MEASUREMENT OF HETEROTROPHIC ACTIVE BIOMASS IN ACTIVATED SLUDGE SYSTEMS

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

1. BACKGROUND

Although significant developments have taken place in both the engineering and technology and the microbiology and biochemistry areas of the activated sludge system for treating mainly domestic wastewaters, these have proceeded on two parallel, but separate paths. Within the engineering and technology, the activated sludge system has become well established, with systems implemented worldwide for the biological removal of carbon (C), nitrogen (N) and/or phosphorus (P). This implementation has been aided by the development of a suite of steady state design and kinetic simulation models which have facilitated optimization of system design and operation. Parallel to these developments, significant advances have been made in the microbiological and biochemical areas of activated sludge. These advances have been driven by the development of new analytical techniques that allow microorganisms to be studied *in situ* in the activated sludge environment. However, there has been little cross-linking or overlap between the engineering and technology and microbiology and biochemistry paradigms. In particular, the information from the microbiology and biochemistry has not been integrated into the engineering and technology paradigm, to enable improved design and optimization. One area that can form a starting point to build bridges between the two paradigm sets is active biomass. The current design and simulation models invariably include active biomasses as fundamental parameters (ordinary heterotrophic organism, OHO, autotrophic organism, AO and phosphate accumulating organism, PAO), yet these parameters remain essentially hypothetical as they have not been measured and favourably compared with theoretical values. Recently, new respirometric based batch test methods have been developed to quantify OHO (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995; Mbewe *et al.*, 1995; Ubisi *et al.*, 1997a,b; Wentzel *et al.*, 1998) and AO (Cronje *et al.*, 2002a,b) active biomass concentrations, with variable success. However, the interpretation and analysis of the data from these tests remains firmly rooted within the engineering and technology modelling structure and independent of the microbiological and biochemical concepts and measurements. To overcome this shortfall, a multi-institutional collaborative research project sponsored by the Water Research Commission (South Africa) was initiated to attempt to refine the batch test methods and to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques. It was hoped that this research would facilitate the development of links and overlap between the two paradigms.

2. PREVIOUS RESEARCH CONTRACTS

In 2000 three parallel Water Research Commission (WRC) research contracts were set up; (1) K5/1178 with the Centre for Water and Wastewater Research at the Durban Institute of Technology (DIT) (3 years; Holder-Snymann *et al.*, 2004), (2) K5/1179 with Water Research Group at the University of Cape Town (UCT) (2 years; Cronje *et al.*, 2002b), and (3) K5/1191 with the Department of Microbiology and Plant

Pathology at the University of Pretoria (UP) 2 years; Cloete and Thantsha, 2003). These research projects investigated measurement of the OHO active biomass parameter, with common objectives:

(1) Measure the OHO active biomass concentration within the engineering and technology paradigm, and
(2) attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

To address these objectives:

(1) UCT operated laboratory-scale activated sludge systems and measured the OHO active biomass within the engineering and technology paradigm,
(2) DIT set up similar laboratory-scale activated sludge systems and measured OHO active biomass within the microbiological and biochemical paradigm, on samples from both the UCT and DIT systems, and
(3) UP measured OHO active biomass in the UCT systems within the microbiological and biochemical paradigms.

Thus, on the same samples both engineering and microbiological/biochemical quantification of the OHO active biomass could be done, enabling a direct comparison and correlation of the different techniques. Furthermore, the systems were run under strictly controlled and defined conditions so that the theoretical values for OHO active biomass could be calculated with the aid of the activated sludge system models, and compared with the measured values derived from the different techniques. It was hoped that this would facilitate development of a common link between the two paradigm sets.

In the three WRC contracts above, the initial focus was on developing and refining the specific experimental techniques at the different institutions. At UCT, quantification of the OHO active biomass concentration within the engineering and technology paradigm was investigated. Initially, the batch test method of Ubisi *et al.* (1997a,b) was evaluated (Cronje *et al.*, 2000, 2002a,b). Initial evaluations gave poor correlations between measured and theoretical values. Accordingly, the batch test method was substantially modified and refined, principally by flocculating the wastewater with aluminium sulphate then filtering it prior to addition to the test. This greatly simplified the test procedure, as the double parallel batch tests of Ubisi *et al.* were reduced to a single batch test. In initial evaluations of this modified batch test on mixed liquor samples drawn from a well defined and controlled laboratory-scale activated sludge system at 10 d sludge age, it was noted that there was (Cronje *et al.*, 2000, 2002a,b):

- Good agreement between theoretical and modified batch test measured OHO active biomass concentrations.

However, this evaluation was limited, and a more extensive evaluation was conducted on mixed liquor samples from parallel *control* and *experimental* (with addition of toilet paper to the influent to alter the mixed liquor composition) Modified Ludzack Ettinger *(*MLE, anoxic-aerobic) activated sludge systems at 10 d sludge age. It was found that there was (Fig 1a) (Beeharry *et al.*, 2001; Cronje *et al.*, 2002b):

- Remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for the mixed liquors drawn from the *control* and *experimental* systems;
- linearity of results with "serial" dilutions; and
- a constant (i.e. independent of volume of mixed liquor added) difference between measured and theoretical values.

The control activated sludge system was then changed from the MLE configuration to a fully aerobic configuration, and modified batch tests continued. It was found that (Fig. 1b) there was (Beeharry *et al.*, 2001; Cronje *et al.*, 2002b):

- A consistent progressive change in behaviour detected by the batch test in changing from the *MLE* (Fig. 1a) to a *fully aerobic* (Fig. 1b) configuration.

It was concluded that these observations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, it was noted that there was a lack of a 1:1 correlation between theoretical and measured values (Fig. 1), and that this requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

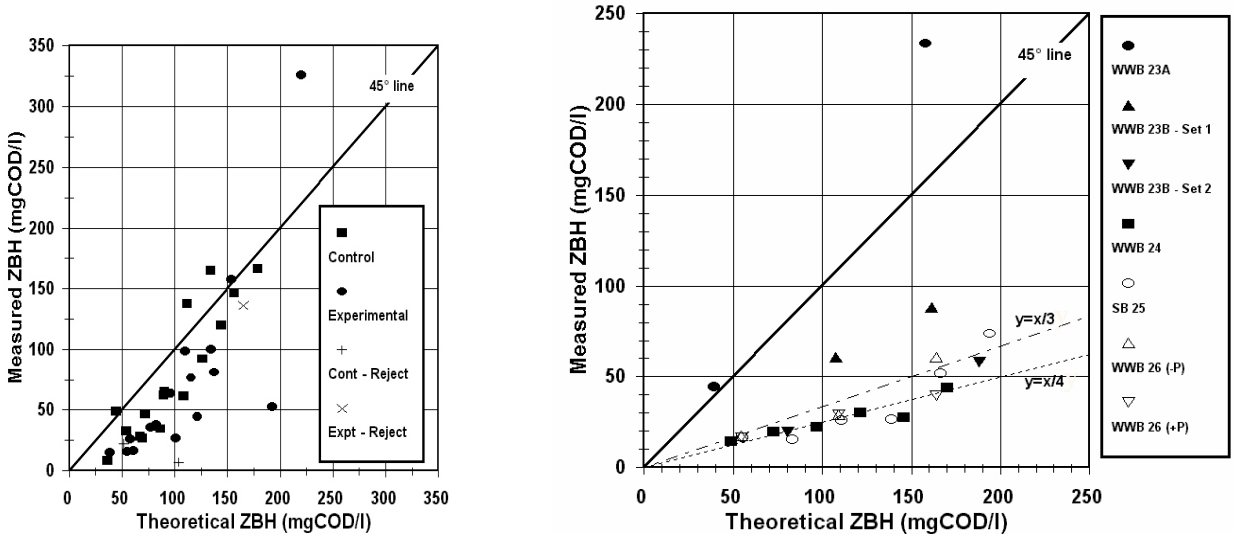

Figure 1: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch tests on mixed liquor drawn from the parent activated sludge systems, (a) both control and experimental anoxic/aerobic MLE and (b) control fully aerobic (Cronje *et al*., 2002; Beeharry *et al.*, 2001). Control system changed from MLE (wastewater batch, WWB, 23A) to aerobic (WWB23B-Set1), with sequence of batch tests 23A → 23B-Set1 → 23B-Set2 → 24 → 25 → 26.

The interpretation and analysis of the data from the batch tests described above remains firmly rooted within the engineering and technology paradigm - the interpretation of the batch test data is based on the same set of models used to calculate the theoretical OHO active biomass concentrations. Independent quantification of the OHO active biomass concentration with the microbiological and biochemical based analytical techniques possibly could provide an alternative independent method to substantiate the active biomass concept. However, little cross-linking exists between the microbiological and biochemical and the engineering and technology paradigms, and deriving compatible data was not possible. To overcome this shortfall, in the three WRC sponsored research projects a first attempt was made to create cross-links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical methods (Cronje *et al.*, 2002a,b), by the three collaborative research partners, UCT, UP and DIT. Various test methods were applied by the different groups to quantify OHO active biomass concentrations, and the results from the test methods compared with each other and with the theoretical OHO active biomass concentrations. The Water Research Group at UCT operated parent MLE and aerobic laboratory-scale activated sludge systems under closely controlled and defined conditions; this enabled the theoretical OHO active biomass concentration to be calculated within the engineering and technology (modelling) paradigm for activated sludge. Additionally, the modified batch test method described above drawn from the parent activated sludge systems. The research group at UP measured the biochemical compound ATP both *in situ* in the laboratory-scale MLE activated sludge systems at 10 and 20 d sludge ages, and during the course of the modified batch tests on mixed liquors drawn from these systems. The research group at DIT used the microbiological technique of a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and AO active biomass concentrations in samples regularly drawn from the laboratory-scale aerobic activated sludge system operated at UCT. From a comparison of the results for OHO active biomass concentrations from the various research groups, it was apparent that (Cronje *et al.*, 2002b):

- The microbiological (DAPI/FISH, Fig. 2a) and biochemical (ATP, Fig. 2b) test methods gave OHO active biomass concentrations that were several orders of magnitude lower than both the theoretical and batch test measured OHO active biomass concentrations.

- The batch tests gave correspondences between measured and theoretical OHO active biomass concentrations that were similar to those described above, i.e. reasonable correspondence for the MLE system (Fig. 2b) and measured values significantly lower than theoretical values for the aerobic system (Fig. 2a).

In examining reasons for the discrepancy in microbiological and biochemical analysis results, the following possibilities were identified:

- For the ATP method applied by UP, it appeared that solids concentrations (i.e. VSS or TSS) interfere in some manner with the ATP measurement method - this would explain the lower values measured in the steady state systems (with higher VSS concentrations) than in the batch tests (with lower VSS

concentrations), and the lower ATP measurements in the 20 d sludge age steady state system (higher VSS concentrations) than in the 10 d sludge age system (lower VSS concentration). **Clearly, this is an aspect that requires investigation.** Hence, it was concluded that the ATP method **as applied** is not a reliable estimate for OHO active biomass concentrations.

- For the DAPI/FISH method applied by DIT, in subsequent investigations it was found that the method of couriering the samples in dry ice caused a significant number of the cells to freeze and hence burst. This would reduce the DAPI/FISH enumerated cell counts significantly, and may be one possible explanation for the low cell counts. **This would be investigated further in the current research project.**

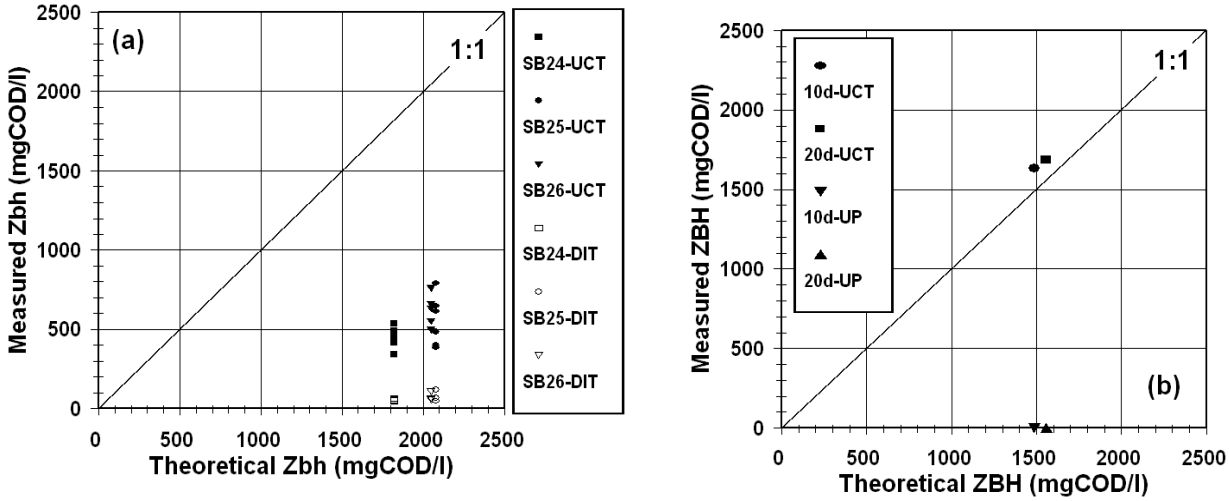

Figure 2: Measured versus theoretical OHO active biomass concentrations in the 10 and 20d sludge age parent laboratory-scale anoxic/aerobic activated sludge systems; measured values from modified batch test method taking account of dilution (UCT) and (a) DAPI/FISH (DIT) and (b) ATP measurements (UP). Measurements on (a) fully aerobic parent system at 10 d sludge age, and (b) parallel MLE parents systems at 10 and 20 d sludge ages.

Although this initial attempt to link the engineering and technology theoretical and batch test measured OHO active biomass concentrations to the values measured with the microbiological and biochemical analytical techniques did not provide even near a close correspondence, it was concluded that, for the first time, the magnitudes of the microbiological and biochemical measurements have been placed within the context of the engineering and technology models. This should help establish a common basis and "language"for the two paradigm sets, to facilitate future exchange of information and development of cross linkages between them.

3. CURRENT RESEARCH CONSULTANCY

In the three WRC contracts above, the initial focus was on developing and refining the specific experimental techniques at the different institutions. Initial exchange of samples occurred, and some results were obtained. However, these results were preliminary only and more detailed research was required. In March 2002, the two year UCT contract ended, whereas the DIT contract still had to run for one more year. Accordingly UCT was granted a research consultancy for one year (K8/453, April 2002 to March 2003), to enable:

(1) Research at UCT to continue into measurement of OHO active biomass within the engineering and technology paradigm.
(2) The laboratory-scale systems to continue to be operated at UCT, to generate samples required by DIT in the final year of their contract for measurement of OHO active biomass within the microbiology and biochemistry paradigm

4. OBJECTIVES AND TASKS OF RESEARCH

The objectives of this research consultancy were to:

(i) Measure the OHO active biomass concentration within the engineering and technology paradigm, and
(ii) attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

To address the objectives above, a number of specific tasks to be addressed by UCT were identified for attention:

Task 1: Operate laboratory-scale systems
Task 2: Conduct batch tests to measure OHO active biomass
Task 3: Calculate OHO active biomass concentrations
Task 4: Harvest and send samples to DIT for microbiological analysis
Task 5: Evaluate engineering and microbiological results

Since the consultancy was a continuation of the previous WRC research contract (K5/1179), the laboratory-scale systems were already in operation, and batch tests being conducted on the mixed liquor from these systems.

5. BATCH TEST FOR MEASUREMENT OF OHO ACTIVE BIOMASS

The principle objective in this part of the research project was to more thoroughly examine the modified batch test procedure and OHO behaviour in the test, in order to explore possible explanations for the inconsistencies in results produced thus far by the test.

To achieve this objective, three main aims were identified (Chapter 3 or Lee *et al.*, 2003):

(1) Re-evaluate the modified batch test method, by repeating the modified batch test procedure and comparing the measured OHO active biomass concentrations with the theoretical values predicted by the steady state model (WRC, 1984). This would establish whether the results of Cronje *et al.* (2000) or Beeharry *et al.* (2001) reported by Cronje *et al.* (2002b) are reproducible, and enable an evaluation of possible causes contributing to variations in correspondence between theoretical and measured values.

(2) In addressing the objective above, it needed to be established whether discrepancies between measured and theoretical OHO active biomass concentrations arise from the activated sludge theory (*i.e.* the theoretical) or from the batch test procedure itself (*i.e.* the measured). Accordingly, the alternative more laborious batch aerobic digestion method of Marais and Ekama (1976) to quantify OHO active biomass would be applied.

(3) Should in application of the modified batch tests, an inconsistency between the measured and theoretical OHO active biomass concentrations be obtained, identify possible causes for this inconsistency. In this regard, during the investigation the importance of OHO behaviour in the modified batch tests became apparent, and the concept of substrate competition between fast and slow growing OHO population groups proposed by Novak *et al.* (1994) was found to be relevant and was examined.

5.1 Parent activated sludge systems

Two laboratory-scale parent Modified Ludzack-Ettinger (MLE) activated sludge systems at 10 and 20 d sludge age and maintained at 20°C served as the source of mixed liquor for the modified batch tests (see Fig. 3.1, system details in Chapter 3). The influent for the parent systems was raw (unsettled) sewage from the Mitchell's Plain Treatment Plant in Cape Town (South Africa). The sewage was collected in batches, stored in 400ℓ stainless steel tanks in a cold room at 4°C and served as feed for both the parent systems and the batch tests for 10 to 14 days. For the parent systems, sewage was diluted with tap water to an influent feed total COD concentration 750 ± 50 mgCOD/ℓ, with influent flow rate of 13.3 or 10 ℓ/d (see Chapter 3 or Lee *et al.*, 2003). Alkalinity was supplemented to the influent to maintain the pH in the aerobic reactor at ± 7.5 Daily monitoring included influent COD, TKN; all reactors nitrite + nitrate; aerobic reactor TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite.

Both the 10 and 20 d sludge age parent systems were operated for 294 days and received 17 batches of sewage. Each sewage batch was accepted as a steady state period, the results for each batch were averaged (after statistical analysis for outliers) and analysed. From this analysis:

- N mass balances were consistent and generally in the range 90 to 110%.

- Generally COD mass balances were not as good as the N mass balances, with 5 out of 17 sewage batches for the 10 day sludge age system and 6 out of 17 sewage batches for the 20 day sludge age system giving mass balances outside the range 90 to 110%.

- From the above, batch tests were conducted during 3 and 2 sewage batches on the 10 and 20 d sludge age mixed liquors respectively. These data were included for analysis, but appropriately marked.

- The influent wastewater mean unbiodegradable soluble COD fractions ($f_{S,us}$) were determined to be 0.043 (sample standard deviation, ± 0.0066) and 0.040 (± 0.0068) for the 10 and 20 d sludge age systems respectively. This difference is not statistically significant at the 95% confidence interval (t-test). The $f_{S,us}$ values are in the range of accepted values, of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The influent wastewater mean unbiodegradable particulate COD fractions ($f_{S,up}$) were determined to be 0.165 (± 0.0295) and 0.148 (± 0.0254) for the 10 and 20 d sludge age systems respectively. This difference is not statistically significant at the 95% confidence interval (t-test). The two $f_{S,up}$ values are higher than the values observed by Ubisi *et al.* (1997a,b) ($f_{S,up}$ = 0.120) and Cronje *et al.* (2000) ($f_{S,up}$ = 0.103) but are similar to the value observed by Beehary *et al.* (2001) ($f_{S,up}$ = 0.161) for the same Mitchell's Plain raw wastewater. The $f_{S,up}$ values of this experiment are in the range of accepted values of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

The consistency in the parent systems data during which batch tests were conducted, lends support to application of the steady state design procedure to the parent systems to calculate the theoretical OHO active biomass concentrations, and to using mixed liquor from the systems in the modified batch tests to measured the OHO active biomass concentrations.

5.2 Modified batch test evaluation

In this research, the modified batch test method of Cronje *et al.* (2000, 2002b) has been extensively evaluated, by conducting a number of batch tests on mixed liquors drawn from the two laboratory-scale MLE activated sludge systems above (35 on mixed liquor from each system) and comparing the measured OHO active biomass concentrations to the theoretical values predicted by the steady state design models. From this evaluation, it was found that:

- In general, %COD recoveries were good, with only 8 out of 35 and 7 out of 35 batch tests on the 10 and 20 d sludge age mixed liquors respectively yielding %COD recoveries < 90%. Excluding outliers, the mean %COD recoveries were 95.2 (sample standard deviation ± 5.3) and 94.8 (± 4.5) % respectively. The %COD recoveries are somewhat lower than that obtained by Cronje *et al.*

(2000) (100.5%), but are similar to those of Beeharry *et al.* (2001) (97.8 and 95.9 %). The good %COD recoveries lend credibility to the experimental data. Rejected batch tests were included for analysis, but appropriately marked, so also batch tests with $R^2 < 0.7$ in the fit to the ℓOUR-time plots.

- For the OHO maximum specific growth rate on SBCOD (K_{MP}), statistical analysis gave mean K_{MP} of 1.37 (± 0.412) and 1.42 (± 0.428) /d for the 10 and 20 day sludge age mixed liquors respectively. The means were not significantly different at 95% confidence interval (t-test), which indicates that sludge age did not have a significant influence on the value for this parameter. The values are larger than that measured by Cronje *et al.* (2000) (0.84 /d), but are close to the values of Beeharry *et al.* (2001) (1.49 and 1.38 /d) and to the value for K_{MP} in the UCT simulation model of 1.35 /d (Dold *et al.*, 1991).

- For the OHO maximum specific growth rate on RBCOD (μ_H), for batch tests on mixed liquors from both parent systems, a clearly discernable trend was noted: As the volume of mixed liquor added to the batch test increased, the value for μ_H decreased. This indicates that one (or more) factor had a dominating influence, which precluded statistical analysis of the data. Re-examination of the data of Beeharry *et al.* (2001) indicated that they obtained, but did not note, a similar trend (see Cronje *et al.*, 2002b). This aspect is examined in more detail below.

In comparing the batch test measured OHO active biomass concentrations with the corresponding theoretical concentrations (Fig. 3):

- The data for both the 10 and 20 d sludge age mixed liquors showed very similar trends and hence the differences between predicted and measured concentrations were not sludge age related.

- Superficially both sludge age comparisons bore a strong resemblance to the data from the preceding contract (Fig. 1a and Cronje *et al.*, 2002b) in that as the theoretical OHO active biomass concentrations increase, the measured values increase virtually parallel to the 1:1 correspondence line, but below it.

- The cause for the difference between predicted and measured OHO active biomass concentrations was not P limitation, since parallel batch tests with and without P supplementation gave near identical results.

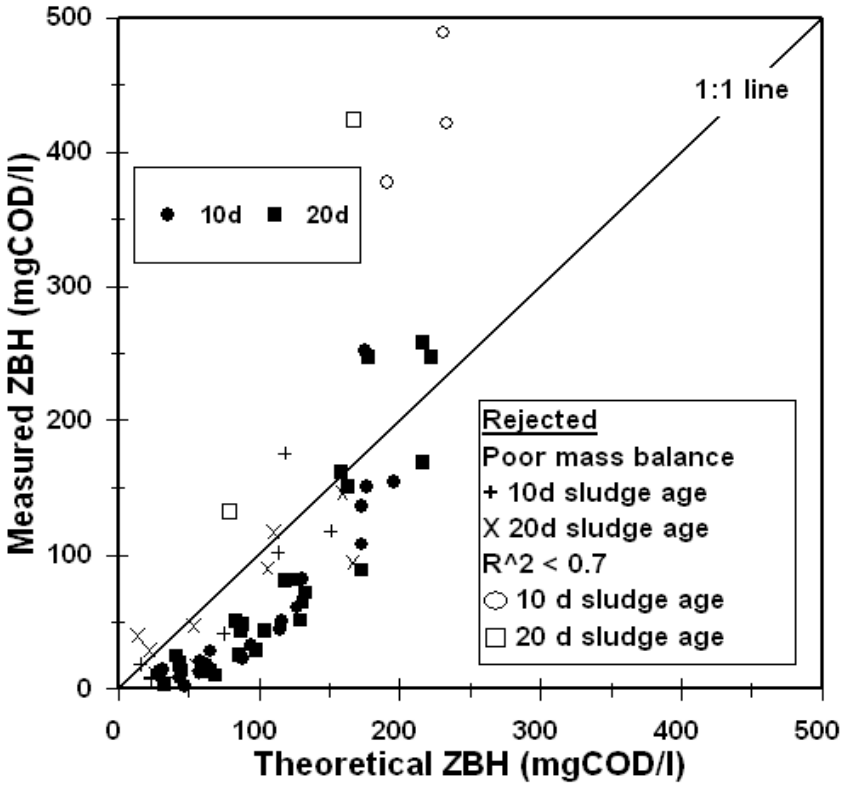

Figure 3: Measured versus theoretical OHO active biomass concentrations ($Z_{BH(0)}$) for mixed liquors from the two parent systems at 10 and 20 d sludge ages.

5.3 Batch aerobic digestion

To determine whether the differences between measured and theoretical OHO active biomass concentrations lay in the activated sludge theory or in the modified batch test procedure, the alternative aerobic batch digestion method of Marais and Ekama (1976) was applied to mixed liquor samples drawn from the 10 and 20 d sludge age MLE activated sludge systems. In total, 2 batch aerobic digestion tests were conducted each on mixed liquor drawn from the 10 and 20 d sludge age parent systems. These tests gave:

- Very close correlation between measured and theoretical OHO active biomass concentrations (Fig. 4).

From this it could be concluded that:

- The close correlation provides substantive support for the OHO active biomass concept as incorporated in the activated sludge models.
- Recognising that the modified batch test is based on the OHO active biomass growth processes (the increase in the concentration in the test needs to be significant compared with the starting concentration) whereas the aerobic batch digestion test is based on the endogenous respiration/"death" processes, it

appears that the cause for the differences between the modified batch test measured and the theoretical concentrations lies in the description and interpretation of the OHO growth processes within the modified batch test itself.

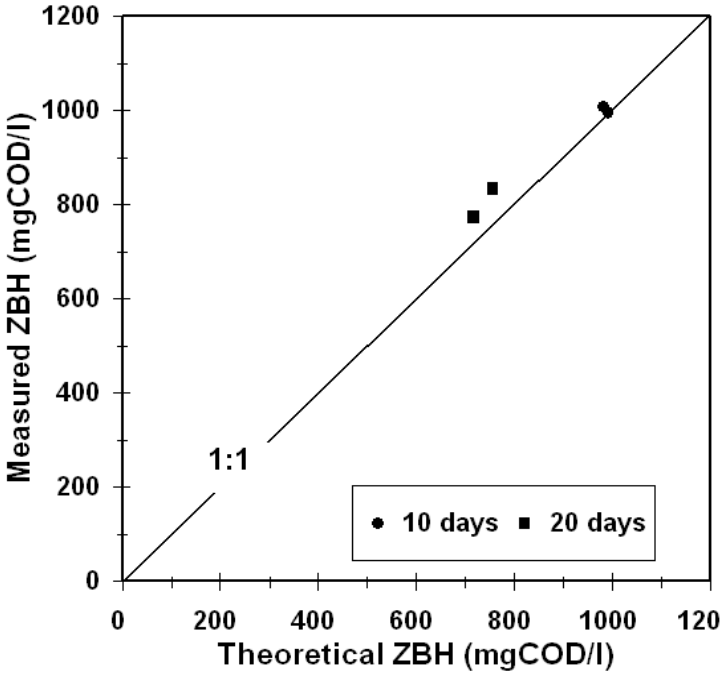

Figure 4: Batch aerobic digestion test measured versus theoretical OHO active biomass concentrations (Z_{BH}) for mixed liquor drawn from the 10 and 20 d sludge age activated sludge systems.

Convincing independent validation of the aerobic batch digestion approach and the model structure for endogenous respiration on which the test is based (and the kinetic model equivalent of death-regeneration) is provided by Van Haandel *et al.* (1998). They operated sequential flow-through aerobic digesters at 1.73, 2.14, 3.00 and 5.63 d and obtained b_H values from both VSS and OUR measurements that are virtually identical to the values of Marais and Ekama (1976) on which the original activated sludge models are based. The data of Van Haandel *et al.* also provides some indirect validation of active biomass concentrations in that there is consistency between the model predicted active fraction in the mixed liquor used as source feed to the digester sequence and that calculated from the aerobic digestion measurements.

5.4 Evaluation of OHO behaviour in modified batch tests

Recognising from the batch aerobic digestion tests above that the variation between batch test measured and theoretical OHO active biomass concentrations was due to the test method and its interpretation focussed attention on the observed response within the modified batch test. The data collected in these tests were examined more closely. Particularly evident was that:

- The OHO maximum specific growth rate on RBCOD (μ_H) appears to be a function of the theoretical OHO active biomass concentration added at the start of the test (Fig. 5). Re-evaluation of the data collected under the preceding contract (Cronje *et al.*, 2002b) indicated that a similar trend was obtained, but not noted. It was noted that the influence of initial substrate to active biomass concentration ratios (in this set of modified batch tests the initial substrate concentration was kept approximately constant) on batch test behaviour and derived kinetics had been observed previously (e.g. Chudoba *et al.*, 1992; Novak *et al.*, 1994; Grady *et al.*, 1996).

- In a plot of $\ell n\ OUR_H$ versus time, the measured data frequently deviated from the fitted linear regression line, showing upward curvature (Fig. 6). This strongly suggested that the net OHO maximum specific growth rates change during the course of the batch test, in agreement with the observations of Pollard *et al.* (1998).

- The observed precipitous drop in OUR implies that the OHO specific growth rates were at their maxima, *i.e.* the changes observed were not due to varying substrate concentrations with time or between batch tests.

- The higher μ_H values (up to about 7/d, Fig. 5) were significantly higher than the range of values accept as default in the UCT kinetic model (1.5 to 3.5/d, Dold *et al.*, 1991).

- The batch test measured OHO active biomass concentrations tended to be consistently lower than the corresponding theoretical values (Fig. 1).

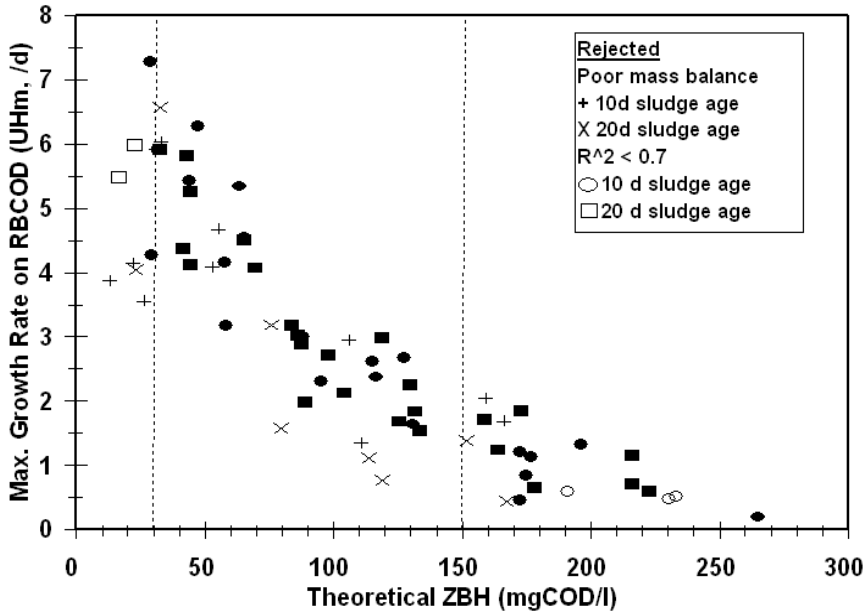

Figure 5: Modified batch test determined OHO maximum specific growth rate on readily biodegradable COD (μ_H) versus the theoretical OHO active biomass concentration at the start of the batch test, $Z_{BH(0)}$.

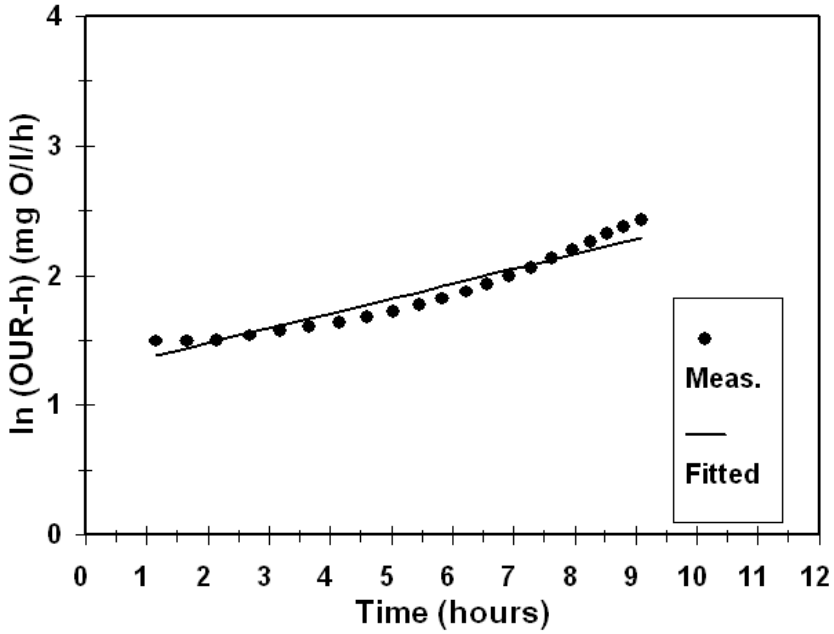

Figure 6: $\ell n(OUR_H)$ versus time profile for modified batch test No. B23, Sewage Batch Test No. 12, with linear regression fit to data.

5.5 Competition kinetic models

To explain observations similar to those above, Novak *et al.* (1994) and Grady *et al.* (1996) proposed substrate competition between different OHO groups as a possible cause. This possibility was investigated, by developing a model for kinetic competition between two OHO populations, one a fast grower and the other a slow grower, based on the concepts of Novak *et al.* (1994). The second OHO group was incorporated into the simplified UCT kinetic model used to analyse the batch test data (Wentzel *et al.*, 1995), as follows (Chapter 3; Lee *et al.*, 2003): (1) The single OHO active biomass was subdivided into two OHO active biomasses, a fast grower (Z_{BH1}) and a slow grower (Z_{BH2}); (2) All OHO mediated processes were duplicated, with the new processes allocated to the second OHO group; (3) The adsorbed SBCOD was split into two, S_{ads1} and S_{ads2}, utilized by Z_{BH1} and Z_{BH2} respectively.

The kinetic model was applied to both the (i) batch tests and (ii) parent systems, using AQUASIM 2.0 (Reichert, 1994). For application to the batch tests, the batch test data with the greatest surety were selected, $30 < Z_{BH(0)} < 150$ mgCOD/ℓ and those with good mass balances (Fig. 3.15). Values for all constants except those related to OHO growth on RBCOD were those of Dold *et al.* (1991). With regard to μ_{Hm} and K_{SH} on RBCOD of Z_{BH1} and Z_{BH2}, these were assumed as 12/d and 3 mgCOD/ℓ and 2/d and 0.1 mgCOD/ℓ respectively, to ensure kinetic competition on RBCOD and that the precipitous drop in OUR could be correctly predicted. Parameters estimated with AQUASIM were initial concentrations of $Z_{BH1(0)}$ (fast grower) and $Z_{BH2(0)}$ (slow grower), and "substrate". To reduce the complexity of parameter estimation, the initial substrate was ascribed to RBCOD only. This restricted the parameter estimation to the period up to the OUR precipitous drop. From the application to the batch tests:

- The model could accurately simulate the OUR_H - time observed in batch tests

(Fig. 7).

- Parameter estimation of the initial batch test $Z_{BH1(0)}$ and $Z_{BH2(0)}$ concentrations gave exceptionally low $Z_{BH1(0)}/(Z_{BH1(0)}+Z_{BH2(0)})$ values, average 1%. However, with time Z_{BH1} increased its proportion significantly and had a marked influence on the predicted OUR_H - time profile. This indicates that the batch test procedure is extremely sensitive to the presence of fast growing OHOs.

- The model could simulate the variety of observations made on the batch tests, including the increase in overall OHO maximum specific growth rates with increasing S_0/Z_{BH} and with time.

- In seeking a source for the fast growing OHOs in the batch test, it was noted that this could not be from the flocculated filtered wastewater added to the batch tests (no observable OUR after 12 hours aeration), and so must be from the mixed liquor drawn from the parent systems.

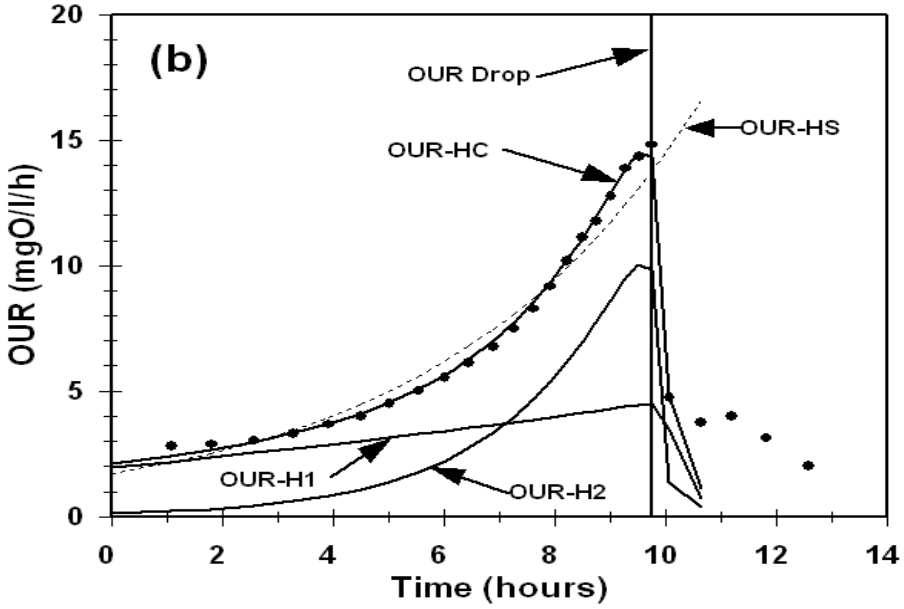

Figure 7: Measured Oxygen Utilisation Rate of OHOs (OUR-m), OUR calculated with the analytical procedure of Wentzel *et al.* (1995) (OUR-HS), OUR simulated with the competition model of kinetic selection (OUR-HC), OUR of the fast grower (OUR-H1) and OUR of the slow grower (OUR-H2). Modified batch tests on mixed liquor drawn from the OHOs 20 days sludge age system, Sewage Batch No.12, Batch Test No. B24.

The competition kinetic model was also applied to simulate the two parent systems, with the same set of kinetic and stoichiometric constants used for the batch tests. Z_{BH1} was accepted to be seeded with the influent wastewater to the parent systems, at 0% - 3% of total COD:

- Z_{BH1} could only be sustained in the parent systems if seeded with the influent.

Seeding was substantiated by the observations of Wentzel *et al.* (1995) and Cronje *et al.* (2000, 2002b) who noted significant fast growing OHOs present in the same raw wastewater as used in this investigation.

- With seeding of Z_{BH1} at concentrations typically measured by Wentzel *et al.* (1995) (< 3% of total COD), the predicted Z_{BH1} proportion of the total OHO active biomass (± 40% at seed of 3% of total COD) was significantly larger than that derived from parameter estimation of the batch test data (1%).

To address the second observation above, the kinetic competition model was modified by removing the processes for growth of Z_{BH1} on SBCOD (model now includes kinetic + metabolic selection). This was considered reasonable, since the original source of Z_{BH1} is seeding with the influent wastewater to the parent systems - this implies growth in the sewer where RBCOD concentrations are very high, which would favour predominantly RBCOD utilization. Simulation of the batch tests and parent systems gave results very similar to the competition only model above, except that for influent Z_{BH1} at 3% of total COD, the predicted proportions of Z_{BH1} in the parent system mixed liquors were < 3% compared to ± 40% for the competition only model above. This former value is very close to those from parameter estimation on the batch test data.

In comparing the various estimates for OHO active biomass concentrations, it was found that the two OHO population kinetic models gave concentrations that were significantly closer to the theoretical values (Fig. 8) than the single OHO population model (Fig. 1). Thus, it could be concluded that:

- The competition hypothesis (in agreement with previous researchers) is one feasible explanation for the observations in the batch tests.

However, the kinetic models developed here are largely hypothetical - insufficient information is available to separate the two OHO populations and quantify the individual kinetic processes. Further, alternative hypotheses could possibly explain the observed behaviours, e.g. physiological adaptation (Daigger *et al.*, 1982). Clearly, this requires further investigation. Such investigations may be facilitated by the microbiologically based analytical techniques, *see below.*

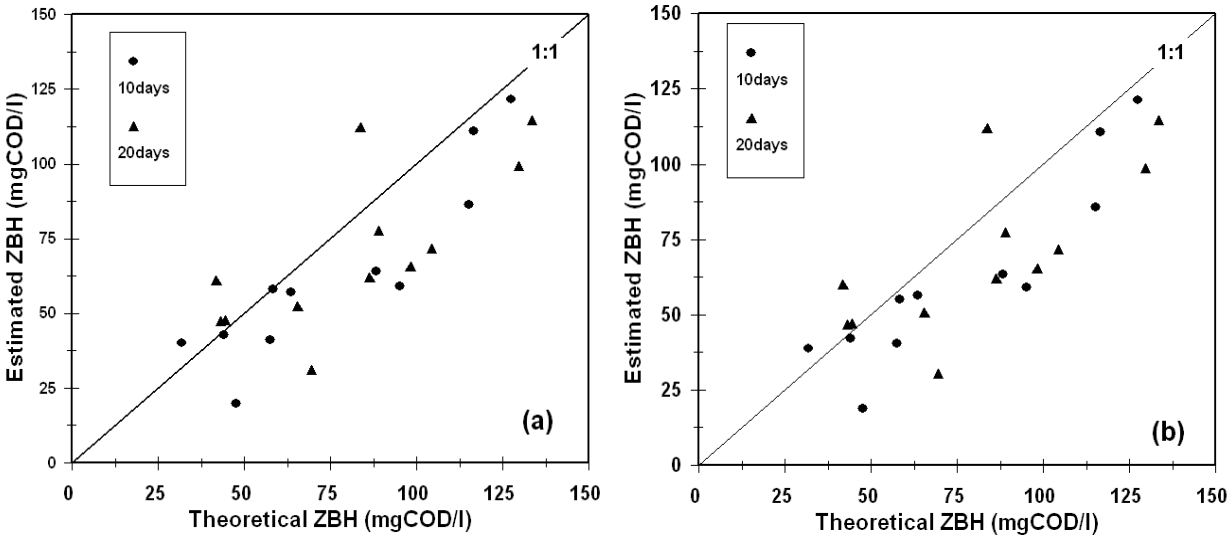

Figure 8 : Plot of the OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] determined by the parameter estimation with the (a) kinetic and (b) kinetic + metabolic competition models versus the theoretical $Z_{BH(0)}$ concentration calculated with the steady-state model (WRC, 1984) for the selected modified batch test data.

6. EVALUATION OF MICROBIOLOGICAL METHODS

In this part of the research, the principle objective was to attempt to link the modified batch test measurements measurements and the defined engineering environment to the new microbiological analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

Since the research project at DIT had a further year to run, to continue investigations into the microbiological measurement of active biomass, this one year consultancy between the WRC and UCT was set up. In this consultancy, the research described in the previous research contract (Cronje *et al.*, 2002b) continued, with UCT operating parallel laboratory-scale anoxic/aerobic MLE activated sludge systems at 10 and 20 d sludge age, conducting modified batch tests on mixed liquors harvested from these systems, and harvesting mixed liquor to send to DIT for microbiological analysis, using a combination of DAPI staining and FISH to determine both OHO and AO active biomass concentrations. From the conclusions arising from the preceding contract, that preserving the samples in ethanol and sending these packed in dry ice to DIT resulted in loss of active biomass due to freezing and bursting of cells, the method for sample preparation was changed. Instead, at UCT the samples were drawn from the laboratory-scale systems and immediately fixed with paraformaldehyde (Chapter 4). The samples were stored at -20°C and couriered in batches to DIT in cooler boxes with ice packs. ***This appeared to resolve the problems with sample exchanges experienced in the previous contract.***

6.1 Parent activated sludge systems

Operation of the two parent MLE activated sludge systems at 10 and 20 d sludge ages continued, and these served as source for mixed liquor for the batch tests and microbiological examinations. From analysis of the parent system data (Chapter 4):

- N mass balances for both systems were consistent and almost invariably in the acceptable range of 90 - 110 %.

- COD mass balances for the 10 d sludge age system were good with only 1 sewage batch marginally less than the range 90 - 110 % (at 89%). However, COD mass balances for the 20 d sludge age system were more variable, with 3 out of 11 sewage batches significantly less than 90 %. These low mass balances were traced to problems with the DO probe, which was replaced. However, additionally during Sewage Batch Nos. 22/02 and 23/02 significant mixed liquor losses occurred due to pipe blockages and reactor overflows. Hence, the data collected during these two sewage batches should be treated with caution.

- The influent wastewater mean unbiodegradable soluble COD fractions ($f_{S,us}$) were determined to be 0.055 (SSD = 0.009) and 0.053 (SSD = 0.017) for the 10 and 20 d sludge age systems respectively. This difference is not significant at the 95% confidence interval (t-test). The values are similar to those determined in Section 5 above (0.043 and 0.040 respectively).

- The influent wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) for the 10 d sludge age system was 0.119 (SSD = 0.042). This value is very similar to that determined by Ubisi *et al.* (1997a,b, $f_{S,up}$ = 0.12) and Cronje *et al.* (2000, 2002b, $f_{S,up}$ = 0.103), but lower than the value determined in Section 5 above ($f_{S,up}$ = 0.165), all on the same Mitchells Plain wastewater. For the 20 d sludge age system, a number of sewage batches gave $f_{S,up}$ values less than zero (17/02, 22/02, 23/02). No active biomass concentration tests were conducted during Sewage Batch No. 17/02, but both batch tests and microbiological analyses were done during Sewage Batch Nos. 22/02 and 23/02. To determine a more realistic theoretical active fraction for these two sewage batches, the positive $f_{S,up}$ values were averaged to give 0.043 (SSD = 0.047), and this value used to calculate the f_{av}. These f_{av} values were used in all subsequent calculations, but it should be noted that the data for these two sewage batches should be treated with caution.

From the discussion above, it is evident that considerable uncertainty exists with respect to the 20 d sludge age system data collected during two sewage batches; both the COD recoveries and the $f_{S,up}$ values calculated were unacceptably low. Accordingly, although this data will be included in subsequent calculations, it needs to be viewed with suspicion.

6.1 Batch tests

With regard to the batch tests (Fig. 9):

- The correspondence between batch test measured and theoretical OHO active biomass concentrations showed close similarity to the data described in Section 5.3.2 above, namely, as the theoretical OHO active biomass concentrations in the batch test increase, the measured values correspondingly increase parallel to the 1:1 correspondence line, but start to increase sharply at the higher OHO active biomass concentrations.

- The similarity in trends between the 10 and 20 d sludge age data confirms the observations above, that any differences are not sludge age related.

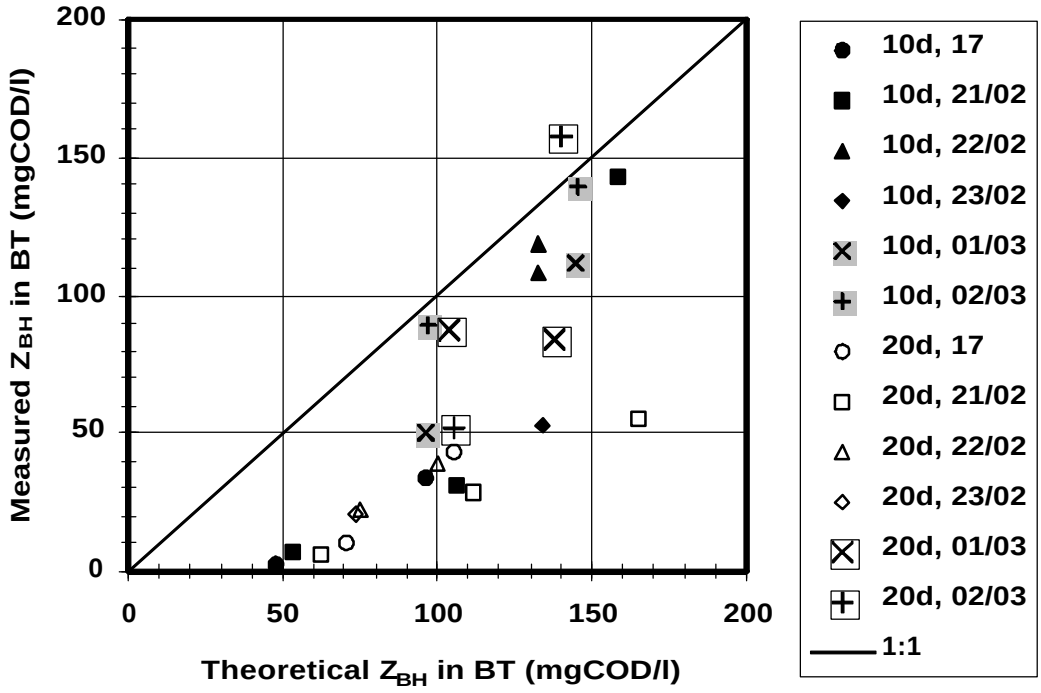

Figure 9: Modified batch tests results; measured versus theoretical OHO active biomass concentration at the start of the batch test [Z_{BH}] for the various sewage batches (SB) for the 10 and 20 d sludge age parent activated sludge systems.

As described and investigated in Section 5 above, it was concluded that:

- The batch test conditions cause the OHO behaviour to change with time in the batch test, to deviate significantly from that in the steady state system. Such behaviour cannot be accommodated in the batch test procedure, with interpretation based on a model with a single OHO population with fixed kinetics.

6.2 Microbiological analysis

From a comparison between DAPI/FISH cell enumeration measured and theoretical OHO active biomass concentrations (Fig. 10):

- There was a significant improvement in the measured OHO active biomass concentrations over those determined under the previous contract (Cronje *et al.*, 2002b). This improvement could be ascribed to the change in sample preparation procedures (changing from ethanol to paraformaldehyde fixing) and the method of couriering the samples to DIT (from dry ice to ice packs).

- Despite the improvement above, the measured OHO active biomass concentrations were generally lower than the corresponding theoretical values.

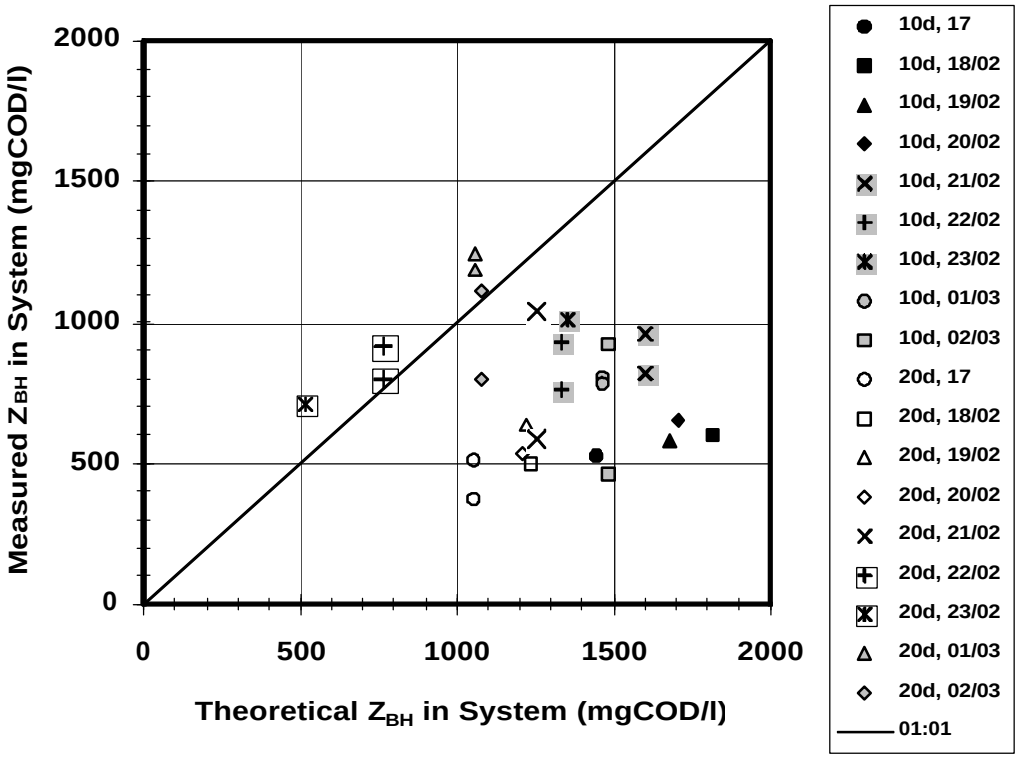

Figure 10: DAPI/FISH enumeration results for OHO active biomass; measured versus theoretical OHO active biomass concentration (Z_{BH}) in the 10 and 20 day sludge age parent activated sludge systems for the various sewage batches.

It was concluded that:

- While the correspondence between measured and theoretical OHO active biomass concentrations is by no means perfect, the microbiological methods show considerable promise, and are providing results that can be correlated directly to the theoretical framework for activated sludge systems developed within the engineering and technology paradigm.

Comparing the batch test and DAPI/FISH measured and the theoretical OHO active biomass concentrations (Figs. 11 and 12):

- Both the batch test and DAPI/FISH methods tend to underestimate the OHO active biomass concentrations compared with the theoretical values.

- The DAPI/FISH method tends to give higher estimates for OHO active biomass concentrations than the batch test method for the 20 d sludge age mixed liquor, but this trend is reversed for the 10 d sludge age mixed liquor. No explanation for this variation is evident.

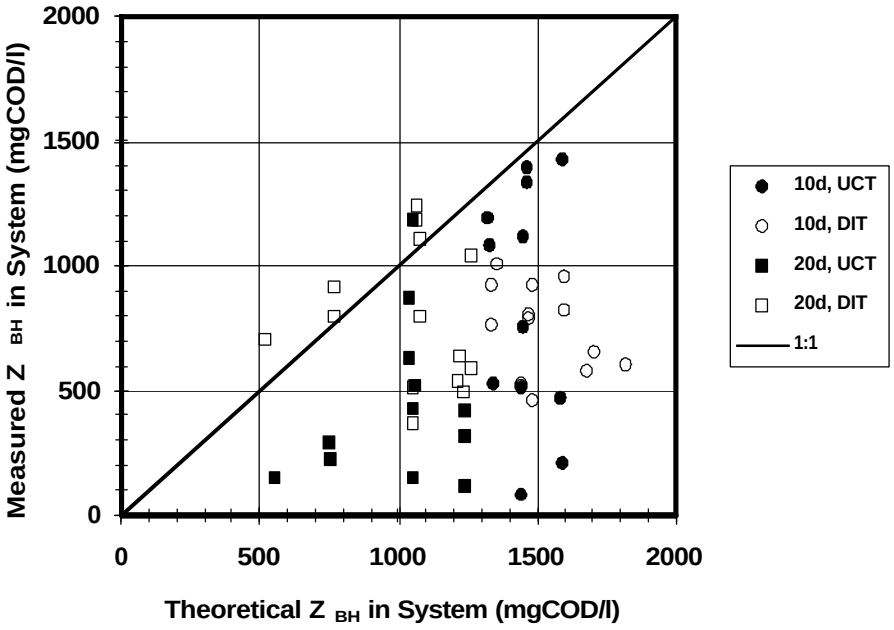

Figure 11: DAPI/FISH (DIT) and batch test (UCT) determined values for OHO active biomass concentrations (Z_{BH}) versus corresponding theoretical values in the 10 and 20 days sludge age parent activated sludge systems.

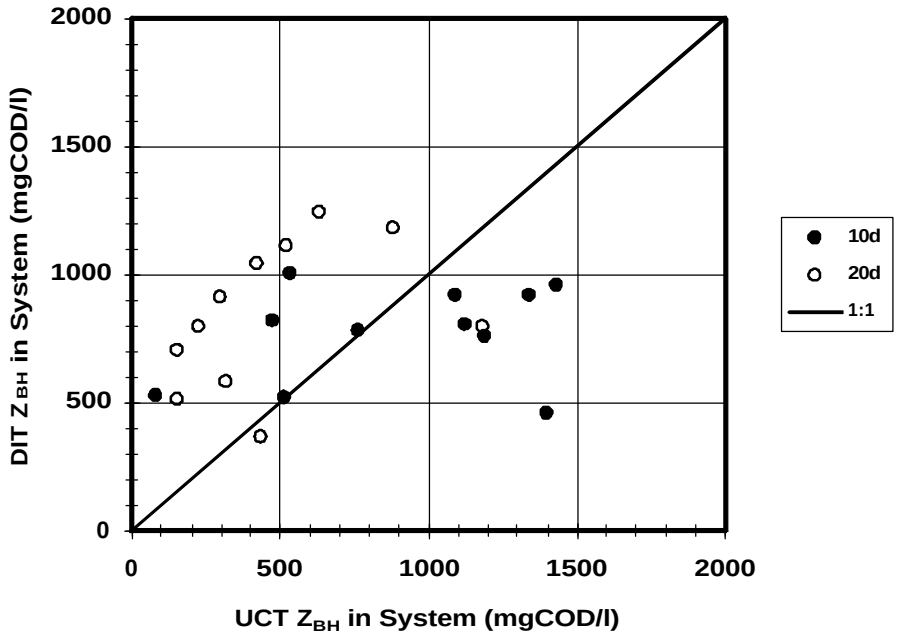

Figure 12: DAPI/FISH (DIT) determined values for OHO active biomass concentrations (Z_{BH}) versus and corresponding batch test (UCT) values in the 10 and 20 days sludge age parent activated sludge systems.

From a comparison between DAPI/FISH cell enumeration measured and theoretical AO active biomass concentrations (Fig. 13):

- Almost invariably the DAPI/FISH determined concentrations are higher than the corresponding theoretical values. One possible explanation identified for this is "double counting" of the AOs with the three probes implemented, and this requires investigation.

- Considerable variation in measured concentrations compared with theoretical values. This would suggest that the resolution of the experimental method is inadequate, and requires investigation.

- However, it was noted that the measured values are of the same order of magnitude as the theoretical values, which is an order of magnitude smaller than the OHO active biomass concentrations, and that this was particularly encouraging.

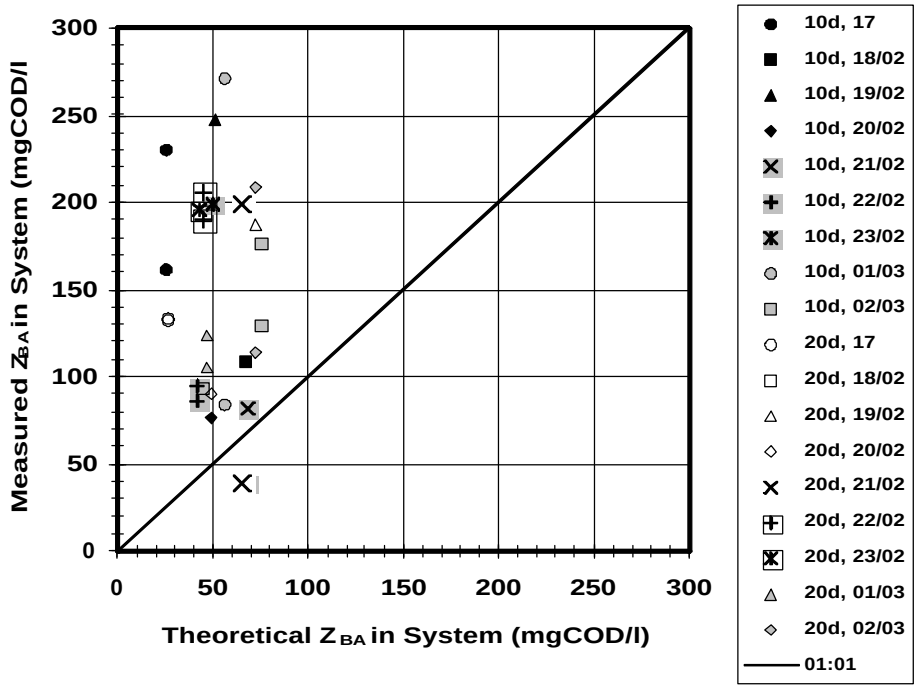

Figure 13: DAPI/FISH enumeration results for AO active biomass; measured versus theoretical AO active biomass concentrations (Z_{BA}) in the 10 and 20 days sludge age parent activated sludge systems for the various sewage batches.

7. CLOSURE

In this and the preceding research projects the main objective has been to address the deficiency in the models for activated sludge systems, of the lack of direct experimental validation of the active biomass concept. This was to be approached by "measuring the OHO active biomass concentration within the engineering and technology paradigm, and to attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiological and biochemistry paradigms".

Within the engineering and technology paradigm, the simple respirometric based modified batch test to quantify OHO active biomass developed in the preceding research contract has been extensively evaluated. In evaluating the modified batch test, it was hoped that the method would provide measured OHO active biomass concentrations that would compare favourably with the theoretical concentrations predicted by the activated sludge models. This would provide independent validation in a simple way of the active biomass concept in the models, and thereby promote confidence in their application. However, similarly to the research in the previous contract (Cronje *et al.*, 2002b), correspondence was not good and it was found that the problem is more complex than originally thought. In examining possible causes for this lack of correspondence, it was noted that the modified batch test method and its interpretation relies on a single OHO population with constant kinetics (Wentzel *et*

al., 1995; Cronje *et al.*, 2000, 2002b). Observations in this and previous research (*e.g.* Daigger *et al.*, 1982; Novak *et al.*, 1994; Grady *et al.*, 1996) suggest that this may not be appropriate, and that the batch test conditions may cause the overall OHO behaviour to deviate significantly from that in the steady state system. This arises because of the requirement that the growth of active biomass in the batch test is significant compared with the initial concentration. Unfortunately, this deviation renders the batch test unsuitable as a simple method to directly quantify the OHO active biomass concentration (and kinetic constants) with sufficient accuracy. The test does, however, hold merit as a tool to investigate OHO population dynamics, as shown by Cronje *et al.* (2002b) and here.

The causes for the lack of correspondence have been shown to lie in the modified batch test itself: Batch aerobic digestion tests and re-interpretation of the modified batch test data with competition kinetic models both provided reasonable correspondence between measured and model predicted OHO active biomass concentrations. ***This does provide independent evidence that substantiates the active biomass concept in the models.*** Convincing independent evidence supporting the aerobic batch digestion approach and the model structure is provided by Van Haandel *et al.* (1998) from aerobic digestion tests on mixed liquors. Also, this data provides indirect validation of active biomass concentrations, in that there is consistency between the model predicted active fraction in the mixed liquor used as source feed to the digesters and that calculated from the aerobic digestion measurements.

Although the competition models needed to be applied to explain the observations in the modified batch tests, it must be remembered that in the parent activated sludge systems, the OHO population is dominated by the slow growing OHO population group, to the extent of near exclusion of the fast growers. Thus, for the activated sludge system the current models incorporating a single OHO population are adequate, provided extremes in dynamic loading are not encountered, *e.g.* with selector reactors (Still *et al.*, 1996). From this work it seems that selector reactors stimulate proliferation in the activated sludge system of the fast growing OHOs, which in the absence of the selector would not be sustained in the system to any significant extent.

In the research aimed at creating links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical methods, a procedure has been developed that enables the quantitative measurements of active cell numbers made with the new microbiological analytical methods to be converted to values that are directly comparable with the activated sludge modelling framework, namely the OHO and AO active biomass VSS or COD concentrations. ***This will enable the information derived from these analytical techniques to be directly applied in assessing, validating and improving mathematical models for activated sludge systems.*** In essence, a common "language" has been established for the two research approaches, to facilitate exchange of information and development of cross-linkages. Initial results from the procedure show considerable promise, but the procedure does require more intensive investigation and refinement.

8. FUTURE WORK

From this investigation the following recommendations can be made:

- In the modified batch test a second plateau in the OUR_H-time plot is observed. Cronje *et al.* (2000) and Beeharry *et al.* (2001) ascribed this second plateau to "soluble" SBCOD that was not removed in the flocculation and filtration of the sewage added to the batch test. In the kinetic models for activated sludge systems, a single SBCOD "type" is accepted, with the same kinetics of hydrolysis/utilization applied to all SBCOD. The existence of a soluble (*i.e.* "non-flocculatable") SBCOD needs to be investigated (e.g. Sollfrank and Gujer, 1991), as this may have an impact on modelling the kinetics of SBCOD hydrolysis/utilization, but only under extreme dynamic loading conditions, e.g. in the contact stabilisation system. Also, other possible explanations for the second OUR plateau need to be evaluated, such as intracellular substrate storage. Majone *et al.* (1999) and Carucci *et al.* (2001) reported evidence of aerobic substrate storage under dynamic conditions. The more fundamental microbiological or biochemical analytical techniques may prove useful in this regard.

- The batch aerobic digestion test provided estimates for OHO active biomass concentrations that agree closely with the activated sludge model theoretical values. However, only 4 such tests were undertaken due to the time consuming nature of the tests (> 10 days in duration). However, more such tests need to be undertaken, to confirm the results obtained here. One issue that would require resolution is that in batch aerobic digestion tests, the measured nitrate concentrations were always higher than the predicted concentrations. This was common to all the batch aerobic digestion tests. An explanation for this poor correlation could not be provided.

- To explain the behaviour observed in the modified batch tests, in this research a competition model was proposed. However, hypotheses alternative to the competition one, also could possibly explain the observed behaviour. For example, Daigger *et al.* (1982) and Grady *et al.* (1996) proposed the concept of "physiological adaptation". In this concept, the relative components of the biomass groups (fast grower and slow grower) do not change, but rather the physiological state of the biomass itself, induced by the change in conditions from the steady state system to the batch test (Grady *et al.*, 1996). Such alternatives also require evaluation.

- In this research, in calibrating the competition models, values had to be assumed for the kinetic parameters for the fast and slow growing OHO population groups (μ_{H1}, μ_{H2}, K_{SH1} and K_{SH2}). With the accepted values, the competition model could reasonably accurately simulate the diverse observed behaviour, *e.g.* change in kinetic rates with time in the batch tests, the effect of initial substrate to active biomass ratio on the kinetics. However, the values for the rate constants were not directly determined. This was not possible because the behaviour of the fast and slow growing OHO population groups could not be separated. This requires investigation.

- Converting from cell counts (the output from the microbiological methods) to VSS (the basic unit of measurement in the engineering and technology models) or COD (VSS converted to COD via a direct measure of the COD/VSS ratio) requires a conversion parameter. The cell count to VSS conversion parameter was determined from averaging the values measured on a number of pure cultures of different species and values in the literature for pilot- and full-scale samples, to be $F_{VB} = 8.49 \times 10^{-11}$ mg VSS/cell; pure cultures were used to minimise (but could not be eliminated) inert and endogenous residue contributions to the VSS measurements (Holder-Snymann *et al.*, 2004). Clearly, the value for this parameter requires further evaluation.

- In quantifying the AO active biomass concentration, a series of three FISH probes was implemented. AO active biomass concentrations measured with these probes were consistently higher than the corresponding theoretical values. One possible cause identified for this was "double counting". Also, it appeared that the resolution in the AO cell counts was inadequate. These aspects require evaluation.

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!	Ms S Mathews	Water Research Commission (Committee Secretary)
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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/abbreviation	Description
ADM	Anaerobic Digestion Model (IWA Taks Group)
AO	Autotrophic organism
ATP	Adenosine tri-phosphate
BEPR	Biological excess phosphorus removal
b_{H20}	OHO specific endogenous respiration rate at 20 °C (/d)
BNR	Biological nutrient removal
BNRAS	Biological nutrient removal activated sludge
C	Carbon
CI	Confidence interval
COD	Chemical oxygen demand
DIT	Durban Institute of Technology
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
f_{av}	Mixed liquor OHO active biomass fraction
f_{CV}	Mixed liquor COD/VSS ratio (mgCOD/mgVSS)
FISH	Flourescent *in situ* hybridisation
f_N	Mixed liquor N/VSS ratio (mgN/mgVSS)
FSA	Free and saline ammonia
$f_{S,up}$	Fraction of influent total COD that is unbiodegradable particulate (mgCOD/mgCOD)
$f_{S,us}$	Fraction of influent total COD that is unbiodegradable soluble (mgCOD/mgCOD)
F_{VB}	VSS per units cell (mgVSS/cell)
IFFD	Intermittently fed fill and draw
K_{MP}	OHO maximum specific growth rate on SBCOD (/d)
K_{SH}	OHO half saturation constant for growth on RBCOD (mgCOD/ℓ)
ML	Mixed liquor
MLE	Modified Ludzack-Ettinger
MLOSS	Mixed liquor organic suspended solids
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
ND	Nitrification denitrification
NDBEPR	Nitrification denitrification biological excess phosphorus removal
NO_2^-	Nitrite
NO_3^-	Nitrate
OHO	Ordinary heterotrophic organisms (non-P removal)
OUR	Oxygen utilization rate
OUR_H	OUR due to OHOs
OUR_N	OUR due to nitrifiers
P	Phosphorus
PBS	Phosphate buffered saline
PHA	Polyhydroxy alkanoate

PS	Parent system
RBCOD	Readily biodegradable COD
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
R_S	Sludge age (d)
S_0/X_0, S_0/Z_{BH}	Initial substrate to OHO active biomass ratio, VSS units, COD units gVSS/mgCOD, mgCOD/mgCOD)
S_{ads1}, S_{ads2}	Adsorbed SBCOD for fast and slow growing OHOs (mgCOD/ℓ)
SBCOD	Slowly biodegradable COD
TKN	Total Kjedahl Nitrogen
TN	Technikon Natal
TSS	Total suspended solids
UCT	University of Cape Town
UP	University of Pretoria
VSS	Volatile suspended solids
WRC	Water Research Commission
WW	Wastewater
Z_{BH}	OHO active biomass concentration (mgCOD/ℓ)
$Z_{BH(0)}$	OHO active biomass concentration at the start of the batch test (mgCOD/ℓ)
Z_{BH1}, Z_{BH2}	Fast and slow growing OHO active biomass concentrations (mgCOD/ℓ)
μ_H	OHO maximum specific growth rate on RBCOD (/d)

Note: Only symbols and abbreviations used in the text are included; those in equations are defined below the appropriate equation.

PRODUCTS DURING CONTRACT PERIOD (APRIL 2002 TO MARCH 2003)

1. PUBLICATIONS

1.1 Published in refereed journals

de Haas DW and Wentzel MC (2002). Calibration of the BIOWIN model for N removal Part 1 - Desktop study. *Water* (AWWA, Australian Water and Wastewater Association), **Sept**, 37-41.

de Haas DW and Wentzel MC (2002). Calibration of the BIOWIN model for N removal Part 2 - Fullscale study. *Water* (AWWA, Australian Water and Wastewater Association), **Nov**., 65-69.

Hu Z-R, Wentzel MC and Ekama GA (2003). Modelling nutrient removal activated sludge systems - a review. *Water Research*, **37**, 3430 - 3444.

Lee BJ, Wentzel MC, Ekama GA and Min KS (2003) Mathematical model application to measure active ordinary heterotrophic organisms in activated sludge mixed liquor. *Korean Society of Water and Wastewater* **17**(5) 827-838 (In Korean).

Lee BJ, Wentzel MC, Ekama GA and Min KS (2004) Abnormal behaviour of ordinary heterotrophic organism active biomass at different substrate/microorganisms ratios in batch test. *Korean Society on Water Quality* **20** (3) 197-205 (In Korean).

1.2 Conferences papers (submitted)

Lee BJ, Wentzel MC and Ekama GA (2004). Measurement and modelling of ordinary heterotrophic active biomass concentrations in anoxic/aerobic activated sludge systems. Submitted to *IWA Activated sludge Population Dynamics Conf.*, Australia, 2005.

1.3 Research reports

Lee B-J, Wentzel MC and Ekama GA (2003). Batch test for measurement of ordinary heterotrophic organism active mass in activated sludge mixed liquor. *Research Report W118*, Dept. Civil Engineering, Univ. of Cape Town, Rondebosch 7701, South Africa.

2. GRADUATES

MSc Lee BJ (2003). Batch test for measurement of ordinary heterotrophic organism active mass in activated sludge mixed liquor (Awarded the Joseph Arenox prize for the best MSc thesis in the Engineering and Built Environment Faculty at the University of Cape Town).

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Although significant developments have taken place in both the engineering and technology and the microbiology and biochemistry areas of the activated sludge system for treating mainly domestic wastewaters, these have proceeded on two parallel, but separate paths. Within the engineering and technology, the activated sludge system has become well established, with systems implemented worldwide for the biological removal of carbon (C), nitrogen (N) and/or phosphorus (P). This implementation has been aided by the development of a suite of steady state design (*e.g.* WRC, 1984; Wentzel *et al.*, 1990; Maurer and Gujer, 1994) and kinetic simulation (*e.g.* Dold *et al.*, 1980, 1991; Van Haandel *et al.*, 1981; Henze *et al.*, 1987; Wentzel *et al.*, 1992; Henze *et al.*, 1995) models which have facilitated optimization of system design and operation. Parallel to these developments, significant advances have been made in the microbiological and biochemical areas of activated sludge. These advances have been driven by the development of new analytical techniques that allow microorganisms to be studied *in situ* in the activated sludge environment, e.g. ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), quinone profiling (Hu *et al.*, 1998), microautoradiography (Nielsen *et al.*, 1998), using florescent probes for ribosomal RNA (Wagner *et al.*, 1994; Water Sci. Technol., 1998). However, there has been little cross-linking or overlap between the engineering and technology and microbiology and biochemistry paradigms. In particular, the information from the microbiology and biochemistry has not been integrated into the engineering and technology paradigm, to enable improved design and optimization. One area that can form a starting point to build bridges between the two paradigm sets is active biomass. The current design and simulation models invariably include active biomasses as fundamental parameters (ordinary heterotrophic organism, OHO, autotrophic organism, AO and phosphate accumulating organism, PAO, see Chapter 2), yet these parameters remain hypothetical as they have not been measured and favourably compared with theoretical values. Recently, new respirometric based batch test methods have been developed to quantify OHO (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995; Mbewe *et al.*, 1995; Ubisi *et al.*, 1997a,b; Wentzel *et al.*, 1998) and AO (Cronje *et al.*, 2002) active biomass concentrations, with variable success. However, the interpretation and analysis of the data from these tests remains firmly rooted within the engineering and technology modelling structure and independent of the microbiological and biochemical concepts and measurements. To overcome this shortfall, a multi-institutional collaborative research project sponsored by the Water Research Commission (South Africa) was initiated to attempt to refine the batch test methods and to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques. It was hoped that this research would facilitate the development of links and overlap between the two paradigms.

1.2 PREVIOUS RESEARCH CONTRACTS

As noted above, one area that can form a starting point to build bridges between engineering and technology and microbiology and biochemistry, is the measurement of OHO active biomass. Accordingly, in 2000 three parallel Water Research Commission (WRC) research contracts were set up; (1) K5/1178 with the Centre for Water and Wastewater Research at the Durban Institute of Technology (DIT) (3 years; Holder-Snymann *et al.*, 2004), (2) K5/1179 with Water Research Group at the University of Cape Town (UCT) (2 years; Cronje *et al.*, 2002), and (3) K5/1191 with the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) 2 years; Cloete and Thantsha, 2003). These research projects investigated measurement of the OHO active biomass parameter, with:

- UCT operating laboratory-scale activated sludge systems and measuring the OHO active biomass within the engineering and technology paradigm,
- DIT setting up similar laboratory-scale activated sludge systems and measuring OHO active biomass within the microbiological and biochemical paradigm, and
- UP measuring OHO active biomass in the UCT systems within the microbiological and biochemical paradigms.

Further, samples from the UCT systems were sent to the collaborators for measurement of OHO active biomass via the microbiological and biochemical techniques. In this manner, on the same samples both engineering and microbiological/biochemical quantification of the OHO active biomass could be done, enabling a direct comparison and correlation of the different techniques. Furthermore, the UCT systems were run under strictly controlled and defined conditions so that the theoretical values for OHO active biomass could be calculated with the aid of the activated sludge system models, and compared with the measured values derived from the different techniques. It was hoped that this would facilitate development of a common link between the two paradigm sets.

In the three WRC contracts above, the initial focus was on developing and refining the specific experimental techniques at the different institutions. At UCT, quantification of the OHO active biomass concentration within the engineering and technology paradigm was investigated. Initially, the batch test method of Ubisi *et al.* (1997a,b) was evaluated (Cronje *et al.*, 2000, 2002a,b). From this evaluation, the batch test procedure was enhanced by:

- Physically removing the OHO active biomass from the wastewater. This was achieved through flocculation of the wastewater with aluminium sulphate, followed by filtration.

This modification greatly simplified the batch test procedure - since the flocculated-filtered wastewater does not contain OHO active biomass, a parallel batch test no longer needed to be conducted to determine the wastewater OHO active biomass, which in the "old" batch test method was subtracted from the mixed liquor + wastewater OHO active biomass to give the mixed liquor OHO active biomass.

The modified batch test method was extensively evaluated by applying the procedure to mixed liquor drawn from a variety of well defined and controlled parent laboratory-scale aerobic and anoxic/aerobic activated sludge systems, operated at 10 and 20d sludge ages and with and without toilet paper dosed to the influent to change the OHO active fraction of the mixed liquor. The batch test measured OHO active biomass concentrations were compared to the corresponding theoretical values predicted from the activated sludge models. Results from these comparisons indicated that some correspondence does exist between theoretical and measured values, but that this correspondence is by no means perfect:

- For mixed liquor drawn from a parent anoxic/aerobic MLE activated sludge system at 10 d sludge age, good correspondence was found between batch test measured and theoretical OHO active biomass concentrations (Cronje *et al.*, 2000).

- For mixed drawn from two parallel parent anoxic/aerobic MLE activated sludge systems at 10 d sludge age, one with toilet paper dosed to the influent and the other without, there was reasonably close correspondence between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor gave an almost linear decrease in OHO active biomass concentration (Beeharry *et al.*, 2001). However, there was a constant (i.e. independent of volume of mixed liquor added) difference between the measured and theoretical values, of approximately 25 mgCOD/ℓ. No explanation for this was apparent.

- For mixed drawn from a parent completely aerobic activated sludge system at 10 d sludge age there was a correlation between the theoretical and measured OHO active biomass concentration values, but the theoretical values were consistently 3 to 4 times those measured (Beeharry *et al.*, 2001).

- In changing the parent 10 d sludge age activated sludge system above from anoxic/ aerobic to aerobic, there was a progressive change in the batch test measured OHO active biomass concentrations, from (2) to (3) above (Beeharry *et al.*, 2001). This would suggest that the change caused a significant change in the mixed liquor. Such a change in population dynamics is to be expected as the population shifts from facultative to obligate aerobic and appears to have been correctly detected by the modified batch test. However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation was not clear.

- In investigating the variability above, one possible cause identified was deficiency of phosphorus (P) in the batch test, due to the pre-flocculation of the wastewater with alum. In examining this, by adding P to one batch test in a set of two parallel batch tests, it was found that the effect of adding P to the batch test was inconsistent, and not entirely conclusive (Beeharry *et al.*, 2001). This aspect was identified as meriting further attention.

In summary for the batch tests, (i) the close correspondence between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the parent anoxic/aerobic (MLE) activated sludge system in (1) above, (ii) the remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the two parallel parent anoxic/aerobic (MLE) activated sludge systems in (2) above, (iii) the linearity of results with "serial" dilutions in all batch tests, and (iv) the consistent progressive change in behaviour detected by the batch test in changing from the MLE to fully aerobic configuration in (4) above all indicated that the batch test method is a valuable tool for examining activated sludge system behaviour. However, it was noted that the lack of a 1:1 correlation between theoretical and measured values **required further investigation**.

The concepts developed above for the batch test method to quantify the OHO active biomass concentration can be applied also to the nitrifying autotrophic organism (AO) active biomass. This is possible because the compound nitrate and its production are uniquely and directly linked to the growth of this population group; some preliminary investigations into this aspect were undertaken (Cronje *et al.*, 2001). Unfortunately, the batch test concept cannot be applied to the phosphorus accumulating organism (PAO) active biomass, or to the OHOs present in biological excess phosphorus removal (BEPR) activated sludge systems. This is because in BEPR activated sludge system mixed liquors, both the OHOs and PAOs are present and the batch test method will not be able to distinguish the contributions of each organism group to the measured OUR.

The interpretation and analysis of the data from the batch tests described above remains firmly rooted within the engineering and technology paradigm - the interpretation of the batch test data is based on the same set of models used to calculate the theoretical OHO active biomass concentrations. Independent quantification of the OHO active biomass concentration with the microbiological and biochemical based analytical techniques possibly could provide an alternative independent method to substantiate the active biomass concept. However, little cross-linking exists between the microbiological and biochemical and the engineering and technology paradigms, and deriving compatible data was not possible. To overcome these shortfalls, in the three WRC sponsored research projects a first attempt was made to create cross-links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical methods (Cronje *et al.*, 2002a,b), by the three collaborative research partners, UCT, UP and DIT. Various test methods were applied by the different groups to quantify OHO active biomass concentrations, and the results from the test methods compared with each other and with the theoretical OHO active biomass concentrations. The Water Research Group at UCT operated laboratory-scale activated sludge systems under closely controlled and defined conditions; this enabled the theoretical OHO active biomass concentration to be calculated within the engineering and technology (modelling) paradigm for activated sludge. Additionally, the modified batch test method described above was run on mixed liquor samples drawn from the parent activated sludge systems. The research group at UP measured the biochemical compound ATP both *in situ* in the laboratory-scale activated sludge systems, and during the course of the modified batch tests. The research group at DIT used the microbiological

technique of a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and AO active biomass concentrations in samples regularly drawn from the laboratory-scale activated sludge systems operated at UCT. From a comparison of the results for OHO active biomass concentrations from the various research groups, it was apparent that (Cronje *et al.*, 2002b):

- The microbiological and biochemical test methods gave OHO active biomass concentrations that were several orders of magnitude lower than both the theoretical and batch test measured OHO active biomass concentrations.

In examining possible reasons for this discrepancy, the following possibilities were identified:

- For the ATP method applied by UP, it appeared that solids concentrations (i.e. VSS or TSS) interfere in some manner with the ATP measurement method - this would explain the lower values measured in the steady state systems (with higher VSS concentrations) than in the batch tests (with lower VSS concentrations), and the lower ATP measurements in the 20 d sludge age steady state system (higher VSS concentrations) than in the 10 d sludge age system (lower VSS concentration). **Clearly, this is an aspect that requires investigation.** One possible avenue is to do serial dilutions of the mixed liquor and measure ATP, thereby to determine the effect of VSS on the ATP test method. What was evident, however, was that the ATP method **as applied** is not a reliable estimate for OHO active biomass concentrations.

- For the DAPI/FISH method applied by DIT, in subsequent investigations it was found that the method of couriering the samples in dry ice caused a significant number of the cells to freeze and hence burst. This would reduce the DAPI/FISH enumerated cell counts significantly, and may be one possible explanation for the low cell counts. **This would be investigated further in the current research project.**

Although this initial attempt to link the engineering and technology theoretical and batch test measured OHO active biomass concentrations to the values measured with the microbiological and biochemical analytical techniques did not provide even near a close correspondence, it did, for the first time, place the magnitudes of the microbiological and biochemical measurements within the context of the engineering and technology paradigm. This should help establish a common basis and "language" for the two paradigm sets, to facilitate future exchange of information and development of cross linkages. In particular, it will make the quantitative information from the new microbiological and biochemical analytical techniques available to possibly improve the engineering and technology based design and simulation models developed for activated sludge systems. This will provide greater surety in the mathematical models for design and operation of the biological nutrient removal activated sludge (BNRAS) system.

1.3 CURRENT RESEARCH CONSULTANCY

In the three WRC contracts above, the initial focus was on developing and refining the specific experimental techniques at the different institutions. Initial exchange of samples occurred, and some results were obtained. However, these results were preliminary only and more detailed research was required. In March 2002, the two year UCT contract ended, whereas the DIT contract still had to run for one more year. Accordingly UCT was granted a research consultancy for one year (K8/453, April 2002 to March 2003), to enable:

- Research at UCT to continue into measurement of OHO active biomass within the engineering and technology paradigm.

- The laboratory-scale systems to continue to be operated at UCT, to generate samples required by DIT in the final year of their contract for measurement of OHO active biomass within the microbiology and biochemistry paradigm

The consultancy would end at the same time that the DIT contract ended (March 2003).

1.4 OBJECTIVES AND AIMS OF RESEARCH

The objectives of this research consultancy were to:

- Measure the OHO active biomass concentration within the engineering and technology paradigm, and

- attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

In terms of these objectives, specific aims identified were:

- To provide greater surety in the mathematical models for design and operation of the biological nutrient removal activated sludge (BNRAS) system.

- To provide a platform for collaborative work between the engineering and microbiological aspects of the BNRAS system.

- To provide a better scientific understanding of the microbiological processes operating in the BNRAS system in the context of defined engineering environments.

- To develop linkages and overlap between the quantitative engineering and qualitative microbiological approaches to understanding biological wastewater treatment systems.

1.5 METHODOLOGY

The Water Research Group at the University of Cape Town were to operate at laboratory-scale two nitrogen removal activated sludge systems receiving real wastewater. The two systems would be operated at different sludge ages of 10 and 20d to generate different heterotrophic active biomass fractions in the mixed liquor. The engineering parameters for these systems would be closely controlled and defined. Nitrogen removal systems were considered because the mathematical models for these appear to be the more consistent. The systems were to be monitored and all the relevant performance parameters measured, and used to make the usual consistency checks, such as COD and N mass balances. These checks provide a means for assessing the accuracy of the measured parameters. Samples would be drawn from these systems and the engineering and technology based batch tests described by Ubisi *et al.* (1997a,b) and Wentzel *et al.* (1998) and modified by Cronje *et al.* (2000, 2002a,b) conducted to determine OHO active biomass concentrations. This experimentally determined parameter would be compared to the hypothetical parameter predicted by steady state design theory (WRC, 1984) and kinetic simulation models (Dold *et al.*, 1991). Additional to these engineering parameters, sludge samples would be sent to collaborators where they would be subject to microbiological and biochemical analysis, using selected microbiological and biochemical techniques. This information was to be used to find ways to integrate the engineering and technology and microbiology and biochemistry paradigms.

1.6 SPECIFIC TASKS

To address the aims above, a number of specific tasks to be addressed by UCT were identified for attention:

Task 1: Operate laboratory-scale systems

The Water Research Group at UCT will operate under controlled laboratory conditions nitrogen removal activated sludge systems receiving real wastewater, at 10d sludge age and at 20d sludge ages. All the engineering parameters will be measured on the systems and with these the usual consistency checks such as COD and N mass balances will be made. These systems will provide the source sludge for the engineering and technology based tests (see below) and the microbiological and biochemical based tests.

Task 2: Conduct batch tests to measure OHO active biomass

Samples will be drawn from the parent laboratory-scale activated sludge systems above, and the batch test method of Ubisi *et al.* (1997a,b), as extended and modified by Cronje *et al.* (2000, 2002b), will be applied to determine OHO active biomass concentrations.

Task 3: Calculate OHO active biomass concentrations

For the parent laboratory-scale activated sludge systems, the theoretical OHO active biomass concentrations predicted by steady state design theory (WRC, 1984) and kinetic simulation models (Dold *et al.*, 1991) will be calculated and compared to the values measured in the batch tests above.

Task 4: Harvest and send samples

Samples will be harvested from the parent laboratory-scale activated sludge systems, and sent to DIT for microbiological and biochemical analysis. In this task, the method for preserving, storing and sending samples to DIT needs to be changed and perfected to prevent sample degradation.

Task 5: Evaluate engineering and microbiological results

The engineering and microbiological results will be evaluated and compared, to look for commonality between the two sets of paradigms.

Since the consultancy was a continuation of the previous WRC research contract (K5/1179), the laboratory-scale systems were already in operation, and batch tests being conducted on the mixed liquor from these systems.

CHAPTER 2

BACKGROUND TO RESEARCH

2.1 INTRODUCTION

Over the past three decades significant advances have be made in the engineering and technology of the single sludge activated sludge system. Worldwide, numerous systems have been successfully designed and implemented at full-scale to include variously biological removal of carbon (C), nitrogen (N) and phosphorus (P). To aid the design and operation of these systems, a suite of steady state design models (*e.g.* WRC, 1984; Wentzel *et al.*, 1990; Maurer and Gujer, 1994) and kinetic simulation models (*e.g.* Dold *et al.*, 1980, 1991; Van Haandel *et al.*, 1981; Henze *et al.*, 1987; Wentzel *et al.*, 1992; Henze *et al.*, 1995) have been developed. In the development of these models, it was recognised that it would not be possible to incorporate the behaviour of specific micro-organism species - the mixed liquor in the activated sludge system contains a wide diversity of different micro-organism species, some of whom for which identification techniques have only recently started becoming available. Instead, micro-organisms that fulfil a particular function in the activated sludge system (*e.g.* aerobic degradation of organics) are grouped together as a single entity, which has been called a "surrogate" organism. This surrogate organism is assigned a set of unique characteristics that reflect the behaviour of the group, but may not reflect the characteristics of any individual organism or species of organisms in the group. A similar approach has been adopted for the "non-organism" components of the activated sludge mixed liquor, *e.g.* inert organics. Together, the surrogate organism and non-organism groups make up the activated sludge mixed liquor organic (volatile) suspended solids (MLOSS) (Cronje *et al.*, 2002b).

In terms of the models (see Ubisi *et al.*, 1997a,b and Cronje *et al.*, 2002b for details), in the non-nitrifying aerobic COD removal activated sludge system, the MLOSS is made up of three components; (1) ordinary heterotrophic organism (OHO) active biomass, (2) endogenous residue and (3) inert material. With the inclusion of nitrification in the system, a fourth component is included; (4) autotrophic organism (AO) active biomass. With the inclusion of denitrification through the incorporation of anoxic reactors, no additional surrogate organism group is included, and the OHO above are considered to be facultative mediating the denitrification process. If biological excess phosphorus removal (BEPR) is included through the incorporation of an anaerobic reactor, additionally, (5) phosphate accumulating organism (PAO) active biomass and (6) this organism group's endogenous residue contributed to the MLOSS (Wentzel *et al.*, 1992; Henze *et al.*, 1995). The active biomass components (surrogate organism groups) of the MLOSS above mediate the relevant biological processes deemed to be of importance; OHO's COD removal and denitrification, AO's nitrification and PAO's BEPR and COD removal.

Thus, in terms of the approach to modelling the activated sludge system, the activated sludge mixed liquor is made up of a number of organic components. Historically though, the MLOSS has been measured as a lumped parameter, via the VSS or COD

test (Standard Methods, 1985). However, from the above, only parts of the MLOSS are active biomasses, and only these parts mediate the relevant biological processes *e.g.* OHOs for COD removal and denitrification. Further, in the models specific rates are expressed in terms of the appropriate active biomass parameters. Acceptance of the active biomass concept met with considerable resistance in the past. However, more recently, with the proliferation of kinetic simulation computer programmes that invariably include active biomass concentrations as parameters (*e.g.* Biowin, Simba, GPX, UCTOLD, UCTPHO), these parameters and the use of specific rates in terms of them, have become much more widely accepted. This acceptance has not been driven by sound scientific proof of the active biomass concept, but rather by the convenience of the computer programmes. It must be remembered that the active biomass concept was a hypothetical construct within the development of the design procedures and kinetic models. Although indirect evidence does provide some support for the active biomass parameters (by consistency between observations and predictions over a wide range of conditions), until recently these have not been directly measured experimentally and compared with the theoretical model values. This deficiency cast a measure of uncertainty on the framework within which the models have been developed and is a weakness in the models. The problem in measurement has been the lack of suitable experimental techniques.

Recently a simple batch test procedure has been developed to quantify the OHO active biomass concentration (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995; Mbewe *et al.*, 1995), based on the concepts incorporated in the models. Evaluation of this batch test method, and comparison of the OHO active biomass concentrations derived from the test with theoretical concentrations from the models has yielded mixed results (Ubisi *et al.*, 1997a,b; Wentzel *et al.*, 1998). Thus, uncertainty around the active biomass concept has largely remained.

Parallel to the developments in the engineering and technology of the activated sludge system, significant advances have been made in the microbiological and biochemical areas. As researchers in these fields have moved away from pure culture work to the activated sludge environment, a number of new analytical techniques have been developed to study microorganisms *in situ,* e.g. ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), quinone profiling (Hu *et al.*, 1998), microautoradiography (Nielsen *et al.*, 1998), using florescent probes for ribosomal RNA (Wagner *et al.*, 1994; Water Sci. Technol., 1998), see Cronje *et al.*, 2002b. While the microbiological and biochemical knowledge and developments have made a considerable contribution to the understanding of the biological nutrient removal activated sludge system, the full potential of these developments has yet to be realised for the system. It remains for the results from these techniques to be integrated with the design and kinetic modelling paradigm. The consequence of this is that the engineering and technology (modelling) paradigm has largely worked independently of the microbiological and biochemical paradigm. To facilitate links and overlap between the two paradigm sets, the new developments in the microbiological and biochemical analytical techniques can be implemented to address the deficiency in the design and kinetic models of the active biomass concept. This should prove possible because, in contrast to the more traditional analytical techniques, the new techniques provide quantitative information, a prerequisite for

modelling. Some initial integration between modelling and these techniques has been started (e.g. Urbain *et al.*, 1998; Wagner *et al.*, 1998), but this is still in its infancy.

As noted above, one area that can form a starting point to build bridges between the engineering and technology and microbiology and biochemistry paradigms, is measurement of OHO active biomass. Accordingly, in April 2000 three parallel WRC research contracts were started; (1) K5/1178 with the Centre for Water and Wastewater Research at the Durban Institute of Technology (DIT) (3 years; Holder-Snymann *et al.*, 2004), (2) K5/1179 with the Water Research Group at the University of Cape Town (UCT) (2 years; Cronje *et al.*, 2002b), and (3) K5/1191 with the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) (2 years; Cloete and Thantsha, 2003). These research projects investigated measurement of the OHO active biomass parameter, with:

- UCT operating laboratory-scale activated sludge systems and measuring the OHO active biomass within the engineering and technology paradigm,

- DIT setting up similar laboratory-scale activated sludge systems and measuring OHO active biomass within the microbiological and biochemical paradigm, and

- UP measuring OHO active biomass in the UCT systems within the microbiological and biochemical paradigms.

Further, samples from the UCT systems were sent to the collaborators for measurement of OHO active biomass via the microbiological techniques. In this manner, on the same samples both engineering and microbiological quantification of the OHO active biomass were done enabling a direct comparison and correlation of the different techniques. Furthermore, the UCT systems were run under strictly controlled and defined conditions so that the theoretical values for OHO active biomass could be calculated and compared with the values measured using the different techniques. It was hoped that this would facilitate development of a common link between the two paradigm sets.

2.2 PREVIOUS RESEARCH PROJECT

2.2.1 Aims and objectives

The principle objectives of the previous research project were to:

- Measure the ordinary heterotrophic organism (OHO) active biomass concentration within the engineering and technology (E & T) paradigm, and

- attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

2.2.2 Measurement of OHO active biomass within E & T paradigm

Batch test development

Prior to the previous research project, Ubisi *et al.* (1997a,b) describe the development of a simple batch test to quantify the OHO active biomass concentration (the procedure is reviewed in detail by Cronje *et al.*, 2002b and Lee *et al.*, 2003). In this test a small sample of mixed liquor is drawn from the activated sludge system and mixed with raw wastewater in a batch reactor and the oxygen utilization rate (OUR) and nitrate and nitrite concentrations monitored with time. In parallel, a similar batch test is conducted on the raw wastewater without mixed liquor addition. From analysis of the OUR and nitrate and nitrite responses of the two parallel tests, the mixed liquor OHO active biomass concentration can be quantified. Wentzel *et al.* (1998) evaluated this batch test method by drawing mixed liquor samples from a well defined laboratory-scale anoxic/aerobic (MLE) activated sludge system operated at 12 and 20 d sludge age. They compared the results from the batch tests with theoretical values for OHO active biomass concentrations from steady state design (WRC, 1984) and kinetic simulation (Dold *et al.*, 1991) models, see Fig. 2.1 (a and b): With the parent system at 12 d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20 d sludge age the agreement was poor, with the theoretical values being about 2 times those measured. Wentzel *et al.* could provide no explanation for this inconsistency, but concluded that the results do indicate that the batch test method may prove to be a valuable tool that can be used to provide greater insight into the behaviour of the aerobic and anoxic/aerobic activated sludge systems.

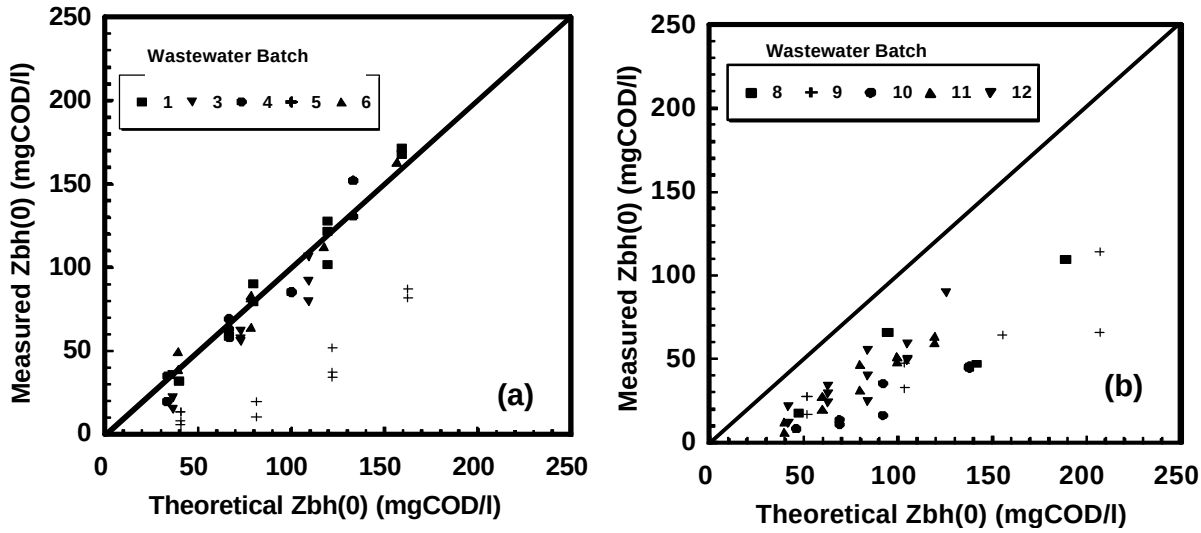

Figure 2.1: Measured vs. theoretical ordinary heterotrophic organism (OHO) active biomass concentration [$Z_{BH(0)}$] in the batch test due to addition of mixed liquor drawn from the parent system at (a) 12d and (b) 20d sludge age (Wentzel *et al.*, 1998).

Batch test evaluation and modification

In the previous research project (K5/1179), initially (for details see Cronje *et al.*, 2000, 2002) the batch test method of Ubisi *et al.* (1997a,b) to quantify the OHO active biomass concentration was extensively evaluated by applying the method to mixed liquor drawn from a well defined and controlled parent laboratory-scale anoxic/aerobic (MLE) activated sludge system operated at 10 d sludge age. From this evaluation, it became evident that the correlation between measured and theoretical OHO active biomass concentrations was poor (see Fig. 2.2), and remarkably similar to that obtained by Wentzel *et al.* (1998) on mixed liquor samples drawn from their system operated at 20 d sludge age, see Fig. 2.1(b).

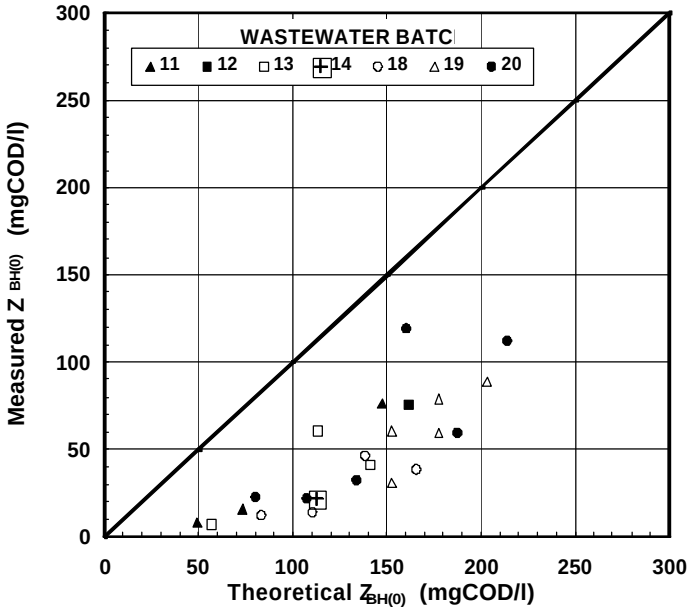

Figure 2.2: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) in the batch test due to addition of mixed liquor drawn from the parent laboratory-scale system operated at 10 d sludge age (Cronje *et al*., 2000, 2002b).

This prompted a detailed investigation into the batch test method. Two sources of potential error in the method were identified (Cronje *et al.*, 2000b, 2002; Beeharry *et al.*, 2001):

- In the batch test method of Ubisi *et al.* (1997a,b), two parallel batch tests are run, one with wastewater only to quantify the wastewater OHO active biomass concentration, and the other with wastewater + mixed liquor to quantify the wastewater + mixed liquor OHO active biomass concentration, with the difference giving the mixed liquor OHO active biomass concentration. In the batch test with mixed liquor + wastewater, the OHO active biomass from the wastewater has a maximum specific growth rate on RBCOD that is much larger than that of the OHO active biomass from the mixed liquor. This causes that the wastewater OHO active biomass dominates the observed OUR response in the batch tests, and thus masks the mixed liquor OHO active biomass OUR

response. This introduces potential errors when the wastewater OHO active biomass is subtracted from the wastewater + mixed liquor OHO active biomass, to give the mixed liquor OHO active biomass.

- In their mixed liquor + wastewater batch test Ubisi *et al.* accepted that the nitrification rate was constant, and accordingly fitted a linear line to the observed increase in nitrate concentration with time. From this linear fit, they determined a constant nitrification OUR which was subtracted from the measured OUR to give the heterotrophic OUR. Examination of the nitrate-time profiles indicated that the increase could be better described by a exponential fit.

To eliminate the potential errors above, it was proposed to:

- Physically remove the OHO active biomass from the wastewater. This was achieved through flocculation of the wastewater with aluminium sulphate followed by filtration.

- Use exponential fits to the nitrate-time profiles to determine nitrification OURs.

In evaluating the proposed flocculation filtration modification to the batch test procedure, it was found that:

- The flocculation filtration procedure effectively removed all active biomass from the wastewater. This was demonstrated by no measurable OUR being observed in separate aerobic batch tests, conducted on the flocculated filtered wastewater only.

The flocculation filtration modification greatly simplified the batch test procedure - since the flocculated-filtered wastewater does not contain OHO active biomass, a parallel batch test no longer needed to be conducted to determine the wastewater OHO active biomass, which in the "old" batch test method was subtracted from the mixed liquor + wastewater OHO active biomass to give the mixed liquor OHO active biomass.

An assessment of the modified batch test procedure using mixed liquor drawn from a well defined parent laboratory-scale MLE activated sludge system operated at 10d sludge age indicated that:

- In general, the modified batch tests yielded good % COD recoveries; the mean % COD recovery for all the batch tests was 100.5% with sample standard deviation of 7.3%. The good % COD recoveries lend credibility to the measurements and the modified batch test procedure.

- The low growth rate of the mixed liquor OHOs (mean maximum specific growth rates on RBCOD, μ_H, and SBCOD, K_{MP}, were both 0.84/d) resulted in extremely flat slopes in regression of the $\ell n(OUR_{H(t)})$-time plots, the slopes being used to calculate OHO active biomass concentrations. The low slope values made the calculation of the OHO active biomass concentration from the

OUR data very sensitive to relatively small changes in the measured slope. This requires that the OURs in the batch test are measured accurately.

- Good agreement existed between the theoretical and modified batch test measured OHO active biomass concentrations, see Fig. 2.3.

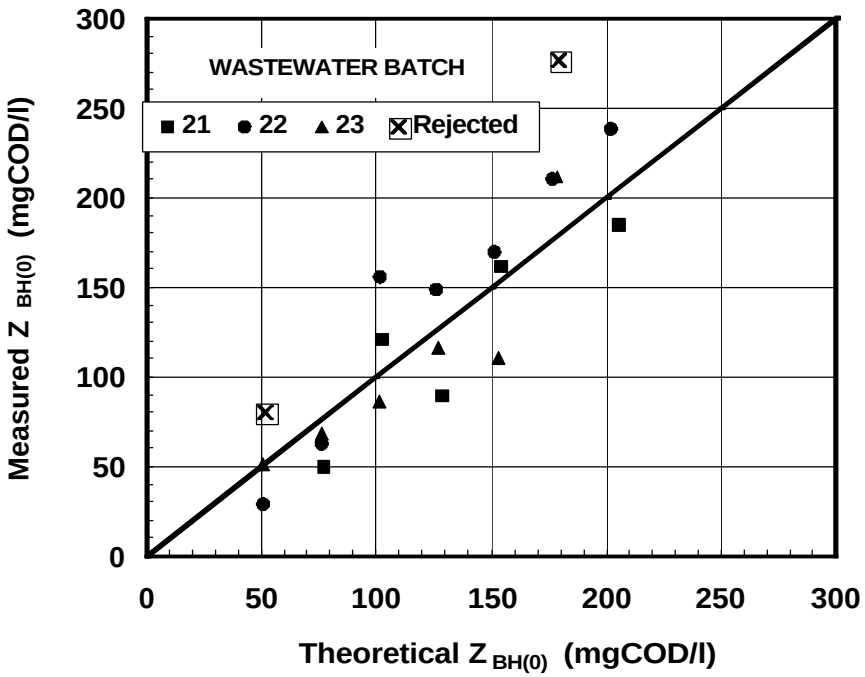

Figure 2.3: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch tests on mixed liquor drawn from parent system at 10d sludge age (Cronje *et al*., 2000, 2002b).

- Taking due account of the different volumes of mixed liquor added to the batch tests, the parent system OHO active biomass concentrations as determined from the batch tests were calculated; the **mean** OHO active biomass concentration value in the parent system measured with the modified batch test method was 1 587 mgCOD/ℓ which compares remarkably closely to the theoretical steady state design value of 1 567 mgCOD/ℓ calculated for the parent system.

- Despite the good agreement between the theoretical and measured OHO active biomass concentrations, correlations for the individual measured versus theoretical OHO active biomass concentrations were variable, due to the sensitivity of the measured OHO active biomass values to the low values measured for the slopes of the ℓn(OUR_H)-time plots. Clearly, a number of tests are required for a reliable estimate.

It was noted above that the derived slopes for the $\ell nOUR_H$-time plots were flat, due to the low maximum specific growth rates of the mixed liquor OHOs, and that this caused individual measurements of OHO active biomass concentration to exhibit variability. To attempt to increase the OHO maximum specific growth rates, a laboratory-scale parent intermittently (batch) fed fill and draw (IFFD) aerobic activated sludge system was operated at 10d sludge age to provide the source mixed liquor for the modified batch tests. In such systems, it has been shown that the batch feeding pattern induces higher maximum specific growth rates (e.g. Ekama *et al.*, 1986). In total 18 modified batch tests were conducted on mixed liquor drawn from this IFFD system (for details see Cronje *et al.*, 2000, 2002b):

- The OUR responses in these batch tests did not conform to the batch test OUR responses with mixed liquor drawn from the continuously fed systems; an initial high OUR was observed which progressively decreased with time ($\approx$ 2 hours) and only then was the characteristic exponential increase in OUR observed.

- Batch tests on mixed liquor and activated sludge system effluent indicated that the OUR response observed was not due to "carry over" with the mixed liquor of stored substrate from the parent system to the batch test.

- Through a process of elimination it was concluded that the shape of OUR response during the initial stages of the batch tests was the result of progressively decreasing substrate utilization/growth rates of the OHO active biomass.

- Due to the uncertainty surrounding the OUR responses, the measured OHO active biomass concentrations showed considerable variability, with the measured values being consistently higher than the theoretical values.

It was therefore apparent that the modified batch test in its present format is unable to provide reliable estimations of the OHO active biomass present in a parent system subjected to cyclic feed/starve conditions, such as in the IFFD system. It appears that the IFFD system induced a behaviour in the mixed liquor that cannot be accommodated within the current activated sludge system modelling theory.

Evaluation of modified batch test

From the research above, particularly encouraging was that the correlation between measured and theoretical OHO active biomass concentrations was good. This indicated that the batch test method holds potential as a valuable tool that can be used to provide greater insight into the activated sludge system. However, the method required more extensive evaluation. Accordingly, a detailed investigation was undertaken into the modified batch test method (for details see Beeharry *et al.*, 2001; Cronje *et al.*, 2002b). The modified batch test was evaluated by drawing mixed liquor from three well defined and controlled parent laboratory-scale activated sludge systems, all operated at 10d sludge age:

- Anoxic/aerobic MLE system fed raw (unsettled) municipal wastewater (termed ***control***).

- Completely aerobic system fed raw (unsettled) municipal wastewater; the system (1) above was changed to completely aerobic to ameliorate excessive bulking by the AA filament *Microthrix parvicella*.

- Anoxic/aerobic system fed a mixture of raw (unsettled) municipal wastewater and macerated toilet paper; the toilet paper was dosed to the influent to change the OHO active biomass fraction of the mixed liquor (termed ***experimental***).

A total of 18 batch tests each were conducted on mixed liquors drawn from the parent control and experimental systems, (1) and (3) above. From an analysis of the batch test results, the following were concluded:

- In interpreting the nitrate and nitrite concentration time profiles observed in their batch tests, both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) found that the ***nitrite*** concentrations were very low, and hence could be neglected. However, in this investigation nitrite concentrations were found to be significant, and hence had to be taken into account in determining the nitrification OUR (OUR_N). This arises because the oxygen requirement to nitrify ammonia-N to nitrite-N (3.43 mgO/mgN) is lower than that for nitrification of ammonia-N to nitrate-N (4.57 mgO/mgN).

- It was found that batch test sample background matrix (the flocculated-filtered wastewater) caused interference with the nitrate analytical method (Technicon Auto Analyzer Method No. 33.68); interference with the nitrite analytical method (Method No. 35.67W) was minimal. Accordingly, all batch test nitrate data were determined using nitrate standards that were made up in the flocculated filtered wastewater, and diluted with distilled de-ionised water in the same ratio as the samples were diluted.

- In their batch tests with wastewater and mixed liquor, Ubisi *et al.* (1997a,b) observed that nitrification caused a linear increase in the nitrate concentration with time. Cronje *et al.* (2000, 2002b) observed that the generation of nitrate in the batch reactor was better represented by an exponential increase. In this experimental investigation, it was observed that the nitrate/nitrite concentrations could be represented by either a linear or an exponential increase. Thus, selecting the type of fit is not general, but must be based on the data for a particular batch test.

- The modified batch tests done using mixed liquor drawn from the two ***MLE*** activated sludge systems yielded good %COD recoveries; for the control system, the mean %COD recovery was 97.8 % with sample standard deviation (SSD) of 6.9 %, for the experimental system it was 95.9 % with SSD of 5.2 %. The good %COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- OHO maximum specific growth rates on SBCOD (K_{MP}) and RBCOD (μ_{HM}) for the control system gave average values of K_{MP} = 1.78 /d (SSD = 0.74) and μ_{HM} = 2.8 /d (SSD = 1.22), and for the experimental system K_{MP} = 1.49 /d (SSD = 0.48) and μ_{HM} = 2.5 /d (SSD = 1.24). Statistically (t-test), at the 95% confidence interval (CI) these average values are not significantly different. The average values are higher than those measured by Cronje *et al.* (2000) (0.84 /d for both), but are close to the default values in the anoxic/aerobic activated sludge simulation model of Dold *et al.* (1991) (K_{MP} = 1.35 /d; μ_{HM} = 1.5 - 3.5 /d).

- Comparing the measured and the theoretical OHO active biomass concentrations (Fig. 2.4), it is apparent that the correlation is remarkably similar for the mixed liquors drawn from the control and experimental systems: It would appear that there is a correlation between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor gave an almost linear decrease in OHO active biomass concentration. However, the values plot virtually parallel to the 45º line (i.e. 1:1 correspondence). This implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between the measured and theoretical values – when the measured OHO active biomass concentration in the batch test is zero, the theoretical OHO active biomass concentration in the batch test is approximately 25 mgCOD/ℓ (i.e. ~ 15% of the theoretical OHO concentration in the parent system). No explanation for this deviation was apparent.

- Although a correlation does exist between the theoretical and measured OHO active biomass concentrations for the range of mixed liquor volumes used in the batch tests (Fig. 2.4), for some wastewater batches individual data points tend to exhibit some variation from the appropriate correlation line. As noted in the earlier research above, this variation can be attributed to the sensitivity of the measured OHO active biomass concentration to the slope of the ℓn (OUR_H) – time plot. This would suggest that a number of batch tests need to be conducted to establish a reasonable estimate for OHO active biomass concentration.

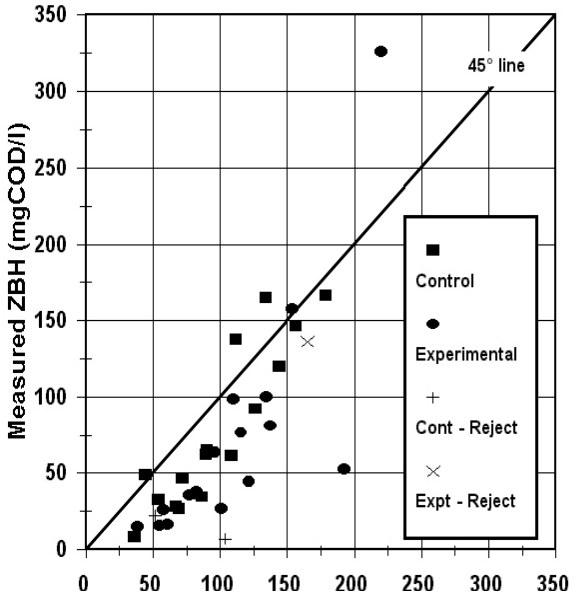

Figure 2.4: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch tests on mixed liquor drawn both control and experimental parent anoxic/aerobic (MLE) activated sludge systems.

In comparing the results from the batch tests on mixed liquor drawn from the two systems, it was noted above that the data for the control and experimental systems are remarkably similar, see Fig. 2.4: Both data sets plot on a line parallel to the 1:1 correspondence (45º) line – as noted above, this implies that there is a constant (i.e. independent of volume of mixed liquor added) difference between measured and theoretical values, of about 25 mgCOD/ℓ. No explanation for this difference could be found. That the two data sets are similar would indicate that the batch test has correctly detected the change in OHO active biomass fraction due to the toilet paper added to the experimental system: The effect of the toilet paper is taken into account automatically in calculating the theoretical OHO active biomass.

Possible phosphorus limitation

In seeking an explanation for the deviation in measured to theoretical OHO active biomass concentrations, it was noted that the preparation of the wastewater for the modified batch tests included flocculating the raw wastewater with aluminium sulphate and then filtering it to remove all the particulate material. It was thought that possibly the aluminium sulphate flocculent removed a large fraction of the available phosphorus (P) required for the growth of the OHO active biomass in the batch test. If true, this would have a direct impact on the OHO active biomass concentration measured in the batch tests, since the growth of OHOs would be restricted by non-availability of P. To evaluate this possibility, the soluble ortho-P concentration of both the *raw* and the *flocculated-filtered* wastewaters were measured on a number of occasions. The soluble ortho-P concentration averaged 12 mgP/ℓ in the *raw* wastewater and 1.6 mgP/ℓ in the *flocculated-filtered* wastewater. Thus, it appeared that P could be a factor limiting growth of the OHO active biomass, which may have caused the deviation between measured and theoretical OHO active biomass

concentrations noted above. Accordingly, it was deemed necessary to further investigate this aspect.

Two sets of modified batch tests using mixed liquor drawn from the *control* activated sludge system only were run in parallel; to the one batch test *flocculated-filtered* wastewater plus mixed liquor were added and to the other, *flocculated-filtered* wastewater plus mixed liquor plus 10 mgP/ℓ *batch reactor* were added. From analysis of the data from these tests, it was concluded that:

- In general, good %COD recoveries were achieved; the mean % COD recovery was 93.9 % with sample standard deviation of 5.5 %. The good % COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- OHO maximum specific growth rates on SBCOD (K_{MP}) and RBCOD (μ_{HM}) were normally distributed for the entire set of data, i.e. there was no difference in values for the batch tests with and without P addition. Average values were K_{MP} = 1.30 /d (SSD = 0.42) and μ_{HM} = 2.0 /d (SSD = 0.77). Statistically (t-test), at the 95% CI these average values are not significantly different from the values measured in the batch tests on mixed liquor drawn from the control or experimental parent systems above. Again, the average values are higher than those measured by Cronje *et al.* (2000, 2002b) (0.84 /d for both), but are close to the default values in the anoxic/aerobic activated sludge simulation model of Dold *et al.* (1991) (K_{MP} = 1.35 /d; μ_{HM} = 1.5 - 3.5 /d).

- The effect of adding P to the batch test was inconsistent, and not entirely conclusive. For some wastewater batches, the effect was negligible, while for others adding P caused either an increase or decrease in the OHO active biomass concentration (e.g. Fig. 2.5). Thus, it appears that the effect of adding P may be dependent on the particular wastewater batch used in the batch test, possibly depending on the P concentration available after flocculation and filtration. With the clarity of hindsight, P should have been supplemented to all subsequent batch tests when this became apparent, but at the time it was thought that the effect of P addition was negligible, so this was not done. This aspect warrants further attention.

Change in system configuration, from anoxic/aerobic to aerobic

In operation of the parent control anoxic/aerobic laboratory-scale system ((1) above), the sludge settleability (DSVI) deteriorated markedly. This forced a change in system configuration to completely aerobic ((2) above). A total of 24 modified batch tests were conducted on mixed liquor drawn from the parent fully aerobic activated sludge system. From an analysis of the data for these tests:

- In general, good % COD recoveries were achieved; the mean %COD recovery was 93.9 % with sample standard deviation of 3.8 %. The good % COD recoveries lend credibility to the reliability of the measurements and the batch test procedure.

- OHO maximum specific growth rates on SBCOD (K_{MP}) and RBCOD (μ_{HM}) gave average values of K_{MP} = 1.38 /d (SSD = 0.48) and μ_{HM} = 2.23 /d (SSD = 0.55). Statistically (t-test), at the 95% confidence interval (CI) these average values are not significantly different from the values measured in the batch tests on mixed liquor drawn from the control anoxic/aerobic parent system ((1) above). Again, the average values are higher than those measured by Cronje *et al.* (2000, 2002b) (0.84 /d for both), but are close to the default values in the anoxic/aerobic activated sludge simulation model of Dold *et al.* (1991) (K_{MP} = 1.35 /d; μ_{HM} = 1.5 - 3.5 /d).

- Comparing the measured OHO active biomass concentrations to the theoretical values (Fig.2. 5), there is a consistent correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured (dotted line Fig. 2.5).

Comparing the data obtained with the mixed liquor drawn from the fully aerobic system (Fig. 2.5) with that from the two *MLE anoxic/aerobic* systems (Fig. 2.4), the trends are completely different: For the anoxic/aerobic system mixed liquor, there is a close correlation between measured and theoretical values, but with a constant difference between the actual values (i.e. the values fall on a line parallel to the 1:1 correlation line); for the fully aerobic system mixed liquor, the measured values are about 1/3 to 1/4 the theoretical values [i.e. the values fall on a line that passes through the (0,0) origin, but which has a reduced slope]. In seeking an explanation for this difference in response, the data collected during WW Batch No. 23 is of interest (Fig. 2.5): For the batch tests conducted during WW Batch No. 23A, the system was operated as an ***MLE*** and the batch test data fall close to or higher than the 1:1 correlation line. The system was then changed to ***fully aerobic***, and shortly thereafter batch tests were conducted. With each successive set of batch tests (23A → 23B-Set 1 → 23B-Set 2 → 24 → 25 → 26), the measured OHO active biomass concentration decreased, to reach the trend line for the fully aerobic system apparent for the batch tests that followed. This would suggest that changing from the anoxic/aerobic to aerobic configuration caused a significant change in the behaviour of the mixed liquor. Such a change in population dynamics is to be expected as the population shifts from facultative to obligate aerobic.

However, why the population did not re-establish to the theoretical values after 3 sludge ages of operation is not clear: It would be expected that with time the data should return to 1:1 correlation line – this clearly did not happen.

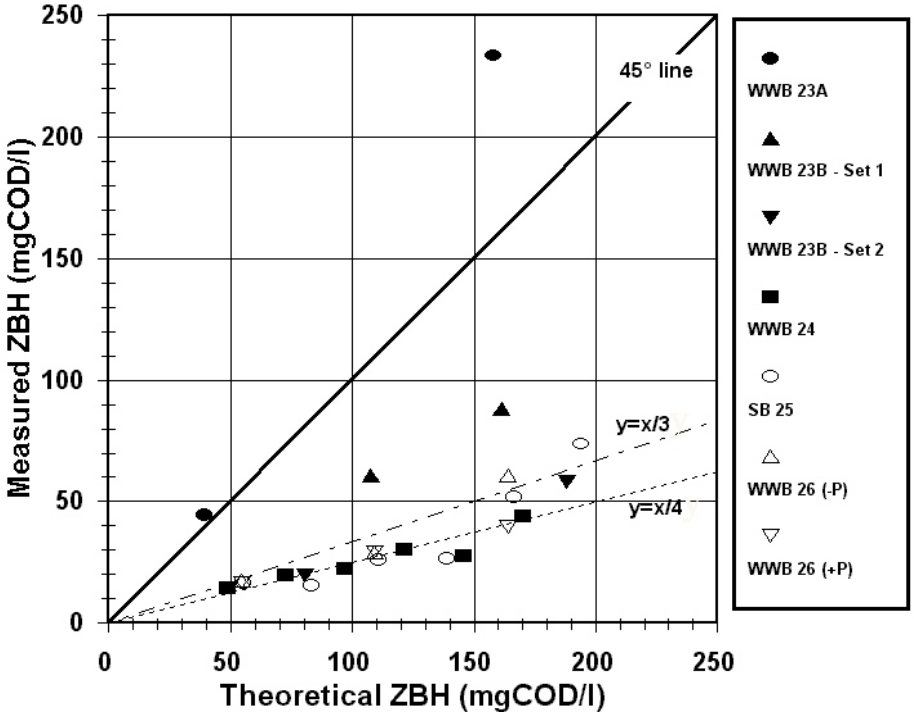

Figure 2.5: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system.

Summary

In summary, (i) the remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from the *control* and *experimental MLE* systems, (ii) the linearity of results with "serial" dilutions, and (iii) the consistent progressive change in behaviour detected by the batch test in changing from the *MLE* to *fully aerobic* configurations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, the lack of a 1:1 correlation between theoretical and measured values requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

2.2.3 Measurement of OHO active biomass within M & B paradigm

One of the objectives in the previous research project was to "attempt to link the batch test measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms" (see **OBJECTIVES** above). To achieve this, collaborative WRC sponsored projects were set up in parallel to the UCT research project, with the

Department of Microbiology and Plant Pathology at the University of Pretoria, UP (K5/1191) and the Centre for Water and Wastewater Research at the Durban Institute of Technology, DIT (K5/1178). In these parallel projects, various test methods were applied by the different groups to quantify OHO active biomass concentrations, and the results from the test methods were compared to each other and to the theoretical OHO active biomass concentrations. The Water Research Group at UCT operated parent aerobic and anoxic/aerobic (MLE) laboratory-scale activated sludge systems under closely controlled and defined conditions; this enabled the theoretical OHO active biomass concentration to be calculated within the engineering and technology (modelling) paradigm for activated sludge. Additionally, the modified batch test method which quantifies OHO active biomass concentration through monitoring OURs in batch reactors (see above) was run on mixed liquor samples drawn from the parent activated sludge systems. The research group at UP measured the biochemical compound ATP both *in situ* in the laboratory-scale anoxic/aerobic activated sludge systems at 10 and 20d sludge ages, and during the course of the modified batch tests on mixed liquors drawn from these systems, as described above. The research group at DIT used the microbiological analytical technique of a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and autotroph active biomass concentrations in samples regularly drawn from the laboratory-scale aerobic activated sludge system operated at UCT. From a comparison of the results for OHO active biomass from the various research groups, it was apparent that (Cronje *et al.*, 2002b):

- The batch test method of UCT gave mixed results:
  - For the parent anoxic/aerobic (MLE) laboratory-scale activated sludge system, there is close correspondence between the batch test measured OHO active biomass concentrations and the theoretical values, see Fig. 2.6.
  - For the parent completely aerobic laboratory-scale activated sludge system, the batch test measured OHO active biomass concentrations are approximately 1/4 the theoretical values, see Fig. 2.7.

  These observations are in agreement with those described in Section 2.2.2 above, where it was noted that when the laboratory-scale activated sludge system was changed from anoxic/aerobic to completely aerobic, there was a progressive decrease in the batch test measured OHO active biomass concentration. No explanation for this decrease could be found.

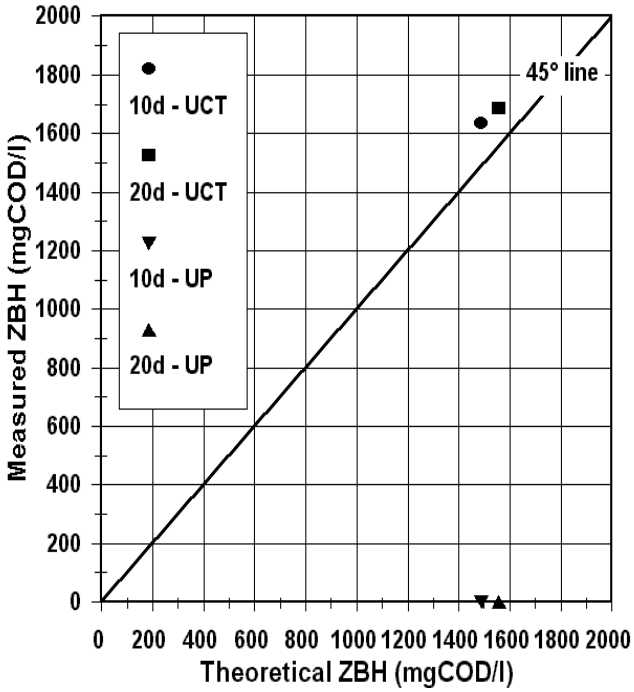

Figure 2.6: Measured versus theoretical OHO active biomass concentrations in the 10 and 20d sludge age parent laboratory-scale anoxic/aerobic activated sludge systems; measured values from batch test method taking account of dilution (UCT) and ATP measurements (UP).

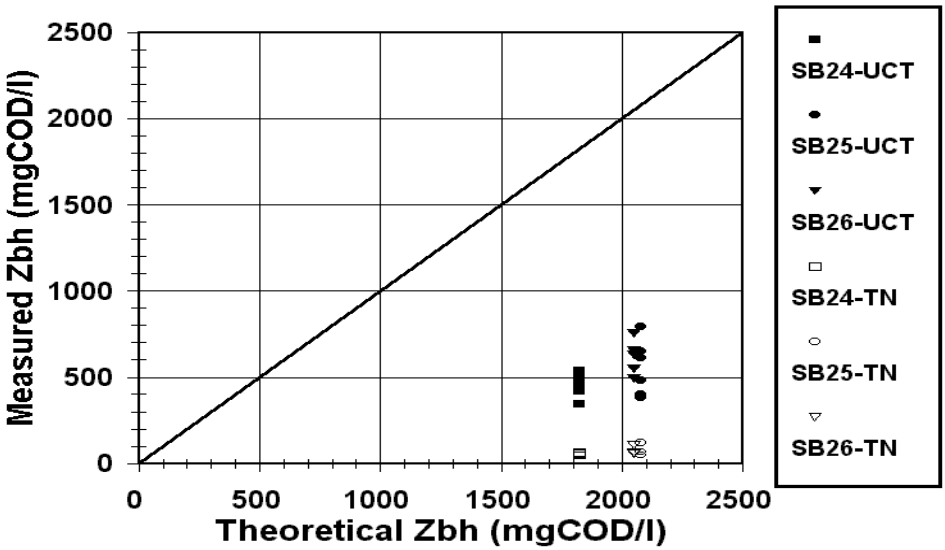

Figure 2.7: Measured versus theoretical OHO active biomass concentrations (Z_{BH}) for mixed liquor in the 10d sludge age parent laboratory-scale completely aerobic activated sludge system; measured values from batch test method taking due account of dilution (UCT) and DAPI/FISH enumeration (DIT).

- The ATP method (see Cloete and Thantsha, 2003 and Cronje *et al.*, 2002b) was applied by UP to samples drawn directly from the aerobic reactor of the laboratory-scale anoxic/aerobic activated sludge systems at 10 and 20d sludge ages above operated at UCT, and during the course of the modified batch test on mixed liquor samples drawn from these systems. Tests were conducted on fresh samples, at UCT. From these experiments, the ATP method gave:

  - OHO active biomass concentrations in the batch tests (0.09957 and 0.04453 mgCOD/ℓ for the mixed liquor from the 10 and 20d sludge age parent system respectively) that are 3 orders of magnitude smaller than both the theoretical (149 and 156 mgCOD/ℓ respectively) and batch test measured (164 and 169 mgCOD/ℓ respectively) values.
  - OHO active biomass concentrations in the parent activated sludge systems that are that 5 to 6 orders of magnitude smaller than both the theoretical and batch test measured values, see Fig. 2.6.
  - OHO active biomass concentrations that are higher in the batch tests (0.09957 and 0.04453 mgCOD/ℓ for the mixed liquor from the 10 and 20d sludge age parent system respectively) than in the corresponding parent activated sludge systems (0.00374 and 0.00160 mgCOD/ℓ respectively), despite the dilution in the batch test of the mixed liquor drawn from the parent systems with flocculated filtered wastewater that exhibits no biological activity.
  - OHO active biomass concentrations that are higher in the 10d sludge age parent activated sludge system (0.00374mgCOD/ℓ), than in the 20d sludge age parent activated sludge system (0.00160mgCOD/ℓ), despite the theoretical (1 489 and 1 558mgCOD/ℓ respectively) and batch test measured (1 636 and 1 687 mgCOD/ℓ respectively) near equivalence in the OHO active biomass concentrations.

  In seeking an explanation for the anomalies above, one possibility identified is that solids concentrations (i.e. VSS or TSS) interfere in some manner with the ATP measurement method - this would explain the lower values measured in the steady state systems (with higher VSS concentrations) than in the batch tests (with lower VSS concentrations), and the lower ATP measurements in the 20d sludge age steady state system (higher VSS concentrations) than in the 10d sludge age system (lower VSS concentration). Clearly, this is an aspect that requires investigation. One possible avenue is to do serial dilutions of the mixed liquor and measure ATP, thereby to determine the effect of VSS on the ATP test method. What is evident, however, is that the ATP method as applied is not a reliable estimate for OHO active biomass concentrations.

- The DAPI/FISH method (see Cronje *et al.*, 2002b) was applied by DIT to samples regularly drawn from the laboratory-scale aerobic activated sludge system at 10d sludge age. Samples were preserved with the 1:1 addition of 98% ethanol and stored at 4°C. Samples were couriered to DIT as a single batch, in a cooler box filled with dry ice. From these experiments, the DAPI/FISH method gave (Fig. 2.7):

- OHO active biomass concentrations that are more than an order of magnitude smaller than the corresponding theoretical values (about 3% of the theoretical values).

- OHO active biomass concentrations that are approximately 1/10 of the modified batch test determined values.

From the above it is evident that the DAPI/FISH determined OHO active biomass concentrations are significantly lower than both the batch test determined values and the theoretical values. In subsequent investigations it was found that the method of couriering the samples in dry ice caused a significant number of the cells to freeze and hence burst. This would reduce the DAPI/FISH enumerated cell counts significantly, and may be one possible explanation for the low cell counts. This was to be investigated further in the current WRC sponsored contracts with Technikon Natal (K5/1178) and UCT (K8/453).

2.3 CLOSURE

Although the initial attempts in the previous WRC contracts to link the engineering and technology theoretical and batch test measured OHO active biomass concentrations to the values measured with the microbiological and biochemical analytical techniques did not provide even near a close correspondence, the research did, for the first time, place the magnitudes of the microbiological and biochemical measurements within the context of the engineering and technology paradigm. This should help establish a common basis and "language" for the two paradigm sets, to facilitate future exchange of information and development of cross linkages. In particular, it should assist in making the quantitative information from the new microbiological and biochemical analytical techniques available to possibly improve the engineering and technology based design and simulation models developed for activated sludge systems. This will provide greater surety in the mathematical models for design and operation of the biological nutrient removal activated sludge (BNRAS) system. This potential led to the continuation of the research, under the existing WRC contract with DIT (K5/1178), and the new consultancy with UCT (K5/453).

CHAPTER 3

BATCH TEST FOR MEASUREMENT OF OHO ACTIVE BIOMASS CONCENTRATION

3.1 INTRODUCTION

From the review of the batch test method and its modification in Chapter 2, in comparing the theoretical OHO active biomass concentrations with those measured in the batch tests and modified batch tests, a variety of correlations have been found, ranging from remarkably close to 1:1 (Wentzel *et al.*, 1998 12d sludge age mixed liquor), through reasonable close to 1:1 (Cronje *et al.*, 2000 10d sludge age mixed liquor) to poor (Wentzel *et al.*, 1998 20d sludge age mixed liquor; Beeharry *et al.*, 2001 10d sludge age mixed liquor). To date, no explanation for this variability in results has been provided. ***Thus, an investigation was initiated, the principle aim of which was to examine more thoroughly the modified batch test procedure, to explore possible explanations for the observed inconsistencies.*** This research is summarised in this Chapter, and described in detail in Lee *et al.* (2003).

3.2 RESEARCH OBJECTIVES

A weakness within the activated sludge steady state design and kinetic simulation models, is the lack of independent validation of the active biomass concept. For validation, independent quantification of active biomass and close correspondence to the theoretical values is required. In this regard, the modified batch test procedure has shown considerable promise to quantify the OHO active biomass. However, results from the batch test are variable, ranging from close correspondence with the theoretical values, to poor.

The principle aim in this part of the research project was to more thoroughly examine the modified batch test procedure and OHO behaviour in the test, in order to explore possible explanations for the inconsistencies in results produced by the test.

To achieve this aim, three primary objectives were identified:

(1) Re-evaluate the modified batch test method, by repeating the modified batch test procedure and comparing the measured OHO active biomass concentrations with the theoretical values predicted by the steady state model (WRC, 1984). This will establish whether the results of Cronje *et al.* (2000) or Beeharry *et al.* (2001) are reproducible, and enable an evaluation of possible causes contributing to variations in correspondence between theoretical and measured values.

(2) In addressing the aim above, it needs to be established whether discrepancies between measured and theoretical OHO active biomass concentrations arise

from the activated sludge theory (*i.e.* the theoretical) or from the batch test procedure itself (*i.e.* the measured). Accordingly, the alternative more laborious batch aerobic digestion method of Marais and Ekama (1976) to quantify OHO active biomass would be applied.

(3) Should in application of the modified batch tests, an inconsistency between the measured and theoretical OHO active biomass concentrations be obtained, identify possible causes for this inconsistency. In this regard, during the investigation the importance of OHO behaviour in the modified batch tests became apparent, and the concept of substrate competition between fast and slow growing OHO population groups proposed by Novak *et al.* (1994) was found to be relevant and was examined.

3.3 RESEARCH APPROACH

Two well-defined and controlled parent laboratory-scale MLE activated sludge systems at 10 and 20 days sludge ages were operated and monitored. These parent systems provided the mixed liquor samples required for measuring OHO active biomass, by means of the modified batch test (objective (1) above) and the batch aerobic digestion test (objective (2) above). The data from these tests and the analytical methods used to interpret the data were thoroughly analysed to identify possible causes for discrepancies between measured and theoretical OHO active biomass concentrations (objective (3) above); this included development of kinetic models to simulate the batch test response.

3.4 RE-EVALUATION OF MODIFIED BATCH TEST

To re-evaluate the modified batch test, the batch test was applied to mixed liquor samples drawn from well defined and controlled parent laboratory-scale activated sludge systems.

3.4.1 Parent system

Two laboratory-scale parent activated sludge systems at 10 and 20 days sludge age and maintained at 20°C served as th source of mixed liquor for the modified batch tests. Both systems were Modified Ludzack-Ettinger (MLE) configurations (see Fig. 3.1), and consisted of an anoxic reactor of 33% of the total system volume, an aerobic reactor of 67% of the total system volume and a secondary settling tank, all in series with an underflow recycle (s-recycle) from the settling tank to the anoxic reactor of 1:1 and from the aerobic reactor to the anoxic reactor (a-recycle) of 2:1 during Periods 1 and 2 or 1:1 during Periods 3 and 4 (see Table 3.1 and Lee *et al.*, 2003). The influent for the parent laboratory-scale activated sludge systems was raw (unsettled) sewage from the Mitchell's Plain Treatment Plant in Cape Town (South Africa). This sewage is primarily domestic, with a small (< 25%) industrial component. This sewage was collected in batches, stored in 400ℓ stainless steel tanks in a cold room at 4°C and served as feed for both the parent systems and the batch tests for 10 to 14 days. For the parent systems, daily sewage was drawn from the storage tanks after thorough mixing and diluted with tap water to give influent feed total COD

concentration 750 ± 50 mgCOD/ℓ, with influent flow rate of 13.3 (Period 1, 2 and 3) or 10 ℓ/d (Period 4). To maintain the pH in the aerobic reactor at ± 7.5, the alkalinity of the influent was increased by 200 mg/ℓ (as $CaCO_3$). Daily monitoring included influent COD, TKN; all reactors nitrite + nitrate; aerobic reactor TSS, VSS, COD and TKN; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985).

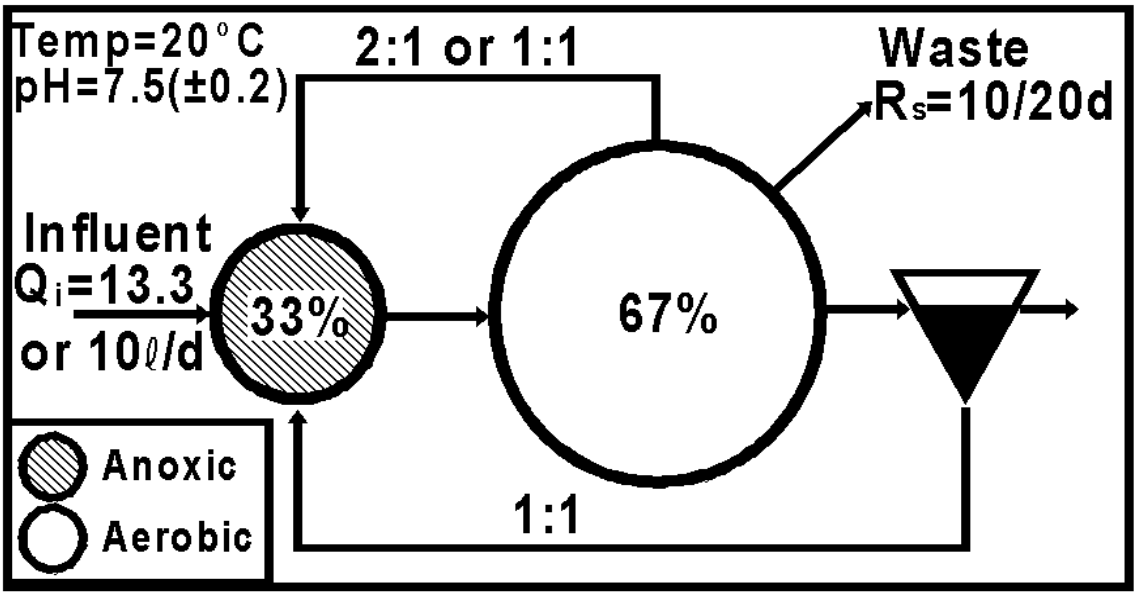

Figure 3.1: Schematic layout and operational data for parent laboratory-scale MLE anoxic/aerobic activated sludge systems.

Table 3.1: Details of changes to the parent laboratory-scale systems operating parameters; AX=anoxic, AE=aerobic.

Period	Dates (2001 - 2002)	Day No	Sewage Batches	Inflow Rate (Qi; ℓ/d)	Recycle ratios (With respect to Qi)		System Volume (ℓ) (33% AX, 67% AE)	
					s-recycle	a-recycle	10days	20days
1	7 Jul~19 Aug	1 ~ 42	1 ~ 3	13.3	0.042361	0.084028	10	10
2	20 Aug~01 Sep	43 ~ 55	4	13.3	0.042361	0.084028	7.8	13.2
3	02 Sep~13 Feb	56 ~ 220	5 ~ 13	13.3	0.042361	0.042361	7.8	13.2
4	14 Feb~28 Apr	221 ~ 294	14 ~ 17	10	0.042361	0.042361	7.8	13.2

Both the 10 and 20 days sludge age parent systems were operated for 294 days and received 17 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town, see Table 3.2. Each wastewater batch was accepted as a steady state period, and the results for each batch were averaged (after statistical analysis for outliers), see Table 3.3.

Table 3.2: Details of the parent laboratory-scale activated sludge systems; sewage batch number, sewage feed dates, days of operation and batch tests conducted. (*indicate Batch Test done with Aerobic Digestion)

Sewage Batch No.	Generic Sew. Batch. No	Date of tests (2001/2002)	Days of operation(d)	Alum Addition[1]	Batch Test	Period
1	SB11/01	7 Jul –24 Jul	1 – 18	No	No	**1**
2	SB12/01	25 Jul – 03 Aug	19 – 28	Yes	No	
3	SB13/01	04 Aug – 19 Aug	29 – 42	No	No	
4	SB14/01	20 Aug – 01 Sep	43 – 55	No	No	**2**
5	SB15/01	02 Sep – 13 Sep	56 – 67	No	Yes	**3**
6	SB16/01	14 Sep – 19 Sep	68 – 73	No	No	
7	SB17/01	20 Sep – 07 Oct	74 – 91	No	Yes	
8	SB18/01	08 Oct – 16 Oct	92 – 100	Yes	Yes	
9	SB19/01	16 Nov – 01 Dec	131 – 146	Yes	Yes	
10	SB20/01	02 Dec – 17 Dec	147 – 162	Yes	Yes	
11	SB21/01	18 Dec – 04 Jan	163 – 180	No	Yes	
12	SB01/02	05 Jan – 17 Jan	181 – 193	No	Yes	
13	SB02/02	30 Jan – 13 Feb	206 – 220	No	Yes	
14	SB03/02	14 Feb – 28 Feb	221 – 235	No	Yes	**4**
15	SB04/02	16 Mar – 26 Mar	251 – 261	No	Yes*	
16	SB05/02	27 Mar – 12 Apr	262 – 278	No	Yes*	
17	SB06/02	13 Apr – 28 Apr	279 – 294	No	Yes	

[1]Alum added to parent system to contain the mixed liquor in the system which was being lost due to poor settleability (see Lee *et al.*, 2003).

Table 3.3 (a): Steady state data for the10 day sludge age parent system; for each sewage (Sew.) batch (steady state period, see Table 3.3) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch	No of tests	TKN (mgN/l)			Nitrite (mgN/ℓ)			Nitrate (mgN/ℓ)			COD (mg/ℓ)			OUR (mgO/ℓ/h)	VSS (mgVSS/ℓ)
		Inf	Eff	ML	Anoxic	Aerobic	Eff	Anoxic	Aerobic	Eff	Inf	Eff	ML		
1	14	57.9 (3.8)	3.1 (0.8)	211 (15)	0.19 (0.45)	0.06 (0.21)	0 (0)	0.2 (0.6)	8.8 (1.4)	10.4 (1.1)	718 (28)	25 (8)	3791 (168)	36.3 (1.9)	2836 (75)
2	7	75.4 (1.7)	3.4 (0.6)	223 (7)	0.89 (0.47)	0.16 (0.26)	0.09 (0.21)	1.8 (1.6)	15.3 (1.7)	17.3 (0.9)	710 (11)	27 (10)	3813 (113)	38.6 (1.2)	2760 (94)
3	7	62.2 (1.7)	3.7 (0.3)	222 (8)	1.04 (0.91)	0 (0)	0 (0)	2.4 (2.4)	14.5 (3.6)	14.6 (3.1)	757 (15)	33 (14)	3923 (96)	32.1 (1.5)	2765 (107)
4	7	90.2 (1.7)	3.0 (1.1)	289 (12)	2.1 (0.3)	0 (0)	0 (0)	14.9 (2.1)	35.2 (2.6)	34.3 (1.2)	712 (33)	33 (7)	5106 (141)	47.5 (2.9)	3510 (129)
5	3	85.1 (1.3)	3.6 (0.7)	293 (16)	2.3 (0.3)	0 (0)	0 (0)	5.8 (1.0)	30.0 (2.8)	27.0 (1.6)	707 (29)	31 (6)	4627 (66)	55.7 (0)	3360 (68)
6	3	66.7 (1.9)	3.2 (0.3)	290 (10)	2.3 (0.3)	0 (0)	0 (0)	0.1 (0.0)	15.8 (0.3)	12.0 (0.3)	735 (13)	26 (3)	4892 (91)	44.6 (1.3)	3458 (113)
7	7	83.2 (3.2)	4.8 (1.1)	272 (6)	0.1 (0.1)	0 (0)	1.3 (0.3)	0.1 (0.1)	19.9 (2.9)	13.4 (0.4)	760 (63)	35 (18)	4633 (130)	60.2 (3.8)	3325 (76)
8	3	59.7 (2.0)	4.2 (0.7)	289 (11)	0 (0)	0 (0)	0.80 (0.10)	0.1 (0.0)	15.4 (0.5)	4.2 (0.8)	677 (28)	18 (9)	4897 (315)	51.2 (2.8)	3469 (143)
9	6	66.5 (1.6)	5.4 (1.1)	265 (20)	0 (0.10)	0 (0)	0.40 (0.20)	0.0 (0.0)	11.5 (1.1)	13.3 (0.7)	687 (23)	49 (16)	4331 (252)	60.6 (6.6)	3291 (127)
10	8	60.8 (2.3)	4.5 (0.8)	235 (19)	0 (0)	0 (0)	0.37 (0.48)	0.0 (0.0)	13.6 (1.1)	6.3 (0.9)	749 (40)	43 (11)	4252 (225)	52.0 (3.9)	3132 (205)
11	9	61.8 (1.3)	4.5 (0.6)	259 (9)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	12.5 (0.7)	13.7 (0.9)	734 (22)	45 (7)	4733 (160)	57.2 (4.2)	3462 (122)
12	8	83.4 (2.2)	4.8 (0.5)	270 (8)	0.52 (0.55)	0 (0)	0.58 (0.39)	0.1 (0.3)	18.8 (1.3)	20.3 (3.4)	749 (26)	51 (6)	4712 (189)	67.8 (6.1)	3521 (173)
13	3	65.1 (0.6)	4.2 (0.3)	270 (7)	0 (0)	0 (0)	1.5 (0.3)	0.0 (0.0)	14.4 (0.2)	8.4 (1.7)	763 (12)	37 (2)	4365 (216)	48.6 (7.6)	3195 (166)
14	9	59.2 (1.7)	4.0 (0.5)	204 (7)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	11.5 (0.8)	12.6 (0.4)	727 (23)	38 (4)	3482 (73)	44.8 (3.2)	2532 (62)
15	6	66.1 (0.5)	3.0 (0.7)	192 (5)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	19.3 (0.3)	19.5 (0.2)	765 (15)	43 (9)	3443 (81)	51.8 (2.9)	2557 (40)
16	11	50.7 (1.3)	4.5 (0.5)	197 (11)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	10.3 (1.4)	10.2 (0.9)	743 (18)	43 (7)	3417 (354)	45.1 (3.9)	2489 (227)
17	8	61.7 (1.6)	4.2 (0.3)	203 (7)	0 (0)	0 (0)	0 (0)	0.0 (0.1)	12.8 (0.9)	13.8 (0.7)	773 (13)	38 (5)	3616 (97)	43.9 (1.0)	2632 (101)

Table 3.3 (b): Steady state data for the 20 day sludge age parent system; for each sewage (Sew.) batch (steady state period, see Table 3.3) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed.

Sew. Batch	No of tests	TKN (mgN/l)			Nitrite (mgN/ℓ)			Nitrate (mgN/ℓ)			COD (mg/ℓ)			OUR (mgO/ℓ/h)	VSS (mgVSS/ℓ)
		Inf	Eff	ML	Anoxic	Aerobic	Eff	Anoxic	Aerobic	Eff	Inf	Eff	ML		
1	14	57.9 (3.8)	2.7 (0.8)	293 (21)	0.60 (0.56)	0 (0)	0.09 (0.24)	1.7 (2.1)	13.2 (3.5)	12.2 (3.0)	718 (28)	21 (8)	5559 (254)	38.4 (1.7)	4074 (229)
2	7	75.4 (1.7)	3.3 (0.5)	328 (6)	0.79 (0.39)	0 (0)	0.44 (0.21)	2.8 (1.4)	20.0 (1.5)	15.1 (1.5)	710 (11)	25 (10)	5737 (138)	44.5 (1.6)	4208 (107)
3	7	62.2 (1.7)	3.7 (0.8)	314 (9)	0.61 (0.54)	1.29 (3.15)	0.38 (0.26)	0.8 (0.8)	12.7 (2.0)	4.9 (1.5)	757 (15)	32 (14)	5703 (235)	38.0 (1.5)	4201 (163)
4	7	90.2 (1.7)	3.2 (0.7)	290 (7)	1.5 (0.3)	0 (0)	0 (0)	11.4 (3.0)	33.2 (4.3)	30.3 (2.3)	712 (33)	30 (9)	5071 (160)	34.9 (1.9)	3647 (66)
5	3	85.1 (1.3)	3.6 (0.5)	276 (6)	1.2 (0.2)	0 (0)	0 (0)	4.9 (1.1)	31.4 (1.8)	31.6 (2.3)	707 (29)	23 (4)	4813 (120)	34.0 (1.8)	3487 (39)
6	3	66.7 (1.9)	4.0 (0.1)	300 (6)	1.2 (0.3)	0 (0)	0 (0)	0.0 (0.1)	18.3 (0.6)	11.7 (0.5)	735 (13)	33 (8)	5087 (176)	33.4 (1.6)	3599 (64)
7	7	83.2 (3.2)	3.9 (0.6)	302 (10)	0.2 (0.2)	0 (0)	1.0 (0.6)	0.3 (0.3)	20.2 (2.6)	18.3 (2.6)	760 (63)	23 (11)	5288 (353)	43.1 (3.6)	3718 (141)
8	3	59.7 (2.0)	3.7 (0.2)	283 (7)	0 (0)	0 (0)	0.5 (0.2)	0.1 (0.1)	16.8 (0.3)	12.9 (1.0)	677 (28)	-4.7 (19)	4877 (142)	34.2 (0.4)	3715 (123)
9	6	66.5 (1.6)	4.2 (0.7)	258 (13)	0.1 (0.1)	0 (0)	0.7 (0.5)	0.0 (0.0)	14.6 (1.0)	12.4 (2.2)	687 (23)	23 (10)	4548 (69)	35.0 (0.5)	3309 (68)
10	8	60.8 (2.3)	4.3 (0.9)	291 (17)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	14.6 (1.1)	11.6 (2.3)	749 (40)	41 (11)	5115 (183)	40.7 (2.5)	3668 (103)
11	9	61.8 (1.3)	5.0 (0.4)	266 (14)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	15.0 (1.0)	15.4 (0.5)	734 (22)	43 (6)	4749 (144)	36.7 (1.4)	3351 (62)
12	8	83.4 (2.2)	4.3 (0.8)	270 (10)	0.81 (0.22)	0 (0)	0.24 (0.01)	0.7 (0.6)	21.1 (0.9)	21.6 (0.8)	749 (26)	37 (6)	4816 (94)	38.2 (0.8)	3407 (53)
13	3	65.1 (0.6)	4.7 (0.5)	269 (19)	0 (0)	0 (0)	0.77 (0.19)	0.0 (0.0)	15.1 (0.3)	12.4 (1.4)	763 (12)	27 (3)	4518 (90)	32.8 (0.8)	3272 (49)
14	9	59.2 (1.7)	3.8 (0.4)	201 (4)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	12.9 (1.0)	13.4 (1.3)	727 (23)	32 (5)	3471 (27)	27.8 (2.6)	2460 (48)
15	6	66.1 (0.5)	2.9 (0.4)	191 (6)	0.24 (0.12)	0 (0)	0 (0)	0.1 (0.0)	20.7 (0.3)	20.7 (0.5)	765 (15)	34 (4)	3474 (83)	31.4 (2.3)	2507 (50)
16	11	50.7 (1.3)	4.5 (0.5)	205 (5)	0 (0)	0 (0)	0 (0)	0.0 (0.0)	11.3 (1.1)	12.3 (0.8)	743 (18)	40 (9)	3634 (69)	30.7 (3.5)	2572 (41)
17	8	61.7 (1.6)	4.1 (0.5)	207 (5)	0.04 (0.07)	0 (0)	0 (0)	0.0 (0.0)	15.3 (0.4)	15.0 (0.7)	773 (13)	36 (5)	3775 (53)	26.1 (1.3)	2667 (70)

Table 3.4 (a): Steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for the 10 days sludge age parent system. Data calculated from Table 3.3 (a) (* indicates data rejected as outliers at 95% confidence interval).

Sew. Batch No.	No of tests	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR	
		N	COD	Unbio. Soluble (fs,us) (mgCOD/mgCOD)	Unbio. Particulate (fs,up) (mgCOD/mgCOD)	COD/VSS (fcv) (mgCOD/mgVSS)	TKN/VSS (fN) (mgN/mgVSS)
1	14	96	93	0.0303	0.1931	1.33	0.075 *
2	7	99	92	0.0345	0.2046	1.34	0.08
3	7	104	79	0.0361	0.1654	1.4	0.081
4	7	102	78	0.0421	0.2037	1.44	0.083
5	3	98	90	0.0387	0.1703	1.37	0.088
6	3	104	84	0.0346	0.1739	1.44	0.086
7	7	99	97	0.0382	0.1345	1.42	0.085
8	3	113	103	0.0060 *	0.2147	1.45	0.087
9	6	91	113	0.039	0.1756	1.38	0.086
10	8	97	92	0.0488	0.1113	1.38	0.078
11	9	96	104	0.0513	0.1794	1.42	0.08
12	8	94	110	0.0487	0.1717	1.4	0.082
13	3	99	85	0.0454	0.1083	1.35	0.086
14	9	95	107	0.0482	0.1625	1.39	0.084
15	6	115	105	0.0477	0.1442	1.41	0.08
16	11	99	106	0.0519	0.1453	1.4	0.083
17	8	97	99	0.0474	0.1515	1.39	0.081
MEAN				**0.0427**	**0.1653**	**1.40**	**0.083**
Std. Deviation				**0.0066**	**0.0295**	**0.03**	**0.003**

Table 3.4 (b): Steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for the 20 days sludge age parent system. Data calculated from Table 3.3 (b) (* indicates data rejected as outliers at 95% confidence interval).

Sew. Batch No.	No of tests	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR	
		N	COD	Unbio. Soluble (fs,us) mgCOD/mgCOD)	Unbio. Particulate (fs,up) (mgCOD/mgCOD)	COD/VSS (fcv) (mgCOD/mgVSS)	TKN/VSS (fN) (mgN/mgVSS)
1	14	99	81	0.0279	0.1123	1.36	0.072 *
2	7	102	86	0.0292	0.128	1.35	0.078
3	7	103	79	0.0363	0.1056	1.35	0.075
4	7	106	85	0.0401	0.187	1.37	0.079
5	3	110	83	0.0425	0.1716	1.37	0.08
6	3	113	94	0.0392	0.1702	1.39	0.083
7	7	96	108	0.0386	0.1707	1.39	0.081
8	3	115	98	-0.0190 *	0.1896	1.30 *	0.076
9	6	91	101	0.0331	0.156	1.35	0.078
10	8	98	110	0.0523	0.1713	1.37	0.079
11	9	103	101	0.0513	0.1448	1.39	0.079
12	8	93	97	0.0471	0.1405	1.38	0.079
13	3	98	87	0.0332	0.1149	1.38	0.084
14	9	95	103	0.0427	0.1353	1.38	0.082
15	6	116	100	0.0415	0.1214	1.36	0.076
16	11	106	113	0.0454	0.1451	1.38	0.08
17	8	102	92	0.0382	0.1429	1.38	0.078
MEAN				**0.0399**	**0.1475**	**1.37**	**0.079**
Std. Deviation				**0.0068**	**0.0254**	**0.01**	**0.003**

With the averaged data, from Ekama *et al.* (1986) and Ubisi *et al.* (1997a,b) the following were calculated, see Table 3.4:

- System COD and N mass balances.
- Influent wastewater unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively).
- Mixed liquor COD/VSS and TKN/VSS ratios (f_{CV} and f_N respectively).
- The OHO active biomass fraction of the mixed liquor organic suspended solids (f_{av}).
- The theoretical OHO active biomass concentration in the steady state system bioreator.

From the results on the parent system:

- N mass balances were consistent and generally in the range 90 to 110%, see Figs. 3.2 (a and b). Sewage batches with N mass balance outside this range were Nos. 8 and 15 for the 10 day sludge age system and Nos. 6, 8 and 15 for the 20 day sludge age system.
- Generally COD mass balances were not as good as the N mass balances, with 5 out of 17 sewage batches for the 10 day sludge age system (Nos. 3, 4, 6, 9 and 13) and 6 out of 17 sewage batches for the 20 day sludge age system (Nos. 1-5, 13 and 16) giving mass balances outside the range 90 to 110%, see Figs. 3.3 (a and b).

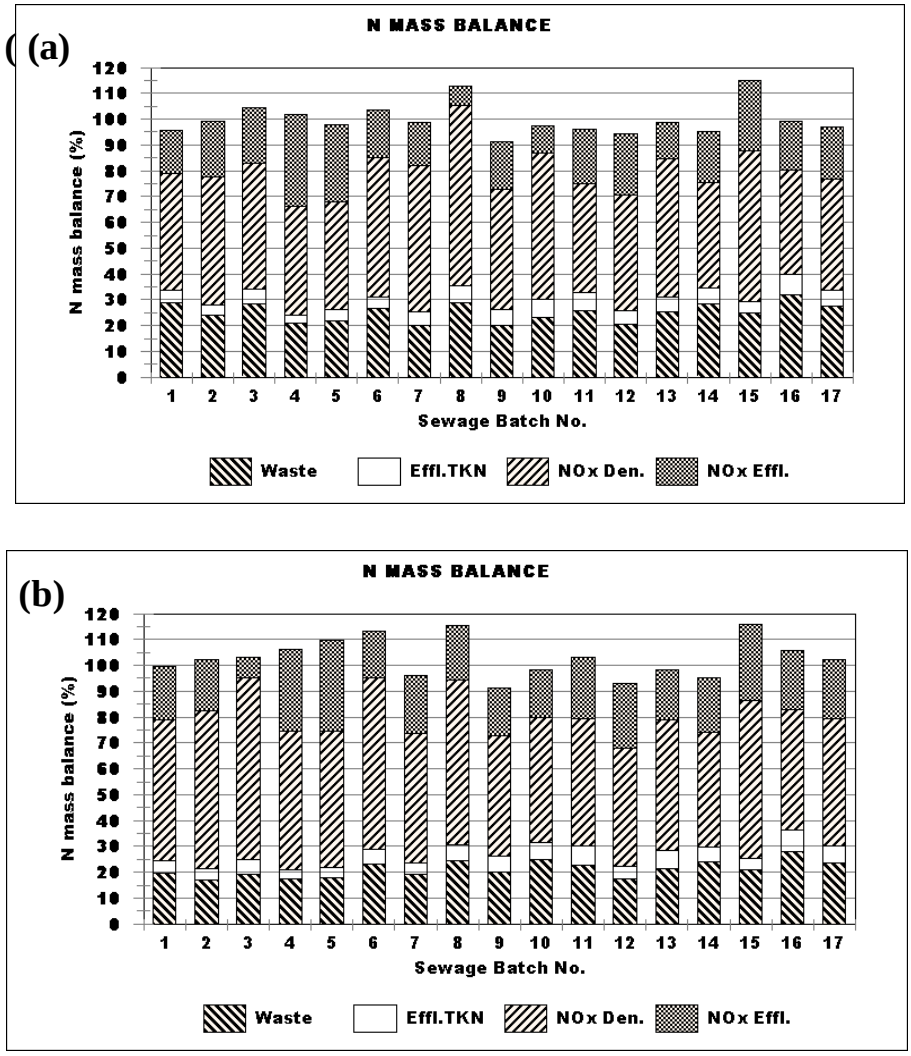

Figure 3.2: Nitrogen (N) mass balance components for the various sewage batches for the (a) 10d sludge age and (b) 20d sludge age parent anoxic/aerobic activated sludge systems: Sludge production (waste), effluent TKN (Effl. TKN), nitrate/nitrite denitrified (NOx Den.) and effluent nitrate/nitrite (NOx effl.).

- From the above, sewage batch Nos. 3, 4, 6, 8, 9, 13 and 15 for the 10 day sludge age system, and 1-6, 8, 13, 15 and 16 for the 20 day sludge age system gave N and/or COD mass balances outside the 90 - 110 % range. Batch test were conducted during sewage batch Nos. 8, 9 and 13, and 5 and 13 respectively. These data will be included for analysis, but will be appropriately marked.

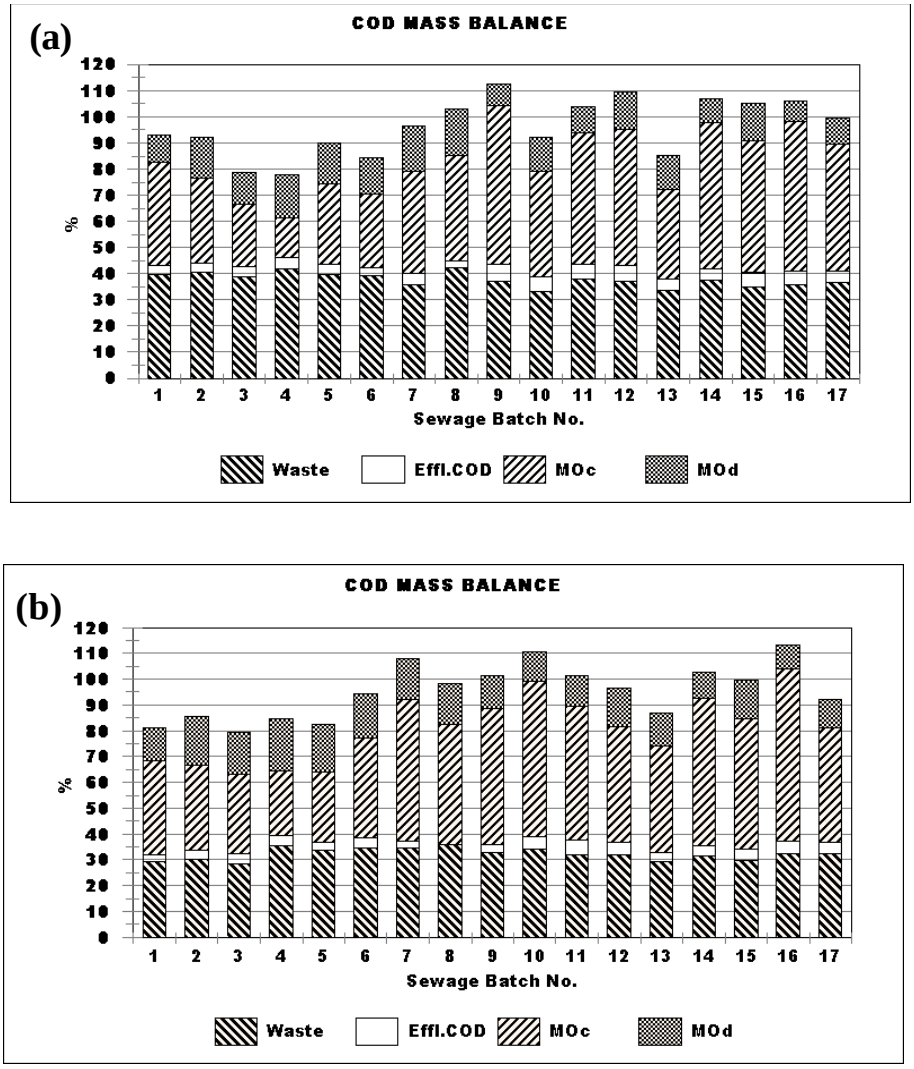

Figure 3.3: COD mass balance components for the various wastewater batches for the (a) 10d and (b) 20d sludge age parent anoxic/aerobic activated sludge systems: Sludge production (waste), effluent COD (Effl. COD), carbonaceous oxygen demand (MOc), equivalent oxygen demand for denitrification (MOd).

- The influent wastewater mean unbiodegradable soluble COD fractions ($f_{S,us}$) were determined to be 0.043 (sample standard deviation, ± 0.0066) and 0.040 (± 0.0068) for the 10 and 20 day sludge age systems respectively, see Tables 3.4 (a and b) respectively. The difference between the two $f_{S,us}$ values is not statistically significant at the 95% confidence interval (t-test). However, the values are lower than the $f_{S,us}$ values obtained by both Ubisi *et al.* (1997a,b) ($f_{S,us}$ = 0.095) and Cronje *et al.* (2000) ($f_{S,us}$ = 0.085) for the same Mitchell's Plain raw wastewater; the values are similar to the $f_{S,us}$ value obtained by Beehary *et al.* (2001) ($f_{S,us}$ = 0.05). Of interest is the fact that both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems; however, Beehary *et al.* (2001) was feeding a COD concentration of 750 ± 50 mg COD/ℓ which is the same as the targeted COD concentration of this investigation. Thus, despite that the $f_{S,us}$ value would be expected to be the same, given that the influent wastewater being treated was the same

and $f_{S,us}$ is a wastewater characteristic, the higher COD concentrations gave lower $f_{S,us}$ values. This observation would imply that dilution of the wastewater (with tap water) used as feed to the laboratory-scale systems has an influence on the $f_{S,us}$ value. The $f_{S,us}$ values in this experiment are, however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The influent wastewater mean unbiodegradable particulate COD fractions ($f_{S,up}$) were determined to be 0.165 (± 0.0295) for the10 day system and 0.148 (± 0.0254) for the 20 day system, see Tables 3.4 (and b) respectively. The $f_{S,up}$ value for the 10 day system is slightly higher than the value for the 20 day system, similar to the $f_{S,us}$ values. However, the difference between the two $f_{S,up}$ values is not statistically significant at the 95% confidence interval (t-test). The two $f_{S,up}$ values are higher than the values observed by Ubisi *et al.* (1997a,b) ($f_{S,up}$ = 0.120) and Cronje *et al.* (2000) ($f_{S,up}$ = 0.103) and are similar to the value observed by Beehary *et al.* (2001) ($f_{S,up}$ = 0.161) for the same Mitchell's Plain raw wastewater. As noted above, both Ubisi *et al.* (1997a,b) and Cronje *et al.* (2000) were feeding a COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems; however, Beehary *et al.* (2001) was feeding a COD concentration of 750 ± 50 mg COD/l which is the same as the targeted COD concentration of this experiment. Thus, despite that the $f_{S,up}$ value would be expected to be the same, given that the influent wastewater being treated was the same, the higher COD concentration gave a higher $f_{S,up}$. The $f_{S,up}$ values of this experiment are, however, in the range of accepted values of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

The consistency in the parent systems data during which batch tests were conducted, lends support to application of the steady state design procedure to the parent systems to calculate the theoretical OHO active biomass concentrations, and to using mixed liquor from the systems in the modified batch tests to measured the OHO active biomass concentrations.

3.4.2 Modified batch tests

To quantify the OHO active biomass concentration in ML drawn from the two parent activated sludge systems (Table 1), the modified batch test procedure of Cronje *et al.* (2002) was followed. Wastewater was drawn from the storage tanks after thorough mixing and diluted to approximately the same COD concentration as that fed to the parent systems (~ 750 mg COD/ℓ). This raw wastewater was pre-heated to 20 °C and then flocculated with alum: 10 mℓ of stock aluminium sulphate [$A\ell(SO_4) \cdot 15\ H_2O$, stock at 50g/ℓ] were added per ℓ wastewater, the mixture was stirred rapidly (~200 rpm) for 2 minutes (rapid mix phase) and then slowly (~1 rpm) for 30 minutes (flocculation and settling phase) and then were allowed to settle (without stirring) for a further 30 minute period. The clear supernatant that developed in the settling cylinders was drawn off and filtered through a glass fibre filter (Whatman's GF/C).

The required volume of mixed liquor was harvested from the aerobic reactor of the relevant parent system and added to the *flocculated-filtered* WW in the batch reactor (Fig. 3.4) maintained at 20 °C, giving a combined volume of 3ℓ. A sample was drawn to obtain the initial total COD concentration. The OUR response in the batch test was continually measured using an automated technique (Randall *et al.*, 1991). Regularly, samples were

drawn from the reactor, immediately filtered (0.45 μm), 2 – 3 drops of $HgCl_2$ were added to the filtrate which was stored for subsequent nitrate and nitrite analysis. At the end of the batch test, a final sample was drawn, macerated and final total COD concentration measured.

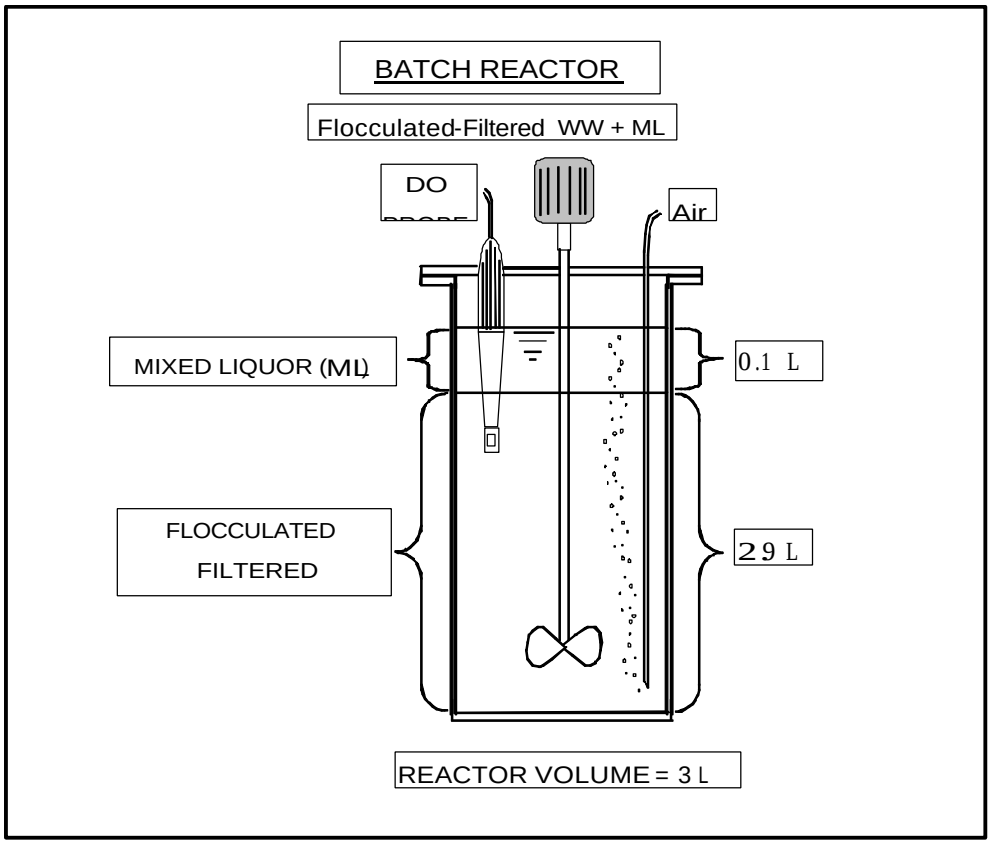

Figure 3.4: Single batch reactor arrangement used to conduct batch tests with mixed liqour (ML) added to flocculated filtered wastewater (WW).

The modified batch test data were analyzed and interpreted using the procedure detailed by Cronje *et al.* (2000), Beeharry *et al.* (2001) and Lee *et al.* (2003), which is based on a simplified UCT model, which consists of a single OHO population. From the batch test data the following was calculated:

- %COD recovery
- OHO active biomass concentration at the start of the batch test, $Z_{BH(0)}$
- OHO maximum specific growth rates on RBCOD (μ_H) and SBCOD (K_{MP})

In total, 35 modified batch tests were conducted on mixed liquor drawn from the 10 day sludge age parent system (Table 3.5), and 35 on that drawn from the 20 day sludge age parent system (Table 3.6). From the batch tests, the following conclusions were drawn (for details see Lee *et al.*, 2003):

- Nitrite concentrations in the modified batch tests were significant, and hence were taken into account in calculating the nitrification OUR.

- In general, %COD recoveries were good, with only 8 out of 35 batch tests on the 10 day sludge age mixed liquor yielding %COD recoveries < 90%, and only 7 out of 35 for the 20 day sludge age mixed liquor. Excluding data for those batch tests that deviated from the "true" normal probability line (4 for 10 days; 3 for 20 days), the mean %COD recoveries were 95.2 (± 5.3) and 94.8 (± 4.5) % for the batch tests on the 10 and 20 day sludge age mixed liquors respectively. The %COD recoveries are somewhat lower than that obtained by Cronje *et al.* (2000) (100.5%), but are similar to those of Beeharry *et al.* (2001) (97.8 and 95.9 %). The good %COD recoveries lend credibility to the experimental data. The rejected batch tests will be included, but appropriately marked, so also batch tests with $R^2 < 0.7$ in the fit to the ℓOUR-time plots.

- For the OHO maximum specific growth rate on SBCOD (K_{MP}), excluding the batch tests with low %COD recoveries, statistical analysis gave mean K_{MP} of 1.37 (± 0.412) and 1.42 (± 0.428) /d for the 10 and 20 day sludge age mixed liquors respectively. The means were not significantly different at 95% confidence interval (t-test), which indicates that sludge age did not have a significant influence on the value for this parameter. The values are larger than that measured by Cronje *et al.* (2000) (0.84 /d), but are close to the values measured by Beeharry *et al.* (2001) (1.49 and 1.38 /d) and to the default value for K_{MP} in the UCT kinetic simulation model of 1.35 /d (Dold *et al.*, 1991). It should be noted that there was some measure of uncertainty in the determination of K_{MP} at the relatively large and small mixed liquor volumes, but this uncertainty was not large.

- For the OHO maximum specific growth rate on RBCOD (μ_H), for batch tests on mixed liquors from both parent systems, a clearly discernable trend was noted: As the volume of mixed liquor added to the batch test increased, the value for μ_H decreased, see Fig. 3.5 (a and b). This indicates that one (or more) factor has a dominating influence, which precluded statistical analysis of the data. Re-examination of the data of Beeharry *et al.* (2001) indicated that they obtained, but did not note, a similar trend (see Cronje *et al.*, 2002b). This aspect is examined in more detail below.

Table 3.5: Results for batch tests on a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML) drawn from the 10d sludge age parent system: Batch test numbers, dates of batch tests, volumes added, COD recoveries, regression data, growth rates, *measured* OHO active biomass concentration present at the start of the batch test ($Z_{BH(0)}$) and in the parent system mixed liquor (ML). The *theoretical* parent system mixed liquor (ML) OHO active biomass concentration and the active biomass concentration present at the start of the batch test are also given.

Sew. Batch No.	Batch test No.	Batch test date	Volume (l)		COD Rec. (%)	Regression			Growth Rate (/d)		ZBH(0) (mgCOD/l)			
			WW	ML		Y-int	Slope	R^2			Measured		Theoretical	
									K_{MP}	μ_H	Batch	ML	Batch	ML
5	A1	11 09	2.70	0.30	92.8	1.542	0.060	0.815	1.18	0.46	108.1	1080.6	172.7	1726.8
7	A2	24 09	2.70	0.30	90.3	2.162	0.080	0.761	1.3	1.30	154.4	1543.6	196.5	1965.1
	A3	29 09	2.80	0.20	104.3	1.714	0.106	0.891	1.51	1.65	81.9	1228	131.0	1965.1
	A4	05 10	2.90	0.10	93.6	1.263	0.215	0.923	1.23	4.55	28.5	854.5	65.5	1965.1
8	A5	12 10	2.70	0.30	96.5	1.987	0.125	0.979	1.93	1.69	93.9	938.6	166.3	1663.3
	A6	19 10	2.80	0.20	100.7	1.854	0.080	0.925	1.18	1.35	117.5	1763	110.9	1663.3
	A7	23 10	2.90	0.10	95.7	0.861	0.235	0.962	1.58	4.67	17.6	528.3	55.4	1663.3
9	A8	26 11	2.70	0.30	93.0	2.482	0.133	0.964	1.76	2.04	146.4	1463.8	159.2	1591.8
	A9	27 11	2.80	0.20	96.5	2.190	0.167	0.928	1.68	2.95	89.8	1346.4	106.1	1591.8
	A10	28 11	2.90	0.10	100.6	1.769	0.218	0.988	1.77	4.08	46.7	1400.2	53.1	1591.8
10	A11	10 12	2.70	0.30	95.8	2.227	0.020	0.379[2]	0.54	0.60	377.6	3776.1	191.2	1912
	A12	11 12	2.80	0.20	104.9	1.733	0.155	0.954	1.65	2.67	60.9	913.1	127.5	1912
	A13	12 12	2.90	0.10	100.8	1.031	0.266	0.993	1.65	5.35	18.7	560.2	63.7	1912
	A14	13 12	2.95	0.05	96.5	0.807	0.266	0.999	1.10	5.91	14.9	893.4	31.9	1912
11	A15	26 12	2.60	0.40	100.2	2.492	0.020	0.581	0.67	0.48	489.4	3670.4	230.4	1728.2
	A16	29 12	2.70	0.30	98.4	1.974	0.080	0.877	1.26	1.21	136.3	1362.5	172.8	1728.2
	A17	30 12	2.80	0.20	93.3	1.381	0.146	0.926	1.51	2.61	45.0	675.2	115.2	1728.2
	A18	31 12	2.90	0.10	90.5	0.459	0.225	0.967	1.86	4.16	12.2	366.9	57.6	1728.2
	A19	05 01	2.95	0.05	85.6	0.878	0.315	0.997	0.90	7.28	13.7	822.1	28.8	1728.2
12	A20	09 01	2.60	0.40	101.8	2.476	0.030	0.594[2]	0.80	0.51	421.6	3161.7	233.4	1750.8
	A21	10 01	2.70	0.30	92.9	2.203	0.040	0.852	0.84	0.84	252.0	2519.8	175.1	1750.8
	A22	11 01	2.80	0.20	93.3	1.538	0.152	0.982	1.90	2.38	50.7	760.5	116.7	1750.8
	A23	14 01	2.90	0.10	92.1	0.759	0.173	0.95	1.58	3.18	20.9	626.4	58.4	1750.8
	A24	15 01	2.95	0.05	83.2	0.306	0.221	0.992	1.65	4.28	10.7	640.4	29.2	1750.8
13	A25	01 02	2.95	0.05	80.2[1]	-0.536	0.252	0.992	0.64	6.03	4.1	245.2	32.9	1973.5
	A26	04 02	2.96	0.04	85.3	0.197	0.189	0.951	1.59	3.56	11.0	825.6	26.3	1973.5
14	A27	15 02	2.40	0.60	96.4	2.688	0***	0***	0.42	0.20	1093.8	5469	265.2	1325.9
	A28	17 02	2.60	0.40	96.6	2.085	0.080	0.985	1.35	1.14	150.8	1131.3	176.8	1325.9
	A29	18 02	2.80	0.20	96.1	0.896	0.178	0.962	1.90	3.00	23.3	349.4	88.4	1325.9
	A30	20 02	2.90	0.10	86.2	0.301	0.275	0.978	1.79	5.42	8.7	261.6	44.2	1325.9
	A31	22 02	2.93	0.07	78.5[1]	0.438	0.261	0.994	0.97	5.91	10.5	449.7	30.9	1325.9
	A32	24 02	2.95	0.05	76.3[1]	1.085	0.172	0.995	0.60	4.14	29.0	1742.8	22.1	1325.9
	A33	25 02	2.97	0.03	74.0[1]	1.362	0.161	0.989	0.62	3.87	40.5	4049.4	13.3	1325.9
17	A34	22 04	2.80	0.20	97.5	0.967	0.128	0.958	1.38	2.31	33.2	497.5	95.2	1427.9
	A35	24 04	2.90	0.10	100.3	-0.837	0.283	0.978	1.14	6.27	2.7	81.6	47.6	1427.9

[1] Rejected on poor COD mass balance.

[2] Rejected on R^2 in fit to ℓnOUR-time plot

*** Approached almost zero due to a very low slope.

Table 3.6: Results for batch tests on a mixture of *flocculated-filtered* wastewater (WW) and mixed liquor (ML) drawn from the 20d sludge age parent system: Batch test numbers, dates of batch tests, volumes added, COD recoveries, regression data, growth rates, *measured* OHO active biomass concentration present at the start of the batch test ($Z_{BH(0)}$) and in the parent system mixed liquor (ML). The *theoretical* parent system mixed liquor (ML) OHO active biomass concentration and the active biomass concentration present at the start of the batch test are also given.

Sew. Batch No.	Batch test No.	Batch test date	Volume(ℓ)		COD Rec. (%)	Regression			Growth Rate (/d)		ZBH(0) (mgCOD/l)			
			WW	ML		Y-int	Slope	R^2			Measured		Theoretical	
									K_{MP}	μ_H	Batch	ML	Batch	ML
5	B1	11 09	2.70	0.30	96.8	1.573	0.027	0.131[2]	0.51	0.77	175.5	1755.2	119.1	1191.2
7	B2	24 09	2.70	0.30	92.9	1.641	0.129	0.910	1.89	1.84	64.6	645.7	131.5	1315
	B3	29 09	2.80	0.20	97.5	1.321	0.141	0.957	1.11	2.89	43.7	654.8	87.7	1315
	B4	05 10	2.90	0.10	91.3	0.916	0.217	0.965	0.56	5.26	20.0	599.9	44.3	1329.1
8	B5	12 10	2.60	0.40	93.7	2.093	0.108	0.972	1.83	1.38	117.7	882.9	151.8	1138.5
	B6	19 10	2.70	0.30	93.9	1.797	0.089	0.910	1.65	1.11	101.8	1018	113.9	1138.5
	B7	23 10	2.80	0.20	91.7	1.489	0.182	0.971	1.80	3.18	41.4	621.2	75.9	1138.5
9	B8	26 11	2.60	0.40	93.8	2.437	0.112	0.979	1.58	1.72	161.6	1211.8	158.8	1190.8
	B9	27 11	2.70	0.30	96.7	2.112	0.172	0.996	1.77	2.99	81.0	810	119.1	1190.8
	B10	28 11	2.80	0.20	97.3	1.970	0.079	0.654[2]	0.93	1.59	132.7	1991	79.4	1190.8
10	B11	10 12	2.60	0.40	100.2	2.379	0.024	0.674[2]	0.74	0.44	424.5	3183.6	167.4	1255.5
	B12	11 12	2.70	0.30	105.1	2.099	0.104	0.960	1.44	1.68	121.6	1216.2	125.6	1255.5
	B13	12 12	2.80	0.20	95.6	1.647	0.174	0.992	1.62	3.18	50.3	755	83.7	1255.5
	B14	13 12	2.90	0.10	93.6	1.136	0.217	0.996	1.45	4.38	24.9	746.3	41.9	1255.5
11	B15	26 12	2.50	0.50	97.9	2.209	0.043	0.860	0.92	0.72	258.4	1550.2	216.1	1296.6
	B16	29 12	2.60	0.40	96.9	1.948	0.128	0.968	1.83	1.85	88.7	664.9	172.9	1296.6
	B17	30 12	2.70	0.30	93.2	1.530	0.148	0.957	1.92	2.26	51.4	514.5	129.7	1296.6
	B18	31 12	2.80	0.20	102.1	0.962	0.178	0.960	1.86	3.02	25.0	374.5	86.4	1296.6
	B19	05 01	2.90	0.10	90.6	1.043	0.282	0.995	1.55	5.82	17.9	537.5	43.2	1296.6
12	B20	09 01	2.50	0.50	90.6	2.268	0.050	0.941	1.22	0.60	247.7	1486	222.6	1335.8
	B21	10 01	2.60	0.40	90.8	2.196	0.045	0.867	1.04	0.66	247.5	1856.6	178.1	1335.8
	B22	11 01	2.70	0.30	95.9	1.637	0.113	0.971	1.80	1.54	71.8	717.7	133.6	1335.8
	B23	14 01	2.80	0.20	94.8	1.252	0.114	0.916	1.37	1.99	48.5	727	89.1	1335.8
	B24	15 01	2.90	0.10	86.6	0.545	0.213	0.967	1.61	4.13	14.0	420.4	44.5	1335.8
13	B25	01 02	2.93	0.07	85.4	-0.752	0.276	0.976	0.67	6.57	3.0	129.9	32.5	1394.3
	B26	04 02	2.95	0.05	80.2[1]	0.027	0.209	0.962	1.58	4.05	8.5	509.8	23.2	1394.3
14	B27	15 02	2.34	0.66	95.4	2.247	0.083	0.984	1.44	1.16	169.2	769.2	216.2	982.7
	B28	17 02	2.50	0.50	95.2	1.945	0.064	0.941	0.92	1.24	150.7	904.5	163.8	982.7
	B29	18 02	2.70	0.30	96.3	1.000	0.157	0.950	1.69	2.71	28.8	288	98.3	982.7
	B30	20 02	2.80	0.20	89.7	0.591	0.238	0.970	1.84	4.51	13.3	198.9	65.5	982.7
	B31	22 02	2.90	0.10	87.9	-0.320	0.307	0.994	2.06	5.92	4.2	127.2	32.8	982.7
	B32	24 02	2.93	0.07	75.9[1]	0.115	0.265	0.970	0.99	5.98	7.5	321.3	22.9	982.7
	B33	25 02	2.95	0.05	72.5[1]	0.929	0.237	0.990	0.81	5.49	18.7	1122.7	16.4	982.7
17	B34	22 04	2.70	0.30	101.0	1.165	0.118	0.945	1.34	2.13	43.1	431.4	104.4	1044.4
	B35	24 04	2.80	0.20	102.8	0.167	0.200	0.953	1.34	4.07	10.2	152.5	69.6	1044.4

[1] Rejected on COD mass balance

[2] Rejected on R^2 in fit to ℓnOUR-time plot

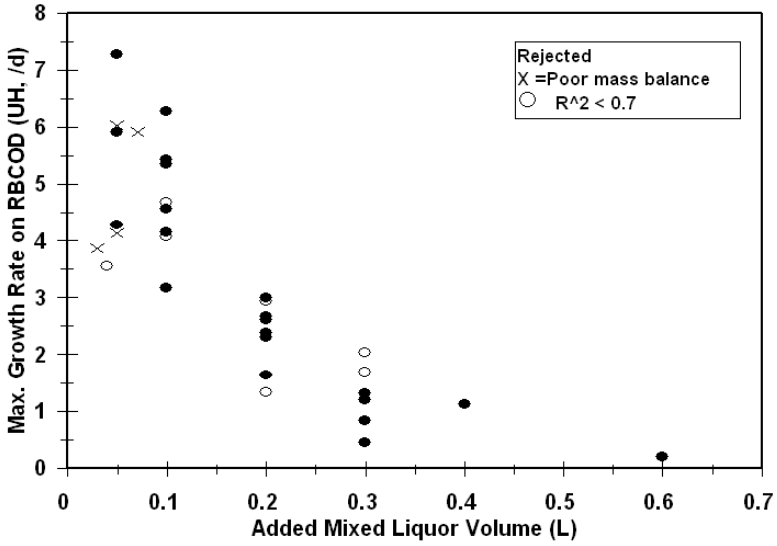

Figure 3.5 (a): OHO maximum specific growth rate on RBCOD (μ_H; /d) versus mixed liquor volume added to batch reactor (V_{ML}; ℓ) for modified batch tests on mixed liquor drawn from the parent 10 day sludge age MLE activated sludge system.

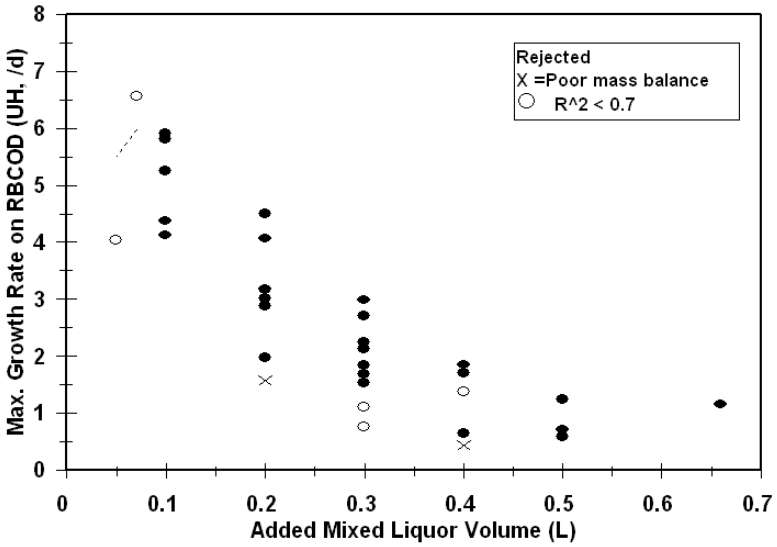

Figure 3.5 (b): OHO maximum specific growth rate on RBCOD (μ_H; /d) versus mixed liquor volume added to batch reactor (V_{ML}; ℓ) for modified batch tests on mixed liquor drawn from the parent 20 day sludge age MLE activated sludge system.

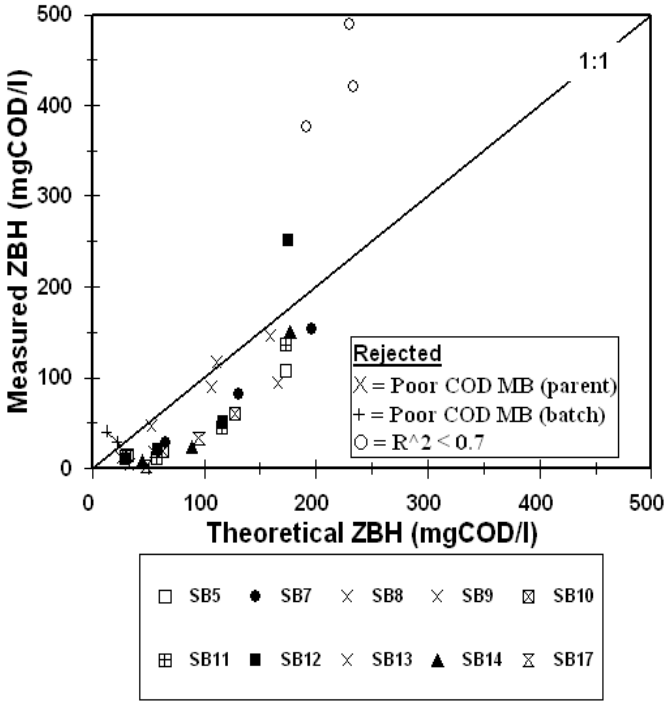

Figure 3.6 (a): Modified batch tests results; measured versus theoretical OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] for the various sewage batches (SB) for the 10 day sludge age parent system.

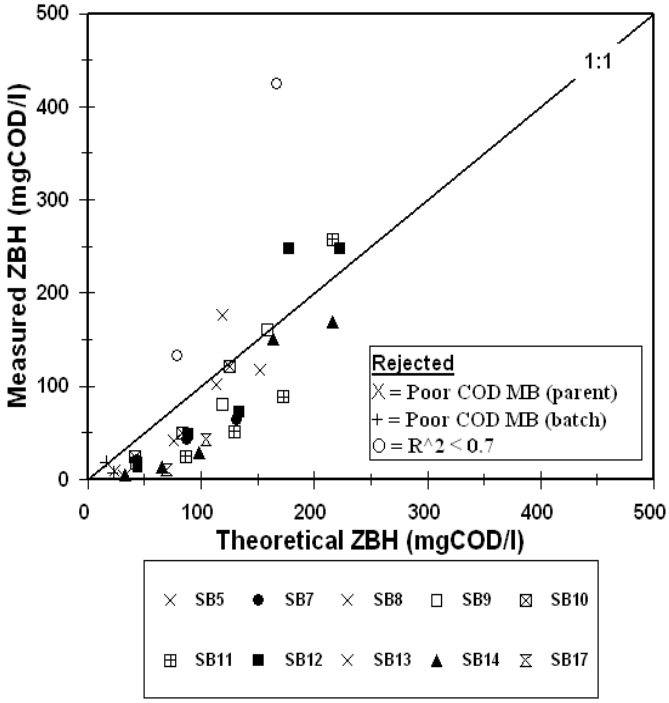

Figure 3.6 (b): Modified batch tests results; measured versus theoretical OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] for the various sewage batches (SB) for the 20 day sludge age parent system.

- In comparing the measured OHO active biomass concentrations at the start of the batch test ($Z_{BH(0)}$) with the corresponding theoretical values, the data for both the 10 and 20 day sludge age mixed liquor showed very similar trends, see Figs. 3.6 (a and b). Also, superficially both comparisons bear a strong resemblance to the data of Beeharry *et al.* (2001) (Fig. 2.4), namely that there is a correspondence, but the values plot parallel to the 1:1 correspondence line. However, if the data is examined more closely, for both the 10 and 20 day mixed liquors three regions could be identified:

  – Theoretical $Z_{BH(0)}$ < ~ 30 mgCOD/ℓ : As the theoretical $Z_{BH(0)}$ increases, the measured values decrease, to approach near zero. However, data in this region exhibit poor COD mass balances. Beeharry *et al.* (2001) did not collect data in this region, so a comparison with their data is not possible.

  – 30 mgCOD/ℓ < Theoretical $Z_{BH(0)}$ < ~ 150 mgCOD/ℓ : As the theoretical $Z_{BH(0)}$ increases, the measured values correspondingly increase virtually parallel to the 1:1 correspondence line, but below it. This trend is near identical to that observed by Beeharry *et al.* (2001), whose data falls primarily in this region.

  – Theoretical $Z_{BH(0)}$ > ~ 150mgCOD/ℓ : As the theoretical $Z_{BH(0)}$ increases, the measured values increase sharply to cross the 1:1 correspondence line. Beeharry *et al.* (2001) collected limited data in this region, but the data available indicates it is consistent with the observation here. Some data in this region exhibit poor R^2 values in the fit to the ℓnOUR-time plot, due to the low slope.

  The similarity in correspondence for the 10 and 20 day sludge age mixed liquors, and to the investigation of Beeharry *et al.* (2001) would suggest that the differences between measured and theoretical OHO active biomass concentrations were not caused by the sludge age itself, but by some other factor(s).

  The cause for the differences was not possible P limitation in the batch test due to pre-flocculation of the sewage with alum, suggested as a possibility by Beeharry *et al.* (2001) (see Chapter 2) and Cronje *et al.* (2002b) - parallel modified batch tests with and without P supplementation gave near identical results. This prompted a more detailed investigation into the modified batch test. It first needed to be established whether the cause for the differences observed above lay in the activated sludge theory (*i.e.* theoretical) or in the modified batch test procedure itself (*i.e.* the measured).

3.5 BATCH AEROBIC DIGESTION TEST

To establish whether the causes for the differences between theoretical and measured OHO active biomass concentrations noted above lay in the activated sludge theory or in the modified batch test procedure, the alternative aerobic batch digestion method of Marais and Ekama (1976) was applied to quantify the OHO active biomass concentration of mixed liquor drawn from both the 10 and 20 day sludge age parent activated sludges systems, see Lee *et al.* (2003).

1.56ℓ and 1.32ℓ of mixed liquor were drawn from the aerobic reactor of the 10 and 20 day sludge age parent MLE systems respectively; for appropriate parent system steady state data, see sewage batch Nos. 15 and 16 in Tables 3.3 and 3.4 respectively. The mixed liquor drawn from the parent system was placed into aerobic batch reactors maintained at 20°C and batch aerobically digested for ~ 10 days. The OUR response in the batch test was measured using an automated technique (Randall *et al.*, 1991), for example see Figs. 3.7 (a and b).

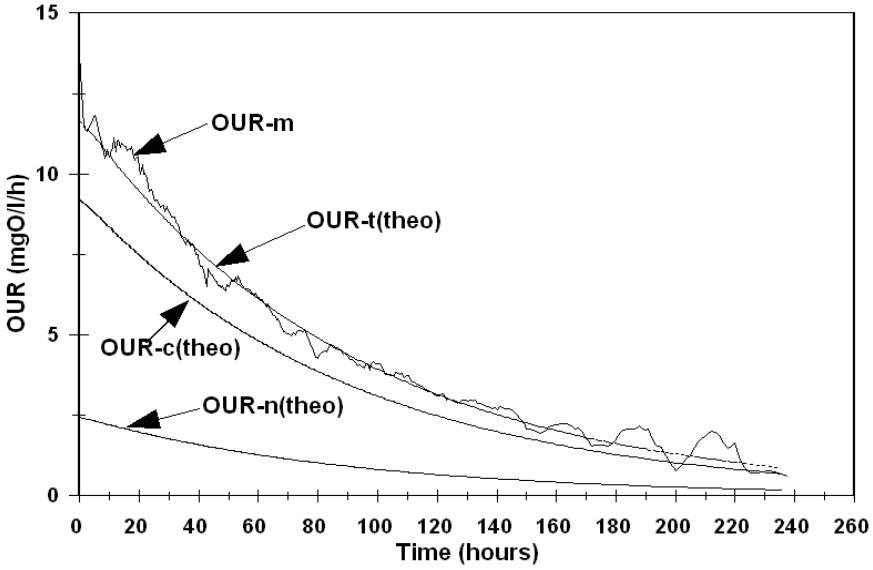

Figure 3.7(a): Measured Oxygen Utilization Rate (OUR-m) and predicted nitrification (OUR-n(theo), carbonaceous (OUR-c(theo)) and total (OUR-t(theo)) oxygen utilisation rate (OUR) responses with time for batch aerobic digestion test on mixed liquor (1.32ℓ) drawn from the aerobic reactor of the 20 day sludge age system. Batch Aerobic Digestion Test No. D2, Sewage Batch No. 16.

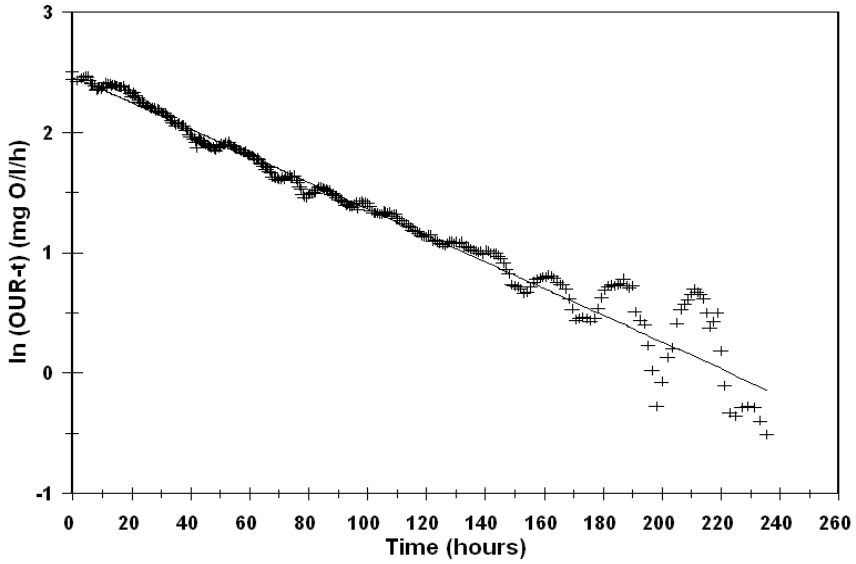

Figure 3.7(b): ℓn [total measured Oxygen Utilization Rate (OUR-t)] versus time for the OUR data in Fig. 3.7(a); Batch Aerobic Digestion Test No. D2, Sewage Batch No. 16.

At the beginning of the test and at the same time on each of the following days, (i) pH was measured and maintained > 7 to ensure complete nitrification and (ii) samples were drawn from the batch reactor, immediately filtered (0.45 μm), 2 – 3 drops of $HgCl_2$ were added to the filtrate which was stored for subsequent nitrate, nitrite and Free and Saline Ammonia (FSA) analysis. For all the batch aerobic digestion tests, the pH was sufficiently high to ensure complete nitrification, indicated by the FSA at constant a low concentrations, *i.e.* FSA did not accumulate during the test, for example see Fig. 3.8.

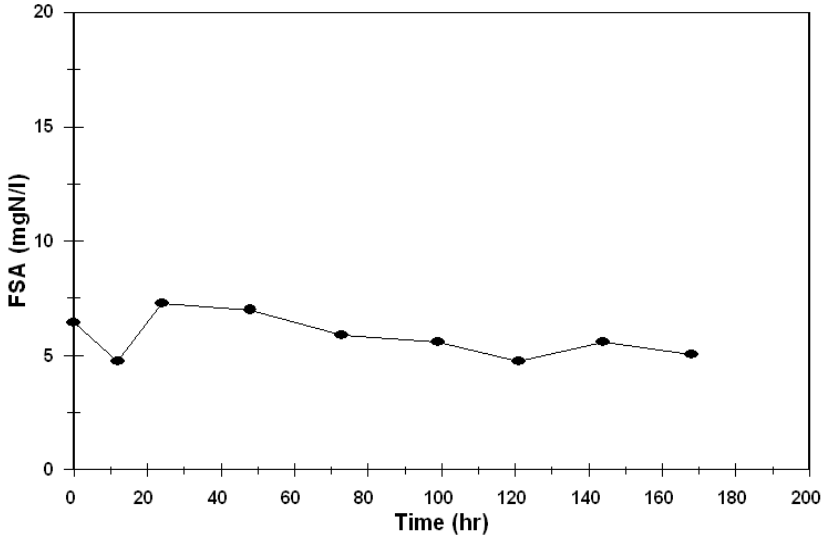

Figure 3.8: Meaured Free and Saline Ammonia (FSA) concentrations versus time for batch aerobic digestion test on mixed liquor from the aerobic reactor of the 20d sludge age system. Batch Aerobic Digestion Test No. D2, Sewage Batch No. 16.

The batch aerobic digestion test data were analyzed and interpreted using the procedure based on Marais and Ekama (1976). From the batch test data the following were calculated.

- Specific endogenous mass loss rate (b_{H20T}) at temperature 20 ^{o}C (/d), from the slope of the ℓn(OUR)-time graph (Fig. 3.7b).

- Initial OHO active biomass concentration ($X_{BH(0)}$) (mgVSS/ℓ), from the y-intercept of the ℓn(OUR)-time graph (Fig. 3.7b).

- Theoretical Nitrate concentrations ($NO_{3(t)}$) with time, for example see Fig. 3.9.

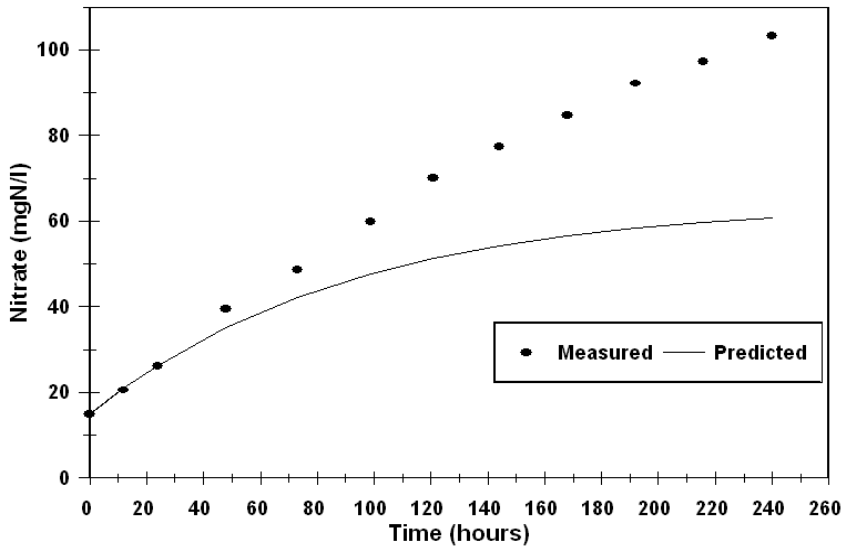

Figure 3.9: Measured and theoretically calculated Nitrate (NO_3) concentrations versus time. Batch Aerobic Digestion Test No. D2, Sewage Batch No. 16.

In total, 2 batch aerobic digestion tests were conducted on mixed liquor drawn from the 10 day sludge age parent system, and 2 on that drawn from the 20 day sludge age parent system. From the batch aerobic digestion tests, the following conclusion were drawn:

- Filtered samples taken at regular intervals during the test showed FSA concentrations were constant, at low values, for example see Fig. 3.8. This implies nitrification was complete, a pre-requisite for analysis of the data.

- Nitrate concentrations with time in the aerobic batch digestion tests were predicted with activated sludge theory. The measured nitrate concentrations were consistently higher than the predicted concentrations during the entire test, for example see Fig. 3.9. No definitive explanation for this inconsistency could be proposed, and this warrants further investigation.

- The high linear regression correlation coefficients ($R^2 > 0.97$) in the fit to the ℓn OUR_t - time plots lends credibility to the experimental data, see Table 3.7.

- For the OHO specific endogenous respiration rate (b_{H20}) values, for the 20 days sludge age system mixed liquor these were 0.22/d and 0.26/d; for the 10 day sludge age system mixed liquor these were 0.31/d and 0.33/d, see Table 3.7. The values for the 20d sludge age system mixed liquor correspond closely to the default value of 0.24/d (Marais and Ekama, 1976; WRC, 1984), but the 10 day sludge age system values are significantly higher. This would imply that sludge age influences b_{H20}, which is contrary to current activated sludge theory. This warrants further investigation.

- A remarkably close correspondence between the theoretical OHO active biomass concentration and that measured in the aerobic batch digestion test was obtained, see Table 3.7 and Fig. 3.10. The differences between measured and predicted values were 1.4% and 8.2% for the 10 and 20 days sludge age parent systems respectively. This close correlation provides substantive support for the OHO active biomass concept incorporated in activated sludge models.

Table 3.7: Results for batch aerobic digestion tests: Sewage (Sew.) batch number, Batch test numbers, dates of batch tests, regression data, measured specific endogenous respiration rate (b_{H20}), *measured* OHO active biomass concentration present the start of the batch test (X_{BHi}) and the *theoretical* parent system mixed liquor (ML) OHO active biomass concentration.

System	Sew. Batch No.	Batch test No.	Date of Test	Regression data			b_{H20} (/d)	X_{BHi} (mgVSS/ℓ)	
				Y-int	Slope	R^2		Meas.	Theo.
10days	15	C1	27 03	2.9029	-0.0129	0.971	0.31	996	993
	16	C2	08 04	2.9768	-0.0137	0.987	0.33	1008	983
20days	15	D1	27 03	2.3453	-0.009	0.979	0.22	834	758
	16	D2	08 04	2.4741	-0.011	0.987	0.26	774	718

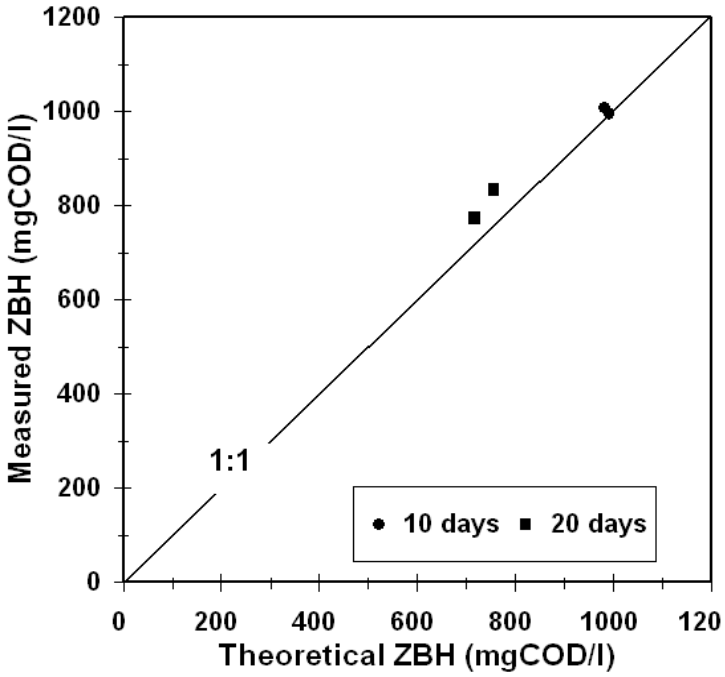

Figure 3.10: Batch aerobic digestion test measured versus theoretical OHO active biomass concentrations (Z_{BH}) for mixed liquor drawn from the 10 and 20 day sludge age activated sludge systems.

In the modified batch tests, which are based on OHO active biomass growth processes, the differences between the measured and theoretical values were large (Fig. 3.6). In contrast, in the batch aerobic digestion tests, which are based on endogenous respiration/"death" processes, the correlation is close. Thus, it appears that the cause for the differences between the modified batch test measured and the theoretical concentrations lies in the description and interpretation of the OHO growth processes within the modified batch test itself. Accordingly, this was examined more closely (Lee *et al.*, 2003).

3.6 DEVELOPMENT OF COMPETITION BASED KINETIC MODELS

3.6.1 Evaluation of OHO behaviour in the modified batch tests

A more detailed investigation into OHO behaviour within the modified batch test was conducted through a detailed analysis of the data collected in the modified batch tests above . In particular, (i) the effect of the ratio substrate concentration to OHO active biomass concentration ($S_0/Z_{BH(0)}$ with active biomass in COD units, or S_0/X_0 in VSS units) and (ii) the validity of the analytical procedures were examined. From this investigation the following general conclusions can be drawn:

- For all observations, the remarkable similarity in the data for modified batch tests on both the 10 and 20 day sludge age mixed liquors substantiates the consistency of the data and the observations made. It also excludes sludge age as the underlying causes for any deviations.

- The OHO maximum specific growth rate on RBCOD (μ_H) appears to be a function of the OHO active biomass concentration added at the start of the test ($Z_{BH(0)}$); as $Z_{BH(0)}$ decreases, μ_H increases, see Fig. 3.11. The OHO maximum specific growth rates on SBCOD (K_{MP}) also exhibit some variation, also apparently linked to the OHO biomass concentration added, but this is not as marked, see Fig. 3.12. Re-examination of the data of Beeharry *et al.* (2001) indicated that they obtained, but did not note, similar trends.

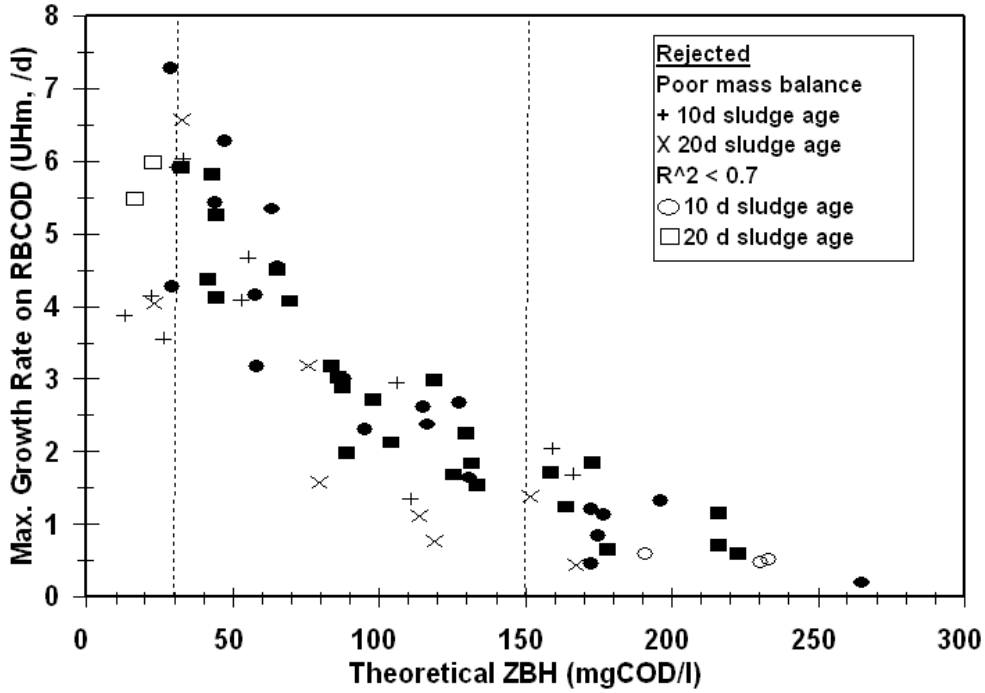

Figure 3.11: Modified batch test determined OHO maximum specific growth rate on readily biodegradable COD (μ_H) versus the theoretical OHO active biomass concentration at the start of the batch test, $Z_{BH(0)}$.

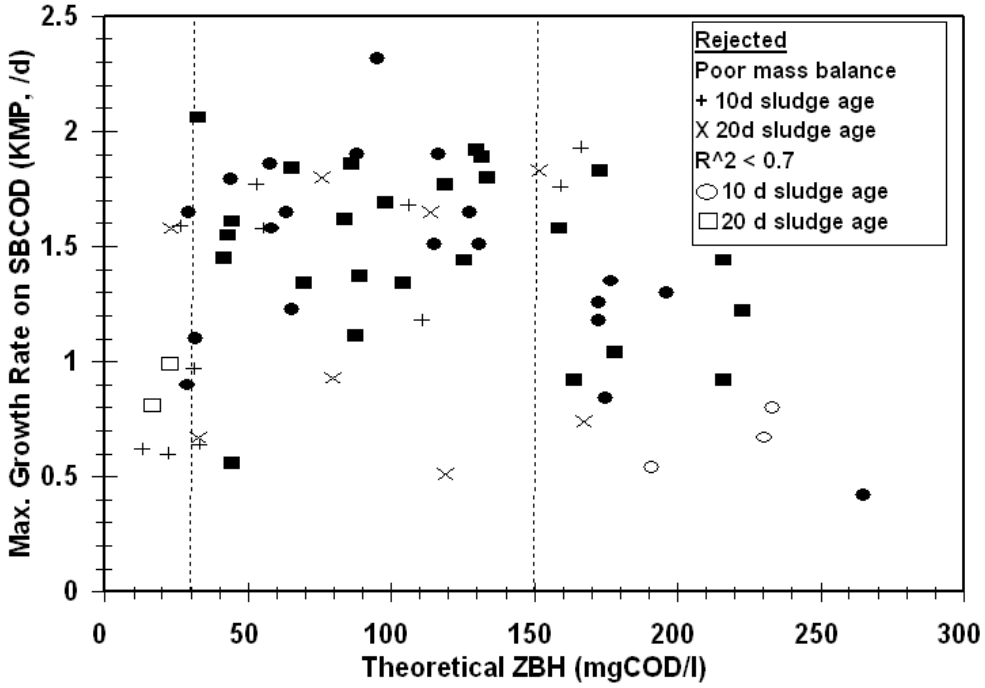

Figure 3.12: Modified batch test determined OHO maximum specific growth rate on slowly biodegradable COD (K_{MP}) versus the theoretical OHO active biomass concentration at the start of the batch test, $Z_{BH(0)}$.

- Similar, though reversed, trends noted above for μ_H and K_{MP} were evident for the effect of initial substrate to OHO active biomass concentration, *i.e.* S_0/Z_{BH} ratio, see Figs. 3.13 (a and b). The influence of S_0/Z_{BH} on batch test behaviour and derived kinetics has been noted previously (e.g. Chudoba *et al.*, 1992; Novak *et al.*, 1994; Grady *et al.*, 1996). With the single OHO population model incorporated in the batch test analytical procedure, it would be expected that μ_{Hm} should remain constant

- In a plot of $\ell n\ OUR_H$ versus time, the measured data deviated from the fitted linear regression line, showing upward curvature, see Fig. 3.14. This strongly suggests that the net OHO maximum specific growth rates ($\mu_{Hm} + K_{MP} - b_H$) change during the course of the batch test, in agreement with the observations of Pollard *et al.* (1998). This is not accommodated in the simplified UCT model used to develop the analytical procedure for the batch test.

- The observed precipitous drop in OUR implies that the OHO specific growth rates were at their maxima, *i.e.* the changes noted above were not directly due to variations in growth rates due to varying substrate concentrations with time or between batch tests.

- The higher OHO μ_{Hm} values were significantly higher than (up to about 7 /d, Fig. 3.11), and outside the range of, values accepted as the default for the UCT kinetic model (1.5 to 3.5 /d, Dold *et al.*, 1991).

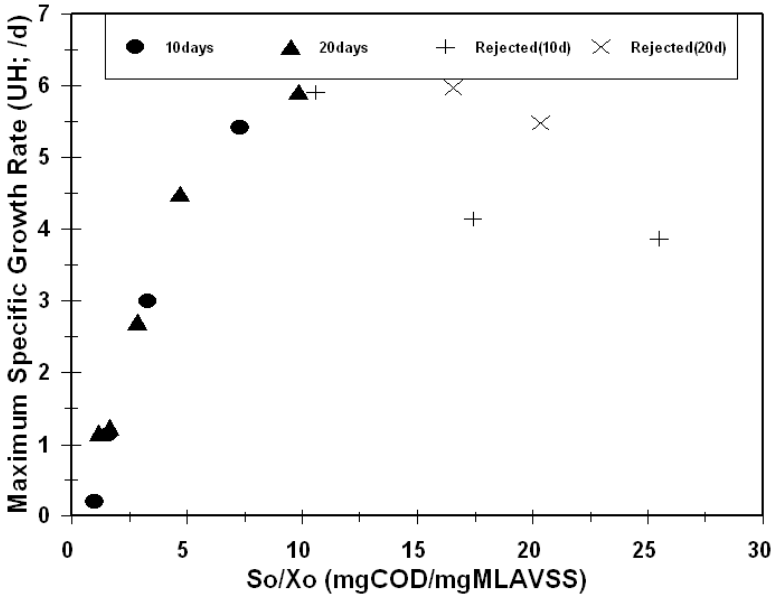

Figure 3.13(a): OHO maximum specific growth rate on readily biodegradable COD (μ_H) versus S_0/X_0 ratio (mgCOD/mgMLAVSS) for the set of the modified batch tests conducted during Sewage Batch No. 14.

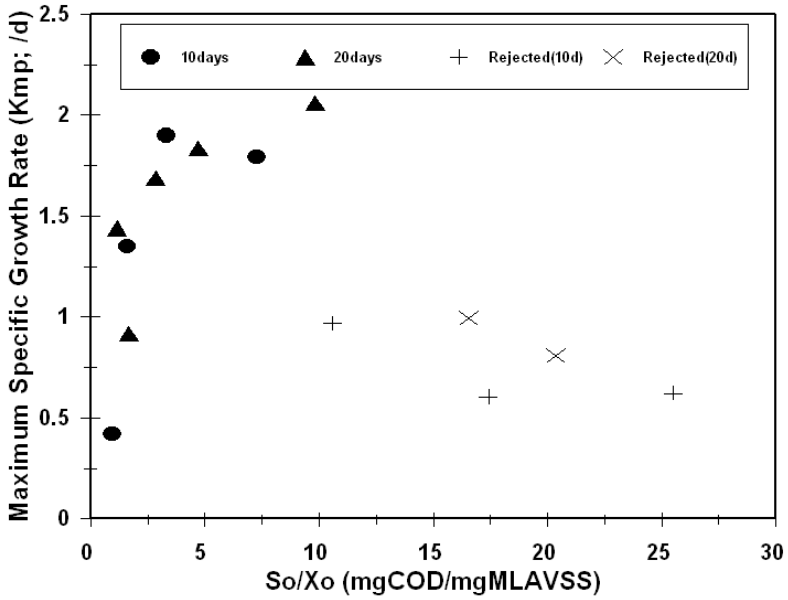

Figure 3.13(b): OHO maximum specific growth rate on slowly biodegradable COD (K_{MP}) versus S_0/X_0 ratio (mgCOD/mgMLAVSS) for the set of the modified batch tests conducted during Sewage Batch No. 14.

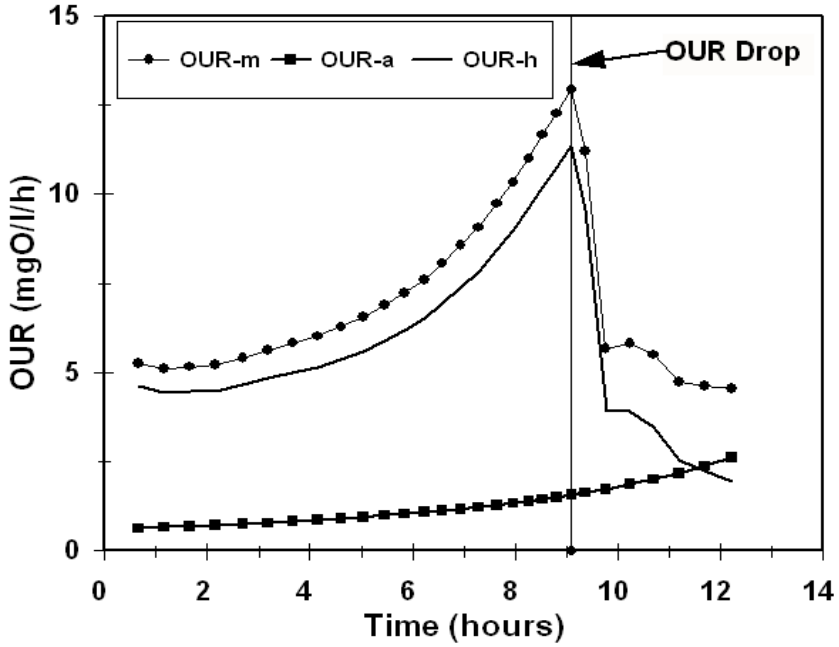

Figure 3.14(a): OUR (measured, m; OHO, h; and AO, a) versus time profile for a representative modified batch test. Modified Batch Test No. B23, Sewage Batch Test No. 12.

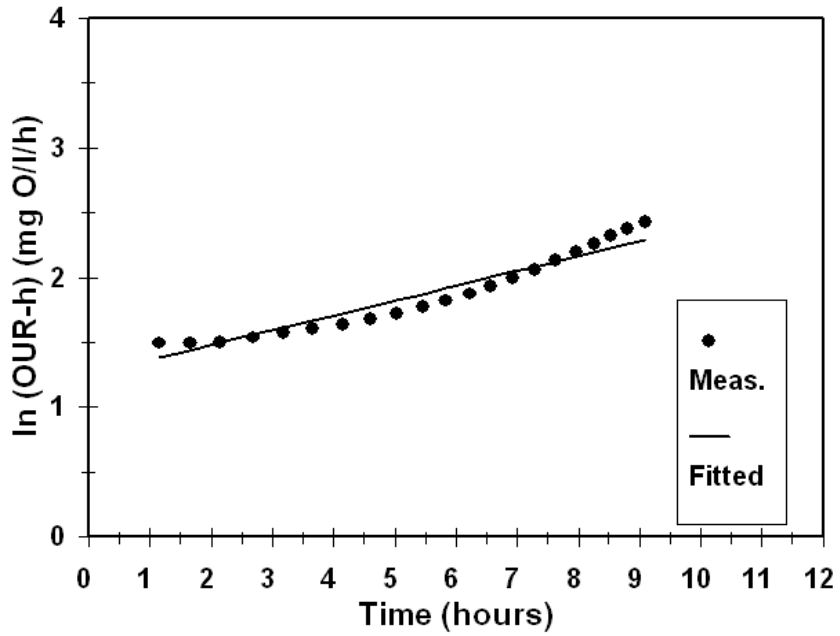

Figure 3.14(b): $\ell n(OUR_H)$ versus time profile for modified batch test in Fig. 3.14 (a). Modified Batch Test No. B23, Sewage Batch Test No. 12.

- The batch test measured OHO active biomass concentrations [$Z_{BH(0)}$] were consistently lower than the corresponding theoretical values (Fig. 3.15). One possible explanation is that the OHO maximum specific growth rates increase with time, as noted above: In the batch test procedure, a single constant OHO maximum specific growth is determined and applied to the start of the test to derive a value for $Z_{BH(0)}$. Thus, if the OHO maximum specific growth rate increases with time, then the value at the start of the test is overestimated and hence $Z_{BH(0)}$ would be underestimated.

The observations above suggest that the OHO maximum specific growth rates increase with time in the batch test. This behaviour cannot be accommodated in the analytical procedure for the batch test which is based on a single OHO population with fixed kinetics.

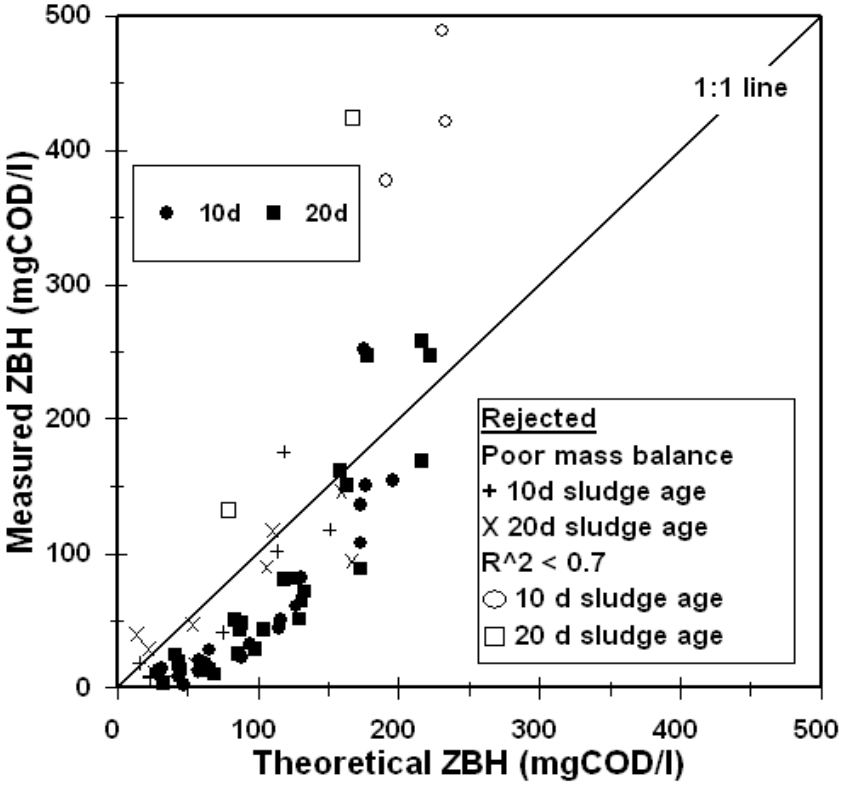

Figure 3.15: Measured versus theoretical OHO active biomass concentrations ($Z_{BH(0)}$) for mixed liquors from parent systems at both 10 and 20d sludge ages.

3.6.2 Development and application of competition based kinetic model

To explain observations similar to those above, Novak *et al.* (1994) and Grady *et al.* (1996) proposed substrate competition between different OHO groups as a possible cause. This possibility was investigated, by developing a kinetic model for competition between two OHO populations, one a fast grower and the other a slow grower, based on the concepts of Novak *et al.* (1994). The second OHO group was incorporated into the simplified UCT kinetic model used to analyse the batch test data (Wentzel *et al.*, 1995), as follows (for details, see Lee *et al.* 2003): (1) The single OHO active biomass was subdivided into two OHO active biomasses, a fast grower (Z_{BH1}) and a slow grower (Z_{BH2}); (2) All OHO mediated processes were duplicated, with the new processes allocated to the second OHO group; (3) The adsorbed SBCOD was split into two, S_{ads1} and S_{ads2}, utilized by Z_{BH1} and Z_{BH2} respectively.

The kinetic model was applied to both the (i) batch tests and (ii) parent systems, using AQUASIM 2.0 (Reichert, 1994). For application to the batch tests, the batch test data with the greatest surety were selected, $30 < Z_{BH(0)} < 150$ mgCOD/ℓ and those with good mass balances (Fig. 3.15). Values for all constants except those related to OHO growth on RBCOD were those of Dold *et al.* (1991). With regard to μ_{Hm} and K_{SH} on RBCOD of Z_{BH1} and Z_{BH2}, these were assumed as 12/d and 3 mgCOD/ℓ and 2/d and 0.1 mgCOD/ℓ respectively, to ensure competition on RBCOD and that the precipitous drop in OUR could be correctly predicted. Parameters estimated with AQUASIM were initial concentrations of $Z_{BH1(0)}$ and $Z_{BH2(0)}$, and "substrate". To reduce the complexity of parameter estimation, the

initial substrate was ascribed to RBCOD only. This restricted the parameter estimation to the period up to the OUR precipitous drop. From the application to the batch tests:

- The model could accurately simulate the OUR_H - time observed in batch tests, see Fig. 3.16, and Lee *et al.*, 2003).

- Parameter estimation of the initial batch test $Z_{BH1(0)}$ and $Z_{BH2(0)}$ concentrations gave exceptionally low $Z_{BH1(0)}/(Z_{BH1(0)}+Z_{BH2(0)})$ values, average 1%, see Table 3.8. However, with time Z_{BH1} increased its proportion significantly and had a marked influence on the predicted OUR_H - time profile (Fig. 3.16). This indicates that the batch test procedure is extremely sensitive to the presence of fast growing OHOs.

- The model could simulate the variety of observations made on the batch tests, including the increase in overall OHO maximum specific growth rates with increasing S_0/Z_{BH} and with time.

- In seeking a source for the fast growing OHOs in the batch test, it was noted that this could not the flocculated filtered WW added to the batch tests (no observable OUR after 12 hours aeration), and so must be from the ML drawn from the parent systems.

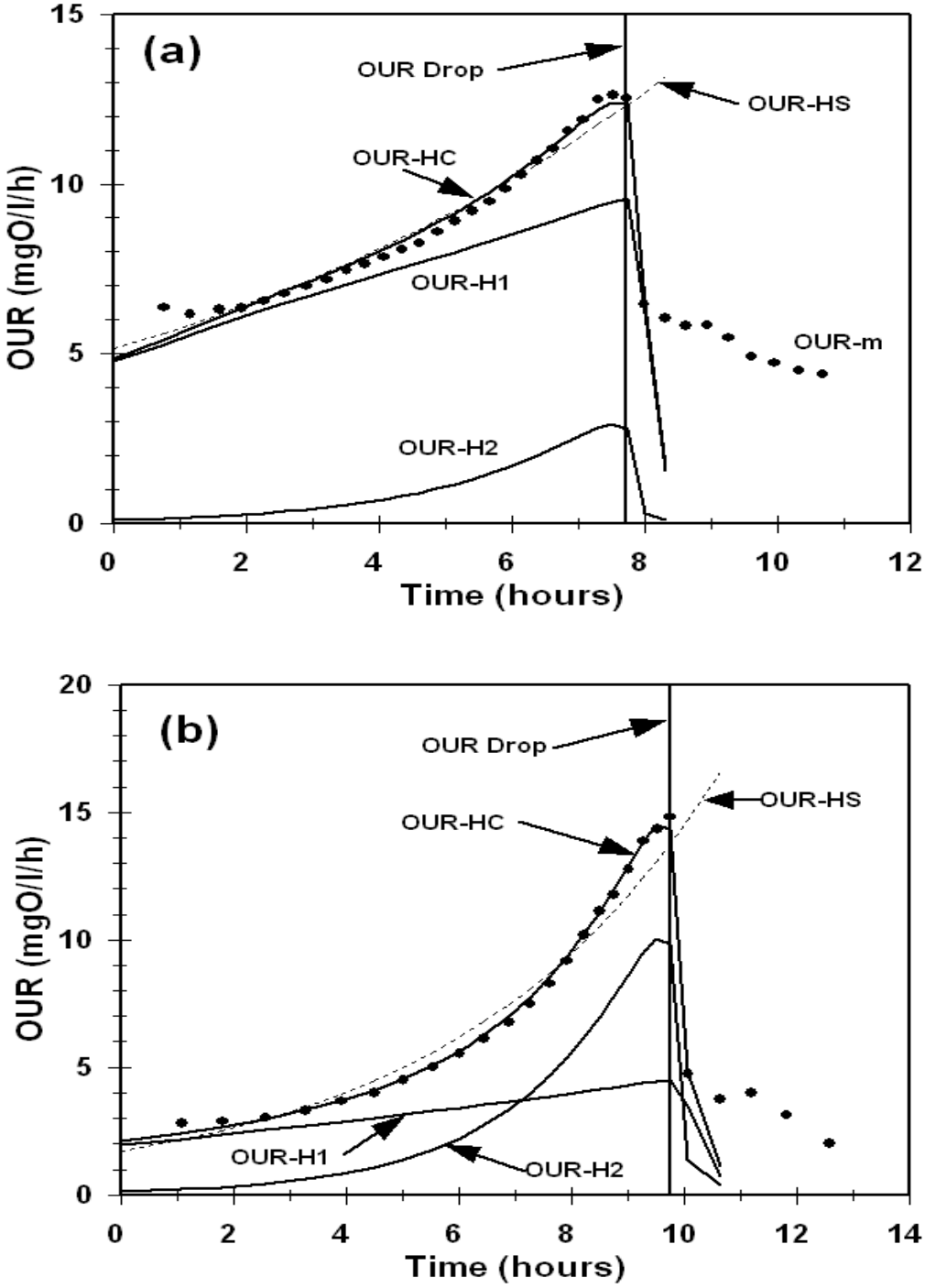

Figure 3.16: Measured Oxygen Uptake Rate of procedure of Wentzel *et al.* (1995) of kinetic selection (OUR-HC), slow grower (OUR-H2) at different $Z_{BH(0)}$(Theo.) = 133.6 and (b) Modified batch tests on mixed system, Sewage Batch No.12, Batch OHOs (OUR-m), OUR calculated by the analytical (OUR-HS), OUR simulated by the competition model OUR of the fast grower (OUR-H1) and OUR of the S_0/X_0 conditions; (a) represents low S_0/X_0 condition, represents high So/Xo condition, $Z_{BH(0)}$(Theo.) = 44.5. liquor drawn from the OHOs 20 days sludge age Test Nos. B22 and B24 for (a) and (b) respectively.

Table 3.8: Results for the parameter estimation with the aid of AQUASIM 2.0 computer program for selected modified batch tests, see Tables 3.5 and 3.6. Also shown are the total OHO active biomass concentrations at the start of the batch test, ($Z_{BH(0)}$) predicted by competition model, from analysis using the procedure developed by Wentzel *et al.* (1995) and described in Chapter 2 (UCT model), and predicted by application of the steady state model (WRC, 1984) to the parent system, taking due account of dilution.

Sew. Batch No.	Batch test No.	Batch test date	Volume (ℓ)		Parameter Estimation				$Z_{BH(0)}$ (mgCOD/ℓ)		
			WW	ML	S_{bi}	$Z_{BH1(0)}$	$Z_{BH2(0)}$	χ^2	Competition Model	UCT Model	Steady-state Model
					mgCOD/ℓ						
10	A12	11 12	2.80	0.20	150.84	1.77	119.97	16.39	121.74	60.9	127.5
	A13	12 12	2.90	0.1	111.69	3.09	53.89	1.93	56.98	18.7	63.7
	A14	13 12	2.95	0.1	70.91	3.20	36.97	0.05	40.17	14.9	31.9
11	A17	30 12	2.80	0.2	137.95	0.94	85.47	3.43	86.41	45	115.2
	A18	31 12	2.9	0.1	135.56	0.70	40.39	5.86	41.09	12.2	57.6
12	A22	11 01	2.8	0.2	193.02	0.81	110.38	46.85	111.19	50.7	116.7
	A23	14 01	2.9	0.10	172.44	0.31	57.69	4.65	58	20.9	58.4
14	A29	18 02	2.80	0.20	184.53	0.46	63.71	15.18	64.17	23.3	88.4
	A30	20 02	2.9	0.1	182.07	0.84	41.95	19.57	42.79	8.7	44.2
17	A34	22 04	2.80	0.20	126.64	0.36	58.91	4.88	59.27	33.2	95.2
	A35	24 04	2.9	0.1	121.40	0.23	19.52	1.24	19.75	2.7	47.6
10	B13	12 12	2.80	0.2	109.96	2.43	110.06	5.86	112.49	50.3	83.7
	B14	13 12	2.90	0.10	72.28	2.95	58.30	0.09	61.25	24.9	41.9
11	B17	30 12	2.7	0.3	141.44	1.23	98.25	2.67	99.48	51.4	129.7
	B18	31 12	2.80	0.2	137.33	0.74	61.50	9.97	62.24	25	86.4
	B19	05 01	2.90	0.10	89.29	4.55	42.79	3.26	47.34	17.9	43.2
12	B22	11 01	2.70	0.3	175.02	0.41	114.31	21.20	114.72	133.6	133.6
	B23	14 01	2.80	0.20	162.99	0.22	77.55	6.28	77.77	48.5	89.1
	B24	15 01	2.90	0.10	166.27	0.52	47.30	7.66	47.82	14	44.5
14	B29	18 02	2.70	0.30	171.32	0.40	65.50	12.07	65.9	28.8	98.3
	B30	20 02	2.80	0.20	198.91	0.71	51.79	1.87	52.5	65.5	65.5
17	B34	22 04	2.70	0.30	121.04	0.33	71.39	5.59	71.72	43.1	104.4
	B35	24 04	2.80	0.20	117.18	0.32	30.71	3.22	31.03	10.2	69.6

Note) A : 10 days sludge age system, B : 20 days sludge age system

The competition kinetic model was also applied to simulate the two parent systems, with the same set of kinetic and stoichiometric constants used for the batch tests. Z_{BH1} was accepted to be seeded with the influent WW to the parent systems, at 0% - 3% of total COD:

- Z_{BH1} could only be sustained in the parent systems if seeded with the influent. Seeding was substantiated by the observations of Wentzel *et al.* (1995) and Cronje *et al.* (2002) who noted significant fast growing OHOs present in the same raw wastewater as used in this investigation.

- With seeding of Z_{BH1} at concentrations typically measured by Wentzel *et al.* (1995) (< 3% of total COD), the predicted Z_{BH1} proportion of the total OHO active biomass (± 40% at seed of 3% of total COD) was significantly larger than that derived from parameter estimation of the batch test data (1%), see Table 3.9 and Figs. 3.17 (a and b).

Table 3.9: Model inputs and results from simulations of the 10 and 20 days sludge age steady state activated systems with the competition model of kinetic selection.

No. of Simulation	% of $Z_{BH1(Inf)}$ in Inf. CODt	Input data			Simulation Results - 10days system				Simulation Results -20days system			
		mgCOD/ℓ			mgCOD/ℓ			%	mgCOD/ℓ			%
		Z_{BH1}	S_{bs}	S_{bp}	Z_{BH1}	Z_{BH2}	Z_E	Z_{BH1}/Z_{BH2}	Z_{BH1}	Z_{BH2}	Z_E	Z_{BH1}/Z_{BH2}
1	0	0	120	480	0.1	1992	988	0	0.2	1383	1366	0
2	0.2	1.5	120	479	46.3	1950	990	2.4	32	1354	1369	2.4
3	0.4	3	120	478	91	1908	992	4.8	62.8	1326	1371	4.7
4	0.6	4.5	120	476	134.3	1896	993	7.2	92.7	1298	1373	7.1
5	0.8	6	120	475	176.3	1830	995	9.6	121.7	1272	1376	9.6
6	1	7.5	120	474	217	1793	997	12.1	149.9	1246	1378	12
7	1.2	9	120	473	256.5	1757	999	14.6	177.2	1221	1381	14.5
8	1.4	10.5	120	472	294.9	1722	1000	17.1	203.7	1197	1383	17
9	1.6	12	120	470	332.2	1688	1002	19.7	229.5	1174	1385	19.5
10	1.8	13.5	120	469	368.5	1656	1004	22.3	254.6	1151	1388	22.1
11	2	15	120	468	403.7	1624	1006	24.9	279	1129	1390	24.7
12	2.2	16.5	120	467	438.1	1593	1007	27.5	302.8	1108	1393	27.3
13	2.4	18	120	466	471.5	1563	1009	30.2	325.9	1087	1395	30
14	2.6	19.5	120	464	504.1	1534	1011	32.9	348.4	1067	1397	32.7
15	2.8	21	120	463	535.9	1506	1013	35.6	370.4	1047	1400	35.4
16	3	22.5	120	462	566.8	1478	1014	38.3	391.9	1028	1402	38.1

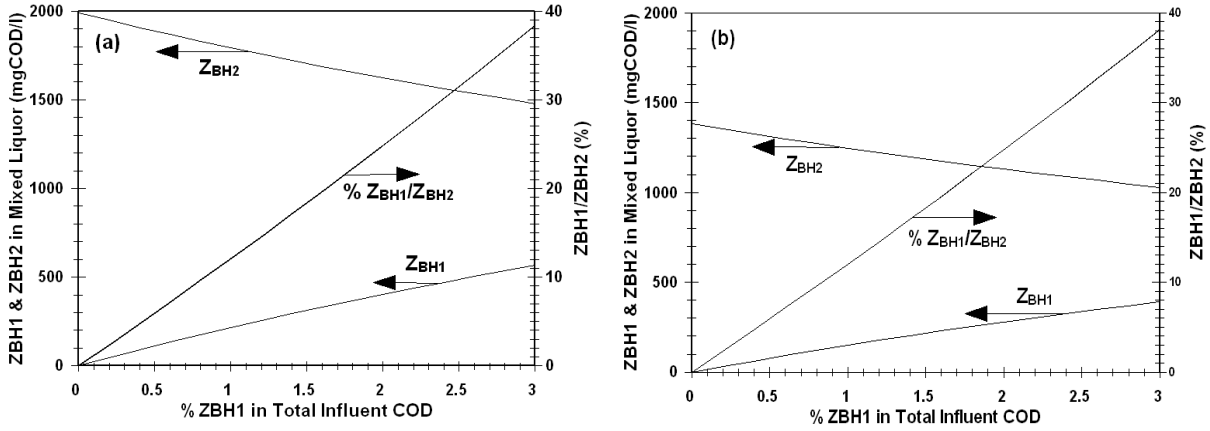

Figure 3.17: Simulated fast growing (Z_{BH1}) and slow growing (Z_{BH2}) OHO active biomass concentrations in mixed liquor and the percentage of Z_{BH1}/Z_{BH2} versus fast growing OHO concentration in the influent, $Z_{BH1(Inf)}$, as a percentage of total influent total COD, for both (a) 10 days sludge age system and (b) 20 days sludge age system, using kinetic competition model.

Table 3.10: Results for the parameter estimation with the aid of AQUASIM 2.0 computer program for the modified batch test data listed in Table 3.5 and 3.6. Also shown are the total OHO active biomass concentrations at the start of the batch test, ($Z_{BH(0)}$) predicted by the combined kinetic and metabolic competition model, from analysis using the procedure developed by Wentzel *et al.* (1995) and described in Chapter 2 (UCT model), and predicted by application of the steady state model (WRC, 1984) to the parent system, taking due account of dilution.

Sew. Batch No.	Batch test No.	Batch test date	Volume (ℓ)		Parameter Estimation				$Z_{BH(0)}$ (mgCOD/ℓ)		
			WW	ML	Sti	$Z_{BH1(0)}$	$Z_{BH2(0)}$	χ^2	Competition Model	UCT Model	Steady-state Model
					mgCOD/ℓ						
10	A12	11 12	2.8	0.2	151.44	1.88	119.53	16.92	121.41	60.9	127.5
	A13	12 12	2.90	0.1	113.20	3.25	53.20	2.39	56.45	18.7	63.7
	A14	13 12	2.95	0.1	71.62	3.39	35.39	0.06	38.78	14.9	31.9
11	A17	30 12	2.80	0.2	138.48	1.02	84.65	3.52	85.67	45	115.2
	A18	31 12	2.9	0.1	137.02	0.77	39.60	6.77	40.37	12.2	57.6
12	A22	11 01	2.8	0.2	193.72	0.88	109.94	48.05	110.82	50.7	116.7
	A23	14 01	2.9	0.1	166.84	0.39	54.73	8.57	55.12	20.9	58.4
14	A29	18 02	2.80	0.20	185.68	0.51	62.93	16.21	63.44	23.3	88.4
	A30	20 02	2.9	0.1	184.78	0.91	41.43	22.63	42.34	8.7	44.2
17	A34	22 04	2.80	0.20	127.22	0.39	58.56	5.22	58.95	33.2	95.2
	A35	24 04	2.9	0.1	122.88	0.25	18.56	1.03	18.81	2.7	47.6
10	B13	12 12	2.8	0.2	110.38	2.54	109.68	6.11	112.22	50.3	83.7
	B14	13 12	2.90	0.10	72.76	3.14	56.96	0.10	60.1	24.9	41.9
11	B17	30 12	2.7	0.3	141.93	1.33	97.39	2.82	98.72	51.4	129.7
	B18	31 12	2.80	0.2	138.44	0.80	61.16	10.67	61.96	25	86.4
	B19	05 01	2.90	0.10	90.44	4.74	42.17	3.85	46.91	17.9	43.2
12	B22	11 01	2.70	0.3	175.32	0.44	114.11	21.54	114.55	133.6	133.6
	B23	14 01	2.80	0.20	163.57	0.25	77.18	5.12	77.43	48.5	89.1
	B24	15 01	2.90	0.10	167.80	0.58	46.44	8.72	47.02	14	44.5
14	B29	18 02	2.70	0.30	172.26	0.45	64.89	12.90	65.34	28.8	98.3
	B30	20 02	2.80	0.20	200.42	0.79	50.01	1.87	50.8	65.5	65.5
17	B34	22 04	2.70	0.30	121.48	0.36	71.35	5.78	71.71	43.1	104.4
	B35	24 04	2.80	0.20	118.57	0.35	30.30	3.79	30.65	10.2	69.6

Note) A : 10 days sludge age system, B : 20 days sludge age system

To address inconsistencies above, the competition model was modified by removing the processes for growth of Z_{BH1} on SBCOD (kinetic + metabolic selection). This was considered reasonable, since the original source of Z_{BH1} is seeding with the influent wastewater to the parent systems - this implies growth in the sewer where RBCOD concentrations are very high, which would favour predominantly RBCOD utilization. Simulation of the batch tests gave results very similar to the competition only model above, cf. Tables 3.8 and 3.10

However, simulation of the steady state systems (Table 3.11) indicated that:

- The predicted proportions of Z_{BH1} in the steady state system mixed liquor were significantly lower that with the kinetic model based on competition only, for influent Z_{BH1} at 3% of total COD, the predicted proportions of Z_{BH1} were < 3% compared to ± 40% for the competition only model above. This former value is very close to those from parameter estimation on the batch test data.

Table 3.11: Model inputs and results from simulations of the 10 and 20 days sludge age steady state activated systems with the competition model of process selection.

No. of Simulation	% of $Z_{BH1(Inf)}$ in Inf. CODt	Input data			Simulation Results - 10days system				Simulation Results - 20days system			
		mgCOD/l			mgCOD/l			%	mgCOD/l			%
		Z_{BH1}	S_{bs}	S_{bp}	Z_{BH1}	Z_{BH2}	Z_E	Z_{BH1}/Z_{BH2}	Z_{BH1}	Z_{BH2}	Z_E	Z_{BH1}/Z_{BH2}
1	0	0	120	480	0	1992	988	0	0	1384	1366	0
2	0.2	1.5	120	479	3.6	1992	990	0.2	2.3	1384	1369	0.2
3	0.4	3	120	478	7.2	1992	992	0.4	4.6	1384	1371	0.3
4	0.6	4.5	120	476	10.9	1992	993	0.5	6.9	1384	1373	0.5
5	0.8	6	120	475	14.5	1992	995	0.7	9.2	1384	1376	0.7
6	1	7.5	120	474	18.1	1992	997	0.9	11.5	1384	1378	0.8
7	1.2	9	120	473	21.7	1992	999	1.1	13.8	1384	1380	1
8	1.4	10.5	120	472	25.4	1992	1000	1.3	16.1	1385	1383	1.2
9	1.6	12	120	470	29	1991	1002	1.5	18.4	1385	1385	1.3
10	1.8	13.5	120	469	32.6	1991	1004	1.6	20.7	1385	1388	1.5
11	2	15	120	468	36.2	1991	1006	1.8	23	1385	1390	1.7
12	2.2	16.5	120	467	40	1991	1007	2	25.3	1385	1392	1.8
13	2.4	18	120	466	43.5	1991	1009	2.2	27.6	1385	1395	2
14	2.6	19.5	120	464	47.1	1991	1011	2.4	29.9	1385	1397	2.2
15	2.8	21	120	463	50.7	1991	1013	2.5	32.2	1386	1400	2.3
16	3	22.5	120	462	54.4	1991	1014	2.7	34.5	1386	1402	2.5

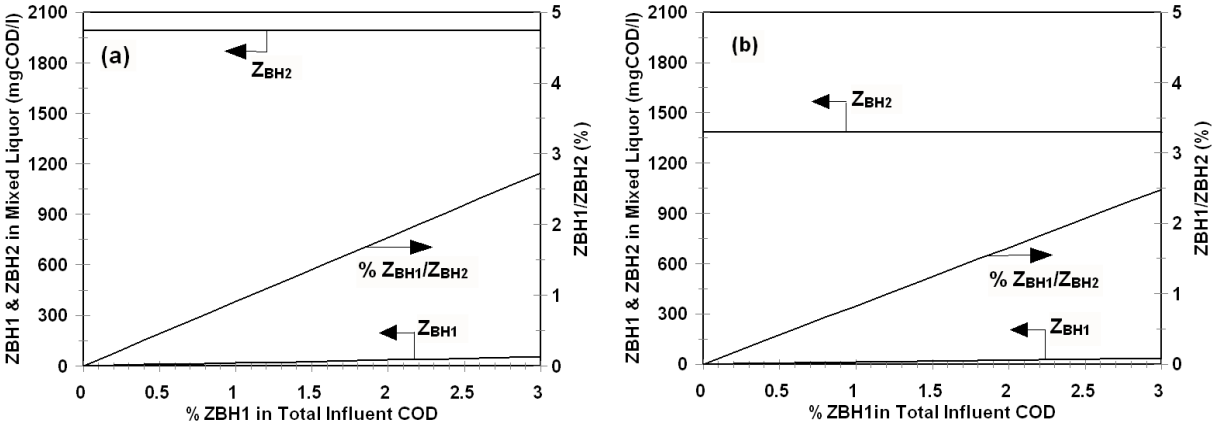

Figure 3.18: Simulated fast growing (Z_{BH1}) and slow growing (Z_{BH2}) OHO active biomass concentrations in mixed liquor and the percentage of Z_{BH1}/Z_{BH2} versus fast growing OHO concentration in the influent, $Z_{BH1(Inf)}$, as a percentage of total influent total COD, for both (a) 10 days sludge age system and (b) 20 days sludge age system, using kinetic + metabolic competition model.

In comparing the various estimates for OHO active biomass concentrations, it was found that both the two OHO population kinetic models (Fig. 3.19) gave concentrations that were significantly closer to the theoretical values than the single OHO population model (Fig. 3.15). Thus, the competition hypothesis (in agreement with previous researchers) is one feasible explanation for the observations in the batch tests. However, the kinetic models developed here are largely hypothetical - insufficient information is available to separate the two OHO populations and quantify the individual kinetic processes. Furthermore, it must be remembered that the kinetic models are a gross practical simplification of the complete OHO community present in activated sludge mixed liquor. This simplification has practical benefits, *e.g.* reduced quantity of input information required, wider generality, but also has limitations. In particular, competitive interactions are difficult to accommodate, where balanced competitive populations are present. Further, alternative hypotheses could possibly explain the observed behaviours. For example, it is possible that the batch conditions induce a change in the substrate utilization/growth kinetics of the OHOs in the batch test with time (*i.e.* physiological adaptation, Daigger *et al.*, 1982). Considering that the OHOs have been developed in a steady state system under substrate (organic C) limiting conditions, and are placed in a batch reactor with excess substrate, adaptation of the OHOs to the batch test conditions (and hence change in kinetics) is not unreasonable. Clearly, this topic requires further investigation. Such an investigation will be facilitated by the new microbiologically based analytical techniques, *e.g.* FISH.

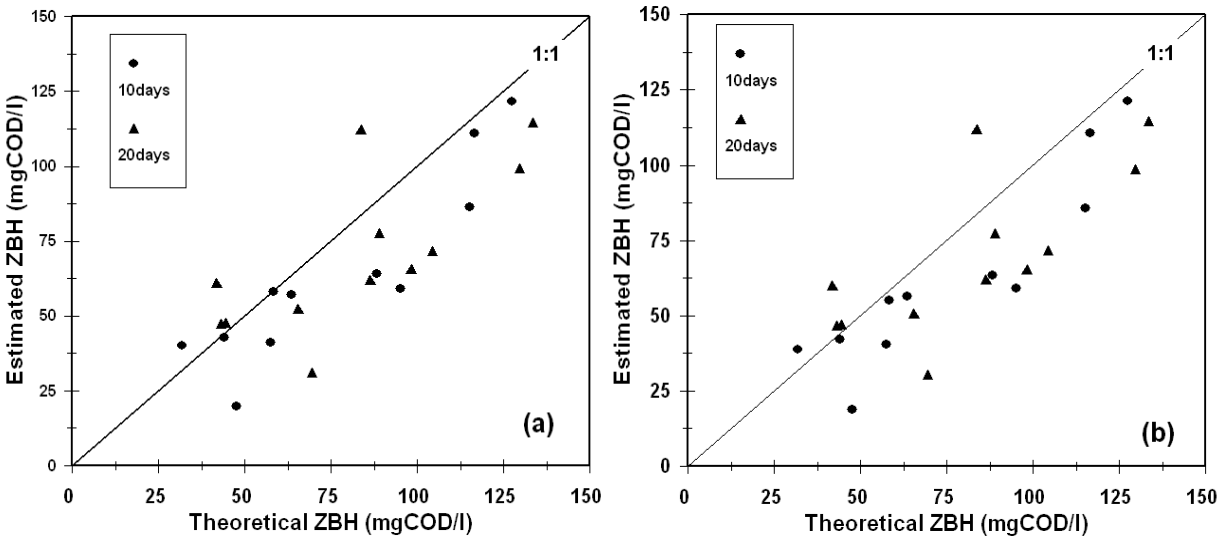

Figure 3.19 : Plot of the OHO active biomass concentration at the start of the batch test [$Z_{BH(0)}$] determined by the parameter estimation with the (a) kinetic and (b) kinetic + metabolic competition models versus the theoretical $Z_{BH(0)}$ concentration calculated with the steady-state model (WRC, 1984) for the selected modified batch test data.

3.7 CLOSURE

In this part of the research project, the modified batch test to quantify OHO active biomass has been extensively evaluated. It was hoped that this respirometric based batch test would provide measured OHO active biomass concentrations that would compare favourably with the theoretical values predicted by the activated sludge models, and hence prove to be a simple, convenient method to quantify the OHO active biomass. Further, agreement between measured and theoretical values would provide independent validation in a simple way of the active biomass concept in the models, and thereby promote confidence in their application. However, similarly to the research in the preceding contract (Cronje *et al.*, 2002b; Beeharry *et al.*, 2001), correspondence was not good and the problem more complex than originally thought. The modified batch test procedure relies on monitoring the increase in OUR with time due to net OHO growth (and associated substrate utilisation) at the maximum rate (and hence is an activity based test). Taking nitrification into account and accepting a single OHO population group with constant kinetics, the increase in the OUR with time is translated through the kinetics for OHO growth into the net specific growth rate of the OHO group. From this and the initial OUR in the test, the initial OHO active biomass concentration can be determined. Thus, in the test the increase in OHO active biomass with time must be significant compared with the initial concentration, so that the change in OUR due to OHO growth can be experimentally quantified. In the test, this is achieved by ensuring high initial substrate to active biomass concentration ratios (i.e. $S_0/Z_{BH(0)}$).

By relying of a single OHO population group with fixed kinetics, the modified batch test and its interpretation are particularly sensitive to any changes in the OHO maximum growth rates as time proceeds in the test, since these kinetics are applied at the start of the test to determine the OHO active biomass concentration. The requirement for significant OHO active biomass growth in the test compared with the initial concentrations (see above) does provide the possibility for population (or kintic) shifts, and hence a change in kinetics with time. From an examination of the batch test data it was concluded that the OHO maximum specific growth rates did indeed increase with time in the batch test. Such behaviour cannot be accommodated in the analytical procedure for the modified batch test which is based on a single OHO population with fixed kinetics. From the observations of Novak *et al.* (1994) and Grady *et al.* (1996), substrate competition between two OHO populations was proposed as a possible cause for the change in OHO maximum specific growth rates. Kinetic models were developed incorporating competition (kinetic only and kinetic + metabolic competition) between two OHO population groups, one a slow growing and the other a fast growing group. From application of these models to the batch tests, although the fast growing group initially may be present at very low concentrations in the batch test (~ 1% of the overall OHO population from the models developed here), due to the significant growth in the batch test the fast grower increases its proportion in the overall OHO population significantly. This change in the proportion of the two OHO groups with time in the test causes the overall kinetics to change, and significantly influences the interpretation of the batch test data. With the competition kinetic models, the improved correlation between measured and predicted OHO active biomass concentrations indicates that the competition hypothesis (in agreement with previous researchers) is one feasible explanation for the observations in the batch tests. However, the kinetic models developed here are largely hypothetical - insufficient information is available to separate the two OHO populations and quantify the individual kinetic processes. Further, alternative hypotheses could possibly explain the observed

behaviours, e.g. physiological adaptation (Daigger *et al.*, 1982). Clearly, this requires further investigation. Such investigations may be facilitated by the microbiologically based analytical techniques, and may be an interesting research topic.

Although considerable success was achieved in improved correlations between theoretical and batch test measured OHO active biomass concentrations, this was through application of the more complex competition based kinetic models. This greatly increases the complexity of the batch test data interpretation, and introduces uncertainty in that calibration of the growth rates of the two competing OHO populations did not prove possible (but the interpretation may be relatively insensitive to this, and requires investigation). Unfortunately this increased complexity renders the batch test unsuitable as a simple method to directly quantify the OHO active biomass concentration with sufficient accuracy (and also to derive estimates for the kinetic constants for OHO maximum specific growth rates). The test does, however, hold merit as a tool to investigate OHO population dynamics, as shown in the previous contract (Cronje *et al.*, 2002) and here. In quantifying the OHO active biomass concentrations, the microbiologically based analytical techniques may prove beneficial, and this is investigated in Chapter 4.

The causes for the lack of correspondence between theoretical and batch test measured OHO active biomass concentrations have been shown to lie in the modified batch test itself: Both the batch aerobic digestion tests and re-interpretation of the modified batch test data with competition kinetic models provided reasonable correspondence between measured and model OHO active biomass concentrations. ***This does provide independent evidence that substantiates the active biomass concept in the models***. Convincing independent validation of the aerobic batch digestion approach and the model structure for endogenous respiration (and the kinetic model equivalent of death-regeneration) is provided by Van Haandel *et al.* (1998). They operated sequential flow-through aerobic digesters at 1.73, 2.14, 3.00 and 5.63 d and obtained b_H values from both VSS and OUR measurements that are virtually identical to the values of Marais and Ekama (1976) on which the original activated sludge models are based. The data of Van Haandel *et al.* also provides some indirect validation of active biomass concentrations in that there is consistency between the model predicted active fraction in the mixed liquor used as source feed to the digester sequence and that calculated from the aerobic digestion measurements.

Although the competition models needed to be applied to explain the observations in the modified batch tests, it must be remembered that in the parent activated sludge systems, the OHO population is dominated by the slow growing OHO population group, to the extent of near exclusion of the fast growers. Thus, for the activated sludge system the current models incorporating a single OHO population are adequate, provided extremes in dynamic loading are not encountered, *e.g.* with selector reactors (Still *et al.*, 1996). From this work it seems that selector reactors stimulate proliferation in the activated sludge system of the fast growing OHOs, which in the absence of the selector would not be sustained in the system to any significant extent.

CHAPTER 4

COMPARISON OF ENGINEERING/TECHNOLOGY MEASUREMENT OF OHO ACTIVE BIOMASS WITH MICROBIOLOGICAL/BIOCHEMICAL MEASUREMENTS

4.1 INTRODUCTION

One of the objectives in this research project was to "attempt to link these (i.e. batch test) measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms" (see Chapter 1, Section 1.4). To achieve this, the UCT research project was run in parallel to the collaborative WRC sponsored project with the Centre for Water and Wastewater Research (CWWR) at the Durban Institute for Technology (DIT) (K5/1178). As part of their research (Holder-Snyman *et al.*, 2004), CWWR applied microbiological analytical methods to quantify ordinary heterotrophic organism (OHO) and autotrophic organism (AO) active biomass concentrations in samples drawn from the laboratory-scale activated sludge systems operated at UCT. The laboratory-scale systems were closely controlled and defined, enabling the theoretical OHO active biomass concentrations in the systems to be calculated. Also, the batch test method to quantify OHO active biomass (Chapter 3) was run by UCT in parallel to the microbiological methods. This allowed the engineering and technology defined theoretical OHO and AO and measured OHO active biomass concentrations to be compared with the microbiological/biochemical quantified OHO and AO active biomass concentrations. This Chapter describes these comparisons.

4.2 METHODS

The University of Cape Town operated two parallel laboratory-scale anoxic/aerobic (MLE) activated sludge systems, at system sludge ages of 10 and 20 d respectively, under controlled and defined conditions. For these systems the theoretical OHO and AO active biomass concentrations could be calculated with the aid of the steady state design model (WRC, 1984). Regularly, samples were drawn from these systems and modified batch tests (Chapter 3) conducted to determine OHO active biomass concentrations. Additionally, in parallel samples were drawn from the systems and couriered to DIT who applied a combination of DAPI staining and Fluorescent *in situ* Hybridisation (FISH) to determine both OHO and AO active biomass concentrations. This enables direct comparison of the theory and various measurements.

4.2.1 Laboratory-scale activated sludge systems

Two laboratory-scale activated sludge systems were operated in parallel, one system at sludge age of 10 d and the other at 20 d. System configurations and operating parameters are summarised in Fig. 4.1, and Table 4.1.

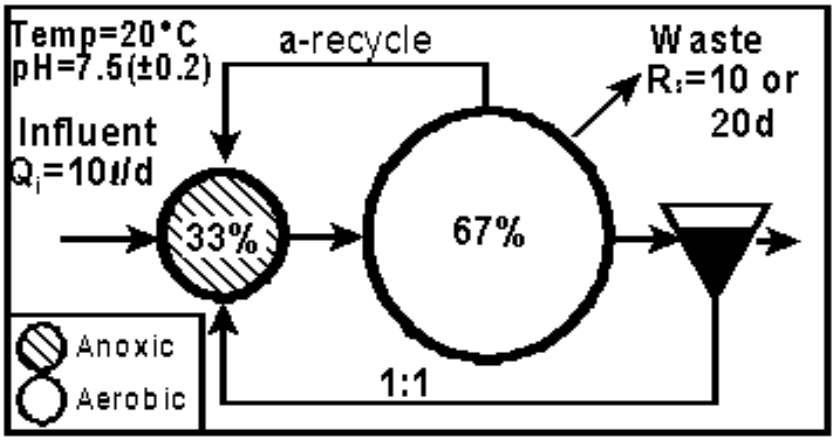

Figure 4.1: Schematic layout and operational data for parent laboratory-scale systems.

Table 4.1: Details of changes to the parent laboratory-scale system operating parameters; AX=anoxic, AE=aerobic.

Period	Dates (2002-2003)	Day No	Sewage Batches[1]	Inflow Rate (Qi; ℓ/d)	Recycle ratios (With respect to Qi)		System Volume (ℓ) (33% AX, 67% AE)	
					s-recycle	a-recycle	10days	20days
4[2]	14 Feb~28 Apr	221 ~ 294	14 ~ 17	10	1:1	1:1	7.8	13.2
5	29 Apr~15 Sep	295 ~ 434	14/02 ~ 16/02	10	1:1	1:1	7.8	13.2
6	16 Sep~29 Sep	435 ~ 448	17/02	10	1:1	1:1	7.8	13.2
7	30 Sep~20 Nov	449 ~ 549	18/02 ~ 20/02	10	1:1	1.2:1 (10d) 1:1 (20d)	7.8	13.2
8	21 Nov~20 Feb	550 ~ 652	21/02 ~ 03/03	10	1:1	1.2:1 (10d) 1:1 (20d)	7.8	13.2

[1]Batch tests during Sewage Batch Nos. 17, 21/02, 23/02, 01/03, 02/03; Microbiological examination during Sewage Batch Nos. 17, 18/02-02/03.
[2]Period 4 corresponds to Period 4 in Chapter 3.

System operation was as briefly described in Chapter 3, and in detail by Lee *et al.* (2003). As described in Chapter 3, for each Sewage Batch the measured data were evaluated for outliers (95% CI) and the remaining data averaged and sample standard deviations (SSD) calculated. System behaviour and performance during the periods samples were drawn for batch tests, and/or for microbiological analysis are summarised in Tables 4.2 (a and b).

Table 4.2 (a): Steady state data for the 10 day sludge age parent system during which samples were drawn for active biomass tests; for each sewage (Sew.) batch (steady state period, see Table 4.1) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed. Inf = influent, Eff = effluent (0.45 μm filtered), OUR = oxygen utilisation rate, VSS = volatile suspended solids.

Sew. Batch	No of tests	COD (mg/ℓ)			TKN (mgN/l)			Nitrite (mgN/ℓ)			Nitrate (mgN/ℓ)			OUR (mgO/ℓ/h)	VSS (mgVSS/ℓ)
		Inf	Eff	ML	Inf	Eff	ML	Anoxic	Aerobic	Eff	Anoxic	Aerobic	Eff		
17	8	773 (13)	38 (5)	3616 (97)	61.7 (1.6)	4.2 (0.3)	203 (7)	0 (0)	0 (0)	0 (0)	0.0 (0.1)	12.8 (0.9)	13.8 (0.7)	43.9 (1.0)	2632 (101)
17/02	12	700 (60)	69 (29)	2369 (209)	63.9 (5.1)	6.6 (3.3)	122 (18)	0 (0)	0 (0)	0 (0)	6.0 (0.9)	23.3 (2.8)	23.7 (2.2)	43 (-)	1550 (132)
18/02	15	749 (23)	53 (13)	2583 (239)	50.5 (2.9)	3.6 (0.6)	121 (12)	0 (0)	0 (0)	0 (0)	2.3 (1.5)	15.3 (0.9)	15.7 (1.0)	42.5 (1.2)	1641 (111)
19/02	9	747 (36)	37 (15)	3159 (242)	54.1 (1.3)	3.5 (0.7)	144 (13)	0 (0)	0 (0)	0 (0)	0.3 (0.5)	10.4 (2.2)	11.9 (3.2)	42.8 (1.5)	1979 (230)
20/02	12	739 (60)	48 (10)	2895 (418)	53.3 (2.3)	3.3 (0.9)	143 (9.6)	0 (0)	0 (0)	0 (0)	0.7 (0.6)	11.1 (2.3)	12.3 (1.6)	42.3 (2.9)	1899 (153)
21/02	11	755 (41)	51 (14)	3439 (351)	68.4 (4.5)	2.6 (0.6)	149 (16)	0 (0)	0 (0)	0 (0)	3.8 (0.6)	18.8 (1.5)	20.2 (1.8)	43 (4.3)	2320 (272)
22/02	8	714 (35)	41 (24)	3409 (190)	49.9 (4.4)	1.8 (0.7)	164 (27)	0 (0)	0 (0)	0 (0)	0.25 (0.13)	10.4 (01.3)	10.6 (01.4)	40.8 (2.1)	2442 (151)
23/02	1	688 (-)	50 (-)	3405 (-)	50.4 (-)	2.4 (-)	147 (-)	0 (-)	0 (-)	0 (-)	0.35 (-)	11.6 (-)	12.3 (-)	40.3 (-)	2308 (-)
01/03	10	741 (49)	71 (42)	3236 (292)	64.0 (3.6)	2.8 (0.6)	174 (31)	0 (0)	0 (0)	0 (0)	1.6 (0.6)	14.6 (1.9)	15.0 (2.3)	37.8 (2.4)	2229 (122)
02/03	12	684 (84)	55 (21)	3235 (562)	74.0 (10)	4.1 (1.9)	174 (15)	0 (0)	0 (0)	0 (0)	6.0 (3.1)	22.7 (2.5)	22.6 (3.3)	41.4 (3.4)	2066 (389)
03/03	8	654 (47)	74 (33)	3024 (304)	51.9 (3.7)	4.3 (1.8)	196 (22)	0 (0)	0 (0)	0 (0)	0.9 (0.6)	9.8 (1.8)	10.4 (1.2)	35.7 (7)	2198 (163)

Table 4.2 (b): Steady state data for the 20 day sludge age parent system during which samples were drawn for active biomass tests; for each sewage (Sew.) batch (steady state period, see Table 4.1) the data have been averaged and the means, sample standard deviations (SSD) and number of tests are listed. Inf = influent, Eff = effluent (0.45 μm filtered), OUR = oxygen utilisation rate, VSS = volatile suspended solids.

Sew. Batch	No of tests	COD (mg/ℓ)			TKN (mgN/l)			Nitrite (mgN/ℓ)			Nitrate (mgN/ℓ)			OUR (mgO/ℓ/h)	VSS (mgVSS/ℓ)
		Inf	Eff	ML	Inf	Eff	ML	Anoxic	Aerobic	Eff	Anoxic	Aerobic	Eff		
17	8	773 (13)	36 (5)	3775 (53)	61.7 (1.6)	4.1 (0.5)	207 (5)	0.04 (0.07)	0 (0)	0 (0)	0.0 (0.0)	15.3 (0.4)	15.0 (0.7)	26.1 (1.3)	2667 (70)
17/02	12	700 (60)	62 (19)	2240 (149)	63.9 (5.1)	6.5 (3.3)	108 (18)	0 (0)	0 (0)	0 (0)	6.1 (0.7)	26.0 (3.0)	24.8 (2.1)	28 (-)	1414 (56)
18/02	15	749 (23)	44 (11)	2607 (439)	50.5 (2.9)	3.4 (0.7)	126 (17)	0 (0)	0 (0)	0 (0)	1.0 (0.8)	12.8 (1.1)	13.3 (1.8)	29.0 (1.6)	1733 (183)
19/02	9	747 (36)	42 (11)	2709 (386)	53.6 (2.1)	3.1 (0.4)	126 (14)	0 (0)	0 (0)	0 (0)	1.1 (0.6)	17.1 (3.1)	18.7 (1.5)	27.8 (1.5)	1596 (204)
20/02	12	739 (60)	52 (18)	2400 (373)	53.3 (2.3)	3.1 (0.3)	128 (11)	0 (0)	0 (0)	0 (0)	0.8 (0.8)	11.9 (2.4)	13.5 (2.2)	27.4 (1.9)	1491 (193)
21/02	11	753 (43)	37 (11)	2540 (300)	68.4 (4.5)	3.0 (0.8)	123 (21)	0 (0)	0 (0)	0 (0)	4.2 (0.5)	20.5 (1.2)	21.5 (1.2)	22.7 (3.1)	1659 (208)
22/02	8	714 (35)	42 (8)	1810 (240)	49.9 (4.4)	1.9 (0.8)	95 (21)	0 (0)	0 (0)	0 (0)	4.8 (1.0)	24.5 (2.0)	19.9 (1.9)	17.8 (0.9)	1183 (138)
23/02	1	688 (-)	29 (-)	1243 (-)	50.4 (-)	2.7 (-)	116 (-)	0 (-)	0 (-)	0 (-)	1.35 (-)	11.9 (-)	14.8 (-)	17.3 (-)	890 (-)
01/03	10	741 (49)	153 (71)	2370 (236)	64.0 (3.6)	21.4 (22.7)	142 (26)	0 (0)	0 (0)	0 (0)	13 (0)	26 (0)	26 (0)	27.5 (13.6)	1560 (123)
02/03	12	684 (84)	79 (30)	2581 (385)	72.0 (6.2)	12.7 (15.3)	158 (20)	0 (0)	0 (0)	0 (0)	10.8 (0.9)	28.7 (3.3)	30.4 (5.6)	25.6 (2.8)	1679 (153)
03/03	8	654 (47)	49 (13)	2719 (328)	51.9 (3.7)	5.5 (1.5)	162 (12)	0 (0)	0 (0)	0 (0)	1.2 (0.3)	10.9 (1.1)	13.1 (2.8)	25.9 (1.4)	1815 (119)

Table 4.3 (a): System steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for the 10 days sludge age parent system. Data calculated from Table 4.2 (a) (* indicates data rejected as outliers at 95% confidence interval).

Sew. Batch No.	No of tests	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR		
		N	COD	Unbio. Soluble (fs,us) (mgCOD/mgCOD)	Unbio. Particulate (fs,up) (mgCOD/mgCOD)	COD/VSS (fcv) (mgCOD/mgVSS)	TKN/VSS (fN) (mgN/mgVSS)	Active fraction (fav)
17[1,2]	8	96	97	0.048	0.152	1.41	0.079	0.399
17/02	12	107	90	0.086*	-0.011*	1.49	0.083	0.705
18/02[2]	15	109	89	0.062	-0.011*	1.55	0.076	0.704
19/02[2]	9	92	99	0.046	0.070	1.58	0.074	0.531
20/02[2]	12	94	97	0.055	0.039	1.50	0.077	0.589
21/02[1,2]	11	95	96	0.051	0.110	1.47	0.065	0.465
22/02[1,2]	8	95	102	0.046	0.156	1.39	0.068	0.392
23/02[1,2]	1	101	105	0.048	0.159	1.46	0.063	0.397
01/03[1,2]	10	92	92	0.056	0.115	1.43	0.079	0.453
02/03[1,2]	12	95	97	0.074	0.116	1.54	0.086	0.458
03/03	8	96	103	0.061	0.156	1.36	0.091	0.386
MEAN				**0.0550**	**0.119**	**1.471**	**0.0765**	
Std. Deviation				**0.0090**	**0.042**	**0.070**	**0.0087**	

[1]Batch tests conducted during these sewage batches
[2]Samples for microbiological analysis during these sewage batches

Table 4.3 (b): System steady state N and COD mass balances, wastewater fractions and mixed liquor parameters for the 20 days sludge age parent system. Data calculated from Table 3.3 (b) (* indicates data rejected as outliers at 95% confidence interval).

Sew. Batch No.	No of tests	MASS BALANCE (%)		WASTEWATER FRACTIONS		MIXED LIQUOR		
		N	COD	Unbio. Soluble (fs,us) mgCOD/mgCOD)	Unbio. Particulate (fs,up) (mgCOD/mgCOD)	COD/VSS (fcv) (mgCOD/mgVSS)	TKN/VSS (fN) (mgN/mgVSS)	Active fraction (fav)
17[1,2]	8	102	90	0.039	0.143	1.39	0.077	0.279
17/02	12	110	89	0.074	-0.003	1.55	0.081	0.517
18/02[2]	15	95	98	0.051	0.016	1.48	0.075	0.473
19/02[2]	9	117	91	0.043	0.027	1.68	0.081	0.451
20/02[2]	12	90	95	0.059	0.002	1.58	0.088	0.505
21/02[1,2]	9	90	72[3]	0.039	0.006	1.51	0.076	0.495
22/02[1,2]	8	91	58[3]	0.048	-0.069 (0.043)[4]	1.50	0.082	0.423[5]
23/02[1,2]	1	94	58[3]	0.036	-0.089 (0.043)[4]	1.37	0.133	0.417[5]
01/03[1,2]	10	107	100	0.178*	0.024	1.43	0.105	0.448
02/03[1,2]	12	110	91	0.089	0.044	1.50	0.102	0.418
03/03	8	95	107	0.055	0.079	1.48	0.092	0.362
MEAN				**0.0533**	**0.043**[4]	**1.497**	**0.0902**	
Std. Deviation				**0.0170**	**0.047**	**0.087**	**0.0174**	

[1]Batch tests conducted during these sewage batches
[2]Samples for microbiological analysis during these sewage batches
[3]Difficulties experienced with DO probe and hence OUR measurement
[4]() = Average value for all positive $f_{S,up}$ values; used in subsequent calculations
[5]f_{av} calulated from average value for $f_{S,up}$

Following the procedures set out in Chapter 3 and in Lee *et al.* (2003), from the averaged data in Tables 4.2 (a and b), the following were calculated:

- System COD and N mass balances.

- Influent wastewater unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively); $f_{S,up}$ was determined with the steady state design procedure (WRC, 1984), see Lee *et al.* (2003) - Cronje *et al.* (2002b) showed that the steady state design procedure and the kinetic simulation models gave near identical results for $f_{S,up}$, and hence the simpler more direct steady state design procedure was used.

- Mixed liquor COD/VSS and TKN/VSS ratios (f_{CV} and f_N respectively).

- The OHO active biomass fraction of the mixed liquor organic suspended solids (f_{av}), with the steady state design procedure (WRC, 1984), see Cronje *et al.* (2002b) and Lee *et al.* (2003).

These values are listed in Tables 4.3 (a and b).

Accepting the parameters in Tables 4.2 and 4.3, the theoretical OHO active biomass concentrations in the two parent laboratory-scale systems could be calculated from the steady state theory (WRC, 1984), and hence also in the batch tests, see Table 4.4. Additionally, from the parameters in Tables 4.2 and 4.3 and the steady state theory (WRC, 1984), the theoretical AO active biomass concentrations in the two parent laboratory-scale systems were also calculated, see Table 4.5. This was necessary since the microbiological measurements included quantifying AO active biomass concentration.

4.2.2 Batch tests

The modified batch test procedure (Chapter 3, Lee *et al.*, 2003) was applied to samples drawn from the two parent laboratory-scale systems. In total 24 batch tests were done over the period when samples were harvested and sent to DIT for analysis, 12 for mixed liquor from each of the parent systems, see Tables 4.4 (a and b).

Table 4.4 (a): Results of modified batch tests with a mixture of flocculated filtered sewage (Sew.) and mixed liquor (ML) drawn from the anoxic/aerobic 10d sludge age parent system: Sew. batch, batch test numbers, date of test, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass concentrations in the batch test ($Z_{BH(0)}$). Also shown are the measured and theoretical $Z_{BH(0)}$ concentrations in the parent system (PS), taking due account of dilution.

MODIFIED BATCH TESTS: 10d SLUDGE AGE PARENT SYSTEM											
Sew. Batch	Batch Test No.	Date of Test	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured		Theoretical	
			ML	Sew.		K_{MP}	μ_{HM}	Batch test	PS	Batch test	PS
17	A34	22/04	0.2	2.8	97.5	1.38	2.31	34	511	96	1442
	A35	24/04	0.1	2.9	100.3	1.14	6.27	2.7	82	48	1442
21/02	A36	24/11	0.3	2.7	105	1.2	1.07	143	1432	159	1586
	A37	27/11	0.2	2.8	122[1]	2.13	2.8	31	470	106	1586
	A38	02/12	0.1	2.9	98	1.92	4.47	7	213	53	1587
22/02	A39	10/12	0.3	2.7	100	1.7	1.22	119	1190	132	1323
	A40	17/12	0.3	2.7	96	1.74	1.02	109	1086	132	1325
23/02	A41	22/12	0.3	2.7	94	2.4	3.65	53	530	134	1338
01/03	A42	07/01	0.3	2.7	95	1.92	1.27	112	1120	145	1446
	A43	14/01	0.2	2.8	91	2.32	1.73	51	758	96	1446
02/03	A44	24/01	0.3	2.7	103	0.95	0.75	140	1396	146	1458
	A45	01/02	0.2	2.8	100	2.15	2.9	89	1338	97	1458

[1]Poor COD mass balance

Table 4.4 (b): Results of modified batch tests with a mixture of flocculated filtered sewage (Sew.) and mixed liquor (ML) drawn from the anoxic/aerobic 20d sludge age parent system: Sew. batch, batch test numbers, date of test, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass concentrations in the batch test ($Z_{BH(0)}$). Also shown are the measured and theoretical $Z_{BH(0)}$ concentrations in the parent system (PS), taking due account of dilution.

MODIFIED BATCH TESTS: 20d SLUDGE AGE PARENT SYSTEM											
Sew. Batch	Batch Test No.	Date of Test	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured		Theoretical	
			ML	Sew.		K_{MP}	μ_{HM}	Batch test	PS	Batch test	PS
17	B34	22/04	0.3	2.7	101	1.34	2.13	43	431	105	1053
	B35	24/04	0.2	2.8	103	1.34	4.07	10	153	70	1053
21/02	B36	24/11	0.4	2.6	98	2.21	2.03	56	417	165	1240
	B37	27/11	0.27	2.73	110	1.72	3.53	29	317	112	1240
	B38	02/12	0.15	2.85	94	2.04	4.74	5.7	114	62	1240
22/02[1]	B39	10/12	0.4	2.6	91	2.41	1.94	39	293	100	751
	B40	17/12	0.3	2.7	98	2.23	2.54	23	225	75	751
23/02[1]	B41	22/12	0.4	2.6	94	2.47	3.17	20	153	73	549
01/03	B42	07/01	0.4	2.6	91	1.69	1.76	84	629	138	1035
	B43	14/01	0.3	2.7	96	1.96	1.28	88	876	104	1035
02/03	B44	24/01	0.4	2.6	101	0.86	0.82	158	1183	141	1054
	B45	01/02	0.3	2.7	100	1.06	1.13	52	521	105	1054

[1]Poor parent system mass balances

From the measured OUR, nitrate and nitrite time data, the following were calculated (see Chapter 3, Cronje *et al.*, 2002b; Lee *et al.*, 2003):

- %COD recovery.
- Nitrification OUR ($OUR_{N(t)}$) for both nitrate and nitrite nitrification (OUR_{NO3} and OUR_{NO2}), from a regression of the nitrate- and nitrite-time concentration profiles (exponential or linear) and using the differential of the fitted equation to determine the rate of nitrification, and hence the associated OUR.
- OHO OUR ($OUR_{H(t)}$), by subtracting the $OUR_{N(t)}$ (for both nitrate and nitrite nitrification) above from the measured $OUR_{M(t)}$.
- The OHO active biomass concentration at the start of the batch test, from linear regression data of the $\ell nOUR_{H(t)}$ versus time plots.

Following the procedure above, the OHO active biomass concentrations in the batch tests were determined and are listed in Tables 4.4(a) and 4.4(b) for the 10 and 20d sludge age mixed liquors respectively. Taking into account the dilution in the batch test (Xmℓ mixed liquor into 3ℓ), the OHO active biomass concentrations in the parent steady state system were calculated and are also listed in Tables 4.4 (a and b).

4.2.3 Microbiological measurements

DAPI and combined DAPI/FISH measurements were done by DIT on samples harvested from the UCT operated parent activated sludge systems (Fig. 4.1, Table 4.1). The cells in the samples were first fixed at UCT (see below) and then couriered to DIT. At DIT, the samples were spotted onto slides. Then, the fixed samples were subject to total cell counts by membrane filtration and staining with DAPI and to combined whole cell hybridisation and DAPI staining.

4.2.3.1 Sampling and sample preparation

Samples were harvested from the aerobic reactor of the parent systems (Fig. 4.1) on the same day or close to the day samples were harvested for the batch tests above. However, although samples were harvested from the parent systems during Sewage Batch Nos. 18/02 - 20/02 for microbiological examination, batch tests could not be conducted during these sewage batches due to construction in the Water Research Laboratory at UCT.

In the previous and current parallel research contracts on OHO active biomass determination (K5/1179 and K5/1178 respectively), the microbiological measurements gave values significantly lower than both the batch test determined values and the theoretical values (Chapter 2, Fig. 2.7 and Cronje *et al.*, 2002b). Accordingly, the procedures for sample preparation and transport to DIT were critically examined: Samples were mixed with 98% ethanol in a 1:1 ratio (25 or 20mℓ sample to 25 or 20mℓ ethanol). Initial sample batches were stored at 4°C and later batches at −20°C. The samples were couriered to DIT as a single batch in a cooler box filled with dry ice. No difference was found in storing the samples at 4°C or at -20°C. However, it was found that the method of couriering the samples in dry ice caused a significant number of the cells to freeze and hence burst. This would significantly reduce the microbiologically determined cell counts, and was proposed as the explanation for the low cell counts (Cronje *et al.*, 2002b). Accordingly, it was decided to do the cell fixation at UCT to overcome this problem. Cell fixation was with paraformaldehyde, as follows:

1. The following solutions were prepared:
   1x phosphate buffered saline (PBS)
   - 130 mM sodium chloride
   - 10 mM sodium phosphate buffer
   - pH 7.2

   3x PBS, reagents at 3x concentration of 1x PBS above

Paraformaldehyde fixative

-65mℓ of double distilled water was heated to 65°C.
-4g of 4% paraformaldehyde was added to the heated water above.
-One drop of 2 M NaOH was added, and the solution stirred rapidly until nearly clarified (1 - 2 minutes).
-The solution was removed from the heat source and 33 mℓ 3x PBS added.
-pH was adjusted to 7.2 with HCl
-Solution was filtered through 0.45µm filter.
-The solution was quickly cooled to 4°C and stored in the refridgerator.
-The fixative solution can be used for up to 24h.

2. Three volumes of paraformaldehyde fixative (30mℓ) were added to one volume of sample (10mℓ), and the mixture held for 1 to 3 h at 4 °C.
3. The solution was pelleted by centrifugation (3 500rpm) and the fixative removed.
4. The cells were washed in 1x PBS, and then resuspended in 1x PBS to the original sample volume (10mℓ) (i.e. to give a final concentration of 10^8 to 10^9 cells/mℓ).
5. Ice-cold 98% ethanol was added in a 1:1 ratio to the above, and mixed.
6. Fixed cells were stored in a freezer at -20°C.

Samples were couriered to DIT in batches in cooler boxes with ice packs. In total, three batches of samples were sent to DIT: Batch 1, Sewage Batch No. 17; Batch 2, Sewage Batch Nos. 18/02, 19/02 and 20/02; Batch 3, Sewage Batch Nos. 21/02, 22/02, 23/02, 01/03, 02/03 (Tables 4.1 (a and b)).

4.2.3.2 Total cell counts by membrane filtration and staining with DAPI

Membrane filtration and DAPI staining (after Porter and Feig, 1980)

Cellulose acetate filters (pore size, 0.22 µm, Millipore) were counter stained with Sudan Black solution (0.3% w/v in 60% ethanol) for 24 h. 900 µℓ of PBS (1x) was added to 10 µℓ activated sludge in a 2 mℓ micro-test tube. 100 µℓ of the non-inionic detergent (Igepal CA-30, Sigma Chemicals) was then added and the test tube contents mixed. 1mℓ of DAPI (0.5 µg/mℓ) was added to 1 mℓ of the activated sludge mixture. The staining was allowed to proceed for ten minutes. The stained cellulose acetate filter and a 0.45µm backing filter was then placed at the base of a 15mℓ filter tower and wet with sterile double distilled water. Quantitatively, the stained activated sludge mixture was transferred to the filter tower under a slight vacuum. After filtration, the excess DAPI stain was removed by washing the filter in the filtering device with sterile double distilled water. The stained cellulose filter was mounted on one drop of a glycerol/PBS mixture (95:5 v/v) on a glass slide. One drop of an anti-fading mounting medium (VECTASHIELD, Vector Laboratories, California) was added to the mounted filter surface before placing the cover slip.

Determination of total cell counts

For the slide with the mounted filter, DAPI fluorescence was detected with a Zeiss Axiolab microscope (Zeiss, Germany) fitted for epifluorescence microscopy with a 50W high-pressure mercury bulb and Zeiss filter set 01. Twenty random fields were selected for cell counts using image analysis software (MACRO) and the mean total cell count determined for the twenty fields. The total cell count was calculated using the following equation:

$$n(MF) = MTCC \cdot MF \cdot DF \qquad (4.1)$$

where:

n(MF) = total cell count (=n(MF) in Eq. (4.2) below)
MTCC = mean total cell count for twenty fields
DF = dilution factor
MF = total number of microscope fields on filter (55703 under 1000x magnification)

4.2.3.3 Whole cell hybridization

Hybridization should be carried out in a properly sealed moisture chamber to prevent evaporative concentration of the hybridization solution which might result in nonspecific binding of the probe to the cells. A 50mℓ polypropylene screw top tube (Corning Glass Works, USA) served as a convenient and portable hybridization chamber. Dual staining of cells with DAPI and flourescent oligonucleotides was achieved with a modified version of the method of Hicks *et al.* (1992), so that cells were stained with DAPI after *in situ* hybridisation, as follows:

1. ***Hybridization buffer*** was prepared with the following concentrations:
   0.9 M sodium chloride
   0.01 % sodium dodecyl sulphate
   20 mM Tris/HCl
   x % formamide (% depends on the particular probe used; Lane, 1985)
   pH 7.2
2. A strip of Whatmans' 3MM paper was soaked in hybridization buffer and placed in the polypropylene tube (hybridization chamber), to maintain moisture content in the chamber and so prevent evaporation.
3. The chamber was allowed to equilibrate for 15min at 46 °C, the hybridization temperature.
4. The fixed cells above were sonicated and then 3 to 5 μℓ spotted onto glass slides. The slides used have a hydrophobic coating with eight glass surface windows. The slides were pre-treated with poly-L-lysine solution and air dried in a 60 °C oven.
5. The spotted slide mounted cells were dehydrated by successive passage through 50, 80 and 98% ethanol washes.
6. For each spot to be hybridized, 9 μℓ hybridization buffer (prewarmed to 46 °C) was mixed with 1 μℓ of fluorescent probe (50ng/μℓ).

7. 10μℓ of hybridization buffer/probe mix was spread on each spot of fixed cells.
8. The slide was quickly transferred to the pre-warmed hybridization chamber and hybridized for 2 h at 46 °C.
9. After hybridization, the slide was removed from the moisture chamber and immediately hybridization was stopped by rinsing the probe from the slide with 2 mℓ washing buffer (20mM Tris/HCl, 0.01% SDS, 5mM EDTA, YM NaCl; the salt concentration in this washing solution was adjusted according to the formula of Lane, 1985) pre-warmed to a wash temperature of 48 °C.
10. The slide was transferred to a polypropylene tube filled with 50 μℓ hybridization buffer and incubated for 20 min at 48 °C.
11. The slides were then dipped in PBS, excess PBS shaken away and air dried. 10 μℓ DAPI was put on each spot, the slides placed in the dark for 10 min, and then washed with PBS.
12. The slides were mounted in VECTASHIELD and viewed with an epifluorescence microscope. Twenty fields were selected randomly for enumeration by image analysis.
13. The active cell count for each probe was determined by using the following equation:

$$\text{Active cell numbers} = \frac{\text{n(probe)}}{\text{n(DAPI)}} \bullet \text{n(MF)} \qquad (4.2)$$

where:

n (probe)	= average number of cells bearing probe conferred fluorescence
n(DAPI)	= average number of cells bearing DAPI conferred fluorescence
n(MF)	= total cell count as obtained by membrane filtration, see Eq. (4.1)

In the procedure above, four flourescent probes were used, EUB338 for all eubacteria (Amann *et al.*, 1990), and NIT3 for all previously sequenced *Nitrobacter* species (Wagner *et al.*, 1996), NEU for most halophilic and halotolerant ammonia oxidisers (Wagner *et al.*, 1995) and Nso190 for ammonia oxidisers in the β subclass of *Proteobacteria* (Mobarry *et al.*, 1996). The AO active biomass was determined from the sum of the NIT3 and NEU probes, and the OHO active biomass from the EUB338 - AO active biomass.

Following the procedures above, the OHO and AO active biomass cells counts in the various samples are shown in Table 4.5.

4.2.3.4 Converting cell counts to COD concentrations

The cell counts were converted to active VSS concentrations by multiplying the cell count by a cell to VSS conversion factor (F_{VB}) of 8.49 x 10^{-11} mgVSS/cell (Holder-Snymann *et al.*, 2004) and taking into account the change in sample volume with storage (original to final volume, Table 4.5) and the 1:1 dilution of the original sample with ethanol (i.e. 2x dilution, DF). Thereafter the measured COD/VSS ratios (f_{CV}, Table 4.5) were used to convert from VSS to COD concentration units, i.e.:

$$Z_{BH} = \frac{\text{active cell numbers} \cdot F_{VB} \cdot f_{CV} \cdot \text{original volume}}{\text{measured vol} \cdot 1000 \cdot DF} \quad (4.3)$$

The COD concentration units for OHO and AO active biomass concentrations are listed in Table 4.5.

Table 4.5 (a): Results from DAPI and FISH enumeration of OHO and AO active biomass concentrations on mixed liquor (ML) drawn from the UCT operated anoxic/aerobic 10d sludge age parent activated sludge system (Fig. 4.1): Sewage (Sew.) batch numbers, date of test, volume (Vol.) of original sample and when analysis done, measured cell counts for OHO (Z_{BH}) and AO (Z_{BA}) active biomasses, and measured and theoretical OHO (Z_{BH}) and AO (Z_{BA}) active biomass concentrations in the parent system (PS), taking due account of dilution.

ANOXIC/AEROBIC PARENT SYSTEM: 10d SLUDGE AGE										
Sew. Batch	Date of Test	Sample Vol. (mℓ)		Cell Counts (cells/mℓ)		Meas. COD/VSS ratio, f_{CV} (mg/mg)	PS Z_{BH} (mgCOD/ℓ)		PS Z_{BA} (mgCOD/ℓ)	
		Original	Final	Z_{BH}	Z_{BA}		Meas.	Theo.	Meas.	Theo.
17	17/04	20	19	2.088e+09	6.4e+08	1.41	525	1442	161	26
	21/04	20	18	1.996e+09	8.7e+08	1.41	529	1442	230	26
18/02	14/10	20	18	2.059e+09	3.7e+08	1.55	602	1818	109	68
19/02	28/10	20	18	1.949e+09	8.3e+08	1.58	581	1677	247	51
20/02	04/11	20	18	2.305e+09	2.7e+08	1.5	652	1705	76	49
21/02	25/11	20	18	3.460e+09	3.0e+08	1.47	960	1599	82	68
	03/12	20	18	2.961e+09	1.4e+08	1.47	821	1599	38	68
22/02	10/12	20	18	2.936e+09	3.6e+08	1.38	764	1336	95	42
	16/12	20	18	3.560e+09	3.3e+08	1.38	927	1336	86	42
23/02	24/12	20	18	3.669e+09	7.2e+08	1.46	1011	1352	199	50
01/03	06/01	20	18	2.980e+09	3.1e+08	1.43	804	1466	83	56
	13/01	20	18	2.915e+09	1.0e+09	1.43	786	1466	271	56
02/03	23/01	20	18	1.593e+09	4.4e+08	1.54	463	1482	129	75
	31/01	20	18	3.180e+09	6.1e+08	1.54	924	1482	176	75

Table 4.5 (b): Results from DAPI and FISH enumeration of OHO and AO active biomass concentrations on mixed liquor (ML) drawn from the UCT operated anoxic/aerobic 20d sludge age parent activated sludge system (Fig. 4.1): Sewage (Sew.) batch numbers, date of test, volume (Vol.) of original sample and when analysis done, measured cell counts for OHO (Z_{BH}) and AO (Z_{BA}) active biomasses, and measured and theoretical OHO (Z_{BH}) and AO (Z_{BA}) active biomass concentrations in the parent system (PS), taking due account of dilution.

ANOXIC/AEROBIC PARENT SYSTEM: 20d SLUDGE AGE										
Sew. Batch	Date of Test	Sample Vol. (mℓ)		Cell Counts (cells/mℓ)		Meas. COD/VSS ratio, f_{CV} (mg/mg)	PS Z_{BH} (mgCOD/ℓ)		PS Z_{BA} (mgCOD/ℓ)	
		Original	Final	Z_{BH}	Z_{BA}		Meas.	Theo.	Meas.	Theo.
17	17/04	20	18	1.411e+09	5.0e+08	1.39	371	1053	132	27
	21/04	20	18	1.950e+09	5.1e+08	1.39	513	1053	133	27
18/02	14/10	20	18	1.789e+09	3.4e+08	1.48	510	1233	94	45
19/02	28/10	20	18	2.013e+09	5.9e+08	1.68	638	1222	187	73
20/02	04/11	20	18	1.796e+09	3.0e+08	1.58	535	1212	90	49
21/02	25/11	20	18	3.664e+09	7.0e+08	1.51	1044	1257	199	65
	03/12	20	18	2.058e+09	1.4e+08	1.51	586	1257	39	65
22/02[1]	10/12	20	18	3.234e+09	7.3e+08	1.5	915	766	206	45
	16/12	20	18	2.822e+09	6.7e+08	1.5	799	766	190	45
23/02[1]	24/12	20	18	2.740e+09	7.6e+08	1.37	708	518	195	43
01/03	06/01	20	18	4.618e+09	4.6e+08	1.43	1246	1062	124	47
	13/01	20	18	4.403e+09	3.9e+08	1.43	1188	1062	105	47
02/03	23/01	20	18	2.829e+09	4.0e+08	1.5	801	1079	114	72
	31/01	20	18	3.931e+09	7.4e+08	1.5	1112	1079	209	72

[1]Poor parent system mass balances

4.3 RESULTS

4.3.1 Parent laboratory-scale activated sludge systems

Steady state behaviour and derived data for the two parent systems are listed in Tables 4.2 and 4.3. From these tables:

- N mass balances for both systems (Tables 4.3 (a and b) respectively) were consistent and almost invariably in the acceptable range of 90 - 110 %. The only exception was Sewage Batch No. 19/02 for the 20 d sludge age system, at 117%. However, all other measurements for this sewage batch were reasonably consistent, and hence on this basis the data were retained for further analysis.

- COD mass balances for the 10 d sludge age system (Table 4.3a) were good with only 1 sewage batch (No. 18/02) marginally less than the range 90 - 110 % (at 89%). However, COD mass balances for the 20 d sludge age system (Table 4.3b) were more variable, with 3 out of 11 sewage batches significantly less than 90 % (Nos. 21/02 = 72%, 22/02 = 59% and 23/02 = 58%). Examination of the data for these sewage batches indicated lower than expected OUR measurements; this was confirmed by changing the DO probe for Sewage Batch No. 01/03 and the subsequent increase in OUR and COD mass balance (to 100%) values. However, additionally during Sewage Batch Nos. 22/02 and 23/02 significant mixed liquor losses occurred due to pipe blockages and reactor overflows; this is evidenced by the lower mixed liquor concentrations (COD, TKN and VSS) for these two sewage batches (Table 4.3b). Hence the data collected during these two sewage batches should be treated with caution.

- The influent wastewater mean unbiodegradable soluble COD fractions ($f_{S,us}$) were determined to be 0.055 (SSD = 0.009) and 0.053 (SSD = 0.017) for the 10 and 20 d sludge age systems respectively (Tables 4.3 (a and b) respectively). As noted in Chapter 3, $f_{S,us}$ is slightly higher for the shorter sludge age system, but this difference is not significant at the 95% confidence interval (t-test). The values are similar to those determined in Chapter 3 (0.043 and 0.040 respectively).

- The influent wastewater mean unbiodegradable particulate COD fraction ($f_{S,up}$) for the 10 d sludge age system was 0.119 (SSD = 0.042). This value is very similar to that determined by Ubisi *et al.* (1997a,b, $f_{S,up}$ = 0.12) and Cronje *et al.* (2000, 2002b, $f_{S,up}$ = 0.103), but lower than the value determined in Chapter 3 ($f_{S,up}$ = 0.165), all on the same Mitchells Plain wastewater. For the 20 d sludge age system, a number of sewage batches gave $f_{S,up}$ values less than zero (17/02, 22/02, 23/02). No active biomass concentration tests were conducted during Sewage Batch No. 17/02, but both batch tests and microbiological analyses were done during Sewage Batch Nos. 22/02 and 23/02. To determine a more realistic theoretical active fraction for these two sewage batches, the positive $f_{S,up}$ values were averaged to give 0.043 (SSD = 0.047), and this value used to calculate the f_{av}. These f_{av} values were used in all subsequent calculations, but it should be noted that the data for these two sewage batches should be treated with caution.

From the discussion above, it is evident that considerable uncertainty exists with respect to the 20 d sludge age system data collected during Sewage Batch Nos. 22/02 and 23/02; both the COD recoveries and the $f_{S,up}$ values calculated were unacceptably low. Accordingly, although this data will be included in subsequent calculations, it will be appropriately marked.

4.3.2 Batch tests

Batch test data are listed in Tables 4.4 (a and b) for the 10 and 20d sludge age parent activated sludge systems respectively . Measured OHO active biomass concentrations at the start of the batch test (Z_{BH}) are compared with the appropriate theoretical OHO active biomass concentrations in Fig. 4.2.

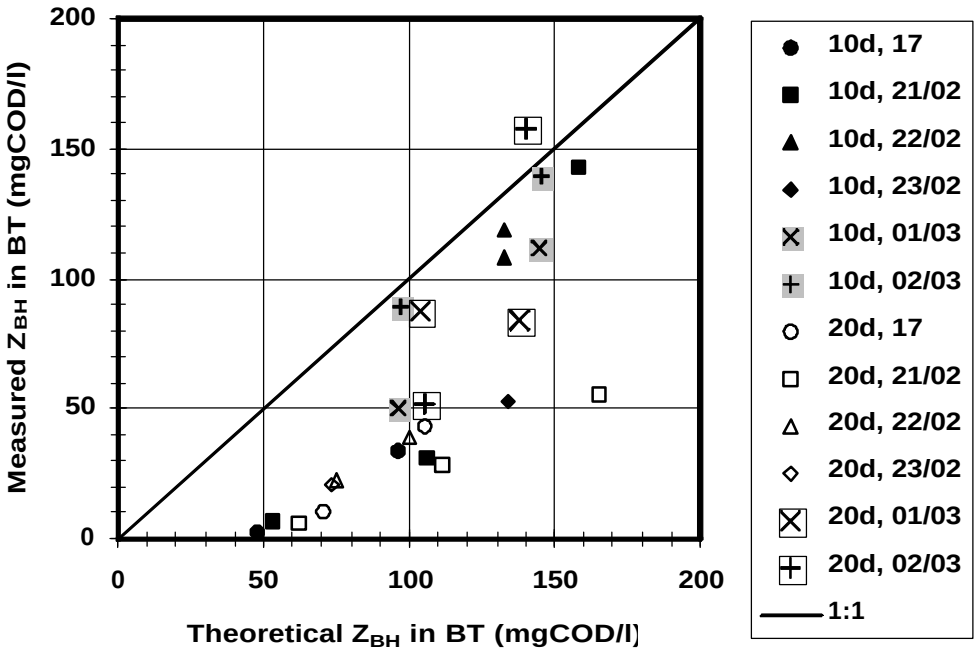

Figure 4.2: Modified batch tests results; measured versus theoretical OHO active biomass concentration at the start of the batch test [Z_{BH}] for the various sewage batches (SB) for the 10 and 20 day sludge age parent activated sludge systems.

From Fig. 4.2, the correspondence between theoretical and batch test measured OHO active biomass concentrations show close similarity to the those in Chapter 3 (Fig. 3.6, range 30 to 150 mgCOD/ℓ), and to the data of Beeharry *et al.* (2001), for both the 10 and 20 d sludge age mixed liquors: As the theoretical values increase, the measured values correspondingly increase parallel to the 1:1 correspondence line, but start to increase sharply at the higher OHO active biomass concentrations. The similarity in the 10 and 20 d sludge age mixed liquor trends confirms the observations in Chapter 3 that any deviations are not sludge age related. Rather, as explored in detail in Chapter 3, the modified batch test method, and its interpretation in terms of the procedures, is particularly sensitive to any changes in the OHO kinetics as time proceeds in the batch test, since these kinetics are applied to the start of the test to determine the OHO active biomass concentration. It was noted that the batch test conditions may cause the overall OHO behaviour to change with time in the batch test, to deviate significantly from that in the steady state system. To explain and model these changes, in Chapter 3 two two OHO population kinetic models were successfully developed. However, it was noted that this may not be the only possible explanation for changes in kinetics with time in the batch test. Whatever the reason,

changes in the kinetics with time can explain the variability in correlations between batch test measured OHO active biomass concentrations and the theoretical values. Unfortunately, the sensitivity of the batch test to kinetic changes with time renders the batch test as an unsuitable method to quantify the OHO active biomass concentration with sufficient accuracy. It was noted, however, that the test does hold merit as a tool to investigate OHO population dynamics, as demonstrated by Beeharry *et al.* (2001) and shown here.

4.3.3 DAPI/FISH cell enumeration

4.3.3.1 OHO active biomass

DAPI/FISH data for OHO active biomass concentrations are listed in Tables 4.5 (a and b) for the 10 and 20 d sludge age parent activated sludge systems respectively. Measured OHO active biomass concentrations in the parent systems (Z_{BH}) are compared with the appropriate theoretical OHO active biomass concentrations in Fig. 4.3.

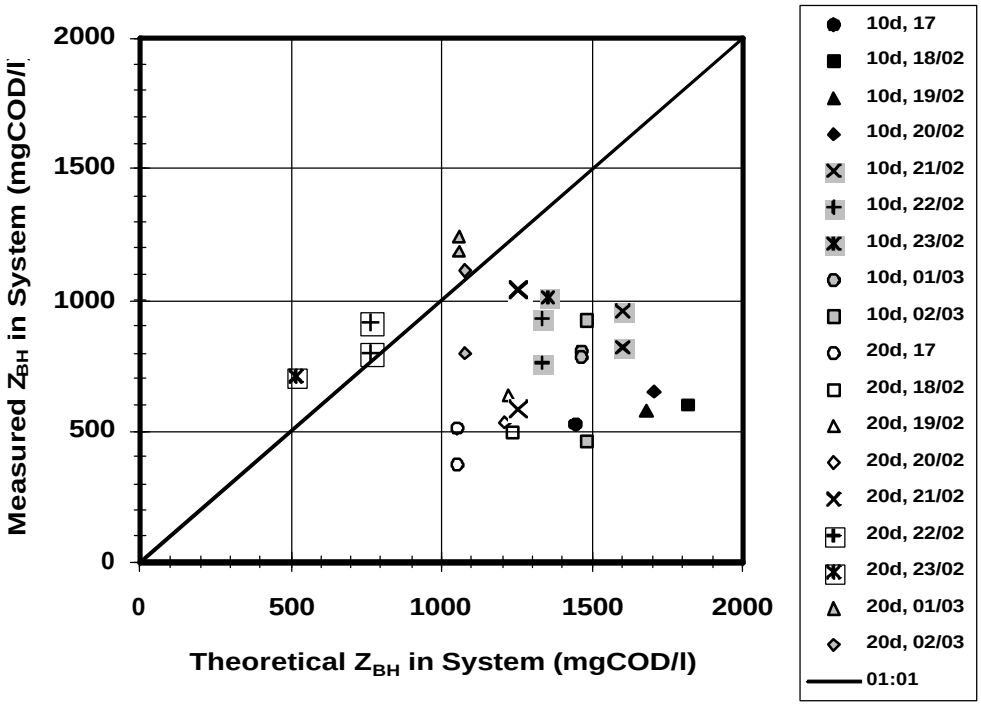

Figure 4.3: DAPI/FISH enumeration results for OHO active biomass; measured versus theoretical OHO active biomass concentration (Z_{BH}) in the 10 and 20 day sludge age parent activated sludge systems for the various sewage batches.

From Fig. 4.3, there is a significant improvement in the DAPI/FISH determined OHO active biomass concentration over those determined under the previous research contract (Fig. 2.7): The measured values are significantly higher than previously, and closer to the theoretical values. This improvement can be directly ascribed to the change in sample preparation procedures (from ethanol preservation to paraformaldehyde fixation) and the method of couriering samples to DIT (from dry ice to ice packs). Further, with each successive batch of samples couriered to DIT

(Batch 1, Sewage Batch No. 17; Batch 2, Sewage Batch Nos. 18/02, 19/02, 20/02; Batch 3, Sewage Batch Nos. 21/02 - 02/03), the determined OHO active biomass concentrations increased, to move closer to the theoretical values. This improvement may be due to refinement and improvement in application of the fixation and/or DAPI/FISH methodologies. While the correspondence between the measured and theoretical OHO active biomass concentrations is by no means perfect, the microbiological methods show considerable promise, and are providing results that can be correlated directly to the theoretical framework for activated sludge systems developed within the engineering and technology paradigm.

4.3.3.2 AO active biomass

DAPI/FISH data for AO active biomass concentrations are listed in Tables 4.5 (a and b) for the 10 and 20d sludge age parent activated sludge systems respectively. Measured AO active biomass concentrations in the parent systems (Z_{BA}) are compared with the appropriate theoretical AO active biomass concentrations in Fig. 4.4.

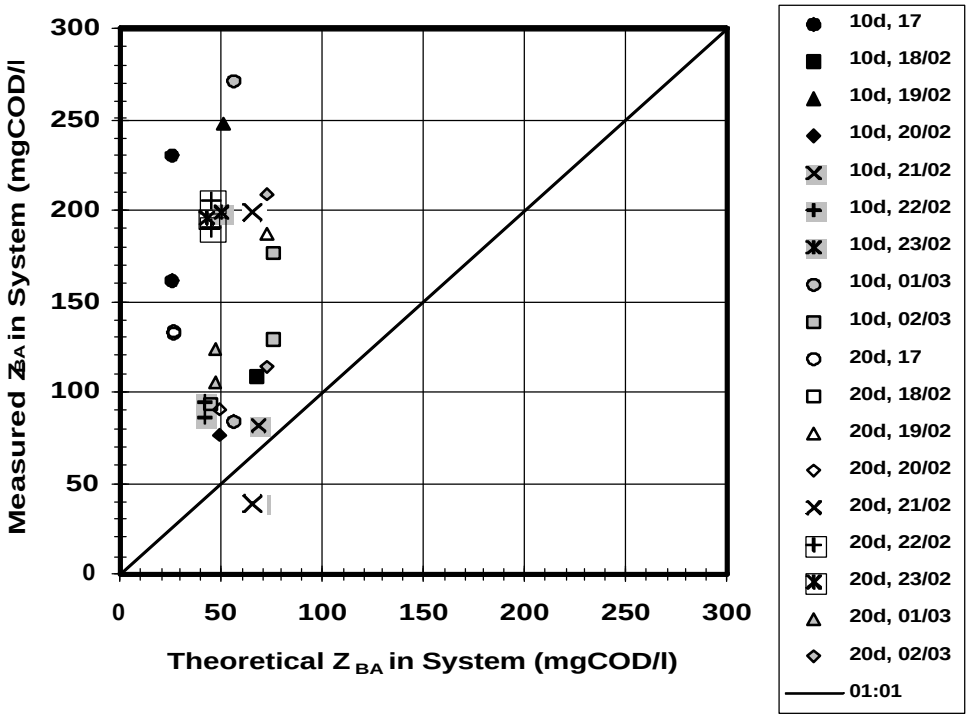

Figure 4.4: DAPI/FISH enumeration results for AO active biomass; measured versus theoretical AO active biomass concentrations (Z_{BA}) in the 10 and 20 days sludge age parent activated sludge systems for the various sewage batches.

From Fig. 4.4, almost invariably the DAPI/FISH determined AO active biomass concentrations are higher than the corresponding theoretical values. Perhaps "double counting" of the AOs occurred with the three probes implemented - this requires investigation. Of particular concern is the wide range of measured values compared with the theoretical values (from 1.5 to 5X). This would suggest that the resolution of

the experimental method is inadequate, and this requires further investigation. However, encouraging is that the measured values are of the same order of magnitude as the theoretical values, which is one order of magnitude smaller than the OHO active biomass concentrations.

4.3.4 Comparison between DAPI/FISH cell enumeration, batch test and theoretical OHO active biomass

DAPI/FISH determined OHO active biomass concentrations are listed in Tables 4.5 (a and b) and batch test determined values in Tables 4.4 (a and b) for the 10 and 20 d sludge age parent activated sludge systems respectively, and the measured values are compared with the theoretical values in Fig. 4.5 and with each other in Fig. 4.6.

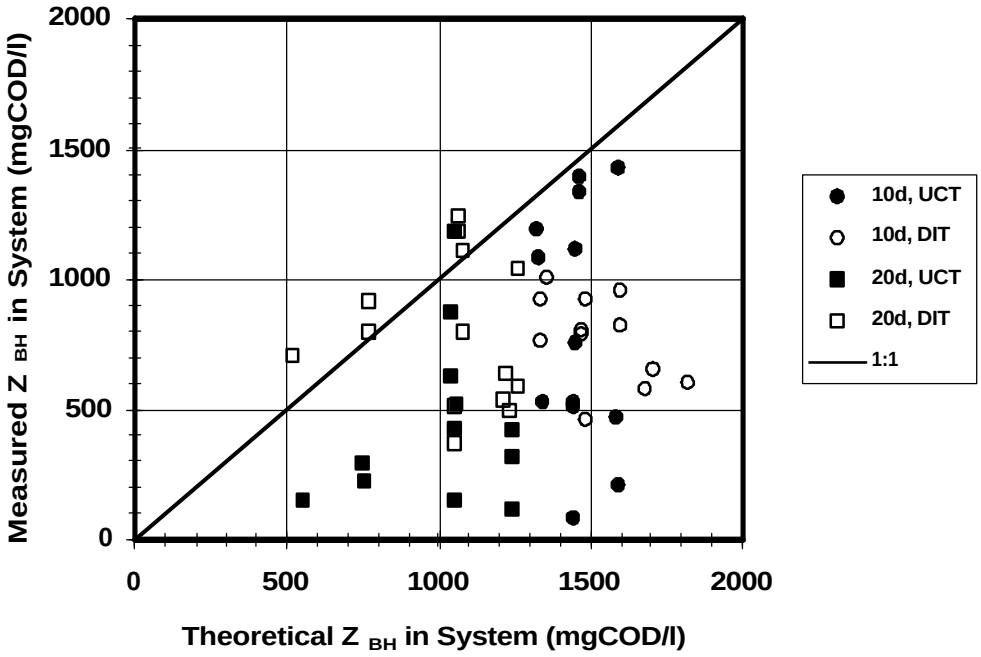

Figure 4.5: DAPI/FISH (DIT) and batch test (UCT) determined values for OHO active biomass concentrations (Z_{BH}) versus corresponding theoretical values in the 10 and 20 days sludge age parent activated sludge systems.

From Figs. 4.5 and 4.6, both the DAPI/FISH and batch test methods tend to underestimate the OHO active biomass concentrations compared with the theoretical values. Of interest is that the batch test tends to give higher OHO active biomass concentrations than the DAPI/FISH method for mixed liquor drawn from the 20 d sludge age parent system, but this trend is reversed for mixed liquor from the 10 d sludge age parent system. No explanation for this variation is evident. However, what is evident is that a procedure has been developed that enables quantitative measurements made with the new microbiological analytical methods to be converted to values that are directly comparable to the modelling framework. This will enable

the information developed with the new analytical methods to be directly applicable to validate and improve models for activated sludge systems.

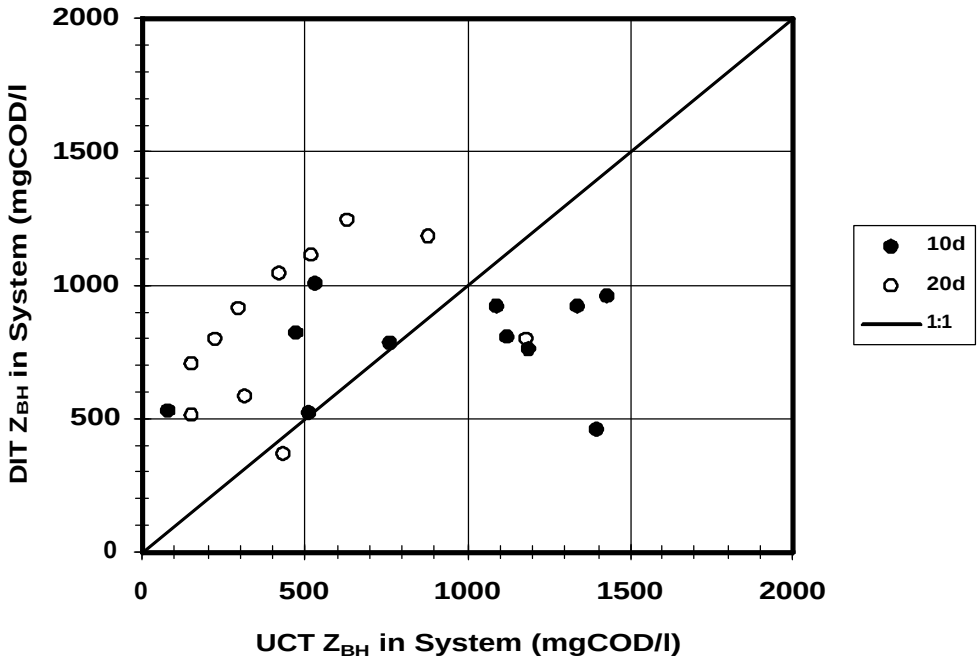

Figure 4.6: DAPI/FISH (DIT) determined values for OHO active biomass concentrations (Z_{BH}) versus and corresponding batch test (UCT) values in the 10 and 20 days sludge age parent activated sludge systems.

4.4 CLOSURE

In this Chapter an attempt has been made to create cross-links between the engineering and technology of activated sludge systems and the microbiological analytical methods. The Water Research Group at UCT operated laboratory-scale activated sludge systems under closely controlled and defined conditions; this enabled the theoretical OHO and AO active biomass concentrations to be calculated within the engineering and technology (modelling) paradigm for activated sludge. Additionally, the modified batch test method (Chapter 3) was run on mixed liquor samples drawn from the parent activated sludge systems. The research group at DIT used a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and AO active biomass concentrations in samples regularly drawn from the laboratory-scale activated sludge systems operated at UCT. From a comparison of the results for OHO active biomass from the various research groups, it is apparent that:

- The batch test method of UCT gave:
  - For the mixed liquors at both 10 and 20 d sludge age, as the theoretical OHO active biomass concentrations in the batch test increase, the

measured values correspondingly increase parallel to the 1:1 correspondence line, but start to increase sharply at the higher OHO active biomass concentrations.
- The similarity in the 10 and 20 d sludge age mixed liquor trends indicates that any deviations are not sludge age related.

The observations above are very similar to those in Chapter 3 and of the preceding research contract (Cronje *et al.*, 2002b), and have been explored in detail in Chapter 3.

- The DAPI/FISH method applied by TN gave:

  - Significantly improved estimates for the OHO active biomass concentrations compared with that obtained in the previous research contract. This improvement could be ascribed to the change in sample preparation procedures (changing from ethanol to paraformaldehyde fixing) and the method of couriering the samples to DIT (from dry ice to ice packs).
  - Despite the improvement above, the measured OHO active biomass concentrations were generally lower than the corresponding theoretical values.

  While the correspondence between measured and theoretical OHO active biomass concentrations are by no means perfect, it appears that the microbiologically based methods are showing considerable promise, and are providing results that can be directly correlated to the theoretical framework for activated sludge systems.

  Comparing the batch test and DAPI/FISH measured and the theoretical OHO active biomass concentrations:

- Both the batch test and DAPI/FISH methods tend to underestimate the OHO active biomass concentrations compared with the theoretical values.

- The DAPI/FISH method tends to give higher estimates for OHO active biomass concentrations than the batch test method for the 20 d sludge age mixed liquor, but this trend is reversed for the 10 d sludge age mixed liquor. No explanation for this variation is evident.

For the AO active biomass concentration, the DAPI/FISH procedure gave:

- Measured concentrations that are almost invariably higher than the theoretical values. Once possible explanation identified for this is “double counting” of the AOs with the three probes implemented, and this requires investigation.

- Considerable variation in measured concentrations compared with theoretical values. This would suggest that the resolution of the experimental method is inadequate, and requires investigation.

Particularly encouraging is that the AO active biomass concentrations are of the same order of magnitude as the theoretical values, which is an order of magnitude smaller that the OHO active biomass concentrations.

Thus, a procedure has been developed that enables quantitative measurements made with the new microbiological analytical methods of active cell numbers to be converted to values that are directly comparable with the activated sludge modelling framework, namely the OHO and AO active biomass concentrations. This will enable the information derived from these analytical techniques to be directly applied in assessing, validating and improving mathematical models for activated sludge systems. Initial results from the procedure show considerable promise, but the procedure does require more intensive investigation and refinement.

In the procedure, converting from cell counts (the output from the microbiological methods) to VSS (the basic unit of measurement in the engineering and technology models) or COD (VSS converted to COD via a direct measure of the COD/VSS ratio) requires a conversion parameter. This conversion parameter was determined by Holder-Snymann *et al.* (2004) from averaging the values they measured on 12 pure cultures of different species, that measured by Sanden *et al.* (1996) on a pure culture of *Nitrobacter sp.* and those measured for mixed liquor samples from a pilot- (Mudaly *et al.*, in press) and a full-scale (Van Munch and Pollard, 1997) plant, to be $F_{VB} = 8.49 \times 10^{-11}$ mg VSS/cell (samples from 15 sources; range 1.14×10^{-10} to 4.35×10^{-11}; sample standard deviation 3.60×10^{-11}). Holder-Snymann *et al.* (2004) used pure cultures to minimise (but could not be eliminated) inert and endogenous residue contributions to the VSS measurements. Clearly, the value for this parameter is central to the inter-linking of the analytical techniques to the models, and requires further evaluation.

CHAPTER 5

DISCUSSION AND FUTURE WORK

5.1 INTRODUCTION

In this research consultancy (K8/453; April 2002 - March 2003) and the preceding research contract (K5/1179; Jan 2000 - Dec 2001; Cronje *et al.*, 2002), it has been endeavoured to address the deficiency in the current steady state design (*e.g.* WRC, 1984; Wentzel *et al.*, 1990; Maurer and Gujer, 1994) and kinetic simulation (*e.g.* Dold *et al.*, 1980, 1991; Van Haandel *et al.*, 1981; Henze *et al.*, 1987; Wentzel *et al.*, 1992; Henze *et al.*, 1995) models for activated sludge systems, of the lack of direct experimental validation of the active biomass concept. The activated sludge system models almost universally include active biomass concentrations explicitly as parameters (OHO, AO and PAO) and the relevant specific rates are expressed in terms of these concentrations (e.g. denitrification in terms of OHO active biomass concentration). The active biomass concentrations form part of the measurable mixed liquor organic concentrations (such as VSS, COD), the other parts being endogenous and inert masses. (Recently, the active biomass concept has been followed in models for other unit processes in wastewater treatment systems, such as that for anaerobic digestion of sewage sludges, ADM 1, Batstone *et al.*, 2002). The activated sludge system models, and the underlying concepts, have attained widespread international acceptance. This acceptance has been achieved through the successful application of the models in design and simulation of a wide variety of activated sludge systems, with the applications facilitated by the general availability of the models in a number of very "user-friendly" computer programmes (e.g. Biowin, WEST, GPX, Simba). However, the underlying fundamental concept of the active biomass (essentially a hypothetical construct of the models) has not been directly validated, only indirectly through consistency between observations and predictions over a wide range of conditions (e.g. Dold *et al.*, 1980, 1991; Alexander *et al.*, 1980; Van Haandel *et al.*, 1981; Warner *et al.*, 1986). Accordingly, to address this deficiency the research funded by the Water Research Commission was initiated, with objective to measure the OHO active biomass concentration. This was to be in terms of the engineering and technology based modelling framework.

In initiating the research, it was recognised that parallel to the developments in the modelling of activated sludge systems and the engineering/technology of this system, significant advances had taken place within the microbiological/biochemical based research into activated sludge systems. In particular, a number of novel analytical techniques had been developed that enabled the study of microorganisms *in situ* within the activated sludge environment, e.g. ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), quinone profiling (Hu *et al.*, 1998), microautoradiography (Nielsen *et al.*, 1998), using florescent probes for ribosomal RNA (Wagner *et al.*, 1994; Water Sci. Technol., 1998). However, it was noted that very little cross-linking was occurring between the engineering/technology and microbiological/ biochemical areas of activated sludge research. To facilitate links, it was noted that the new developments in the microbiological and biochemical analytical techniques can be implemented to address the deficiency in the engineering and technology based models for activated sludge systems, of the active biomass

concept. This should prove possible because, in contrast to the more traditional analytical techniques, the new techniques provide quantitative information, a prerequisite for modelling. Hence, the information from the microbiological/biochemical studies may have direct beneficial application in modelling of the activated sludge system. Accordingly, collaborative parallel research projects were initiated with the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) (K5/1191; Jan 2000- Dec 2001) and the Centre for Water and Wastewater Research at the Durban Institute of Technology (K5/1178; Jan 2000 - March 2003), see Chapter 1. In these projects the OHO active biomass concentration was to be measured with the new microbiological/ biochemical analytical techniques for comparison with the measurements made in terms of the engineering and technology based modelling framework. This would establish a common basis and "language" for the two research approaches, to facilitate exchange of information and development of cross-linkages.

5.2 RESEARCH OBJECTIVES

The main research objectives for the preceding contracts and this consultancy were to:

(1) Measure the ordinary heterotrophic organism (OHO) active biomass concentration within the engineering and technology (E & T) paradigm, and
(2) attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiology and biochemistry paradigms.

5.3 MODIFIED BATCH TEST FOR OHO ACTIVE BIOMASS CONCENTRATION

With regard to objective (i) above, a simple respirometric based batch test procedure had been developed to quantify the OHO active biomass concentration (Kappeler and Gujer, 1992; Wentzel *et al.*, 1995; Mbewe *et al.*, 1995), based on the concepts incorporated in the models. Initial evaluation of this batch test method, and comparison of the OHO active biomass concentrations derived from the test with theoretical concentrations from the models had yielded mixed results (Ubisi *et al.*, 1997a,b; Wentzel *et al.*, 1998). Hence, the research started by examining this test procedure more closely.

5.3.1 Previous research contract

Under the previous research contract considerable progress was made in modifying and refining the batch test method of Ubisi *et al.* (1997a,b) to measure the OHO active biomass concentration, principally by flocculating and filtering the wastewater prior to addition to the test. This greatly simplified the test procedure, as the double parallel batch tests of Ubisi *et al.* were reduced to a single batch test. In evaluation of this modified batch test on mixed liquor samples drawn from well defined and controlled laboratory-scale activated sludge systems, it was noted that there was (Cronje *et al.*, 2002):

- Remarkable similarity in the correlation between theoretical and measured OHO active biomass concentrations for mixed liquor drawn from parallel *control* and *experimental*

(with addition of toilet paper to the influent to alter the mixed liquor composition) Modified Ludzack Ettinger *(MLE*, anoxic-aerobic) activated sludge systems;

- linearity of results with "serial" dilutions; and

- a consistent progressive change in behaviour detected by the batch test in changing from a *MLE* to a *fully aerobic* configuration.

It was concluded that these observations all indicate that the batch test method is a valuable tool for examining activated sludge system behaviour. However, it was noted that there was a lack of a 1:1 correlation between theoretical and measured values, and that this requires further investigation. In this regard, the possibility of P limitation due to aluminium sulphate flocculation of the wastewater should be examined more closely.

5.3.2 Current research contract

The observations above led to the research in this area of this consultancy.

5.3.2.1 Modified batch test evaluation

In this research, the modified batch test method has been extensively evaluated, by conducting a number of batch tests on mixed liquors drawn from laboratory-scale MLE activated sludge systems at 10 and 20 d sludge age (35 on mixed liquor from each system) and comparing the measured OHO active biomass concentrations to the theoretical values predicted by the steady state design models. From this evaluation, it was found that:

- The data for both the 10 and 20 d sludge age mixed liquors showed very similar trends and hence the differences between predicted and measured concentrations were not sludge age related.

- Superficially both sludge age comparisons bore a strong resemblance to the data from the preceding contract (Cronje *et al.*, 2002) in that as the theoretical OHO active biomass concentrations increase, the measured values increase virtually parallel to the 1:1 correspondence line, but below it (Figs. 3.6a and b).

- The cause for the difference between predicted and measured OHO active biomass concentrations was not P limitation, since parallel batch tests with and without P supplementation gave near identical results.

5.3.2.2 Batch aerobic digestion

To determine whether the differences between measured and theoretical OHO active biomass concentrations lay in the activated sludge theory or in the modified batch test procedure, the alternative aerobic batch digestion method of Marais and Ekama (1976) was applied to mixed liquor samples drawn from the 10 and 20 d sludge age MLE activated sludge systems. These tests gave very close correlation between measured and theoretical OHO active biomass concentrations (Fig. 3.10). From this it could be concluded that:

- The close correlation provides substantive support for the OHO active biomass concept as incorporated in the activated sludge models

- Recognising that the modified batch test is based on the OHO active biomass growth processes (the increase in the concentration in the test needs to be significant compared with the starting concentration) whereas the aerobic batch digestion test is based on the endogenous respiration/"death" processes, it appears that the cause for the differences between the modified batch test measured and the theoretical concentrations lies in the description and interpretation of the OHO growth processes within the modified batch test itself.

5.3.2.3 Evaluation of OHO behaviour in modified batch tests

This focussed attention on the observed response within the modified batch test. The data collected in these tests were examined more closely. Particularly evident was that:

- The OHO maximum specific growth rate on RBCOD (μ_H) appears to be a function of the theoretical OHO active biomass concentration added at the start of the test (Fig. 3.11). Re-evaluation of the data collected under the preceding contract indicated that a similar trend was obtained, but not noted. It was noted that the influence of initial substrate to active biomass concentration ratios (in this set of modified batch tests the initial substrate concentration was kept approximately constant) on batch test behaviour and derived kinetics had been observed previously (e.g. Chudoba *et al.*, 1992; Novak *et al.*, 1994; Grady *et al.*, 1996).

- In a plot of $\ell n\ OUR_H$ versus time, the measured data frequently deviated from the fitted linear regression line, showing upward curvature (Fig. 3.14). This strongly suggested that the net OHO maximum specific growth rates change during the course of the batch test, in agreement with the observations of Pollard *et al.* (1998).

- The observed precipitous drop in OUR implies that the OHO specific growth rates were at their maxima, *i.e.* the changes observed were not due to varying substrate concentrations with time or between batch tests.

- The higher μ_H values (up to about 7/d, Fig. 3.11) were significantly higher than the range of values accept as default in the UCT kinetic model (1.5 to 3.5/d, Dold *et al.*, 1991).

- The batch test measured OHO active biomass concentrations tended to be consistently lower than the corresponding theoretical values (Fig. 3.15).

It was concluded that the observations above suggest that:

- The OHO maximum specific growth rates increase with time in the batch test, and that this behaviour cannot be accommodated in the analytical procedure for the batch test which is based on a single OHO population with fixed kinetics.

5.3.2.4 Competition kinetic models

To explain observations similar to those above, Novak *et al.* (1994) and Grady *et al.* (1996) proposed substrate competition between different OHO groups as a possible cause. This possibility was investigated, by developing a model for kinetic competition between two OHO populations, one a fast grower and the other a slow grower, based on the concepts of Novak *et al.* (1994). The second OHO group was incorporated into the simplified UCT kinetic model used to analyse the batch test data (Wentzel *et al.*, 1995), as follows (Chapter 3; Lee *et al.*, 2003): (1) The single OHO active biomass was subdivided into two OHO active biomasses, a fast grower (Z_{BH1}) and a slow grower (Z_{BH2}); (2) All OHO mediated processes were duplicated, with the new processes allocated to the second OHO group; (3) The adsorbed SBCOD was split into two, S_{ads1} and S_{ads2}, utilized by Z_{BH1} and Z_{BH2} respectively.

The kinetic model was applied to both the (i) batch tests and (ii) parent systems, using AQUASIM 2.0 (Reichert, 1994). For application to the batch tests, the batch test data with the greatest surety were selected, $30 < Z_{BH(0)} < 150$ mgCOD/ℓ and those with good mass balances (Fig. 3.15). Values for all constants except those related to OHO growth on RBCOD were those of Dold *et al.* (1991). With regard to μ_{Hm} and K_{SH} on RBCOD of Z_{BH1} and Z_{BH2}, these were assumed as 12/d and 3 mgCOD/ℓ and 2/d and 0.1 mgCOD/ℓ respectively, to ensure kinetic competition on RBCOD and that the precipitous drop in OUR could be correctly predicted. Parameters estimated with AQUASIM were initial concentrations of $Z_{BH1(0)}$ and $Z_{BH2(0)}$, and "substrate". To reduce the complexity of parameter estimation, the initial substrate was ascribed to RBCOD only. This restricted the parameter estimation to the period up to the OUR precipitous drop. From the application to the batch tests:

- The model could accurately simulate the OUR_H - time observed in batch tests (Fig. 3.16).

- Parameter estimation of the initial batch test $Z_{BH1(0)}$ and $Z_{BH2(0)}$ concentrations gave exceptionally low $Z_{BH1(0)}/(Z_{BH1(0)}+Z_{BH2(0)})$ values, average 1%. However, with time Z_{BH1} increased its proportion significantly and had a marked influence on the predicted OUR_H - time profile (Fig. 3.17). This indicates that the batch test procedure is extremely sensitive to the presence of fast growing OHOs.

- The model could simulate the variety of observations made on the batch tests, including the increase in overall OHO maximum specific growth rates with increasing S_0/Z_{BH} and with time.

- In seeking a source for the fast growing OHOs in the batch test, it was noted that this could not the flocculated filtered wastewater added to the batch tests (no observable OUR after 12 hours aeration), and so must be from the mixed liquor drawn from the parent systems.

The competition kinetic model was also applied to simulate the two parent systems, with the same set of kinetic and stoichiometric constants used for the batch tests. Z_{BH1} was accepted to be seeded with the influent wastewater to the parent systems, at 0% - 3% of total COD:

- Z_{BH1} could only be sustained in the parent systems if seeded with the influent. Seeding was substantiated by the observations of Wentzel *et al.* (1995) and Cronje *et al.* (2002) who noted significant fast growing OHOs present in the same raw wastewater as used in this investigation.

- With seeding of Z_{BH1} at concentrations typically measured by Wentzel *et al.* (1995) (< 3% of total COD), the predicted Z_{BH1} proportion of the total OHO active biomass (± 40% at seed of 3% of total COD) was significantly larger than that derived from parameter estimation of the batch test data (1%).

To address the second observation above, the kinetic competition model was modified by removing the processes for growth of Z_{BH1} on SBCOD (model now includes kinetic + metabolic selection). This was considered reasonable, since the original source of Z_{BH1} is seeding with the influent wastewater to the parent systems - this implies growth in the sewer where RBCOD concentrations are very high, which would favour predominantly RBCOD utilization. Simulation of the batch tests and parent systems gave results very similar to the competition only model above, except that for influent Z_{BH1} at 3% of total COD, the predicted proportions of Z_{BH1} in the parent system mixed liquors were < 3% compared to ± 40% for the competition only model above. This former value is very close to those from parameter estimation on the batch test data.

In comparing the various estimates for OHO active biomass concentrations, it was found that the two OHO population kinetic models gave concentrations that were significantly closer to the theoretical values (Fig. 3.19) than the single OHO population model (Fig. 3.15). Thus, it could be concluded that:

- The competition hypothesis (in agreement with previous researchers) is one feasible explanation for the observations in the batch tests.

However, the kinetic models developed here are largely hypothetical - insufficient information is available to separate the two OHO populations and quantify the individual kinetic processes. Further, alternative hypotheses could possibly explain the observed behaviours, e.g. physiological adaptation (Daigger *et al.*, 1982). Clearly, this requires further investigation. Such investigations may be facilitated by the microbiologically based analytical techniques, *see below.*

5.4 EVALUATION OF MICROBIOLOGICAL METHODS

5.4.1 Previous research contracts

In the previous collaborative research projects (K5/1179 UCT, K5/1191 UP, K5/1178 DIT) attempts were made to link the batch test measurements and the defined engineering environment to the new biochemical and microbiological analytical technique results. In these parallel projects, various test methods were applied by the different research groups to quantify OHO active biomass concentrations, and the results from the test methods were compared with each other and with the theoretical OHO active biomass concentrations. The Water Research Group at UCT operated parent aerobic and anoxic/aerobic (MLE) laboratory-scale activated sludge systems under closely controlled and defined conditions; this enabled

the theoretical OHO active biomass concentration to be calculated within the engineering and technology (modelling) framework for activated sludge. Additionally, the modified batch test method to quantify the OHO active biomass concentration was run on mixed liquor samples drawn from the parent activated sludge systems. The research group at UP measured the biochemical compound ATP both *in situ* in the laboratory-scale systems, and during the course of the modified batch tests. The research group at DIT used the microbiological analytical technique of a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and AO active biomass concentrations in samples regularly drawn from the activated sludge systems operated at UCT. From a comparison of the results for OHO active biomass from the various research groups, it was apparent that:

- The microbiological and biochemical test methods gave OHO active biomass concentrations that were several orders of magnitude lower than both the theoretical and batch test measured OHO active biomass concentrations.

In examining possible reasons for this discrepancy, the following possibilities were identified:

- For the ATP method applied by UP, it appears that solids concentrations (i.e. VSS or TSS) interfere in some manner with the ATP measurement method - this would explain the lower values measured in the steady state systems (with higher VSS concentrations) than in the batch tests (with lower VSS concentrations), and the lower ATP measurements in the 20d sludge age steady state system (higher VSS concentrations) than in the 10d sludge age system (lower VSS concentration). It was concluded that the ATP method **as applied** is not a reliable estimate for OHO active biomass concentrations.

- For the DAPI/FISH method applied by DIT, it was noted that in subsequent investigations it was found that the method of couriering the samples in dry ice caused a significant number of the cells to freeze and hence burst. This would reduce the DAPI/FISH enumerated cell counts significantly, and may be one possible explanation for the low cell counts. **This was identified as requiring further investigation.**

Although this initial attempt to link the engineering and technology theoretical and batch test measured OHO active biomass concentrations to the values measured with the microbiological and biochemical analytical techniques did not provide even near a close correspondence, it was concluded that, for the first time, the magnitudes of the microbiological and biochemical measurements have been placed within the context of the engineering and technology models. This should help establish a common basis and "language"for the two paradigm sets, to facilitate future exchange of information and development of cross linkages between them.

5.4.2 Current research consultancy

Since the research project at DIT had a further year to run, to continue investigations into the microbiological measurement of active biomass, this one year consultancy between the WRC and UCT was set up. In this consultancy, the research described above continued, with UCT operating parallel laboratory-scale anoxic/aerobic MLE activated sludge systems at 10 and 20

d sludge age, conducting modified batch tests on mixed liquors harvested from these systems, and harvesting mixed liquor to send to DIT for microbiological analysis, using a combination of DAPI staining and FISH to determine both OHO and AO active biomass concentrations. From the conclusions arising from the preceding contract, that preserving the samples in ethanol and sending these packed in dry ice to DIT resulted in loss of active biomass due to freezing and bursting of cells, the method for sample preparation was changed. Instead, at UCT the samples were drawn from the laboratory-scale systems and immediately fixed with paraformaldehyde (Chapter 4, Section 4.2.3.1). The samples were stored at -20°C and couriered in batches to DIT in cooler boxes with ice packs. This appeared to resolve the problems with sample exchanges experienced in the previous contract.

5.4.2.1 Batch tests

With regard to the batch tests (Fig. 4.2):

- The correspondence between batch test measured and theoretical OHO active biomass concentrations showed close similarity to the data described in Section 5.3.2 above, namely, as the theoretical OHO active biomass concentrations in the batch test increase, the measured values correspondingly increase parallel to the 1:1 correspondence line, but start to increase sharply at the higher OHO active biomass concentrations.

- The similarity in trends between the 10 and 20 d sludge age data confirms the observations above, that any differences are not sludge age related.

As described and investigated above, it was concluded that:

- The batch test conditions cause the OHO behaviour to change with time in the batch test, to deviate significantly from that in the steady state system. Such behaviour cannot be accommodated in the batch test procedure, with interpretation based on a model with a single OHO population with fixed kinetics.

5.4.2.2 Microbiological analysis

From a comparison between DAPI/FISH cell enumeration measured and theoretical OHO active biomass concentrations (Fig. 4.3):

- There was a significant improvement in the measured OHO active biomass concentrations over those determined under the previous contract (Fig. 2.7). This improvement could be ascribed to the change in sample preparation procedures (changing from ethanol to paraformaldehyde fixing) and the method of couriering the samples to DIT (from dry ice to ice packs).

- Despite the improvement above, the measured OHO active biomass concentrations were generally lower than the corresponding theoretical values.

It was concluded that:

- While the correspondence between measured and theoretical OHO active biomass concentrations is by no means perfect, the microbiological methods show considerable promise, and are providing results that can be correlated directly to the theoretical framework for activated sludge systems developed within the engineering and technology paradigm.

Comparing the batch test and DAPI/FISH measured and the theoretical OHO active biomass concentrations (Figs. 4.5 and 4.6):

- Both the batch test and DAPI/FISH methods tend to underestimate the OHO active biomass concentrations compared with the theoretical values.

- The DAPI/FISH method tends to give higher estimates for OHO active biomass concentrations than the batch test method for the 20 d sludge age mixed liquor, but this trend is reversed for the 10 d sludge age mixed liquor. No explanation for this variation is evident.

From a comparison between DAPI/FISH cell enumeration measured and theoretical AO active biomass concentrations (Fig. 4.4):

- Almost invariably the DAPI/FISH determined concentrations are higher that the corresponding theoretical values. One possible explanation identified for this is "double counting" of the AOs with the three probes implemented, and this requires investigation.

- Considerable variation in measured concentrations compared with theoretical values. This would suggest that the resolution of the experimental method is inadequate, and requires investigation.

- However, it was noted that the measured values were of the same order of magnitude as the theoretical values, which is an order of magnitude smaller than the OHO active biomass concentrations, and that this was particularly encouraging.

5.5 CLOSURE

In this and the preceding research projects the main objective has been to address the deficiency in the models for activated sludge systems, of the lack of direct experimental validation of the active biomass concept. This was to be approached by "measuring the OHO active biomass concentration within the engineering and technology paradigm, and to attempt to link these measurements and the defined engineering environment to the new microbiological and biochemical analytical techniques, to create links and even overlap between the engineering and technology and microbiological and biochemistry paradigms".

Within the engineering and technology paradigm, the simple respirometric based modified batch test to quantify OHO active biomass developed in the preceding research contract has been extensively evaluated. In evaluating the modified batch test, it was hoped that the

method would provide measured OHO active biomass concentrations that would compare favourably with the theoretical concentrations predicted by the activated sludge models. This would provide independent validation in a simple way of the active biomass concept in the models, and thereby promote confidence in their application. However, similarly to the research in the previous contract (Cronje *et al.*, 2002), correspondence was not good and the problem more complex than originally thought. In examining possible causes for this lack of correspondence, it was noted that the modified batch test method and its interpretation relies on a single OHO population with constant kinetics (Wentzel *et al.*, 1995; Cronje *et al.*, 2000, 2002). Observations in this and previous research (*e.g.* Daigger *et al.*, 1982; Novak *et al.*, 1994; Grady *et al.*, 1996) suggest that this may not be appropriate, and that the batch test conditions may cause the overall OHO behaviour to deviate significantly from that in the steady state system. This arises because of the requirement that the growth of active biomass in the batch test is significant compared with the initial concentration. Unfortunately, this deviation renders the batch test unsuitable as a simple method to directly quantify the OHO active biomass concentration (and kinetic constants) with sufficient accuracy. The test does, however, hold merit as a tool to investigate OHO population dynamics, as shown by Cronje *et al.* (2002) and here.

The causes for the lack of correspondence have been shown to lie in the modified batch test itself: Batch aerobic digestion tests and re-interpretation of the modified batch test data with competition kinetic models both provided reasonable correspondence between measured and model predicted OHO active biomass concentrations. ***This does provide independent evidence that substantiates the active biomass concept in the models.*** Further, although the competition models needed to be applied to explain the observations in the modified batch tests, it must be remembered that in the parent activated sludge systems, the OHO population is dominated by the slow growing OHO population group, to the extent of near exclusion of the fast growers. Thus, for the activated sludge system the current models incorporating a single OHO population are adequate, provided extremes in dynamic loading are not encountered, *e.g.* with selector reactors (Still *et al.*, 1996). From this work it seems that selector reactors stimulate proliferation in the activated sludge system of the fast growing OHOs, which in the absence of the selector would not be sustained in the system to any significant extent.

In the research aimed at creating links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical methods, a procedure has been developed that enables the quantitative measurements of active cell numbers made with the new microbiological analytical methods to be converted to values that are directly comparable with the activated sludge modelling framework, namely the OHO and AO active biomass VSS or COD concentrations. ***This will enable the information derived from these analytical techniques to be directly applied in assessing, validating and improving mathematical models for activated sludge systems.*** In essence, a common "language" has been established for the two research approaches, to facilitate exchange of information and development of cross-linkages. Initial results from the procedure show considerable promise, but the procedure does require more intensive investigation and refinement.

5.6 FUTURE WORK

From this investigation the following recommendations can be made:

- In the modified batch test a second plateau in the OUR_H-time plot is observed. Cronje *et al.* (2000) and Beeharry *et al.* (2001) ascribed this second plateau to "soluble" SBCOD that was not removed in the flocculation and filtration of the sewage added to the batch test. In the kinetic models for activated sludge systems, a single SBCOD "type" is accepted, with the same kinetics of hydrolysis/utilization applied to all SBCOD. The existence of a soluble (*i.e.* "non-flocculatable") SBCOD needs to be investigated (e.g. Sollfrank and Gujer, 1991), as this may have an impact on modelling the kinetics of SBCOD hydrolysis/utilization, but only under extreme dynamic loading conditions, e.g. in the contact stabilisation system. Also, other possible explanations for the second OUR plateau need to be evaluated, such as intracellular substrate storage. Majone *et al.* (1999) and Carucci *et al.* (2001) reported evidence of aerobic substrate storage under dynamic conditions. The more fundamental microbiological or biochemical analytical techniques may prove useful in this regard.

- The batch aerobic digestion test provided estimates for OHO active biomass concentrations that agree closely with the theoretical values. However, only 4 such tests were undertaken due to the time consuming nature of the tests (> 10days in duration). More such tests need to be undertaken, to confirm the results obtained here. One issue that would require resolution is that in batch aerobic digestion tests, the measured nitrate concentrations were always higher than the predicted concentrations. This was common to all the batch aerobic digestion tests. An explanation for this poor correlation could not be provided.

- To explain the behaviour observed in the modified batch tests, in this research a competition model was proposed. However, hypotheses alternative to the competition one, also could possibly explain the observed behaviour. For example, Daigger *et al.* (1982) and Grady *et al.* (1996) proposed the concept of "physiological adaptation". In this concept, the relative components of the biomass groups (fast grower and slow grower) do not change, but rather the physiological state of the biomass itself, induced by the change in conditions from the steady state system to the batch test (Grady *et al.*, 1996). Such alternatives also require evaluation.

- In this research, in calibrating the competition models, values had to be assumed for the kinetic parameters for the fast and slow growing OHO population groups (μ_{H1}, μ_{H2}, K_{SH1} and K_{SH2}). With the accepted values, the competition model could reasonably accurately simulate the diverse observed behaviour, *e.g.* change in kinetic rates with time in the batch tests, the effect of initial substrate to active biomass ratio on the kinetics. However, the values for the rate constants were not directly determined. This was not possible because the behaviour of the fast and slow growing OHO population groups could not be separated. This requires investigation.

- Converting from cell counts (the output from the microbiological methods) to VSS (the basic unit of measurement in the engineering and technology models) or COD (VSS converted to COD via a direct measure of the COD/VSS ratio) requires a conversion

parameter. The cell count to VSS conversion parameter was determined from averaging the values measured on a number of pure cultures of different species and values in the literature for pilot- and full-scale samples, to be F_{VB} = 8.49 x 10^{-11} mg VSS/cell; pure cultures were used to minimise (but could not be eliminated) inert and endogenous residue contributions to the VSS measurements (Holder-Snymann *et al.*, 2004). Clearly, the value for this parameter requires further evaluation.

- In quantifying the AO active biomass concentration, a series of three FISH probes was implemented. AO active biomass concentrations measured with these probes were consistently higher than the corresponding theoretical values. One possible cause identified for this was "double counting". Also, it appeared that the resolution in the AO cell counts was inadequate. These aspects require evaluation.

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