentrations of nitrite (> 1 mgN/ℓ) were noted in the Denox reactor as in the 2nd anoxic reactor of the experimental system so for some unknown reason the Denox reactor was not removing the nitrite, and by implication the nitric oxide. After installing the Denox reactor in the 2RND system, the DSVI decreased from 280 mℓ/g to 130 mℓ/g in

under 30 days but afterwards showed the same inability to remove the nitrite as was observed in the MUCT system, leading to increasing DSVIs. From the above preliminary results, it appears that the strategy holds promise, but more research needs to be done to confirm and establish credibility for the strategy.

9. CONCLUSIONS AND RECOMMENDATIONS

From laboratory experimental investigations conducted under the previous research contract, it was concluded that selector reactors, whether aerobic or anoxic by kinetic selection or anaerobic by metabolic selection, were unable to control the AA filament proliferation and therefore were an unfruitful control strategy to adopt for this kind of bulking. This finding required the selector effect approach to be set aside and consequently placed the research back into an exploratory phase to seek and establish a new direction and framework for research in this area. The establishment of a new direction of research was the principal objective of this research contract.

From the observations of an extensive laboratory research investigation seeking to determine causes for and establish control strategies against low F/M filament proliferation in which fully aerobic, fully anoxic and intermittent aeration single reactor N removal systems, 2 reactor anoxic aerobic ND systems and MUCT N & P removal systems were operated under a wide range of conditions. It was concluded that a major factor influencing low F/M filament bulking was the continuous alternation between anoxic and aerobic conditions in the systems, this alternation inducing a competitive disadvantage to the floc–formers against the AA filaments. From an examination of the experimental data and a survey of denitrification pathways in the microbiological and biochemical literature, it was hypothesized that this advantage arises because the filaments reduce NO_3^- to NO_2^- whereas floc–formers reduce NO_3^- to N_2 and in the process accumulate NO which exerts an inhibitory effect on their aerobic oxygen utilization rate (OUR) in the subsequent aerobic conditions. While the hypothesis could not be directly verified because this would fall into specialized microbiological and biochemical experimentation, indirect confirmation of the hypothesis was obtained from 4 sources:

1) Batch tests on sludge harvested from intermittent aeration and 2 reactor ND and MUCT N & P removal systems showed inhibition of aerobic OUR;

2) Nitrate reduction tests on activated sludge samples with high and low DSVI

(many and few low F/M filaments respectively) indicated that in the former, NO_2^- tended to accumulate whereas in the latter N_2 gas accumulated;

3) Two Modified UCT systems operated to have high or low nitrate and nitrite concentrations in the influent to the aerobic reactor showing high and low DSVIs respectively; and

4) Most of the experimental data observed in the exploratory investigation.

These results provide strong evidence that the proposed hypothesis for the cause of AA filament bulking in N and N & P removal systems has merit.

From the proposed hypothesis, two control strategies were devised and implemented on laboratory scale 2 reactor ND and MUCT N & P removal systems. The first, which comprised installing a small denitrifying (Denox) reactor between the anoxic and aerobic reactors initially appeared to be successful in that after installation it promoted low DSVIs in the systems, but was unable to consistently maintain low DSVIs in N and N & P removal systems, indicating that further experimental testing and verification is required. The second, which comprised designing and operating a Modified UCT system such that low nitrate and nitrite concentrations (< 2 mgNO_3–N/ℓ and $< 0{,}5$ mgNO_2–N/ℓ) from the 2nd anoxic to the aerobic reactor was maintained, showed excellent promise because it cured as well as controlled the AA filament proliferation (DSVI < 80 mℓ/g).

Although the experimental investigations conducted in this research contract have produced a deeper understanding of the AA filament behaviour in N and N & P removal systems and have given some interesting insights into the problem, more research is required before AA filament proliferation can be effectively controlled in full scale N and N & P removal plants. This research is being conducted in a follow–up 3 year research contract (1993–1995), the objects of which are:

1) Devise tests and refine specific AA bulking control strategies on laboratory–scale biological N and N & P removal systems that flow from the present understanding of the proposed cause of AA filament bulking.

2) Confirm the hypothesis for the cause of AA filament bulking by examining the experimental data collected to date and conducting further experiments. For

example, operate MUCT systems at low temperatures (13°C) to inhibit nitrification and evaluate the effect of this on AA filament bulking. This will help elucidate the often observed temperature dependence of sludge settleability and bulking (possibly via the temperature dependence of nitrification and denitrification) and provide valuable kinetic information on nitrification, denitrification (by dosing nitrate and/or nitrite to the systems) and biological excess P removal.

3) To develop and design AA bulking control methods for full-scale implementation, and integrate these methods into the design procedures of biological N and N & P removal systems.

4) Write a series of publications and present a number of seminars and talks to disseminate the information so that it can be adopted by plant operators and designers.

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GLOSSARY

DEFINITION OF TERMS

AA	Anoxic-aerobic; new group name for most of the low F/M filamentous organisms
Arch.Microbiol.	Archives of Microbiology – a journal
AVSS	Active Volatile Suspended Solids. The volatile suspended solids (VSS) comprise active organisms and inert organic mass. The active organism mass is the live biological mass which performs the biological reactions; the inert mass originates from two sources (i) from inert organic material in the influent and (ii) endogenous residue. The active fraction of the VSS is a function of the sludge age of the system and sewage characteristics. It is an empirical estimate that has found acceptability because of the consistency it brings to kinetic rates observed in activated sludge systems, e.g. based on active mass the specific endogenous mass/respiration rate and specific denitrification rates are constant with sludge age from 3 to 72 days. Because of this consistency, the readily biodegradable COD (RBCOD) uptake rates also are reduced to specific rates with respect to AVSS so that rates in different sludge age systems can be compared. For details on calculation of the AVSS, see Marais and Ekama, 1976 and Ekama, Dold and Marais, 1986.
Bacteriol.Rev.	Bacteriological Reviews – an annual review journal in Bacteriology
Biochim.Biophys.Acta	Biochimica Biophysica Acta – one of the major biochemical/microbiological journals
COD	Chemical oxygen demand
DO	Dissolved oxygen
DSVI	Diluted sludge volume index, a modified SVI sludge settleability test [see Ekama and Marais (1984). Two improved sludge settleability parameters, IMIESA, 9, 6, 20–25 for method]
d	day
FA	fully aerobic; an activated sludge system with all its sludge under aerobic conditions
FX	fully anoxic; an activated sludge system not aerated at all, but receiving dosed nitrate to create anoxic conditions
H.hydrossis	*Haliscomenobacter hydrossis*, one of the filamentous organisms in the AA (low F/M) group
h	hour

IA	intermittent aeration; a single reactor activated sludge system with aeration on for a specified time to create aerobic condition and aeration off for the remaining time to create anoxic (and possibly anaerobic) conditions
g	gram
J.Appl.Bact.	Journal of Applied Bacteriology
J.Bacteriol.	Journal of Bacteriology
low F/M	low food to micro–organism ratio; equivalent to low load factor, or low loading rate or long sludge age
ℓ	litre, the unit measure for volume
MUCT	Modified University of Cape Town system for biological removal of nitrogen and phosphorus
M. parvicella	*Microthrix parvicella;* one of the filamentous organisms in the AA (low F/M) group
MLVSS	mixed liquor volatile suspended solids; same as VSS, the organic part of the suspended solids in activated sludge plants
MLSS	mixed liquor suspended solids; the organic and inorganic suspended solids in activated sludge plants, also referred to as Total Suspended Solids
N	nitrogen; all nitrogen concentrations i.e. nitrate, nitrite or TKN are expressed as mgN/ℓ
NO_3–N; NO_2–N	nitrate and nitrite respectively as N
NO; N_2O	nitric oxide and nitrous oxide respectively, the two gaseous denitrification intermediates between NO_2 and N_2
N_2	dinitrogen gas, the end product of denitrification
N & P	nitrogen and phosphorus; applied to activated sludge plants incorporating simultaneous biological N and P removal
OUR	oxygen utilization rate; mass oxygen utilized per unit reactor volume per unit time, or per unit VSS mass per unit time, e.g. mgO/(gVSS.h)
P	phosphorus; all phosphorus concentrations are total phosphorus concentrations and expressed as mgP/ℓ
RBCOD	readily biodegradable COD component of the influent COD
SBCOD	slowly biodegradable COD component of the influent COD
TKN	Total Kjeldahl nitrogen

TKN/COD	ratio of the influent TKN and COD concentrations – a useful term comparing the quantity of nitrate that is going to be generated by nitrification from the influent TKN (called nitrification capacity) with the quantity of organic material (influent COD) available for denitrification
UCT	University of Cape Town activated sludge system for biological removal of nitrogen and phosphorus
Wat.Pollut.Control	Journal of the erstwhile Institute for Water Pollution Control, now Institute for Water and Environmental Management (IWEM)
Wat.Sci.Tech.	Water, Science and Technology, a journal of the International Association for Water quality (IAWQ)
WRC	Water Research Commission, a water research co–ordination and funding agency in south Africa. Executive Director Mr P E Odendaal, P O Box 824, Pretoria, 0001
0092, 0041 0914, 0803 1851, 021N	Six different filamentous organism types of activated sludge, the first 5 common in biological N & P and N removal plants and therefore sorting into the AA (low F/M) group, the last arising mainly with septic wastewaters (i.e. high sulphides) as happens occasionally in the UCT Water Research Laboratory with refrigeration breakdowns
2RND	Two reactor nitrification denitrification activated sludge systems, i.e. ones wherein the aerobic and anoxic zones are in 2 separated reactors either with anoxic reactor first followed by the aerobic reactor (i.e. predenitrification or primary anoxic reactor) or with aerobic reactor first, followed by t he anoxic reactor (i.e. post–denitrification or secondary anoxic reactor)

Table 2: Summary of experimental work from Bulking Contract period 1989–1992.

System Type	Aerobic Mass Fraction	DO Conc (mgO/l)	Cycles /d	NH_4^+ Addition to Influent	NO_3^-/NO_2^- Dosing	Sludge Age (d)	Substrate Type	Steady-State DSVI (ml/g)	Dominant Filament	Selector Reactor	Reference
	30 -40	< 2,0	72	_	NO_3^-	15	Artificial	400-600	*H. hydrossis*	_	Casey *et al.*(W83)
	"	< 2,0	72	_	NO_3^-	15	Artificial SBCOD	500	*H. hydrossis*	_	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	>1000	*H. hydrossis*	_	"
	"	0,2<DO<0,5	72	_	_	15	Municipal	160	Type 0092	_	"
	"	0,2<DO<1,0	72	_	_	15	Municipal	180	Type 0092	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal	250	Type 0041	_	"
	"	0,2<DO<3,0	72	_	_	15	Municipal	280	Type 021N	_	"
	"	0,2<DO<1,0	72	_	NO_3^-	15	Municipal	180	Type 0041	_	"
	"	0,2<DO<1,0	72	_	NO_3^-+NO_2^-	15	Municipal	200	*M. parvicella*	_	"
	"	0,2<DO<3,0	72	_	NO_3^-+NO_2^-	15	Municipal	200	*M. parvicella*	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal RBCOD	240/400	Type 021N/ *H. hydrossis*	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal SBCOD	240/400	Type 021N/ *M. parvicella*	_	"
	"	0,2<DO<2,0	72	_	NO_3^-	15	Municipal RBCOD in anoxic	300	*H. hydrossis*	_	"
N Removal	"	< 2,0	72	_	NO_3^-	15	Municipal RBCOD in aerobic	<100	Type 0041	_	"
IAND Systems	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	<250	*H. hydrossis*	2 aerobic	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	400	Type 1701	2 anoxic	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	400	Type 1701	3 anoxic	"
	"	< 2,0	72	_	NO_3^-	20	Municipal	>400	Type 0092	_	Warburton *et al.*(W65)
	"	< 2,0	72	_	NO_3^-	15	Municipal	>400	Type 0092	_	"
	"	< 2,0	72	_	NO_3^-	10	Municipal	>200	Type 0092	_	"
	"	< 2,0	72	_	_	10	Municipal	>200	Type 0092	_	"
	"	> 2,0	48	_	_	15	Artificial	80	Type 0092/ *Thiothrix sp.*	_	Ketley *et al.*(W68)
	"	> 2,0	48	_	NO_3^-	15	Municipal	400	*M. parvicella* Type 0803	_	"
	"	> 2,0	3	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	2	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	1	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	0,33	_	NO_3^-	15	Municipal	400		_	"
	"	1,0<DO<3,0	3	_	_	15	Municipal	190	Type 0041	_	De Villiers *et al.*(W81)
	"	1.0<DO<3,0	3	+	_	15	Municipal	220	Type 0041	_	"

Table 2: Summary of experimental work from Bulking Contract period 1989–1992.

System Type	Aerobic Mass Fraction (%)	DO Conc (mgO/l)	Cycles /d	NH_4^+ Addition to Influent	NO_3^-/NO_2^- Dosing	Sludge Age (d)	Substrate Type	Steady-State DSVI (ml/g)	Dominant Filament	Selector Reactor	Reference
N Removal IAND Systems cont'd	30-40	< 2,0	144	_	NO_3^-	10	Municipal	>400	*M. parvicella*	_	Warburton *et al.* (W65)
	70-80	< 2,0	144	_	NO_3^-	10	Municipal	<200	*M. parvicella*	_	"
	30-40	< 2,0	144	_	NO_3^-	10	Municipal	300-600	*M. parvicella*	_	"
	30-40	< 2,0	144	_	NO_3^-	8	Municipal	300-400	*M. parvicella*	_	"
	30-40	< 2,0	144	_	NO_3^-	7,5	Municipal	150	*M.parvicella H. hydrossis*	_	"
	30-40	< 2,0	144	_	NO_3^-	6	Municipal	250	*H. hydrossis*	_	"
	30-40	< 2,0	144	_	NO_3^-	5	Municipal	<200	*H. hydrossis*	_	"
Aerobic	100	> 2,0	_	_	NO_3^-	15	Artificial	<100	Type 0041	_	Casey *et al.* (W83)
	100	0,2<DO<0,5	_	_	_	15	Municipal	<100	*H. hydrossis*	_	"
	100	0,2<DO<2,0	_	_	_	15	Municipal	<100	*H. hydrossis*	_	"
	100	3,0<DO	_	_	_	15	Municipal	<100	Type 021N	_	"
Anoxic	Nil	_	_	_	NO_3^-	15	Municipal	80-130	Type 0092	_	Ketley *et al.* (W68)
	Nil	_	_	_	NO_2^-	15	Municipal	80	Type 0092	_	Casey *et al.* (W83)
	Nil	_	_	_	NO_3^-	15	Municipal SBCOD	130	Type 0092	_	"
	Nil	_	_	_	NO_3^-	15	Artificial	200	*H. hydrossis*	_	"
N Removal 2RND Systems	30	2,0<DO	_	_	NO_3^-	15	Artificial	400	Type 1851	_	Hulsman *et al.* (W73)
	45	2,0<DO<4,0	_	_	NO_3^-	15	Artificial	100	*H. hydrossis*/ Type1851	_	"
	45	2,0<DO<4,0	_	_	NO_3^-	15	Municipal	100	Type 0092/ *H. hydrossis*	_	"
	30	2,0<DO<4,0	_	_	NO_3^-	15	Municipal	150	Type 021N	_	"
	33	1,0<DO<3,0	_	_	_	15	Municipal	<200	Type 0803	_	De Villiers *et al.* (W81)
	33	1,0<DO<3,0	_	+	_	15	Municipal	370	Type 0041	_	"
	45	2,0<DO<4,0	_	_	NO_3^-	15	Municipal	<150	Type 1851	_	Hulsman *et al.* (W73)
	45	2,0<DO<4,0	_	_	NO_3^-	15	Municipal	100	*H. hydrossis*/ Type 0041	_	"
N & P Removal MUCT Systems	33	DO>2,0	_	_	_	20	Municipal	>200	Type 0092	_	Casey *et al.* (W83)
	65	DO>2,0	_	_	_	20	Municipal	<150	Type 0092	_	"
	33	DO>2,0	_	_	_	20	Municipal	150	Type 0092	_	"
	33	DO>2,0	_	+	_	20	Municipal	250-280	Type 0092	_	"
	33	DO>2,0	_	_	NO_2^-	20	Municipal	220	Type 0092	_	"
	20	2,5<DO<4,0	_	_	_	20	Municipal	70-100	Type 0092	_	Musvoto *et al.* (W77)
	20	2,5<DO<4,0	_	_	NO_3^-	20	Municipal	160	Type 0092	_	"
	20	2,5<DO<4,0	_	_	NO_2^-	20	Municipal	>160	Type 0092	_	"

EXECUTIVE SUMMARY / BESTUURSOPSOMMING

DEVELOPMENT AND EVALUATION OF SPECIFIC CONTROL METHODS FOR AMELIORATING LOW F/M FILAMENT BULKING

UNIVERSITY OF CAPE TOWN
(Department of Civil Engineering)

WRC Report No. 286/1/94

UNIVERSITY OF CAPE TOWN
Department of Civil Engineering
(Water Research Group)

FINAL REPORT
on the
FOUR YEAR RESEARCH CONTRACT (1989–1992)
into
DEVELOPMENT AND EVALUATION OF SPECIFIC CONTROL METHODS FOR AMELIORATING LOW F/M FILAMENT BULKING
for the
WATER RESEARCH COMMISSION

by
Casey T G, Wentzel M C, Ekama G A, Lakay M T and Marais GvR

WRC Report 286/1/94
ISBN No. 1 86845 076 7
ISBN Set No. 1 86845 078 3

Research Report No. W 82
August, 1993

Final Report to the Water Research Commission on a 4–year research contract (1989–1992) into development and evaluation of specific control methods for ameliorating low F/M filament bulking

SYNOPSIS

Following the finding in the previous research contract that the selector effect did not control low F/M filament bulking[1] in N and N & P removal systems, new approaches for dealing with this common problem in N and N & P removal plants needed to be developed. In this research contract a number of directions were explored to try to identify the cause for the low F/M filament bulking; if the cause could be understood, then it becomes possible to devise strategies for the control of the low F/M filament proliferation.

From the results of an experimental investigation over 3 years in which various laboratory[2] N and N & P removal plants were operated (all at 20° C) to examine the effect of:

(i) readily biodegradable or slowly biodegradable COD only as feed,
(ii) the aerobic mass fraction,
(iii) frequency of exposure to anoxic and aerobic conditions,
(iv) nitrate and nitrite concentrations in the anoxic zone or during the anoxic period,
(v) DO concentration in the aerobic zone or during the aerobic period,
(vi) sludge age,
(vii) fully aerobic and fully anoxic conditions, and
(viii) differences in the anoxic–aerobic condition in intermittently aerated single reactor and 2 reactor anoxic aerobic systems,

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[1] Caused principally by filaments 0092, *Microthrix parvicella*, 0041, 0675, 0914 and 185l.

[2] In full–scale N and N & P removal systems, *M. Parvicella* is frequently associated with significant scum formation on the reactor surfaces, particularly when the reactors are designed with subsurface inter–reactor connections allowing the foam to accumulate on the surface. In the laboratory N and N & P removal systems, *M. Parvicella* was frequently identified in the mixed liquor and often found to be the dominant filament in particular in the N removal systems. However, even though the laboratory systems also had subsurface inter–reactor connections scum formation was not observed in the investigation. The reason for this difference in *M. Parvicella* behaviour between laboratory and full–scale N and N & P removal systems is not clear and not dealt with in this investigation.

it was concluded that the single most important factor influencing low F/M filament proliferation was alternating anoxic–aerobic conditions with significant concentrations of nitrate and/or nitrite (>5 mgNO_3–N/ℓ and >2 mgNO_2–N/ℓ) present at the commencement of aerobic conditions. Under these conditions the activated sludge is forced to switch between aerobic and anoxic metabolic pathways in which nitrate/nitrite and oxygen respectively serve as terminal electron acceptors, this switching conferring some competitive advantage onto the filaments or some disadvantage onto the floc–formers. As a consequence of the direct influence of anoxic–aerobic conditions on low F/M filament proliferation irrespective of sludge age, and a virtual absence of these filaments under fully aerobic or fully anoxic conditions, these filaments were renamed anoxic–aerobic (AA) filaments as a name more descriptive of the conditions under which they proliferate. From the observations and a review of the microbiological and biochemical literature on facultative heterotrophic denitrification pathways, a hypothesis for the cause of the AA filaments was developed, viz:

If denitrification is not complete upon commencement of aerobic conditions the facultative heterotrophic floc–formers, which denitrify nitrate completely to dinitrogen gas, are inhibited in the oxygen uptake oxidase cytochromes under the aerobic conditions by denitrification intermediates in particular nitric oxide (NO) accumulated under the previous anoxic conditions. In contrast, the AA filaments, which reduce nitrate only as far as nitrite, will not be inhibited in their oxygen uptake cytochromes because they do not accumulate the inhibiting NO intermediates from the nitrite to dinitrogen gas step.

Because the hypothesis is at a microbiological and biochemical level, it could not be directly experimentally verified in this investigation i.e. it could not be tested that floc–formers denitrify to dinitrogen gas whereas the AA filaments only as far as nitrite. This aspect will need to be taken up by microbiologists and biochemists in specialist pure culture work. However, convincing indirect evidence in support of the hypothesis was obtained in the investigation from

1) The literature survey which demonstrates that inhibition of oxidase cytochrome by denitrification intermediates is a recognized phenomenon by microbiologists and biochemists.

2) Specifically designed batch tests on sludges harvested from bulking and

non–bulking N and N & P removal activated sludge systems in demonstrating the existence of the inhibition.

3) In intermittent aeration single reactor and two–reactor anoxic–aerobic N removal systems, when nitrate and nitrite concentrations were low (< 2 mgNO_3–N/ℓ and <0,5 mgNO_2–N/ℓ) in the anoxic reactor upstream of the aerobic or at the time aerobic conditions commenced, the systems demonstrated a significantly reduced level of filaments and lower DSVIs compared with systems that had high nitrate and nitrite concentrations at commencement of aerobic conditions; this feature was particularly notable in Modified UCT N & P removal systems.

4) Most of the experimental results observed in the exploratory investigation.

The implications of the above conclusions regarding AA filament bulking on the design and operation of N and N & P removal plants still need to be examined, but it would appear that these plants need to be designed and operated such that the nitrate/nitrite recycled to the anoxic reactor(s) should be fully denitrified before re–entering to the aerobic zone or period.

Further investigations with the objective of (1) confirming the new framework for understanding AA filament bulking, and (2) examining its implications on design and operation are being conducted in a new three year (1993–1995) follow–up research contract with the Water Research Commission, the 4th in a series since 1983.

Final Report to the Water Research Commission on a 4 year research contract (1989–1992) into development and evaluation of specific control methods for ameliorating low F/M filament bulking

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ACKNOWLEDGEMENTS

The writers wish to express their gratitude to the following persons for their contribution to the research work reported here:

- Mr Taliep Lakay – Laboratory Technical Assistant, for his invaluable help in running the experimental laboratory systems, analytical equipment, stores, and being the helping hands required at the right time and right place.

- Mrs Heather Bain – Clerical and Administrative Assistant, for so cheerfully and unquestioningly typing and re–typing the seemingly unending drafts, attending to the accounts and seeing to all the clerical details that we so easily overlook.

The contribution of these two persons is not that of support only – they are vital members of the research team.

- The staff of the Civil Engineering Workshop and Laboratory, Messrs E von Guerard, C Nicholas and D Botha, Principal, Senior and Senior Technical Officers respectively, and N Hassen, Technical Assistant, for construction and maintenance of the laboratory equipment.

- Mr Dougie Swartz, Departmental Assistant, for his help in the Water Research Laboratory.

All of the experimental work conducted under this research contract was done by the following post graduate students:

Mr Tim Casey, Research Officer, who conducted a large part of the experiments, and inspired the many others done by the MSc students listed below, for his PhD degree.

Ms Eustina Musvoto and Messrs Charles Warburton, Dave Ketley, Andrew Hulsman and Michael de Villiers who undertook the many experiments which examined the effect of the various system design and operating parameters on AA filament bulking for their MSc degrees.

It is the dedication and effort of these students that produced the large body of experimental data out of which flowed the new framework for understanding the AA filamentous bulking problem in nutrient removal plants.

A special word of gratitude and appreciation is expressed to Mrs Mara Segal and Mrs Lee Boyd, Principal and Senior Professional Officers respectively of the Johannesburg Scientific Services Department at Cydna Laboratory, for doing so willingly and capably all the filament identifications throughout the 4 year contract period.

Acknowledgement is due to the members of the Steering Committee of the project who guided the research work during the 4 year period:

Dr S A (Steve) Mitchell – Water Research Commission (Chairman)
Dr L H (Laurraine) Lötter – Johannesburg Scientific Services Department
Mr A R (Tony) Pitman – Johannesburg Wastewater Department
Mr G (Gerhard) Offringa – Water Research Commission
Dr J J (Koos) Barnard – Department of Water Affairs
Prof W A (At) Pretorius – Pretoria University
Mr H G J (Henk) Beekman – Cape Town City Engineer's Department
Mr P W (Piet) Weideman – Water Research Commission (Committee Secretary)
Mr F (Fanus) Venter – Division of Water Technology, CSIR

Gratitude is expressed to the Water Research Commission and Foundation for Research Development for financial support of the research.

Finally the writers express their appreciation to all their colleagues and associates in the field for their willingness to inform us of their experiences and observations on full-scale plant behaviour. We value this contact with practice, not only for the information it provides, but also for the sobering reminders of the magnitude and urgency of the bulking problem.

PAPERS, REPORTS AND OTHER CONTRIBUTIONS PUBLISHED DURING CONTRACT PERIOD (January 1989 to December 1992)

A. PAPERS PUBLISHED

A.1 Journals and Conference

1. Gabb DMD, Still DA, Ekama GA, Jenkins D and Marais GvR (1991). The selector effect on filamentous bulking in long sludge age activated sludge systems. Wat.Sci.Tech. 23(4/6-2), Kyoto, 867-877.

2. Casey TG, Wentzel MC, Loewenthal RE, Ekama GA and Marais GvR (1992). A hypothesis for the cause of low F/M filament bulking in nutrient removal activated sludge systems. Water Research, 26(6), 867-869.

3. Musvoto EV, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993). The effect of incomplete denitrification on anoxic-aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. Presented at first IAWQ ASPD Specialist Conference, Paris, Sept. 1993. (To appear in Wat.Sci.Tech.)

4. Casey TG, Wentzel MC, Ekama GA, Loewenthal RE and Marais GvR (1993). An hypothesis for the causes and control of anoxic-aerobic (AA) (low F/M) filament bulking in biological N and N & P removal activated sludge systems. Presented at first IAWQ ASPD Specialist Conference, Paris, Sept. 1993. (To appear in Wat.Sci.Tech.)

5. Gabb DMD, Ekama GA, Jenkins D and Marais GvR (1989). Evaluation of bulking control methods for long sludge age activated sludge systems. Procs. 1st biennial WISA conference, Cape Town, March 1989.

6. Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). Causes and control of low F/M filament bulking in nutrient removal activated sludge systems. Procs. Two-day Workshop on prevention and control of bulking activated sludge, Perugia, Italy, June 1992.

B. REPORTS PUBLISHED

1. W 65[3] Warburton CA, Lakay MT, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1991). The effect of sludge age and aerobic mass fraction on low F/M filament bulking in intermittent aeration nitrogen removal systems.

2. W 68[3] Ketley DA, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1991). The effect of fully anoxic conditions and frequency of exposure to aerobic and anoxic conditions on the growth of low F/M filaments in nitrogen removal systems.

3. W 73[3] Hulsman A, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). The effect of type, size, position and recycle ratio of the anoxic

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[3] Research Report Number, obtainable from Department of Civil Engineering, University of Cape Town, Rondebosch 7700, Cape, South Africa. Attention Prof G A Ekama. Fax 0927 21 650 2603.

zone on low F/M filament bulking in nitrogen removal activated sludge systems.

4. W 77[3] Musvoto EV, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1992). The effect of large anoxic fractions and concentration of nitrate and nitrite in the primary anoxic zone in low F/M filament sludge systems.

5. W 81[3] De Villiers ME, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993). The effect of nitrate and nitrite concentrations at the commencement of aerobic conditions on AA (low F/M) filament bulking in N removal activated sludge systems.

6. W 82[4] Casey TG, Ekama GA, Wentzel MC, Lakay TM and Marais GvR (1993). Development and evaluation of specific control methods for ameliorating low F/M filament bulking. Summary final report to Water Research Commission on 4 year (1989–1992) research contract K5/286. WRC 286/1/93, P O Box 824, Pretoria, 0001. (This report)

7. W 83[4] Casey TG, Wentzel MC, Ekama GA, Lakay MT and Marais GvR (1993). Causes and control of anoxic-aerobic (AA) filament bulking in biological N and N & P removal systems. Detailed final report to Water Research Commission on 4 year (1989–1992) research contract K5/286, WRC 286/2/93, P O Box 824, Pretoria 0001.

[4] Reports obtainable from Water Research Commission, P O Box 824, Pretoria, 0001, South Africa. Attention Dr S Mitchell. Fax 0927 12 331 2565.

Final Report to the Water Research Commission on a 4 year research contract (1989–1992) into development and evaluation of specific control methods for ameliorating low F/M filament bulking

SUMMARY REPORT

1. OBJECTIVES

This 4 year research contract from 1989 to 1992 is the third in a consecutive series which commenced in 1983. The first of these contracts (1983–84) was an exploratory study and survey of the extent and severity of the bulking problems in South African activated sludge plants and identification of the principal filamentous organisms causing the problems. In this work, which surveyed 110 activated sludge plants, the majority of which were long sludge nitrification plants incorporating denitrification either intentionally (in identified non–aerated zones) or unintentionally (in poorly mixed "dead" zone) and 26 of which were biological N & P removal plants, the principal dominant filamentous organisms were the so–called low F/M ones (i.e. 0092, 0041, *Microthrix parvicella*, 0675, 9014 and 1851) (Blackbeard *et al.*, 1985).

In the second contract, the promoted non–specific and specific control strategies for low F/M filaments were evaluated. The main non–specific strategy, chlorination, so named because it is effective for any filamentous organism type, was found to be successful without significant influence on nitrification, denitrification and biological P removal. However, the non–specific methods treat only the symptoms of bulking, i.e. kills the filaments, and does not eliminate the causes for the proliferation of the filaments. Consequently the promoted specific control method for low F/M filaments was evaluated. This method, called the selector effect, involves modifying the activated sludge reactor in such a way as to impose alternating feed starve conditions and a readily biodegradable COD concentration gradient on the sludge by introducing plug–flow conditions, or selector reactors, which are small reactors receiving the influent and underflow recycle ahead of the main reactor. It was found that when selector reactors, either aerobic or anoxic, were installed on intermittent aeration nitrification–denitrification activated sludge systems (mimicing Carousel or Orbal plants), the selector effect induced by the selector reactor did not control the low F/M filament proliferation. Although failure of the selector effect to control the low F/M filament proliferation was disappointing, it was reassuring because it brought consistency with regard to the effect of the anaerobic reactor. This reactor

in effect also functioned as a selector in that the RBCOD was taken up in it by floc-formers (in this case the polyP organisms that effect biological excess P removal) and it was noted in laboratory and full-scale N & P removal plants that the anaerobic reactor did not control low F/M filament proliferation because these systems seemed prone to low F/M filament bulking. Curiously, the major influence that appeared to ameliorate the low F/M filament proliferation was continuous aeration. This was effective in rapidly (in 2 to 3 weeks) reducing the low F/M filament population and improving sludge settleability, but it also eliminated the conditions required for biological N & P removal. The investigation concluded that alternative specific control methods for low F/M filament proliferation in N and N & P removal systems would need to be found (Gabb *et al.*, 1989).

The 4 year research contract results presented in this report commenced at the termination of the 2nd bulking contract described above. The finding that the selector effect did not control the low F/M filament proliferation placed the research back into an exploratory phase. As a consequence the central task of this research contract was to establish and pursue new directions of research that held promise of controlling the low F/M filament proliferation without compromising biological N & P removal. By considering the implications of the research conducted in the previous contract, *inter alia*:

i) the low F/M filaments appear not to require influent RBCOD for proliferation,
ii) anoxic-aerobic conditions appear to stimulate low F/M filament proliferation,

a wide-ranging experimental investigation was initiated to determine the influence of a range of factors on low F/M filament proliferation. The experimental results obtained in this investigation and conclusions gleaned from them forms the central activity of this research contract.

2. RESEARCH DIRECTIONS AND SYSTEMS OPERATED

In order to establish which conditions in N and N & P removal systems promote (or inhibit) low F/M filament proliferation, the following system factors were examined:

1. Readily biodegradable COD (RBCOD) or slowly biodegradable COD (SBCOD) only as influent. (Casey *et al.*, Report W 83)

2. Fully aerobic and fully anoxic conditions. (Ketley *et al.*, W 68)

3. Differences in alternating anoxic–aerobic conditions caused by (i) intermittent aeration in a single reactor, or (ii) separate anoxic and aerobic reactors in single sludge systems. (Hulsman *et al.*, W 73; de Villiers *et al.*, W 81)

4. The magnitude of the aerobic mass fraction, i.e. (i) the fraction of the day the sludge mass is under aerobic conditions, or (iii) the proportion of the mass of sludge in the system under aerobic conditions. (Warburton *et al.*, W 65; Ketley *et al.*, W 68; Musvoto *et al.*, W 77)

5. The magnitude of the sludge age. (Warburton *et al.*, W 65)

6. The nitrate and nitrite concentrations during the anoxic periods. (Warburton *et al.*, W 65; Musvoto *et al.*, W 77; de Villiers *et al.*, W 81; Casey *et al.*, W 83)

7. The frequency of alternation between anoxic and aerobic conditions. (Warburton *et al.*, W 65; Ketley *et al.*, W 68; Hulsman *et al.*, W 73)

8. The dissolved oxygen (DO) concentration in fully aerobic systems or during the aerobic period or zone of anoxic aerobic systems. (Casey *et al.*, W 83)

In the experiments to examine the above factors (except sludge age), three basic types of long sludge age (15–30 d) laboratory scale activated sludge systems were operated at 20° C:

(1) continuously fed single reactor systems fed either real sewage, artificial sewage or real sewage separated into its soluble and particulate fractions by 0,45μm membrane filtration, operated either fully aerobic (FA), fully anoxic (FX) or anoxic–aerobic by intermittent aeration (IA) with varying aerobic mass fractions and frequencies of alternation between anoxic and aerobic conditions,

(2) continuously fed two reactor (separate) anoxic–aerobic nitrification–denitrification (2RND) pre– and post–denitrification systems fed real or artificial sewage with varying aerobic mass fractions and frequencies of alternation between anoxic and aerobic conditions by adjustment of the mixed liquor recycle ratio,

(3) continuously fed modified UCT N & P removal systems fed real sewage with varying influent TKN/COD ratios and varying anoxic mass fractions between 20 and 65% (with constant anaerobic mass fraction of 15% giving unaerated mass fractions between 35 and 80%).

The effect of sludge age at 20, 15, 10, 8, 7, 6 and 5 days was examined in single reactor intermittently aerated ND systems fed real sewage with 30% aerobic mass fraction.

3. RESULTS FROM THE INVESTIGATION

From monitoring the behaviour of the above types of systems, the following significant observations were made (details given in references cited and summarized in Table 2 on pages 26 and 27):

3.1 In fully anoxic (FX) systems with effluent NO_3^- concentrations > 5 mgN/ℓ (i.e. by NO_3^- dosing) and in fully aerobic systems with high DO (>2 mgO/ℓ) and low DO (0,2<DO<0,5 mgO/ℓ), all fed real sewage, the low F/M filaments (0041, *H.hydrossis*, 0092, *M.parvicella*, 1851) were present but *did not* proliferate and low DSVIs (<80 mℓ/g) were obtained. When the effluent NO_3^- was <5 mgN/ℓ in the fully anoxic systems due to cessation or insufficient nitrate dosing, the sludge settleability became very poor by floc dispersion, not by filament proliferation, presumably due to the unavailability of sufficient external electron acceptors on a continuous basis. In contrast, in the intermittent aeration (IA) systems (30% aerobic fraction) with either high or low DO concentration during the aerobic period, the low F/M filaments *did* proliferate causing high DSVIs (> 200 mℓ/g). These observations indicated that (i) alternation between anoxic and aerobic conditions was necessary for low F/M filament proliferation and (ii) the low DO conditions in the IA system as it moves between anoxic and aerobic conditions did not appear to play a role in promoting low F/M filament proliferation (Casey *et al.*, 1993; Ketley *et al.*, 1991).

3.2 In the intermittent aeration (IA) systems fed artificial sewage, low F/M filaments, in particular *H.hydrossis* and 1851, but also 0092, 0675 and 0041, proliferated both with RBCOD alone, SBCOD alone and with mixtures of RBCOD and SBCOD as feed. With RBCOD alone, excessively high DSVIs (>1000 mℓ/g) were quickly reached (in days) whereas with SBCOD alone the

DSVIs increased but not to such very high values (DSVI ≈ 500 mℓ/g) and much more slowly (weeks). These effects could be interchanged on a pair of IA systems by interchanging the RBCOD only and SBCOD only feeds between them. In doing this a number of times with both systems receiving the same mass of COD daily and neither nitrate limited, it was observed that increases in DSVI (which occurred when switching to RBCOD only as feed) were accompanied by increases in effluent nitrate + nitrite concentrations. This seemed unusual because RBCOD is more rapidly degradable and therefore is able to induce a higher denitrification potential than SBCOD (Casey *et al.*, 1993; Ketley *et al.*, 1991).

These observations demonstrate that low F/M filaments can grow on SBCOD only and confirmed the earlier conclusion that the selector effect was unable to control low F/M proliferation; selectors stimulate the preferential uptake of RBCOD by floc–formers and the SBCOD passes through the selectors to the main intermittently aerated reactor. Further it was noted in these experiments that periods of high DSVI were accompanied by corresponding periods of increased effluent NO_3^- concentrations. This seemed to indicate that sludge with large populations of low F/M filaments did not denitrify to the same degree as the well settling sludges.

3.3 In the intermittent aeration (IA) systems fed real or artificial sewage, the aerobic fraction and the nitrate concentration during the anoxic period of the anoxic–aerobic cycle influenced the proliferation of the low F/M filaments (*M.parvicella*, 0092, 1851, 0041 and *H.hydrossis*). The aerobic fraction had a strong influence with proliferation reaching a maximum (highest DSVI 300 to 400 mℓ/g for real sewage) at aerobic mass fractions between 30 to 40%, decreasing as the aerobic mass fraction became greater and smaller to fully aerobic and fully anoxic conditions (DSVI ~80 mℓ/g). At aerobic mass fractions between 30 and 40%, (i) the nitrate + nitrite concentration during the anoxic period had only a mild influence, with proliferation generally increasing as the nitrate concentration increased above 5 mgN/ℓ and (ii) the frequency of alternation between anoxic and aerobic conditions from 72 times per day to once per day did *not* significantly influence the DSVI and therefore low F/M filament proliferation (Warburton *et al.*, 1991; Ketley *et al.*, 1991).

3.4 In the two–reactor anoxic–aerobic (2RND) pre– and post–denitrification

systems fed either real or artificial sewage with aerobic mass fractions from 45% to 30%, the size of the aerobic mass fraction had a significant effect on the level of filament proliferation. With an aerobic mass fraction of 30% and fed artificial substrate the DSVI was high (400 mℓ/g) but with a higher aerobic mass fraction of 45%, the DSVI was considerably lower (100 mℓ/g). For systems with an aerobic mass fraction of 30 or 45% and fed municipal sewage, the DSVI's were low (<150 mℓ/g), values being somewhat lower than expected given the IA system results cited above. With an aerobic mass fraction of 45%, manipulating the a–recycle ratio such that the frequency of exposure to anoxic and aerobic conditions changed from once per day to 15 times per day and dosing nitrate into the anoxic reactor resulting in high concentrations of nitrate in the outflow of the anoxic reactor (>5 mgN/ℓ) did not lead to low F/M filament proliferation (Hulsman *et al.*, 1992).

In repeating the experiments with a pair of the 2RND predenitrification systems fed real sewage, supplementing the influent with ammonium, instead of dosing nitrate into the anoxic reactor, *did* cause low F/M filament proliferation and high DSVIs (>200 mℓ/g); in the control system which did not receive dosed ammonium, the DSVI remained low (<100mℓ/g). These results were reproducible by switching the ammonium dose between the two systems (De Villiers *et al.*, 1993; Casey *et al.*, 1993). This second experiment with 2RND systems is difficult to reconcile with the first because insofar as nitrate loads on the anoxic reactor is concerned, it should make little difference whether the nitrate is dosed directly into the reactor or generated by nitrification provided the frequency of exposure remains unchanged. In both cases, i.e. with nitrate dosing and ammonium supplementation, high concentrations of nitrate + nitrite (> 5mgN/ℓ) were observed in the outflow of the anoxic reactor, yet in the former case low F/M filaments did not proliferate whereas in the latter case they did.

3.5 In the Modified UCT systems fed real sewage it was found possible to manipulate the sludge settleability (DSVI), and hence low F/M filament proliferation (generally 0092 dominant, 0041 and *M.parvicella* secondary and *Thiothrix*, 021N, *H.hydrossis* and 1851 incidental and occasional irrespective of filament abundance and DSVI), between high values (>200 mℓ/g) and low values (<120 mℓ/g) by:

(i) Manipulating the anoxic sludge mass fraction. In a pair of MUCT systems, one with a high anoxic mass fraction (15% anaerobic, 20% first anoxic and 32% second anoxic and 33% aerobic), the DSVI was high (200–250 mℓ/g); in the other, with a low anoxic mass fraction (15% anaerobic, 20% first anoxic and 32+33 = 65% aerobic) the DSVI tended to be low (100–150 mℓ/g). This experiment was prompted by the work on N removal systems mentioned above which indicated that the greater the aerobic mass fraction from 35%, the lower the DSVI and shows that this also applies to N & P removal systems (Casey *et al.*, 1993).

(ii) Manipulating the influent TKN concentration with ammonium dosing to the influent. At low TKN/COD ratio (no ammonium dosing) the concentration of nitrate generated to be denitrified by the anoxic reactors was such that the nitrate and nitrite concentrations leaving the anoxic reactors was very low (< 5mgN/ℓ). At high TKN/COD ratios (with ammonium dosing) complete denitrification in the anoxic reactors (mainly the second anoxic) was no longer possible leading to high concentrations of nitrate and nitrite leaving the anoxic reactor (> 10 mgN/ℓ). With ammonium dosing, the DSVI increased (from 100 to 280 mℓ/g) and without ammonium dosing, the DSVI decreased (from 250 to 170 mℓ/g) (Casey *et al.*, 1993).

(iii) Manipulating the nitrate concentration to be denitrified by dosing nitrate to the second anoxic reactor: In a pair of MUCT systems with very large anoxic mass fraction (15% anaerobic, 20% first anoxic, 45% second anoxic and 20% aerobic) to obtain a low DSVI [see (i) above], one receiving dosed nitrate, the other not, the DSVI increased (from 60 mℓ/g to 160 mℓ/g) from the time nitrate dosing commenced at a rate which caused each of the nitrate and nitrite concentrations in the outflow of the second anoxic reactor to increase from <0,1 mgN/ℓ to >5 mgNO_3^-–N/ℓ and >1 mgNO_2^-–N respectively. In the system with no nitrate dosing, the nitrate and nitrite concentrations in the outflow of the second anoxic reactor were each <0,1 mgN/ℓ and the DSVI remained low (~70 mℓ/g) (Musvoto *et al.*, 1992).

(iv) Manipulating the nitrite concentrations in the outflow of the secondary anoxic reactor: In a pair of MUCT systems with large anoxic sludge mass

fraction (15% anaerobic, 20% first anoxic, 33% second anoxic and 32% aerobic), one receiving ammonium supplemented to the influent, the other not, the DSVI in the former was high (250 mℓ/g) whereas that in the latter was low (<150 mℓ/g). To check whether or not the ammonium supplementation was an indirect way of increasing the nitrite concentration in the outflow of the second anoxic reactor, nitrite was dosed into the second anoxic reactor of the system not receiving ammonium supplementation. Upon commencement of dosing, the DSVI increased sharply from 130 to 220 mℓ/g and upon termination of dosing after 40 days (2 sludge ages), the DSVI sharply decreased to <150 mℓ/g. Without nitrite dosing the nitrite concentration in the second anoxic reactor was low (<1,0 mgN/ℓ) but during dosing the concentration increased to 8 mgN/ℓ while that in the ammonium supplemented system was between 1 and 3 mgN/ℓ (Casey *et al.*, 1993, Musvoto *et al.*, 1992).

In the discussion of the experimental results above, only those relating to the system operating parameters, sludge settleability and filament identification were presented. In the detailed reports cited a range of additional data is given such as COD and N mass balances over the systems, COD, nitrification, denitrification, N removal and biological P removal (where applicable) performances, as well as kinetic rates of nitrate and nitrite denitrification and oxygen utilization calculated from the system data or from ancillary batch tests on sludge harvested from the systems.

4. CONCLUSIONS FROM THE EXPLORATORY INVESTIGATION

From the above experiments on N and N & P removal systems throughout which filaments common to N and N & P removal plants were observed in greater or lesser quantities (i.e. 0092, *M.parvicella*, 0041, *Thiothrix*, 021N, *H.hydrossis* and 1851) it was concluded that a major factor influencing filament proliferation was intermittent aeration, causing the organisms to be alternately exposed to aerobic conditions (where oxygen serves as terminal electron acceptor) and anoxic conditions (where NO_3^- or NO_2^- serve as terminal electron acceptor), provided complete reduction of NO_3^- and denitrification of NO_2^- did not take place. From this two conclusions emerged: (i) That the name low F/M filaments was no longer appropriate and because the conditions for their proliferation appeared to be closely linked to anoxic–aerobic conditions, they were renamed anoxic–aerobic (AA) filaments; (ii) that the cause for the AA filament proliferation lay in the *requirement for the sludge mass to switch between aerobic and anoxic metabolic*

pathways, this switching providing some competitive advantage to the filamentous organisms or disadvantage to the floc–forming organisms. With these conclusions as a basis, attention was focused on facultative heterotrophic denitrification pathways.

5. LITERATURE REVIEW OF DENITRIFICATION PATHWAYS

Payne (1973) proposed the general denitrification pathway

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

nitrate → nitrite → nitric oxide → nitrous oxide → nitrogen gas

Initially denitrification was considered a strictly anoxic process, occurring only in the total absence of oxygen. However, subsequently it has been demonstrated quite convincingly in pure cultures that denitrification can continue under aerobic conditions, albeit at a lower rate [Pichinoty and d'Ornano (1961), Showe and De Moss (1968), Krul and Veeningen (1977), Robertson and Kuenen (1984)]. Pure culture studies have also demonstrated that one or more of the intermediates in the denitrification pathway have an inhibitory effect on the aerobic utilization of substrate with oxygen as terminal electron acceptor. Krul (1976) in pure culture studies on a denitrifying organism isolated from activated sludge, cultured under anoxic conditions and tested under aerobic conditions, concluded that the accumulation of the intermediate nitric oxide (NO) during denitrification caused a measurable and prolonged inhibition of oxygen utilization under subsequent aerobic conditions. Curiously, this inhibition could be demonstrated for a pure culture of an isolate from activated sludge but not for a mixed culture of activated sludge.

Some controversy arose as to whether the inhibitory effect was due to NO_2^- or NO, but recent work has concluded that the inhibitory effect is due to NO and not NO_2^-. However, the degree of inhibition is exacerbated by the presence of NO_2^- and NO_3^- (Kučera *et al.*, 1987; Carr and Ferguson, 1990).

6. HYPOTHESIS (EXPLANATION) FOR AA FILAMENT BULKING

The findings of the literature review together with the experimental results described earlier provided the basis for an explanation for the proliferation of AA filamentous organisms in N and N & P removal systems:

When denitrification is not complete under anoxic conditions, floc–formers are inhibited in their oxygen uptake cytochromes (oxidases) under subsequent

aerobic conditions by the remaining denitrification intermediates accumulated under the anoxic conditions; this inhibition of floc–formers places them at disadvantage for substrate uptake and utilization against the filamentous organisms. The denitrification intermediate causing the inhibition is nitric oxide (NO).

For this hypothesis to be valid requires the AA filaments to denitrify only as far as NO_2^- so that they do not accumulate NO, and the floc–formers to denitrify completely to N_2 gas and thereby accumulate NO under certain conditions. The literature review was regarded as providing sufficient evidence that NO inhibition of oxidases under aerobic conditions is a known phenomenon. Therefore to verify the hypothesis, it was required to be shown that (1) filaments denitrify only to NO_2^- and floc–formers to dinitrogen gas and (2) oxygen uptake inhibition is manifest in bulking activated sludge. Because the first proof requires specialized biochemical and microbiological techniques, this could not be conclusively tested in this project and is a question that would need to be taken up by microbiologists and biochemists in pure culture work. Consequently only indirect evidence for the hypothesis could be collected. This evidence is briefly presented below; for details see Casey *et al.* (1993) and Musvoto *et al.* (1992).

7. SOME EXPERIMENTAL EVIDENCE SUPPORTING THE HYPOTHESIS

7.1 Demonstration of Inhibition

To determine whether or not inhibition of oxygen utilization (and correspondingly substrate utilization) takes place in activated sludge subjected to alternating anoxic aerobic conditions, a series of batch tests was conducted on sludge harvested from the anoxic reactor of the 2RND system operated by de Villiers *et al.* (1993). In these tests the maximum specific OUR was measured (Ekama *et al.*, 1986, Randall *et al.*, 1991) upon sewage addition, with different anoxic or aerobic pretreatment conditions.

7.1.1 *Anoxic denitrification*

Figure 1 shows that inhibition of OUR was induced in the sludge after a 2 hr anoxic period with NO_2^- present during both anoxic and aerobic periods ($\approx$ 25,0 mgN/ℓ at the start of the aerobic period). Inhibition was less marked in a sludge subjected to the same conditions but with less NO_2^- present (5,5 mgN/ℓ). Almost no inhibition was measured in a sludge subjected to the same conditions with only 0,1 mgN/ℓ NO_2^- present. These batch tests show that (1) inhibition of OUR in the presence of

NO_2^- is observed and (2) the degree of inhibition is directly related to the concentration of NO_2^- at the commencement of aerobic conditions. However, it was not clear whether the inhibition results from the NO generated by NO_2^- denitrification under anoxic conditions or under aerobic conditions.

7.1.2 *Aerobic denitrification*

To check whether or not activated sludge from the 2RND system exhibited denitrification of NO_2^- under aerobic conditions, aerobic batch tests were conducted on specially prepared sludge samples. In the preparation, first, virtually all of the NO_3^- and NO_2^- were removed from the sludge by diluting with tap water, settling and decanting the supernatant 3 successive times. Then the sludge was held anoxic in the presence of about 120 mgCOD/ℓ sewage in order to denitrify any remaining NO that might be present in the organisms. After 2 h, during which thiourea was added (10 mg/ℓ) to inhibit NO_2^- formation by *Nitrosomonas*, aeration was commenced ($2{,}0 < DO < 4{,}0$ mgO/ℓ). After 1 h aeration, 20 mgNO_2^-–N/ℓ batch volume was added. After a further 1 h aeration, 360 mgCOD/ℓ (final batch volume) sewage was added and the OUR, NO_3^- and NO_2^- concentrations measured with time. Figure 2 shows that OUR inhibition is exhibited. In a similar test but with NO_3^- addition (20 mgN/ℓ) instead of NO_2^-, no inhibition is exhibited. These tests seem to indicate that NO inhibition under aerobic conditions does take place with NO_2^-, (the NO apparently produced by aerobic denitrification of NO_2^-) but not with NO_3^-. In a control batch test, in which no NO_2^- or NO_3^- was added, no inhibition was exhibited. The results of these batch tests were reproducible with sludges from intermittently aerated N and Modified UCT N & P removal systems.

In the batch tests presented so far, it appears that during the aerobic period after sewage addition the inhibition is relieved, reflected in a steadily increasing maximum specific OUR, in some cases leveling off at a constant value before the precipitous decrease in OUR when the RBCOD has been depleted. The relief of OUR inhibition possibly arises because the presence of significant quantities of RBCOD under aerobic conditions accelerates the $NO \rightarrow N_2O \rightarrow N_2$ part of the denitrification pathway so that the NO produced from NO_2^- denitrification does not accumulate.

7.1.3 *Effect of RBCOD on OUR inhibition by NO*

To check if OUR inhibition takes place in the presence of significant quantities of RBCOD, an aerobic batch test was conducted in which NO_2^- was added after the

sewage addition but while RBCOD was still present, rather than before sewage addition when only SBCOD (principally generated from organism death and lysis) is present as in the previous batch experiments. In this test no inhibition was noted, and it was concluded that the presence of RBCOD (in sufficient quantity) prevented or relieved the inhibition. From this it seemed reasonable to accept the suggestion above that the RBCOD accelerates the NO $\rightarrow$ N_2 steps of the pathway in such a way that NO no longer is accumulated, is reasonable.

7.2 Determination of the extent of NO_3^- reduction and denitrification under anoxic conditions by filaments and floc–formers

With the experiments above, it was demonstrated that OUR inhibition, hypothesized to be by NO, takes place in the presence of NO_2^- in switching from anoxic to aerobic conditions. For the proposed explanation to be acceptable, it needed to be shown even superficially that floc–formers denitrify from NO_3^- to N_2 gas, and so are susceptible to OUR inhibition by accumulated NO, whereas the low F/M filaments reduce NO_3^- to NO_2^- only, and therefore do not accumulate NO and so are not susceptible to this inhibition. Clearly this is an experiment that needs to be taken up by microbiologists and biochemists, but for the purposes of testing the hypothesis, sludge samples from a fully anoxic *(FX)* system (low DSVI) and the 2RND system on which the batch tests above were done (high DSVI), both fed real sewage, were subjected to a nitrate reduction test, a test which allows the generation of NO_2^- and/or N_2 gas to be determined. The sample with the high DSVI (many low F/M filaments) showed an accumulation of NO_2^- with no N_2 gas being detected in 8 out of 10 tests. The sample with the low DSVI (few low F/M filaments) accumulated N_2 gas, but no NO_2^- accumulated in 8 out of 10 tests. From this it is reasonable to accept that qualitatively, filaments tend to reduce NO_3^- to NO_2^- only, whereas floc–formers denitrify NO_3^- to N_2 gas. This observation lends credibility to the proposed hypothesis for low F/M filament proliferation. With a reasonable hypothesis for low F/M filament proliferation in N and N & P removal systems, attention was directed at devising strategies for the control of these filaments in the systems.

7.3 The effect of incomplete denitrification – an example in a biological N & P removal system (ex Musvoto *et al.*, 1992)

7.3.1 *Experimental Set–up*

Two identical Modified UCT systems (MUCT 1 and MUCT 2) for biological N and P removal were set up. Their anoxic mass fraction was very large at 65% (1st

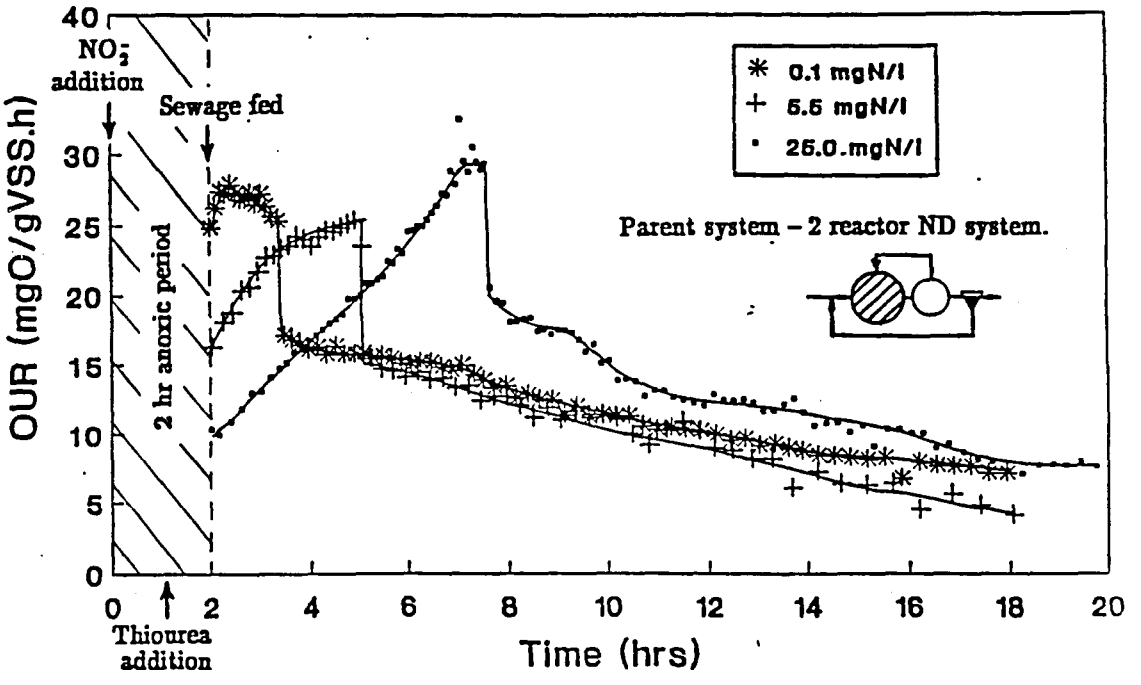


Fig 1: *Oxygen utilization rate [OUR, in mgO/(gVSS.h)] with time under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2 reactor ND system with a 2hr anoxic period prior to the aerobic test and with varying nitrite concentrations at the start of the aerobic test, demonstrating the initial but gradually declining inhibitory effect of NO_2^- on maximum specific OUR (*0,1, + 5,5 and · 25 NO_2^--N/ℓ).*

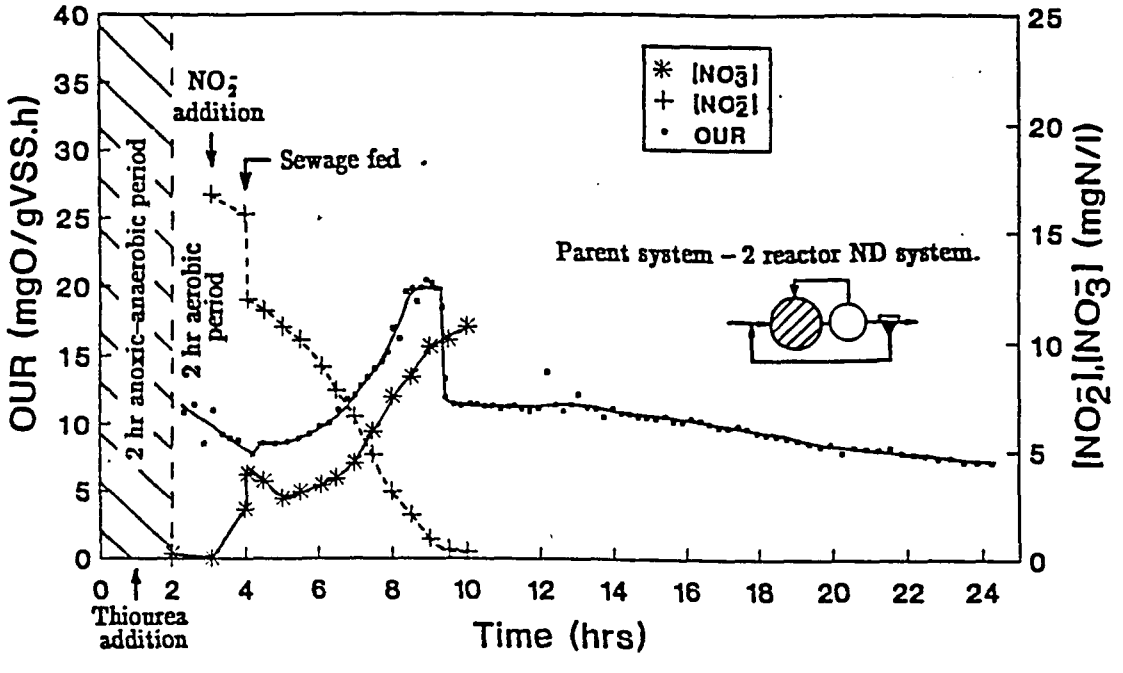


Fig 2: *Oxygen utilization rate [· OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (+NO_2^- and *NO_3^-, in mgN/ℓ) with time under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2 reactor ND system with a 2hr anoxic–anaerobic period prior to a 2hr aerobic period during which NO_2^- was added (20 mgN/ℓ), prior to the aerobic test.*

anoxic 20%, 2nd anoxic 45%) to ensure even at high TKN/COD ratios (up to 0,11 mgN/mgCOD) complete denitrification in the second anoxic reactor could be achieved at a fixed mixed liquor (aerobic to 2nd anoxic) recycle ratio of 3:1 and, as a result, produce a nitrate and nitrite free inflow to the aerobic reactor. Both systems were started up with an AA (low F/M) bulking sludge from other laboratory MUCT systems containing typical filaments for these systems, i.e. 0092, 0041, *M.parvicella* and some *H.hydrossis*. Both were operated identically at 20 days sludge age and each fed 10ℓ/d of the same Mitchell's Plain (Cape) unsettled real sewage diluted with tap water to a concentration of 1000 mgCOD/ℓ. The MUCT 1 system was operated for 340 days; for 111 days from day 129 to 240, *nitrate* was dosed into the 2nd anoxic reactor at a rate of 720 mgNO_3–N/d (72 mgN/ℓ influent). The MUCT 2 system was operated for 169 days from day 171 to day 340; for 50 days from day 290 to 340 *nitrite* was dosed into the second anoxic reactor at a rate of 900 mgNO_2–N/d (90 mgN/ℓ influent). The two systems were monitored almost daily for influent and effluent COD, TKN, nitrate, nitrite and total P concentrations, individual reactor nitrate, nitrite and total P concentrations, aerobic reactor MLSS and MLVSS concentrations, oxygen utilization rate and sludge settleability in terms of DSVI. Filament identifications were done every 3 to 4 weeks. Regular batch tests were conducted on sludge harvested from the systems to measure the nitrate and nitrite denitrification rates so that the nitrate load on the anoxic reactors could be compared with their denitrification potential.

7.3.2 *Experimental Results*

With nitrate dosing. *In the MUCT 1 system before nitrate dosing, the DSVI decreased from a start up value of 164 to 80 mℓ/g* in 128 days. The nitrate and nitrite concentrations in the outflow of the second anoxic reactor were very low i.e. <0,5 mgNO_3–N/ℓ and <0,2 mgNO_2–N/ℓ (Fig 3). The filaments were of the AA (low F/M) type i.e. 0092 and 0914 dominant with 0041, *M.parvicella*, *H.hydrossis* and 0803 present (Table 1). After commencement of nitrate dosing the DSVI increased slowly reaching 176 mℓ/g in 111 days. The nitrate and nitrite concentrations in the outflow of the second anoxic reactor increased to between 2 and 10 mgNO_3–N/ℓ and between 1,5 and 3 mgNO_2–N/ℓ respectively. The increase in nitrite concentration with nitrate dosing is acceptable because in this investigation as well as in those of Clayton *et al.* (1989) and Stern and Marais (1974), it was found that nitrite was formed at a slow rate of 0,060 mgNO_2–N/(mgAVSS.d) while nitrate was being denitrified, and that only when the nitrate concentration reaches low values (<1 mgNO_3–N/ℓ) did nett nitrite removal

commence. The same filaments as earlier were identified to be dominant and present. After cessation of nitrate dosing the DSVI declined from 176 to 91 mℓ/g in 69 days. A few days after cessation of nitrate dosing, the nitrate and nitrite concentrations in the outflow of the second anoxic reactor decreased to similarly low values (<0,5 mgNO_3–N/ℓ and <0,2 mgNO_2–N/ℓ respectively) as observed earlier before nitrate dosing. The same low F/M filaments as earlier were identified as dominant and present.

With nitrite dosing. In the MUCT 2 system before nitrite dosing, the DSVI decreased slowly from a start–up value of 131 to 90 mℓ/g in 118 days (Fig 4). The concentrations of nitrate and nitrite in the outflow of the second anoxic reactor were very low <0,2 mgNO_2–N/ℓ and <0,7 mgNO_3–N/ℓ. The dominant filaments were of the AA (low F/M) type i.e. 0092 dominant with *M.parvicella*, 0041 and *H.hydrossis* present (Table 2). From commencement of nitrite dosing the DSVI initially increased sharply from 90 to 116 mℓ/g in 11 days and thereafter more slowly, reaching 174 mℓ/g, 39 days later. The nitrite concentration in the outflow of the second anoxic reactor increased to between 10 and 20 mgNO_2–N/ℓ. The same filaments as earlier were identified to be dominant and present. The effect of withdrawing the nitrite dose was not investigated.

7.3.3 *Other Observations*

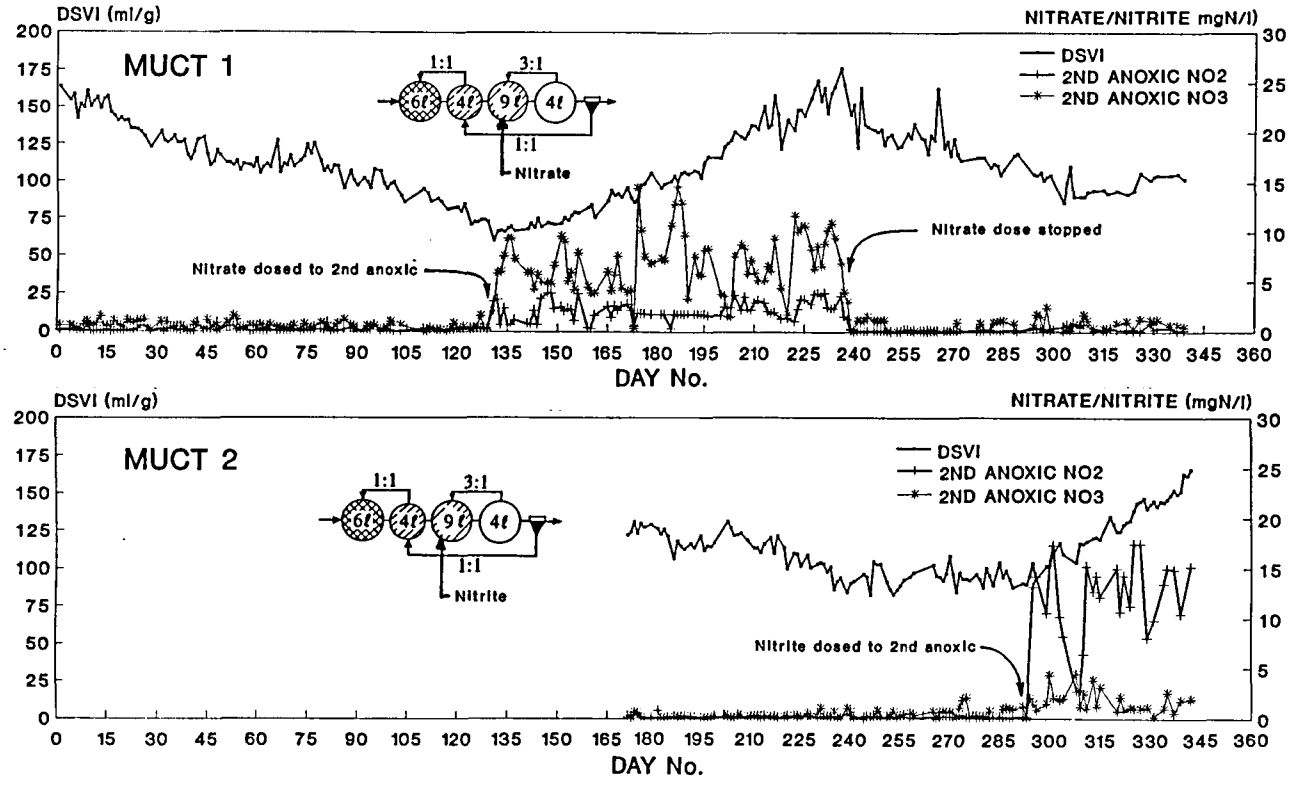
VSS mass. The two systems produced between 15 to 30% more VSS than expected in terms of the steady state design model of Wentzel *et al.* (1990) and WRC (1984). The VSS mass in the system was found to be dependent on the DSVI (or bulking); initially the VSS increased as the DSVI decreased in the absence of nitrate or nitrite dosing, then decreased as the DSVI increased with dosing and then increased again as the DSVI decreased upon withdrawal of the dosing. Although not so strongly connected to AA filament bulking as in this investigation, Clayton *et al.* (1989) also observed significantly increased sludge mass in MUCT systems compared to single or 2–reactor N removal systems at the same sludge age receiving the same wastewater and daily COD mass load (Warburton *et al.*, 1991).

Denitrification rates. The nitrate and nitrite denitrification rates observed were 0,296 mgNO_3–N/(mgAVSS.d) and 0,247 mgNO_2–N/(mgAVSS.d); the former is 24% higher than that observed by Clayton *et al.* (1989) and about 3 times higher than the second rate of denitrification (K_2) in N removal systems; due to an absence of earlier determinations, the nitrite rate could not be compared. In batch tests with

MUCT1 FILAMENT IDENTIFICATION					
DAY No.	DSVI	DOMINANT FILAMENT	SECONDARY FILAMENT	OTHER FILAMENTS PRESENT	RELATIVE AMOUNT OF FILAMENT
61	105	0092	021N	0041 *M.parvicella* *H.hydrossis*	Common to V.common
119	82	0092	021N	*H.hydrossis* *M.parvicella* 0041	V.common
181	96	0914	0092 Beggiatoa	*M.parvicella* 0041 *H.hydrossis* *Flexibacter*	Common
202	126	0092	*M.parvicella*	0803 0041 *H.hydrossis*	Abundant
237	165	0092	0041	*M.parvicella* 0803 O21N	Common
270	129	0092	021N	0803 0041 *M.parvicella*	V.common
308	91	0092	021N	*M.parvicella* 0041, 0675 *H.hydrossis* *Thiothrix* sp.	Common to v.common

Table 1: Filament identifications and DSVI for MUCT 1 and MUCT 2 systems during the investigation. Both systems operated at 20 days sludge age and 20° C.

MUCT2 FILAMENT IDENTIFICATION					
DAY No.	DSVI	DOMINANT FILAMENT	SECONDARY FILAMENT	OTHER FILAMENTS PRESENT	RELATIVE AMOUNT OF FILAMENT
181	122	0092	021N	*M.parvicella* 0041 *H.hydrossis*	Common
202	127	0092	0041	021N *H.hydrossis*	V.common
237	94	0092	0041	*H.hydrossis* *M.parvicella*	Common
270	84	0092	*H.hydrossis*	*M.parvicella* 0041 O21N	Common to V.common
308	116	0092	021N 0675	*M.parvicella*	V.common to abundant



Figs 3 and 4: Sludge settleability (in DSVI, mℓ/g) and nitrate and nitrite concentration in the 2nd anoxic reactor for MUCT 1 (Fig 1, top) and MUCT 2 (Fig 2, bottom). Note the increase in these parameters upon commencement of nitrate dosing to MUCT 1 on day 129 and nitrite dosing to MUCT 2 on day 291. Also the decrease in these parameters in MUCT 1 upon cessation of nitrate dosing to MUCT 1.

high nitrate and nitrite concentrations (>10 mgN/ℓ), nett nitrite denitrification commenced only when the nitrate concentration reached low concentrations (<1 $mgNO_3$–N/ℓ); at high nitrate concentrations, the nitrite concentration increased at a rate of about 0,060 $mgNO_2$–N/(mgAVSS.d).

8. A PROPOSED STRATEGY FOR LOW F/M FILAMENT BULKING CONTROL IN N AND N & P REMOVAL SYSTEMS

In the batch tests described above (Figs 1–2), not only was OUR inhibition demonstrated but also it appeared that, in the presence of RBCOD the inhibition was progressively relieved. In the parent N and N & P removal system from which the sludges were harvested, this relief probably does not take place because RBCOD is not available in sufficient quantity upon entry into the aerobic reactor. If a system modification could be devised in which RBCOD is available (in sufficient quantity) at the end of the anoxic reactor to reduce the accumulated NO in the floc–formers, then the floc–formers would enter the aerobic reactor in a more competitive condition against the low F/M filaments. This proposal was implemented by installing a small (containing 4% of system sludge mass) completely mixed reactor between the anoxic and aerobic zones of the MUCT and 2RND systems (De Villiers *et al.*, 1993; Casey *et al.*, 1993). These reactors, called Denox for convenience, received influent RBCOD by diverting 10% of the daily influent flow to them. Although from the batch tests it would appear logical to have made these reactors aerobic, it was decided to make them anoxic because it was thought that this would deplete the accumulated NO more rapidly.

With a pair of MUCT systems with 52,5% anoxic mass fraction (15% anaerobic) after installing a Denox reactor on one of the systems, the DSVI decreased from 150 mℓ/g to 100 mℓ/g in 30 days while the DSVI in the control MUCT system (which received supplemented NH_4^+) increased from 180 to 230 mℓ/g. Transferring the Denox reactor to the control system, caused the DSVI to decline from 230 to 120 mℓ/g in 25 days while that in the experimental system increased from 100 to 170 mℓ/g. Curiously, after this the DSVI of the control system (with the Denox reactor) began to increase slowly and after about 30 days the DSVI of both systems was around 140 to 180 mℓ/g. During this last period similarly high concentrations of nitrite (> 1 mgN/ℓ) were noted in the Denox reactor as in the 2nd anoxic reactor of the experimental system so for some unknown reason the Denox reactor was not removing the nitrite, and by implication the nitric oxide. After installing the Denox reactor in the 2RND system, the DSVI decreased from 280 mℓ/g to 130 mℓ/g in

under 30 days but afterwards showed the same inability to remove the nitrite as was observed in the MUCT system, leading to increasing DSVIs. From the above preliminary results, it appears that the strategy holds promise, but more research needs to be done to confirm and establish credibility for the strategy.

9. CONCLUSIONS AND RECOMMENDATIONS

From laboratory experimental investigations conducted under the previous research contract, it was concluded that selector reactors, whether aerobic or anoxic by kinetic selection or anaerobic by metabolic selection, were unable to control the AA filament proliferation and therefore were an unfruitful control strategy to adopt for this kind of bulking. This finding required the selector effect approach to be set aside and consequently placed the research back into an exploratory phase to seek and establish a new direction and framework for research in this area. The establishment of a new direction of research was the principal objective of this research contract.

From the observations of an extensive laboratory research investigation seeking to determine causes for and establish control strategies against low F/M filament proliferation in which fully aerobic, fully anoxic and intermittent aeration single reactor N removal systems, 2 reactor anoxic aerobic ND systems and MUCT N & P removal systems were operated under a wide range of conditions. It was concluded that a major factor influencing low F/M filament bulking was the continuous alternation between anoxic and aerobic conditions in the systems, this alternation inducing a competitive disadvantage to the floc–formers against the AA filaments. From an examination of the experimental data and a survey of denitrification pathways in the microbiological and biochemical literature, it was hypothesized that this advantage arises because the filaments reduce NO_3^- to NO_2^- whereas floc–formers reduce NO_3^- to N_2 and in the process accumulate NO which exerts an inhibitory effect on their aerobic oxygen utilization rate (OUR) in the subsequent aerobic conditions. While the hypothesis could not be directly verified because this would fall into specialized microbiological and biochemical experimentation, indirect confirmation of the hypothesis was obtained from 4 sources:

1) Batch tests on sludge harvested from intermittent aeration and 2 reactor ND and MUCT N & P removal systems showed inhibition of aerobic OUR;

2) Nitrate reduction tests on activated sludge samples with high and low DSVI

(many and few low F/M filaments respectively) indicated that in the former, NO_2^- tended to accumulate whereas in the latter N_2 gas accumulated;

3) Two Modified UCT systems operated to have high or low nitrate and nitrite concentrations in the influent to the aerobic reactor showing high and low DSVIs respectively; and

4) Most of the experimental data observed in the exploratory investigation.

These results provide strong evidence that the proposed hypothesis for the cause of AA filament bulking in N and N & P removal systems has merit.

From the proposed hypothesis, two control strategies were devised and implemented on laboratory scale 2 reactor ND and MUCT N & P removal systems. The first, which comprised installing a small denitrifying (Denox) reactor between the anoxic and aerobic reactors initially appeared to be successful in that after installation it promoted low DSVIs in the systems, but was unable to consistently maintain low DSVIs in N and N & P removal systems, indicating that further experimental testing and verification is required. The second, which comprised designing and operating a Modified UCT system such that low nitrate and nitrite concentrations (< 2 mgNO_3–N/ℓ and $< 0{,}5$ mgNO_2–N/ℓ) from the 2nd anoxic to the aerobic reactor was maintained, showed excellent promise because it cured as well as controlled the AA filament proliferation (DSVI < 80 mℓ/g).

Although the experimental investigations conducted in this research contract have produced a deeper understanding of the AA filament behaviour in N and N & P removal systems and have given some interesting insights into the problem, more research is required before AA filament proliferation can be effectively controlled in full scale N and N & P removal plants. This research is being conducted in a follow–up 3 year research contract (1993–1995), the objects of which are:

1) Devise tests and refine specific AA bulking control strategies on laboratory–scale biological N and N & P removal systems that flow from the present understanding of the proposed cause of AA filament bulking.

2) Confirm the hypothesis for the cause of AA filament bulking by examining the experimental data collected to date and conducting further experiments. For

example, operate MUCT systems at low temperatures (13°C) to inhibit nitrification and evaluate the effect of this on AA filament bulking. This will help elucidate the often observed temperature dependence of sludge settleability and bulking (possibly via the temperature dependence of nitrification and denitrification) and provide valuable kinetic information on nitrification, denitrification (by dosing nitrate and/or nitrite to the systems) and biological excess P removal.

3) To develop and design AA bulking control methods for full-scale implementation, and integrate these methods into the design procedures of biological N and N & P removal systems.

4) Write a series of publications and present a number of seminars and talks to disseminate the information so that it can be adopted by plant operators and designers.

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GLOSSARY

DEFINITION OF TERMS

AA	Anoxic–aerobic; new group name for most of the low F/M filamentous organisms
Arch.Microbiol.	Archives of Microbiology – a journal
AVSS	Active Volatile Suspended Solids. The volatile suspended solids (VSS) comprise active organisms and inert organic mass. The active organism mass is the live biological mass which performs the biological reactions; the inert mass originates from two sources (i) from inert organic material in the influent and (ii) endogenous residue. The active fraction of the VSS is a function of the sludge age of the system and sewage characteristics. It is an empirical estimate that has found acceptability because of the consistency it brings to kinetic rates observed in activated sludge systems, e.g. based on active mass the specific endogenous mass/respiration rate and specific denitrification rates are constant with sludge age from 3 to 72 days. Because of this consistency, the readily biodegradable COD (RBCOD) uptake rates also are reduced to specific rates with respect to AVSS so that rates in different sludge age systems can be compared. For details on calculation of the AVSS, see Marais and Ekama, 1976 and Ekama, Dold and Marais, 1986.
Bacteriol.Rev.	Bacteriological Reviews – an annual review journal in Bacteriology
Biochim.Biophys.Acta	Biochimica Biophysica Acta – one of the major biochemical/microbiological journals
COD	Chemical oxygen demand
DO	Dissolved oxygen
DSVI	Diluted sludge volume index, a modified SVI sludge settleability test [see Ekama and Marais (1984). Two improved sludge settleability parameters, IMIESA, 9, 6, 20–25 for method]
d	day
FA	fully aerobic; an activated sludge system with all its sludge under aerobic conditions
FX	fully anoxic; an activated sludge system not aerated at all, but receiving dosed nitrate to create anoxic conditions
H.hydrossis	*Haliscomenobacter hydrossis*, one of the filamentous organisms in the AA (low F/M) group
h	hour

IA	intermittent aeration; a single reactor activated sludge system with aeration on for a specified time to create aerobic condition and aeration off for the remaining time to create anoxic (and possibly anaerobic) conditions
g	gram
J.Appl.Bact.	Journal of Applied Bacteriology
J.Bacteriol.	Journal of Bacteriology
low F/M	low food to micro–organism ratio; equivalent to low load factor, or low loading rate or long sludge age
ℓ	litre, the unit measure for volume
MUCT	Modified University of Cape Town system for biological removal of nitrogen and phosphorus
M. parvicella	*Microthrix parvicella;* one of the filamentous organisms in the AA (low F/M) group
MLVSS	mixed liquor volatile suspended solids; same as VSS, the organic part of the suspended solids in activated sludge plants
MLSS	mixed liquor suspended solids; the organic and inorganic suspended solids in activated sludge plants, also referred to as Total Suspended Solids
N	nitrogen; all nitrogen concentrations i.e. nitrate, nitrite or TKN are expressed as mgN/ℓ
NO_3–N; NO_2–N	nitrate and nitrite respectively as N
NO; N_2O	nitric oxide and nitrous oxide respectively, the two gaseous denitrification intermediates between NO_2 and N_2
N_2	dinitrogen gas, the end product of denitrification
N & P	nitrogen and phosphorus; applied to activated sludge plants incorporating simultaneous biological N and P removal
OUR	oxygen utilization rate; mass oxygen utilized per unit reactor volume per unit time, or per unit VSS mass per unit time, e.g. mgO/(gVSS.h)
P	phosphorus; all phosphorus concentrations are total phosphorus concentrations and expressed as mgP/ℓ
RBCOD	readily biodegradable COD component of the influent COD
SBCOD	slowly biodegradable COD component of the influent COD
TKN	Total Kjeldahl nitrogen

TKN/COD	ratio of the influent TKN and COD concentrations – a useful term comparing the quantity of nitrate that is going to be generated by nitrification from the influent TKN (called nitrification capacity) with the quantity of organic material (influent COD) available for denitrification
UCT	University of Cape Town activated sludge system for biological removal of nitrogen and phosphorus
Wat.Pollut.Control	Journal of the erstwhile Institute for Water Pollution Control, now Institute for Water and Environmental Management (IWEM)
Wat.Sci.Tech.	Water, Science and Technology, a journal of the International Association for Water quality (IAWQ)
WRC	Water Research Commission, a water research co–ordination and funding agency in south Africa. Executive Director Mr P E Odendaal, P O Box 824, Pretoria, 0001
0092, 0041 0914, 0803 1851, 021N	Six different filamentous organism types of activated sludge, the first 5 common in biological N & P and N removal plants and therefore sorting into the AA (low F/M) group, the last arising mainly with septic wastewaters (i.e. high sulphides) as happens occasionally in the UCT Water Research Laboratory with refrigeration breakdowns
2RND	Two reactor nitrification denitrification activated sludge systems, i.e. ones wherein the aerobic and anoxic zones are in 2 separated reactors either with anoxic reactor first followed by the aerobic reactor (i.e. predenitrification or primary anoxic reactor) or with aerobic reactor first, followed by t he anoxic reactor (i.e. post–denitrification or secondary anoxic reactor)

Table 2: Summary of experimental work from Bulking Contract period 1989–1992.

System Type	Aerobic Mass Fraction	DO Conc (mgO/l)	Cycles /d	NH_4^+ Addition to Influent	NO_3^-/NO_2^- Dosing	Sludge Age (d)	Substrate Type	Steady-State DSVI (ml/g)	Dominant Filament	Selector Reactor	Reference
	30 -40	< 2,0	72	_	NO_3^-	15	Artificial	400-600	*H. hydrossis*	_	Casey *et al.*(W83)
	"	< 2,0	72	_	NO_3^-	15	Artificial SBCOD	500	*H. hydrossis*	_	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	>1000	*H. hydrossis*	_	"
	"	0,2<DO<0,5	72	_	_	15	Municipal	160	Type 0092	_	"
	"	0,2<DO<1,0	72	_	_	15	Municipal	180	Type 0092	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal	250	Type 0041	_	"
	"	0,2<DO<3,0	72	_	_	15	Municipal	280	Type 021N	_	"
	"	0,2<DO<1,0	72	_	NO_3^-	15	Municipal	180	Type 0041	_	"
	"	0,2<DO<1,0	72	_	NO_3^-+NO_2^-	15	Municipal	200	*M. parvicella*	_	"
	"	0,2<DO<3,0	72	_	NO_3^-+NO_2^-	15	Municipal	200	*M. parvicella*	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal RBCOD	240/400	Type 021N/ *H. hydrossis*	_	"
	"	0,2<DO<2,0	72	_	_	15	Municipal SBCOD	240/400	Type 021N/ *M. parvicella*	_	"
	"	0,2<DO<2,0	72	_	NO_3^-	15	Municipal RBCOD in anoxic	300	*H. hydrossis*	_	"
N Removal	"	< 2,0	72	_	NO_3^-	15	Municipal RBCOD in aerobic	<100	Type 0041	_	"
IAND Systems	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	<250	*H. hydrossis*	2 aerobic	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	400	Type 1701	2 anoxic	"
	"	< 2,0	72	_	NO_3^-	15	Artificial RBCOD	400	Type 1701	3 anoxic	"
	"	< 2,0	72	_	NO_3^-	20	Municipal	>400	Type 0092	_	Warburton *et al.*(W65)
	"	< 2,0	72	_	NO_3^-	15	Municipal	>400	Type 0092	_	"
	"	< 2,0	72	_	NO_3^-	10	Municipal	>200	Type 0092	_	"
	"	< 2,0	72	_	_	10	Municipal	>200	Type 0092	_	"
	"	> 2,0	48	_	_	15	Artificial	80	Type 0092/ *Thiothrix sp.*	_	Ketley *et al.*(W68)
	"	> 2,0	48	_	NO_3^-	15	Municipal	400	*M. parvicella* Type 0803	_	"
	"	> 2,0	3	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	2	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	1	_	NO_3^-	15	Municipal	400		_	"
	"	> 2,0	0,33	_	NO_3^-	15	Municipal	400		_	"
	"	1,0<DO<3,0	3	_	_	15	Municipal	190	Type 0041	_	De Villiers *et al.*(W81)
	"	1.0<DO<3,0	3	+	_	15	Municipal	220	Type 0041	_	"

Table 2: Summary of experimental work from Bulking Contract period 1989–1992.

System Type	Aerobic Mass Fraction (%)	DO Conc (mgO/l)	Cycles /d	NH_4^+ Addition to Influent	NO_3^-/NO_2^- Dosing	Sludge Age (d)	Substrate Type	Steady-State DSVI (ml/g)	Dominant Filament	Selector Reactor	Reference
N Removal IAND Systems cont'd	30-40	< 2,0	144	–	NO_3^-	10	Municipal	>400	*M. parvicella*	–	Warburton *et al.* (W65)
	70-80	< 2,0	144	–	NO_3^-	10	Municipal	<200	*M. parvicella*	–	"
	30-40	< 2,0	144	–	NO_3^-	10	Municipal	300-600	*M. parvicella*	–	"
	30-40	< 2,0	144	–	NO_3^-	8	Municipal	300-400	*M. parvicella*	–	"
	30-40	< 2,0	144	–	NO_3^-	7,5	Municipal	150	*M.parvicella H. hydrossis*	–	"
	30-40	< 2,0	144	–	NO_3^-	6	Municipal	250	*H. hydrossis*	–	"
	30-40	< 2,0	144	–	NO_3^-	5	Municipal	<200	*H. hydrossis*	–	"
Aerobic	100	> 2,0	–	–	NO_3^-	15	Artificial	<100	Type 0041	–	Casey *et al.* (W83)
	100	0,2<DO<0,5	–	–	–	15	Municipal	<100	*H. hydrossis*	–	"
	100	0,2<DO<2,0	–	–	–	15	Municipal	<100	*H. hydrossis*	–	"
	100	3,0<DO	–	–	–	15	Municipal	<100	Type 021N	–	"
Anoxic	Nil	–	–	–	NO_3^-	15	Municipal	80-130	Type 0092	–	Ketley *et al.* (W68)
	Nil	–	–	–	NO_2^-	15	Municipal	80	Type 0092	–	Casey *et al.* (W83)
	Nil	–	–	–	NO_3^-	15	Municipal SBCOD	130	Type 0092	–	"
	Nil	–	–	–	NO_3^-	15	Artificial	200	*H. hydrossis*	–	"
N Removal 2RND Systems	30	2,0<DO	–	–	NO_3^-	15	Artificial	400	Type 1851	–	Hulsman *et al.* (W73)
	45	2,0<DO<4,0	–	–	NO_3^-	15	Artificial	100	*H. hydrossis*/ Type1851	–	"
	45	2,0<DO<4,0	–	–	NO_3^-	15	Municipal	100	Type 0092/ *H. hydrossis*	–	"
	30	2,0<DO<4,0	–	–	NO_3^-	15	Municipal	150	Type 021N	–	"
	33	1,0<DO<3,0	–	–	–	15	Municipal	<200	Type 0803	–	De Villiers *et al.* (W81)
	33	1,0<DO<3,0	–	+	–	15	Municipal	370	Type 0041	–	"
	45	2,0<DO<4,0	–	–	NO_3^-	15	Municipal	<150	Type 1851	–	Hulsman *et al.* (W73)
	45	2,0<DO<4,0	–	–	NO_3^-	15	Municipal	100	*H. hydrossis*/ Type 0041	–	"
N & P Removal MUCT Systems	33	DO>2,0	–	–	–	20	Municipal	>200	Type 0092	–	Casey *et al.* (W83)
	65	DO>2,0	–	–	–	20	Municipal	<150	Type 0092	–	"
	33	DO>2,0	–	–	–	20	Municipal	150	Type 0092	–	"
	33	DO>2,0	–	+	–	20	Municipal	250-280	Type 0092	–	"
	33	DO>2,0	–	–	NO_2^-	20	Municipal	220	Type 0092	–	"
	20	2,5<DO<4,0	–	–	–	20	Municipal	70-100	Type 0092	–	Musvoto *et al.* (W77)
	20	2,5<DO<4,0	–	–	NO_3^-	20	Municipal	160	Type 0092	–	"
	20	2,5<DO<4,0	–	–	NO_2^-	20	Municipal	>160	Type 0092	–	"