CAUSES AND CONTROL OF LOW F/M FILAMENT BULKING IN NUTRIENT REMOVAL ACTIVATED SLUDGE SYSTEMS

by

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EXECUTIVE SUMMARY

Specific control of filamentous bulking in biological N and N & P removal plants has been undertaken principally at laboratory scale by the Water Research Group (WRG) at UCT under this and the previous contracts with the Water Research Commission (WRC) (K5/542 and K5/286 respectively).

This research has shown that the promoted specific control method of selectors, which stimulate removal of influent readily biodegradable COD in anaerobic, anoxic or aerobic selectors by metabolic or kinetic selection, are not successful for controlling bulking in biological N&P removal plants. It was found that the conditions that stimulate biological N removal are conducive to bulking on nutrient removal plants - stated simply (but not completely) that if denitrification is not complete (nitrate and nitrate concentrations >2 mgN/ℓ) at the time conditions switch from anoxic to aerobic, then proliferation of low F/M (renamed Anoxic-Aerobic to more accurately describe the conditions under which they proliferate) filaments takes place.

In this project two 18 month laboratory scale investigations were undertaken. In the first two Modified University of Cape Town (MUCT) nitrification denitrification (ND) biological excess P removal (BEPR) systems were operated at 12 and 20°C at 12 days sludge age and in the second a Modified Ludzack Ettinger (MLE) ND and a UCT NDBEPR system at 30°C and 10 days sludge age. In all four systems the anoxic reactors preceding the aerobic reactors were underloaded with nitrate so that very low concentrations of nitrate and nitrite would be present at the transition from anoxic to aerobic conditions. Sludge settleability and filamentous organisms also were regularly determined and identified. Because the changes in filamentous organism populations and abundance and sludge settleability are such slow processes, the opportunity was taken in these investigations to also measure the temperature sensitivity of the ND and BEPR biological nutrient removal processes. Indeed, reading this report, it would appear that this was the main objective of the investigation because so much more information is given on this aspect than on the filamentous organisms and sludge settleability. The reason this was done, in particular the ND process rates is because the nitrate and nitrite concentrations, and hence the AA filament bulking if the hypothesis holds merit, are dependent on these two processes.

The filaments most frequently dominant in both the MLE, MUCT and UCT systems were, types 0092, 0041, *Microthrix parvicella* and type 1851. Type 021N and *Haliscomenobacter hydrossis* were also frequently observed but not at dominant levels. Apart from type 021N, these filaments are classified as typical of the low F/M category (Jenkins *et al.*, 1984) and are almost always observed in laboratory (Ekama *et al.*, 1996) and full scale (Blackbeard *et al.*, 1986, 1988) ND and NDBEPR systems. During the changes in DSVI in the systems, the filament types did not change significantly and therefore the different DSVIs were caused by

essentially the same filament types at different levels of abundance. The nitrate plus nitrite concentrations (NO_x, mgN/ℓ) in the anoxic reactors of the systems were generally low (< 1.0 mgN/ℓ) because the anoxic reactors were underloaded with nitrate, except when sewage batches with high TKN/COD ratios (>0.10 mgN/mgCOD) were fed to the systems which occurred sporadically during the investigations. The DSVI in the MUCT systems operated at 12 and 20°C conformed to expectation that when the NO_x concentration entering the aerobic reactor was high (> 1mgN/ℓ), the DSVI increased and *visa versa*. After a long period of low NO_x concentration, the DSVI was low at about 100 mℓ/g. In the MLE and UCT systems operated at 20 and 30°C, the outcome is less clear. While some periods during this investigation show decreasing DSVI with low anoxic NO_x concentrations in the systems, and would therefore support the AA filament hypothesis, other periods are in conflict with this, e.g. decreasing DSVI with high anoxic NO_x concentrations.

A large body of laboratory scale evidence of an indirect nature supporting this alternative explanation for bulking in nutrient removal plants has been accumulated in the two research contracts. Some direct support for the 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 nitrate to nitrite but could not denitrify nitrate to nitrogen gas. Also, increasingly more indirect supportive evidence from full scale nutrient removal plants in Europe is appearing in the literature - in particular at the recent Activated Sludge Population Dynamics Specialized Conference (*Water Sci Technol* **29**(7) 1994) (see Ekama, 1994). The observations of Eikelboom (1994) and Foot *et al* (1994) summarize this anecdotal evidence particularly well (see Section 1 below): The problem being worse in spring, worse with settled wastewater, an inverse relationship between total filament length and effluent total oxidized nitrogen, the efficacy of selectors decreasing if denitrification is permitted to take place in the main reactor (due to underaeration or plant load increase) are all factors that influence the denitrification performance of the plant and increase the likelihood of significant nitrate and nitrate concentrations being present at the transition from anoxic to aerobic conditions and therefore provides indirect support for the bulking hypothesis. Although not unequivocally, this was shown to be the case in the two laboratory investigations undertaken under this contract at 12, 20 and 30°C.

In order to establish the merits of the AA filament bulking hypothesis, two types of full scale studies and one labaratory study need to be undertaken: The first fullscale study with identical parallel modules, one operated to produce a good settling sludge in terms of the bulking hypothesis and the other module to produce a poor settling sludge; the second full scale study to evaluate full scale plant operating data to see whether or not the mechanisms implicated in the hypothesis are operative in plants with good and bad settling sludges and the laboratory scale study with an intermittently aerated single reator system operated with a redox controller, which would can be set such that nitrate and nitrite concentrations are always low before the aerobic (aeration) conditions commence. Proposals to do such full scale studies, the first at Mitchell's Plain WWTP, Western Cape in a tripartite project between the Cape Metropolitan Council, UCT and the WRC and the second between Stewart Scott Inc. and the WRC, have been approved by the WRC. Also, preparations to undertake the laboratory scale study at UCT have been made and a proposal submitted to the WRC. The work at full scale will be difficult due to the continuously varying conditions under normal plant operating conditions, but it will demonstrate whether or not the AA filament bulking hypothesis has merit for bulking control in fullscale nutrient removal activated sludge systems.

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Papers, reports and other contributions published during contract period
(January, 1993 to December 1996)

1. Refereed Papers

Casey TG, Wentzel MC, Loewenthal RE, Ekama GA and Marais GvR (1994) A hypothesis for the causes and control of anoxic-aerobic (AA) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 203-212.

Musvoto EV, Casey TG, Ekama GA, Wentzel MC and Marais GvR (1994) The effect of incomplete denitrification on anoxic-aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(4), 295-299.

Casey TG, Ekama GA, Wentzel MC and Marais GvR (1995) Filamentous organism bulking in nutrient removal activated sludge systems Paper 1: An historical overview of causes and control. *Water SA*, **21**(3), 321-338.

Still DA, Ekama GA, Wentzel MC, Casey TG and Marais GvR (1996) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 2: Stimulation of the selector effect under aerobic conditions. *Water SA*, **22**(2), 97-118.

Ekama GA, Wentzel MC, Casey TG and Marais GvR (1996a) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 3: Stimulation of the selector effect under anoxic conditions. *Water SA*, **22**(2), 119-126.

Gabb DMD, Ekama GA, Jenkins D, Wentzel MC, Casey TG and Marais GvR (1996a) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 4: System configurations and operating conditions to develop low F/M filament bulking sludges at laboratory-scale. *Water SA*, **22**(2), 127-137.

Gabb DMD, Ekama GA, Jenkins D, Wentzel MC, Casey TG and Marais GvR (1996b) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 5: Experimental examination of aerobic selectors in anoxic-aerobic systems. *Water SA*, **22**(2), 127-137.

Ekama GA, Wentzel MC, Casey TG and Marais GvR (1996b) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 6: Review, evaluation and consolidation of results. *Water SA*, **22**(2), 147-152.

2. Papers submitted for publication

Lakay MT, Hulsman A, Ketley DA, Warburton CA, De Villiers ME, Casey TG, Wentzel MC and Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 7: Exploratory experimental investigations. (Submitted to *Water SA*).

Musvoto EV, Lakay MT, Casey TG, Wentzel MC and Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 8: The effect of nitrate and nitrite. (Submitted to *Water SA*).

Casey TG, Wentzel MC and Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 9: Review of biochemistry of heterotrophic respiratory metabolism. (Submitted to *Water SA*).

Casey TG, Wentzel MC and Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 10: Metabolic behaviour of heterotrophic facultative aerobic organisms under aerated/unaerated conditions. (Submitted to *Water SA*).

Casey TG, Wentzel MC and Ekama GA. Filamentous organism bulking in nutrient removal activated sludge systems. Paper 11: A biochemical/ microbiological model for proliferation of anoxic-aerobic (AA) filamentous organisms. (Submitted to *Water SA*).

3. Conference Papers

Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993) Causes and control of low F/M filamentous bulking in nutrient removal activated sludge systems. *Procs. International Workshop on prevention and control of bulking activated sludge*; Eds. Jenkins D, Ramadori R and Cingolani L, Luigi Bazzucchi Centre, Perugia.

Mellin HKO, Rintala J, Karsisto S, Viitasaari M, Wentzel MC, Ekama GA and Marais GvR (1995) Integrated treatment of a cold municipal wastewater and a thermophilically pretreated effluent from a paper mill. *Procs. 5th IAWQ Symposium on forestry industry wastewater*, Vancouver, June, 6pp.

The first two refereed papers were presented at the 1st IAWQ Activated Sludge Population Dynamics (ASPD) Specialist Seminar in Paris, September, 1993.

4. Articles

Ekama GA (1994) A selection of impressions and statements from papers and posters presented at 1st IAWQ ASPD Conference (Paris, 1993), on filamentous bulking, *IAWQ ASPD Newsletter*, 7(1), 16-19.

Casey TG, Wentzel MC, Loewenthal RE, Ekama GA and Marais GvR (1994) Reply to "Is incomplete denitrification a key factor influencing anoxic-aerobic filamentous organism proliferation in DN/N activated sludge systems" by Chudoba P. (1994), *IAWQ ASPD Newsletter*, 17(1), 12-14.

Casey TG, Ekama GA, Wentzel MC and Marais GvR (1993) Causes and control of low F/M filamentous bulking in nutrient removal activated sludge systems, *Water Sewage and Effluent*, **13**(4), 10-26.

5. Reports

Casey TG, Wentzel MC, Ekama GA and Marais GvR (1994) Development and evaluation of specific control methods for ameliorating low F/M filament bulking. Final Report on 4-year (1989-1992) Water Research Commission (WRC) contract K5/286, WRC, Report No. 286/1/93 (UCT Research Report No. W82), WRC, P O Box 824, Pretoria, 0001, South Africa.

Casey TG, Wentzel MC, Ekama GA and Marais GvR (1994) Causes and control of low F/M filamentous bulking in long sludge age nutrient removal activated sludge systems, Final Report to WRC, No. 286/2/93 (UCT Research Report No. W83), WRC, P O Box 824, Pretoria, 0001, South Africa.

*de Villiers ME, Casey TG, Wentzel MC and Ekama GA (1994) The effect of nitrate and nitrite concentrations on low F/M filament bulking in nitrogen removal activated sludge systems. Research report No. W81, Dept. of Civil Eng., Univ of Cape Town, Rondebosch, 7701, RSA.

*Pilson RA, Ekama GA, Wentzel MC and Casey TG (1995) The effect of temperature on denitrification kinetics and biological excess phosphorus removal in nutrient removal activated sludge systems in temperate climates (12°C - 20 °C), Research Report No. W86, Dept. Of Civil Eng., Univ of Cape Town, Rondebosch, 7701, RSA.

*Mellin HKO, Wentzel MC and Ekama GA (1995) The effect of temperature on denitrification kinetics and biological excess phosphorus removal in nutrient removal activated sludge systems in tropical climates (20°C - 30°C), Research Report No. W91, Dept. of Civil Eng., Univ of Cape Town, Rondebosch, 7701, RSA.

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Causes and control of low F/M filament bulking in nutrient removal activated sludge systems
Final report on the four year research contract K5/542 (1993-1996) for the Water Research Commission

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1 SUMMARY REPORT

1.1 Filament Identification and Causes of Bulking

Jenkins *et al.* (1984a) attempted to define the wastewater characteristics, system design parameters and operating conditions conducive to proliferation of different filamentous organism types from surveys of filamentous organisms in USA activated sludge plants. The outcome of this work is set out in Table 1. Of the 29 recognised filament types, only 11 are of major importance as a result of their widespread occurrence and dominance in activated sludge plants. Ten of the 15 filament types listed in Table 1 account for about 90% of the bulking incidents in the surveyed plants and five broad categories of causes were identified, i.e. (i) low dissolved oxygen (DO) concentration, (ii) low Food/Micro-organism (F/M) ratio, (iii) septic/high sulphide wastewater, (iv) nutrient deficiency and (v) low pH. Ranking of filaments in order of frequency of occurrence in different countries is given in Table 2. The lists of the United States and Europe (see also ATV, 1989) are similar in that the top 6 to 8 most frequently occurring filaments are from 4 of the 5 causative categories, but both differ substantially from those for South Africa and Australia where 6 of the top 8 filaments are all low F/M ones. At the time of the USA and European surveys (early 80s), the plants were principally aerobic with relatively short sludge ages (high F/M ratios). In contrast, South African plants have long sludge ages (20-30 days) and usually incorporate anoxic-aerobic or anaerobic-anoxic-aerobic zones for biological N and N&P removal. Therefore, while the filament populations that develop in the USA and European plants can be expected to similar, the significant differences in environmental factors imposed on the organisms in South African long sludge age biological N and N&P removal plants clearly give rise to considerably different filament populations. From the predominance of low F/M filaments in Australian plants it seems that these plants are similar to those in South Africa. However, as Europe and the USA increasingly implement biological nutrient removal (BNR) plants, it can be expected that the problem filaments will become more predominantly the low F/M group. It is mainly for this reason that the research relating to causes and control of this filament group, which has formed the essential part of this research contract, is reviewed below in some detail and includes the research conducted under this and previous contracts.

While the grouping of filaments into causative categories was a significant advance towards understanding the causes and control of filamentous bulking, Strom and Jenkins (1984) cautioned against rigorous application of the approach in that "at present most of the filament types can only be categorised to a few broad groupings of associated conditions", implying that at a specific treatment plant, conditions may be present other than those listed in Table 1, which can change the filament types. Nevertheless, this association between filament type and causative condition has found considerable practical application and identification of these indicators has come to be regarded as the first step in ameliorating sludge bulking problems (Jenkins *et al.*, 1984a, 1993; Wanner, 1994a,b). However research conducted over the past decade since publication of the valuable work of Jenkins *et al.* (1984a,b), which includes the research conducted under this contract, have led to refinements in Table 1, in particular in the low F/M category.

Table 1: Dominant filament types indicative of conditions causing activated sludge bulking (Jenkins *et al.*, 1984a).

Causative Conditions	Indicative filament types
Low F/M ratio	*M.parvicella*, types 0041, 0675, 0092, 0581, 0961, 0803, 021N, *Haliscomenobacterhydrossis*, *Nocardia* spp., 0914*, 1851.*
Low dissolved oxygen	type 1701, *Sphaerotilus natans*, *H.hydrossis.*
Presence of sulphide	*Thiothrix* spp., *Beggiatoa* spp.; type 021N.
Low pH	Fungi.
Nutrient deficiencies	*S.natans*, *Thiothrix* spp., type 021N and possibly *H.hydrossis*, and types 0041 and 0675.

* Included in the low F/M group by Blackbeard *et al.* (1986, 1988) due to their frequent dominance with the other low F/M filaments.

Table 2: Comparison of dominant filamentous organisms in bulking sludges from activated sludge plants in different countries.

	Ranking in order of prevalence				
Filamentous organism	USA[1]	Holland[2]	West[3] Germany	Austr-alia[4]	South Africa[5]
Nocardia sp.	1	-	-	6	7
Type 1701	2	5	8	16	8
Type 021N	3	2	1	7	13
Type 0041	4	6	3	1	6
Thiothrix sp.	5	19	-	14	15
Sphaerotilus natans	6	7	4	13	15
Microthrix parvicella	7	1	2	2	2
Type 0092	8	4	2	3	1
Haliscomenobacter hydrossis	9	3	6	4	12
Type 0675	10	-	-	1	4
Type 0803	11	9	10	12	8
Nostocoida limicola	12	11	7	5	10
Type 1851	13	12	-	9	3
Type 0961	14	10	9	11	14
Type 0581	15	8	-	-	16
Beggiatoa sp.	16	18	-	-	-
Fungi	17	15	-	-	-
Type 0914	18	-	-	8	5

1. Richard *et al.* (1982) and Strom and Jenkins (1984): 525 samples from 270 treatment plants.
2. Eikelboom (1977): 1 100 samples from 200 treatment plants.
3. Wagner (1982): 3 500 samples from 315 treatment plants.
4. Seviour *et al.* (1993): 50 samples from 50 treatment plants
5. Blackbeard *et al.* (1986): 96 samples from 96 treatment plants.

1.2 Specific Control of Low F/M Filament Bulking

Specific control of the filaments in the low F/M category is of major importance - this is the largest filamentous organism group and not only do these filaments cause practically all of the bulking problems in South African N and N & P removal plants but also increasingly cause bulking problems in European plants as biological nutrient removal systems are implemented there (Pujol *et al.*, 1991; Andreasen and Sigvardsen, 1993; Rossetti *et al.*, 1994; Eikelboom, 1994; Kunst and Reins, 1994; Foot *et al.*, 1994; Seviour *et al.*, 1994, Casey *et al.*, 1995).

Although the most ubiquitous, the low F/M category unfortunately is rather vaguely defined. No explicit definition for low F/M is specified so it presumably arose mainly to distinguish between high and low F/M ratio plants. The difficulty in clearly defining this category is that at long sludge ages (low F/M), a wide range of different operating conditions and configurations is possible, each of which may stimulate proliferation of different filaments. With the introduction of N and N & P removal plants, the plant configurations and operational conditions have changed drastically compared with the conventional high-rate fully aerobic systems prevalent in Europe and the USA before the 90s. In the N and N&P removal systems other factors such as unaerated mass fraction, frequency of alternation between anoxic and aerobic conditions, low DO concentrations at the anoxic-aerobic transition, anoxic or aerobic reactor nitrate and nitrite concentrations may be more important than the F/M (sludge age) in causing bulking. Casey *et al.* (1992,1993,1994) have found this to be so and have renamed the low F/M category Anoxic- Aerobic (AA), a name more descriptive of the conditions that apparently lead to their proliferation. A brief overview of the research and development in low F/M filament bulking control is given below commencing with the selection criterion of Chudoba *et al.* (1973a,b; 1974) which has featured prominently in this.

1.3 Chudoba's Selection Criterion

Chudoba *et al.* (1973a,b;1974) proposed an organism selection criterion as an explanation for the occurrence or non-occurrence of filamentous bulking. This criterion is based on competition between floc-formers and filaments for the mutually limiting *soluble* substrate as follows: In the Monod formulation for the specific rate of growth of organisms, filaments have lower values for both the maximum specific growth rate (μ_H) and the half saturation coefficient (K_s) than floc-formers. Consequently at low substrate concentrations the filaments have a higher specific growth rate than the floc-formers and at high substrate concentrations, a lower specific growth rate.

Over the past 15 years this selection criterion has provided a framework for research into the causes of bulking and its control by specific methods. Results reported by a number of investigators who have measured the Monod constants of various filaments and floc-formers, appear to fit within the structure of the selection criterion: Van Den Eynde *et al.* (1982a,b) showed that in general, organisms with high μ_H rates have high K_s values and ones with low μ_H rates have low K_s values. Slijkhuis (1983) measured the μ_H of *M. parvicella* one of the principal filaments causing low F/M bulking to be 1.6/d; this is considerably lower than a μ_H of 4.33/d measured by Richard *et al.* (1982) for a floc-former isolated from activated sludge.

Palm *et al.* (1980) extended the selection criterion to incorporate limiting nutrients; for some filaments (the low DO ones), the limiting nutrient apparently is oxygen whereas for others, the limiting nutrient is the soluble substrate concentration surrounding the organism, as originally

conceived by Chudoba *et al.* (1973a,b). With regard to low DO bulking, Hao *et al.* (1983) and Lau *et al.* (1984a,b) confirmed the work of Palm *et al.* (1980). From dual species studies they showed that low DO filaments (*S. natans*, type 1701) and floc-formers can be selectively grown by manipulating the DO concentration - if high, the floc-former dominates; if low, the filament dominates.

With regard to bulking in long sludge age (low F/M) systems, Chudoba *et al.* (1973a,b) tested the selection criterion with pure soluble substrates; they controlled the substrate concentration surrounding the organism by having different configurations for the activated sludge system. In a single reactor completely mixed system, the substrate concentration was low throughout the reactor whereas in a multi-reactor plug flow system the substrate concentration was high in the upstream section and low in the downstream section. They found that in aerobic single reactor completely mixed systems, filamentous organisms proliferated causing bulking whereas in aerobic multi-reactor plug flow systems filamentous organisms did not proliferate and a good settling sludge was maintained. From this work, Chudoba *et al.* (1973b) developed the selector reactor for filamentous bulking control, the purpose of the selector being to remove the influent readily biodegradable (RB) COD while at a high concentration before the main aeration reactor. Although the filament categorization into 5 causative groups was not yet developed - this only emerged in 1984 as mentioned above - it is worth noting that even though the systems operated by Chudoba *et al.* (1973a,b) were low F/M, the dominant filament causing the bulking was *not* a low F/M one but a low DO one, i.e. *S. natans*.

The work of Chudoba *et al.* (1973a,b) stimulated research into the control of bulking in low F/M (long sludge age) systems. Most of this research was conducted on fully aerobic systems at laboratory scale with real or synthetic sewage as influent. In this research it was found that good settling (non-bulking) sludges were produced in systems with (i) compartmentalization of the aeration reactor while maintaining continuous feeding of waste water (ii) batch or intermittent feeding to completely mixed aeration basins and (iii) small mixing reactors (aerobic or anoxic selectors) ahead of the main completely mixed aeration reactor receiving the influent and underflow streams (Chudoba *et al.*, 1974; Houtmeyers, 1978; Goronszy, 1979; Goronszy and Barnes, 1979; Houtmeyers *et al.*, 1980; Verachtert *et al.*, 1980; Barnes and Goronszy, 1980; Van Den Eynde *et al.*, 1982a,b; Eikelboom, 1982; Rensink *et al.*, 1982; Grau *et al.*, 1982; Lee *et al.*, 1982; Jenkins *et al.*, 1983; Wu *et al.*, 1984; Chiesa and Irvine, 1985; Daigger *et al.*, 1985; Ekama and Marais, 1986; Still *et al.*, 1985; Van Niekerk, 1985; Shao, 1986; Van Niekerk *et al.*, 1987,1988; Shao and Jenkins, 1989; Gabb *et al.*, 1991; Still *et al.*, 1996; Ekama *et al.*, 1996a). A common feature in all this work was that the alternating feed-starve conditions that these modifications created stimulated in the sludge a selector effect i.e. a high soluble (<0.45μm) or RBCOD uptake rate and a concomitantly high oxygen (if aerobic) or nitrate (if anoxic) utilization rate (OUR or NUR). These rates were 2 to 3 times higher than those in comparable sludges without alternating feed-starve conditions. Curiously, in very few of these experiments, which were mainly laboratory scale investigations, did low F/M filaments proliferate in the low F/M systems. The filaments that did tend to proliferate in the control systems i.e. those without a selector effect, were *S. natans*, *Thiothrix* and type 021N, indicating that the selector effect controlled proliferation of these filaments. Wanner *et al.* (1987a) called this kind of bulking control kinetic selection. In their investigation Still *et al.* (1985,1996), Gabb *et al.* (1991) and Ekama *et al.* (1996b) found that invariably when low F/M filament bulking sludges (DSVI> 250 mℓ/g) from full scale N removal plants were brought to the laboratory to start up fully aerobic low

F/M (long sludge age) systems, the low F/M filaments declined in both the experimental and control systems. It therefore appeared that aeration had a far greater influence on the low F/M filaments than the selector effect. This raised the question of the appropriateness of the system modification approach to create a selector effect for controlling low F/M filaments.

1.4 Developments in Specific Control of Low F/M Filaments - a New Hypothesis

In the bulking research reviewed above, it appears that controlling bulking in low F/M systems was the focus rather than controlling bulking specifically by low F/M filaments and the success of the selector effect in controlling bulking by *S. natans*, *Thiothrix* or type 021N in low F/M systems may have led to the notion that this approach would control low F/M filaments. Long sludge age (low F/M) systems often nitrify and if mixing is not adequate unintentional (but not unwelcome) denitrification takes place in the main aeration reactor (Barnard and Hoffmann, 1986). Gabb *et al.* (1989,1991, 1996a) and Casey *et al.* (1992,1993,1994) have shown that these conditions are conducive to low F/M filament proliferation. Therefore in the surveys of full scale plants of Strom and Jenkins (1984) on which Table 1 is based, it is not unlikely that nitrification-denitrification conditions in the low F/M plants could have been the reason for the prevalence of low F/M filaments rather than the low F/M conditions *per se*.

Gabb *et al.* (1989) showed that the proliferation of *S. natans* and possibly also *Thiothrix* was a consequence of inadvertent artifacts introduced in their laboratory systems i.e. seeding from attached growths on influent feed line walls. This artifact may also have been present in many of the investigations cited above because of the prevalence of *S. natans* and *Thiothrix* in these fully aerobic low F/M systems. In contrast Gabb *et al.* (1996a) showed that the absence of low F/M filaments from their fully aerobic low F/M systems was *not* the result of such inadvertent artifacts. In a laboratory scale MUCT biological N&P removal system, they demonstrated that low F/M filaments proliferated and caused bulking while in parallel fully aerobic systems, either batch or continuously fed with the same wastewater, started up with the MUCT system bulking sludge and operated at the same sludge age, the low F/M filaments declined. Indeed, the only laboratory systems in which the low F/M filaments proliferated in the same way as full scale plants were the N&P removal ones (N removal ones were not operated). This observation highlighted the perplexing question that if anaerobic reactors in biological N&P removal plants could not control low F/M filament bulking, then how can aerobic or anoxic selectors? Anaerobic reactors in biological N&P removal plants serve the same function as aerobic or anoxic selectors - they stimulate removal of influent RBCOD by floc formers; in the N&P removal system the floc-formers happen to be the poly-phosphate accumulating organisms which mediate the biological excess P removal (BEPR), a mechanism which Wanner *et al.* (1987b) calls metabolic selection. Full scale N&P removal plant surveys (Blackbeard *et al.*, 1988) and the above and other laboratory scale experiments (Lakay *et al.*, 1988; Musvoto *et al.*, 1994) have shown unequivocally that anaerobic reactors do not control low F/M filament proliferation because these plants so often have low F/M bulking sludges even when they exhibit very good BEPR (i.e. the anaerobic reactor stimulated complete removal of influent RBCOD - see Fig. 1 and Table 3).

In order to grow low F/M filaments in laboratory scale systems other than the N&P removal ones, Gabb *et al.* (1996a) operated single reactor completely mixed long sludge age systems with intermittent aeration to mimic ditch-type simultaneous nitrification-denitrification extended aeration activated sludge plants. With intermittent aeration (3-4 min aerobic in a 10 min cycle),

the low F/M filaments proliferated but switching to continuous aeration they declined in the absence of a selector effect. Having developed a laboratory system in which low F/M filament bulking could be induced, allowed the effect of selectors on the low F/M filaments to be tested. Gabb *et al.* (1996b) installed a correctly sized two compartment aerobic selector ahead of an intermittently aerated main reactor of a long sludge age system. Even though the selector induced a selector effect, the low F/M filaments continued to proliferate at bulking levels (DSVI >350 mℓ/g).

The research discussed above indicates that selectors in their most general form i.e. preferential uptake of influent RBCOD by floc-formers through kinetic selection (aerobic or anoxic selectors) or metabolic selection (anaerobic reactors) was not effective for controlling bulking by low F/M filaments in N and N&P removal systems and resolved the apparent inconsistency between aerobic (and anoxic) selectors and anaerobic reactors/selectors. This placed the research back into an exploratory phase and in a follow up investigation Casey *et al.* (1992,1993,1994) evaluated the effect of the following different factors on low F/M filament bulking in various laboratory N and N&P removal systems such as single reactor intermittently aerated, two-reactor pre- and post-denitrification and MUCT systems - (i) RBCOD or slowly biodegradable (SB) COD only as feed obtained from artificial wastewater and real wastewater separated into its soluble and particulate fractions by membrane filtration, (ii) fully aerobic and fully anoxic conditions, (iii) DO and nitrate/nitrite concentrations in the aerobic and anoxic zones or periods, (iv) the aerobic fraction (% of time sludge mass is aerobic), (v) frequency of alternation between anoxic and aerobic conditions (vi) differences in alternating anoxic-aerobic conditions caused by intermittent aeration in single reactors and in compartmentalized anoxic-aerobic reactor systems and (vii) sludge age. From these experiments, they concluded that the major factor influencing low F/M filament bulking was alternating anoxic-aerobic conditions forcing the heterotrophic organisms to switch between DO and nitrate as terminal electron acceptors. From an examination of the microbiological and biochemical literature on aerobic and anaerobic (denitrification) respiratory pathways and their experimental results, they formulated a hypothesis for the cause of low F/M filament bulking viz.; if denitrification is not complete under anoxic conditions (i.e. all nitrate and nitrite not denitrified) the floc-formers, which denitrify nitrate fully to nitrogen gas via nitrite, nitric oxide and nitrous oxide, are inhibited in their oxygen uptake under subsequent aerobic conditions by the denitrification intermediates accumulated under the preceding anoxic conditions, in particular nitric oxide (NO). This inhibition of the floc formers under aerobic conditions provides advantage to the filaments which reduce nitrate only as far as nitrite and therefore are not inhibited in their oxygen uptake by nitric oxide (Lakay *et al.*, in prep; Musvoto *et al.*, in prep; Casey *et al.*, in prep a,b,c).

1.5 Laboratory and full-scale plant support for the New Hypothesis

The above hypothesis as an alternative to the selector approach for explaining the causes and control of low F/M (AA) filament bulking is a recent development and therefore still needs thorough evaluation and verification. However, a significant body of information providing indirect support for this alternative conceptualization of the low F/M filament bulking problem is emerging not only from laboratory scale systems (Casey *et al.*, 1993,1994; Musvoto *et al.*, 1994; Chudoba, 1994; Lakay *et al.*, in prep; Musvoto *et al.*, in prep) but also from full scale plant observations. In this respect the conference proceedings of the 1st ASPD Specialist Group make interesting reading (for details see Ekama, 1994) eg.

1) In a laboratory scale MUCT system with a 20% aerobic mass fraction, Musvoto *et al.* (1994) showed that when the nitrate and nitrite concentrations entering the aerobic zone exceeded 0.5 and 0.2 mgN/l respectively, then the low F/M (AA) filaments proliferated to cause bulking (DSVI>150 mℓ/g), but when the concentrations were below these values, then low F/M filaments declined (DSVI< 80 mℓ/g) (see Fig. 1 and Table 3).

2) From a survey of Danish plants, Andreasen and Sigvardsen (1993) concluded that the "first survey at nutrient removal plants show a higher percentage with SVI >150 mℓ/g and especially at some plants which include BEPR which seem to have constant high SVI with filaments *M. parvicella*, 0041 and 0803."

3) From a survey of Rome's plants, Rossetti *et al.* (1994) found that "systems operating with alternating aeration conditions (like Carousel type) with short anoxic-aerobic cycles show permanent high levels of low F/M filaments with the main filament being *M. parvicella*."

4) From a survey of German plants, Kunst and Reins (1994) state that "in the last years a lot of treatment plants with bio-P removal went into operation and people thought that anaerobic reactors in the treatment plants would be able to prevent sludge bulking - the experience in technical practice shows this didn't happen."

5) From practical experience in operating long sludge age plants in South England, Foot *et al.* (1994) concluded that (i) there is an inverse relationship between the total filament length (TFL) and the total oxidized nitrogen (TON) concentration in the effluent - the higher the TON concentration, the lower the TFL and *vice versa*, and (ii) "the widespread occurrence of this species (*M. parvicella*) in WWTPs would tend to indicate that it is not so much the substrate (sewage characteristics) which are important as the configuration and operation of the plant."

6) From 10 years experience with bulking in Dutch full scale plants, Eikelboom (1993,1994) concludes that (i) "development of *M. parvicella* shows a distinct seasonal pattern with highest DSVIs in spring and lowest in autumn", (ii) "*M. parvicella* grows better in Carousel type systems than in other extended aeration plants and it is worse with settled sewage", (iii) the usefulness of selectors for controlling *M. parvicella* decreases as the overall load on the plant increases", (iv) "after introduction of nutrient removal conditions, the DSVI increased in 60% of these plants and *M. parvicella* was dominant in 87% of them" so that (v) "the application of BNR methods will even increase the dominating position of this organism", and (vi) "the ultimate effect of selectors for control of *M. parvicella* is insufficient and unpredictable so far. In Holland over 80 selectors have been incorporated in full-scale plants. Comparing the results with 15 years ago, it seems that the percentage of plants with bulking has not significantly changed with application of selectors".

While most of these statements and conclusions indirectly support the research on low F/M (AA) filament bulking reviewed above, those of Foot *et al.* (1994) and Eikelboom (1994) are particularly pertinent; (i) the problem being worst in spring (also noted by Kunst and Reins, 1994), (ii) worse with settled wastewater, (iii) the inverse relationship between the TFL and effluent TON, and (iv) the efficacy of aerobic selectors decreasing if denitrification is permitted to take

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

Table 3: Filament identifications and DSVI for MUCT1 and MUCT2 biological N&P removal systems both operated at 20 days sludge age and 20°C (from Musvoto *et al.*, 1994)

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

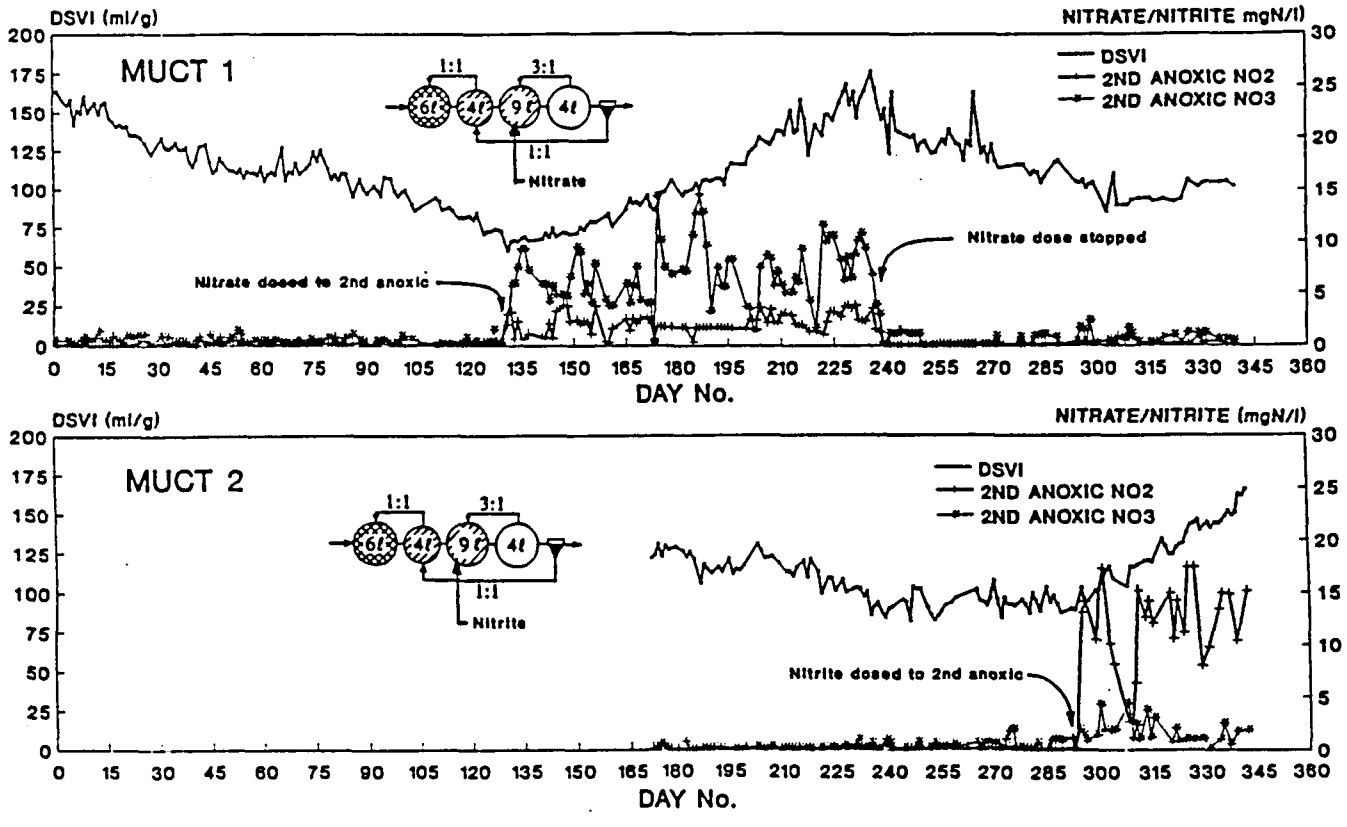

Fig. 1: Sludge settleability (in DSVI, mℓ/g) and nitrate and nitrite concentrations in the 2nd anoxic reactors of MUCT1 (top) and MUCT2 (bottom). Note the increase in these concentrations and concomitantly the increase in DSVI upon commencement of nitrate dosing to MUCT1 on day 129 and nitrite dosing to MUCT2 on day 291; also the decrease in these concentrations in the 2nd anoxic reactor of MUCT1 upon cessation of nitrate dosing to MUCT1 and the concomitant decline in DSVI.

place in the main aeration reactor (due to either under aeration or increased plant load), all influence the denitrification performance of the plant and increase the likelihood of significant nitrate and nitrite concentrations being present at the transition from anoxic to aerobic conditions. A detailed exposition of the AA filament bulking hypothesis is presented by Casey *et al.* (in prep. a,b,c). Further laboratory system results obtained during this contract are given the synopses of the experimental investigations below.

1.6 References

Andreasen K. and Sigvardsen L. (1993) Optimisation of flow pattern in nutrient removal plants to prevent filamentous bulking. *Procs. 1st Int. Spec. Conf. on Microorganisms in Activated Sludge and Biofilm Processes*, 27-28 Sept. 1993, Paris, 361-363.

ATV (Abwassertechnische Vereinigung) (1989) ATV-Working Report: Prevention and control of bulking sludge and scum. *Korrespondenz Abwasser*, **36**(13), 165-177.

Barnard J.L. and Hoffmann J.R. (1986) Selecting aerators for nutrient removal plants. *Water Sewage and Effluent*, **26**, 6-15.

Barnes D. and Goronszy M.C. (1980) Continuous intermittent wastewater systems for municipal and industrial effluents. *Public Health Engineer*. **8**, 20.

Blackbeard J.R., Ekama G.A. and Marais G.vR. (1986). Identification of filamentous organisms in nutrient removal activated sludge plants in South Africa. *Water Pollut. Control*, **85**(1), 90-100.

Blackbeard J.R., Gabb D.M.D., Ekama G.A. and Marais G.vR. (1988) Identification of filamentous organisms in nutrient removal activated sludge plants in South Africa. *Water SA*, **14**(1), 29-34.

Casey T.G., Wentzel M.C., Loewenthal R.E., Ekama G.A. and Marais G.vR. (1992) A hypothesis for the cause of low F/M filament bulking in nutrient removal activated sludge systems. *Water Research*, **26**(6), 867-869.

Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.vR. (1993) Causes and control of low F/M filamentous bulking in nutrient removal activated sludge systems. *Procs. International Workshop on prevention and control of bulking activated sludge*; Eds. Jenkins D., Ramadori R. and Cingolani L., Luigi Bazzucchi Centre, Perugia.

Casey T.G., Wentzel M.C., Loewenthal R.E., Ekama G.A. and Marais G.vR. (1994) A hypothesis for the causes and control of anoxic-aerobic (AA) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 203-212.

Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.vR. (1995) Filamentous organism bulking in nutrient removal activated sludge systems Paper 1: An historical overview of causes and control. *Water SA*, **21**(3), 321-338.

Casey T.G., Wentzel M.C. and Ekama G.A. (In prep, a) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 9: Review of biochemistry of heterotrophic respiratory metabolism. (Submitted to Water SA)

Casey T.G., Wentzel M.C. and Ekama G.A. (In prep, b) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 10: Metabolic behaviour of heterotrophic facultative aerobic organisms under aerated/unaerated conditions. (Submitted to Water SA).

Casey T.G., Wentzel M.C. and Ekama G.A. (In prep, c) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 11: A biochemical/ microbiological model for proliferation of anoxic-aerobic (AA) filamentous organisms. (Submitted to

Water SA)
Chiesa S.C. and Irvine R.L. (1985) Growth and control of filamentous microbes in activated sludge: An integrated hypothesis. *Water Research*, **19**(4), 471-479.
Chudoba J., Ottova V. and Madera V. (1973a) Control of activated sludge filamentous bulking I. Effect of hydraulic regime or degree of mixing in an aeration tank. *Water Research*, 7(9), 1163-1182.
Chudoba J., Grau P. and Ottova V. (1973b) Control of activated sludge filamentous bulking II. Selection of micro-organisms by means of a selector. *Water Research*, 7(10),1398-1406.
Chudoba J., Blaha H. and Madera V. (1974) Control of activated sludge filamentous bulking III - Effect of sludge loading. *Water Res.*, **8**(4), 231-237.
Chudoba P. (1994) Is incomplete denitrification a key factor influencing anoxic-aerobic filamentous organisms proliferation in DN/N activated sludge systems? *IAWQ ASPD Newsletter*, 7(1), 6-15.
Daigger G.T., Robbins M.H. and Marshall B.R. (1985) The design of a selector to control low F/M filamentous bulking. *Journal WPCF*, **56**(3), 220-226.
Eikelboom D.H. (1977) Identifications of filamentous organisms in bulking activated sludge. *Prog. Wat. Technol.*, **8**(6), 153-167.
Eikelboom D.H. (1982) Biosorption and prevention of bulking sludge by means of high floc loading (Chapter 6), *In: Bulking of activated sludge: Preventative and remedial methods.* Eds: Chambers B. and Tomlinson E.J., Ellis Horwood Ltd., Chichester, UK, 90-110.
Eikelboom D. H. (1993) Invloed van P- en N-verwijdering op de bezinkeigenschappen van actiefslib. TNO rapport IMW R93/074, Instituut voor Milieuwetenschappen, TNO, Postbus 6011, 2600 JA, Delft, Holland.
Eikelboom D.H. (1994) The *Microthrix parvicella* puzzle. Selectors for bulking control at domestic plants in the Netherlands. *Water Sci.Technol.*, **29**(7), 273-279.
Ekama G.A. and Marais G.vR. (1986) The implications of the IAWPRC hydrolysis hypothesis on low F/M bulking. *Water Sci. Technol.*, **18**(6), 11-19.
Ekama G.A. (1994) A selection of impressions and statements from papers and posters presented at 1st IAWQ ASPD Conference (Paris, 1993), on filamentous bulking, *IAWQ ASPD Newsletter*, 7(1), 16-19.
Ekama G.A., Wentzel M.C., Casey T.G. and Marais G.vR. (1996a) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 3: Stimulation of the selector effect under anoxic conditions. *Water SA*, **22**(2), 119-126.
Ekama G.A., Wentzel M.C., Casey T.G. and Marais G.vR. (1996b) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 6: Review, evaluation and consolidation of results. *Water SA*, **22**(2), 147-152.
Foot R.J., Robinson M.S. and Forster C.F. (1994) Systematic activated sludge bulking and foam control. *Water Sci. Technol.*, **29**(7), 213-220.
Gabb D.M.D., Ekama G.A., Jenkins D. and Marais G.v.R (1989) The incidence of *Sphaerotilus natans* in laboratory-scale activated sludge systems. *Water Sci. Technol.*, **21**(6/7), 29-41.
Gabb D.M.D., Still D.A., Ekama G.A., Jenkins D. and Marais G.vR. (1991) The selector effect on filamentous bulking in long sludge age activated sludge systems. *Water Sci. Technol.*, **23**(4/6-2), 867-877.
Gabb D.M.D., Ekama G.A., Jenkins D., Wentzel M.C., Casey T. G. and Marais G.v.R (1996a) Filamentous organism bulking in nutrient removal activated sludge systems.

Paper 4: System configurations and operating conditions to develop low F/M filament bulking sludges at laboratory-scale. *Water SA*, **22**(2), 127-137.

Gabb D.M.D., Ekama G.A., Jenkins D., Wentzel M.C., Casey T. G. and Marais G.v.R (1996b) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 5: Experimental examination of aerobic selectors in anoxic-aerobic systems. *Water SA*, **22**(2), 127-137.

Goronszy M.C. (1979) Intermittent operation of the extended aeration process for small systems. *Journal WPCF*, **51**(2), 274-287.

Goronszy M.C. and Barnes D. (1979) Continuous single vessel activated sludge treatment of diary wastes. *Proc. 87th Am. Inst. Chem. Eng.*, Boston, MA.

Grau P., Chudoba J. and Dohanyos M. (1982) Theory and practice of accumulation-regeneration approach to the control of activated sludge filamentous bulking (Chapter 7) . In: *Bulking of activated sludge: Preventative and remedial methods.* Eds: Chambers B. and Tomlinson E.J., Ellis Horwood Ltd., Chichester, UK, 111-122.

Hao O.J., Richard M.G. and Jenkins D. (1983) The half-saturation coefficient for dissolved oxygen: A dynamic model for its determination and its effect in dual species competition. *Biotechnol. and Bioeng.*, **XXV**, 403-416.

Houtmeyers J. (1978) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. *Agricultura* (Belgium), **26**, 1.

Houtmeyers J., Van Den Eynde E., Poffe R. and Verachtert H. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. 1. Influence of process parameters. *European J Appl. Microbiol. Biotechnol.*, **9**, 63.

Jenkins D., Parker D.S., Van Niekerk A.M., Shao Y.J. and Lee S.E. (1983) Relationship between bench scale and prototype activated sludge systems. In: Schmidtke N.W. and Smith D.W. (eds.) *Scale-up of water and wastewater treatment processes.* Butterworth Publishers. 307.

Jenkins D., Richard M.G. and Daigger G.T. (1984a) *Manual on the causes and control of activated sludge bulking and foaming.* Published by Water Research Commission, PO Box 824, Pretoria, 0001, RSA.

Jenkins D., Richard M.G. and Neethling J.B. (1984b). Causes and control of activated sludge bulking. *Water Pollut. Control*, **83**(4), 455-472.

Jenkins D., Daigger G.T. and Richard M.G. (1993) Manual on the causes and control of activated sludge bulking and foaming. 2nd Edn., Lewis Publishers, Chelsea, MI., USA.

Kunst S. and Reins M. (1994) Practical investigations on bulking and foaming in activated sludge plants with biological phosphorus removal. *Water Sci. Technol.*, **29**(7), 289-294.

Lakay M.T., Wentzel M.C., Ekama G.A. and Marais G.vR. (1988) Bulking control with chlorination in a nutrient removal activated sludge system. *Water SA,* **14**(1), 35-42.

Lakay M.T., Hulsman A., Ketley D.A., Warburton C.A., De Villiers M.E., Casey T.G., Wentzel M.C. and Ekama G.A. (In prep) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 7: Exploratory experimental investigations (Submitted *Water SA*).

Lau A.O., Strom P.F. and Jenkins D. (1984a). Growth kinetics of *Sphaerotilus natans* and a floc former in pure and dual continuous culture. *Journal WPCF,* **56**(1), 41-51.

Lau A.O., Strom P.F. and Jenkins D. (1984b). The competitive growth of floc-forming and filamentous bacteria: A model for activated sludge bulking. *Journal WPCF,* **56**(1), 52-61.

Lee S-E., Koopman B.L., Jenkins D. and Lewis R.F. (1982). The effect of aeration basin configuration on activated sludge bulking at low organic loading. *Water Sci. Technol.*, **14**(6/7), 407-427.

Musvoto E.V., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.vR. (1994) The effect of incomplete denitrification on anoxic-aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(4), 295-299.

Musvoto E.V., Lakay M.T., Casey T.G., Wentzel M.C. and Ekama G.A. (In prep) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 8: The effect of nitrate and nitrite. (Submitted to Water SA).

Palm J.C., Jenkins D. and Parker D.S. (1980) Relationship between organic loading, dissolved oxygen concentration and sludge settleability in the completely-Mixed activated sludge process. *Journal WPCF,* **52**(10), 2484-2506.

Pujol R., Duchene P.H., Schetrite S. and Canler J.P. (1991) Biological foams in activated sludge plants: Characterization and situation. *Water Research*, **25**(11), 1399-1404.

Rensink J.H., Donker H.J.G.W. and Ijwema T.S.J. (1982) The influence of feed pattern on sludge bulking (Chapter 9). In: *Bulking of activated sludge: Preventative and remedial methods.* Eds: Chambers B. and Tomlinson E.J., Ellis Horwood Ltd., Chichester, UK, 147-163.

Richard M.G., Jenkins D., Hao O. J. and Shimizu G. (1982). The isolation and characterization of filamentous micro-organisms from activated sludge bulking. Rept No. 81-2, Sanitary Eng. Env. Health Research Lab., University of California, Berkeley, Ca.

Rossetti S., Carucci A. and Rolle E. (1994) Survey on the occurrence of filamentous organisms in municipal wastewater treatment plants related to their operating conditions. *Water Sci. Technol.*, **29**(4), 305-308.

Seviour E.M., Williams C.J., Seviour R.J., Soddell J.A. and Lindrea K.C. (1993) The identification of filamentous bacteria in Australian activated sludge plants. *Proc. Int. workshop on prevention and control of bulking activated sludge,* Perugia, Italy, 22-23 June 1992, 163-165.

Seviour E.M., Williams C., De Grey B., Soddell J.A., Seviour R.J. and Lindrea K.C. (1994) Studies on filamentous bacteria from Australian activated sludge plants, *Water Research*, **28**(11), 2335-2342.

Shao Y.J. (1986) The mechanism and design of anoxic selectors for the control of low F/M filamentous bulking. Ph.D. thesis, Univ. of California, Berkeley, CA.

Shao Y.J. and Jenkins D. (1989) The use of anoxic selectors for the control of low F/M activated sludge bulking. *Water Sci. Technol.*, **21**(6/7), 606-619.

Slijkhuis H. (1983) The physiology of the filamentous bacterium *Microthrix parvicella.* Ph.D. Thesis, Agricultural College of Wageningen, Holland.

Still D.A., Blackbeard J.R., Ekama G.A. and Marais G.vR. (1985) The effect of feeding pattern on sludge growth rate and sludge settleability. Research Report W55, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7700, Cape, RSA

Still D.A., Ekama G.A., Wentzel M.C., Casey T.G. and Marais G.vR. (1996) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 2: Stimulation of the selector effect under aerobic conditions. *Water SA*, **22**(2), 97-118.

Strom P.F. and Jenkins D. (1984). Identification and significance of filamentous organisms in activated sludge. *Journal WPCF*, **56**(5), 449-459.

Van Den Eynde E.J. Houtmeyers J. and Verachtert H. (1982a) Relation between substrate feeding pattern and development of filamentous bacteria in activated sludge. In: *Bulking of activated sludge: Preventative and remedial methods* (Chapter 8). Eds: Chambers B. and Tomlinson E.J., Ellis Horwood Ltd., Chichester, UK, 128-142.

Van Den Eynde E.J., Vries L. and Verachtert H. (1982b) Relationship between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. III. Application with industrial wastewaters. *Eur. J. Appl. Microbiol. Biotechnol.*, **15**, 246.

Van Niekerk A.M. (1985) Competitive growth of flocculant and filamentous microorganisms in activated sludge systems. Ph.D. Thesis, Dept Civil Eng., Univ. of California, Berkeley.

Van Niekerk A.M., Jenkins D. and Richard M.G. (1987) The competitive growth of *Zoogloea* ramigera and type 021N in activated sludge and pure culture - A model for low F/M bulking. Paper presented at IWPC Bienn. Conf., Port Elizabeth, May 1987.

Van Niekerk A.M., Jenkins D. and Richard M.G. (1988) A mathematical model of the carbon-limited growth of filamentous and floc-forming organisms in low F/M sludge. *Journal WPCF*, **60**(1), 100-106.

Verachtert H., Van Den Eynde E., Poffe R. and Houtmeyers J. (1980) Relations between substrate feeding pattern and development of filamentous bacteria in activated sludge processes. II. Influence of substrate present in the influent. *Eur. J. Appl. Microbiol Biotechnol.*, **9**, 137-149.

Wagner F. (1982) Study of the causes and prevention of sludge bulking in Germany. In: *Bulking of activated sludge: Preventative and remedial methods*. Eds. Chambers B. and Tomlinson E.J., Ellis Horwood Ltd., Chichester, UK, 29-40.

Wanner J., Ottova V. and Grau P. (1987a) Effect of an anaerobic zone on settleability of activated sludge. In: *Advances in water pollution control* (IAWPRC), *biological phosphorus removal from wastewaters.* Ed. Ramadori R. Pergamon, Oxford, England, 155-164.

Wanner J., Chudoba J., Kucman K. and Proskel (1987b) Control of activated sludge filamentous bulking - VII: Effect of anoxic conditions. *Water Research,* **21**(12), 1447-1451.

Wanner J. (1994a) The implementation of bulking control in the design of activated sludge systems. *Water Sci. Technol.*, **29**(7), 193-202.

Wanner J. (1994b). *Activated sludge bulking and foaming control.* Technomic Publishing Co., Lancaster, Pa.

Wu Y.C., Hsieh H.N., Carey D.F. and Ou K.C. (1984) Control of activated sludge bulking. *J. Env. Eng. Div. ASCE.*, **110**(2), 472-491.

2 SYNOPSES OF DETAILED REPORTS - Pilson *et al.* (1995)

Pilson RA, Ekama GA , Wentzel MC and Casey TG (1995) The effect of temperature on dentrification kinetics and biological excess phosphorus removal in nutrient removal activated sludge systems in temperate climates (12°C - 20 °C), Research Report No. W86, Dept. of Civil Eng., Univ of Cape Town, Rondebosch, 7701, RSA.

2.1 Scope and objectives of investigation

Filamentous bulking in nutrient (N & P) removal activated sludge systems is a problem of considerable magnitude - three quarters of 45 plants surveyed were found to have bulking sludges to the extent that sludge settleability (DSVI) was adversely affected. If filamentous organism proliferation could be controlled and thereby sludge settleability improved to below DSVI of 100 mℓ/g, then in addition to factors such as additional aeration capacity, between 50% and 75% more wastewater could be treated in existing nutrient removing activated sludge plants.

Anoxic-aerobic (AA) or low F/M filaments appear to proliferate in activated sludge plants that incorporate biological nitrogen removal. From earlier research, Casey *et al.* (1992, 1993, 1994) showed that the cause for AA filament proliferation lay in the denitrification behaviour of the N removal systems. They hypothesized that filamentous and floc-forming organisms have different denitrification behaviour - the former reducing nitrate only as far as nitrite whereas the latter reducing nitrate all the way to nitrogen gas via the denitrification intermediates nitrite, nitric oxide (NO) and nitrous oxide (N_2O). If nitrate and nitrite removal to nitrogen gas is not complete in the anoxic reactor, then, when conditions become aerobic, the accumulated denitrification intermediates, in particular NO, inhibit oxygen uptake in the floc-formers. The filaments do not experience this inhibition because by reducing nitrate only to nitrite, no denitrification intermediates accumulate in their cytoplasmic membrane and consequently they can successfully compete against the floc-formers and proliferate in the N removal systems. If denitrification is complete, no residual intracellular denitrification intermediates remain in the floc-formers. Therefore when conditions become aerobic, the floc-formers are not inhibited in their oxygen uptake and can successfully compete against the filamentous organisms which cause the bulking.

In full scale N removal plants, filamentous bulking has been observed to be a seasonal problem being worst at the start of Spring. This observation conforms to the above explanation for AA filament proliferation because the reduced wastewater temperature in the winter slows down the denitrification rates making incomplete denitrification in the anoxic reactor more likely. This aspect was examined in detail in this investigation, the specific objectives of which were:

(1) To examine the AA filament response in a system without nitrification, i.e. without nitrate to denitrify, bulking should not take place.

(2) To observe the biological nutrient (N & P) removal at low (12°C) temperature and in particular, to delineate the denitrification kinetics and rates in nutrient removal activated sludge systems at low temperatures (12°C).

(3) To examine the AA filament response at low (12°C) in relation to the denitrification performance.

Two identical modified UCT systems were set up at the same sludge age (20 days) and received the same real unsettled wastewater, one Experimental at 12°C and one Control at 20°C. The low temperature did not terminate nitrification and so after about 3 months operation, the sludge age of both systems was reduced to 12 days, which was maintained for the remainder of the 433 day investigation. The reduction in sludge age initially had the desired effect in the Experimental system - i.e. nitrification stopped but gradually over the rest of the investigation period, nitrification improved. By the end of the investigation it was virtually complete again. Nitrification was complete throughout the investigation period in the Control (20°C) system.

Cessation of nitrification in the Experimental system had the unexpected result that filamentous organisms *Haliscomenobacter hydrossis* and type 0803 proliferated excessively to cause DSVIs above 1000 mℓ/g. This was ameliorated by dosing nitrate into the anoxic reactor to simulate nitrification but at a controlled rate to ensure that complete denitrification could be achieved in the anoxic reactor. The sludge settleability improved and reduced to 200 mℓ/g. With the sludge settleability in the Experimental system restored to a normal value (110 mℓ/g) by day 330, objective 1 was set aside and the investigation continued by addressing objectives 2 and 3. This was accomplished by monitoring the Experimental and Control systems on an almost daily basis for the 433 day investigation, and from day 150, conducting anoxic and aerobic batch tests on sludge harvested from the two (parent) systems. Altogether 34 anoxic and 2 aerobic batch tests were conducted on each system, the former to delineate the denitrification kinetics and rates and the latter to determine the maximum specific growth rate of the nitrifiers. The results obtained from these experiments are as follows:

2.2 Overall system performance

1. On average over the 433 day investigation the N balances in the Experimental (12°C) and Control (20°C) were good - 99% and 94%. The COD balances were lower - 84% for both systems - but of a similar magnitude to earlier research on MUCT N & P removal systems.

2. The average percentage COD removal was 92% and 93% at 12°C and 20°C respectively. The average percentage N removal at 20°C (Control system) was 77%, with 55% of the N nitrified and denitrified, 22% incorporated in sludge mass and 4% and 19% leaving the system with the effluent as TKN and nitrate respectively. The percentage N removal at 12°C was much poorer and more variable due to the intentional retardation of nitrification; at its lowest it was 29%, but this gradually improved to 75% at the end of the investigation when nitrification was again virtually complete.

2.3 Biological excess P removal

3. The temperature effect on the Biological Excess Phosphorus Removal (BEPR) was small, on average 11,0 and 12,0 mgP/ℓ at 12°C and 20°C respectively. However, the BEPR at 20°C was only 60% of that expected in terms of the Wentzel *et al.* (1990) model for the measured influent RBCOD concentration and system design parameters. This reduced BEPR was also noted by Kaschula *et al.* (1993) and Musvoto *et al.* (1992, 1994) in similar laboratory MUCT systems. No explanation for this reduced BEPR compared to that observed by Wentzel *et al.* (1990) can be advanced (see 3.6 below).

2.4 Nitrification

4. From observations on the Experimental system which nitrified partially, maximum specific growth rate of the *Nitrosomonas* at 12°C (μ_{nm12}) was 0,36/d. From aerobic batch tests on sludge harvested from the Experimental and Control (20°C) systems μ_{nmT} values of 0,31/d at 12°C and 0,67/d at 20°C were obtained, which yields a θ temperature sensitivity value of 1,10. For the Experimental system, the parent system and batch test μ_{nm12} value compare well. Also the θ value of 1,10 for the batch test measured μ_{nm12} and μ_{nm20} compares reasonably well with the 1,123 value normally accepted for design (WRC, 1984) considering only 2 aerobic batch tests were conducted on each system. These results confirm the validity of the functional form of the nitrification model used for design of nutrient removal systems. The difficulty remains estimating the μ_{nm20} value for the particular wastewater. In this respect, the usually recommended values of around 0,33 to 0,45/d appear rather conservative in the light of 0,67/d measured for the Mitchell's Plain purely domestic wastewater.

2.5 Denitrification

5. From 34 anoxic batch tests conducted on each system to delineate the denitrification kinetics and rates it was established that significant nitrite denitrification does not take place until the nitrate concentration has been reduced to below 1 $mgNO_3^-$-N/ℓ. While nitrate is being denitrified, a slow accumulation or reduction of nitrite was observed - invariably an accumulation at a rate of about 1/10th of the nitrate denitrification rate at 20°C [i.e. 0,0215 $mgNO_2^-$-N/(mgAHVSS.d) compared with 0,1941 $mgNO_3^-$-N/(mgAHVSS.d)], but at 12°C an accumulation for the first half of the batch tests [day 110-300; mean 0,0402 $mgNO_2^-$-N/(mgAHVSS.d)] and a reduction for the second half of the batch tests [day 300-433; mean 0,0662 $mgNO_2^-$-N/(mgAHVSS.d)].

6. The mean nitrate denitrification rates (appropriately corrected for nitrite accumulation or reduction) K'_{2T} at 20°C and 12°C were 0,1812 ±0,0076 and 0,1567 ±0,0069 $mgNO_3^-$-N/(mgAHVSS.d) respectively giving a temperature sensitivity coefficient θ of 1,018. These K'_{2T} rates, attributable to the utilization of slowly biodegradable COD in the anoxic reactor, are specified in terms of the facultative heterotrophic active organism concentration (AHVSS) which excludes the polyphosphate accumulating organisms. The rate at 20°C is somewhat lower than K'_{220} denitrification in nutrient (N & P) removal systems observed by Clayton *et al.* (1989, 1991) - 0,224 $mgNO_3^-$-N/(mgAVSS.d)[1]. It was

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[1]The rates of Clayton *et al.* (1989) and Musvoto *et al.* (1992) are defined in terms of AVSS, which accepts that all the biodegradable COD is utilized by ordinary (facultative) heterotrophic organisms so that theoretically the active organism mass (AVSS) comprises only these ordinary heterotrophs. In this investigation the denitrification rates are defined in terms of AHVSS, which accepts that both polyP and ordinary heterotrophs comprise the active VSS mass but only the ordinary heterotrophic ones contribute to the denitrification. Defining Clayton's and Musvoto's rates in terms of AHVSS would increase their rates in terms of AVSS by about 10%. In addition an active fraction of 0,24 mgAVSS/mgVSS was used by Clayton *et al.* (1989) compared to values of 0,33 and 0,30 mgAHVSS/mgVSS used at 20°C and 12°C respectively in this investigation.

noted that the single most significant factor influencing the K_2' rate was the estimate of the active organism fraction - this appears to be influenced by the magnitude of the unaerated sludge mass of the system - the higher this fraction, the lower the AHVSS or AVSS fraction and the higher the specific denitrification rate. Despite these variations the K'_2 denitrification rate is significantly greater and its temperature sensitivity significantly lower than the equivalent rate (K_2) in N removal systems i.e. K_{220} - 0,101 mgNO_3^--N/(mgAVSS.d) with its temperature sensitivity coefficient θ = 1,08.

7. From this investigation, the nitrate (and nitrite) denitrification rate utilizing slowly biodegradable COD (K'_{2T}) at 12°C is 14% lower than at 20°C viz. 0,181 and 0,157 mgNO_3^--N/(mgAHVSS.d) at 20°C and 12°C respectively. However, the accumulation of nitrite at 20°C had the effect of reducing the nitrate denitrification rate while the reduction of nitrite at 12°C had the effect of increasing the denitrification rate. For the purposes of conservative design therefore, it is recommended that the denitrification rate at 12°C not be adjusted to account for nitrite accumulation. This rate is therefore 0,149 mgNO_3^--N/(mgAHVSS.d) which is 22% lower than the value of 0,181 mgNO_3^--N/(mgAHVSS.d) for 20°C. This gives a temperature sensitivity coefficient of θ=1,025 which is somewhat larger than the previously quoted value of 1,018 (see 6 above). Therefore a reduction in wastewater temperature in winter causes the denitrification potential of the primary anoxic reactor upstream of the aerobic reactor to decrease, and if this decrease is below the nitrate load on the anoxic reactors, incomplete denitrification will take place. This creates conditions conducive for AA filament proliferation and a progressively deteriorating sludge settleability (increasing DSVI) results.

8. In the absence of nitrate (<1 mgNO_3^--N/ℓ), the nitrite denitrification is approximately as fast as the nitrate denitrification rate viz. 0,20 and 0,15 mgNO_2^--N/(mgAHVSS.d) at 20°C and 12°C. This observation compares well with the results of Musvoto *et al.* (1992) who also observed this at 20°C.

2.6 Sludge settleability and filamentous organisms

9. In the Control system (20°C), the DSVI of the sludge was between 130 and 150mℓ/g for the first 250 days decreasing to 100 mℓ/g between days 250 and 300. The filamentous organisms were in decreasing order of prevalence 0092, 0041, 0803, 012N, *Microthrix parvicella.* Throughout this period the nitrate and nitrite concentrations entering the aerobic reactor were very low - <0,5 mgN/ℓ - confirmed by the batch tests in that for all the batch tests (at 20°C) conducted during this time the denitrification potential of the anoxic reactor exceeded the nitrate load. From day 300 to day 375, the influent TKN concentration progressively increased from 100 to 140 mgN/ℓ, causing the effluent nitrate concentration to increase from 10 to 30 mgN/ℓ. At the same time, the nitrate and nitrite concentrations entering the aerobic reactor increased from very low values to 10 and 2 mgN/ℓ respectively and concomitantly the DSVI of the sludge increased to 200mℓ/g caused by a proliferation of AA filaments 0092 and 0041. On day 375, the effluent TKN concentration declined to 100 mgN/ℓ which resulted in very low concentrations again entering the aerobic reactor and the DSVI progressively declined from 200 to 160 mℓ/g at the end of the investigation (day 433).

10. The above interactions between influent TKN, nitrate and nitrite concentrations entering the aerobic reactor and DSVI, conform to hypothesized cause of AA filament bulking by Casey *et al.* (1994).

11. The Experimental system (12°C) DSVI declined to around 110 mℓ/g by day 330 after nitrate dosing to the second anoxic reactor was started on day 245 to simulate nitrification in the aerobic reactor. As in the Control system (see 8 above), the DSVI ceased decreasing and started increasing from 110 mℓ/g on day 350 to around 200 mℓ/g on day 380 when the influent TKN concentration increased over the period from day 325 to day 375. The filamentous organisms were 0803, 021N and 0092. The increases in TKN concentration caused the nitrate and nitrite concentrations entering the aerobic reactor to increase from less than 1 mgN/ℓ to over 20 and 2 mgN/ℓ respectively. From day 380, the influent TKN concentration decreased to below 100 mgN/ℓ, but the nitrite concentration remained above 2 mgN/ℓ up to day 420, after which it decreased below 0,5 mgN/ℓ. As a consequence the DSVI remained high at around 200 mℓ/g until the end of the investigation (day 433).

2.7 Conclusion

12. The observed interactions between influent TKN concentration, nitrate dosing into the anoxic reactor upstream of the aerobic reactor, nitrate and nitrite concentrations entering the aerobic reactor and DSVI at 12°C also confirm the hypothesized cause of AA filament bulking of Casey *et al.* (1992).

2.8 References

Casey T.G., Wentzel M.C., Loewenthal R.E., Ekama G.A. and Marais G.vR. (1992) A hypothesis for the cause of low F/M filament bulking in nutrient removal activated sludge systems. *Water Research*, **26**(6), 867-869.

Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.vR. (1993) Causes and control of low F/M filamentous bulking in nutrient removal activated sludge systems. *Procs. International Workshop on prevention and control of bulking activated sludge*; Eds. Jenkins D., Ramadori R. and Cingolani L., Luigi Bazzucchi Centre, Perugia.

Casey T.G., Wentzel M.C., Loewenthal R.E., Ekama G.A. and Marais G.vR. (1994) A hypothesis for the causes and control of anoxic-aerobic (AA) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 203-212.

Clayton J.A., Ekama G.A., Wentzel M.C. and Marais G.vR. (1989). Denitrification kinetics in biological nitrogen and phosphorus removal activated sludge systems. Research Report W63, Dept. Of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Clayton J.A., Ekama G.A., Wentzel M.C. and Marais G.v.R. (1991). Denitrification kinetics in biological nitrogen and phosphorus removal systems treating municipal wastewaters. *Water Sci. Technol.*, **23**(4/6-2), 1025-1035.

Kaschula W.A., Ekama G.A., Palmer S.H., Wentzel M.C. and Birch R.R. (1993). The effect of alternative detergent builders on the nutrient removal activated sludge sewage treatment process. Research Report W78, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Musvoto E.V., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.VR. (1992). The effect of

a large anoxic mass fraction and concentrations of nitrate and nitrite in the primary anoxic zone on low F/M filament bulking in nutrient removal activated sludge systems. Research Report W77, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Musvoto E.V., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.v.R. (1994) The effect of incomplete denitrification on anoxic-aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 295-299.

Wentzel M.C., Dold P.L., Ekama G.A. and Marais G.v.R. (1990) Biological excess phosphorus removal - Steady state process design. *Water SA*, **16**(1), 29-48.

WRC (1984). *Theory, design and operation of nutrient removal activated sludge processes.* Water Research Commission, PO Box 824, Pretoria 0001, South Africa. ISBN 0908356137.

3 SYNOPSES OF DETAILED REPORTS - Mellin *et al.* (1995)

Mellin HKO, Wentzel MC and Ekama GA (1995) The effect of temperature on denitrification kinetics and biological excess phosphorus removal in nutrient removal activated sludge systems in tropical climates (20°C - 30 °C), Research Report No. W91, Dept. of Civil Eng., Univ of Cape Town, Rondebosch, 7700, RSA.

3.1 Scope and objectives of investigation

The main objective of this investigation was to evaluate activated sludge biological nutrient removal (BNR) performance at elevated temperatures for possible application of nitrification denitrification (ND) and ND biological excess phosphorus removal (NDBEPR) systems to municipal wastewater treatment in the equatorial and tropical regions or to combined treatment of municipal and anaerobically (thermophilic) pretreated paper and pulp industry wastewaters in the very cold northern forested regions. To accomplish this objective a ND Modified Ludzack Ettinger (MLE) system and a NDBEPR University of Cape Town (UCT) system were operated at 30°C and 10 days sludge age for a period of 582 days. During the investigation 41 sewage batches, each lasting about two weeks, of real sewage from the Mitchells Plain municipal wastewater treatment plant (Western Cape, South Africa) were fed to the systems. The two systems were sampled and tested almost daily for Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN), Free and Saline Ammonia (FSA), nitrate, nitrite, Total Phosphorus, Volatile Settleable Solids (VSS), Total Settleable Solids (TSS), pH, Oxygen Utilization Rate (OUR) and diluted sludge volume index (DSVI) in the influent, anaerobic, anoxic and aerobic reactors and effluent as appropriate. Microscopic analysis of the sludge mixed liquor was undertaken every four weeks during the latter half of the investigation to identify the filamentous organisms. Also, in order to determine the kinetic rates of nitrification, denitrification and readily biodegradable COD (RBCOD) conversion to Volatile Fatty Acids (VFA), aerobic, anoxic and anaerobic batch tests were conducted at 30°C on sludge harvested from the two systems. These kinetic rates at 30°C were then compared with equivalent process rates measured in 20°C ND and NDBEPR systems operated in parallel as part of this investigation or in separate investigations before and after this one. From the 20°C and 30°C process rates, the temperature sensitivity coefficients θ of some of the biological kinetic processes were determined for the 20 to 30°C range and compared with those determined in earlier investigations in the 12 to 20°C range.

Examining the average experimental results of each of the 41 sewage batches, it was noticed that during the winter months (May to June) the UCT system showed a very poor P removal performance. The data were therefore divided into 3 long term periods with long term period I including sewage batches 1 to 23 (360 days), long term period II including sewage batches 24 to 33 (130 days) and long term period III including sewage batches 34 to 41 (92 days). The poor P removal performance occurred during period II, with periods of good (though not "normal", see 16 below) P removal during periods I and III. The UCT system BEPR performance was therefore evaluated with the aid of steady state and dynamic simulation models for period I only. These and the other results from this investigation are summarized in Sections 3.2 to 3.9 below.

3.2 System COD and N removal performance (see Table 4)

1. Over the 582 day investigation the average COD balance in the MLE and UCT systems were 94 and 92% respectively, and the average N balance 80% and 82% respectively. Although considerably lower than 100%, these are acceptable and similar to COD and N balances observed in other investigations on nutrient removal systems.

Table 4: Comparison of MLE and UCT system performance at 30°C and 20°C receiving the wastewater from the same source (Mitchells Plain, Western Cape, South Africa).

Parameter Temperature Sludge age (days)	MLE 30°C 10	MLE* 20°C 12	UCT 30°C 10	UCT** 20°C 10
%COD balance %N Balance	94 80	91 90	92 82	92 88
%COD removal %COD in unfiltered effluent %COD in waste sludge %COD in denitrification %COD in oxygen utilized %COD unaccounted for	94.3 5.7 36.0 10.4 41.9 6.0	89.9 10.1 32.7 10.1 8.1 9.0	93.8 6.2 37.8 9.2 38.8 8.0	94.1 6.9 37.4 12.8 34.3 8.6
%N removal %TKN in unfiltered effluent %Nitrate in effluent %N in waste sludge %N in denitrification %N unaccounted for	85.3 4.2 10.5 25.8 39.5 20.0	72.7 7.6 19.8 24.7 37.8 10.1	79.2 4.3 16.5 26.1 35.1 18.0	77.6 4.2 18.2 21.7 44.6 11.3
Filtered effluent COD concentration (mgCOD/*l*) Unbiodegradable soluble COD fraction (f_{us}) Unfiltered effluent COD concentration (mgCOD/*l*) COD of effluent suspended solids (mgCOD/*l*)	36.4 0.050 41.2 4.8	- 0.094 54.4 -	38.5 0.054 44.2 5.7	46.4 0.058 - -
Filtered effluent TKN concentration (mgN/*l*) Effluent FSA concentration (mgN/*l*) Unbiodegradable soluble Organic N concentration Unbiodegradable soluble Organic N fraction (f_{nu}) Unfiltered effluent TKN concentration (mgN/*l*) TKN of effluent suspended solids (mgN/*l*)	2.86 2.2 0.7 0.010 3.36 0.5	- - - - 3.48 -	3.05 2.3 0.7 0.010 3.50 0.45	2.21 - - 0.033 - -
Mass TSS wasted (mgTSS wasted/d)/(mgCOD load/d) Mass TSS in system (mgTSS in system)/(mgCOD load/d) Mass VSS wasted (mgVSS wasted/d)/(mgCOD load/d) Mass VSS in system (mgVSS in system)/(mgCOD load/d)	0.278 2.78 0.245 2.45	0.258 3.09 0.226 2.72	0.316 3.16 0.260 2.60	0.327 3.27 0.266 2.66
Unbiodegradable particulate COD fraction (f_{up}) COD/VSS ratio (f_{cv}) TKN/VSS ratio (f_n) VSS/TSS ratio (f_i) P Removal (mgP/*l*)	0.155 1.460 0.100 0.873 5.5	0.114 1.449 0.102 0.876 -	0.149 1.442 0.095 0.824 11.3	0.062 1.48 0.10 0.812 19.6

* Data from Ubisi *et al.* (1997) from a MLE ND system at 12 days sludge age and 25% anoxic mass fraction, which also was system (recycle, 2:1) denitrification limited.

** Data from Sneyders *et al.* (1997) from identical UCT NDBEPR system.

2. The percentage COD removal in the MLE system was 94.3%. Of the 100% influent COD, 5.7% passed out of the system via the unfiltered effluent, 36.0% via the waste sludge, 10.4% via nitrate denitrified and 41.9% via oxygen utilized with 6.0% unaccounted for. The percent COD removal in the UCT system was 93.8%. Of the 100% influent COD, 6.2% passed out of the system via the unfiltered effluent, 37.8% via the waste sludge, 9.2% via nitrate denitrified and 38.8% via oxygen utilized with 8.0% unaccounted for.

3. The overall average 0.45 μm membrane filtered effluent COD concentrations from the MLE and UCT systems were 36.4 and 38.5 mgCOD/ℓ respectfully, giving unbiodegradable soluble COD fractions (f_{us}) of 0.050 and 0.054 respectively. The overall average unfiltered effluent COD concentration from the MLE and UCT systems were 41.2 and 44.2 mgCOD/*l* respectively. The difference between the unfiltered and filtered COD concentrations was therefore 5 mgCOD/ℓ from both systems and is the COD of the suspended solids in the effluent. These results at 30°C are very similar to those typically obtained at 20°C.

4. The percentage N removal in the MLE system was 85.3%. Of the 100% influent TKN, 10.5%, 4.2 % and 3.3% passed out of the system via the unfiltered effluent as nitrate, TKN and FSA respectively, 25.8% via the waste sludge and 39.5% as N_2 gas via denitrification with 20.0% unaccounted for. The percentage N removal in the UCT system was 79.2%. Of the 100 % influent TKN, 16.5%, 4.3% and 3.3% passed out of the system via the unfiltered effluent as nitrate, TKN and FSA respectively, 26.1% via the waste sludge and 35.1% as N_2 gas via denitrification with 18.0% unaccounted for. The higher % N removal in the MLE system compared with that in the UCT system arises because the MLE system anoxic mass fraction and mixed liquor recycle ratio (i.e. 50% and 4:1) were higher than those of the UCT system (i.e. 35% and 2:1). Denitrification kinetics details are given in Sections 3.4 and 3.5 below.

5. The overall average 0.45 μm membrane filtered effluent TKN concentrations from the MLE and UCT systems were 2.86 and 3.05 mgN/ℓ of which 2.2 and 2.3 mgN/*l* is FSA respectively. Hence, the unbiodegradable soluble organic N concentrations (N_{ousi}) from both systems was 0.7 mgN/*l* giving an unbiodegradable soluble TKN fraction (f_{nu}) of 0.010. The overall average unfiltered effluent TKN concentrations from the MLE and UCT systems were 3.36 and 3.50 mgN/*l* respectively. The difference between the unfiltered and filtered TKN concentrations was therefore 0.5 mgN/ℓ from both systems and is the TKN of the suspended solids in the effluent (see 4 above). While the TKN results at 30°C are very similar to those typically obtained at 20°C, the FSA is considerably higher and the organic N considerably lower than at 20°C (see 12 and 13 below).

6. The masses of COD, TKN and TP fed to both systems daily as well as the system volumes were the same. Hence the TSS and VSS sludge production and reactor masses are directly comparable. In the UCT system, the TSS and VSS sludge production and reactor masses [i.e. 0.316 (mgTSS/d)/(mgCOD/d), 0.260 (mgVSS/d)/(mgCOD/d), 3.16 mgTSS/(mgCOD/d), 2.60 mgVSS/(mgCOD/d)

respectively] were 12.6% and 6.5% higher compared with those in the MLE system [i.e. 0.278 (mgTSS/d)/(mgCOD/d), 0.245 (mgVSS/d)/(mgCOD/d), 2.78 mgTSS/(mgCOD/d), 2.45 mgVSS/(mgCOD/d) respectively]. The higher TSS and VSS in the UCT system is due to BEPR - the polyphosphate accumulating organisms (PAOs) generate more VSS per COD utilized than the ordinary heterotrophs (OHOs) and also have a much higher inorganic content due to the presence of internally accumulated P (Wentzel *et al.*, 1990). For the same wastewater characteristics at 10 days sludge age and 20°C (temperature does not influence this result very much), the BEPR model of Wentzel *et al.* (1990) predicts a 23.6% TSS and 8.3% VSS increase due to BEPR compared to ND systems (no BEPR). A VSS/TSS ratio of PAOs (f_{iG}) of 0.69 instead of the "standard" Wentzel *et al.* (1990) BEPR model value of 0.46 mgVSS/mgTSS gives the observed 12.6 %TSS increase over the MLE system. This higher value of f_{iG} = 0.69 confirms at least qualitatively that the PAOs in the UCT system of this investigation contained less P per mgVSS ($f_{XBG,P}$) than those in the enhanced PAO cultures of Wentzel *et al.* (1989) (see 16 below).

7. To determine the unbiodegradable particulate COD fraction (f_{up}) of the sewage fed to the MLE system, the appropriate f_{up} value was selected so that the system VSS mass calculated with the ND model of WRC (1984) was equal to that measured using the measured influent characteristics of the sewage (f_{us}, S_{ti}) and the system parameters as input. To do this the endogenous mass loss rate of the OHOs at 20°C (b_{H20}) was adjusted to 30°C with $b_{H30} = 0.24(1.029)^{(T-20)} = 0.32$ /d. Because the model assumes all the biodegradable COD is utilized, this procedure fractionates the VSS mass into its hypothetical constitutive components viz. active OHOs, endogenous residue of OHOs and unbiodegradable particulate VSS (X_i). This fractionation is also required to determine the specific denitrification rates K_1 and K_2 in terms of mgNO_3-N/(mgOHOAVSS.d) (see 14 below). An overall average f_{up} value of 0.155 was found for the MLE system. This value is reasonably close to other f_{up} values obtained at 10 and 20 days sludge age and 20°C in previous (Warburton *et al.*, 1991; Mbewe *et al.*, 1995) and concurrent (Ubisi *et al.*, 1995) investigations using the same wastewater viz 0.135, 0.11 and 0.12 respectively. Also, the 30°C COD/VSS (f_{cv}) and TKN/VSS (f_n) ratios of the mixed liquor were similar to the 20°C values viz. f_{cv} = 1.46 mgCOD/mgVSS and f_n = 0.095 at 30°C mgN/mgVSS compared with 1.48 and 0.10 at 20°C (WRC, 1984). These results indicate that the WRC (1984) ND model is consistent in the temperature range 20-30°C and can be used for estimating sludge production and oxygen demand at 30°C with reasonable confidence. The ND aspects of the WRC (1984) models are given in Sections 3.3 and 3.4 below.

8. To determine the unbiodegradable particulate COD fraction (f_{up}) of the sewage fed to the UCT system, the appropriate f_{up} value was selected so that the system VSS mass calculated with the BEPR model of Wentzel *et al.* (1990) was equal to that measured using the measured readily biodegradable (RB) COD concentration and influent characteristics of the sewage (f_{us}, S_{ti}) and the system parameters as input. To do this the endogenous mass loss rates of the OHOs and PAOs at 20°C (b_{H20} and b_{G20}) and the OHO RBCOD conversion to VFA rate (K) were adjusted to 30°C with

$b_{H30} = 0.24(1.029)^{(T-20)} = 0.32$ /d and $b_{G30} = 0.04(1.029)^{(T-20)} = 0.053$ /d and $K_{30} = 0.06(1.029)^{(T-20)} = 0.08$ /d (see Table 7 below). This procedure fractionated the VSS mass into its hypothetical constitutive components viz. OHOs, PAOs, endogenous residue of OHOs and PAOs and unbiodegradable particulate VSS (X_i). This fractionation also is required to evaluate the P removal (see 16 below) and to check the specific denitrification and RBCOD conversion to VFA rates in the UCT system (see 17 below). An overall average f_{up} value of 0.149 was found for the UCT system, which is similar to that found for the MLE system. After reconciling the calculated VSS mass in the UCT system with that measured, the calculated P removal for the standard PAO P content ($f_{XBG,P}$) of 0.38 mgP/mgPAOAVSS was higher than that measured (14.3 versus 12.9 mgP/ℓ) for long term period I. One of two Wentzel *et al.* (1990) model parameters could be decreased to decrease the calculated P removal; either (1) the conversion rate (K) of RBCOD to VFA, which decreases the proportion of the RBCOD obtained by the PAOs and hence decreases their mass in the system, or (2) the P content of the PAOs, $f_{XBG,P}$. Approach 2 does not affect the calculated VSS mass and fractionation. Approach 1 decreases the calculated VSS mass and results in a higher f_{up} and OHO concentration, which in turn affects the measured specific denitrification rates (see 15 below). Because the RBCOD conversion rate was measured to be close to the BEPR model value of 0.07/d (see 17 below) and the f_{up} value similar to that found for the MLE system, it was decided to accept approach 2. This approach is also the most appropriate for design because in design situations the active PAO mass will be calculated using the Wentzel *et al.* (1990) model from the influent RBCOD concentration with the "standard" conversion rate of K=0.06 ℓ/(mgOHOAVSS.d) at 20°C. An overall average $f_{XBG,P}$ value of 0.280 mgP/mgPAOAVSS set the calculated P removal equal to that measured in the UCT system (12.9 mgP/ℓ). In contrast, the overall average P removal in the MLE system was 5.5 mgP/*l*.

9. Comparing the $f_{up} = 0.149$ found for the UCT system at 30°C with f_{up} values obtained in other investigations on NDBEPR systems with the same wastewater at 20°C, a wide range in f_{up} values from 0.04 to 0.32 was found. Because reasonably consistent f_{up} values for the same wastewater are expected, and indeed found to be so for the MLE systems, there must be factors that influence sludge production in NDBEPR systems that are not taken account of in the steady state and dynamic simulation models. One such factor appears to be the sludge settleability. It has been found in earlier investigations (Musvoto *et al.*, 1992, 1994; Casey *et al.*, 1994 a,b) that as the DSVI, and hence the AA (low F/M) filament abundance, increased so the VSS mass in the NDBEPR system decreased and *visa versa*. The peculiar aspect of this phenomenon is that the MLE system, which had an overall higher DSVI than that of the UCT system (see 26 below), did not exhibit this variation in VSS mass with DSVI. No explanation for this unusual behaviour in the UCT system can be advanced (see 15 below).

3.3 Nitrification in the MLE and UCT systems at 30°C

10. Accepting that the endogenous respiration rate of nitrifiers b_A at 30°C is given by $b_{A30} = 0.04(1.029)^{(T-20)} = 0.053$ /d, an average maximum specific growth rate of

nitrifiers μ_{nm} at 30°C μ_{nm30} of 1.03 /d was determined from the 13 aerobic batch tests on the MLE system. The μ_{nm30} rate determined for the parallel 20°C MLE system of this investigation from 2 batch tests was 0.85 /d. This 20°C rate far exceeds any earlier μ_{nm20} rate measured at 20°C in the UCT Water Research Laboratory and because it was obtained from only 2 batch tests, this very high μ_{nm20} rate was not taken into account in the further evaluation.

11. From the 14 aerobic batch tests conducted on the UCT system at 30°C, an average μ_{nm30} rate of 0.81 /d was determined. From the 6 aerobic batch tests conducted on the parallel and identical UCT system at 20°C operated by Sneyders *et al.* (1997), a μ_{nm20} of 0.30 /d was obtained. These μ_{nm30} and μ_{nm20} rates give a temperature sensitivity coefficient $\theta_{\mu nm} = 1.10$, which is close to the "standard" value of 1.123. Although Pilson *et al.* (1995) measured different μ_{nm} rates in their MUCT systems at 12°C and 20°C (i.e. $\mu_{nm12} = 0.31$ /d and $\mu_{nm20} = 0.67$ /d), they obtained the same $\theta_{\mu nm}$ coefficient of 1.10. The temperature sensitivity for the μ_{nm} and b_A rates of nitrifiers therefore can be seen to be the same in the 20 to 30°C range as in the 12 to 20°C range. Hence in nitrification design, the difficulty remains to establish the μ_{nm20} rate for the particular wastewater and system under consideration because this rate, which governs the required minimum sludge age of the system (R_{sm}) to ensure nitrification, varies from wastewater to wastewater and to some extent for different systems.

12. Both MLE and UCT systems had a sludge age (R_s = 10d,) about 4 times longer than the minimum required for nitrification (R_{sm}) viz. $R_{sm30} = 1/[(1-f_{xt})\mu_{nm30} - b_{A30}] = 1/[(1-0.5)0.81 - 0.053] = 2.8$d where f_{xt} is the total unaerated mass fraction of the systems (i.e. 0.50). Hence nitrification can be considered to be as complete as possible from a kinetic point of view. The effluent FSA concentration of 2.2 mgN/*l* is therefore not a consequence of poor nitrification but a consequence of the nitrification kinetic response to the higher temperature. The minimum effluent FSA concentration is governed by the half saturation coefficient of the nitrifiers (K_{nT}) - the higher the K_{nT}, the higher the effluent FSA concentration. At 20°C, $K_{n20} \approx 1.0$ mgN/*l* and the effluent FSA is usually < 1.0 mgN/*l*. At 30°C, K_{n30} seems to be much higher at about 7 mgN/*l* to give an effluent FSA concentration of around 2.2 mgN/*l* (see 13 below).

13. The half saturation coefficient for nitrification K_n at 30°C, K_{n30}, was determined from simulation studies with the ND UCTOLD and NDBEPR UCTPHO activated sludge dynamic simulation models. The effluent FSA concentration from the 30°C MLE and UCT systems were 2.2 and 2.3 mgN/*l* respectively (see 5 above). The model default temperature sensitivity coefficient for K_n , θ_{Kn} , in the temperature range 12 to 22°C (i.e. 1.123) was found to be too low for the simulation models to predict 2.2 and 2.3 mgFSA-N/*l* and needed to be increased to 1.215. Hence, while the θ values for the μ_{nm} and b_A rates are similar in the 12 to 22°C and 20 to 30°C ranges, that for K_n is significantly increased in the 20 to 30°C range from 1.123 to 1.215. The K_{nT} coefficient affects only the effluent FSA concentration; it does not significantly affect the minimum sludge age for nitrification, the nitrate concentration generated by nitrification and the nitrification oxygen demand.

3.4 Denitrification in the MLE system at 30°C

14. The anoxic reactor of the MLE system was generally underloaded with nitrate throughout the investigation (see 26 below). Therefore the system denitrification was recycle limited and the system denitrification rate [27.3 mgN/*l* influent giving a system denitrification rate of 0.088 $mgNO_3$-N/(mgOHOAVSS.d)], was so much lower than the biological denitrification rates (K). The biological rates K_1 (due to utilization of influent RBCOD) and K_2 (due to utilization of SBCOD) at 30°C were determined in anoxic batch tests on sludge harvested from the MLE system. The mean K_1 and K_2 rates at 30°C from 17 anoxic batch tests were 0.821 and 0.430 $mgNO_3$-N/(mgOHOAVSS.d) respectively. The "standard" K_1 and K_2 rates at 20°C and their temperature sensitivity coefficients θ_{K1} and θ_{K2} in the 12 to 20°C range determined in previous investigations were 0.720 and 0.101 $mgNO_3$-N/(mgOHOAVSS.d) and $\theta_{K1} = 1.20$ and $\theta_{K2} = 1.080$. These "standard" values give 30°C K_{130} and K_{230} rates of 4.46 and 0.218 $mgNO_3$-N/(mgOHOAVSS.d) respectively. The measured K_{130} rate is much lower than 4.46 (i.e. 0.821) and the measured K_{230} rate is much higher than 0.218 (i.e. 0.43). From the measured K_{130} and "standard" K_{120} rates, a θ_{K1} of 1.03 is obtained. The actual K_1 rate and θ_{K1} coefficient usually are not important in the design of ND systems (except for design of anoxic selectors) because the influent RBCOD usually is utilized in a very short time (< 1h) and so would be completely utilized in a normal anoxic reactor. The θ_{K2} coefficient based on the measured K_{230} and "standard" K_{220} is 1.15 which is very high. It is difficult to reconcile the very fast K_{230} rate with the earlier K_{220} and θ_{K2} measurements. Even though the very high measured K_{230} rate is based on 17 batch tests, because the K_{220} rate and its θ_{K2} coefficient are so important in ND system design, it is recommended that the standard K_{220} rate and its θ_{K2} coefficient are accepted until the very high K_{230} rate of 0.43 $mgNO_3$-N/(mgOHOAVSS.d) can be confirmed with additional tests.

3.5 Denitrification in the UCT system at 30°C

15. The anoxic reactor of the UCT system also was underloaded with nitrate with the result that the system nitrate removal [24.2 mgN/*l* influent giving a system denitrification rate of 0.133 $mgNO_3$-N/(mgOHOAVSS.d)] also was much lower than the biological denitrification rate K'_2. The biological denitrification rate at 30°C due to utilization of SBCOD, K'_{230}, was determined in 15 anoxic batch tests on sludge harvested from the UCT system. The K_1 rate attributable to utilization of influent RBCOD was absent in the UCT system due to RBCOD conversion to VFA and VFA uptake by PAOs in the preceding anaerobic reactor. Also the K'_{230} rate is expressed in terms of the OHOs only i.e. $f_{av,OHO}$ x Measured VSS concentration where the $f_{av,OHO}$ is the OHO active fraction of the VSS and is a result obtained from the integrated f_{up} determination for the sewage batch during which the anoxic batch tests were done (see 8 above). The mean K'_{230} rate at 30°C was 0.254 $mgNO_3$-N/(mgOHOAVSS.d). The K'_{220} rate determined from 10 batch tests on the parallel UCT system at 20°C as part of this investigation was 0.181 $mgNO_3$-N/(mgOHOAVSS.d). Later Sneyders *et al.* (1997) determined a K'_{220} rate at 20°C of 0.071 $mgNO_3$-N/(mgOHOAVSS.d) on the same 20°C UCT system which is less

than half of the rate measured earlier at 20°C in this investigation. Comparing the K'_{2T} rates obtained in this and in 5 other investigations, it was concluded that the K'_2 rate is inversely proportional to the $f_{av,OHO}$ which in turn is itself inversely proportional to f_{up}. No explanation for this consistent but wide variation in the K'_{2T} rate observed in NDBEPR systems can be advanced (see 9 above). The variation in f_{up}, which appears to be the basic varying parameter on which the variation in $f_{av,OHO}$ and K'_2 depend, causes the K'_2 rate to vary much more than temperature because the K'_{212} and K'_{230} rates determined by Pilson *et al.* (1995) at 12°C and in this investigation at 30°C, fall close to the K'_2 - $f_{av,OHO}$ and $f_{av,OHO}$ - f_{up} trend lines for 20°C. It would appear therefore that the temperature sensitivity coefficient for K'_2, $\theta_{K'2}$, is very small, around 1.035 in the 12 to 20°C range and even smaller at 1.018 in the 20 to 30°C range.

3.6 BEPR in the UCT system at 30°C

16. Accepting that the endogenous respiration rates of the OHOs and PAOs (b_H and b_A) and the RBCOD conversion to VFA (K) rates have temperature sensitivity coefficients of 1.029 each (see 8 above), the theoretically predicted BEPR by the Wentzel *et al.* (1990) BEPR model at 30°C was found to be significantly higher than that observed for long term period I viz. 14.3 mgP/*l* compared with 12.9 mgP/*l*. In order to match the predicted BEPR to that observed, the P content of the PAOs ($f_{XBG,P}$) was reduced from the "standard" model value of 0.38 mgP/mgPAOAVSS to 0.28. Lower than expected BEPR has also been observed in a number of earlier investigations at 20°C viz. Musvoto *et al.* (1992), Kaschula *et al.* (1993) and Pilson *et al.* (1995) and appears to be associated with significant P uptake in the anoxic reactor. Significant P uptake under anoxic conditions was confirmed in this investigation in the anoxic batch tests. In investigations where P uptake took place mainly (>95%) in the aerobic reactor viz. Wentzel *et al.* (1985, 1989), Clayton *et al.* (1989, 1991) and Sneyders *et al.* (1997), the BEPR was found to be "normal" i.e. $f_{XBG,P}$ = 0.38 mgP/mgPAOAVSS. It appears that for reasons not clear yet at this stage that denitrifying PAOs, which have a P content lower than their aerobic counter parts or grow less biomass from the same VFA taken up in the anaerobic reactor, find a niche in the NDBEPR system. Biochemical assays have indicated that some PAOs can denitrify (Lötter, 1985; Lötter *et al.*, 1986). Also, laboratory anaerobic-anoxic BEPR systems have been operated successfully (Kuba *et al.*, 1993) and in other studies significant anoxic P uptake has been observed in NDBEPR systems at laboratory scale (Kerrn-Jespersen and Henze, 1993), pilot scale (Bortone *et al.*, 1996; Sorm *et al.*, 1996) and full scale (Kuba *et al.*, 1997). Denitrification by PAOs is included in the biochemical models of Wentzel *et al.* (1986, 1991) and Kuba *et al.* (1996) but is not included in the current NDBEPR simulation models such as IAWQ ASM No2 (Henze *et al.*, 1995) and UCTPHO (Wentzel *et al.*, 1992). *Therefore anoxic P uptake behaviour cannot be realistically simulated with current NDBEPR models and this will significantly effect the comparison between the UCTPHO model predictions and the observed experiment results of this investigation at 30 °C where significant anoxic P uptake was observed (see 19 below).* Proposals to include PAO denitrification into the simulation models have been made (Mino *et al.*, 1995; Barker and Dold, 1997) and

it would be interesting to compare the anoxic/aerobic P uptake BEPR behaviour in "conventional" NDBEPR systems with that observed in the DEPHANOX system, which is designed to maximize anoxic P uptake (Bortone *et al.*, 1996; Sorm *et al.*, 1996) to see if the BEPR in these latter systems also is lower.

17. The RBCOD to VFA conversion rate by the OHOs in the anaerobic reactor K was determined with anaerobic batch tests on sludge harvested from the UCT system. From 15 such batch tests a mean K rate = 0.070 *l*/(mgOHOAVSS.d) was determined. Accepting the 20°C rate of 0.060 *l*/(mgOHOAVSS.d) measured by Wentzel *et al.* (1985) gives a temperature sensitivity coefficient θ_K of 1.016. This is a low value and indicates that the RBCOD to VFA conversion K rate is not very sensitive to temperature. Interestingly, a similarly low θ value is obtained for the K'_2 denitrification rate, which also is mediated by the OHOs (see 14 above). [The OHO active fraction $f_{av,OHO}$ to calculate the specific K rate was based on a θ_K of 1.029 (see 8 above) making K_{30} = 0.08 *l*/(mgOHOAVSS.d). However, a lower value of 0.07 was actually found. The RBCOD conversion to VFA was therefore somewhat overpredicted, but the difference between a K_{30} rate of 0.08 or 0.07 *l*/(mgOHOAVSS.d) is so small that the VSS fractionation based on a K_{30} = 0.07 was not recalculated.]

3.7 Simulation of MLE and UCT system performance at 30°C

18. The wastewater characteristics determined either from direct measurement or with the aid of the steady state ND model (WRC, 1984) and the MLE system operating conditions were given as input to the UCTOLD dynamic simulation model. The model default stoichiometric and kinetic constants and temperature coefficients were retained except for the two Monod constants associated with nitrification. The measured maximum specific growth rate of nitrifiers at 30°C (μ_{nm30}) was standardized to 20°C with the standard $\theta_{\mu nm}$ = 1.123 and given as input to the model; K_{n20} = 1.0 mgN/*l* was retained but its θ value, θ_{Kn}, was increased from 1.123 to 1.215 (see 13 above, and Section 3.9 below). The predicted MLE system response was then compared with that observed. A very good correlation was found for all the measured parameters, except for the aerobic reactor oxygen utilization rate (OUR), which was predicted to be 43.5 mgO/(*l*.h) whereas that measured was 38.3 mgO/(*l*.h). The reason for the higher predicted OUR is that the model is based on a 100% COD and N balance, whereas experimentally 92% COD and 80% N balance was obtained (see 1 above). Because the influent unbiodegradable particulate COD fraction (f_{up}) was calculated from the measured VSS mass in the system with the aid of the WRC (1984) ND model (which is essentially a simplified subset of the UCTOLD dynamic ND simulation model see Section 3.9 below), the lower than 100% observed COD and N balances will reflect in the OUR. In this respect, a good correlation between predicted and measured response is expected because to a large extent the simulation model was calibrated to the observed results because some wastewater characteristics and kinetic constants were calculated from the measured results.

19. The measured and predicted VSS concentrations were 1893 and 1893 mgVSS/*l*,

the (i) anoxic, (ii) aerobic and (iii) effluent nitrate and nitrite concentrations were (i) 0.6 and 0.0 mgN/*l*, (ii) 5.5 and 6.7 and (iii) 6.0 and 6.7 mgN/*l* respectively, and the measured and predicted FSA concentration were 2.1 and 2.2 mgN/*l* respectively. The UCTOLD ND simulation model therefore predicts the MLE system observed results at 30°C very well provided the appropriate sewage characteristics and kinetics constants are given as input. However, it must be remembered that the anoxic reactor was underloaded with nitrate. Predicting the nitrate concentrations in the various reactors is therefore not a test of the denitrification kinetics in the model; provided the kinetic rates are fast enough, these concentrations are governed mainly by the recycle ratio for underloaded reactors.

20. As for the MLE system, the wastewater characteristics determined either from direct measurement or with the aid of the steady state BEPR model of Wentzel *et al.* (1990) and the UCT system operating conditions were given as input to the UCTPHO NDBEPR dynamic simulation model. Also, the model default stoichiometric and kinetic constants and temperature coefficients were retained except for the μ_{nm20} and θ_{Kn} like for the MLE system (see 18 above and Table 7 below). It should be noted that in the UCTPHO model, the P content of the PAOs ($f_{XBG,P}$) is not an input stoichiometric constant - it arises from the process rates of P release and uptake and PAO organism growth, which, when go to completion, result in a net PAO content of 0.38 mgP/mgPAOAVSS. As for the MLE system, a very good correlation was obtained for all the measured parameters relating to COD and N removal, except for the aerobic reactor OUR. Because the f_{up} was calculated from the measured VSS mass in the system with the aid of the BEPR model of Wentzel *et al.* (1990) (which is essentially a simplified subset of the UCTPHO dynamic ND simulation model see Table 7 below), the lower than 100% observed COD and N balances result in the significantly higher predicted OUR than observed viz. 41.1 versus 37.8 mgO/(*l*.h). It should be noted that because the VSS sludge production per mass COD load is higher for the UCT system than the MLE system due to BEPR, the OUR is lower in the UCT system compared with that in the MLE system. This is apparent both theoretically (Wentzel and Ekama, 1997) and experimentally - the MLE and UCT predicted OUR is 43.5 and 41.1 mgO/(*l*.h) respectively and the MLE and UCT system measured OUR is 38.3 and 37.8 mgO/(*l*.h).

21. The measured and predicted VSS concentrations were 1959 and 2039 mgVSS/*l*, the (i) anaerobic (ii) anoxic, (iii) aerobic and (iv) effluent nitrate and nitrite concentrations were (i) 0.2 and 0.0 mgN/*l*, (ii) 0.7 and 0.0 mgN/*l* (iii) 7.6 and 9.5 and (iv) 8.4 and 9.5 mgN/*l* respectively. As for the MLE system, because the anoxic reactor was underloaded with nitrate, this good correlation between predicted and observed results is mainly the consequence of the recycle ratio and therefore not a test of the denitrification kinetics in the model. Because of the calibration of the nitrification kinetic constants (μ_{nm20} and θ_{Kn}) the predicted and observed FSA concentrations were very close at 2.2 and 2.3 mgN/*l* respectively. The COD and ND components of the UCTPHO BEPR simulation model therefore predicted the UCT system results at 30°C very well provided the appropriate sewage characteristics and kinetics constants were given as input.

22. For any wastewater, the wastewater characteristics, including the μ_{nm20} rate, applicable to it have to be given as input to the UCTOLD and UCTPHO models - these models cannot be expected to give a good correlation between experimental and predicted results if this is not done. Therefore, the measure of reliability of the models is the measure whereby the values of the kinetic and stoichiometric constants have to be adjusted to achieve a good correlation. In this investigation very few kinetic constants and their temperature sensitivity coefficients needed to be adjusted - only θ_{Kn} was changed from 1.123 to 1.215 to correctly predict the effluent FSA concentration for both the MLE and UCT systems. The default values for all the other kinetic and stoichiometric constants and their θ coefficients (except θ_{Kn}) relating to COD removal and ND were not required to be changed to obtain a good correlation between experimental and observed results. It is mainly in the BEPR component of UCTPHO that a poor correlation between experimental and predicted results is obtained.

23. Although the difference between the predicted and observed BEPR is relatively small i.e. 14.3 and 12.9 mgP/*l* respectively for long term period I, the BEPR component of UCTPHO model predicts completely different P concentrations to those observed for the reactors. As mentioned above (see 16 above), the steady state and dynamic state BEPR models are based on experimental BEPR performance associated with predominantly aerobic P uptake, whereas in the UCT system, significant anoxic P uptake took place. This therefore makes a comparison between predicted and experimental BEPR performance invalid. Comparison of the BEPR behaviour of the UCTPHO model is therefore confined to experimental results which manifest the same predominantly (>90%) aerobic P uptake behaviour.

3.8 Sludge settleability and filamentous organisms

24. The filament most frequently dominant in both the MLE and UCT systems were, in descending order, type 0092, *Microthrix parvicella* and type 1851. Type 021N and *Haliscomenobacter hydrossis* were also frequently observed but not at dominant levels. Apart from type 021N, these filaments are classified as typical of the low F/M category (Jenkins *et al.*, 1984) and are almost always observed in laboratory (Ekama *et al.*, 1996) and full scale (Blackbeard *et al.*, 1986, 1988) ND and NDBEPR systems. During the changes in DSVI in the MLE and UCT systems, the filament types did not change significantly and therefore the different DSVIs were caused by essentially the same filament types at different levels of abundance.

25. The AA (low F/M) filament bulking hypothesis of Casey *et al.* (1994a,b) describes how an AA filament bulking sludge may be the result of nitrate and nitrite "leakage" from the anoxic to the aerobic reactor of ND and NDBEPR systems. If these concentrations are high (> 1 mgN/*l*) in conjunction with a low aerobic mass fraction (<0.70), then a bulking sludge with a high DSVI (> 150 m*l*/g) would prevail.

26. The nitrate plus nitrite concentrations (NO_x, mgN/*l*) in the anoxic reactor of the MLE and UCT were generally low (< 1.0 mgN/l) because the anoxic reactors were underloaded with nitrate, except for the sewage batches with high TKN/COD ratios

(>0.10 mgN/mgCOD) which occurred mostly during long term period II. The overall mean DSVI in the MLE and UCT systems were 235 and 201 ml/g (see Table 5).

Table 5: Mean DSVI and anoxic reactor nitrate plus nitrite (NO_x) concentrations in the MLE and UCT systems at 30°C during long term periods I, II, III and IV (overall).

System	Parameter	Overall (IV)	Period I	Period II	Period III
MLE	DSVI (ml/g)	235	270	204	183
	NO_x (mgN/l)	1.13	0.68	2.58	0.50
UCT	DSVI (ml/g)	201	213	201	171
	NO_x (mgN/l)	2.06	1.34	4.71	0.64

27. While some periods during the investigation show decreasing DSVI with low anoxic NO_x concentrations in the MLE and UCT systems, e.g. from sewage batch 13 to 22, and would therefore support the AA filament hypothesis, other periods are in conflict with this, e.g. decreasing DSVI with high anoxic NO_x concentrations. The results from this investigation therefore do not give unequivocal support for the AA filament bulking hypothesis of Casey *et al.* (1994a,b).

3.9 Closure

In this investigation an inquiry into the kinetic behaviour of the MLE and UCT systems at 30°C has been undertaken. This inquiry was facilitated by application of both the steady state and dynamic simulation models (i) to laboratory scale steady state MLE and UCT activated sludge systems and (ii) in the interpretation of the data collected from an extensive series of anaerobic, anoxic and aerobic batch tests conducted on sludges harvested from the two systems both at 20 and 30°C. The models provided a defined structure within which the data could be consistently evaluated, and enabled the results to be compared on the same basis with similar results collected from systems operated in earlier investigations in the laboratory.

In application of the models to the steady state systems, one question that arose was whether the steady state models could be applied without significant error: In the steady state models it is assumed that a number of biological processes have essentially progressed to completion so that the kinetic relationships can be replaced with more simple stoichiometric ones. In particular, it is assumed that the organism synthesis processes on biodegradable COD are complete (both for OHOs and PAOs), so that the kinetics of synthesis are excluded in the steady state models. To asses this assumption, accepting the same wastewater characteristics (derived from the experimental data) the appropriate steady state and dynamic models were applied to the laboratory MLE and UCT system results for long term period I. Both models provide estimations for the active organism (OHO and PAO where appropriate) and VSS masses that are remarkably similar (see Table 6). This confirmed that the simplified steady state models could be applied to evaluate the experimental data.

Table 6: Comparison of steady (or stationary) state solutions for OHO and PAO active organism and VSS concentrations predicted by the WRC (1984) steady state and UCTOLD (Dold *et al.*, 1991) dynamic simulation ND models and the Wentzel *et al.* (1990) steady state and UCTPHO (Wentzel *et al.*, 1992) dynamic simulation NDBEPR models with experimental VSS concentrations measured during long term period I in the MLE and UCT systems operated in this investigation at 30°C.

Wastewater characteristic input data:
Influent COD (S_{ti}) concentration = 751 mgCOD/*l*
Unbiodegradable soluble COD fraction of influent (f_{us}) = 0.050
Unbiodegradable particulate COD fraction of influent (f_{up}) = 0.154

Model	VSS mgVSS/l	OHO Active Concentrations			PAO Active Concentrations		
		f_{avOHO}	mgCOD/*l*	mgAVSS/*l*	f_{avPAO}	mgCOD/*l*	mgAVSS/*l*
ND System response: MLE System							
WRC (1984)	1849	0.347	950	642	-	-	-
UCTOLD	1893	0.345	968	654	-	-	-
Measured	1892	-	-	-	-	-	-
NDBEPR System response: UCT System							
Wentzel *et al.* (1990)	2004[1]	0.267	792	535	0.145	431	291
UCTPHO	2039[1]	0.273	824	577	0.146	439	297
Measured	1959	-	-	-	-	-	-

[1]The reason for the higher predicted VSS concentrations than measured is because the MLE system f_{up} fraction (0.154) was given as input to the NDBEPR models instead of the UCT system f_{up} fraction (0.149) for long term period I; had the UCT system f_{up} fraction been given as input, the predicted VSS concentration would have been as close as measure as for the ND system.

From the data and model application, it is evident that:

1) Stoichiometric constants obtained from experimental work in the temperature range 12 to 22°C e.g. sludge production and oxygen demand (with underlying organism yield, endogenous residue and influent unbiodegradable particulate COD fraction) remain valid for the temperature range 20 to 30°C.

2) All kinetic processes proceed more rapidly at 30°C than at 20°C.

The above implies that for systems operated over a temperature range 20 to 30°C, the systems can be designed at 20°C with the models and constants which are well established for 20°C and the system performance (in terms of effluent quality) will not be poorer at 30°C. To asses the expected performance at 30°C, it would appear that the structure of the models remains valid. Some information was developed on the temperature sensitivity of the kinetic constants of key processes such as nitrification, denitrification and RBCOD conversion to VFA in BEPR. A detailed comparison of the temperature sensitivity coefficients in the 20 to 30°C range with coefficients determined in earlier investigations in the 12 to 20°C range is presented in the detailed report (Mellin *et al.*, 1998).

If the system design has been set for 20°C, it is evident from this study that all the principal

kinetic processes will be essentially complete at 30°C except the endogenous respiration ones, e.g. heterotrophic COD degradation and autotrophic nitrification, and in essence the system will be overdesigned for 30°C. If the design is to be done at 30°C, then, for the MLE ND system, the models with the temperature sensitivities validated or determined in this investigation can be used for the design with reasonable confidence. However, for the UCT NDBEPR system, the same confidence could not be established, but this is not due to a lack of confidence in the temperature sensitivity coefficients. Rather, there is considerable uncertainty surrounding the specific denitrification rate K'_2. This uncertainty is not unique to this investigation at 30°C; it has been noted in several other investigations with NDBEPR systems at 20°C also and is an aspect that needs further research attention.

3.10 References

Barker P.S. and Dold P.L. (1997) General model for biological nutrient removal activated - sludge systems: Model presentation, *Water Environment Research*, **69**(5), 969-984.

Blackbeard J.R., Ekama G.A. and Marais G.vR. (1986). A survey of filamentous bulking and foaming in activated sludge plants in South Africa. *Water Pollut. Control*, **85**(1), 90-100.

Blackbeard J.R., Gabb D.M.D., Ekama G.A. and Marais G.vR. (1988). Identification of filamentous organisms in nutrient removal activated sludge plants in South Africa. *Water SA*, **14**(1), 29-34.

Bortone G., Saltarelli R., Alonso V., Sorm R., Wanner J. and Tilche A. (1996) Biological anoxic phosphorus removal - The DEPHANOX process. *Water Sci. Technol.*, **34**(½), 119-128.

Casey T.G., Wentzel M.C., Ekama G.A., Loewenthal R.E. and Marais G.v.R. (1994a). An hypothesis for the cause and control of anoxic-aerobic (AA) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 203-212.

Casey T.G., Wentzel M.C., Ekama G.A., Lakay M.T. and Marais G.vR. (1994b). Causes and control of anoxic-aerobic (AA) filament bulking in biological N and N & P removal systems. Final report to Water Research Commission on 4 year (1989 - 1992) research contract K5/286. WRC 286/2/93, P O Box 824, Pretoria, 0001.

Clayton J.A., Ekama G.A., Wentzel M.C. and Marais G.vR. (1989). Denitrification kinetics in biological nitrogen and phosphorus removal activated sludge systems. Research Report W63, Dept. Of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Clayton J.A., Ekama G.A., Wentzel M.C. and Marais G.v.R. (1991). Denitrification kinetics in biological nitrogen and phosphorus removal systems treating municipal wastewaters. *Water Sci. Technol.*, **23**(4/6-2), 1025-1035.

Ekama G.A., Wentzel M.C., Casey T.G. and Marais G.vR. (1996) Filamentous organism bulking in nutrient removal activated sludge systems. Paper 6: Review, evaluation and consolidation of results. *Water SA*, **22**(2), 147-152.

Jenkins D., Richard M.G. and Daigger G.T. (1984) *Manual on the causes and control of activated sludge bulking and foaming*. Water Research Commission, PO Box 824, Pretoria, 0001, South Africa.

Kaschula W.A., Ekama G.A., Palmer S.H., Wentzel M.C. and Birch R.R. (1993). The effect of alternative detergent builders on the nutrient removal activated sludge

sewage treatment process. Research Report W78, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Kerrn-Jespersen J.P. and Henze M. (1993) Biological phosphorus uptake under anoxic and oxic condition. *Water Research*, **27**(4), 617-624.

Kuba T., Smolders G.J.F., van Loosdrecht M.C.M. and Heinen J.J. (1993) Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. *Water Sci. Technol.*, **27**(5/6), 241-252.

Kuba T., Murnleitner E., van Loosdrecht M.C.M. and Heinen J.J. (1996) A metabolic model for the biological phosphorus removal by denitrifying organisms. *Biotech. Bioeng.*, **52**, 685-695.

Kuba T., van Loosdrecht M.C.M., Brandse F.A. and Heinen J.J. (1997) Occurrence of denitrifying phosphorus removing bacteria in modified UCT-type wastewater treatment plants. *Water Research*, **31**(4), 777-786.

Lötter L.H. (1985). The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. *Water Sci. Technol.*, **17**(11/12), 127-139.

Lötter L.H., Wentzel M.C., Ekama G.A. and Marais G.v.R. (1986). A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic/anoxic/aerobic and aerobic systems. *Water SA*, **12**(4), 203-208.

Mbewe A., Wentzel M.C. and Ekama G.A. (1995). Characterization of the carbonaceous material in municipal wastewaters. Research Report W84, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Mino T., Liu W.T., Kurisu F. and Matsuo T. (1995) Modelling glycogen storage and denitrification capability of microorganisms in enhanced biological phosphate removal processes. *Water Sci. Technol.*, **31**(2), 25-34.

Musvoto E.V., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.VR. (1992). The effect of a large anoxic mass fraction and concentrations of nitrate and nitrite in the primary anoxic zone on low F/M filament bulking in nutrient removal activated sludge systems. Research Report W77, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Musvoto E.V., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.v.R. (1994) The effect of incomplete denitrification on anoxic-aerobic (low F/M) filament bulking in nutrient removal activated sludge systems. *Water Sci. Technol.*, **29**(7), 295-299.

Pilson R.A., Wentzel M.C. and Ekama G.A. (1995). The effect of temperature on denitrification kinetics and biological excess phosphorus removal in nutrient removal activated sludge plants in temperate climates (12°C - 20°C). Research Report W86, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Sneyders M.J., Wentzel M.C. and Ekama G.A. (1997) Treatability of unstabilized landfill leachate in a nutrient removal activated sludge system. Research Report W95, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Sorm R., Bortone G., Saltarelli R., Jenicek P., Wanner J. and Tilche A. (1996) Phosphate uptake under anoxic conditions and fixed film nitrification in nutrient removal activated sludge system. *Water Research*, **30**(7), 1573-1584.

Ubisi M.F., Wenzel M.C. and Ekama G.A. (1997). Organic and inorganic components of activated sludge mixed liquor. Research Report W94, Dept of Civil Eng., Univ of Cape Town, Rondebosch, 7701, Cape, South Africa.

Warburton C.A., Lakay M.T., Casey T.G., Ekama G.A., Wentzel M.C. and Marais G.vR.

(1991). The effect of sludge age and aerobic mass fraction on low F/M filament bulking in intermittent aeration nitrogen removal systems. Research Report W65, Dept. of Civil Eng., Univ. of Cape Town, Rondebosch, 7701, Cape, South Africa.

Wentzel M.C., Dold P.L., Ekama G.A. and Marais G.v.R. (1985) Kinetics of biological phosphorus release. *Water Sci. Technol.*, **17**(11/12), 57-71.

Wentzel M.C., Lötter L.H., Loewenthal R.E. and Marais G.v.R. (1986) Metabolic behaviour of *Acinetobacter* spp. in enhanced biological phosphorus removal - A biochemical model. *Water SA*, **12**(4), 209-224.

Wentzel M.C., Ekama G.A., Loewenthal R.E., Dold P.L. and Marais G.v.R. (1989) Enhanced polyphosphate organism cultures in activated sludge systems. Part II: Experimental behaviour. *Water SA*, **15**(2), 71-88.

Wentzel M.C., Dold P.L., Ekama G.A. and Marais G.v.R. (1990) Biological excess phosphorus removal - Steady state process design. *Water SA*, **16**(1), 29-48.

Wentzel M.C., Lötter L.H., Ekama G.A., Loewenthal R.E. and Marais G.v.R. (1991) Evaluation of biochemical models for biological excess phosphorus removal. *Water Sci. Technol.*, **23**(4/6-2), 567-576.

Wentzel M.C., Ekama G.A. and Marais G.v.R. (1992) Processes and modelling of nitrification denitrification biological excess phosphorus removal systems - A review. *Water Sci. Technol.*, **25**(6), 59-82

Wentzel MC and Ekama GA (1997) Principles in the design of single sludge activated sludge systems for the biological removal of carbon, nitrogen and phosphorus. *Water Environment Research,* **69**(7),1222-1231.

WRC (1984). *Theory, design and operation of nutrient removal activated sludge processes.* Water Research Commission, PO Box 824, Pretoria 0001, South Africa. ISBN 0908356137.

4 CONCLUSIONS AND RECOMMENDATIONS

Specific control of filamentous bulking in biological N and N & P removal plants has been undertaken principally at laboratory scale by the Water Research Group (WRG) at UCT under this and the previous contracts with the Water Research Commission (WRC) (K5/542 and K5/286 respectively).

This research has shown that the promoted specific control method of selectors, which stimulate removal of influent readily biodegradable COD in anaerobic, anoxic or aerobic selectors by metabolic or kinetic selection, are not successful for controlling bulking in biological N&P removal plants. It was found that the conditions that stimulate biological N removal are conducive to bulking on nutrient removal plants - stated simply (but not completely) that if denitrification is not complete (nitrate and nitrate concentrations >2 mgN/ℓ) at the time conditions switch from anoxic to aerobic, then proliferation of low F/M (renamed Anoxic-Aerobic to more accurately describe the conditions under which they proliferate) filaments takes place.

A large body of laboratory scale evidence of an indirect nature supporting this alternative explanation for bulking in nutrient removal plants has been accumulated in the two research contracts. Some direct support for the 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 nitrate to nitrite but could not denitrify nitrate to nitrogen gas. Also, increasingly more indirect supportive evidence from full scale nutrient removal plants in Europe is appearing in the literature - in particular at the recent Activated Sludge Population Dynamics Specialized Conference (*Water Sci Technol* **29**(7) 1994) (see Ekama, 1994). The observations of Eikelboom (1994) and Foot *et al* (1994) summarize this anecdotal evidence particularly well (see Section 1 above): The problem being worse in spring, worse with settled wastewater, an inverse relationship between total filament length and effluent total oxidized nitrogen, the efficacy of selectors decreasing if denitrification is permitted to take place in the main reactor (due to underaeration or plant load increase) are all factors that influence the denitrification performance of the plant and increase the likelihood of significant nitrate and nitrate concentrations being present at the transition from anoxic to aerobic conditions and therefore provides indirect support for the bulking hypothesis. Although not unequivocally, this was shown to be the case in the two laboratory investigations undertaken under this contract at 12 and 30°C.

In order to establish the merits of the AA filament bulking hypothesis, two types of full scale studies and one labaratory study need to be undertaken: The first fullscale study with identical parallel modules, one operated to produce a good settling sludge in terms of the bulking hypothesis and the other module to produce a poor settling sludge; the second full scale study to evaluate full scale plant operating data to see whether or not the mechanisms implicated in the hypothesis are operative in plants with good and bad settling sludges and the laboratory scale study with an intermittently aerated single reator system operated with a redox controller, which would can be set such the nitrate and nitrite concentrations are always low before the aerobic (aeration) conditions commence. Proposals to do such full scale studies, the first at Mitchell's Plain WWTP, Western Cape in a tripartite project between the Cape Metropolitan Council, UCT and the WRC and the second between

Stewart Scott Inc. and the WRC, have been approved by the WRC. Also, preparations to undertake the laboratory scale study at UCT have been made and a proposal submitted to the WRC. The work at full scale will be difficult due to the continuously varying conditions under normal plant operating conditions, but it will demonstrate whether or not the AA filament bulking hypothesis has merit for bulking control in fullscale nutrient removal activated sludge systems.

References

Ekama G.A. (1994) A selection of impressions and statements from papers and posters presented at 1st IAWQ ASPD Conference (Paris, 1993), on filamentous bulking, *IAWQ ASPD Newsletter*, 7(1), 16-19.

Eikelboom D.H. (1994) The *Microthrix parvicella* puzzle. Selectors for bulking control at domestic plants in the Netherlands. *Water Sci. Technol.*, **29**(7), 273-279.

Foot R.J., Robinson M.S. and Forster C.F. (1994) Systematic activated sludge bulking and foam control. *Water Sci. Technol.*, **29**(7), 213-220.

Tandoi V., Rossetti S., Blackall L.L. and Majone M. (1997) Some physiological properties of an Italian isolate of *Microthrix parvicella*. *Procs. 2nd Intnl. Conf on Microorganisms in activated sludge and biofilm processes*. Ed: Jenkins D and Hermanowicz SW, 15-22, 21-23 July, Berkeley, CA.

5 GLOSSARY - DEFINITION OF TERMS AND ABBREVIATIONS

AA	Anoxic-aerobic; new group name for most of the low F/M filamentous organisms
AHVSS	Active heterotrophic volatile suspended solids i.e. that part of the Active VSS (AVSS) that is ordinary heterotrophic organisms that do not take part in the BEPR.
ASPD IAWQ	Activated sludge population dynamics specialist group of the
ATV	Abwassertechnische Vereinigung; the German wastewater association.
AVSS	Active Volatile Suspended Solids. The volatile suspended solids (VSS) comprise active organisms and inert organic mass. The active organism mass is the live biological mass which performs the biological reactions; the inert mass originates from two sources (i) from inert organic material in the influent and (ii) endogenous residue. The active fraction of the VSS is a function of the sludge age of the system and sewage characteristics. It is an empirical estimate that has found acceptability because of the consistency it brings to kinetic rates observed in activated sludge systems, e.g. based on active mass the specific endogenous mass/respiration rate and specific denitrification rates are constant with sludge age from 3 to 72 days. Because of this consistency, the readily biodegradable COD (RBCOD) uptake rates also are reduced to specific rates with respect to AVSS so that rates in different sludge age systems can be compared. For details on calculation of the AVSS, see Marais Ekama, 1976 and Ekama, Dold and Marais, 1986. In fully aerobic and anoxic-aerobic systems the AVSS comprises (aside from the autotrophic nitrifiers) only ordinary heterotrophs organisms; in BEPR plants the AVSS includes the polyphosphate accumulating organisms also, and then care needs to be taken that the different kinetic reactions are ascribed to the particular organism group mediating it.
BEPR	Biological excess phosphorus removal
BNR	Biological nutrient removal
COD	Chemical oxygen demand
DO	Dissolved oxygen
DSVI	Diluted sludge volume index, a modified SVI sludge settleability test [see Ekama and Marais (1984). Two improved sludge settleability parameters, *IMIESA*, **9**, 6, 20-25 for method]
d	day
et al.	and others
F/M	Food to Microorganism ratio
FRD	Foundation for Research Development
g	gram

H.hydrossis	*Haliscomenobacter hydrossis*, one of the filamentous organisms in the AA (low F/M) group
h	hour
IAWQ	International Association on Water Quality
K_s	Half saturation coefficient in the Monod equation
K_{2T}, K_{220}	Specific denitrification rate constant in mgNO_3-N/(mgAVSS.d) for N removal systems
K'_{2T}, K'_{220}	Specific denitrification rate constant in mgNO_3-N/(mgAHVSS.d) for N&P removal systems
low F/M	low food to micro-organism ratio; equivalent to low load factor, or low loading rate or long sludge age
ℓ	litre, the unit measure for volume
mℓ	Millilitre
MUCT	Modified University of Cape Town system for biological removal of nitrogen and phosphorus
M. parvicella	*Microthrix parvicella*; one of the most ubiquitous and problematic filamentous organisms in the AA (low F/M) group
MLVSS	mixed liquor volatile suspended solids; same as VSS, the organic part of the suspended solids in activated sludge plants
MLSS	mixed liquor suspended solids; the organic and inorganic suspended solids in activated sludge plants, also referred to as Total Suspended Solids
N	nitrogen; all nitrogen concentrations i.e. nitrate, nitrite or TKN are expressed as mgN/ℓ
NO_3-N; NO_2-N	nitrate and nitrite respectively as N
NO; N_2O	nitric oxide and nitrous oxide respectively, the two gaseous denitrification intermediates between NO_2 and N_2
NUR	Nitrate utilization rate
N_2	Dinitrogen gas, the end product of denitrification
N & P	nitrogen and phosphorus; applied to activated sludge plants incorporating simultaneous biological N and P removal
OUR	oxygen utilization rate; mass oxygen utilized per unit reactor volume per unit time, or per unit VSS mass per unit time, e.g. mgO/(gVSS.h)
P	phosphorus; all phosphorus concentrations are total phosphorus concentrations and expressed as mgP/ℓ
RBCOD	readily biodegradable COD component of the influent COD
RSA	Republic of South Africa
SBCOD	slowly biodegradable COD component of the influent COD
STR	Scientific and Technical Report series of the IAWQ
TFL	Total filament length
TKN	Total Kjeldahl nitrogen
TKN/COD	ratio of the influent TKN and COD concentrations - a useful term comparing the quantity of nitrate that is going to be generated by nitrification from the influent TKN (called nitrification capacity) with the quantity of organic material (influent COD) available for

TON	Total oxidized nitrogen i.e. nitrate + nitrite concentrations
UCT	University of Cape Town activated sludge system for biological removal of nitrogen and phosphorus
USA	United States of America
WRC	Water Research Commission, a water research co-ordination and funding agency in South Africa. Executive Director Mr P E Odendaal, P O Box 824, Pretoria, 0001
WWTP	Wastewater treatment plant
0092, 0041 0914, 0803 1851, 0675 021N	Seven different filamentous organism types of activated sludge, the first 6 common in biological N&P and N removal plants and therefore sorting into the AA (low F/M) group, the last arising mainly with septic wastewaters (i.e. high sulphides) as happens occasionally in the UCT Water Research Laboratory with long sewage storage periods (> 3 weeks) or refrigeration breakdowns.
μm	micrometers (10^{-6} meters)
μ_H	Maximum specific growth rate of heterotrophs in Mond equation
θ	Arrhenius temperature coefficient.

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

Table 3: Filament identifications and DSVI for MUCT1 and MUCT2 biological N&P removal systems both operated at 20 days sludge age and 20°C (from Musvoto *et al.*, 1994)

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

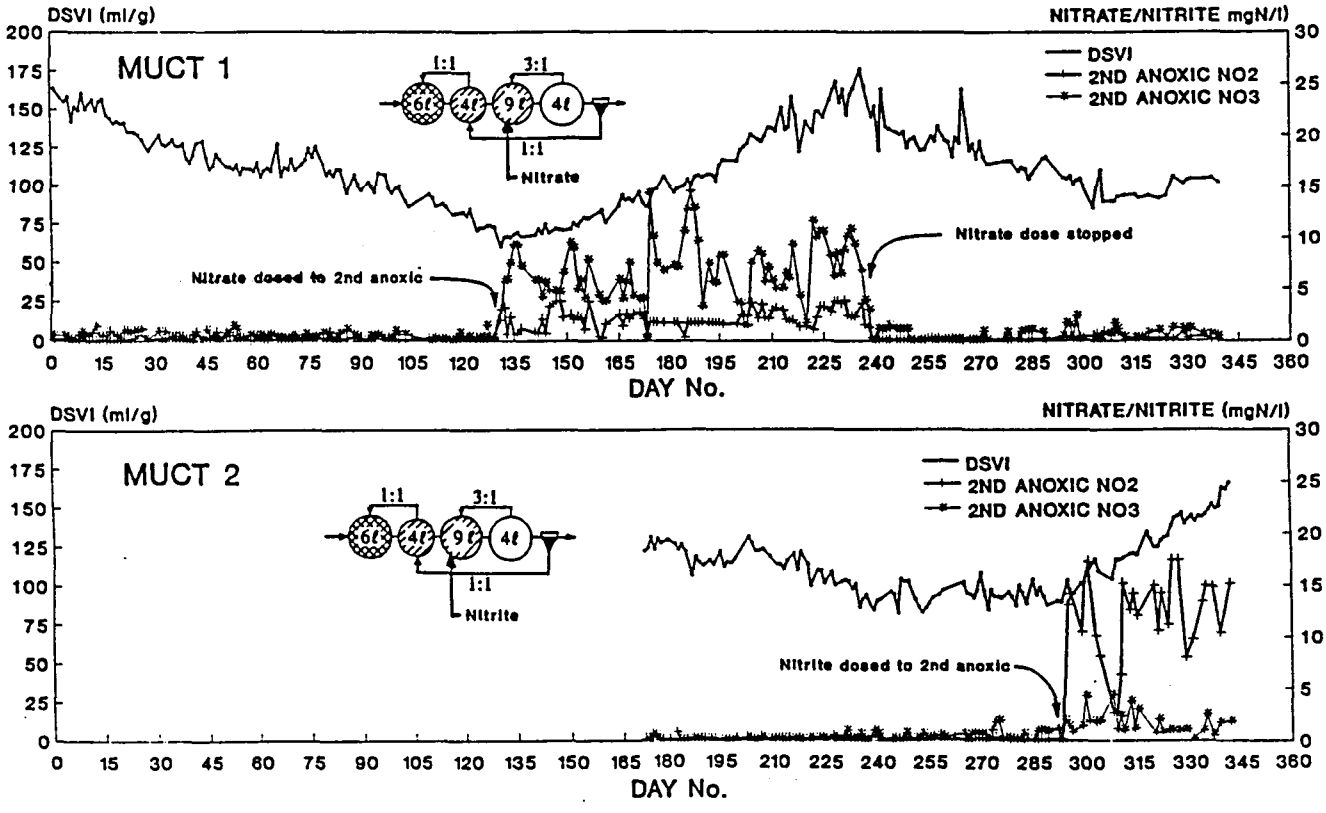

Fig. 1: Sludge settleability (in DSVI, mℓ/g) and nitrate and nitrite concentrations in the 2nd anoxic reactors of MUCT1 (top) and MUCT2 (bottom). Note the increase in these concentrations and concomitantly the increase in DSVI upon commencement of nitrate dosing to MUCT1 on day 129 and nitrite dosing to MUCT2 on day 291; also the decrease in these concentrations in the 2nd anoxic reactor of MUCT1 upon cessation of nitrate dosing to MUCT1 and the concomitant decline in DSVI.