Filamentous organism bulking in nutrient removal activated sludge systems

Paper 7: Exploratory experimental investigations

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Abstract

This paper describes an experimental investigation into the causes of proliferation of low F/M filaments in activated sludge system configurations which expose the mixed liquor to alternating anoxic-aerobic conditions. Using laboratory-scale anoxic-aerobic systems fed defined artificial substrate or municipal sewage, a combination of conditions is identified which results in low F/M filament proliferation; i.e. exposure of sludge to alternating anoxic-aerobic conditions with slowly biodegradable COD (SBCOD) present under both anoxic and aerobic conditions, with an aerobic mass fraction of 30 to 40% of the total, and nitrite (NO_2^-) present at a concentration greater than about 1.0 mgN/ℓ when conditions change from anoxic to aerobic.

List of symbols

a	=	anoxic-aerobic recycle ratio
AOOs	=	ammonia oxidising organisms
COD	=	chemical oxygen demand
DO	=	dissolved oxygen
DSVI	=	diluted sludge volume index
E_h	=	reduction-oxidation (redox) potential
F/M	=	food to micro-organism ratio
FID	=	filament identification
IAND	=	intermittently aerated nitrification-denitrification
IAWQ	=	International Association for Water Quality
MLE	=	modified Ludzack-Ettinger
MLSS	=	mixed liquor suspended solids
MUCT	=	modified University of Cape Town
mV	=	millivolts
NDBEPR	=	nitrification-denitrification biological excess phosphorus removal
NO_x^-	=	combined nitrate and nitrite concentration
NOOs	=	nitrite oxidising organisms
RBCOD	=	readily biodegradable COD
s	=	underflow recycle ratio (Q_r/Q_i)
SBCOD	=	slowly biodegradable COD
t	=	time
TKN	=	total Kjeldahl nitrogen
UCT	=	University of Cape Town
VSS	=	volatile suspended solids

Introduction

In the majority of countries in which sludge settleability in activated sludge systems is sufficiently poor to affect plant performance, application of selector reactor technology has become the promoted method for control of filament proliferation (Pujol and Canler, 1994; Kruit et al., 1994; Eikelboom, 1994). However, in the preceding papers in this series, it was concluded that induction of the selector effect, either through the application of selector reactors (whether anaerobic, anoxic or aerobic) or via intermittent feeding (sequencing batch reactors) does not control low F/M filaments in intermittent aeration systems (such as Carousel and Orbal type plants) for biological nitrogen (N) removal, or in multi-reactor anaerobic-anoxic-aerobic systems (such as UCT and Bardenpho systems) for biological N and phosphorus (P) removal. These findings placed research in the field back into an exploratory stage and consequently a new research direction needed to be established. In this regard a wide ranging experimental programme was initiated, the main emphasis of which was to investigate aspects identified by Ekama et al. (1996a) as possibly having an influence on proliferation of low F/M filamentous organisms. These aspects were examined and are described in this paper under six sections as follows:

- RBCOD or SBCOD as influent substrate.
- RBCOD as substrate under aerobic and anoxic conditions.
- Continuous aerobic and continuous anoxic conditions.
- Magnitude of the aerobic mass fraction in anoxic-aerobic systems.
- Sludge age.
- Differences between alternating anoxic-aerobic nitrification-denitrification (ND) conditions caused by intermittent aeration in a single reactor (IAND) configuration, or by separate anoxic-aerobic reactors in a 2-reactor (2RND) configuration, i.e.:
  - frequency of alternation between anoxic and aerobic conditions;
  - availability of the RBCOD and SBCOD fractions of the influent under aerobic and anoxic conditions;
  - DO concentration in the aerobic period or reactor;
  - NO_3^-/NO_2^- concentrations in the anoxic period or reactor.

This paper summarises the key features of the experimental investigation into the six aspects listed above. For a detailed description of the research the reader is referred to Casey et al. (1994a, b); Hulsman et al. (1992); Ketley et al. (1991); Warburton et al. (1991); De Villiers et al. (1994).

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Experimental investigation

The objective of the investigation was to experimentally determine the influence of each of the aspects listed above on low F/M filamentous organism proliferation. This was achieved through the operation of laboratory-scale anoxic-aerobic activated sludge systems, using three types of system configuration:

- Single-reactor, either continuously aerobic, continuously anoxic or intermittently aerated anoxic-aerobic nitrification-denitrification (IAND).
- Two-reactor anoxic-aerobic, nitrification-denitrification (2RND), either pre-denitrification (modified Ludzack-Ettinger, MLE) or post-denitrification (Wuhrmann).
- Multi-reactor anaerobic-anoxic-aerobic nitrification-denitrification biological excess phosphorus removal (NDBEPR), in the MUCT configuration.

The single-reactor and two-reactor nitrogen removal systems were operated at 15 d sludge age, and the multi-reactor systems were operated at 20 d sludge age. All experimental work was conducted at 20°C.

The experimental programme was divided into two parts. In the first part, experiments were conducted using a defined artificial substrate as influent and in the second part using municipal sewage as influent. The objectives in using a defined substrate as influent were twofold: Firstly, a substrate of known composition would eliminate variations in composition associated with municipal sewage, which could affect low F/M filamentous organism growth. Secondly, the flexible composition of the substrate would allow the effect of the proportions of influent readily biodegradable soluble COD (RBCOD) and slowly biodegradable particulate COD (SBCOD) and influent nitrogen (organic and ammonia) on filament proliferation to be evaluated.

As will become evident, even though the use of defined substrate provided considerable insight into the causes of filamentous organism bulking, its use also had a complicating effect in that a number of the filamentous organisms which developed with the defined substrate have not been found to be problematic in full-scale South African N and N&P plants. Additionally, the DSVI values which developed with the defined substrate as influent usually were considerably higher than DSVI values which developed under similar conditions with municipal sewage as influent. For these reasons a degree of circumspection was necessary in evaluating results from the experimental work conducted with the defined substrate.

For details of daily experimental data, COD and nitrogen mass balances, operating parameters and filament identifications, the reader is directed to the appropriate detailed reports cited in the text.

Experimental results and discussion

RBCOD or SBCOD as influent substrate

In Ekama et al. (1996a), it was concluded that neither kinetic selection (aerobic or anoxic selectors) nor metabolic selection (anaerobic reactors) control low F/M filament proliferation. Since with kinetic and metabolic selection, RBCOD is taken up preferentially by floc-formers, the continued proliferation of low F/M filaments appears to indicate that they are able to compete for and utilise SBCOD for growth. To examine the role of SBCOD in the development of low F/M filamentous organism populations, the results of experiments conducted with defined artificial substrate and municipal sewage are described below.

Defined artificial substrate

In experiments conducted with defined substrate (see Gabb, 1988 for details of substrate development and Casey et al., 1994b for details of composition) two substrate types were used, one termed RBCOD (determined by physical and biological methods to contain 80-85% RBCOD) and one termed SBCOD (80-85% SBCOD). Each substrate type was fed separately to an IAND system (Systems A and B) operated at a 15 d sludge age with nitrate added continuously by drip-feeding to ensure a continuous presence of nitrate under non-aerated (anoxic) conditions. The systems were operated for a period of 185 d, during which time the feed to each system was switched twice, at regular intervals, between RBCOD and SBCOD, that is, the substrate types fed to Systems A and B were changed from SBCOD to RBCOD and back to SBCOD for System A, and from RBCOD to SBCOD and back to RBCOD for System B.

For both systems, the DSVI increased substantially following a change from SBCOD to RBCOD substrate (from ≈500 to ≈1 000 mℓ/g) and decreased similarly following a change from RBCOD to SBCOD substrate as shown in Fig. 1 for System A and in Fig. 2 for System B. In the two systems, the dominant filaments were either *Haliscomenobacter hydrossis* or type 1851 or both, and the secondary filaments were the low F/M types 0092 and 0041. Although the dominant filaments are not the ones found most often as the dominant types in South African N and N&P removal systems, they are nevertheless classified by Jenkins et al. (1984, 1993) as low F/M. As indicated earlier, their presence in Systems A and B, and not the usual low F/M filaments found in South African plants, may be a consequence of the composition of the substrate rather than the conditions in the systems *per se*, because, as will become evident in this paper, identical systems fed municipal sewage developed filaments typical of South African N and N&P removal plants.

From these results it was concluded that although the filaments - both the dominant *H. hydrossis* and type 1851 and the secondary types 0092 and 0041 - could proliferate to a greater extent and much more rapidly with RBCOD than with SBCOD, growth with SBCOD nevertheless was appreciable and sufficient to cause bulking conditions. This was an expected result since it was noted that low F/M filaments can proliferate with SBCOD because low F/M filaments proliferate in intermittently aerated ND systems with aerobic selectors (Gabb et al., 1996b), laboratory MUCT systems (Gabb et al., 1996a) and full-scale NDBEPR systems (Ekama et al., 1996b) in which RBCOD from the influent is not available in the aerobic or anoxic zones.

An interesting result noted during these experiments concerns the change in combined NO_3^- and NO_2^- concentration (NO_x^-) in the effluent with change in substrate type. [*Note: The notation NO_x^- is used to denote the sum of the concentrations of NO_2^- and NO_3^-, in mgN/ℓ. Because the concentration of NO_2^- was not measured separately in these experiments, the individual concentrations of NO_3^- and NO_2^- could not be calculated*].When the DSVI increased as a consequence of the change from SBCOD to RBCOD substrate, the effluent NO_x^- concentration increased and when the DSVI decreased as a consequence of the change from RBCOD to SBCOD substrate, the effluent NO_x^- concentration in the effluent also decreased. Figures 1 and 2 illustrate the changes in concentration of NO_x^- with change in DSVI for Systems A and B respectively. This is contrary to expectation from RBCOD and SBCOD utilisation kinetics embodied in the IAWQ (Henze et al., 1987) and UCT models (Dold et al., 1991); the expectation is that the system fed the RBCOD, which is utilised at equal rates under anoxic and aerobic conditions (see Still et al., 1996 and Ekama et al., 1996b), should produce lower effluent NO_x^- concentrations than the system fed

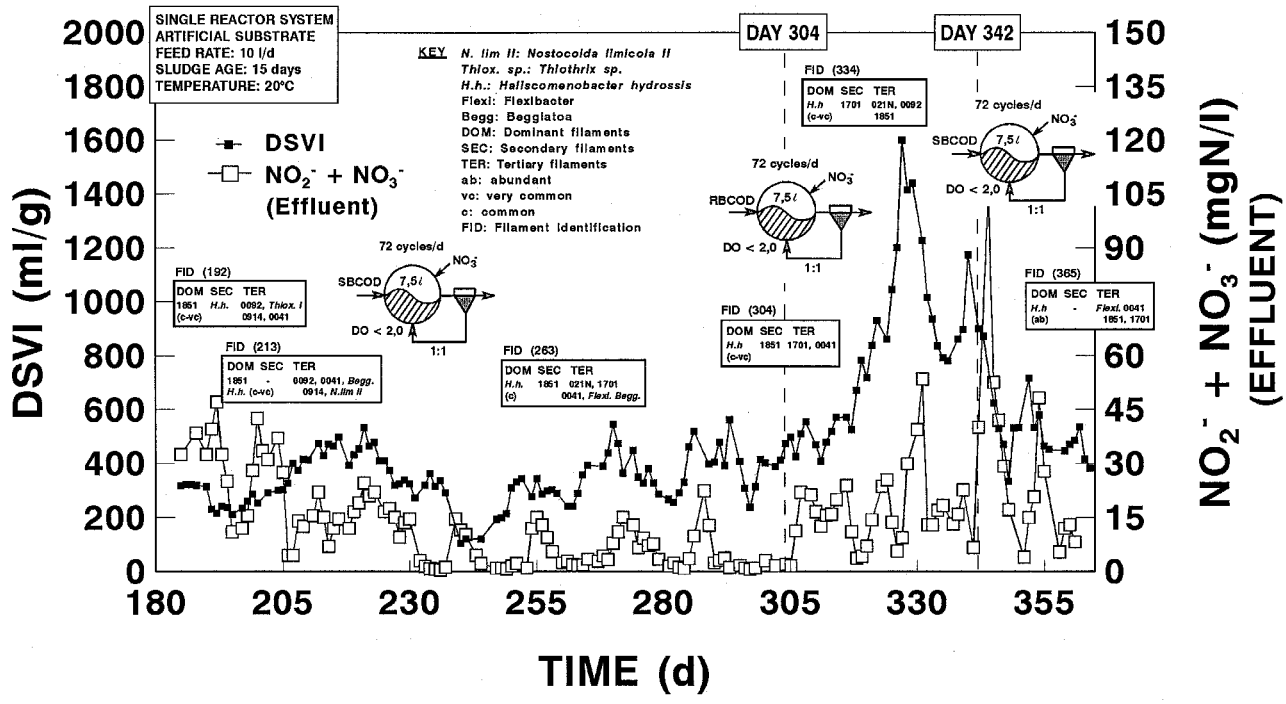


Figure 1

Sludge settleability as DSVI (mℓ/g) and effluent nitrate + nitrite concentration (mgN/ℓ) with time (in days) for System A, alternatingly fed the RBCOD and SBCOD fractions of a defined artificial substrate

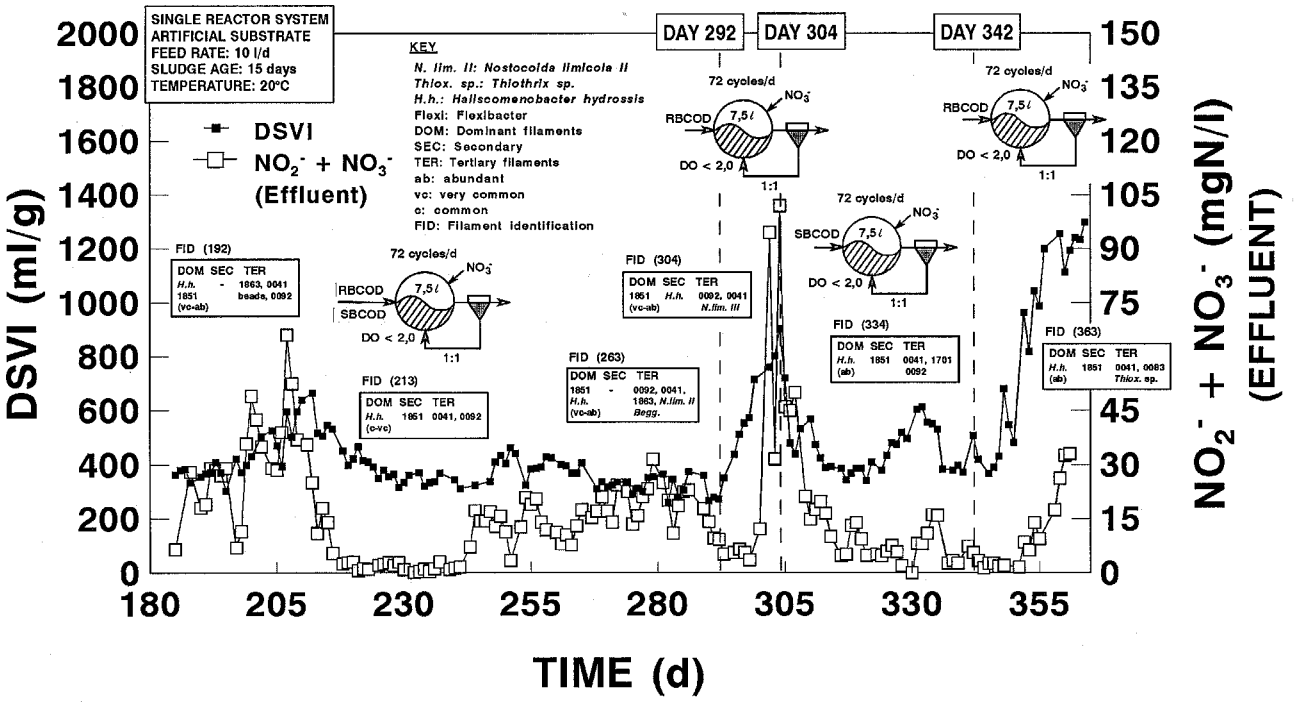


Figure 2

Sludge settleability as DSVI (mℓ/g) and effluent nitrate + nitrite concentration (mgN/ℓ) with time (in days) for System B, alternatingly fed the RBCOD and SBCOD fractions of a defined artificial substrate

SBCOD, which is utilised more slowly (by $^{2}/_{3}$) under anoxic conditions compared with aerobic conditions. Consequently, the difference in the NO_x^- concentration from the RBCOD and SBCOD fed systems lies in some other cause. An interrelationship that is apparent from Figs. 1 and 2 is one between the effluent NO_x^- concentration and the quantity of filamentous organisms present in the sludge as indicated by the DSVI value. In support of this as a possible interrelationship, it can be seen from Figs. 1 and 2 that

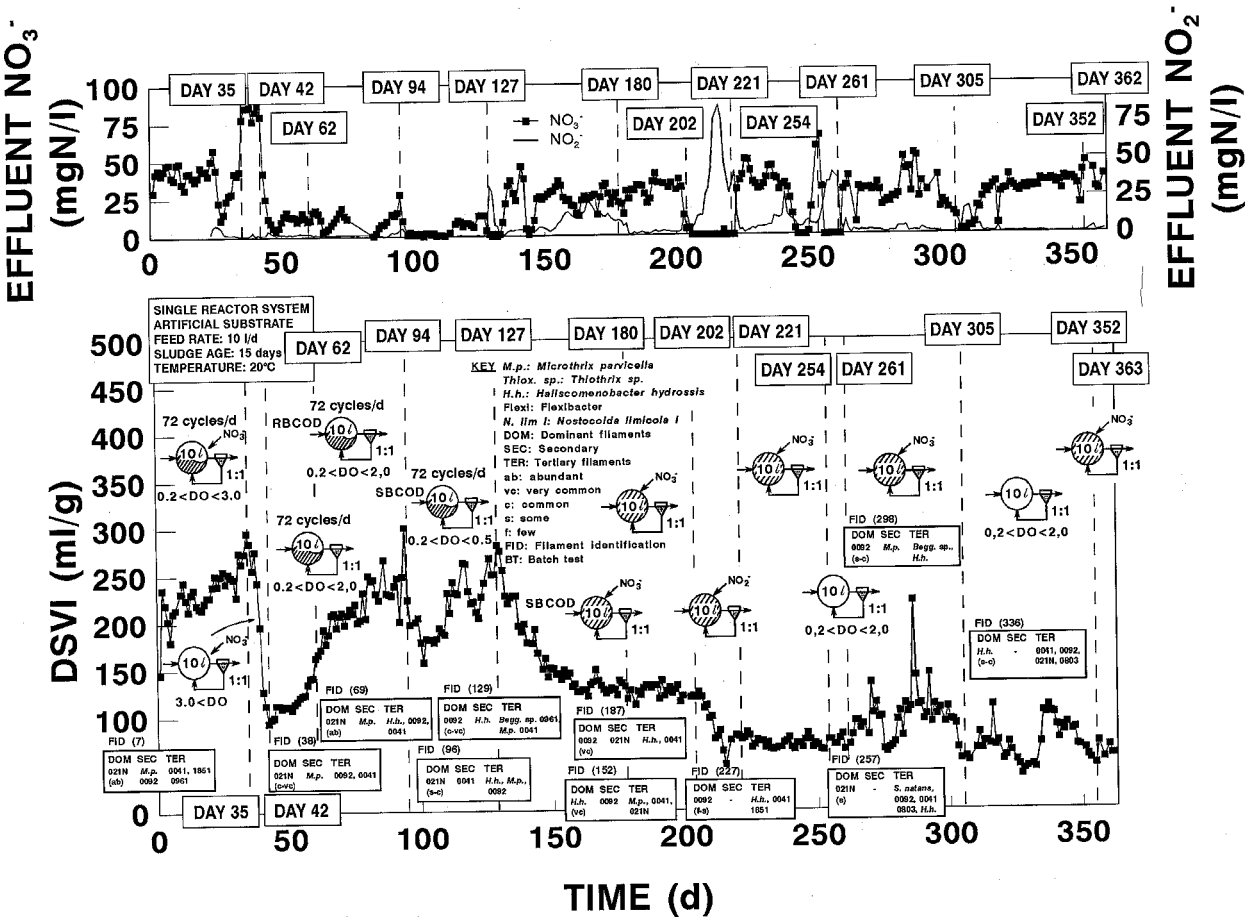


Figure 3
Sludge settleability as DSVI (mℓ/g), effluent nitrate (NO_3^-) and nitrite (NO_2^-) concentrations and changes in system operation with time (d) for System 1

during periods of steady state operation, small daily changes in DSVI resulted in corresponding small changes in NO_x^- concentration in the effluent. At the time, no explanation could be given for these unexpected results; however, they did provide one of the clues that contributed to formulating the hypothesis for low F/M filament bulking described in Casey et al. (1999a).

Municipal sewage

To examine the effect of the SBCOD and RBCOD fractions of municipal sewage on filament proliferation, municipal sewage was subjected to ultrafiltration (0.1µm) to produce RBCOD and SBCOD fractions (the former with unbiodegradable soluble COD and the latter with unbiodegradable particulate COD). The ultrafiltration method is described by Casey et al. (1994b). The RBCOD and SBCOD fractions were alternatingly fed to intermittently aerated single-reactors (Systems 1 and 2) operated at 15 d sludge age, the systems being fed with each substrate for periods not less than 2 sludge ages. The changes in DSVI of Systems 1 and 2 are illustrated in Figs. 3 and 4 respectively.

From Day 1 to Day 42, the systems were operated to test whether each demonstrates behaviour similar to systems operated previously; i.e., development of high DSVI values under intermittent aeration conditions (Day 1 to 34) and development of low DSVI values under continuous aeration conditions (Day 35 to 41) (see Gabb et al., 1996a). From Day 1 to Day 34 both systems were fed complete municipal sewage (not ultrafiltered) under intermittent aeration conditions (0.2 < DO < 3.0 mgO/ℓ) and the DSVI values were in the range 200 to 300 mℓ/g and increasing with time (Figs. 3 and 4). From Day 35 to Day 41 the systems were operated continuously aerobic (DO > 3.0 mgO/ℓ) and the DSVI values decreased from 250 to 300 mℓ/g to less than 100 mℓ/g. Following the reintroduction of intermittent aeration (0.2 < DO < 2.0) on Day 42, the DSVI values of both systems increased to above 150 mℓ/g by Day 62. These results conform to the results of systems operated earlier (see Gabb et al., 1996a). On Day 62 the municipal sewage (10 ℓ at 500 mgCOD/ℓ) fed to each system was replaced with the sewage ultrafiltrate RBCOD fraction (20 ℓ at 250 mgCOD/ℓ) to System 1 and the sewage ultrafiltrate SBCOD fraction (10 ℓ at 500 mgCOD/ℓ) to System 2 [*Note: It was not possible to produce a higher concentration of the RBCOD component of the wastewater and consequently, to ensure the same COD load on both systems, the volume of RBCOD (filtrate) fed to the system daily was increased*]. From Day 62 to 93, the DSVI value in System 1 increased from 160 to 250 mℓ/g and in System 2 increased from 160 to 350 mℓ/g. On Day 94, the feed type was interchanged between the two systems. In System 1, in which the RBCOD was replaced with SBCOD, the DSVI remained high (200 to 270 mℓ/g) up to Day 126. In System 2, in which the SBCOD was replaced with RBCOD, the DSVI initially decreased to 120 mℓ/g but then increased to 400 mℓ/g by Day 126.

In System 1, between Day 1 and Day 126, the dominant filament was type 021N. The secondary filament was *Microthrix parvicella* from Day 1 to Day 69, whereafter it was type 0041 on Day 96 and *H. hydrossis* on Day 129 (see Fig. 3). In System 2 on

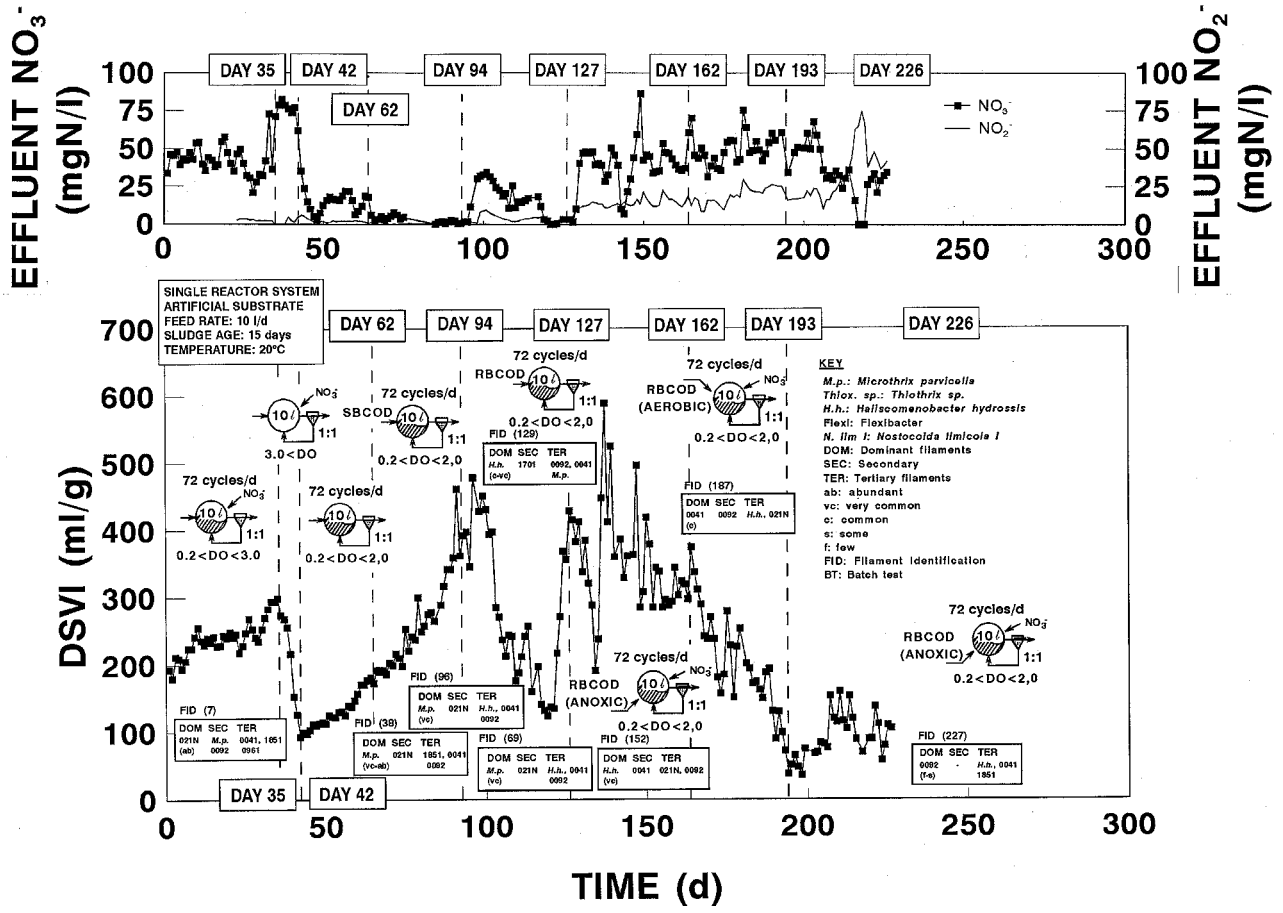


Figure 4
Sludge settleability as DSVI (mℓ/g), effluent nitrate (NO_3^-) and nitrite (NO_2^-) concentrations and changes in system operation with time (d) for System 2

Day 7, the dominant filament was type 021N, and thereafter was *M. parvicella* until Day 129 at which time *H. hydrossis* became dominant. The cause of the changes in DSVI between Days 94 and 127 were not apparent at this stage, but it is interesting to note that the decrease and subsequent increase in DSVI is associated with a change in dominant filament type, i.e. *H. hydrossis* became dominant over *M. parvicella* in the time period between the filament identifications (FIDs) conducted on Days 96 and 129. The secondary filaments were *M. parvicella* and type 0092 on Day 7, whereafter it was type 021N until Day 129 when type 1701 was secondary (see Fig. 4).

From the results it would appear that low F/M filaments *H. hydrossis*, type 021N, *M. parvicella*, types 0041, 0092, 1851 and 0803 can proliferate with either RBCOD or SBCOD only as substrate. Furthermore, it should be noted that, as in the experiments with defined artificial substrate, filaments belonging exclusively to the low F/M filament group (i.e. *M. parvicella* and types 0092, 1851 and 0803) although not often dominant, did nevertheless proliferate sufficiently with SBCOD to cause bulking. Those filaments which appeared to proliferate to greater numbers with RBCOD, i.e. *H. hydrossis* and type 021N are not exclusively classified as low F/M filaments, *H. hydrossis* is also included in the low DO and nutrient deficient groups, and type 021N in the septic wastewater and nutrient deficient groups (see Jenkins et al., 1984, 1993; Casey et al., 1995). It was concluded that although the sludges from the two systems had a significant presence of the filaments *H. hydrossis* and type 021N which normally are not dominant in full-scale N and N & P removal systems, the low F/M types *M. parvicella*, 0041, 0092, 1851 and 0803, could proliferate sufficiently with SBCOD to produce bulking sludges (DSVI > 150 mℓ/g), which confirmed the earlier results with the defined substrate.

RBCOD as influent substrate fed under either aerobic or anoxic conditions

In the above experiments, the RBCOD and SBCOD sewage substrates were fed throughout the intermittent aeration cycle and it was not possible to determine which (if either) of the periods (aerobic or anoxic), would provide an environment more conducive to filament proliferation. To examine this aspect more closely, System 2 (formerly fed municipal sewage RBCOD throughout the intermittent aeration cycle) was operated from Day 127 with the same sewage RBCOD but fed only during the anoxic period. To maintain anoxic conditions, nitrate was dosed continuously over the full anoxic-aerobic cycle (see Fig. 4). The DSVI remained high (300 mℓ/g) over a period of more than 2 sludge ages (35 d) up to Day 161 with *H. hydrossis* as dominant filament. On Day 162 the feeding pattern was changed and RBCOD was fed during the aerobic period only, while continuous nitrate dosing was maintained. During the following 31 d (2 sludge ages), the DSVI decreased from 300 to 50 mℓ/g. On Day 193 the feeding pattern was again changed and the RBCOD was fed during the anoxic period of the cycle while nitrate was continued to be dosed to the system. The DSVI increased from 50 to 100 mℓ/g until Day 226 when the

experiment was terminated.

In these experiments it was not possible to confirm the earlier observed relationship between filament proliferation (DSVI) and effluent nitrate concentration (Systems A and B, Figs. 1 and 2) because switching the RBCOD feed between the anoxic and aerobic periods (which influences the degree of denitrification) masked changes in effluent NO_3^- and NO_2^- concentration. It was expected that the effluent nitrate concentration would be much higher where feed was discharged to the anoxic period, but this was not observed (Fig. 4). From these results it appears that the supply of RBCOD under aerobic conditions ameliorates low F/M filament proliferation, in particular that due to *H. hydrossis*. In contrast, the supply of RBCOD under anoxic conditions promoted the proliferation of the low F/M filaments, in particular *H. hydrossis* which was the dominant filament. The reason for the roughly constant effluent NO_x^- concentration was that although the total COD of the ultrafiltered wastewater was around 250 mg/ℓ, the biodegradable portion of the wastewater was low (100 to 110 mg/ℓ), consequently, the system VSS concentration declined progressively to a very low value (≈500 mgVSS/ℓ) at which point the viability of the system was suspect. Therefore, while the results are interesting, little substantive information can be gained from this experiment.

Continuous aerobic, continuous anoxic conditions

Continuous aerobic conditions

The effect of continuous aerobic conditions on low F/M filament proliferation in IAND systems was re-examined (see Ketley et al., 1991; for earlier work see Gabb et al., 1996a), but this time the systems were fed not only municipal sewage but also defined artificial substrate. For the systems fed defined substrate (no graphs shown in interests of brevity), under intermittent aeration conditions, the sludges developed DSVIs > 800 mℓ/g, but when the systems were continuously aerated for 20 d the DSVI decreased to 100 mℓ/g. After the reintroduction of intermittent aeration, the DSVI increased to 800 mℓ/g over a period of 35 d. During the periods of filament growth under intermittent aeration conditions, the dominant filaments were *H. hydrossis* and type 1851. By comparison, for the systems fed municipal sewage (Systems 1 and 2 discussed above - see Figs. 3 and 4), the intermittent aeration conditions resulted in an increase in DSVI from 200 to 300 mℓ/g from Day 1 to Day 34. Changing both systems to continuous aeration on Day 35 resulted in a decrease in DSVI from around 250 mℓ/g to below 100 mℓ/g in 7 d and with re-introduction of intermittent aeration to both systems on Day 42, the DSVIs again increased, from below 100 mℓ/g to above 150 mℓ/g in just over a sludge age (Figs. 3 and 4). Ketley et al. (1991) also investigated the effect of continuous aerobic conditions on bulking sludges developed in three IAND systems fed municipal sewage. In each system under intermittent aeration conditions, the low F/M filament *M. parvicella* was dominant and resulted in a DSVI ≈400 mℓ/g. Changing the aeration pattern from intermittent (30 to 35% aerobic) to continuous (100% aerobic) caused amelioration of filament proliferation (DSVI ≈100 mℓ/g). All these experimental results are in agreement with those described by Gabb et al. (1996a,b) that low F/M filament proliferation (in particular *M. parvicella*) is ameliorated by continuous aeration and promoted with intermittent aeration.

Continuous anoxic conditions

Following the experiments conducted on System 1 to investigate the roles of municipal sewage RBCOD and SBCOD in low F/M filament proliferation described above (Days 1 to 126), this system was used from Day 127 to examine the effect of completely anoxic conditions on low F/M filament proliferation (see Fig. 3). Under fully anoxic conditions, nitrate was dosed in sufficient quantity to exceed the denitrification potential of the system so that nitrate would be present at all times (> 10 mgNO_3^--N/ℓ). On Day 127, the intermittent aeration conditions with SBCOD as substrate were changed to completely anoxic conditions with SBCOD as substrate and the DSVI decreased from 270 to 130 mℓ/g between Days 127 and 179, a period of about three and a half sludge ages. During a subsequent period of twelve sludge ages to Day 363, during which time the system was fed complete (unseparated) municipal sewage and operated either as completely aerobic or completely anoxic (with either nitrate or nitrite as electron acceptor through dosing), the DSVI remained around 100 mℓ/g at all times. These various operating conditions for System 1 were not imposed solely for testing the effect of fully anoxic and fully aerobic conditions on filamentous organism proliferation, but to provide a source sludge for batch tests for evaluating the effect of the changes between these conditions on denitrification behaviour (Casey et al., 1999b).

Ketley et al. (1991) also investigated the effect of continuous anoxic conditions on bulking sludges developed in IAND systems fed municipal sewage. Following the imposition of continuous anoxic conditions with excess nitrate supplied as electron acceptor, the DSVI decreased from ≈200 to ≈90 mℓ/g over a period of three sludge ages. Growth of the filaments *M. parvicella*, type 1851 and type 0092, which were prolific under intermittent aeration conditions, were suppressed under continuous anoxic conditions.

Magnitude of the aerobic mass fraction

From the work described above, it can be concluded that systems which are continuously aerobic or continuously anoxic, develop sludges with low DSVIs, but IAND systems, in which the sludge alternates between anoxic and aerobic conditions, develop sludges with high DSVIs. In the IAND systems the aerobic period was 30 to 40% of the total, but the effect of the aerobic mass fraction in IAND systems on DSVI was not clear. This aspect was examined by determining the steady-state DSVI values established at different aerobic mass fractions. Figure 5 illustrates the relationship between steady-state DSVI and average percentage aerobic time (equal to % aerobic mass fraction) for periods of steady-state operation of an IAND system fed defined artificial substrate (System B). The results indicate that the highest DSVI values develop at 30 to 40% aerobic mass fraction - aerobic mass fractions greater than 40% and less than 30% result in lower DSVI values.

The effect of variation in the aerobic sludge mass fraction on low F/M filament proliferation was examined also on IAND and MUCT systems fed municipal sewage: In two IAND systems, both operated at a sludge age of 10 d, a 70% aerobic mass fraction system had a much lower DSVI than a 30% aerobic mass fraction system (Warburton et al., 1991). Experiments were conducted to examine the effect on DSVI of variation in aerobic mass fraction for two MUCT N&P removal systems (Systems 3 and 4, see Fig. 6). With an aerobic mass fraction of 33% (3rd reactor anoxic), System 3 developed a DSVI ≈ 250 mℓ/g between Days 52 and 126. On Day 127, the aerobic mass fraction was increased to 65% (3rd reactor aerobic) and the DSVI decreased from ≈250 mℓ/g to ≈150 mℓ/g. Similarly, System 4 developed a DSVI between 100 and 150 mℓ/g with an aerobic mass fraction of 65% (3rd reactor aerobic) from Days 52 to 126. On Day 127, the aerobic mass fraction was reduced to 35% (3rd reactor anoxic) and the DSVI increased to between 200 and 230 mℓ/g up to Day 185 when the experiment was terminated. In both systems, when the DSVI was high, the dominant filament was type 0092 which is classified exclusively as low F/M (Jenkins

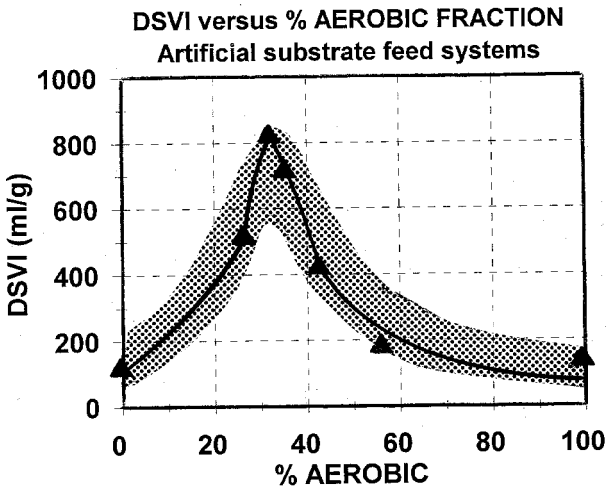
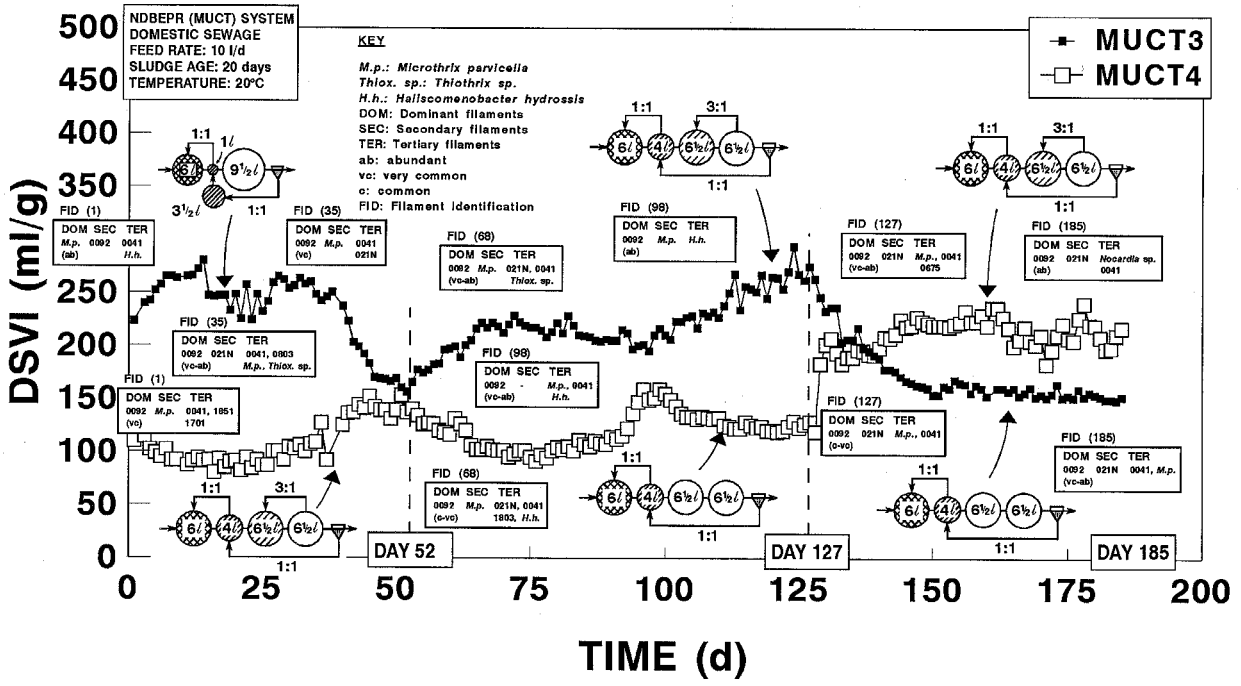


Figure 5 (right)
Sludge settleability as DSVI with percentage aerobic mass (%) for steady-state periods of operation for System B, a single-reactor intermittently aerated system fed defined artificial substrate

Figure 6 (bottom)
Sludge settleability as DSVI (mℓ/g) and changes in system operation with time (d) for two multi-reactor NDBEPR systems, operated in the MUCT configuration (MUCT 3 and MUCT 4) with different aerobic and anoxic mass fractions



et al., 1984, 1993). For these multi-reactor N&P removal systems fed municipal sewage, the relationship between DSVI and percentage aerobic mass fraction is in agreement with those for single-reactor nitrogen removal (IAND) systems fed defined substrate (Fig. 5) and real sewage (Warburton et al., 1991).

From these results it can be concluded that aerobic mass fractions between 30 and 40% result in the highest DSVIs. While there is agreement on the trend of the relationship between aerobic mass fraction and DSVI for defined substrate and real sewage, quantitatively, defined substrates result in generally much higher DSVIs than real sewage for the same system operating conditions.

Sludge age

In examining the effect of sludge age on filament proliferation, Warburton et al. (1991) found that for an IAND system fed real sewage with a 30% aerobic mass fraction and containing a high proportion of low F/M filamentous organisms (DSVI > 300 mℓ/g), decreasing the sludge age from 20 d to 10 d had little effect on DSVI, filament proliferation and filament type (i.e. *M. parvicella*, type 0092, type 0675 and type 0041 were the dominant filaments at both sludge ages). Decreasing the sludge age further, from 10 to 7 and then to 5 d, also had little effect on filament type, but proliferation was reduced; the DSVI declined from > 300 mℓ/g to around 120 mℓ/g. In order to ensure sufficient availability of nitrate for denitrification for the system with 30% aerobic mass fraction and at sludge ages < 10 d (under which conditions nitrification was no longer complete), excess nitrate was dosed continually.

From these results it would appear that reducing sludge age below 10 d ameliorates low F/M filament bulking, an observation also noted by Foot et al. (1994). However, while this strategy brings relief to the secondary settling tank, reducing not only the DSVI but also the reactor MLSS concentration, its applicability is limited where low effluent ammonium concentrations are required, due to its adverse impact on nitrification.

Differences in sludge settleability between IAND and 2RND systems with apparently similar operating conditions

During the first part of the experimental programme in which defined substrate was used as feed, single reactor IAND systems

(30 to 40% aerobic fraction) invariably developed sludges with high DSVI values (400 to 600 mℓ/g) caused by principally low F/M filaments including *H. hydrossis* and type 1851 (Casey et al., 1994b). In contrast, two-reactor (anoxic-aerobic) nitrification-denitrification (2RND) systems (30 to 40% aerobic fraction) fed the same substrate, whether they were operated in a pre-denitrification anoxic-aerobic (modified Ludzack-Ettinger) mode or in a post-denitrification aerobic-anoxic (Wuhrmann) mode, invariably developed sludges with comparatively lower DSVI values (150 to 200 mℓ/g) and lower levels of low F/M filaments (Hulsman et al., 1992). **This observation served as the point of departure for a new research direction**. The objective was to determine the condition which allowed low F/M filaments to proliferate extensively in IAND systems, but not in 2RND systems with apparently similar conditions. With this as the foremost objective, the feeding patterns and aeration conditions to which sludge is exposed in IAND and 2RND systems were compared and the following differences were noted:

- In an IAND system the frequency of exposure of sludge to alternating anoxic-aerobic cycles is high (> 30/d) as a result of the large number of intermittent aeration cycles per day, but in a 2RND system this frequency is low (< 5/d) as a consequence of the low a- (aerobic-anoxic) and s- (sludge return) recycle ratios.
- In an IAND system, the influent is fed to the aerobic and anoxic periods in proportion to the aerobic and anoxic mass fractions, but in 2RND systems the influent is fed into the anoxic reactor only (for pre-denitrification MLE systems), or into the aerobic reactor only (for post-denitrification Wuhrmann systems). Thus in IAND systems, both RBCOD and SBCOD are fed to the aerobic and anoxic zones, whereas in 2RND systems the RBCOD will be utilised exclusively under anoxic or aerobic conditions depending on the system type (pre- or post-denitrification).
- In an IAND system, when the air is switched off, the DO concentration changes progressively from a high value (DO ≈2.0 mgO/ℓ) to zero, then through a period of low DO as a result of biological action until conditions are anoxic, but in a 2RND system the DO concentration is relatively constant in the aerobic reactor (DO > 2.0 mgO/ℓ) and is zero in the anoxic reactor and the change between the two conditions takes place rapidly.
- In an IAND system, the NO_2^- and NO_3^- concentrations in the anoxic period decrease progressively as a result of denitrification, but in a 2RND system operating at steady-state, the NO_2^- and NO_3^- concentrations are relatively constant in the anoxic reactor.

Each of these differences was examined experimentally by modifying the 2RND system to conform as closely as possible to the IAND system:

- increasing frequency of exposure to anoxic conditions by increasing the a-recycle ratio;
- splitting the influent feed to both aerobic and anoxic reactors in proportion to their mass fractions;
- installing a small unaerated reactor in the a-recycle stream to provide a low DO zone between the aerobic and anoxic reactors; and
- dosing ammonia to the influent to vary the nitrate concentration (via nitrification) in the system.

Where appropriate the above four aspects were also examined in IAND and MUCT systems. Upon examination of the results, only one difference appeared to have a significant effect on low F/M filament proliferation, that is, the concentrations of NO_3^- and NO_2^- in the anoxic period of an IAND system and in the anoxic reactor of a 2RND system. To avoid a lengthy discussion of the experimental work conducted on aspects described above which did not have a substantial effect on filament proliferation, only the experimental work relating to the concentration of NO_3^- and NO_2^- in the anoxic zone is described in this paper; experiments relating to the other differences (i.e. frequency of exposure to anoxic-aerobic conditions, availability of RBCOD under anoxic and aerobic conditions, and concentration of DO) are described only briefly, details of the experiments are given by Casey et al. (1994b); Hulsman et al. (1992); Ketley et al. (1991); De Villiers et al. (1994).

Frequency of exposure of sludges to aerobic-anoxic conditions

If filaments do benefit from exposure to alternating anoxic-aerobic cycles (a conclusion drawn from the finding that intermittent aeration systems bulk but completely aerobic and completely anoxic systems do not), then the benefit may be proportionally increased by exposure to increased numbers of anoxic-aerobic cycles. Experiments on this aspect were conducted both with IAND and 2RND systems.

- Ketley et al. (1991) examined IAND systems with aerobic periods of approximately 30% and anoxic-aerobic cycle lengths varying between 20 min (14 min anoxic, 6 min aerobic) and 3 d (2 d anoxic, 1 d aerobic). All systems had NO_3^- addition during the anoxic period and all systems developed similar bulking sludges (DSVI > 150 mℓ/g), the dominant filaments being *M. parvicella* and type 0803.
- Hulsman et al. (1992) examined 2RND systems with aerobic mass fractions comprising 30 to 40% of the total, one system with a low (3:1) a-recycle ratio, the other system with a high (>30:1) a-recycle ratio. The objective of the high a-recycle ratio in the 2RND system was to provide a frequency of exposure of the sludge to alternating anoxic-aerobic conditions similar to IAND systems. The systems with the low and the high a-recycle ratios both developed non-bulking sludges (DSVI < 150 mℓ/g).

The results of Ketley et al. (1991) and Hulsman et al. (1992) indicated that the frequency of exposure of sludge to alternating anoxic-aerobic conditions does not influence the proliferation of low F/M filaments; IAND systems bulked irrespective of the number of anoxic-aerobic cycles, and 2RND systems did not bulk irrespective of the number of anoxic-aerobic cycles. However, it should be cautioned that these results are specific to the wastewater characteristics and operating conditions of these systems. It will be seen later in this paper that different wastewater characteristics and operating conditions result in bulking sludges in 2RND systems.

Availability of RBCOD under aerobic and anoxic conditions in a 2RND (MLE) configuration

In a 2RND system, all the substrate feeds into the anoxic reactor in an MLE configuration and into the aerobic reactor in a Wuhrmann configuration. In these reactors, for an average domestic sewage and anoxic/aerobic mass fractions of 70:30, all the RBCOD is utilised completely in the first reactor. The only substrate available in the aerobic reactor of an MLE configuration and in the anoxic

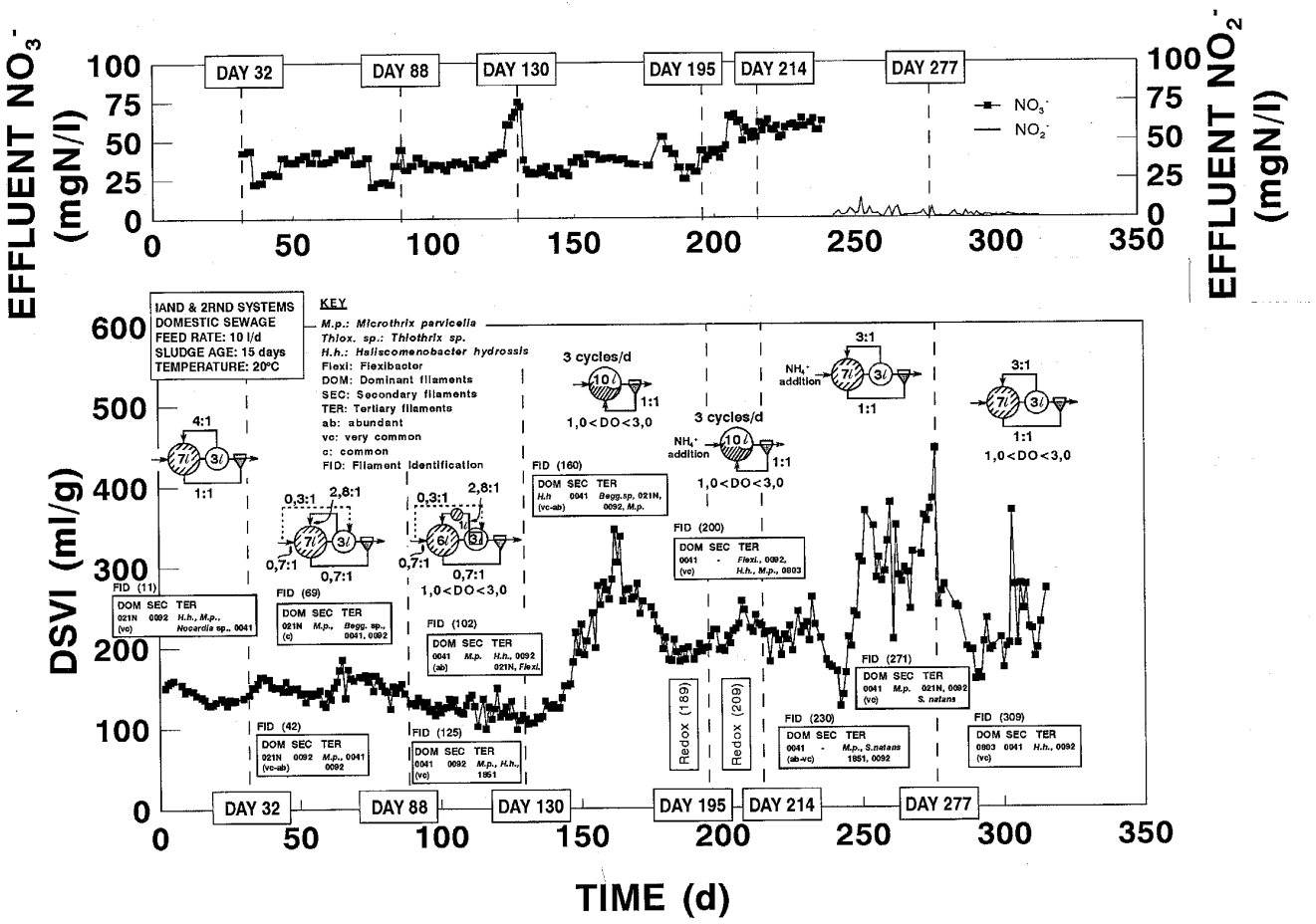


Figure 7

Sludge settleability as DSVI (mℓ/g), effluent nitrate (NO_3^-) and nitrate (NO_2^-), and changes in system operation with time (d) for System 5 in which the effect of differences between IAND and 2RND configurations were examined

reactor of a Wuhrmann configuration is SBCOD. In IAND systems, substrates feed continuously into the system throughout the intermittent aeration cycle; thus in these systems, RBCOD and SBCOD are available in both the anoxic and aerobic periods. In the experiments described above, it was shown that IAND systems develop high DSVIs, whereas the 2RND systems develop low DSVIs. Further, in **RBCOD as substrate under either aerobic or anoxic conditions** above it was demonstrated that the conditions (aerobic or anoxic) under which RBCOD is utilised appear to influence the degree of filament proliferation, although the filaments which developed (principally *H. hydrossis*) were not the low F/M types which dominate in full-scale N and N&P removal plants in South Africa (types 0092, *M. parvicella*, 0041, 0675). On the basis of these results, experiments were conducted with a 2RND system to examine the effect on filament proliferation of the availability of RBCOD under both anoxic and aerobic conditions.

- A 2RND system (System 5) was operated for 31d as a modified Ludzack-Ettinger (MLE) configuration, during which a steady-state DSVI value of about 130 mℓ/g was achieved (Fig. 7). From Day 32 to Day 87, influent sewage was split between the anoxic and aerobic zones in proportion to their respective mass fractions, in order to approximate the feeding pattern of an IAND system. The DSVI remained at 150 mℓ/g throughout this period.

From the results it can be concluded that in 2RND systems fed sewage (≈20% RBCOD) the environmental condition (aerobic or anoxic) in which the RBCOD was utilised *did not* influence the growth of low F/M filaments. However in the IAND systems (Systems 1 and 2) fed the RBCOD fraction of sewage (≈50% RBCOD) (see Figs. 3 and 4 above), the environmental condition in which the RBCOD was utilised *did* influence the growth of low F/M filaments. If the reason for the difference in filament proliferation between IAND and 2RND systems (IAND - high DSVI; 2RND - low DSVI) is not a consequence of the system configuration, it can be concluded that a combination of the proportion of RBCOD in the influent and the environmental condition under which the RBCOD is available affects filament proliferation. That is, for a substrate containing low proportions (< 30%) of RBCOD, the zone under which the substrate is available does not significantly influence filament proliferation, whereas for a substrate comprising a high proportion (>70%) of RBCOD, the zone under which the RBCOD is available does influence the extent of proliferation of filamentous organisms. High concentrations of RBCOD under anoxic conditions result in high DSVIs, and high concentrations of RBCOD under aerobic conditions result in low DSVIs.

Concentration of DO during the aerobic period of an IAND system and in the aerobic reactor of a 2RND system

In an IAND system, when the air is switched on at the start of the aeration cycle, the sludge is exposed to a high DO concentration (> 2.0 mgO/ℓ) and when the air is switched off, the sludge is exposed

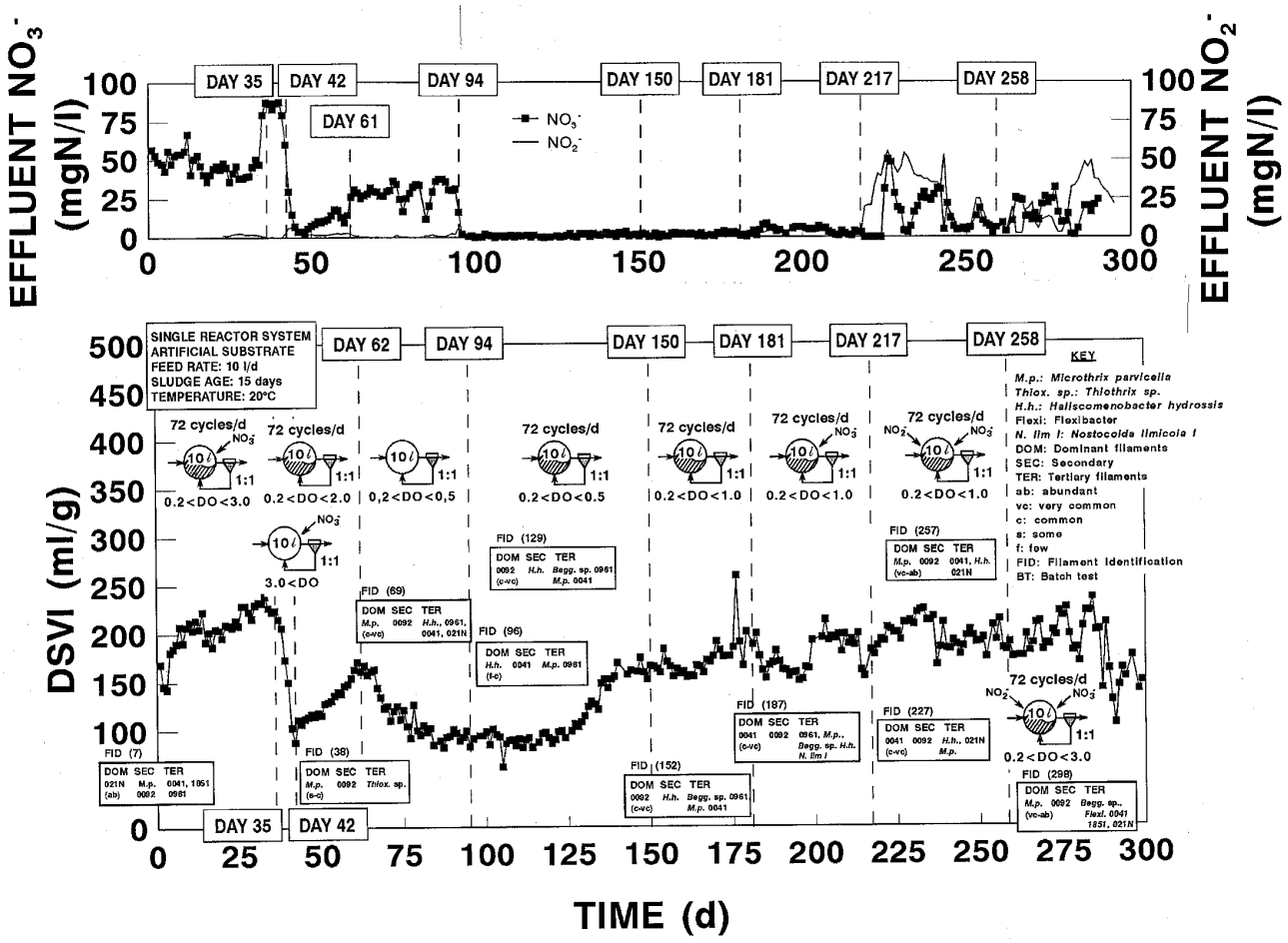


Figure 8

Sludge settleability as DSVI (mℓ/g), effluent nitrate (NO_3^-) and nitrate (NO_2^-), and changes in system operation with time (d) for System 6 in which the concentration of DO was varied in the aerobic period of the intermittently aerated cycle

to a decreasing concentration of DO as a result of biological action, followed by a period of very low DO, and finally to zero DO when oxygen is depleted. In a 2RND system, the sludge is exposed in the aerobic reactor to a constant high DO concentration (> 2.0 mgO/ℓ) for the entire residence time and then in the anoxic reactor the sludge is exposed to conditions in which oxygen is absent. There is no zone in the 2RND system between the aerobic and anoxic reactors in which the sludge is exposed to a low, or decreasing DO concentration. This would suggest that perhaps low DO concentration or decreasing DO concentration are linked to filament proliferation.

To examine the effect of low DO conditions:

- A 15 d sludge age single-reactor IAND system fed real sewage (System 6) was operated. The changes in DSVI and aeration pattern for this system are shown in Fig. 8. Following periods of intermittent aeration (Days 1 to 34) and continuous aeration (Days 35 to 39) (as for Systems 1 and 2), System 6 had continuous (24 h/d) low DO (0.2 < DO < 0.5) for 2 sludge ages (Days 61 to 93), and then intermittent aeration (35% aerobic, 65% anoxic) for 3 sludge ages (Days 94 to 149), the aerobic part of the cycle being maintained at low DO (0.2 < DO < 0.5). With continuous aeration at low (DO < 0.5 mgO/ℓ) or high DO (DO > 2 mgO/ℓ), the DSVI was low, but with intermittent aeration with low or high DO in the aerobic period, the DSVI was high. The filamentous organisms causing the high DSVI during the intermittent aeration periods were types 0092 and 0041.

From the results of these experiments it was concluded:

- Low F/M filamentous organisms (*M. parvicella*, types 0092, 0041, 0675) do not proliferate under continuously aerated conditions irrespective of whether the concentration of DO is low (0.2 < DO < 0.5 mgO/ℓ), or high (0.5 < DO < 2.0 mgO/ℓ).
- To proliferate, low F/M filamentous organisms require alternating anoxic-aerobic periods.
- For intermittent aeration conditions (35% aerobic, 65% anoxic) the higher the peak DO concentration (up to 3.0 mgO/ℓ) during the aerobic period, the greater the proliferation of low F/M filamentous organisms (see Gabb et al., 1996b).
- For a change from intermittent aeration to continuous aeration conditions, the higher the DO concentration during continuous aeration, the more rapid the reduction in DSVI.

To examine the effect of a decreasing DO concentration in a 2RND system:

- An MLE 2RND system (System 5) was operated from Day 32 to Day 87, with aerated and unaerated mass fractions of 30 and 70% respectively, with the feed split between the aerobic and anoxic reactors in proportion to the respective mass fractions (i.e. 3 ℓ/d to the aerobic reactor and 7 ℓ/d to the anoxic reactor) as shown in Fig. 7 (see De Villiers et al., 1994). The DSVI of the system remained between 140 and 170 mℓ/g. On Day 88, a small (1 ℓ) unaerated reactor was placed in the a-recycle

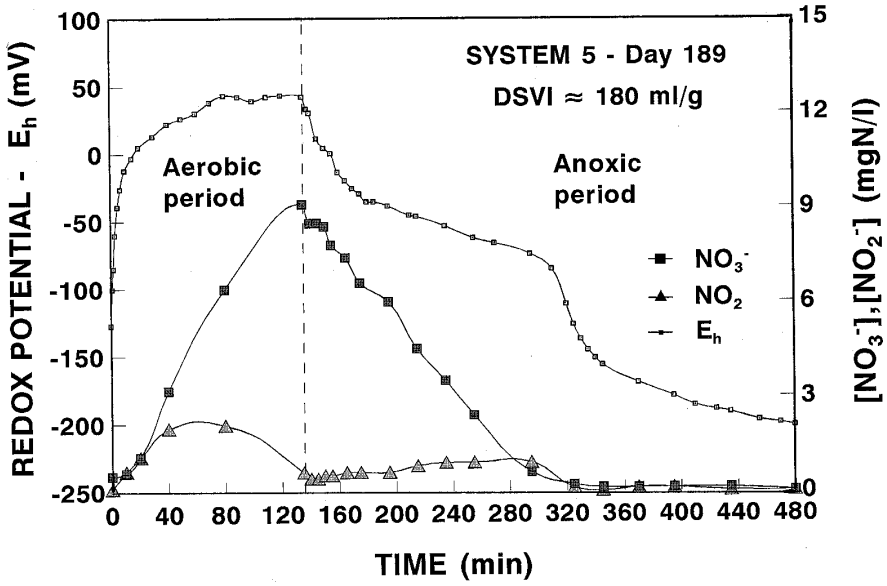


Figure 9
Redox potential (E_h in mV) and nitrate (NO_3^-) and nitrate (NO_2^-) concentrations with time (min) for one 8 h aerated-unaerated cycle of System 5 on Day 189

stream between the aerobic and anoxic reactors. To maintain the same total system volume and aerobic mass fractions, the size of the anoxic reactor was decreased by 1 ℓ from 7 ℓ to 6 ℓ. The concentration of DO in the small unaerated reactor was very low (< 0.5 mgO/ℓ), providing a low DO environment between the aerobic and anoxic reactors, thereby avoiding a rapid change in DO concentration when the sludge is recycled from the aerobic to the anoxic reactor. It was expected that the DO in the a-recycle would lead to a low DO concentration in the small reactor and closely approximate the period of low DO in the IAND system. However, during the almost three sludge ages this system was operated (Days 88 to 129), the DSVI decreased slightly, from about 125 mℓ/g to about 100 mℓ/g, in comparison to the high DSVI values which develop in IAND systems with apparently similar conditions.

From the results of this experiment, it was concluded that low F/M filament proliferation in IAND configurations is not a consequence of low or decreasing DO concentration between the aerobic and anoxic periods.

Changes in the concentration of nitrate in the anoxic period of IAND and 2RND systems

A further difference between IAND and 2RND systems concerns the concentrations of nitrate in the anoxic period of an IAND system and in the anoxic reactor of a 2RND system. In an IAND system, the nitrate concentration at the beginning of the anoxic period is high as a result of nitrate generated by nitrification during the preceding aerobic period. During the anoxic period, the nitrate concentration decreases as a result of denitrification, and depending on the amount of nitrate generated in the aerobic period, the nitrate concentration may or may not become zero before the next aerobic period commences. This is the case for IAND systems with long anoxic (e.g. 5 h) periods. However, in systems with short (e.g. 15 min cycles), little variation in nitrate concentration is apparent between the beginning and end of the anoxic period. In a 2RND system operating at steady state, the nitrate concentration in the anoxic zone remains essentially constant, either at zero or at some positive value depending on whether or not the nitrate load imposed by the recycles is respectively less than or greater than the anoxic reactor's denitrification potential.

In the intermittent aeration system, changes in the concentration of NO_3^- and NO_2^- in the anoxic period and changes in DO in the aerobic period are reflected in changes in the reduction-oxidation (redox) potential (E_h) in those zones. Facultative organisms have a hierarchy for utilisation of electron acceptors (i.e. O_2, NO_3^-, NO_2^-) and one means of differentiating between the electron acceptors is via the redox potential of the surrounding liquid which is established by the concentrations of the various electron acceptors. That is, under aerobic conditions, when O_2, NO_3^- and NO_2^- are present, the redox potential is high, and the first choice electron acceptor, O_2 is utilised. As oxygen is consumed, the redox potential decreases and when the oxygen is depleted (or at very low concentration) the organisms switch to the use of the second-, and third-choice electron acceptors, NO_3^- and NO_2^- respectively. As each of these is consumed, the redox potential decreases until anaerobic conditions (i.e. no O_2, NO_3^- or NO_2^-) are reached.

In IAND and 2RND systems, there is a major difference in the redox potential in the aerobic and anoxic zones. For IAND systems, the scenario described above is applicable, but for 2RND systems a constant redox potential is established in each of the anoxic and aerobic reactors and the organisms, in moving between the two zones, experience a large and sudden change in redox potential.

To examine the differences in redox potential between IAND and 2RND systems, the redox potential (E_h) and NO_3^- and NO_2^- concentrations were measured in the anoxic and aerobic reactors of a 2RND system and throughout the anoxic-aerobic cycle of an IAND system with three cycles (2¼ h aerobic, $5^3/_4$ h anoxic) per day. The IAND system was operated with three (8 h) anoxic-aerobic daily cycles as opposed to about 72 (20 min) daily cycles in order to simulate the operating conditions in the 2RND system with a 3:1 recycle between the aerobic and anoxic reactors, and allow any changes in redox potential and NO_3^- and NO_2^- concentration to become apparent and measurable.

- Redox potential was measured in the anoxic and aerobic reactors of System 5 (see Fig. 7) between Days 88 and 130. In the anoxic zone, the average value was -81 mV and in the aerobic zone, the average value was +48 mV.
- After establishing the above redox potentials, System 5 (DSVI 120 mℓ/g) was changed to an IAND system on Day 130. The DSVI increased over an initial period of 30d to 300 mℓ/g before decreasing to and stabilizing at a DSVI of 180 to 200 mℓ/g by Day 195. On Day 189, redox potential (E_h) was measured throughout one 8 h intermittent aeration cycle and the concentrations of NO_3^- and NO_2^- were measured at intervals of approximately 20 min during the same cycle and are illustrated in Fig. 9.

  Following the introduction of oxygen at the start of the aerobic period (see Fig. 9), the value of E_h increased from -130 mV and attained a value of +40 mV at the end of the 2¼ h

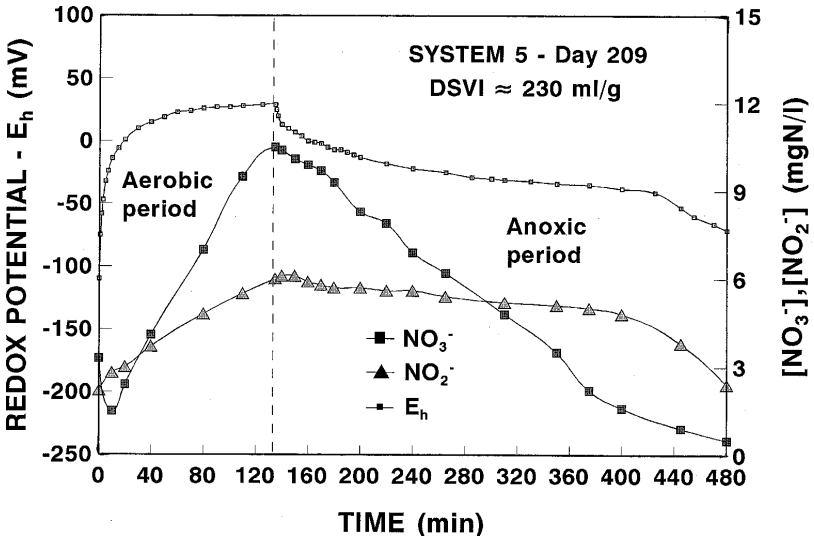


Figure 10

Redox potential (E_h in mV) and nitrate (NO_3^-) and nitrate (NO_2^-) concentrations with time (min) for one 8 h aerated-unaerated cycle of System 5 on Day 209

aerobic period. During the same period the concentration of NO_2^- increased from zero to 2.0 mgN/ℓ after 80 min due to the initial faster nitrification of NH_4^+ to NO_2^- by ammonia oxidising organisms (AOOs) than the rate of NO_2^- to NO_3^- by nitrite oxidising organisms (NOOs). Thereafter the NO_2^- concentration decreased to 0.6 mgN/ℓ at the end of the aerobic period as a result of a decreased nitrification rate of NH_4^+ to NO_2^- by AOOs. [*Note: It is generally accepted that under steady-state aerobic conditions, the growth rate of NOOs is faster than that of AOOs as and nitrite concentrations will be low. For the experimental set-up described here, it is surmised that the anoxic zone that precedes the aerobic zone affects the nitrification rate of NOOs to a greater extent than it affects AOOs, the result being an increase in nitrite concentration. This has support from pure culture work (Ulken, 1963; Schöberl and Engel 1964; Painter, 1970) in which the NOO was considered more sensitive to oxygen depletion than the AOO*]. The concentration of NO_3^- increased from 0.5 mgN/ℓ to 9.0 mgN/ℓ during the same period.

During the first $2^3/_4$ h of the unaerated period, during which time the concentration of NO_3^- decreased from 9.0 mgN/ℓ to less than 1.0 mgN/ℓ as a result of denitrification of NO_3^-, E_h decreased from ≈+40 mV to ≈ -80 mV. During the same period the concentration of NO_2^- increased from 0.5 mgN/ℓ to 0.9 mgN/ℓ as a consequence of the denitrification of NO_3^- to NO_2^- proceeding at a slightly faster rate than the denitrification of NO_2^-. When the nitrate concentration reached a value less than 1 mgN/ℓ (at which time the nitrite concentration was at its maximum value, i.e. ≈1 mgNO_2^--N/ℓ), net nitrite reduction commenced and proceeded simultaneously with nitrate reduction, and E_h initially decreased rapidly, from -80 mV to about -160 mV in 30 min and then more slowly, from -160 mV to about -200 mV over the next 140 min .

As a means of ensuring that the anoxic period had sufficient NO_3^- to avoid anaerobic conditions, NH_4^+ in the form of a solution of ammonium chloride was added to the influent from Day 195 to provide adequate NO_3^- (generated by nitrification of the NH_4^+) during the aerobic period. Addition of NH_4^+ increased the influent TKN/COD ratio from 0.11 to 0.12 mgN/mgCOD. During the 9 d following the E_h, NO_3^- NO_2^- test shown in Fig. 9, the DSVI increased from ≈200 to 220 mℓ/g. On Day 204 the influent TKN/COD ratio was further increased to 0.14 mgN/mgCOD, by increasing NH_4^+ addition to the influent. Between Days 204 and 214, the DSVI increased further from 220 mℓ/g to 240 mℓ/g. On Day 209, a second 8 h anoxic/aerobic cycle was monitored for E_h, NO_3^- and NO_2^- and the results are plotted in Fig. 10. Although the trend in the E_h, NO_3^- and NO_2^- parameters during the aerated period are similar to the first test conducted on Day 189 (Fig. 9), the values of the parameters at the end of the unaerated period (start of the aerobic period) are markedly different: Whereas in the test conducted on Day 189 the nitrate and nitrite concentrations were zero and E_h was -200 mV at the end of the unaerated period, for the test conducted on Day 209, the nitrate and nitrite concentrations were 0.5 and 2.4 mgN/ℓ respectively and the E_h did not decrease below -75 mV during the unaerated period.

Although the original objective of the experiments was to compare the values of E_h measured for the 2RND system with the values measured for the IAND systems, an apparently more significant aspect was identified in comparing the plots of E_h, NO_3^-, NO_2^- and DSVI for the two tests conducted on the IAND system, i.e. one before and one after the addition of NH_4^+ to the influent.

To summarise, during the period in which NH_4^+ was added to the influent (Day 195 to Day 213), the DSVI was high (between 220 and 240 mℓ/g) and increasing (Fig. 7) and the concentrations of NO_3^- and NO_2^- at the end of the anoxic period were high (0.5 and 2.4 mgN/ℓ respectively). In contrast, during the period in which NH_4^+ was not added to the influent (Day 183 to Day 194), the DSVI was lower (180 to 200 mℓ/g) and had decreased from 300 mℓ/g, and the concentrations of NO_3^- and NO_2^- were zero at the end of the anoxic period. Although there appeared to be a relationship between the DSVI of the system and the concentrations of NO_3^- and NO_2^- at the end of the anoxic period, the mechanism by which the one affected the other was unclear at this stage.

Although only two cycles were monitored intensively, it is concluded that the results were typical of the two operating periods, for the following reasons:

- During operation of IAND System 5, with a TKN/COD ratio of 0.11 mgN/mgCOD between Days 183 and 203, daily samples were taken at the end of the anoxic period of one of the three daily 8 h cycles; the concentration of NO_2^- in the majority of samples was less than 0.4 mgN/ℓ.
- After the TKN/COD ratio of the influent to System 5 was increased to 0.14 mgN/mgCOD on Day 204, the concentration of NO_2^- of the majority of the samples taken at the end of the anoxic period was high; between 2.0 and 4.0 mgN/ℓ from Days 204 to 214.

These steady-state measurements indicated that the observations made during the two intensive 24 h monitoring periods of System 5 described above (Days 189 and 209) were a good reflection of the changes in NO_3^- and NO_2^- concentrations throughout the three 8 h daily cycles.

The finding that the system with NO_3^- and NO_2^- present

throughout the anoxic period (DSVI ≈230 mℓ/g) had a greater tendency toward low F/M filament proliferation (type 0041, *M. parvicella* and type 0092) than the system in which NO_3^- and NO_2^- had been reduced to zero by the end of the anoxic period (i.e. the system was partially anaerobic, DSVI ≈180 mℓ/g) appeared to be significant and the results of experiments conducted to examine this aspect are described in the next paper in this series (Musvoto et al., 1999). Two reasons prompted further research into this aspect: all other areas of investigation had been examined, without major indications as to the cause of filament proliferation; and a relationship between the NO_x^- concentrations and filament proliferation had considerable inferential support from earlier experimental work. In particular:

- Addition of the RBCOD and SBCOD components of defined substrate to Systems 1 and 2 resulted in increases and decreases respectively in DSVI, which were associated with increases and decreases in NO_x^- in the system.
- With the direct addition of NO_3^- to System 2, it was concluded that the concentration of NO_x^- had a significant effect on the type and extent of filament proliferation.
- With municipal sewage, variation in the aerobic and anoxic mass fractions of two MUCT systems (Systems 5 and 6) resulted in variations in DSVI, and also resulted in variation in the concentration of NO_x^- in the anoxic reactor preceding the aerobic reactor.
- Warburton et al. (1991) demonstrated that with two IAND systems at 15 d sludge age, to which nitrate was alternatively added and removed, generally, the DSVI increased with increase in nitrate concentration in the effluent and decreased with decrease in nitrate concentration in the effluent.

From these experiments it was clear that some relationship exists between the degree of low F/M filament proliferation and the presence of nitrate and nitrite in the anoxic zone/reactor. However, it was unclear whether the relationship was one of cause or effect.

Closure

From the review of earlier filamentous bulking research (Ekama et al., 1996b), six areas were identified which required experimental investigation to determine their influence on low F/M filament bulking. In this paper the results of an exploratory experimental investigation into these six specific areas are reported.

At this point it is not the intention to evaluate all the information from the investigation for implications regarding the specific conditions required by individual filament types for proliferation as this was not the intention of the exploratory investigation reported here. Rather, the objective of the investigation was to eliminate the factors which have only minor or negligible influence on low F/M filament bulking and to clarify the areas that appear to have a major influence, thereby highlighting the direction that further investigations should take to establish the cause(s) of the low F/M filament bulking problem.

Of the six areas listed for investigation, those that were found to have a minor or no influence on low F/M filament proliferation were:

- Sludge age (provided this is > 10 d).
- Frequency of alternation between anoxic and aerobic conditions.
- Concentration of DO in fully aerobic systems or during the aerobic period or zone of anoxic-aerobic systems.

Factors which apparently have a major influence on low F/M filament bulking were:

- Continuous aerobic and continuous anoxic conditions; these control filament proliferation to low DSVI values (≈100 mℓ/g).
- Alternating anoxic-aerobic conditions (30-40% aerobic, 60-70% anoxic); these conditions result in maximum proliferation of low F/M filaments (*M. parvicella*, *H. hydrossis*, and types 0092, 0041, 1851, 0803); aerobic mass fractions increasingly less than 30% and increasingly more than 40% result in progressively lower DSVI values.
- Reactor configuration; filamentous organisms proliferate in systems with sludges exposed to alternating anoxic-aerobic conditions whether in single reactor, two reactor, or multireactor systems in which nitrate and/or nitrite are present throughout the anoxic period (IAND) or in the anoxic reactor just prior to the aerobic reactor (2RND, MUCT), i.e. nitrate and/or nitrite are present at concentrations exceeding 5 and/or 1 mgN/ℓ respectively, when the sludge is exposed to aerobic conditions.

In the execution and in the analysis of the experimental results discussed in this paper, some factors were identified which were considered to be consequences of the bulking conditions rather than causes of it.

- Systems with sludge exposed to alternating anoxic-aerobic conditions in which the DSVI increases, appeared to demonstrate lower COD mass balances than systems with sludge exposed to continuous aerobic conditions which have low DSVI values.
- Systems with sludge exposed to alternating anoxic-aerobic conditions which develop a high DSVI produce a reduced sludge mass (VSS), and systems which develop sludges with a low DSVI produce an increased sludge mass.

An explanation for these observations emerges from the biochemical/microbiological (bulking) model developed in Casey et al. (1999a) based on a review of the biochemistry of heterotrophic respiratory metabolism (Casey et al., 1999c).

The next paper in this series (Musvoto et al., 1999) examines more closely the role of nitrate and nitrite in low F/M filament bulking. This direction of research is a result of findings that the factors which appear to have a major influence on sludge settleability, appear also to be associated with changes in the concentration of nitrate and nitrite in the system.

Acknowledgements

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