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A HYPOTHESIS FOR THE CAUSES AND CONTROL OF ANOXIC–AEROBIC (AA) FILAMENT BULKING IN NUTRIENT REMOVAL ACTIVATED SLUDGE SYSTEMS

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ABSTRACT

From laboratory research and a literature review of the biochemical pathways of aerobic–facultative heterotrophic organisms, an hypothesis is proposed for the proliferation of anoxic–aerobic (AA) filamentous organisms in nitrification–denitrification (ND) and nitrification–denitrification biological excess phosphorus removal (NDBEPR) systems. In activated sludge, under anoxic conditions floc-forming organisms execute the denitrification of nitrate (NO_3^-) through each of the denitrification intermediates to dinitrogen (N_2), in the process of which the intermediate nitric oxide (NO) is accumulated intracellularly. Intracellular NO is inhibitory to the utilization of oxygen in the subsequent aerobic zone. In contrast, the filamentous organisms execute only part of the denitrification pathway, i.e. the reduction of NO_3^- to NO_2^-; they do not accumulate NO and hence are not inhibited in the subsequent aerobic zone. Thus in anoxic–aerobic systems, floc–formers are placed at a disadvantage in the aerobic zone giving an advantage to the filaments in the competition for substrate. Experimental evidence to support this hypothesis is presented and a tentative proposal of a strategy for control of AA filament proliferation is described and tested experimentally.

KEYWORDS

Low F/M filaments, AA filaments, filamentous bulking, floc–former, activated sludge, anoxic, aerobic, intermittent aeration, denitrification.

INTRODUCTION

Jenkins *et al.* (1984) categorized the filamentous organisms implicated in activated sludge bulking by the condition that overtly appears to give rise to the proliferation of that category of organism. They listed five conditions – low DO, low F/M, septic wastewater, nutrient deficiency, and low pH. This association between filament category and causative condition implies that if the causative condition is removed the associated filaments will decline in number and bulking will be ameliorated. The causative condition ascribed to the filaments which usually proliferate in ND and NDBEPR systems is low F/M. Historically, control of low F/M filament proliferation had been to increase the F/M ratio, implemented by incorporation of a selector reactor (Chudoba *et al.*, 1973). However, in a review of investigations into the efficacy of anoxic and aerobic selectors, Gabb *et al.* (1991) concluded that no conclusive evidence exists that low F/M filaments are controlled by this approach. This implied that the low F/M condition associated with these filaments is not a dominant cause of their proliferation; consequently other causes were sought.

In experiments with fully aerated, fully anoxic and intermittently aerated single reactor continuously fed activated sludge systems which received real and synthetic sewage, Gabb *et al.* (1989) and Casey *et al.* (1990, 1991) found that the low F/M filaments (*Microthrix parvicella* and types 0092, 0041,

1851, and 0675) did not proliferate in fully aerobic or fully anoxic low F/M systems, but proliferated in intermittent aeration systems. Because the low F/M condition did not appear to influence the bulking significantly, whereas alternating anoxic–aerobic conditions did, the filamentous organisms responsible were renamed anoxic–aerobic (AA) filaments. It was still unclear, however, as to why the switching of the sludge between anoxic and aerobic states induces AA filament proliferation.

This paper describes briefly, (1) experimental initiatives that led to (2) an hypothesis on bulking by AA filaments, (3) experimental evidence supporting this hypothesis and (4) proposed procedures for amelioration of AA filament growth. A brief outline of this hypothesis with some experimental verification has been presented in a short communication by Casey *et al.* (1992b).

BACKGROUND RESEARCH

It was noted above that earlier work by Gabb *et al.* (1989), and Casey *et al.* (1990, 1991) established that AA filaments proliferate under intermittent aeration conditions, but not under fully aerobic or fully anoxic conditions. However they did not establish which factors in intermittent aeration promote filament proliferation. In work with a defined artificial substrate fed to intermittently aerated nitrification–denitrification (IAND) systems, it was found that maximum filamentous organism proliferation occurred when the aerobic mass fraction comprised between 30 and 35% of the total, as shown in Fig 1. IAND systems with municipal sewage as influent and operated with different aerobic mass fractions, produced results similar to the IAND systems fed artifical substrate, although the DSVIs were considerably lower with the municipal sewage (Casey *et al.*, 1990, 1991). However, a two–reactor nitrification–denitrification (2RND) system operated with a 30% aerobic mass fraction did not bulk (Hulsman *et al.*, 1992). This raised the question as to why IAND and 2RND systems operated under apparently identical conditions (i.e. aerated/unaerated mass fractions, influent wastewater, temperature and sludge age) exhibited markedly dissimilar sludge settleability – high DSVI for the IAND system (≈ 200 mℓ/g), and low DSVI for the 2RND system (≈ 150 mℓ/g). Possible causes for the disparate behaviour were sought in the following differences between the two systems: (1) frequency of alternation of sludge between anoxic and aerobic zones, i.e., 2RND – low (1–3 times/d), IAND – high (≈ 72 times/d); (2) influent feed distribution between the aerobic and anoxic zones, i.e., 2RND – anoxic reactor only, IAND – feed split between anoxic and aerobic zones in proportion to the aerobic–anoxic mass fractions; (3) concentration of dissolved oxygen in aerobic zone, i.e., 2RND – constant, IAND – variable.

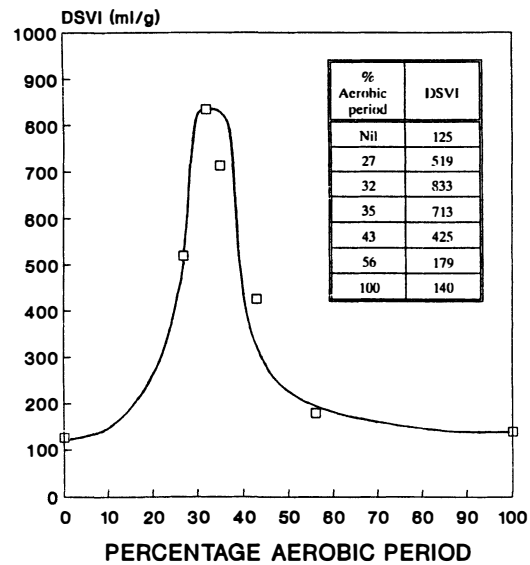

Fig 1: Dilute sludge volume index (DSVI, in mℓ/g) with percentage aerobic period for a long sludge age (15d) IAND system fed artificial defined substrate.

Noting the differences listed above, appropriate modifications were made to the configuration of a 2RND system and its operation in order to approximate the IAND condition, with the objective of identifying the factor that promotes filament proliferation in IAND systems, viz., (1) The a–recycle, between the anoxic and aerobic reactors, was increased from 1:1 to >30:1; this did not increase the low DSVI (≈ 150 mℓ/g) (Hulsman *et al.*, 1992). (2) The a–recycle was changed back to 1:1 and the influent feed was split into 2 streams, in the same proportion as the aerated/unaerated mass fractions, and these fed to the respective aerobic and anoxic reactors; this did not increase the low DSVI (≈ 150 mℓ/g). (3) With the feed split a small unaerated reactor (10% of total mass fraction) was included in the a–recycle in order to simulate the gradual reduction of DO in the aerobic period of an IAND system; the DSVI did not increase but decreased from ≈ 150 mℓ/g to ≈ 120 mℓ/g. It was concluded that none of the noted differences between 2RND and IAND systems could account for the differences in AA filament proliferation. Because of this lack of success in developing a bulking sludge in the 2RND system, attention was directed to the IAND system. An IAND system was set up using the sludge from the 2RND system, with the same aerobic mass fraction (30%), sludge age (15 days), number of anoxic–aerobic alternations (three daily 8 hr intermittent aeration cycles – $5^3/_4$ hrs anoxic, $2^1/_4$ hrs aerobic). Background reading into the biochemical behaviour of organisms under anoxic–aerobic changes indicated that some of the denitrification intermediates have an effect on subsequent aerobic growth. Accordingly the study of the IAND system included measurement of the redox potential (E_h), and NO_3^- and NO_2^- throughout the cycle. After the system attained steady–state, the DSVI was ≈ 180 mℓ/g. The changes throughout the cycle in E_h, NO_3^- and NO_2^- are shown in Fig 2. To study the effect of changes in the three variables, ammonium (NH_4^+) was added to the influent to increase the TKN/COD ratio from ≈ 0.11 to ≈ 0.14 mgN/mgCOD. The DSVI increased from 180 to 230 mℓ/g; changes throughout the cycle in E_h, and NO_3^- and NO_2^- are shown in Fig 3. Before the increase in TKN/COD ratio, NO_3^- and NO_2^- were absent at the end of the anoxic period; after the increase, NO_3^- and NO_2^- were present at the end of the anoxic period (compare Figs 2 and 3). It was concluded that the increase in DSVI appeared to be linked to the presence of NO_3^- and NO_2^- at the end of the anoxic period. However it was not clear as to whether, NO_3^- or NO_2^-, had the greater influence, and experiments were conducted to determine this.

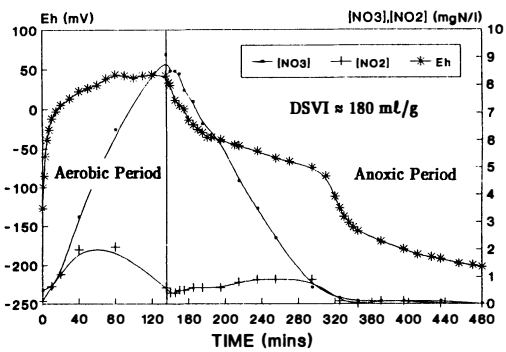

Fig 2: Redox potential (E_h, in mV) and nitrate and nitrite concentrations (NO_3^- and NO_2^-,in mgN/ℓ) with time, during an 8hr intermittent aeration cycle of a long sludge age (15d) IAND system with a DSVI ≈ 180 mℓ/g.

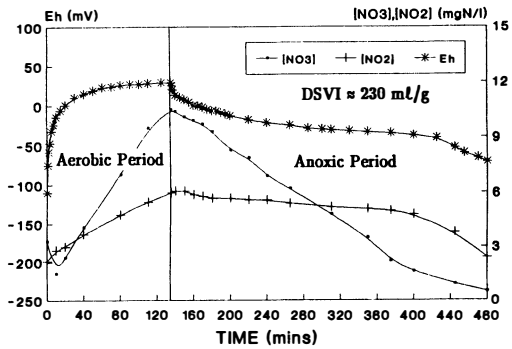

Fig 3: Redox potential (E_h, in mV) and nitrate and nitrite concentrations (NO_3^- and NO_2^-, in mgN/ℓ) with time, during an 8hr intermittent aeration cycle of a long sludge age (15d) IAND system with a DSVI ≈ 230 mℓ/g.

To study the effect of NO_2^- on AA filament proliferation, two identical MUCT configurations were set up; one, a control, was operated with a high TKN/COD ratio ≈ 0.13 (NH_4^+ added to the influent), the other with a low ratio ≈ 0.09 (no NH_4^+ added to the influent). In the control, the NO_2^- in the 2nd anoxic reactor ranged between 0.5 and 1.5 mgN/ℓ and the DSVI between 200 and 250 mℓ/g. For the system with the low TKN/COD ratio, the NO_2^- in the 2nd anoxic reactor ranged between zero and 0.4 mgN/ℓ and the DSVI between 100 and 150 mℓ/g (see Fig 4). After 62 days operation, NO_2^- was added by continuous drip feeding to the 2nd anoxic reactor of the low TKN/COD system. The NO_2^- in the 2nd anoxic reactor increased to a high but variable value (1–10 mgN/ℓ) and the DSVI increased rapidly to between 200 and 230 mℓ/g. After 35 days, NO_2^-

addition was terminated; the NO_2^- in the 2nd anoxic reduced to ≈ 0.3 mgN/ℓ and the DSVI declined within 3 days to 170 mℓ/g and within 25 days to 150 mℓ/g.

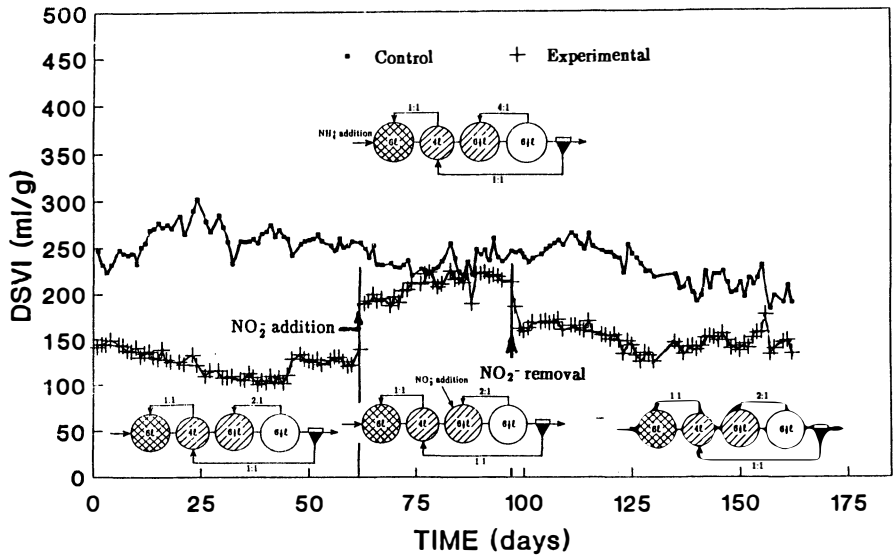

Fig 4: Dilute sludge volume index (DSVI, in mℓ/g) with time, for two MUCT systems (Control and Experimental)to which ammonium (NH_4^+) was added to the influent of the Control system and NO_2^- was added to and removed from the 2nd anoxic reactor of the Experimental system.

With regard to the role of NO_3^- in AA filament proliferation in MUCT systems, Musvoto *et al.* (1992) examined separately the effect of the NO_2^- and NO_3^- concentrations in the 2nd anoxic reactor. They concluded that although NO_3^- concentration in the second anoxic reactor had an effect on DSVI, the effect of NO_2^- concentration was significantly stronger. In both sets of experiments with NO_2^- addition to MUCT systems, the rapid increases in DSVI resulted from the proliferation of the AA filament, type 0092.

The experimental investigations above indicated that the causative conditions for AA filament bulking in ND and NDBEPR systems appear to be a result of (1) alternating anoxic and aerobic conditions, and (2) the presence of certain denitrification intermediates in the anoxic zone before the subsequent aerobic zone. The alternating anoxic–aerobic conditions would indicate that both the filaments and floc–formers which develop in these systems are aerobic–facultative heterotrophs. Very little was understood of the basic behaviour of this type of organism since the mechanisms which allow facultative organisms to switch between anoxic and aerobic metabolism are biochemical in nature. An extensive review was undertaken of the respiratory biochemistry of aerobic–facultative heterotrophs. From this study it was possible to develop a biochemical model for aerobic–facultative heterotrophs and using this model, to develop an hypothesis to explain AA filament bulking.

BIOCHEMICAL MODEL FOR AEROBIC–FACULTATIVE HETEROTROPHS

The metabolic pathways for denitrification have been studied extensively by microbiologists and biochemists, with particular emphasis being placed on identification of the intermediates generated by the reactions, and on characterization of the enzymes which mediate the reactions. A comprehensive conceptual model of the biochemical mechanisms exhibited by aerobic–facultative heterotrophs has been developed and will be presented in a future paper. For the purposes of this paper the model is presented with only the biochemical detail necessary for its understanding.

Payne (1973) proposed a denitrification pathway in which each of the nitrogen oxides (NO_3^-, NO_2^-, NO, and N_2O) are reduced at separate and specific enzyme complexes called reductases, i.e.:

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

nitrate → nitrite → nitric oxide → nitrous oxide → dinitrogen

Pure culture studies have demonstrated that one or more of the denitrification intermediates generated under anoxic conditions have an inhibitory effect on the utilization of substrate under subsequent aerobic conditions, i.e., with oxygen as terminal electron acceptor. Krul (1976) in pure culture studies on a denitrifying organism (isolated from activated sludge), cultured under anoxic conditions and tested under aerobic conditions, concluded that the intermediate NO accumulates intracellularly during denitrification of NO_3^- and NO_2^- under anoxic conditions and that this causes a measurable and prolonged inhibition of oxygen utilization under subsequent aerobic conditions. The inhibition results from the interaction of NO with the enzyme responsible for oxygen reduction, i.e., cytochrome oxidase. Krul (1976) demonstrated this inhibition for a pure culture of an isolate from activated sludge, not for the activated sludge mixed culture.

The degree of inhibition due to NO is exacerbated by the presence of NO_2^- and NO_3^-. Kučera *et al.*, (1987), and Carr and Ferguson (1990) attributed this to the maintenance of a high level of NO from denitrification of NO_3^- and NO_2^-.

Initially denitrification was considered a strictly anoxic process, occurring only in the total absence of oxygen. However, later in pure cultures it was demonstrated that denitrification can continue under aerobic conditions, albeit at a lower rate (Pichinoty and d'Ornano, 1961; Showe and De Moss, 1968; Krul and Veeningen, 1977; Robertson and Kuenen, 1984). The continuation of denitrification under aerobic conditions takes place when intracellular NO, accumulated under anoxic conditions, is present under subsequent aerobic conditions. The intracellular NO interacts with cytochrome oxidase, inhibiting aerobic substrate utilization and in the process stimulates production of more NO through reduction of NO_2^- under aerobic conditions.

HYPOTHESIS FOR AA FILAMENT BULKING

With the model of aerobic–facultative biochemical behaviour as a basis, the following hypothesis for the proliferation of AA filaments in ND and NDBEPR systems is proposed.

In these systems, the majority of heterotrophic organisms can be classified by their morphological characteristics as either filamentous or floc–forming organisms. The floc–forming organisms are aerobic–facultative; under aerobic conditions they utilize oxygen and under anoxic conditions they denitrify NO_3^- or NO_2^- to N_2 as described by the denitrification pathway outlined earlier. The filamentous organisms are also aerobic–facultative but are nitrate reducers only; under anoxic conditions they reduce NO_3^- to NO_2^- only.

When sludge is exposed to alternating anoxic–aerobic conditions in which NO_3^- and/or NO_2^- are present *throughout* the anoxic period, floc–formers denitrify NO_3^- and NO_2^- through each of the denitrification intermediates to the end–product N_2, in which process some level of intracellular NO is established. When a floc–former with intracellular NO is subjected to aerobic conditions, the NO inhibits the utilization of oxygen (and concomitantly the utilization of substrate). Furthermore, while NO is present under aerobic conditions, the floc–formers continue to respire with NO_2^- (i.e. aerobic denitrification), albeit at a much reduced rate to that under anoxic conditions. For filamentous organisms, under anoxic conditions NO is not an intermediate and hence does not accumulate intracellularly; consequently these organisms are not inhibited in their utilization of substrate under subsequent aerobic conditions. When sludge is exposed to alternating anoxic–aerobic conditions in which NO_3^- and/or NO_2^- are *not* present at the end of the anoxic period (i.e. all NO_3^- and NO_2^- have been denitrified completely to N_2), no intracellular NO is present in the floc–formers and under subsequent aerobic conditions substrate utilization is not inhibited.

With intracellular NO present, and inhibition induced, floc–formers are placed at a disadvantage with respect to the filaments in competition for substrate under aerobic conditions. Consequently, the filamentous organisms utilize a greater proportion of the substrate under aerobic conditions than they would if the floc–formers were not inhibited. With floc–forming organisms inhibited, the filamentous organisms increase their relative mass in the sludge with each exposure to aerobic conditions, leading to the condition referred to as a bulking sludge.

EXPERIMENTAL EXAMINATION OF THE HYPOTHESIS

To determine whether or not inhibition of oxygen utilization (and correspondingly substrate utilization) takes place in activated sludge subjected to switching from anoxic to aerobic conditions, a series of batch tests was conducted on sludge harvested from the anoxic reactor of a 2RND system. In these tests, different anoxic or aerobic pretreatment conditions were imposed and subsequently sewage was added, the batch test aerated, and the OUR measured. In the absence of inhibition, Ekama *et al.* (1986) showed that on addition of sewage which contains readily biodegradable COD (RBCOD) and slowly biodegradable COD (SBCOD) the OUR increases virtually instantaneously to some maximum value. This increase is principally due to the RBCOD fraction. The OUR remains at this maximum level until the RBCOD is depleted, at which point there is a precipitous decrease to a level governed by SBCOD and nitrification.

Anoxic denitrification. To determine the effect on OUR of NO_2^- denitrified under prior anoxic conditions, NO_2^- was added at the start of a two hour anoxic period of the batch test. After this period sewage was added, the system aerated and the OUR measured with time. In a series of 3 tests different amounts of NO_2^- were added at the beginning of the anoxic period, such that at the beginning of the aerobic period approximately 25.0, 5.5 and 0.1 $mgNO_2^-$–N/ℓ were present. On aeration, with addition of substrate, the OURs measured are shown plotted in Fig 5. Inhibition is indicated by a lowered initial but increasing OUR. Inhibition was significant with 25.0, less so with 5.5 and relatively insignificant with 0.1 $mgNO_2^-$–N/ℓ at the start of the aerobic period. These batch tests demonstrate that (1) due to the presence of NO_2^- in the anoxic period, inhibition of OUR is induced and (2) the degree of inhibition is directly related to the concentration of NO_2^- at the commencement of aerobic conditions. From the review of the denitrification pathways, the observed OUR inhibition results from the presence of intracellular NO originating from the denitrification of NO_2^-; the higher the concentration of external NO_2^-, the higher the concentration of intracellular NO, and the greater the inhibition of OUR.

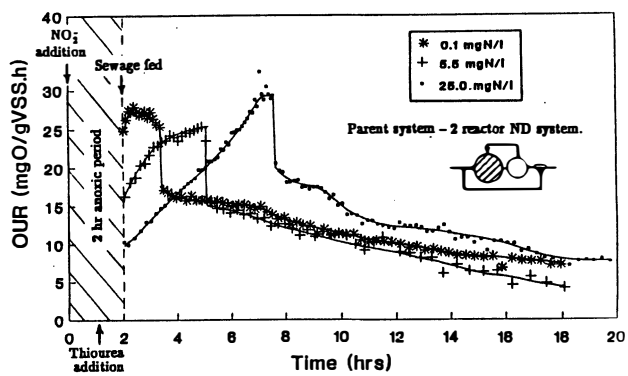

Fig 5: Oxygen utilization rate [OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (NO_2^- and NO_3^-, in mgN/ℓ) with time, under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2RND system with a 2hr anoxic period prior to the aerobic test and with varying nitrite concentrations at the start of the aerobic test, demonstrating the inhibitory effect of NO_2^- on maximum specific OUR.

Aerobic denitrification. To determine whether activated sludge from the 2RND system exhibited denitrification of NO_2^- under aerobic conditions in the absence of RBCOD, aerobic batch tests were conducted on specially prepared sludge samples from the anoxic reactor. In the preparation, first, virtually all of the NO_3^- and NO_2^- were removed from the sludge by diluting with tap water, settling and decanting the supernatant 3 successive times. Then the sludge was held anoxic in the presence of about 120 mgCOD/ℓ sewage in order to denitrify any remaining NO. After 2 hrs of anoxic conditions, during which thiourea was added (10 mg/ℓ) to inhibit NO_2^- formation by *Nitrosomonas*, aeration commenced ($2.0 < DO < 4.0$ mgO/ℓ). After 1 hr aeration when all the RBCOD had been utilized and only SBCOD was present, 20 $mgNO_2^-$–N/ℓ final batch volume was added. After a further 1 hr aeration, 360 mg/ℓ (final batch volume) sewage was added and the OUR, NO_3^- and NO_2^- concentrations measured. Figure 6 shows that OUR inhibition is exhibited. A control batch test with identical conditions except no NO_2^- addition exhibited no OUR inhibition; this indicates

that the inhibition in the batch test with NO_2^- addition must have been induced during the one hr aeration period after NO_2^- addition with only SBCOD present. In a similar test but with NO_3^- (20 mgN/ℓ) instead of NO_2^- addition, no inhibition is exhibited (Fig 7). These tests indicate that NO inhibition under aerobic conditions takes place with NO_2^-, (the NO apparently produced by aerobic denitrification of NO_2^-) but not with NO_3^- (aerobic denitrification of NO_3^- does not occur). The results of these batch tests were reproduced with sludges from IAND systems and Modified UCT (MUCT) NDBEPR systems. Results such as the finding that NO_2^- but not NO_3^- can be denitrified under aerobic conditions can be explained adequately only through the comprehensive conceptual biochemical model for aerobic–facultative heterotrophs.

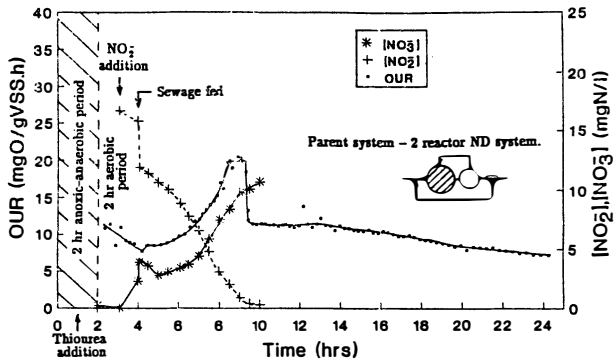

Fig 6: Oxygen utilization rate [OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (NO_2^- and NO_3^-, in mgN/ℓ) with time, under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2RND system with a 2hr anoxic–anaerobic period prior to a 2hr aerobic period during which NO_2^- was added (20 mgN/ℓ), prior to the aerobic test.

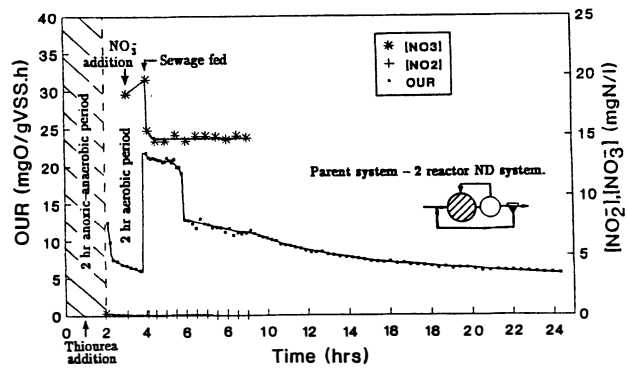

Fig 7: Oxygen utilization rate [OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (NO_2^- and NO_3^-, in mgN/ℓ) with time, under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2RND system with a 2hr anoxic–anaerobic period prior to a 2hr aerobic period during which NO_3^- was added (20 mgN/ℓ), prior to the aerobic test.

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

To check if OUR inhibition takes place in the presence of significant quantities of RBCOD, an aerobic batch test was conducted in which NO_2^- was added after the sewage addition but while RBCOD still was present, rather than before sewage addition when only SBCOD is present (from organism death) as in the previous batch experiments. In this test, the results of which are shown in

Fig 8, no inhibition was noted, and it was concluded that the presence of RBCOD (in sufficient quantity) under aerobic conditions prevents or relieves the inhibition. This supports the proposal above, that RBCOD accelerates the NO → N_2 steps of the pathway such that NO is not accumulated.

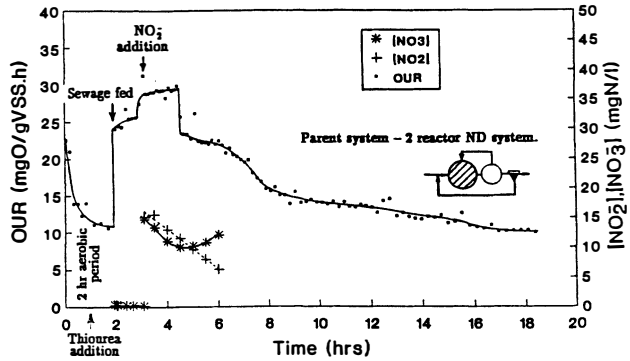

Fig 8: Oxygen utilization rate [OUR, in mgO/(gVSS.h)] and nitrite and nitrate concentrations (NO_2^- and NO_3^-, in mgN/ℓ) with time, under aerobic batch conditions (nitrification inhibited) on sludge harvested from a 2RND system with a 2hr aerobic period prior to the batch test and NO_2^- added (15 mgN/ℓ) after sewage addition, demonstrating the lack of inhibition induced under aerobic conditions in the presence of sufficient quantities of RBCOD.

Determination of the extent of NO_3^- reduction and denitrification under anoxic conditions by filaments and floc-formers. With the experiments above, it was demonstrated that the OUR in sludges switching between anoxic and aerobic conditions is inhibited under certain conditions. From the experimental results and the model of aerobic-facultative organisms, this inhibition is due to the presence of intracellular NO, arising either from aerobic or anoxic denitrification. In the hypothesis for bulking by AA filaments, it has been proposed that the inhibition acts on the floc-formers and not on the filaments. For the proposed explanation to be acceptable, it needs to be shown that (1) floc-formers denitrify from NO_3^- to N_2, and so are susceptible to OUR inhibition by accumulated NO, whereas the AA filaments reduce NO_3^- to NO_2^- only, and consequently do not accumulate NO and so are not susceptible to inhibition.

To test this, sludge samples from a fully anoxic system (low DSVI) and a 2RND system (high DSVI), both fed municipal sewage, were subjected to a nitrate reduction test, a test which determines the generation of NO_2^- and/or N_2 from reduction and denitrification respectively of NO_3^-. The sample with the high DSVI (many AA filaments) showed an accumulation of NO_2^- with no N_2 being detected in 8 out of 10 tests. The sample with the low DSVI (few AA filaments) accumulated N_2 but no NO_2^- in 8 out of 10 tests. These results *could* be interpreted as indicative of the parent system N removal behaviour, in that sludges developed in systems with increased unaerated mass fractions (i.e. the fully anoxic system) would develop a greater proportion of denitrifying organisms such that the extent of formation of N_2 from NO_3^- would be increased both in the parent system and in the nitrate reduction test. However, in neither of the two parent systems was the generated NO_2^- mass greater than 10% of the NO_3^- mass denitrified to N_2, indicating that the unaerated mass fraction has little effect on whether NO_2^- accumulates through incomplete denitrification of NO_3^-. Thus the results were interpreted as supportive of the hypothesis that the denitrification pathway is mediated to different extents by filaments (NO_3^- → NO_2^-) and floc-formers (NO_3^- → N_2).

CONTROL PROCEDURES FOR AA FILAMENT BULKING

From the experimental work presented in this paper, one aspect of the hypothesis that has significant support is that if the intracellular NO is reduced to near zero in the anoxic zone then the floc-formers will not be inhibited under subsequent aerobic conditions and significant bulking is unlikely to occur. However, NO itself cannot serve as a control or design parameter because it cannot be measured readily. Thus NO_2^- must serve as the surrogate parameter because NO_2^- is the source of NO through the process of denitrification. In a design situation, estimation of NO_2^- concentration is still uncertain as the denitrification kinetics of this parameter have not been

established. To prevent high concentrations of NO_2^- prior to the aerobic zone, the most appropriate solution at present is to design the anoxic mass fraction/a–recycle aspects of the system such that the denitrification potential of the anoxic reactor is greater than the mass of NO_3^- and NO_2^- recycled to it, i.e. complete denitrification in the anoxic reactor is achieved. In the event that inflexible system configurations or unusual influent wastewater characteristics do not practically allow for removal of all NO_3^- and NO_2^- recycled to the anoxic reactor, other provisions can be incorporated by which NO can be reduced as much as possible.

(1) Inclusion of a small aerobic reactor between the anoxic and main aerobic reactor to which a fraction of the influent sewage is fed – it was shown earlier in the paper in aerobic batch tests, that inhibition is relieved in the presence of adequate RBCOD. This proposal is currently under investigation.

(2) Inclusion of a small anoxic reactor between the anoxic and the main aerobic reactor to which a fraction of the influent sewage is fed – consideration of the biochemical model denitrification pathways would suggest that in the presence of a readily assimilable substrate, NO does not accumulate intracellularly and inhibition will not be induced. This proposal has been investigated (Casey *et al.*, 1992a) and the experiments are described below.

An anoxic (DENOX) reactor comprising 4% of the total sludge mass of an MUCT system was added between the 2nd anoxic reactor and the aerobic reactor, and 10% of the influent feed was introduced to the DENOX reactor as shown in Fig 9. Between days 50 and 76, the DSVI decreased from > 150 mℓ/g to < 100 mℓ/g. During the same period, the DSVI of a control system increased from > 160 mℓ/g to > 220 mℓ/g. Following removal of the DENOX reactor from the experimental system and addition of the DENOX to the control system, the DSVIs of the two systems initially acted in accordance with expectation; the DSVI of the system now without the DENOX reactor increased from < 100 mℓ/g to ≈ 170 mℓ/g in 20 days (up to day 97) and the DSVI of the DENOX system decreased from > 220 mℓ/g to ≈ 110 mℓ/g in 20 days. On Day 97 the DSVIs of the systems began to deviate from the expected behaviour. The DSVI of the DENOX system increased to between 150 and 170 mℓ/g and the DSVI of the other system decreased to ≈ 100 mℓ/g. At present, no definitive judgement can be made as to the cause of this deviant behaviour, but one possible explanation is that the changes in DSVI were a consequence of septic sewage – the septic sewage filament 021N was identified as the second most common filamentous organism (type 0092 was dominant). However, from the experimental results obtained thus far, the DENOX reactor appears to hold promise as a strategy for control of AA filaments.

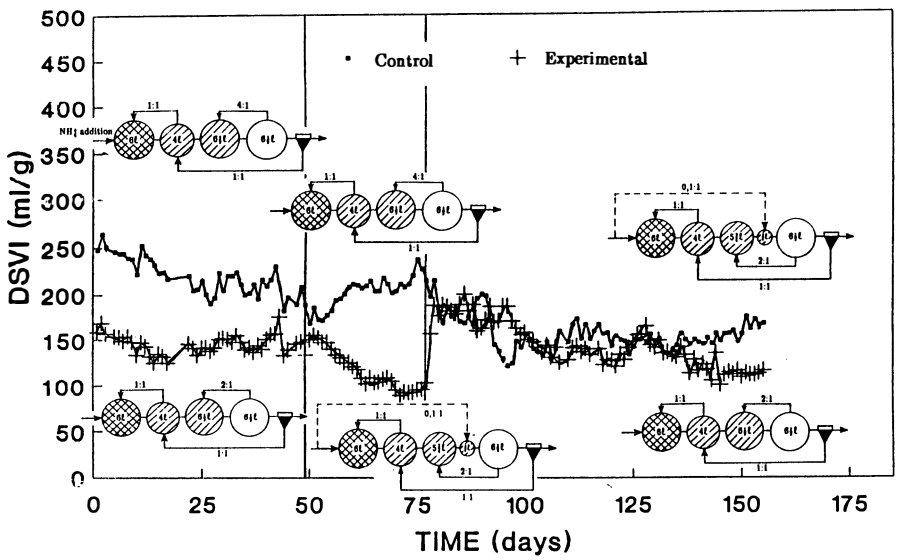

Fig 9: Dilute sludge volume index (DSVI, in mℓ/g) with time, for two MUCT systems (Control and Experimental) to which a small denitrification (DENOX) reactor was added between the 2nd anoxic and aerobic reactors.

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