

# Filamentous organism bulking in nutrient removal activated sludge systems

## Paper 11: A biochemical/microbiological model for proliferation of anoxic-aerobic (AA) filamentous organisms

TG Casey, MC Wentzel and GA Ekama\*

University of Cape Town, Department of Civil Engineering, Rondebosch 7701, Cape, South Africa

### Abstract

A model is presented that describes the competitive growth behaviour of floc-forming and anoxic-aerobic (AA) filamentous organisms in long sludge age nitrogen (N) and nitrogen and phosphorus (N&P) removal activated sludge systems. The model, referred to as the bulking model, establishes the potential for filamentous organism proliferation under various aeration and substrate feeding regimes. Based on the principles of the model, system configuration and operational procedures are proposed for the amelioration of bulking and are tested experimentally. To examine the general applicability of the bulking model it is tested by applying it to the experiments described by Lakay et al. (1999) and Musvoto et al. (1999). [Note: Throughout description of the bulking model, the terms filamentous and filaments refer specifically to AA filaments (formerly designated low F/M filaments), which are associated with poorly settling sludges in long sludge age N and N&P removal systems].

### List of symbols

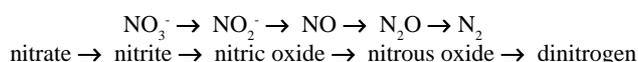
AA	=	anoxic-aerobic filaments
ADP	=	adenosine diphosphate
AOOs	=	ammonia oxidising organisms
ATP	=	adenosine triphosphate
COD	=	chemical oxygen demand
Cyt	=	cytochrome
DSVI	=	dilute sludge volume index (mL/g)
DO	=	dissolved oxygen (mgO/L)
ETP	=	electron transport pathway
FAD	=	flavin adenine dinucleotide
FeS	=	iron-sulphur complexes
FMN	=	flavomononucleotide
NAD	=	nicotinamide adenine dinucleotide
NaR	=	nitrate reductase
ND	=	nitrification-denitrification system
NDBEPR	=	nitrification-denitrification biological excess phosphorus removal
NiR	=	nitrite reductase
NOOs	=	nitrite oxidising organisms
NOR	=	nitric oxide reductase
N <sub>2</sub> OR	=	nitrous oxide reductase
Q	=	ubiquinone
RBCOD	=	readily biodegradable COD
SBCOD	=	slowly biodegradable COD
TKN	=	total Kjeldahl nitrogen (mgN/L)
VSS	=	volatile suspended solids (mg/L)
2RND	=	2 reactor nitrification denitrification system

### Introduction

The objective of the conceptual biochemical model for aerobic facultative heterotrophic organism respiration developed by Casey et al. (1999a, b) was to establish a basis by which the mechanisms of respiration of the facultative organism mass in activated sludge could be understood. In this paper, the biochemical model is applied to filamentous and floc-forming organisms, to develop a microbiological model for substrate competition by these organisms as a means of explaining the proliferation of AA (low F/M) filaments in N and N&P removal systems. Implicit to the formulation of the AA filament bulking model is the assumption of the biochemical model, that both filamentous and floc-forming organisms are aerobic facultative heterotrophic organisms. Filamentous organisms that are considered to be AA filaments are those that proliferate in N and N&P removal systems and sort into Jenkins et al. (1984) low F/M group, viz. *Microthrix parvicella*, and types 0092, 0041, 0675, 1851, 0914 and 0803.

### Statement of hypothesis

In activated sludge systems, floc-formers and filaments compete for mutually growth-limiting substrate. Under completely aerobic or completely anoxic conditions, the floc-formers outcompete the filaments for substrate due to higher substrate utilisation rates, and filament growth is restricted. In ND and NDBEPR activated sludge systems, competition between filamentous and floc-forming organisms for mutually growth-limiting substrate is influenced by inhibition of substrate utilisation by floc-formers under aerobic conditions. Under anoxic conditions, in utilisation of substrate the floc-formers execute the denitrification of nitrate through each of the denitrification intermediates to dinitrogen as follows:



\* To whom all correspondence should be addressed.

☎(021) 650-2588; fax (021) 689-7471; e-mail ekama@engfac.uct.ac.za

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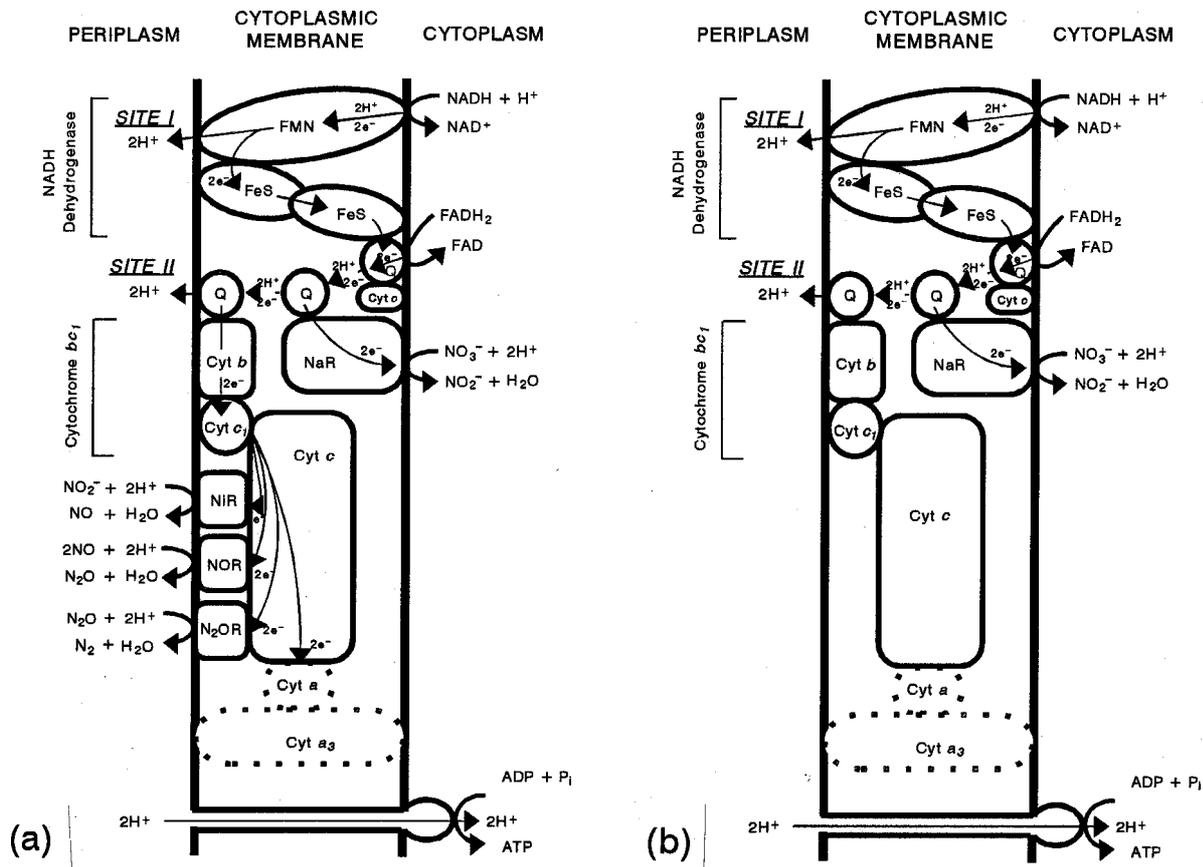
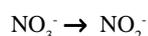


Figure 1  
Models of the ETP for (a, left) floc-forming and (b, right) filamentous organisms under steady-state anoxic conditions

In the absence of, or at low concentrations of readily biodegradable substrate, the intermediate nitric oxide is accumulated intracellularly in the floc-formers. In the subsequent aerobic zone, intracellular nitric oxide inhibits the utilisation of oxygen by floc-forming organisms as a result of the interaction of nitric oxide with one of the enzymes specific to aerobic respiration, the cytochrome oxidase, cytochrome *o*. Under these conditions, floc-forming organisms are inhibited in aerobic respiration. The inhibition of aerobic respiration causes electrons to be redirected to the reductases for nitrite, nitric oxide, and nitrous oxide (aerobic denitrification) and this continues while nitrite remains available. Thus, under aerobic conditions nitrite maintains the intracellular accumulation of nitric oxide, and thereby its inhibitory effect, and higher concentrations of nitrite exacerbate the inhibitory effect. High nitrite concentrations are a result of the inhibitory effect of aerobic conditions on the denitrifying organisms, leading to accumulation of nitrite under anoxic conditions; and the inhibitory effect of unaerated conditions on the nitrifying organisms, in particular nitrite oxidising organisms (NOOs), leading to accumulation of nitrite under aerobic conditions. The inhibition of aerobic respiration and the phenomenon of aerobic denitrification in floc-formers results in lower substrate utilisation rates, and lower nett energy yields.

In contrast to floc-formers, the AA filamentous organisms are nitrate reducers and execute only part of the denitrification pathway, i.e. the reduction of nitrate to nitrite as follows:



They do not accumulate nitric oxide under anoxic conditions and hence are not inhibited in aerobic respiration in the subsequent aerobic zone.

Thus, in systems in which sludge is exposed to alternating anoxic-aerobic conditions with nitrite present in the anoxic zone, floc-formers are placed at a disadvantage in the competition for substrate under subsequent aerobic conditions and filaments gain an advantage. With continued exposure to alternating anoxic-aerobic conditions with nitrite present, filaments proliferate with time.

### Examination of the hypothesis

The implications of the hypothesis for organism (AA filament and floc-former) growth under different environmental conditions are determined by examining the effect of the following conditions: steady-state anoxic conditions, steady-state aerobic conditions, and alternating anoxic-aerobic conditions.

### Steady-state anoxic conditions

Under anoxic conditions in which oxygen is absent and one or both of the ionic nitrogen oxides, nitrate or nitrite is available, a major difference between the AA filaments and floc-formers is in their anoxic respiratory pathways, as described above.

For the floc-formers the ETP is modelled as shown in Fig. 1a: Of the oxidases, only cytochrome *o* is synthesised and cytochrome *aa<sub>3</sub>* either is absent, or present as a basal level; of the reductases,

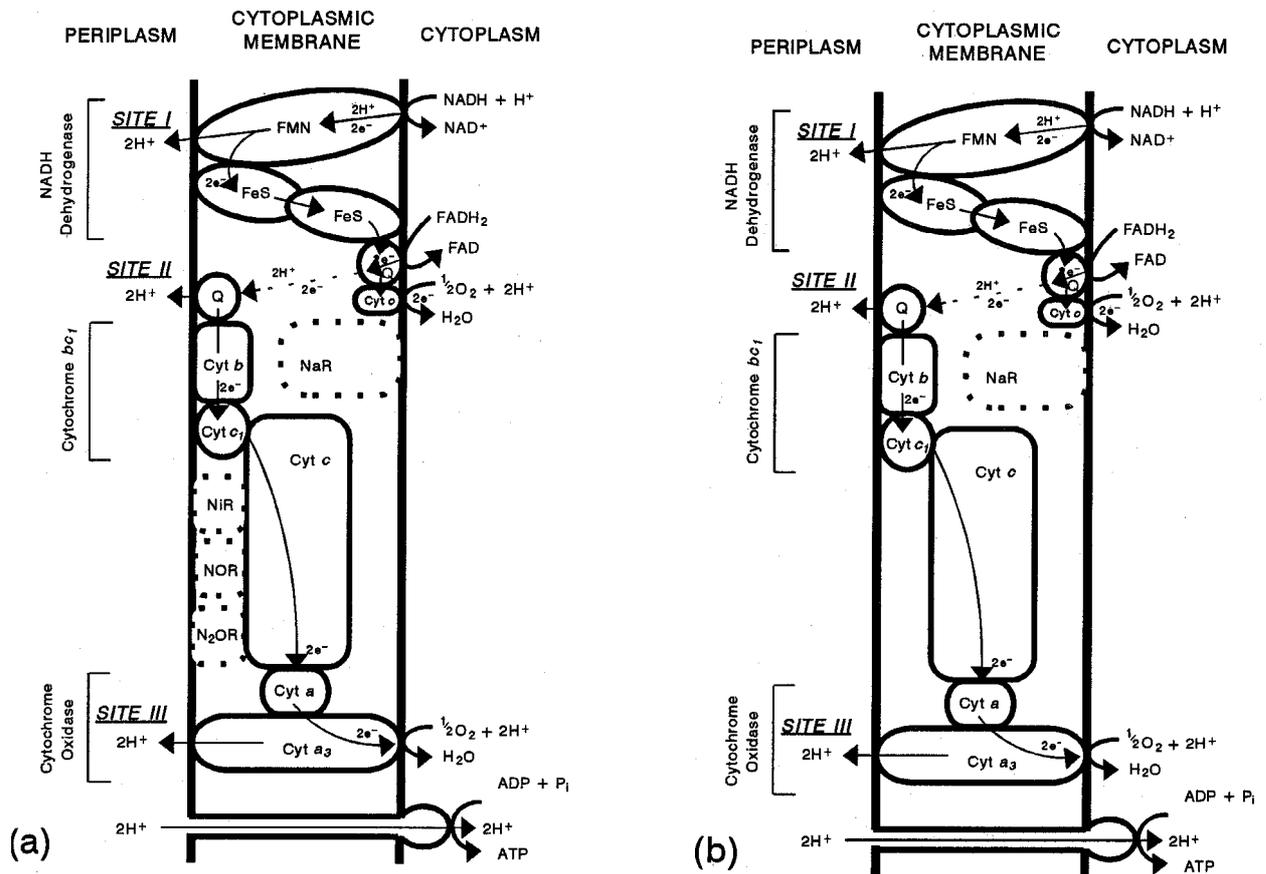


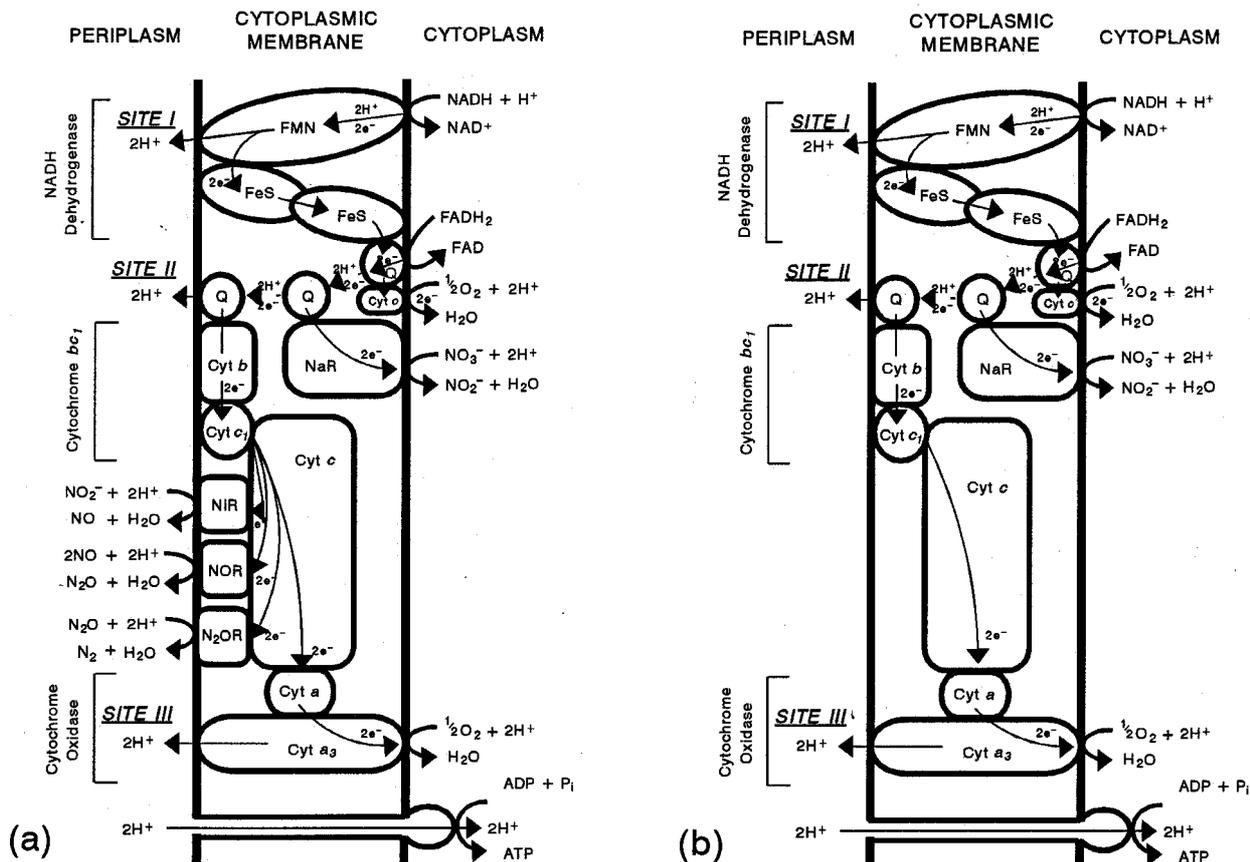
Figure 2 Models of the ETP for (a, left) floc-forming and (b, right) filamentous organisms under steady-state aerobic conditions

all are synthesised to their maximum level. For the AA filaments the ETP is modelled as shown in Fig. 1b: Of the oxidases, only cytochrome *o* is synthesised, and cytochrome *aa<sub>3</sub>* either is absent or present at a basal level; of the reductases, only nitrate reductase is synthesised as illustrated in Fig. 1b.

Under conditions in which nitrate and nitrite are present in sufficient quantities so as not to be limiting, the theoretical energetic yields of the ETP of the two groups of organisms are equal. Electrons, transferred to nitrate-, nitrite-, nitric oxide-, or nitrous oxide reductase for the floc-formers, or to nitrate reductase for the filaments, pass two proton-pumping (energy conserving) sites, Sites I and II. Therefore, under conditions in which nitrate is not limiting, the ability to execute the denitrification pathway through each of the intermediates does not endow the floc-formers with an energetic benefit in comparison with the filaments. Under conditions in which nitrate is limiting and substrate is in excess, the ability of floc-formers to utilise each of the sequentially produced denitrification intermediates as an electron acceptor endows them with greater energetic benefit than the filaments (for which only nitrate can be utilised as electron acceptor). In conclusion, under steady-state anoxic conditions with sufficient nitrate present, the floc-formers gain no energetic advantage over the filaments for each nitrate molecule reduced, and the low proportion of filaments which develop under steady-state (continuous) anoxic conditions (see Lakay et al., 1999) is a consequence of a lower specific substrate utilisation/growth yield for filaments than floc-formers.

### Steady-state aerobic conditions

Under aerobic conditions with a DO concentration greater than 2 mgO/l, the filaments and floc-formers utilise oxygen in preference to nitrate and nitrite as electron acceptor. [Note: For the purposes of the model, it is assumed that the filaments and floc-formers switch from nitrate/nitrite to oxygen as electron acceptor at about the same concentration of DO. However, equal justification could be given to the argument that as a consequence of the protrusion of the filaments from the flocs, the filaments switch from nitrate/nitrite to oxygen at a lower concentration of DO than the floc-formers (oxygen at low concentrations may not penetrate into the floc). This would provide an advantage to the filaments under conditions of frequent anoxic-aerobic alternations]. For floc-formers and filaments, the ETPs of which are modelled as shown in Figs. 2a and b respectively, both of the oxidases cytochrome *o* and cytochrome *aa<sub>3</sub>* are synthesised, cytochrome *aa<sub>3</sub>* to its maximum level and cytochrome *o* to a considerably reduced level ( $\approx 30\%$  of the level under steady-state anoxic conditions), and the reductases are synthesised at a basal level for both floc-formers and filaments respectively. For both floc-formers and filaments, the mechanisms of electron transfer to the complexes and finally to oxygen during respiration are the same as described in the biochemical model for facultative heterotrophs under aerobic conditions (Casey et al., 1999b). In their transfer to oxygen, the greater proportion of electrons pass to cytochrome *aa<sub>3</sub>*, in the process of which all three proton-pumping (energy conserving) sites, Sites I, II and III are passed. Under steady-state aerobic conditions,



**Figure 3**  
Models of the ETP for (a, left) floc-forming and (b, right) filamentous organisms under conditions of alternation between anoxic (60-70%) and aerobic (30-40%) conditions

theoretically, floc-formers gain no energetic advantage over the filaments for each oxygen molecule reduced, and it is concluded that the low proportion of filaments which develop under steady-state continuous aerobic conditions (see experimental results in Still et al., 1996, Ekama et al., 1996 and Lakay et al., 1999) result from a lower specific substrate utilisation/growth yield for filaments than floc-formers.

### Alternating anoxic-aerobic conditions

#### Level of synthesis of enzymes of the ETP

In the preceding two sections, the status of the ETP under steady-state (continuous) aerobic and steady-state anoxic (continuous) conditions is described. As demonstrated by Casey et al. (1999b), maximum synthesis of the oxidases may take up to three sludge ages when conditions change between anoxic and aerobic conditions. However, in ND and NDBEPR systems the residence times in the anoxic and aerobic zones are in the order of hours, and neither the oxidases nor the reductases will be synthesised at their maximum levels. Consequently, the ETPs of the filaments and the floc-formers are conceptualised as having all the complexes present but at lower levels than under steady state anoxic or steady state aerobic conditions. The ETPs of the floc-formers and filaments are modelled as illustrated in Figs. 3a and 3b respectively for conditions under which filaments proliferate, i.e. conditions in which the sludge is subject to alternating anoxic-aerobic conditions (30 to 40 % aerobic), with nitrite present throughout the anoxic zone or

period. The oxidases and reductases are present at a level intermediate between the levels under steady-state aerobic and steady-state anoxic conditions, depending on the proportion of exposure of the organisms to aerobic and anoxic conditions. For the denitrifying reductases, the level of each reductase increases with time under anoxic conditions and decreases with time under aerobic conditions. For the oxidases under anoxic conditions, the level of cytochrome  $aa_3$  decreases and cytochrome  $o$  increases, and under aerobic conditions, the level of cytochrome  $aa_3$  increases and cytochrome  $o$  decreases.

#### Activity of enzymes of the ETP

Activation of reductases of filaments and floc-formers during a change from short (hours) aerobic to anoxic conditions is a consequence of the absence of oxygen. Activation of the oxidases during a change from anoxic to aerobic conditions is a consequence of the presence of oxygen. Inactivation of the reductases during a change from anoxic to aerobic conditions is due to the presence of oxygen. These mechanisms apply to both filaments and floc-formers.

#### Effect on inhibition of aerobic respiration of biodegradability of substrate

For long sludge age ND and NDBEPR systems fed municipal sewage, the majority of substrate available under aerobic and anoxic conditions is SBCOD (70 to 90% for the majority of South African wastewaters). This has implications for the floc-formers with respect to intracellular accumulation of nitric oxide generated

by denitrification of nitrite under anoxic and aerobic conditions. Nitric oxide does not accumulate through denitrification with readily biodegradable substrate under anoxic or aerobic conditions but does accumulate through denitrification with slowly biodegradable substrate under both conditions.

#### ***Inhibition of floc-former substrate utilisation under aerobic conditions***

Of particular interest to this model is a mechanism described at length in Casey et al. (1999b); when facultative organisms are subjected to a change from anoxic to aerobic conditions with nitrite present, they are inhibited in their utilisation of substrate by accumulated intracellular nitric oxide. It is not the intention here to describe in detail the biochemical mechanisms that contribute to inhibition, but rather to indicate the applicability of the mechanisms of inhibition of substrate utilisation under aerobic conditions to filamentous and floc-forming organisms.

The mechanism for inhibition of substrate utilisation under aerobic conditions by nitric oxide is applicable to floc-formers, but not to filaments. This is a consequence of the postulate that under anoxic conditions that precede the aerobic conditions, filaments reduce nitrate to nitrite only, do not produce nitric oxide and are not inhibited under subsequent aerobic conditions. In contrast, floc-formers denitrify nitrate to the end-product dinitrogen through each of the intracellular denitrification intermediates, nitrite, nitric oxide, and nitrous oxide, with nitric oxide accumulating with utilisation of slowly biodegradable substrate. Under subsequent aerobic conditions, intracellular nitric oxide inhibits aerobic respiration. As a consequence, the floc-formers cannot compete with filaments for substrate. In the biochemical model described by Casey et al. (1999b), facultative organisms inhibited in aerobic respiration transfer electrons to the denitrification pathway and denitrify nitrite under aerobic conditions. Similarly, floc-formers inhibited in aerobic respiration denitrify nitrite via the same mechanisms, with an accompanying reduction in substrate utilisation rate and energetic yield with nitrite as electron acceptor compared with oxygen as electron acceptor (as indicated in the biochemical model, nitrate is not denitrified under aerobic conditions due to impermeability of the cytoplasmic membrane to nitrate, preventing access to its reductase).

As described in the biochemical model for facultative respiration, a requirement for inhibition of aerobic respiration is the presence of intracellular nitric oxide during a change from anoxic to aerobic conditions. *However, intracellular nitric oxide cannot be directly or easily monitored; but because nitrite is the precursor to nitric oxide in the denitrification pathway, in the bulking model, nitrite is used as an indicator of the presence of nitric oxide.* In the absence of nitrite during a change from anoxic to aerobic conditions, floc-formers are not inhibited and compete for substrate, and filaments gain no advantage under aerobic conditions. The biochemical interactions that take place during a change to aerobic conditions from anoxic conditions in which nitrite is absent, are described extensively in the biochemical model for facultative organism respiration and apply to the floc-formers. A requirement to maintain inhibition of floc-formers under aerobic conditions is the presence of nitrite and low rate of RBCOD ( $e^-$ ) addition in the aerobic zone. The conditions that cause nitrite to be present at concentrations sufficient to induce and maintain inhibition are described below.

#### ***Cause of high nitrite concentrations***

A condition necessary for filament proliferation is a concentration of extracellular nitrite under anoxic conditions adequate to estab-

lish an intracellular concentration of nitrite and nitric oxide in floc-formers under anoxic conditions such that during a change in conditions from anoxic to aerobic, cytochrome *o* activity is inhibited. To maintain inhibition under aerobic conditions, a sufficient concentration of extracellular nitrite ( $>1.0 \text{ mgNO}_2\text{-N/l}$ ) is required to maintain the intracellular concentration of nitric oxide (from aerobic denitrification) at a level at which inhibition of cytochrome *o* activity is maintained.

The objective of this section is to postulate causes for the high nitrite concentrations ( $>1 \text{ mgNO}_2\text{-N/l}$ ) measured in the aerobic zones of ND and NDBEPR systems.

In anoxic-aerobic activated sludge systems there are two sources of nitrite. In the anoxic zone, nitrite is the product of the reduction of nitrate by nitrate reducing (filamentous) and denitrifying (floc-forming) organisms. In the aerobic zone, nitrite is the product of nitrification of ammonium by the ammonia oxidising organisms (AOOs) species of nitrifying organisms. Concerning the source of nitrite under anoxic conditions, it is generally accepted that under steady-state (continuous) anoxic conditions, the rate of nitrite reduction is equal to the rate of nitrite production (such that nitrite does not accumulate). This is based on the results of denitrification batch tests conducted on sludge developed under completely anoxic conditions with high concentrations of nitrate ( $>10 \text{ mgNO}_3\text{-N/l}$ ) in which nitrite remains at relatively low concentrations ( $<1 \text{ mgNO}_2\text{-N/l}$ ) (see Fig. 16 in Casey et al., 1999b and Ekama and Wentzel, 1999). It was established by Casey et al. (1999b) that for denitrifying organisms grown under anoxic conditions subjected to aerobic conditions, oxygen affects the synthesis of the reductases in the order, nitrous oxide-, nitric oxide-, nitrite-, and then nitrate reductase. Thus, for denitrifying organisms subjected to frequent changes between anoxic and aerobic conditions, the nitrogen oxide reductases are not synthesised to the levels achieved under steady-state anoxic conditions; this effect is least pronounced for nitrate reductase, but is increasingly more pronounced for each successive reductase, from nitrate reductase to nitrous oxide reductase. Thus, under the anoxic period of alternating anoxic-aerobic systems, nitrate reductase is synthesised at a level closer to its anoxic steady-state level than is nitrite reductase resulting in an increase in nitrite concentration during reduction of nitrate under the anoxic period during alternating anoxic-aerobic conditions.

Concerning the source of nitrite under aerobic conditions, it is well established that under steady-state aerobic conditions in which ammonium is not limiting, the rate of nitrification of nitrite to nitrate by NOOs is generally faster than the rate of nitrification of ammonium to nitrite by AOs and nitrite does not accumulate. However, unaerated (anoxic or anaerobic) conditions inactivate the two groups of nitrifying organisms AOs and NOOs such that their rates of oxidation are reduced under subsequent aerobic conditions, and of the two groups, NOOs are more sensitive to low concentrations of DO or the absence of DO than are AOs. As a consequence, under the aerobic conditions of alternating anoxic-aerobic systems nitrite is not nitrified to nitrate at the same rate as it is produced and it accumulates under alternating anoxic-aerobic conditions.

Conceivably, both production of nitrite under aerobic conditions, and production of nitrite under anoxic conditions can contribute to the pool of extracellular nitrite in systems with alternating anoxic-aerobic conditions. Under different system design and operating conditions and with variation in wastewater characteristics, the relative contribution to nitrite by these two sources can change.

## Model evaluation and experimental testing

In the following section, specific implications of the model are experimentally tested and some of the major experimental observations made during the exploratory phase of the experimental programme (Lakay et al., 1999; Musvoto et al., 1999) are examined with the model. All of the factors that were identified as influencing AA filament bulking are now conceptualised into the framework of the bulking hypothesis to demonstrate that the hypothesis can explain the observed behaviour of the experimental systems.

### Effect of presence and absence of nitrite on proliferation of filaments

Under alternating anoxic-aerobic conditions, the presence of nitrite during a change from the anoxic to the aerobic zone should result in the proliferation of filaments, whereas the absence of nitrite under the same conditions would result in reduced filament proliferation. The results of experiments conducted to examine this aspect are discussed in Lakay et al. (1999) and Musvoto et al. (1999) and the effect on AA filament of three aspects concerned with aeration and anoxic/aerobic conditions are discussed below:

### Aeration pattern and anoxic/aerobic mass fractions

**(i) Continuous aerobic and continuous anoxic conditions resulted in low DSVI (< 150 ml/g) values and systems incorporating 30 to 40 % aerobic mass fraction developed high DSVI (> 150 ml/g) values (Lakay et al., 1999, Fig. 5)**

In the exploratory experimental programme described by Lakay et al. (1999), a relationship was established between DSVI and aerobic mass fraction during steady-state operation of System 2 (see Lakay et al., 1999, Fig. 5). For aerobic mass fractions decreasing from 30%, the DSVI decreases. At aerobic mass fractions between 30 and 40% the DSVI attains maximum values, and at aerobic mass fractions increasing from 40% and decreasing from 30%, the DSVI decreases. Further support that this is an observation for systems in general comes from the work of Ketley et al. (1991), Musvoto et al. (1992) and Warburton et al. (1991).

At low aerobic mass fractions (resulting in low DSVI values) three effects combine to promote floc-former growth. Firstly, the associated large anoxic mass fraction (>70%) provides a denitrification potential in excess of that required to denitrify the nitrate and nitrite produced in the system (for an influent TKN/COD ratio  $\approx 0.1$  mgN/mgCOD). Under these conditions, nitrate or nitrite would not be present at the end of the anoxic period of intermittently aerated systems, or in the anoxic reactor of 2-reactor, or multi-reactor systems. Thus, intracellular nitric oxide would not accumulate in the floc-formers under anoxic conditions and they would not be inhibited under subsequent aerobic conditions. Secondly, if nitrite were to be present under the anoxic zone or period of the system with the small aerobic mass fraction, or if the floc-formers do contain intracellular nitric oxide and are inhibited when subjected to aerobic conditions, the small aerobic residence time (< 30% of the total residence time) does not allow the filaments sufficient residence time to gain an advantage. Thirdly, at high anoxic mass fractions (> 70%), the nitrogen oxide reductases are synthesised to a greater extent than at lower anoxic mass fractions and the difference between the levels of each of the reductases under these conditions and their level under steady-state (continuous) anoxic conditions is not significant. As a

consequence of the three effects described above, little nitric oxide or nitrite accumulate intracellularly, and inhibition of aerobic respiration of floc-forming organisms is insignificant and proliferation of AA filaments is also insignificant.

For systems with high aerobic mass fractions, a similar analysis of the levels of the oxidases, the reductases, and the levels of intracellular nitric oxide can be conducted. At high aerobic mass fractions (resulting in low DSVI values), for the floc-formers the denitrifying enzymes are synthesised at a low level, little intracellular nitric oxide is produced and there is little interaction between nitric oxide and the oxidases, resulting in little inhibition of floc-former aerobic respiration and therefore little proliferation of AA filaments.

For systems with 30 to 40% aerobic mass fraction (resulting in high DSVI values), the denitrification potential of the system is exceeded by the nitrate and nitrite load. The denitrifying reductases are synthesised at sufficiently high levels that intracellular nitric oxide accumulates, and cytochrome *o* is synthesised at sufficiently high levels that interaction between nitric oxide and cytochrome *o* inhibits floc-former aerobic respiration, allowing AA filaments to proliferate.

**(ii) With continuous aerobic low DO ( $0.2 < DO < 0.5$  mg/l) conditions, low DSVI (< 100 ml/g) values developed (Lakay et al., 1999, Fig. 8, Days 63 to 94)**

Under conditions of low continuous DO, the oxidases cytochrome *aa<sub>3</sub>* and cytochrome *o* are synthesised at an intermediate level, as are the reductases. For the low DO conditions, it is assumed that both filaments and floc-formers respire aerobically (i.e. with oxygen as electron acceptor). Interaction between nitric oxide and cytochrome *o* results in some redirection of electrons to the denitrification pathway for the floc-formers, however cytochrome *aa<sub>3</sub>* is available (albeit at a low level) for aerobic respiration and although it is not present at as high a level as under continuous high DO conditions, a significant proportion of the oxygen available under the low DO conditions would be reduced at that oxidase. Comparing the yield of the filament respiring on oxygen under the low DO conditions with the floc-formers that would be slightly inhibited and would therefore respire partially on nitrate, the concentration of DO under the low DO conditions would not afford the filaments an energetic advantage and a low DSVI would result.

**(iii) With intermittent aeration conditions, the higher the DO during the aerobic period the higher the DSVI (Lakay et al., 1999, Fig. 8, Days 1 to 62)**

For intermittent aeration with low DO ( $0.2 < DO < 0.5$  mgO/l) in the aerobic period, a low DSVI results, for similar reasons as for continuous low DO systems described above; i.e. the extent of inhibition of aerobic respiration of the floc-formers is low because although the level of synthesis of cytochrome *o* is higher under low DO conditions than under high DO conditions, synthesis of the nitrogen oxide reductases is affected less than under high DO conditions and little nitric oxide accumulates. In addition, the low DO concentration (in which oxygen is limiting) does not give the aerobically respiring filaments sufficient advantage over the floc-formers, which are only partially inhibited by the very small amount of nitric oxide produced under anoxic conditions. For intermittent aeration systems with increasingly higher DO in the aerobic zone, two mechanisms contribute to increased inhibition. Firstly, the increased concentration of DO inhibits synthesis of the

reductases and nitric oxide accumulates. Secondly filaments gain an increasing advantage in respiring under aerobic conditions because oxygen is not limiting. This allows filaments to proliferate to a greater extent at increasingly higher DO concentrations in the aerobic zone of intermittently aerated systems.

A common condition in the above experiments is that when nitrite concentrations greater than about 0.5 mgN/l were measured in the anoxic zone preceding the aerobic zone as a result of either a high TKN/COD ratio or through direct addition of nitrite, the DSVI increased or was high. In the absence of nitrite, the DSVI decreased or was low.

### Substrate biodegradability

#### (i) RBCOD fed throughout the intermittent aeration sequence resulted in high DSVI values (Lakay et al., 1999, Fig. 3 Days 63 to 94; Fig. 4, Days 95 to 127)

This result was unexpected since it was expected that the rate of supply of electrons from RBCOD would be sufficient to remove any accumulated intracellular nitric oxide. Relief of inhibition due to a high rate of supply of electrons was noted in batch test experiments illustrated by Figs. 7 to 9 of Casey et al. (1999b). When RBCOD was fed to the system shown in Fig. 4 of Lakay et al. (1999), the DSVI initially decreased rapidly as expected (proliferation of the AA filament *M. parvicella* was reduced), but the DSVI then increased due to growth of *H. hydrossis*, a filament categorised as low DO, a category of filaments to which the model does not apply.

#### (ii) SBCOD fed throughout the intermittent aeration sequence resulted in high DSVI values (Lakay et al., 1999, Fig. 3, Days 95 to 127, Fig. 4, Days 63 to 94)

Under conditions of high nitrite concentration entering the aerobic zone, a low rate of supply of electrons from SBCOD would be insufficient to remove the intracellular accumulation and inhibitory effect of nitric oxide and floc-formers would be inhibited.

### Frequency of alternation between aerobic and anoxic conditions

Ketley et al. (1991) demonstrated that in an anoxic-aerobic system, irrespective of the frequency of alternation of sludge between the zones (from 72 cycles/d to 1 cycle/3 d), the DSVI remained high. A similar result is noted by Lakay et al. (1999) in comparing the intermittently aerated systems in their Fig. 7 (3 cycles/d) and their Fig. 8 (72 cycles/d), the DSVI values of which were high for both systems.

With nitrite and SBCOD present under alternating anoxic-aerobic conditions, the floc-formers are inhibited. Floc-former inhibition is maintained with time under aerobic conditions until the denitrifying enzymes producing and reducing nitric oxide degrade, and the oxidase (cytochrome *aa<sub>3</sub>*) which is not inhibited is fully synthesised, i.e. 1 to 3 sludge ages. Thus, under conditions in which the sludge is subjected to alternating anoxic-aerobic conditions, unless the aerobic period is longer than the time required to synthesise cytochrome *aa<sub>3</sub>* above a certain level, inhibition of floc-formers is maintained and bulking results.

### Relationship between sludge settleability (in DSVI), nitrite concentration in the 2nd anoxic reactor, volatile suspended solids (VSS)

In experiments described in Musvoto et al. (1999) to examine the effect on DSVI of ammonium addition to the influent, increases and decreases in nitrite concentration in the 2nd anoxic reactor were accompanied by increases and decreases respectively in DSVI (see their Fig. 1). Also, when the 2nd anoxic nitrite concentration and DSVI increased, the VSS decreased, and when the 2nd anoxic nitrite concentration and DSVI decreased, the VSS increased (see their Figs. 2 and 3). These results can be explained through the bulking model - high nitrite concentrations result in inhibition of floc-formers. Interaction of nitric oxide with cytochrome oxidase *o* results in redirection of electrons to nitrite reductase, and resultant aerobic denitrification will yield only two-thirds the energy of uninhibited aerobic respiration. The consequence is a reduction in cell yield and an overall reduction in sludge mass.

### A difference in the relative extents of reduction of nitrate by floc-formers and filaments

If floc-formers denitrify nitrate through each of the denitrification intermediates to the end-product dinitrogen and filaments reduce nitrate to the end-product nitrite, then under anoxic conditions a sludge containing a high proportion of filaments (high DSVI) should accumulate nitrite ( $\text{NO}_2^-$ ), and a sludge containing a high proportion of floc-formers (low DSVI) should produce greater quantities of dinitrogen ( $\text{N}_2$ ).

Sludge samples from two different systems were subjected to a nitrate reduction test, a test that determines the production of  $\text{NO}_2^-$  from reduction of  $\text{NO}_3^-$  and production of  $\text{N}_2$  from denitrification of  $\text{NO}_3^-$ : a fully anoxic system with a low DSVI (Day 253 in System 1 of Lakay et al. (1999); and a 2RND system with a high DSVI (70% unaerated mass fraction) (Day 228 in System 5 of Lakay et al., 1999). The sample with the high DSVI (many filaments) accumulated  $\text{NO}_2^-$ , but did not accumulate  $\text{N}_2$  in 8 out of 10 tests. The sample with the low DSVI (few filaments) accumulated  $\text{N}_2$  but did not accumulate  $\text{NO}_2^-$  in 8 out of 10 tests. The results are interpreted as supportive of the hypothesis that the denitrification pathway is mediated to different extents by filaments ( $\text{NO}_3^- \rightarrow \text{NO}_2^-$ ) and floc-formers ( $\text{NO}_3^- \rightarrow \text{N}_2$ ). While this result could be explained in other ways, the above explanation is the most obvious for the parent systems and test conditions. For example, the results could be interpreted as indicative of the parent system conditions; sludges that develop in systems with no aerated mass fraction (i.e. fully anoxic systems) may be expected to produce a larger proportion of denitrifying organisms and a greater proportion of nitrate reducing organisms than systems with aerated mass fractions (i.e. the anoxic-aerobic 2RND systems). Thus, in systems incorporating an aerobic mass fraction, the extent of formation of  $\text{NO}_2^-$  from  $\text{NO}_3^-$  both in the parent system and in the nitrate reduction test would be expected to be more than in systems that have a low, or no aerobic mass fraction. However, in neither of the two parent systems was the generated  $\text{NO}_2^-$  mass greater than 10% of the  $\text{NO}_3^-$  mass denitrified to  $\text{N}_2$ , indicating that the aerated mass fraction did not develop a population that influenced the extent of denitrification. Therefore the conclusion would appear to be valid that floc-formers can denitrify  $\text{NO}_3^-$  through each of the intermediates to  $\text{N}_2$ , but filaments can reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  only. Some direct support for this hypothesis is provided by Tandoi et al. (1997) - they found that an isolate of *M. parvicella* from an Italian activated sludge (AS)

plant, which was identical to Australian isolates, could reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  but could not denitrify  $\text{NO}_3^-$  to  $\text{N}_2$ .

### **Inhibition of floc-former respiration and filament proliferation results in reduced cell yield**

From the hypothesis it was concluded that inhibition of floc-former aerobic respiration results in aerobic denitrification of nitrite. Casey et al. (1999b) demonstrated that substrate utilisation through denitrification of nitrite produces about two-thirds the energetic yield of aerobic substrate utilisation. This should result in a lower cell yield for floc-formers and a lower net sludge mass production in systems in which floc-formers are inhibited and filaments proliferate.

Experiments were not conducted expressly to investigate this aspect. However, in examining the results of systems in which a high DSVI developed (floc-formers inhibited) (MUCT systems 1 to 4 in Musvoto et al., 1999; see also Musvoto et al., 1994), the relationship between DSVI and VSS was investigated and is described above.

In summary, for sludge subjected to alternating anoxic-aerobic conditions with nitrite present, floc-former aerobic respiration is inhibited by interaction of nitric oxide with cytochrome oxidase *o*, filaments proliferate, and the DSVI increases. Electrons (originating from COD) are then directed to nitrite reductase and under aerobic conditions nitrite is reduced. Because of the lower yield of respiration with nitrite compared to respiration with oxygen, the VSS of the sludge is less than under conditions in which inhibition of floc-former aerobic respiration is not induced (i.e. under continuous aerobic conditions, or under alternating anoxic-aerobic conditions in which nitrite is absent). Under conditions in which floc-formers are not inhibited, electrons are not redirected to nitrite reductase and nitrite is not utilised under aerobic conditions.

### **Control procedures for AA filament bulking**

In summary, experimental examination of the implications of the model and application of the model to a range of experimental results provide support for the validity of the model. To assist in development of procedures for control of filament proliferation, the major aspects of the model can be devolved to four main principles, which in combination result in filament proliferation:

- (1) Alternating anoxic-aerobic conditions result in development of both the respiratory oxidases and the reductases in the facultative floc-formers and filaments.
- (2) In alternating anoxic-aerobic systems the absence of high concentrations of readily biodegradable substrate and the presence of nitrite and slowly biodegradable substrate under anoxic conditions results in the accumulation of intracellular nitric oxide in floc-formers during denitrification.
- (3) In the aerobic zone of alternating anoxic-aerobic systems, accumulated nitric oxide results in inhibition of floc-formers, and a high concentration of nitrite under aerobic conditions maintain floc-former inhibition.
- (4) Filamentous organisms (which reduce nitrate only as far as nitrite in the denitrification pathway) do not produce nitric oxide and are not inhibited under aerobic conditions.

The ultimate objective in the investigation was to formulate a procedure by which AA filament bulking in ND and NDBEPR systems could be ameliorated. To this end, methods have been developed for avoiding, preventing or removing one or more of the

conditions listed above, which in combination result in filamentous organism bulking. With reference to the principal aspects of the model listed above, attention is directed at points (1), (2) and (3), but not (4). Regarding (4), the physiology of the filaments (or floc-formers) cannot be altered. Regarding (1), a necessity for nitrogen removal is that sludge be exposed to a combination of aerobic (for nitrification) and anoxic (for denitrification) conditions. However, to limit bulking the aerobic mass fraction range 30 to 40% should be avoided (as illustrated in Fig. 5 of Lakay et al., 1999). With reference to points (2) and (3), the bulking model proposes that if the nitrite concentration is reduced to near zero in the anoxic zone, or if intracellular nitric oxide is significantly reduced under aerobic or anoxic conditions, then the floc-formers will not be inhibited and significant bulking is unlikely to occur.

In a design situation, estimation of  $\text{NO}_2^-$  concentration is still uncertain as the denitrification kinetics of this parameter have not been well established for the activated sludge process. To prevent high concentrations of  $\text{NO}_2^-$  prior to the aerobic zone in ND and NDBEPR systems, the most appropriate solution at present is to design the anoxic mass fraction or the aerobic-anoxic (a) recycle of the system such that the denitrification potential of the system is greater than the mass of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  recycled to it, i.e. complete denitrification in the anoxic zones is achieved. This can be accomplished by increasing the un-aerated mass fraction, or reducing the a-recycle.

In the event that inflexible system configurations do not allow for reduction of the a-recycle, or unusual influent wastewater characteristics do not practically allow for removal of all  $\text{NO}_3^-$  and  $\text{NO}_2^-$  recycled to the anoxic reactor or zone, attention is directed not at the extracellular  $\text{NO}_2^-$ , but at intracellular  $\text{NO}$ , and provisions can be made for its reduction under both aerobic and anoxic conditions. These are:

- Inclusion of a small aerobic reactor immediately upstream of the main aerobic reactor to which a fraction of the influent sewage or another readily biodegradable organic material is fed. From the biochemical model, intracellular  $\text{NO}$  is reduced rapidly under aerobic conditions with RBCOD and inhibition is not induced. Experiments with aerobic batch tests (see Casey et al., 1999b) in which inhibition is relieved in the presence of sufficient quantities of RBCOD offer support for this proposal.
- Inclusion of a small anoxic reactor immediately upstream of the main aerobic reactor to which a fraction of the influent sewage is fed - from the biochemical model, intracellular  $\text{NO}$  is reduced rapidly under anoxic conditions with RBCOD and inhibition is not induced under subsequent aerobic conditions.

It should be noted that no laboratory- or pilot-scale experimental work has been conducted to directly test the role of a small high rate denitrification reactor upstream of the main aerobic reactor.

### **Closure**

A wide-ranging experimental programme (Ekama et al., 1996, Lakay et al., 1999 and Musvoto et al., 1999) investigating at laboratory-scale the problem of filamentous organism bulking (formerly low F/M bulking - now renamed AA bulking), resulted in the development of a biochemical model describing the fundamental respiratory mechanisms of facultative heterotrophic organisms (Casey et al., 1999a). Application of the biochemical model to filamentous and floc-forming facultative organisms provided

the means to develop a model that explains AA filamentous organism proliferation or non-proliferation. The model was evaluated against experimental data and shown to provide an explanation for observations relating to AA filament bulking. From the conceptual model, proposals for measures by which the bulking problem can be reduced or eliminated were identified.

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