ACTIVITY OF HETEROTROPHIC AND AUTOTROPHIC BIOMASS IN BNR ACTIVATED SLUDGE

Report to the Water Research Commission on the Project *"Measurement of heterotrophic and autotrophic organism active biomass in biological nutrient removal activated sludge systems"*

by

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EXECUTIVE SUMMARY

1. MOTIVATION FOR THE RESEARCH

To comply with more stringent effluent legislation, over the past two decades significant advances have been made in the areas of engineering (design) and technology (implementation and operation) of the single sludge activated sludge system. Activated sludge systems have been successfully designed and implemented at full-scale to progressively include the biological removal of carbon (C), nitrogen (N) and phosphorus (P). This implementation has been aided by the development of 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; Barker and Dold, 1997). These models are based on a common conceptualization of the processes acting in the activated sludge system. In terms of this group of models, in the bioreactor of the non-nitrifying aerobic activated sludge system, the mixed liquor organic (volatile) suspended solids (MLOSS) is made up of three components; (1) ordinary heterotrophic organism (OHO) active biomass, (2) endogenous residue and (3) inert material. In the nitrifying aerobic and anoxic/aerobic activated sludge systems, a fourth component is included; (4) autotrophic organism (AO) active biomass. All four MLOSS components settle out in the secondary settling tank and are returned to the bioreactor via the underflow recycle; these components leave the system via the waste flow. If an anaerobic reactor is included to stimulate biological excess phosphorus removal (BEPR), additionally (5) phosphate accumulating organism (PAO) active biomass and (6) this organism group's endogenous residue will contribute to the MLOSS (Wentzel *et al*., 1992; Henze *et al*., 1995). The active biomass components of the MLOSS 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.

Historically the MLOSS has been measured as a lumped parameter, via the VSS or COD test (Standard Methods, 1985). Specific rates for the biological processes (e.g. denitrification; oxygen utilization) often were (and still are) expressed in terms of this lumped parameter. However, from the above, only parts of the MLOSS are active biomasses, and only these parts mediate the relevant biological processes, e.g. OHO's for COD removal and denitrification. Accordingly, the specific rates for the relevant (and associated) biological processes should be expressed in terms of the appropriate active biomass concentration to allow a meaningful comparison of rates measured in different systems. 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. However, 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 active biomass exists only hypothetically within the structure 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, e.g. Dold *et al*., 1980, 1991; Alexander *et al*., 1980; Van Haandel *et al*., 1981; Warner *et al*., 1986), these have not been directly measured experimentally and compared to the hypothetical model values. This deficiency casts a measure

of uncertainty on the entire 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 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 had mixed results (Ubisi *et al.*, 1997; Wentzel *et al*., 1998). Thus, uncertainty around the active biomass concept 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 *insitu,* 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). 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 have yet to be realised for the system. It remains for the results of 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 engineering and technology paradigm 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 the measurement of OHO active biomass. This research project investigates measurement of the OHO active biomass parameter within the engineering and technology paradigm. If OHO active biomass can be successfully quantified within this paradigm and agreement obtained between the measurements and the theoretical modelling values, this will provide a basis for comparison with the quantitative data arising from the new microbiological and biochemical analytical techniques. This will facilitate development of a common link between the two paradigm sets. In this research project, an initial investigation into comparing the engineering and technology and the microbiological and biochemical measurement of OHO active biomass also is undertaken. This report summarises these investigations; details are reported by Cronje *et al.* (2000) and Beeharry *et al.* (2001).

2. AIMS AND OBJECTIVES

The objectives of this research project were to:

(i) Measure the ordinary heterotrophic organism (OHO) active biomass concentration within the engineering and technology (E & T) 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.

3. MEASUREMENT OF OHO ACTIVE BIOMASS WITHIN E & T PARADIGM

Ubisi *et al*. (1997a,b) describe the development of a simple batch test to quantify the OHO active biomass concentration. 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 (summarised in Chapter 4). 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 activated sludge system operated at 12 and 20 days 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. 1 (a and b): With the parent system at 12d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d 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.

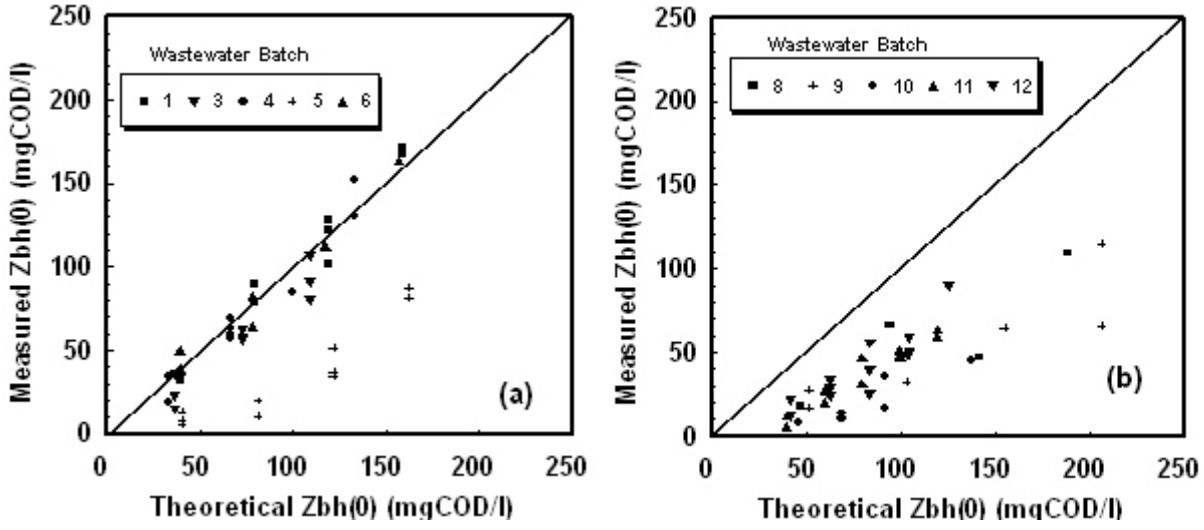

Figure 1: Measured vs. theoretical ordinary heterotrophic organism (OHO) active biomass concentration [$Z_{BH(0)}$] in the batch test due to addition of mixed liquor drawn from the parent system at (a) 12d and (b) 20d sludge age (Wentzel *et al*., 1998).

3.1 Batch test evaluation and modification

In this research project, initially (summarised in Chapter 5; for details see Cronje *et al*., 2000) 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 MLE activated sludge system operated at 10d sludge age. From this evaluation, it became evident that the correlation between measured and theoretical OHO active biomass concentrations was poor (see Fig. 2), and remarkably similar to that obtained by Wentzel *et al*. (1998) on mixed liquor samples drawn from their system operated at 20d sludge age, see Fig. 1(b).

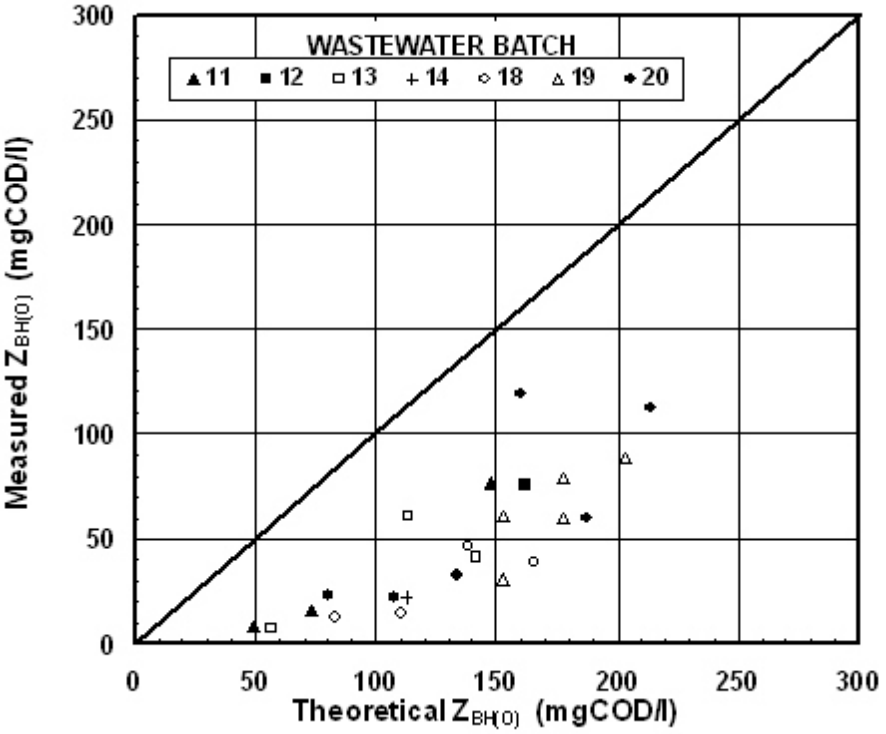

Figure 2: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) in the batch test due to addition of mixed liquor drawn from the parent laboratory-scale system operated at 10d sludge age.

This prompted a detailed investigation into the batch test method. Two sources of potential error in the method were identified:

- 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 the 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.

- Using 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. 3.

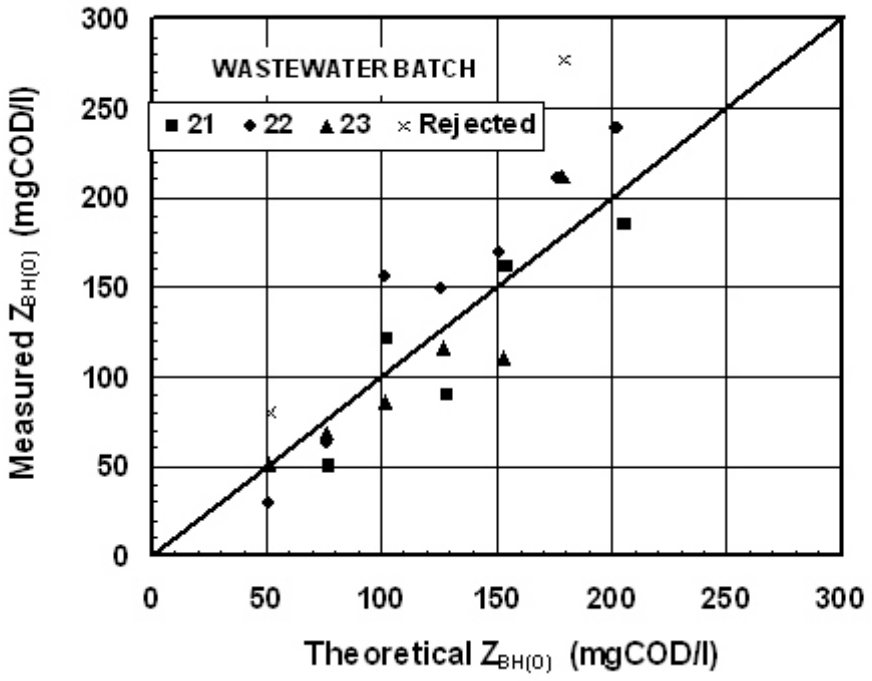

Figure 3: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch test data; theoretical values for WW Batch No. 23 using average active fraction from WW Batch Nos. 21 and 22.

- 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 ℓnOUR_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 system (Chapter

5; for details see Cronje *et al.*, 2000):

- 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 (≈ 2 hours) and only then was the characteristic exponential increase in OUR observed.

- 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 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.

3.2 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 (summarised in Chapter 6, for details see Beeharry *et al.*, 2001). 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:

(1) Anoxic/aerobic MLE system fed raw (unsettled) municipal wastewater (termed ***control***).
(2) 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*.
(3) 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 liquor 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 (3.43 mgO/mgN) is lower than that for nitrification of ammonia-N to nitrate (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 the batch tests with wastewater and mixed liquor conducted by Ubisi *et al*. (1997a,b), they observed that nitrification in these batch tests caused a linear increase in the nitrate concentration with time. Cronje *et al*. (2000) (Chapter 5) 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, Chapter 5) (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. 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 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. 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

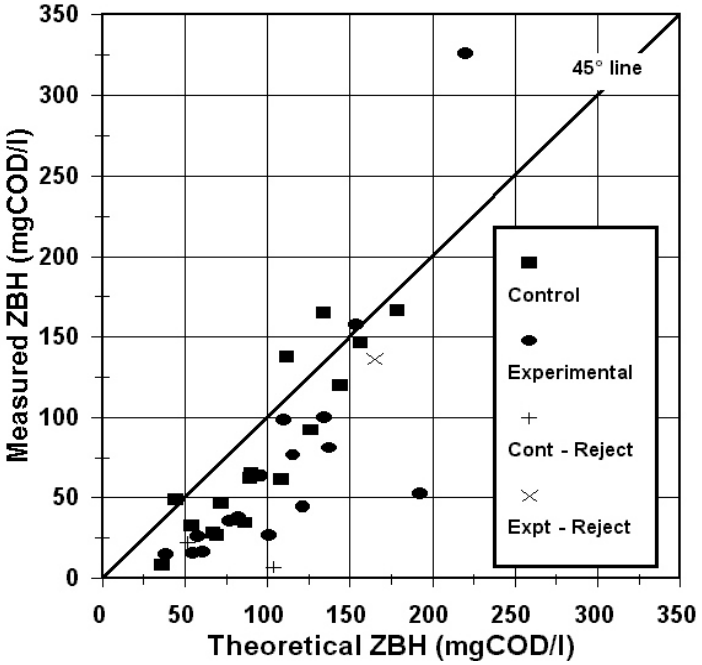

Figure 4: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch tests on mixed liquor drawn both control and experimental parent anoxic/aerobic (MLE) activated sludge systems.

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 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. 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.

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. 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.

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 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 phosphorus. 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, phosphorus 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* was 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 system above. Again, the average values are higher than those measured by Cronje *et al*. (2000, Chapter 5) (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 an increase or decrease in the OHO active biomass concentration. 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.

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 ((3) 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, Chapter 5) (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. 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. 5).

Comparing the data obtained with the mixed liquor drawn from the fully aerobic system (Fig. 5) with that from the two *MLE anoxic/aerobic* systems (Fig. 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. 5): For the batch test conducted during WW Batch No. 23A, the system was operated as an ***MLE*** and the batch test data falls 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, 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.

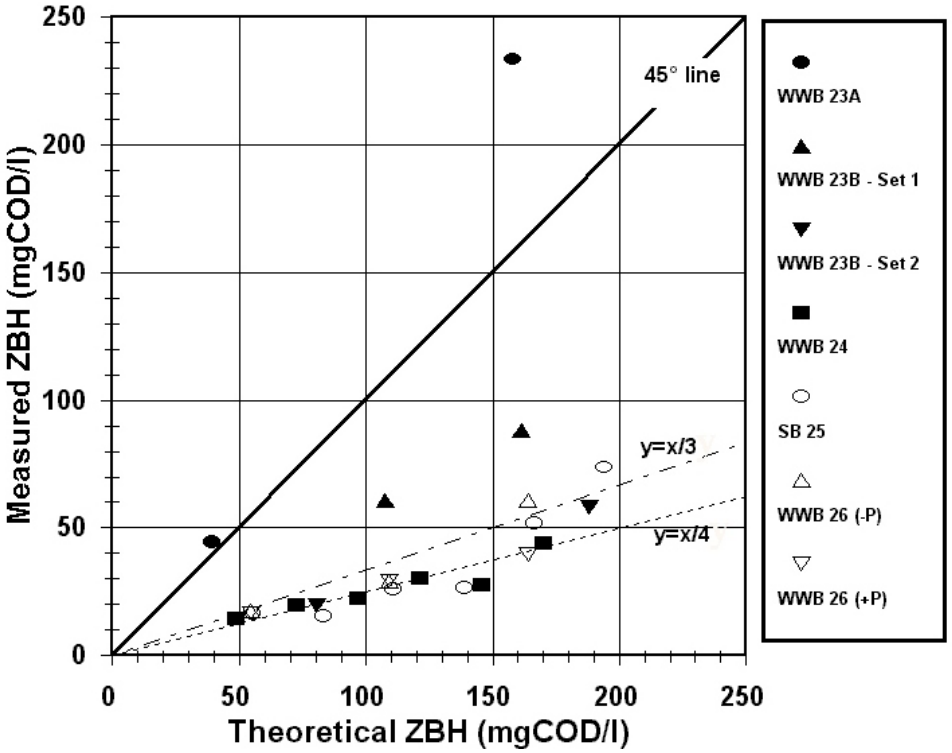

Figure 5: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system.

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.

4. MEASUREMENT OF OHO ACTIVE BIOMASS WITHIN M & B PARADIGM

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 **1. 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 (K5/1191) and the Centre for Water and Wastewater Research at the Technikon Natal (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 (Chapter 7). 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 Section 3 above and Chapters 5 and 6) 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 in Section 3 above. The research group at TN 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 is apparent that:

- 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. 6.
  - For the parent aerobic laboratory-scale activated sludge system, the batch test measured OHO active biomass concentrations are approximately 1/4 the theoretical values, see Fig. 7.

  These observations are in agreement with those in Section 3 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.

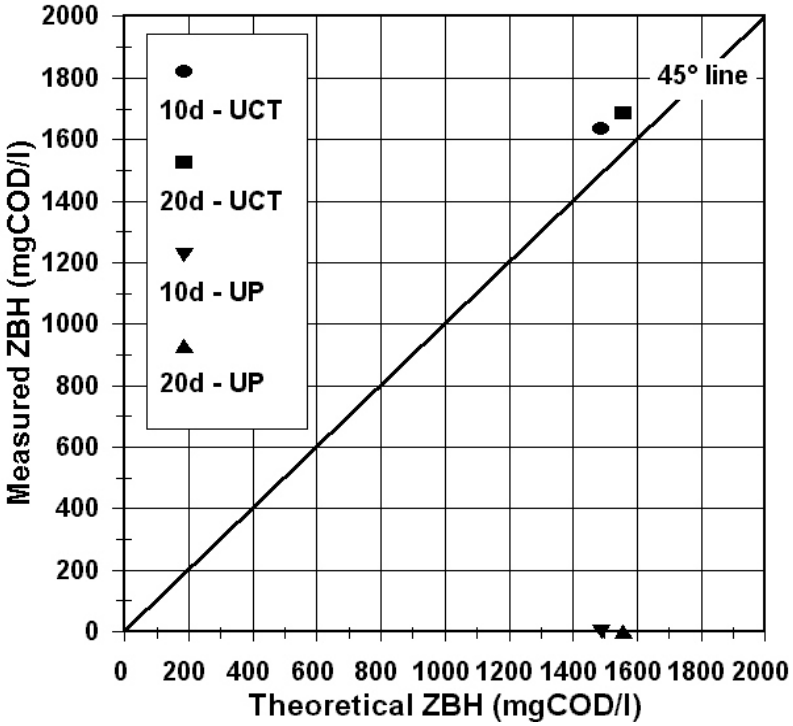

Figure 6: Measured versus theoretical OHO active biomass concentrations in the 10 and 20d sludge age parent laboratory-scale activated sludge systems; measured values from batch test method taking account of dilution (UCT) and ATP measurements (UP).

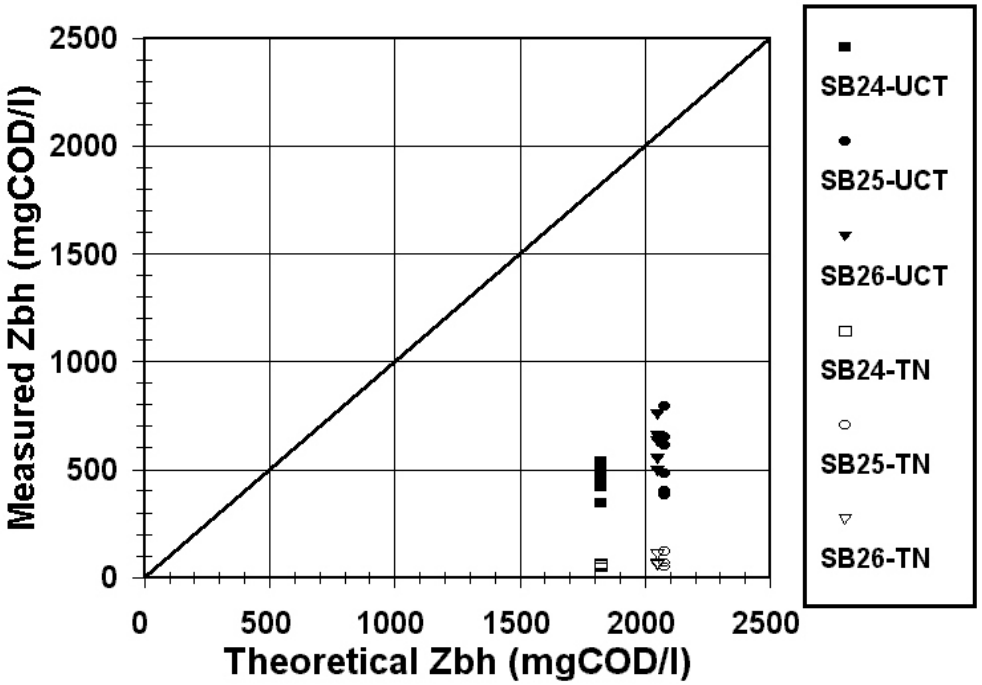

Figure 7: Measured versus theoretical OHO active biomass concentrations (Z_{BH}) for mixed liquor in the 10d sludge age parent laboratory-scale aerobic activated sludge system; measured values from batch test method taking due account of dilution (UCT) and DAPI/FISH enumeration (TN).

- The ATP method 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. 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 was applied by TN 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 TN as a single batch, in a cooler box filled with dry ice. From these experiments, the DAPI/FISH method gave (Fig. 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 has been 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 is being investigated further in WRC sponsored contracts with Technikon Natal (K5/1178) and UCT (K8/453).

5. DISCUSSION AND FUTURE WORK

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 C, N and/or P. This implementation has been aided by the development of a suite of steady state and kinetic simulation models which have facilitated optimization of design and operation. Parallel to this development, 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 and 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 phosphorus accumulating organism, PAO), yet these parameters remain hypothetical as they have not been measured and favourably compared to theoretical values. Recently, new batch test methods have been developed to quantify OHO active biomass concentrations (Kappeler and Gujer, 1992; Wentzel *et al*., 1995; Mbewe *et al*., 1995; Ubisi *et al*., 1997a,b; Wentzel *et al.*, 1998). However, the interpretation and analysis of the data from these tests remains firmly rooted within the engineering and technology paradigm. No cross-linking exists with the microbiological and biochemical paradigms.

In this research project, quantification of the OHO active biomass concentration within the engineering and technology paradigm has been investigated. The batch test method of Ubisi *et al*. (1997a,b) above has been evaluated. In this batch test procedure, 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 this evaluation it became apparent that:

- In the batch test with mixed liquor + wastewater, the OHO active biomass from the wastewater 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.

To overcome this difficulty, the batch test procedure was modified, 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 simplifies the batch test procedure - since the flocculated-filtered wastewater does not contain OHO active biomass, a parallel batch test no longer needs 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 has been 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 have been compared to the corresponding theoretical values predicted from the activated sludge models. Results from these comparisons indicate that the some correlation does exist between theoretical and measured values, but that this correlation is by no means perfect:

1. For mixed liquor drawn from a parent anoxic/aerobic activated sludge system at 10d sludge age, good correspondence between batch test measured and theoretical OHO active biomass concentrations exists (Section 3.1).

2. For mixed drawn from two parallel parent anoxic/aerobic activated sludge systems at 10d sludge age, one with toilet paper dosed to the influent and the other without, there is reasonably close correspondence between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor give an almost linear decrease in OHO active biomass concentration (Section 3.2). However, there is 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.

3. For mixed drawn from a parent aerobic activated sludge system at 10d sludge age there is a close correlation between the theoretical and measured OHO active biomass concentration values, but the theoretical values are approximately 3 to 4 times those measured (Section 3.2).

4. In changing from the parent anoxic/aerobic to aerobic activated sludge system at 10d sludge age above, there was a progressive change in the batch test measured OHO active biomass concentrations, from (2) to (3) above (Section 3.2). 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 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 is not clear.

5. 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 (Section 3.2). For some wastewater batches, the effect was negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. 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. **Clearly, this aspect deserves 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 single parent anoxic/aerobic (MLE) activated sludge system, (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, (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 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.

The concepts developed in this research project 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 have been 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 PAO contribution to the measured OUR can not be isolated because the PAOs do not have a unique compound associated with their growth - the PAO mediated P release and uptake are not growth associated processes.

As noted earlier, 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 substantiate the active biomass concept. However, little cross-linking exists between the microbiological and biochemical and the engineering and technology paradigms. To overcome this shortfall, in this research project a first attempt has been made to create cross-links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical

methods (Section 4). This was achieved through collaborative WRC sponsored research projects, with the Water Research Group at the University of Cape Town (UCT), the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) and the Centre for Water and Wastewater at the Technikon Natal (TN): 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 which quantifies OHO active biomass concentration through monitoring OURs in batch reactors 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 TN used the microbiological 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 activated sludge systems operated at UCT. From a comparison of the results for OHO active biomass concentrations from the various research groups, it is apparent that:

- The microbiological and biochemical test methods gave OHO active biomass concentrations that are 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 have been 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). **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.

- For the DAPI/FISH method applied by TN, in subsequent investigations it has been 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 is being investigated further.**

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 has not provided even near a close correspondence, it has, for the first time, placed the magnitudes of the microbiological and biochemical measurements within the context of the engineering and technology paradigm. This will 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. 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.

The research in this contract has initiated the development of cross-linkages between the engineering and technology concepts for activated sludge systems and the newly developed microbiological and biochemical analytical techniques. This research should be continued under future WRC guided projects.

6. PUBLICATIONS AND OTHER PRODUCTS DURING CONTRACT PERIOD

6.1 Publications

6.1.1 Chapters in books

Cronje GL, Beeharry AO, Wentzel MC and Ekama GA (2002). Active biomass in activated sludge mixed liquor. In: Modern scientific tools in bioprocessing, developments in water science series. Eds: Wilderer P and Wuertz S, Elsevier, Amsterdam, ISBN 0-444-51006-0, 439 - 444.

6.1.2 Refereed Articles published

Cronje GL, Beeharry AO, Wentzel MC and Ekama GA (2001). Active biomass in activated sludge mixed liquor. *Water Research*, **36**, 439 - 444.

6.1.3 Published Conference Proceedings

Beeharry AO, Cronje GL, Lee BJ, Wentzel MC and Ekama GA (2002). Measurement of active biomass in activated sludge mixed liquor. Proceedings 7th Water Institute of Southern Africa conference and exhibition, 19 - 23 May, Durban, Paper 25, ISBN 1-86845-844-X.

Wentzel MC, Ubisi MF, Lakay MT and Ekama GA (2000). Inorganic component of activated sludge mixed liquor. Proceedings 6th biennial Water Institute of Southern Africa conference and exhibition, 28 May to 1 June, Suncity.

Wentzel MC, Mbewe A, Lakay MT and Ekama GA (2000). Evaluation of a modified flocculation filtration method to determine wastewater readily biodegradable COD. Proceedings 6th biennial Water Institute of Southern Africa conference and exhibition, 28 May to 1 June, Suncity.

6.1.4 Published in non-refereed journals

Wentzel MC, Mbewe A, Lakay MT and Ekama GA (2001). Evaluation of a modified flocculation filtration method to determine wastewater readily biodegradable COD. *Chemical Technology*, **March/April**, 21-23.

6.1.5 Refereed articles accepted

Wentzel MC, Ubisi MF, Lakay MT and Ekama GA (2001). Inorganic component of activated sludge mixed liquor. Accepted by *Water Research*.

6.1.6 Research Reports

W102 Cronje GC, Wentzel MC and Ekama GA (2000) Measurement of active heterotrophic organism concentration in nitrification-denitrification activated sludge systems.

W112 Beeharry AO, Wentzel MC and Ekama GA (2001) Evaluation of batch test for measurement of active biomass in activated sludge mixed liquor.

6.2 Graduates - Degrees awarded

MSc GL Cronje "Measurement of active biomass in activated sludge mixed liquor" (Dec 2000)

MSc AO Beeharry "Evaluation of batch test for measurement of active biomass in activated sludge mixed liquor" (Dec 2001)

7. CAPACITY BUILDING

Capacity building included:

- Exchanges and collaborations between UCT and Natal Technikon
  - transfer of understanding and knowledge on microbiological/biochemical tests from Natal Technikon to UCT
  - transfer of skills in operation and analysis of laboratory-scale activated sludge systems from UCT to Natal Technikon

- Exchanges and collaborations between UCT and University of Pretoria
  - transfer of understanding and knowledge on ATP based tests from University of Pretoria to UCT
  - transfer of skills on analysis of laboratory-scale activated sludge systems from UCT to University of Pretoria

- Post-graduate degrees awarded
  - 2 MSc - Mr GL Cronje (Namibian) and Mr AO Beeharry (Muaritian)
  - Previously disadvantaged students from South Africa are difficult to attract into post-graduate degrees, as they are readily able to secure employment in industry

- Undergraduate involvement
  - As part of their BSc degree, students in the Department of Civil Engineering at UCT must complete a 9 week thesis project - 2 students have completed theses in this area

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- Dr G Offringa — Water Research Commission
- Dr J du Preez — Water Research Commission (Committee Secretary)
- Ms S Mathews — Water Research Commission (Committee Secretary)
- Mrs CM Smit — Water Research Commission (Committee Services)
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TABLE OF CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/abbreviation	Description
AO	Autotrophic organism
ATP	Adenosine tri-phosphate
BEPR	Biological excess phosphorus removal
BNR	Biological nutrient removal
BNRAS	Biological nutrient removal activated sludge
C	Carbon
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
e-	Electron
f_{av}	Mixed liquor OHO active biomass fraction
f_{CV}	Mixed liquor COD/VSS ratio (mgCOD/mgVSS)
FDA	Flourescein diacetate
f_N	Mixed liquor N/VSS ratio (mgN/mgVSS)
FISH	Flourescent *insitu* hybridisation
$f_{S,up}$	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)
INT	2-(*p*-codophenyl)-5-phenyl tetrazolium chloride
ISS	Inorganic suspended solids
K_{MP}	OHO maximum specific growth rate on SBCOD (/d)
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
NAD	Nicotinamide adenosine dinucleotide (oxidised)
NADH	Nicotinamide adenosine dinucleotide (reduced)
ND	Nitrification denitrification
NDBEPR	Nitrification denitrification biological excess phosphorus removal
NO_3^-	Nitrate
OD	Optical density
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
PAO	Phosphate accumulating organisms
PHA	Polyhydroxy alkanoate

PS	Parent system
PolyP	Polyphosphate
RBCOD	Readily biodegradable COD
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
R_S	Sludge age (d)
SBCOD	Slowly biodegradable COD
SS	Steady state
S_{bi}	Influent biodegradable COD concentration (mgCOD/ℓ)
S_{bpi}	Influent slowly biodegradable COD concentration (mgCOD/ℓ)
S_{bsi}	Influent readily biodegradable COD concentration (mgCOD/ℓ)
S_{bsai}	Influent short-chain fatty acid concentration (mgCOD/ℓ)
S_{bsfi}	Influent fermentable readily biodegradable COD concentration (mgCOD/ℓ)
S_{ti}	Influent total COD concentration (mgCOD/ℓ)
S_{ui}	Influent unbiodegradable COD concentration (mgCOD/ℓ)
S_{upi}	Influent unbiodegradable particulate COD concentration (mgCOD/ℓ)
S_{usi}	Influent unbiodegradable soluble COD concentration (mgCOD/ℓ)
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
Y_{ZA}	AO active biomass yield (mgCOD/mgCOD)
Y_{ZH}	OHO active biomass yield (mgCOD/mgCOD)
Z_{BH}	OHO active biomass concentration (mgCOD/ℓ)
$Z_{BH(0)}$	OHO active biomass concentration at the start of the batch test (mgCOD/ℓ)
μ_H	OHO maximum specific growth rate on RBCOD (/d)
2°	Secondary

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

CHAPTER 1

INTRODUCTION

1.1 MOTIVATION FOR THE RESEARCH

To comply with more stringent effluent legislation, over the past two decades significant advances have been made in the areas of engineering (design) and technology (implementation and operation) of the single sludge activated sludge system. Activated sludge systems have been successfully designed and implemented at full-scale to progressively include the biological removal of carbon (C), nitrogen (N) and phosphorus (P). This implementation has been aided by the development of 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; Barker and Dold, 1997). These models are based on a common conceptualization of the processes acting in the activated sludge system. In terms of this group of models, in the bioreactor of the non-nitrifying aerobic activated sludge system, the mixed liquor organic (volatile) suspended solids (MLOSS) is made up of three components; (1) ordinary heterotrophic organism (OHO) active biomass, (2) endogenous residue and (3) inert material. In the nitrifying aerobic and anoxic/aerobic activated sludge systems, a fourth component is included; (4) autotrophic organism (AO) active biomass. All four MLOSS components settle out in the secondary settling tank and are returned to the bioreactor via the underflow recycle; these components leave the system via the waste flow. If an anaerobic reactor is included to stimulate biological excess phosphorus removal (BEPR), additionally (5) phosphate accumulating organism (PAO) active biomass and (6) this organism group's endogenous residue will contribute to the MLOSS (Wentzel *et al*., 1992; Henze *et al*., 1995). The active biomass components of the MLOSS 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.

Historically the MLOSS has been measured as a lumped parameter, via the VSS or COD test (Standard Methods, 1985). Specific rates for the biological processes (e.g. denitrification; oxygen utilization) often were (and still are) expressed in terms of this lumped parameter. However, from the above, only parts of the MLOSS are active biomasses, and only these parts mediate the relevant biological processes, e.g. OHO's for COD removal and denitrification. Accordingly, the specific rates for the relevant (and associated) biological processes should be expressed in terms of the appropriate active biomass concentration to allow a meaningful comparison of rates measured in different systems. 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. However, 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 active biomass exists only hypothetically within the structure 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, e.g. Dold *et al*., 1980, 1991; Alexander *et al.*, 1980; Van Haandel *et al*., 1981; Warner *et al*., 1986), these have not been directly measured experimentally and compared to the hypothetical model values. This deficiency casts a measure of uncertainty on the entire 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 OHO active biomass concentration (Kappeler and Gujer, 1992; Wentzel *et al*., 1995; Mbewe *et al*., 1995). This batch test method was modified by Wentzel *et al*. (1998) and used to quantify the OHO active biomass concentration of mixed liquor samples drawn from a well-defined parent laboratory-scale anoxic/aerobic activated sludge system operated at 12 and 20d sludge age. The measured OHO active biomass concentrations were in close agreement with those calculated theoretically for the parent system at 12d sludge age, but were about ½ the theoretical values for the system at 20d sludge age. While the good correspondence at 12d sludge age provides substantive direct evidence supporting both the models and the experimental method, reasons for the poor correspondence at 20d sludge age need to be found. Wentzel *et al*. were not able to provide an explanation for this inconsistency, so the uncertainty around the active biomass concept 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). 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 have yet to be realised for the system. It remains for the results that these techniques provide to be integrated with the design and kinetic modelling theory. 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 engineering and technology paradigm 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 the measurement of OHO active biomass. This research project investigates measurement of the OHO active biomass parameter within the engineering and technology paradigm. If OHO active biomass can be successfully quantified within this paradigm and agreement obtained between the measurements and the theoretical modelling values, this will provide a basis for comparison with the quantitative data

arising from the new microbiological and biochemical analytical techniques. This will facilitate development of a common link between the two paradigm sets.

1.2 OBJECTIVES AND AIMS OF RESEARCH

The objectives of this research project are 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.

In terms of these objectives, specific aims identified are:

1) To provide greater surety in the mathematical models for design and operation of the biological nutrient removal activated sludge (BNRAS) system.
2) To provide a platform for collaborative work between the engineering and microbiological aspects of the BNRAS system.
3) To provide a better scientific understanding of the microbiological processes operating in the BNRAS system in the context of defined engineering environments.
4) To develop linkages and overlap between the quantitative engineering and qualitative microbiological approaches to understanding biological wastewater treatment systems.

1.3 METHODOLOGY

The Water Research Group at the University of Cape Town will operate at laboratory-scale nitrogen removal activated sludge systems receiving real wastewater. The engineering parameters for these systems will be closely controlled and defined. Nitrogen removal systems will be considered first because the mathematical models for these appear to be the more consistent. The systems will 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 will 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) conducted to determine OHO active biomass. This experimentally determined parameter will 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 will be sent to collaborators where they will be subject to microbiological and biochemical analysis, using the array of modern microbiological and biochemical techniques. In this aspect it is vitally important that the analytical techniques provide quantitative information, because the engineering paradigm is rooted in quantitative measurements. This information will be used to find ways to integrate the engineering and technology and microbiology and biochemistry paradigms.

When satisfactory results are obtained for OHO active biomass, the investigation can be expanded to include AO, facultative denitrifying OHO and PAO.

1.4 SPECIFIC TASKS

To address the aims above, a number of specific tasks have been identified for completion:

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

Ubisi *et al*. (1997a,b) describe the development of a simple batch test to quantify the OHO active biomass concentration. 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 activated sludge system operated at 12 and 20 days 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 Figs 1.1 (a and b). From the comparison they concluded that the results obtained were both encouraging and perplexing. With the parent system at 12d sludge age (Fig 1.1a), the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d sludge age (Fig 1.1b) 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.

Samples will be drawn from the parent laboratory-scale activated sludge system, and the batch test method above applied to determine OHO active biomass concentrations. These will be compared to the theoretical OHO active biomass concentrations predicted by steady state design theory (WRC, 1984) and kinetic simulation models (Dold *et al*., 1991). The batch test method will be refined and modified to resolve the inconsistency between experimental and theoretical OHO active biomass concentrations.

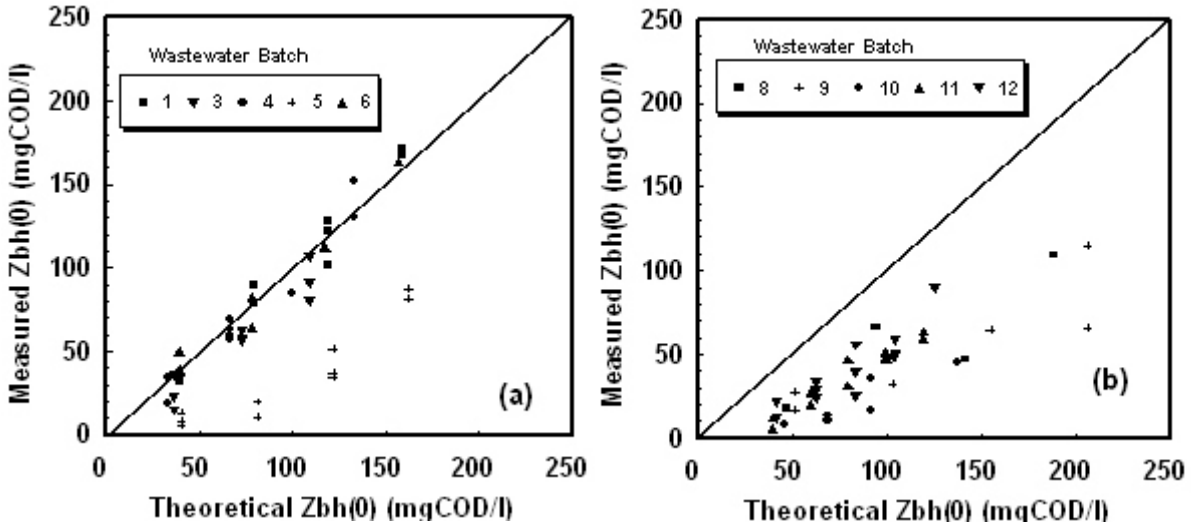

Figure 1.1. Measured vs. theoretical ordinary heterotrophic organism (OHO) active biomass concentration [$Z_{BH(0)}$] in the batch test due to addition of mixed liquor drawn from the parent system at (a) 12d and (b) 20d sludge age (Wentzel *et al.*, 1998).

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 research collaborators for microbiological and biochemical analysis.

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.

CHAPTER 2

THE ACTIVE BIOMASS CONCEPT

2.1 INTRODUCTION

To optimize the design and operation of the single sludge activated sludge system, over the past two decades a number of steady state design models (e.g. Marais and Ekama, 1976; WRC, 1984; Wentzel *et al*., 1990; Scheer and Seyfried, 1993; Maurer and Gujer, 1994) and kinetic simulation models (e.g. Dold *et al*., 1980, 1991; Van Haandel *et al*., 1981; Henze *et al*., 1987, 1995; Wentzel *et al*., 1992; Gujer *et al*., 1995) have been developed, to progressively include aerobic COD removal and nitrification, anoxic denitrification and anaerobic/anoxic/aerobic biological excess phosphorus removal. These models enable system design and operational parameters to be readily identified, provide guidance in selecting values for these parameters and quantify the expected behaviour of the system.

This group of models are based, to a large degree, on a common conceptual framework which has been developed from an understanding of the mechanisms operating in the activated sludge system, particularly from an understanding of the interactions between the components making up the mixed liquor in the bioreactor and the influent wastewater. In this Chapter, this conceptual framework will be outlined briefly, to provide an overview of the current understanding of the processes that operate in the bioreactor and give rise to the various mixed liquor components, and how these processes relate to the influent wastewater. From this overview, the importance of the mixed liquor component ordinary heterotrophic organism (OHO) active biomass will become evident.

2.2 FATE OF INFLUENT WASTEWATER IN ACTIVATED SLUDGE SYSTEM

The activated sludge system comprises a biological reactor and a secondary settling tank. Irrespective of whether or not biological N and/or P removal are included, many different biological and physical processes take place in the biological reactor, and the physical process sedimentation takes place in the secondary settling tank. These processes form the basis for subdividing the influent wastewater, carbon (C), nitrogen (N) and phosphorus (P) materials into subfractions (see Fig 2.1). On entry of the influent into the biological reactor, the particulate materials, which include both settleable and suspended (non-settleable or colloidal), organic and inorganic materials, are enmeshed (a biologically mediated flocculation) and become part of the activated sludge mixed liquor. The soluble materials, both organic and inorganic, remain in solution. In the biological reactor, the bacteria present will act on the biologically utilizable material, termed *biodegradable*, whether organic or inorganic, soluble or particulate, and transform these to other compounds or products, either gaseous, soluble or particulate: The gaseous products escape to the atmosphere, the particulate products become (or remain) part of the mixed liquor solids and the soluble products become (or remain) dissolved in solution. The non-biologically utilizable material, termed *unbiodegradable*, will not be transformed and will

remain in either the soluble or particulate form. Following from the processes in the bioreactor, the first major division of the influent is based on whether the material is *biodegradable* or *unbiodegradable*, see Fig 2.1.

After biological treatment the flow passes from the biological reactor to the secondary settling tank. In the secondary settling tank, the particulate materials making up the mixed liquor (whether organic or inorganic, biodegradable or unbiodegradable) settle out and are returned to the biological reactor. The particulate components of the mixed liquor entering the settling tank are thus retained in the system. All the soluble components of the mixed liquor (whether organic or inorganic, biodegradable or unbiodegradable) cannot settle out and escape with the effluent, see Fig 2.1.

The settling behaviour in the secondary settling tank therefore forms the basis for subdividing the influent *unbiodegradable* material into subfractions: The influent unbiodegradable material passes unmodified through the biological reactor to the secondary settling tank; ideally all the particulate (and colloidal) material settles out in the secondary settling tank and these constituents are therefore termed *unbiodegradable particulate*, the soluble constituents cannot settle out so that these constituents are termed *unbiodegradable soluble*, see Fig 2.1. With regard to the influent *biodegradable* material, because a substantial amount of this material has been biologically transformed in the biological reactor preceding the secondary settling tank, it cannot be subdivided into subfractions based on its behaviour in the secondary settling tank; subdivision of the biodegradable material is based on the rates of transformation/utilization by the bacteria in the biological reactor (see below).

From the above, in terms of the conceptualization of the activated sludge system behaviour, the wastewater constituents are characterized (1) biologically, i.e. as biodegradable (biologically utilizable) or unbiodegradable (non-biologically utilizable) material, and (2) physically, i.e. as soluble or particulate material. Therefore, for the models based on fundamentals of behaviour, it is necessary to divide the influent constituents into at least three components:

- biodegradable
- unbiodegradable soluble
- unbiodegradable particulate.

This general wastewater characterization structure (see Fig 2.2) conforms to the biological degradation and physical solid/liquid separation processes that take place in the activated sludge system.

From the description above:

(1) The unbiodegradable soluble component of the influent passes unmodified through the bioreactor to the secondary settling tank, and passes out in the secondary settling tank overflow to appear in the effluent; therefore, this influent component has no influence on the mixed liquor solids, but does influence the effluent quality.

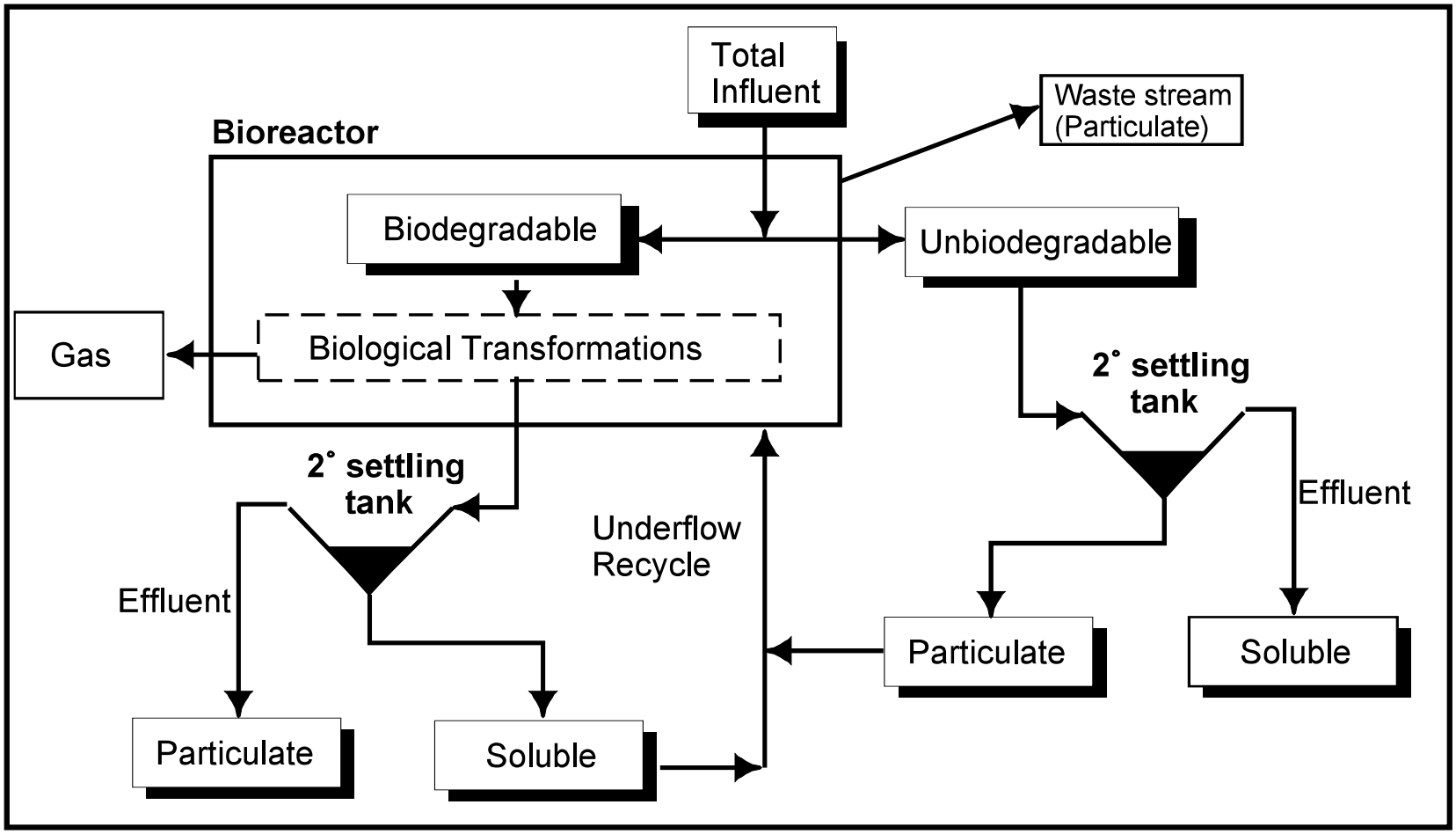

Figure 2.1: Division of influent carbon (C), nitrogen (N) and phosphorus (P) materials according to the biological and physical processes in the activated sludge system.

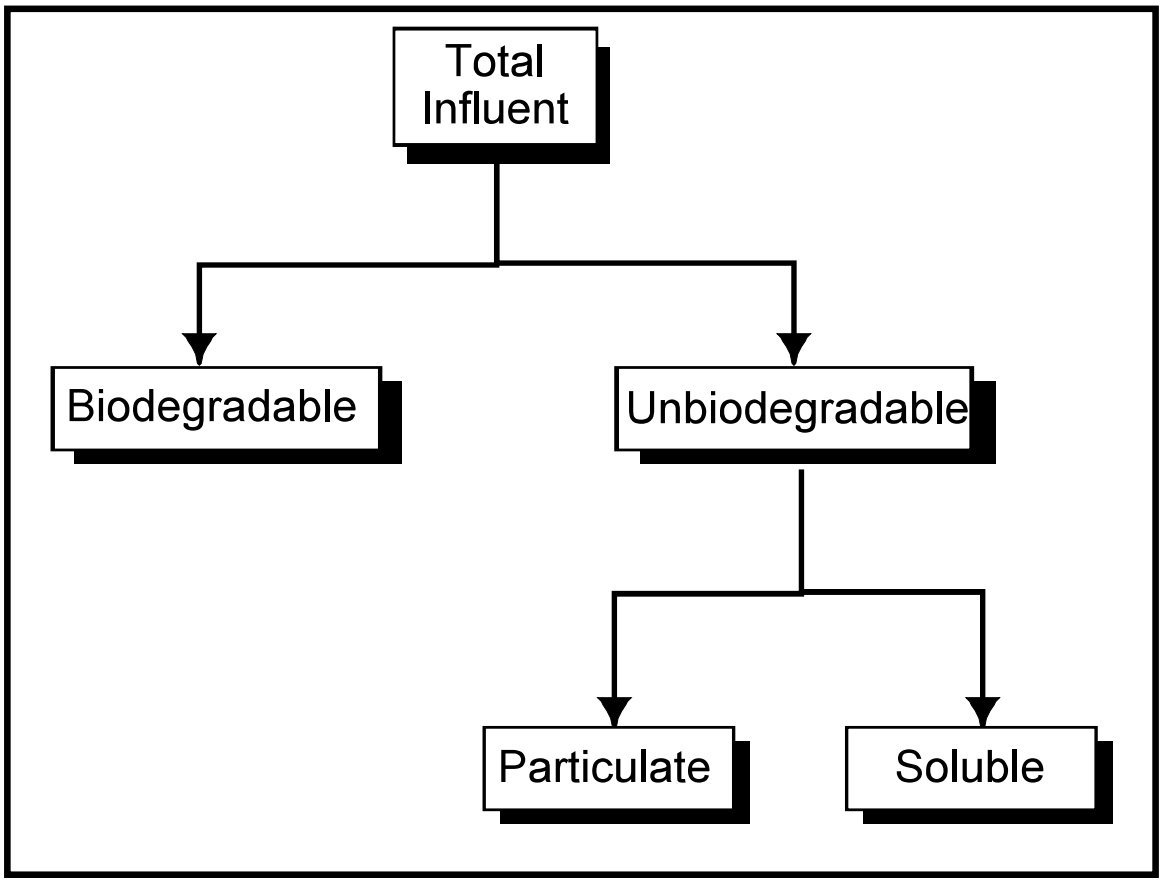

Figure 2.2: The general wastewater characterisation structure for carbon (C), nitrogen (N) and phosphorus (P).

(2) The unbiodegradable particulate component of the influent, on entry into the bioreactor is enmeshed in the mixed liquor mass and will settle out in the secondary settling tank to be retained in the system; thus this influent component has a direct influence on the mixed liquor solids in the reactor, but no influence on the effluent quality. The unbiodegradable particulate component leaves the system with the mixed liquor wasted to maintain the sludge age.

(3) The biodegradable component of the influent is transformed by biological action in the bioreactor to other compounds or products, either gaseous, soluble or particulate; the particulate compounds/products will settle out in the secondary settling tank to be retained in the system and will therefore influence the mixed liquor solids in the reactor; the soluble components will not settle out in the secondary settling tank and so will pass out in the effluent while the gaseous components will escape to the atmosphere - these latter two components will not, therefore, influence the mixed liquor solids.

Thus, the mixed liquor solids present in the bioreactor will be comprised of components arising directly from the influent and from biological transformations of the influent. This conceptualization of the fate of the influent components in the activated sludge system can be applied to any material in the influent, organic or inorganic. Of particular interest to this discussion is the organic carbonaceous material. The carbonaceous material usually is quantified by means of the chemical oxygen demand (COD) test, which measures the electron, or equivalently energy, donating capacity of organic material, so that in effect the conceptualization of the fate of the influent is applied to the electrons (e^-) in the carbonaceous material.

2.3 BIOLOGICAL TRANSFORMATIONS OF THE ORGANIC CARBONACEOUS MATERIAL IN THE BIOREACTOR

As noted above, the biodegradable components of the influent wastewater are biologically transformed in the bioreactor to other components or products, either gaseous, soluble or particulate. In the steady state design and dynamic kinetic simulation models, the biological transformations of the organic carbonaceous materials are explicitly included, and directly influence the organic solids in the bioreactor. It is important, therefore, to gain a basic understanding of the approach adopted in the models to describe these transformations.

2.3.1 Organism groups

In the bioreactor of the activated sludge system a wide diversity of organism species have been identified. However, in both the steady state design and dynamic kinetic simulation models it has been recognized that including a complete description of every organism species would be impractical. Instead microorganisms that fulfill a particular function in the activated sludge system 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. Thus, for modelling of activated sludge systems the "organizational level" (Odum, 1971) that is followed is the mass behaviour of a population or group of selected organisms; these populations or groups are identified and selected based on identifiable functions. The principle organism groups, their functions and the zones in which these functions are performed are summarized in Table 2.1. ***This research project considers only the aerobic and anoxic/aerobic activated sludge systems.*** Thus, two organism groups need to be taken into account; (i) heterotrophic organisms unable to accumulate polyP, termed ordinary heterotrophic organisms (OHO) and (ii) autotrophic organisms (AO) mediating nitrification, termed autotrophs or nitrifiers. The biological transformation mediated by these two organism groups are described below.

Table 2.1: Principle organism groups included in models for activated sludge systems, their functions and the zones in which these functions are performed.

ORGANISM	BIOLOGICAL PROCESS	ZONE
1. Ordinary heterotrophs - OHO (unable to accumulate polyP)	• COD removal (organic degradation; DO uptake)	Aerobic
	• Ammonification (organicN→NH_4^+)	Aerobic
	• Denitrification (organic degradation; NO_3^-→NO_2^-→N_2)	Anoxic
	• Fermentation (F-RBCOD→SCFA)	Anaerobic
2. PolyP heterotrophs - PAO (accumulate polyP)	• P release (SCFA uptake; PHA storage)	Anaerobic
	• P release (SCFA uptake; PHA storage)	Anoxic
	• P uptake (PHA degradation; denitrification?)	Anoxic
	• P uptake; P removal (PHA degradation; DO uptake)	Aerobic
3. Autotrophs - AO (nitrifiers)	• Nitrification (NH_4^+→NO_2^-→NO_3^-; DO uptake)	Aerobic

2.3.2 "Ordinary" heterotrophic organisms (OHO)

The heterotrophic organisms obtain both energy and carbon from complex organic compounds. This is a diverse group of organisms, which, given sufficient time and appropriate environmental conditions, will utilize every type of organic material. The group is ubiquitous and in any given situation those members of the group that derive maximum benefit from the specific organic material and environmental conditions will develop. As the organic source and environmental conditions change, so associated changes in the heterotrophic organism species take place.

As noted above, for the purpose of modelling the activated sludge system, the heterotrophic organism species are grouped together as "surrogate" which is assigned a unique set of characteristics reflecting the behaviour of the group. This group of organisms mediates the processes of COD removal and denitrification. Conceptually, in the models this "surrogate" heterotroph is subject to two main biological processes; (i) synthesis or growth and (ii) endogenous mass loss/death.

(i) Synthesis/growth

For the heterotrophic organisms, the organic material in the influent serves two functions: (a) it is the supply of materials which are transformed into new cell materials, and (b) it is the supply of energy to effect these transformations. In terms of the models, on entry into the bioreactor some of the influent organic materials are transformed (via a number of biochemical pathways, collectively called anabolism) to new heterotroph cell material. Accepting the general formulation for protoplasm as $C_5H_7O_2N$ (McCarthy 1964), with glucose as an example substrate the synthesis reaction can be summarized as:

$$5\ C_6H_{12}O_6 + 6\ NH_3 \rightarrow 6\ C_5H_7O_2N + 18\ H_2O \qquad (2.1)$$

This synthesis/growth process requires energy. The energy is obtained as follows: Some of the organic molecules are split by the heterotrophs to give hydrogen ions, electrons and carbon dioxide. For example, consider the organic molecule glucose:

$$C_6H_{12}O_6 + H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^- \qquad (2.2)$$

Because it releases electrons the organic molecule is termed an electron donor and on yielding the electrons, the molecule is said to be oxidized. The electrons (and protons) are captured by the heterotrophs, and transferred via an internal sequential set of oxidation reduction (redox) reactions eventually to a molecule which can accept them; this molecule is called the terminal electron acceptor. In this series of redox reactions, free energy is released (i.e. energy that is available to do work) which is captured by the organism (collectively this process is called catabolism). The energy captured by the organism principally is used by the organism for the transformation reactions that synthesize new cell mass, Eq. (2.1). From bioenergetics it is possible to determine the amount of glucose that must be oxidized to synthesize one mole of new cell mass (WRC, 2001).

In the oxidation of substrate, if oxygen is present (aerobic conditions) the terminal electron acceptor is oxygen (O_2) which is reduced to water (H_2O), i.e.

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O \qquad (2.3)$$

If oxygen is absent, but nitrate (or nitrite) is present (anoxic conditions), the nitrate (NO_3^-) (or nitrite, NO_2^-) serves as terminal electron acceptor and is reduced to nitrogen gas (N_2), giving rise to the denitrification process:

$$NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O \qquad (2.4)$$

Thus, of the original biodegradable organic material present, a part is oxidized to yield free energy. This free energy is utilized by the heterotrophs to “reorganize” the remaining organic material into new cell mass, the free energy being lost as heat in the re-organization. Noting that the heterotrophic organisms use electron transfer (redox) reactions to generate the free energy, the transformation of the biodegradable organic material can best be traced by monitoring the transformations in electrons. This has the advantage, *inter alia*, that, due to the indestructibility and hence conservation of electrons, electron mass balances can be conducted. Accordingly, of

the electrons present in the biodegradable organic material (measured via the chemical oxygen demand (COD) test, see WRC, 1984), a fraction are transformed into new heterotrophic cell mass, and the remainder pass to the terminal electron acceptor, oxygen if aerobic and nitrate if anoxic.

Now, the ratio of electrons captured in new cell mass to the electrons present in the biodegradable substrate is termed the yield (Y_{ZH}), i.e.

$$Y_{ZH} = \frac{e^- \text{ in synthesized material}}{e^- \text{ in biodegradable material}} \tag{2.5a}$$

Accepting the COD test as a measure of the electrons, then

$$Y_{ZH} = \frac{\text{COD in synthesized material}}{\text{COD in biodegradable material}} \tag{2.5b}$$

And, from a mass balance on electrons (COD)

COD of biodegradable material = COD of synthesized material + e^- to electron acceptor.

For activated sludge systems with the wide diversity of biodegradable organics in the influent and heterotrophs in the bioreactor, it has been found that the yield can be accepted to be approximately constant, at Y_{ZH} = 0.666 mgCOD/mgCOD (Dold *et al*., 1980; 1991). In other words, for every biodegradable organic COD utilized, a constant fraction will be transformed to heterotrophic active biomass, and the balance of the e^- will pass to the electron acceptor.

A question that needs to be addressed is how much of the influent biodegradable material will be transformed in the bioreactor, within the residence time in the system. This is a problem of kinetics or rates. In considering the kinetics of biodegradable organic material transformation, it is subdivided into two components, readily biodegradable (soluble) COD (S_{bsi}) and slowly biodegradable (particulate) COD (S_{bpi}), see Fig 2.3. This division is based on observed biological responses of activated sludge mixed liquor to domestic wastewater (Dold *et al*., 1980; Van Haandel *et al.*, 1981), that is, the division is a biokinetic one: Under dynamic loading of activated sludge (short sludge age cyclic loading, plugflow reactors, batch tests) two distinct rates of utilization of domestic wastewater biodegradable COD substrate were apparent with either oxygen (Dold *et al*., 1980; Ekama *et al*., 1986) or nitrate (Van Haandel *et al*., 1981; Ekama *et al*., 1986) as electron acceptor (aerobic or anoxic conditions respectively). A fraction (called readily biodegradable COD, RBCOD) was taken up rapidly by the sludge and metabolized, giving rise to a high oxygen or nitrate utilization rate respectively. The other fraction (called slowly biodegradable COD, SBCOD) was taken up much more slowly and metabolized, giving rise to oxygen or nitrate utilization rates about 1/10 of the rate with RBCOD.

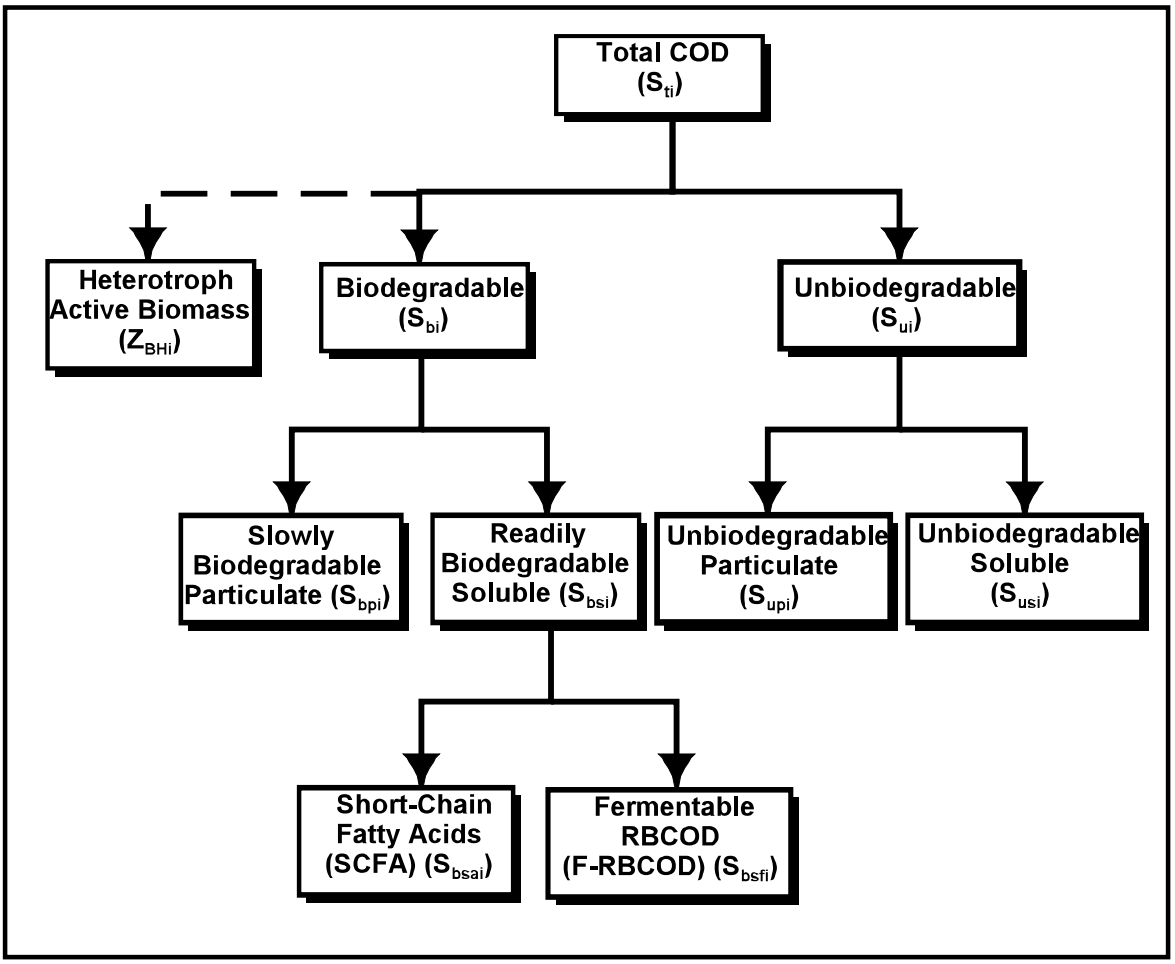

Figure 2.3: The division of the influent COD into its different constituents.

To explain these observations, the RBCOD was hypothesized to consist of simple *soluble* molecules that can be absorbed readily by the organism and metabolized for energy and cell synthesis, whereas the SBCOD was assumed to be made up of *particulate/colloidal/complex* organic molecules that require extra cellular adsorption and enzymatic breakdown (hydrolysis) prior to absorption and utilization. The hypothesized difference in molecule size between RBCOD and SBCOD has been used to classify the RBCOD as a biodegradable soluble COD and the SBCOD as a biodegradable particulate COD. Since the RBCOD is soluble, it is exposed to biological treatment only as long as the liquid remains in the reactor, i.e. for the hydraulic retention time which is relatively short (~ 6 - 24h). However, the rate of RBCOD utilization is high and for sludge ages greater than about 3 days the concentration of RBCOD in the effluent is negligible even though the hydraulic retention time is relatively short. Accordingly, for completely aerobic systems it can be safely assumed that all the RBCOD will be utilized in the system. For the SBCOD, the extracellular breakdown (hydrolysis) is slow and forms the limiting

rate in the utilization of SBCOD. Although the rate of SBCOD utilization is relatively slow, the SBCOD does not appear in the effluent. This is because on entry of the influent into the bioreactor, the SBCOD becomes enmeshed in the mixed liquor, settles out in the secondary settling tank and is retained in the system. Therefore, the particulate biodegradable organics (SBCOD) are exposed to biological treatment for as long as the solid (settleable) material is retained in the system, i.e. for the sludge age. Thus, even though the utilization of the SBCOD is around 10 times slower than that for the RBCOD, because the sludge age in most activated sludge systems is usually more than 10 times longer than the hydraulic retention time, the SBCOD is completely utilized also. From simulation studies using dynamic kinetic models (Dold *et al*., 1991) all the SBCOD is completely utilized for sludge ages greater than about 2 to 3 days and temperatures greater than about 20°C (5 to 6 days at 14°C). Accordingly, for most activated sludge systems it is sufficient to assume all the SBCOD will be utilized in the system.

Thus, for activated sludge systems at 20°C for sludge ages greater than about 3 days all the biodegradable organic material in the influent, whether soluble or particulate, will be transformed in the bioreactor by biological action mediated by the heterotrophic active biomass. The products of this transformation are:

(1) Gaseous - carbon dioxide (only considered if the fate of the carbon is traced) and nitrogen, produced if nitrate acts as terminal electron acceptor, Eq. (2.4), i.e. anoxic conditions.

(2) Soluble - water, produced when oxygen acts as terminal electron acceptor, Eq. (2.3), i.e. aerobic conditions

(3) Particulate - new heterotrophic active biomass.

The particulate heterotrophic active biomass will settle out in the secondary settling to be retained in the system and thus will be another component of the mixed liquor solids. Since the influent SBCOD is virtually totally utilized for systems with sludge ages > 3d at 20°C, the SBCOD will not be a significant component of the mixed liquor solids, and hence can be neglected.

(ii) Endogenous mass loss/death

The endogenous mass loss phenomenon is the loss of active organism mass with time. The phenomenon can be observed as a reduction in organism active mass when the organism population is aerated with no substrate added, for example, in the aerobic digestion of mixed liquor. The phenomenon also manifests itself as a decrease in specific organism yield with increase in sludge age. In the models, two conceptual approaches have been followed to describe this phenomenon; endogenous respiration and death-regeneration.

Endogenous respiration

This approach is the simpler and is followed in the steady state design models. In the approach, active organism mass is lost at a constant specific rate. Of the active organism mass that is lost, a part (the biodegradable fraction, about 80%) is oxidized to provide energy for maintenance of the active mass remaining and the balance (the unbiodegradable fraction, about 20%) remains as a particulate unbiodegradable organic fraction accumulating in the system, and is called

endogenous residue. The oxidation of the biodegradable part of the active mass lost gives rise directly to an endogenous oxygen consumption under aerobic conditions, and indirectly to a nitrate demand (denitrification) under anoxic conditions. Whether the active mass oxidized is some "deep" storage material or from the death of individual organisms is of little practical importance in these models, but conceptually death of individual organisms would seem more likely; on organisms death the biodegradable part of the organism is oxidized by those remaining for maintenance energy, and the unbiodegradable part accumulates as a particulate endogenous residue. The simplicity of this approach allows analytical solutions to be readily determined.

Death-regeneration

This approach is followed in the dynamic kinetic simulation models. In the death-regeneration approach an attempt is made to separate out the processes which take place during the organism's "death phase". Disappearance of live active mass is hypothesized to be due to the net effect of death (natural or predation) and regeneration of organisms: On death the cell material is released through lysis; a part is particulate unbiodegradable endogenous residue (about 8%); the remaining part (about 92%) is biodegradable and adds to the slowly biodegradable COD (SBCOD) which passes through the path of adsorption, hydrolysis and synthesis of new cell mass (i.e. regeneration) described above. The synthesis of new cell mass gives rise to an associated aerobic oxygen or anoxic nitrate demand. Thus, in death-regeneration the oxygen or nitrate demand arises in fact from the energy requirements for resynthesis of organism active mass (regeneration) from the SBCOD liberated from organism death. The main implication of this approach is that "maintenance energy" *per se* (the oxygen/nitrate requirement for maintenance) is considered to be so small that it can be lumped with, and completely swamped by, the oxygen/nitrate demand for resynthesis of new cell mass from the lysed biodegradable substrate.

Comparing the two approaches, for the mixed cultures present in the activated sludge system predation is likely to be a significant cause for death of organisms, "liberating" substrate for synthesis of new cell mass (predator and others). Thus, conceptually the death-regeneration would appear superior. However, provided all the biodegradable COD has been depleted and a terminal electron acceptor (oxygen or nitrate) is continually available, with the appropriate selection of constants the two approaches give the same nett result, i.e. same loss of heterotrophic active biomass, utilization of oxygen/nitrate and generation of endogenous residue (Dold *et al*., 1980, 1991). If a terminal electron acceptor is not available, then the endogenous-respiration approach is deficient and the death-regeneration approach preferable. Also, the two approaches do deviate slightly when significant biodegradable COD is present, in for example, batch tests.

Irrespective of whether the endogenous respiration or the death-regeneration approach is followed, in both a part of the organism active mass that is lost is unbiodegradable particulate which will settle out in the secondary settling to accumulate in the system as endogenous residue. Thus, the endogenous residue will be another component of the mixed liquor solids.

2.3.3 Autotrophic organisms (AO)

In the activated sludge system, the autotrophic organisms of importance are the nitrifiers. These organisms mediate the process of nitrification, whereby free and saline ammonia is oxidized to nitrite and nitrate. It is generally accepted that the nitrification is due to two specific groups of autotrophic bacteria, the *Ammonia Oxidisers* and the *Nitrite Oxidisers*. Since these organisms are autotrophs, they obtain the carbon required to form cellular material from carbon dioxide. Their energy requirements are obtained by oxidizing ammonia to nitrite and nitrate. *Ammonia Oxidisers* utilizes ammonium (NH_4^+) as electron donor and oxygen as electron acceptor; the ammonium is oxidized to nitrite and the oxygen reduced to water, i.e.

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \qquad (2.6)$$

Nitrite oxidisers uses nitrite as electron donor and oxygen as terminal electron acceptor; the nitrite is oxidized to nitrate and the oxygen reduced, i.e.

$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \qquad (2.7)$$

The nitrifiers can only execute these two reactions if oxygen is present, and are thus obligate aerobes.

Thus, the full nitrification process takes place in two steps. However, the rate of conversion of ammonium to nitrite by *Ammonia Oxidisers* is very much slower than the rate of conversion of the nitrite to nitrate by *Nitrite Oxidisers*; usually as fast as the nitrite is formed it is nitrified to nitrate. The rate limiting step in the nitrification sequence, therefore, is that due to the *Ammonia Oxidisers* - only the kinetics of this organism needs to be considered. As a consequence, in the models the two stage nitrification process is approximated as a single stage process with the ammonium being converted directly to nitrate, i.e.

$$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+ \qquad (2.8)$$

This step is modelled as being mediated by a single nitrifying organism group, whose kinetics and characteristics closely approximate the *Ammonia Oxidisers* (since this organism group mediates the rate limiting step).

Part of the energy released in the single nitrification process is used for synthesis of new nitrifier organism active mass (growth), i.e. accepting $C_5H_7O_2N$ as a generalized formulation for nitrifier protoplasm (McCarty, 1964), then:

$$5\ CO_2 + NH_4^+ + 2\ H_2O \rightarrow C_5H_7O_2N + 5\ O_2 + H^+ \qquad (2.9)$$

Recognizing that ammonium serves as the "substrate" for the nitrifiers, the specific yield is expressed in terms of the ammonium utilized, i.e.

$$Y_{ZA} = \frac{\text{COD of nitrifiers formed}}{NH_4^+\text{-N utilized}} \qquad (2.10)$$

The nitrifier active biomass that is formed in the activated sludge system is important in considering the mixed liquor solids, because it is particulate and so will settle in the secondary settling tank to be retained in the system. The nitrifier active biomass, therefore, will be one component making up the mixed liquor solids.

As with the heterotroph active biomass, the nitrifier active biomass also is subject to endogenous mass loss/death. Although conceptually for consistency, this endogenous mass loss/death should result in the generation of an endogenous residue, compared to the other mixed liquor fractions the relative amount of endogenous residue generated by the autotrophs is so small that it is of little practical importance and can be neglected.

2.3.4 Phosphate accumulating organisms (PAO)

The phosphate accumulating organisms (PAO) are a specific group of heterotrophic organisms able to accumulate P intracellularly in long chains called polyphosphate (polyP). In terms of the models, only those heterotrophic organisms ***exhibiting*** this behaviour in the activated sludge system are included in the PAO group. To stimulate the P behavioural patterns typical of the surrogate PAO group (P release, uptake and excess removal) requires the presence of an anaerobic reactor, with the influent wastewater fed to this reactor. ***This research project will not consider activated sludge systems that include anaerobic reactors, and hence quantifying the PAOs does not fall within the ambit of the current research project.***

2.4 ACTIVATED SLUDGE MIXED LIQUOR ORGANIC COMPONENTS

From the discussion above, the mixed liquor in the bioreactor of nitrifying aerobic and anoxic/aerobic activated sludge systems is comprised of a number of organic components (see Fig 2.4):

(1) Inert material - derived from the unbiodegradable particulate organics present in the influent.

(2) Heterotroph active biomass - synthesized in the bioreactor from the biodegradable organics present in the influent.

(3) Endogenous residue - generated from the unbiodegradable portion of the heterotrophic (and autotrophic) active biomass that is lost in the endogenous respiration/death-regeneration process.

(4) Autotrophic active biomass - synthesized in the bioreactor in the nitrification of ammonia to nitrate.

All four mixed liquor organic solids components settle out in the secondary settling tank and are returned to the bioreactor via the underflow recycle; these components leave the activated sludge system via the waste flow.

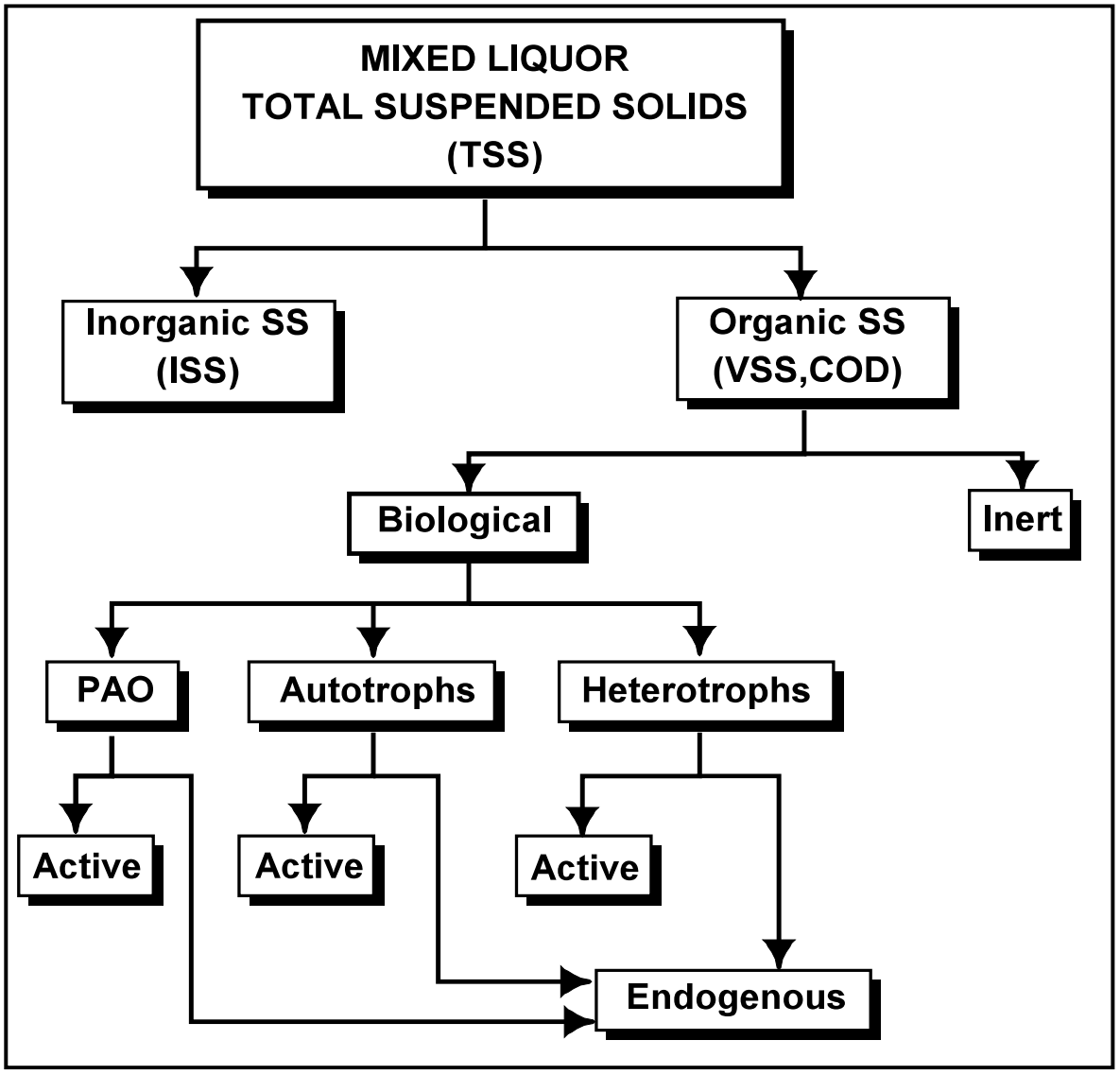

Figure 2.4: The organic and inorganic components of the mixed liquor in the bioreactor of the biological nutrient removal activated sludge system (note that in this project the phosphorus accumulating organisms, PAOs, are not considered).

For activated sludge systems receiving "typical" municipal wastewaters (influent TKN/COD < 0.12 mgN/mgCOD) the autotrophic active biomass component of the mixed liquor organic solids is very small compared to the other three components (< 2% of the total) - the substrate source for the autotrophs (NH_4^+) is very much less than that for the heterotrophs (COD) and their specific yield is very much lower (autotrophs Y_{ZA} = 0.15 mgCOD/mgN; heterotrophs Y_{ZH} = 0.666 mgCOD/mgCOD, Dold *et al*., 1980, 1991). Accordingly, in considering the components making up the mixed liquor organic solids, from a mass point of view with very little error the autotrophic active biomass can be neglected, so that the number of organic components reduces to three; (i) inert, (ii) endogenous residue and (iii) heterotrophic active biomass. However, if the nitrification process itself is being considered, then the autotroph active biomass is of fundamental

importance and must be taken into account, because in the kinetic simulation models the nitrification rates are expressed in terms of the autotrophic active biomass; in contrast, in the steady state design models, the autotrophic active biomass is not required for calculating nitrification. This research project focusses on the OHO active biomass; the autotrophic active biomass will be considered in a future project. Thus, autotrophic active biomass does not need to be considered.

Accepting that the autotroph active biomass can be neglected, a schematic diagram showing the origin of the mixed liquor organic fractions is shown in Fig 2.5. For the three mixed liquor organic solids components, two, the inert material and endogenous residue, are inactive and do not participate in any of the biological processes. The heterotrophic active biomass component, on the other hand, mediates the biodegradation processes of COD removal and denitrification. Thus, the rates for these processes are directly related to the amount of heterotrophic active biomass present. Accordingly, the specific rates should be expressed in terms of this parameter to allow a meaningful comparison of the rates measured in different systems. Although with the proliferation of computer programmes for the models, the heterotrophic active biomass seems to be almost universally accepted, it remains hypothetical within the structure of these models, because it has not been measured directly, primarily due to the lack of suitable simple measurement techniques (see Chapter 3 for a review of experimental techniques). This deficiency has cast some measure of doubt on the framework within which the steady state design and kinetic simulation models have been developed. ***One of the main objectives of this research project is to develop a technique to measure the heterotrophic active biomass, and to compare the measured values to those calculated theoretically.***

2.5 CLOSURE

From the discussion in this Chapter, two important deficiencies can be identified in the current steady state design and dynamic simulation models:

(1) The heterotrophic active biomass, which is the component of the bioreactor mixed liquor that mediates the biodegradation processes of COD removal and denitrification, has not been measured.

(2) The autotrophic active biomass, which is the component of the biorector mixed liquor that mediates the biological process of nitrification, has not been measured.

This research project endeavours to address the deficiency in heterotrophic active biomass measurement; autotrophic active biomass will be considered in a future project.

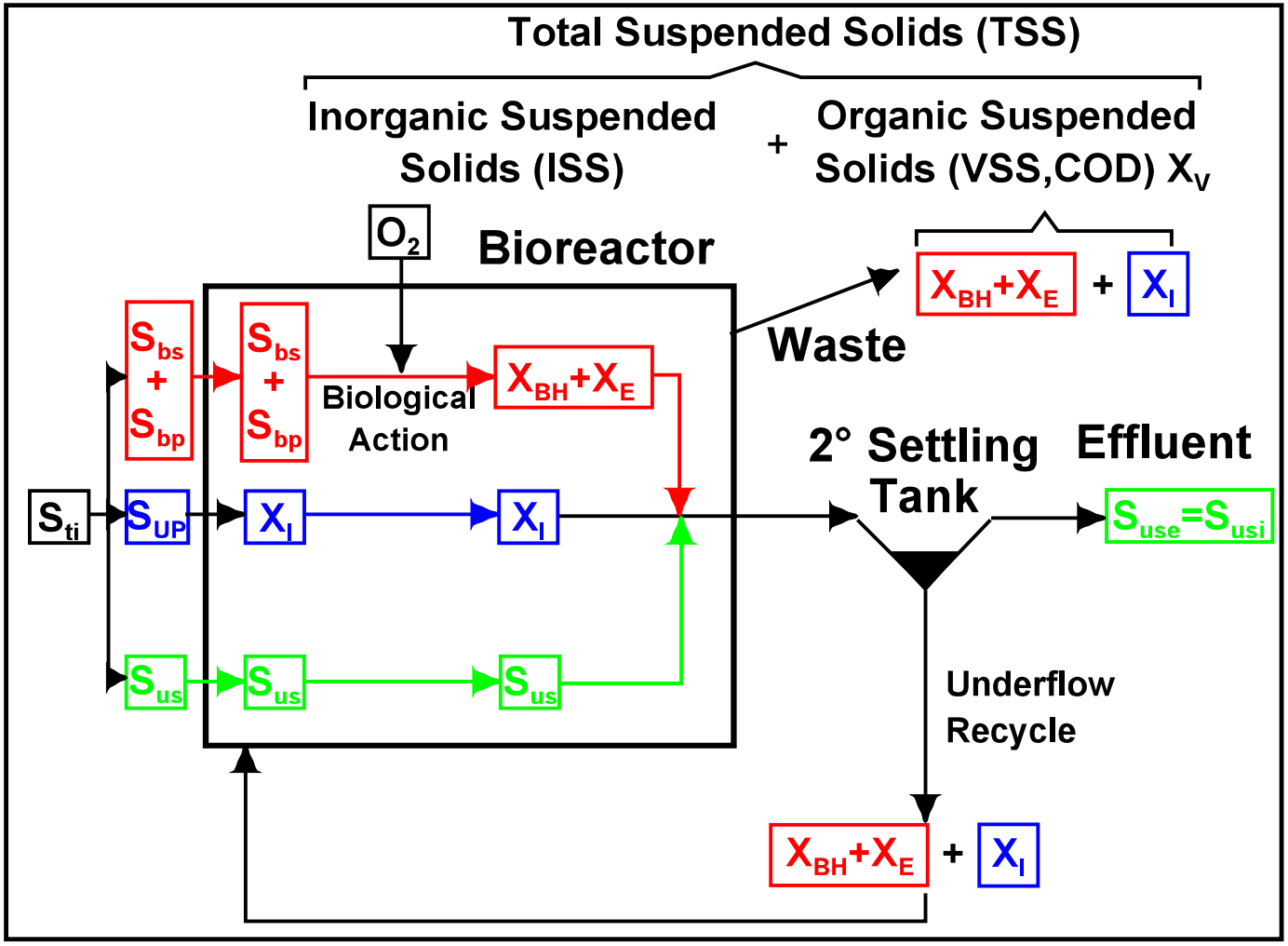

Figure 2.5: Schematic representation of the transformations of the influent COD fractions within the activated sludge system.

CHAPTER 3

EXISTING METHODS FOR QUANTIFYING HETEROTROPHIC ACTIVE BIOMASS

3.1 INTRODUCTION

In Chapter 2 the central role in the steady state design procedures and kinetic simulation models of the mixed liquor component ordinary heterotrophic organism (OHO) active biomass has been highlighted. Further, the need for accurate quantification of this mixed liquor component has been demonstrated. In this Chapter, existing methods or methods with potential to quantify the OHO active biomass in activated sludge or similar systems will be reviewed, to identify their strengths and weaknesses.

3.2 MEASUREMENT METHODS

A variety of methods (both direct and indirect) have been developed to attempt to experimentally quantify the parameters loosely termed "biomass". However, as will become evident in the review, the "biomass" parameter does not necessarily relate directly to the OHO active biomass in the steady state design procedures and kinetic simulation models for activated sludge and similar systems. This deficiency limits possible application of a number of the methods.

3.2.1 Weight

Weight has been widely used as a measure of biomass, either by direct measurement or by the use of indirect measurements such as optical density/turbidity.

3.2.1.1 Direct measurement

The dry weight per unit of volume is readily obtained by separating the solid materials from the liquid by, for example, centrifugation and then drying the solids at 105°C and weighing in a tared container; the dry weight is termed total suspended solids (TSS), Standard Methods (1985). Also, the volatile or organic solids weight can be obtained by combusting the dried sample at 600°C; the mass that combusts is termed the volatile suspended solids (VSS), Standard Methods (1985). Alternatively, the COD of the solid material can be measured (Standard Methods, 1985). These methods are widely used to quantify the mixed liquor in the activated sludge system.

3.2.1.2 Optical density (OD)

A simple technique proposed to measure biomass is to use optical density. The optical density (OD) of a growth culture is measured with a spectrophotometer at 450 nm (Jensen *et al*., 1988; Jørgensen *et al*., 1992). In parallel, samples of the growth culture are centrifuged at 4 000 rpm for 10 minutes. The sediment/pellet is dried for 24 hours at 105°C, then weighed to determine

the growth culture dry weight. A calibration curve to determine the conversion of OD to dry weight is made (a conversion factor of 250 ng/mℓ per absorbance unit is typically obtained). Absorbance of the sample to be quantified is measured and then converted to dry weight using the calibration curves. The dry weight is used as an approximation of biomass.

3.2.1.3 Summary

For activated sludge mixed liquor the weight determined with these methods will include all three organic components, i.e. active, endogenous and inert (see Chapter 2), and the dry weight (TSS) will additionally include the inorganic component. Thus, these types of tests will not be capable of isolating OHO active biomass.

3.2.2 Total cell count

The number of cells in a population can be measured by counting under the microscope, a method called the direct microscopic count (Brock and Madigan, 1988). Two kinds of count are done, either on samples dried on slides or on samples in liquid. With liquid counts, special counting chambers are used consisting of a slide with a grid marked on the surface, the volume above each grid being precisely measured. The number of cells per grid is counted under the microscope, this giving the number of cells per chamber volume.

Direct microscopic counting has a number of limitations: (1) The method is tedious, (2) living cells are not distinguished from dead, or inert/endogenous material, (3) small cells are difficult to see under the microscope and probably are missed, (4) precision is difficult to achieve, and (5) with the flocs from activated sludge it is difficult to separate out individual organisms. Thus, the method is not suitable to quantify OHO active biomass.

3.2.3 Viable cell count

In the total cell count described above, one limitation identified was that both living and dead cells are counted. To distinguish the living cells, viable cell counting methods have been developed. A viable cell may be defined as one that is able to divide and produce off-spring, i.e. replicate. The most usual way to perform a viable count is to determine the number of cells in the sample capable of forming colonies on a “suitable” medium. For this reason, the viable cell count also has been called the plate or colony count. Measurements of the number of cells capable of replication can be correlated to the weight of biomass. The viable count and the relation between viable numbers of cells and the weight of the biomass has been used as a basis for estimating the OHO active biomass in biological wastewater treatment systems (Gaudy and Gaudy, 1980; Droste and Sanchez, 1983).

Several techniques have been used in estimating the viable count, but the most common are: (1) colony count using solid media, (2) membrane filter and (3) null-point dilution in liquid medium.

3.2.3.1 Colony count using solid media

In this method, a solid medium is used to determine the number of cells capable of forming colonies. The three main types of colony count using solid media are: (1) Pour plate, (2) spread

plate and (3) spot plating. The methods differ principally in how the medium is inoculated with the sample: In pour plating, the sample is mixed with the melted agar medium and then allowed to cool, in spread plating the sample is spread evenly over the surface of a solid agar plate, while in spot plating a micro-pipette is used to add a very small discrete volume to the solid agar plate. The pour plate method is perhaps the most common plating method. In all the plating methods, the number of colonies that develop on the plate must not be too large. Thus, to obtain the correct colony numbers, the sample usually must be pre-diluted. Several ten-fold (serial) dilutions are commonly used.

The assumption made in all the solid media methods is that each visible colony grows from a single cell. Therefore, if cells are flocculated they must be thoroughly dispersed before conducting the test. For activated sludge system mixed liquor, often it is difficult to disperse the cells without influencing their viability. Thus, to retain viability less harsh dispersal methods are used, and the counts are expressed as the number of colony forming units, not as viable cells. Spread and spot plating usually have some advantage over pour plating because (i) agar plates contaminated during the pouring of agar can be discarded, and this eliminates counting errors, (ii) in pour plating the organism must be able to withstand the temperature of the melted medium, and (iii) because all colonies will be in the same plane in spread and spot plating, counting is easier. The spread and spot plating methods also have been found to be more serviceable (Gaudy *et al*., 1963).

In all plating techniques, the number of colonies obtained on the plate will depend not only on the inoculum sample size, but also on the suitability of the culture medium, the incubation conditions and the length of incubation. Despite these limitations, and the others listed above, these methods have been widely used; although for the activated sludge system, the plating methods have been more commonly used for organism identification than for viable cell counts.

3.2.3.2 Filter membrane

In this method a sample is poured over a membrane filter, preferably marked with grids to facilitate counting of colonies that develop. The filter paper then is placed on an absorbent pad containing nutrients, the pad being of such thickness that the paper will take up approximately 2 mℓ of the nutrient solution. The nutrient in the pad diffuses to the cells on the filter. The filter paper may also be placed on an agar plate. Since large volumes can be passed through the filter, the method can be used for dilute suspensions. Although this method offers some advantages over the solid media plating methods described above (e.g. easier to apply), because it also relies on colony growth on a selected medium it experiences a number of the same limitations. Furthermore, the method has inherent increased costs associated with it (Gaudy and Gaudy, 1980).

3.2.3.3 Null-point dilution in liquid medium

The basis of this method is to determine the dilution factor for a sample that no longer will provide sufficient seed of microorganisms to permit growth in fresh liquid media, i.e. the sample is diluted serially and the presence/absence of microorganisms determined. Any convenient quantitative measurement of growth can be used to detect the presence/absence of organisms in the liquid medium, e.g. gas formation has been used in a standard test for coliform organisms

(Gaudy and Gaudy, 1980). However, because the measurement method is preselected, certain organisms may be excluded. The viable count in the original sample is estimated from the dilution and presence/absence results using the appropriate probability theory (Gaudy and Gaudy, 1980). The method usually is applied to estimate the concentration of a specific organism type, and is applicable to samples that contain very few organisms; both these factors limit possible application to determine the OHO active biomass in activated sludge mixed liquor.

3.2.3.4 Summary

All the tests to detect viable cells described above rely on the ability of the organisms to replicate (plating and membrane techniques) or exhibit a specific metabolic activity (null-point dilution) in an artificial medium. This will cause the tests to be selective - only those organisms with the ability to replicate/metabolize on the artificial substrates will be included. For example, it has been estimated that less than 10% of the organisms present in activated sludge mixed liquor will be cultured on the agar plates used as a standard in the plating techniques (Cloete and Steyn, 1988). Furthermore, dispersion of the cells in the activated sludge floc (a requirement in the tests) is difficult without reducing cell viability. These factors limit possible application of this type of test to determine activated sludge mixed liquor OHO active biomass.

3.2.4 Epifluorescence microscopy

To overcome the problems associated with culturing organisms to determine viable numbers (described above), techniques have been developed to count viable cells by microscopic examination of samples. The total cell count using microscopy has been described earlier; the principle deficiency identified with this method was that it could not distinguish living cells from dead. To get around this problem, the organisms can be stained with any one of a variety of fluorescent dyes specific for living cells, and the fluorescing cells counted under the microscope; the method is termed cell epifluorescence microscopy. Fluorescent dyes are used as they aid in microscopic counting. As an alterative to cell counts, biomass volume can be determined (Andreottola *et al*., 2001). Both the cell count and biomass volume can be converted to biomass by using conversion factors such as weight of carbon per cell or cell volume (typically 310fg μm^{-3}, Fry, 1990). Converting from the derived weight of C to a dry weight or a COD requires a assumed cell formulation (Andreottola *et al*., 2001).

3.2.4.1 Acridine orange (AO) direct count

Acridine orange (AO) is a fluorescent dye that is commonly used; this dye stains any organism containing DNA (i.e. any living organism); cells staining green with AO are generally viable - AO binds to nucleic acids, with the resultant RNA-AO complexes fluorescing orange-red while DNA-AO complexes fluoresce green (Porter and Feig, 1980).

Briefly the method is: The samples are diluted with phosphate buffer to give a bacterial count of ~ 100 bacterial cells per microscopic field. A sample (0.1 mℓ) is placed in a filter tower, and 1 mℓ of 0.1% acridine orange (AO) added. The sample is incubated for 2 minutes and 3 to 5 mℓ of 0.1M phosphate buffer added and the sample filtered. The damp filter is placed on a drop of immersion oil on a glass-slide. Immersion oil and a cover slip are then added on top of the filter. The sample is examined at 1 000X (oil immersion) magnification and the number of green

fluorescing bacteria counted to determine active biomass. The AO method is mainly used in water treatment (Albat *et al*., 1986), but Bitton *et al*. (1993) have used the method for total bacterial counts in samples of non-chlorinated activated sludge effluent.

The method has a number of deficiencies for application to activated sludge mixed liquor: (i) The method yields inconsistent cell fluorescence; the fluorescence does not differentiate microbial cells on the basis of metabolic activity or viability (APHA *et al*., 1989; ASTM, 1985), (ii) the method is tedious, (iii) with the organisms in activated sludge mixed liquor binding in flocs, counting of individual cells is difficult - dispersion of the cells is a problem, as discussed earlier, and (iv) the colour of fluorescence depends on the moisture content of the filter paper; Bitton *et al*. (1993) found that addition of moisture to filter papers could change some cells fluorescence from orange-red to green.

3.2.4.2 DAPI direct count

The technique is similar to the AO direct count above, but with the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980). DAPI is a cell permanent strain, specifically binding to double stranded DNA which results in an approximately 20 fold fluorescence enhancement. When excited with light, the stain bound to DNA fluoresces bright blue, while non-DNA bound stain fluoresces weak yellow. Factors influencing the method are as for AO above.

3.2.4.3 SYBR-Green I direct count

Another epifluorescence microscopy technique is that with the flourescent dye SYBR-Green I which offers a brighter flourescent signal (Andreottola *et al*., 2001). However, this stain permeates all cells, both viable and dead.

3.2.4.4 Combination stains

Andreottola *et al*. (2001) have proposed using a combination of two flourescent stains to distinguish dead and viable organisms: SYBR-Green I is used to stain both dead and viable cells, while Propidium is used to stain dead cells only, with viable cells given by the difference.

3.2.4.5 Summary

Epifluorescence microscopy has a number of potential deficiencies for application to activated sludge mixed liquor: (i) The method can yield inconsistent cell fluorescence, (ii) the method is complex, expensive and tedious, (iii) with the organisms in activated sludge mixed liquor binding in flocs, counting of individual cells is difficult - dispersion of the cells is a problem, as discussed earlier, (iv) small volumes are used which may not be representative of the “true” sample and (v) converting from cell or cell biovolume to cell dry weight or COD requires assumed conversion factors.

3.2.5 Flow cytometry

As an alternative to epiflourescent staining and direct microscopy counting above, Andreottola *et al.* (2001) used flow cytometry to "count" cells stained with a combination of flourescent stains. The advantage of the method is that large numbers of cells can be counted in a short period (1 000 cells/s). However, the method still retains the difficulties associated with flourescent stains above.

3.2.6 Measurement of biochemical compounds

Due to the difficulties associated with the counting of organisms (directly via microscopy or indirectly via plating), various methods have been developed to measure quantitatively key compounds in organism's biochemical pathways and to relate these in some manner to organism mass. The two most commonly measured compounds are adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD).

3.2.6.1 Adenosine triphosphate (ATP) measurement

In this method the amount of adenosine triphosphate (ATP) is used as the indicator of microbial biomass. The ATP has the advantage of being a non-conservative constituent of the living cell which is directly related to the energy-growth process (Postage and Hunter, 1962; Holm-Hansen and Booth, 1966; Chappelle and Levin, 1968; Patterson *et al.*, 1970; Weddle and Jenkins, 1971; Kucknerowicz *et al.*, 1979; Roe *et al.*, 1982; Jensen *et al.*, 1988 and Jørgensen *et al.*, 1992). ATP is an energy-carrier molecule in micro-organisms, has a rapid turnover and is lost very rapidly from dead or dormant organisms. In addition, its concentration remains relatively constant and independent of the growth rate in living cells (Franzen and Binkley, 1961; Forrest, 1965; D'Eustachio and Levin, 1967; Weddle and Jenkins, 1971). Hence, the total amount of ATP measured should provide an estimate of the number of living active micro-organisms; one µg of ATP is equivalent to about 250 µg of carbon in living organisms. In the test, a sample is treated to extract ATP, and the ATP of the extract is measured. A number of sensitive methods are available for measuring ATP (Brock and Madigan, 1988). The most common method involves the measurement of light produced in the luciferin - luciferase reaction: Luciferin and luciferase are obtained from firefly lanterns and the amount of light produced when the enzyme, luciferase, acts on the substrate, luciferin, is proportional to the amount of ATP present. Thus, the ATP extracted from the sample is mixed with luciferin and luciferase and the light emission in the reaction is measured using a scintillation spectrophotometer. The light emission is proportional to the ATP present, so that from the light emission the ATP can be determined from a calibration curve. Accepting a constant ATP per unit biomass, the biomass concentration then can be calculated.

Nelson and Lawrence (1980) applied the ATP measurement method to mixed liquor drawn from a laboratory-scale completely mixed fill and draw activated sludge system receiving a synthetic wastewater. The biological solids retention time (= sludge age, R_S) in the system was varied from 0.5 to 12 days. They found that the microbial viability (measured via the ATP) of the activated sludge mixed liquor volatile suspended solids (MLVSS) exhibits a functional relationship with R_S: Expressed as % viability of the MLVSS, it is close to 100% at low values of R_S and decreases to an approximate constant value at high R_S values; this type of behaviour is typical of the activated

sludge system. Nelson and Lawrence (1980), confirmed from their study that the ATP pool level for a 100% viable culture of activated sludge is in reasonable agreement with many previously reported results for pure cultures of bacteria, and that the viable percentage of MLVSS varied with the value of R_S in a manner similar to the variations described by Postage and Hunter (1962), Weddle and Jenkins (1971) and Upadhyaya and Eckenfelder (1975). In activated sludge studies using domestic sewage as substrate, Weddle and Jenkins (1971) reported a lower viable percentage (10-20%) than found in the Nelson and Lawrence (1980) study (40-50%) at the larger values of R_S which are typical of normal process operation. Nelson and Lawrence (1980) proposed that the lower viable percentages reported in the studies treating domestic sewages are due to accumulation of non-biodegradable MLVSS which are originally present in the influent wastewater.

Summary

The ATP measurement method requires sophisticated equipment and analytical techniques which are not widely available. This will cause the method to be unsuitable for general routine application. Furthermore, because ATP turns over rapidly in metabolizing cells, the levels of ATP in a single cell can vary depending upon the conditions that the cell is subjected to, e.g. concentrations of substrate, oxygen. For example, under starvation conditions the ATP levels reduce to low values (Brock and Madigan, 1988). Since the method is based on the assumption that the ATP level per unit organism remains constant (to convert ATP to biomass concentrations), the ATP may not be a good measure of biomass, but may rather be a measure of a combination of organism activity and biomass. However, the method does appear to hold promise and has been shown to correlate to the engineering concepts of activated sludge behaviour.

3.2.6.2 Nicotinamide Adenine Dinucleotide (NADH)

This test is very similar to that for ATP, with nicotinamide adenine dinucleotide (NADH) being measured instead. NADH is the electron and proton carrier molecule in organisms and its metabolism is an indicator of metabolic activity. The NADH measurement is based upon the principle that NADH, which is found in all living cells, fluoresces at 460 nm when radiated with light at 340 nm (Armiger *et al*., 1986; 1993), the intensity of fluorescence being proportional to the NADH present. Measurement of NADH has been proposed as a method to control biological nutrient removal plant (BNR) processes (Armiger *et al*., 1990; 1991; Yang *et al*., 1991). The environmental conditions of the activated sludge determine the metabolic pathways by which NADH is constantly recycled from the oxidised to the reduced form. Specifically, in BNR processes the reduction state of the activated sludge varies as the mixed culture flows from the anaerobic zone to the anoxic zone to the aerobic zone. The biological activity in each zone is defined as the reduction state of the activated sludge times the viable cell population. By constantly measuring the fluorescence from the NADH, it is possible to monitor changes in the biological activity of the activated sludge system.

Summary

NADH measurement has potential more as an indicator of biological activity than as a method to quantify OHO active biomass.

3.2.7 Measurement of biochemical reactions

This group of tests involves measurement of the "activity" of key biochemical reactions, by monitoring the changes in substrates or products of the selected reaction. Two examples of this type of test are given below.

3.2.7.1 Fluorescein diacetate (FDA) hydrolysis

In this method the ability of the mixed liquor to hydrolyse flourescein diacetate (FDA) is monitored. FDA can be quantified by measuring light absorbance at 450 nm. Specific volumes (50 mℓ) of mixed liquor are centrifuged at 5 000 rpm for 5 minutes (Jensen *et al*., 1988; Jørgensen *et al*., 1992). The pellet is resuspended in 5 mℓ $NaHPO_4$ buffer and homogenised for two minutes by heavy stirring (Jensen *et al*., 1988). A 4.5 mℓ volume of the resuspension is placed in a 10 mℓ flask containing 0.1 mℓ EDTA and 0.4 mℓ of a solution of protein synthesis inhibitors. A 25 µℓ volume of FDA solution is added, the flask incubated on a rotating axis for 45 minutes at room temperature. After incubation the reaction is terminated by transferring to 3 mℓ of acetone (Schnürer and Roswall, 1982). The mix is then vortexed and centrifuged at 5 000 rpm for 5 minutes. The absorbance of the supernatant at 450 nm is measured with a spectrophotometer, the absorbance quantifying the FDA. Autoclaved samples are treated in the same way to serve as blanks, the difference in absorbance between the samples and blanks quantifying the FDA that has been hydrolysed. The FDA hydrolysis results are converted, using a conversion factor of 10, to determine the biomass in the sample. Thus, this method assumes that the FDA hydrolysis per unit of viable organisms is essentially constant.

Summary

As a method for OHO active biomass measurement, this technique has serious deficiencies as the values obtained are generally higher than suspended solids measurements; the opposite is expected, as it has been found that not all bacteria are able to hydrolyse FDA (Leach, 1981a,b; Chrzanowski *et al*., 1984).

3.2.7.2 Dehydrogenase enzyme activity

This method measures the activity of the dehydrogenase enzyme using fluorescence microscopy. It is based on the principle that the electron transport system of respiring organisms reduce 2-(*p*-codophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan (Zimmermann *et al*., 1978; Droste and Sanchez, 1983). Respiring bacteria deposit accumulated INT-formazan as optically dense, dark red intracellular spots which can be examined by light microscopy; the amount of INT-formazan deposited corresponding to the intensity of the respiration. By combining formazan detection with acridine orange epifluorescence microscopy (see above), a method is then obtained which allows discrimination of bacteria from detritus, and differentiation between respiring and non-respiring cells (dehydrogenase enzyme activity) (Droste and Sanchez, 1983).

Summary

Although the method allows the determination of the total and active (cells with formazan) number of bacteria from the same sample, the method, however, fails to differentiate between

active OHO and autotrophic organisms (AO). Also, the method fails to distinguish formazan deposits in small bacteria due to interference from the structure (pore openings) of the filter paper on which the microorganisms are collected (Droste and Sanchez, 1983) and the method is thus conservative. Furthermore, since acridine orange epifluorescence is required, the problems detailed above for this method apply here also. Thus, the method cannot be used for routine OHO active biomass quantification.

3.2.8 Determination of deoxyribonucleic acid (DNA) content

In this method, deoxyribonucleic acid (DNA), which constitutes the genetic material of organisms, is extracted from the activated sludge mixed liquor and the amount extracted is measured. This is used to derive an estimate for the number of active organisms present; it is assumed that each organism has a constant known amount of DNA (Weddle and Jenkins, 1971).

In using measured cellular constituents (e.g. Protein, carbohydrates, ATP, DNA) to calculate active biomass, a requirement is that the quantity of the constituent per unit active biomass remains constant. However, this may not be true in activated sludge mixed liquor because, (1) some of the measured components are not exclusively found in the biomass, and (2) the nutritional conditions of the activated sludge micro-organisms are not constant; depending on the sludge loading rate (SLR) micro-organisms contain different amounts of storage polymers (Liesbekind and Dohmann, 1994). Although the nutritional condition of the activated sludge may differ, the genome size (i.e. DNA) probably does not; thus a proportionality factor between DNA and the number of micro-organisms present can be found. Micro-organism genomes contain (with some exceptions) approximately 4 to 5×10^6 base pairs (bp) (Liesbekind and Dohmann, 1994); for example *E.coli* has 4.35×10^6 bp (Schlegel, 1985). Since activated sludge does not represent a pure culture, but is a bioceonosis of several hundreds or thousands of different micro-organisms species, an average genome size of 4.5×10^6 bp per microorganism can be assumed.

The DNA method relies on reliable extraction of the DNA. However, extraction of the DNA is not without problems: Iron has a significant effect on the amount of acid extractable DNA; Hall and Axelrod (1977) showed that in pure cultures of *Asperigillus nidulans* trace quantities of cellular ferric iron (5.6 mg/ℓ) inhibited complete DNA extraction with perchloric acid at 70°C. Iron is a common component of activated sludge, sometimes reaching concentration levels as high as 40 mg/ℓ. Temperature and the technique of washing with EDTA solution also have a significant effect on the measured DNA content. For these and other reasons, the conventional method of biomass determination using DNA (Thomanetz, 1982, Obst and Holzatel-Pschorr, 1988), can only detect up to half the actual biomass DNA present in most activated sludge systems (Raebel and Schliert, 1980), depending on the presence/absence of substances and conditions that inhibit DNA extraction.

Despite the DNA extraction problems, in a general study on biomass characterization of activated sludge, Thomanetz (1982) described and tested 17 methods for living biomass estimation and biomass activity determination and found that the best method to determine living biomass is via the determination of the DNA content, because the method was comparatively simple, quick and reproducible.

Liesbekind and Dohmann (1994) applied the method to activated sludge mixed liquor using acid

extraction of DNA, quantitative determination of the deoxyribose sugar by a colour reaction with diphenylamine, calibration of the colour reaction with standard DNA, and mathematical conversion of the measured DNA into biomass and found that the conventional DNA method is strongly affected by unknown activated sludge constituents and in particular iron. They found that washing the sludges with EDTA first improved DNA extraction, but concluded that there still is no surety as to whether all the DNA is successfully extracted.

Summary

The method, described in detail by Liesbekind and Dohmann (1994) is complicated, tedious and requires sophisticated equipment to extract DNA. Furthermore, the extraction of all DNA is problematic and depends on the presence/absence of substances and conditions that inhibit its extraction - there is uncertainty on whether all the DNA is extracted from activated sludge mixed liquid. Also, the conversion of the measured DNA to the OHO active biomass parameter used in the steady state design and kinetic simulation models is unclear. Thus this method is not practical for general routine application.

3.2.9 Molecular identification of activated sludge bacteria using rRNA/DNA

This method seeks to identify bacteria by detecting nucleic acid sequences common to the targeted bacteria. The most common nucleic acid sequences targeted are ribosomal RNA (rRNA) (e.g. Amann *et al*., 1990a,b, 1991; Wagner *et al*., 1993). Ribosomal RNAs (rRNA) are selected because they possess qualities that cause them to be suitable for discerning evolutionary relationships between bacteria: rRNAs are ancient molecules, functionally constant, universally distributed and moderately well conserved across broad phylogenetic distances. They are also readily purified from organisms without the use of cloning procedures (Brock and Madigan, 1988). There are three rRNA molecules, which in procaryotes have sizes 5S, 16S and 23S. The small size of 5S rRNA (~ 120 nucleotides) limits the information contained in the molecule, and so limits its use. However, the large rRNAs, 16S and 23S (containing approximately 1 500 and 3 000 nucleotides respectively) contain several regions of highly conserved sequence useful for proper sequence alignment, yet have sufficient sequence variability in other regions to show phylogenetic diversity. Of the two large rRNAs, 16S RNA is more experimentally manageable than 23S RNA, and so has been used extensively (it has been termed small subunit, SSU, rRNA).

Exploiting the above properties of rRNA, a number of techniques have been developed for bacterial identification, and to estimate proportions of specific or functional groups of bacteria in a sample. It is not the intention here to provide an exhaustive review of these techniques, but rather to provide a very simplified overview of some of these:

(1) rRNA sequence analysis

This technique involves sequencing the 16S rRNA. A number of methods are used to do this. For example: The rRNA is extracted from the bacteria of interest. A small DNA oligonucleotide primer (15 - 20 nucleotides in length) complementary in base sequence to some highly conserved section of the 16S rRNA molecule, is added. The enzyme reverse transcriptase (adds to the primer nucleotides which are complimentary to the rRNA) is added with 32P - labelled deoxyadenosine triphosphate and the other unlabelled deoxyribonucleotides. The mixture then is divided into four portions, and to each a small

amount of different 2', 3' dideoxynucleotide is added. The enzyme reverse transcriptase will read the rRNA and make a DNA copy interrupted at various points by the incorporation of the dideoxynucleotide. The fragments are then sequenced by electrophoresis and autoradiography. From knowledge of the complementary DNA sequence, the sequence of the original 16S rRNA can be deduced. Once the sequence is known, it can be compared to known sequences of known bacteria, and the sample bacteria identified or placed in the correct phylogenetic group.

(2) rDNA gene sequencing

The principle is the same as for the rRNA sequence analysis, except that the DNA gene coding for the 16S rRNA is sequenced. Also, instead of using the enzyme reverse transcriptase to make a complimentary copy of the nucleotide sequence, the enzyme polymerase is used to make an identical copy.

(3) In situ hybridization

In this technique an oligonucleotide compliment (called a probe) is manufactured for a specific bacterial 16S rRNA sequence. On being combined with the sample, the oligonucleotide probe will hybridize with its compliment rRNA sequence. On hybridization, the paired oligonucleotide can be caused to fluoresce and this fluorescence can be viewed under a microscope. The technique is known as fluorescent *in situ* hybridization (FISH). By careful selection of the oligonucleotide probe, the probe can be hybridized to any desired target sequence. Since some areas of the rRNA sequence are common to specific species, while others are common to genus, sub groups, groups, subphyla, etc., specific bacteria or functional groups can be identified. Also, by comparative tests the proportion of a specific bacteria or group relative to other groups (e.g. proportion of a species relative to a genus) can be determined.

The rRNA/DNA based methods are gaining increasing popularity for application to activated sludge mixed liquor. For example, Blackall (1994) applied rDNA gene sequencing to investigate filamentous bacteria in the stable dark viscous foam on the activated sludge aeration basin surfaces, and found that the diversity of the filamentous organisms in the foam increased with time. Similar studies were carried out on *Nocardia amarae* and *Nocardia pinensis* (now reclassified as *Gordona amarae* and *Skermania pinensis* respectively), both prominent foaming filaments in Australia. Genomine DNA was isolated from strains of *N.amarae* and *N.pinensis*. The 16S rDNA was amplified by the polymerize chain reaction and sequenced using an automated DNA sequencing machine. The sequences were compared and regions that could be exploited for oligonucleotide probes highlighted. Regions in the evolutionary conserved 16S rDNA gene were highlighted as possible contenders for an oligonucleotide probe for *in situ* identification and quantification of these bacteria in activated sludge plants. Good yields of unsheared, genomic DNA were obtained with all bacterial strains studied; sequences of 16S rDNA of *N.pinensis* strains were identical, whilst those for *N.amarae* varied in a couple of positions (Blackall, 1994).

Using FISH, Wagner *et al*. (1994) compared the results from *in situ* rRNA oligonucleotide probes with those from culturing samples on nutrient rich media and found large discrepancies. They ascribed these discrepancies to the selectivity of the media and culture conditions. They successfully developed probes for *Acinetobacter*, and found that the probe results indicated that these organisms were present in BEPR systems at significantly lower levels than indicated by

culturing techniques. Further, they demonstrated the application of probes to study the filamentous organism *Sphaerotilus natans*. They concluded that oligonucleotide probes will provide a tool that will greatly enhance knowledge of the ecology and phylogeny of wastewater organisms.

Summary

For the rRNA/DNA based methods, these are complex and analytically tedious requiring sophisticated equipment and considerable expertise. At present, the methods appear more suited for bacteria identification and the study of particular organism species or groups, than for quantification of total OHO active biomass in terms of the total mass in the activated sludge system. However, the methods appear to hold promise to provide quantitative data on active biomass, and this requires further investigation.

3.2.10 Batch test method

Kappelar and Gujer (1992) describe a simple batch test to quantify heterotrophic active biomass in activated sludge mixed liquor; a small quantity of mixed liquor is mixed with centrifuged wastewater and the oxygen utilization rate (OUR) response is monitored with time. From the observed exponential increase in the OUR, the initial OUR in the batch test can be determined, which can be used to derive an estimate for the heterotrophic active biomass concentration. Wentzel *et al*. (1995) and Mbewe *et al*. (1995) modified and extended this method for application to the characterization of municipal wastewaters: The batch test was conducted on unsettled municipal wastewater *without* the addition of activated sludge mixed liquor. From the OUR-time response (for example, see Fig 3.1) and a flocculated-filtered COD measurement at the end of the test, the wastewater heterotrophic active biomass, readily biodegradable COD (RBCOD) and unbiodegradable soluble COD (USCOD) could be determined. Mbewe *et al*. (1995) found that the RBCOD and USCOD measured in the batch test correlate closely to that measured via conventional methods, see Fig. 3.2. However, they were not able to evaluate the results for wastewater heterotrophic active biomass, since no conventional tests were available. They did note that measurements appeared to reflect operational changes at the wastewater treatment plant where the wastewater was collected - at the treatment plant, due to operational problems with sludge handling unit processes, on occasion waste activated sludge mixed liquor was discharged into the sewer at a point upstream of where the wastewater was collected; the batch test method could correctly detect the increase in OHO active biomass during these periods.

Ubisi *et al*. (1997a,b) extended this simple batch test method to quantify the OHO active biomass concentration in an activated sludge system. 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 where the oxygen utilization rate (OUR) and nitrate and nitrite concentrations are 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.

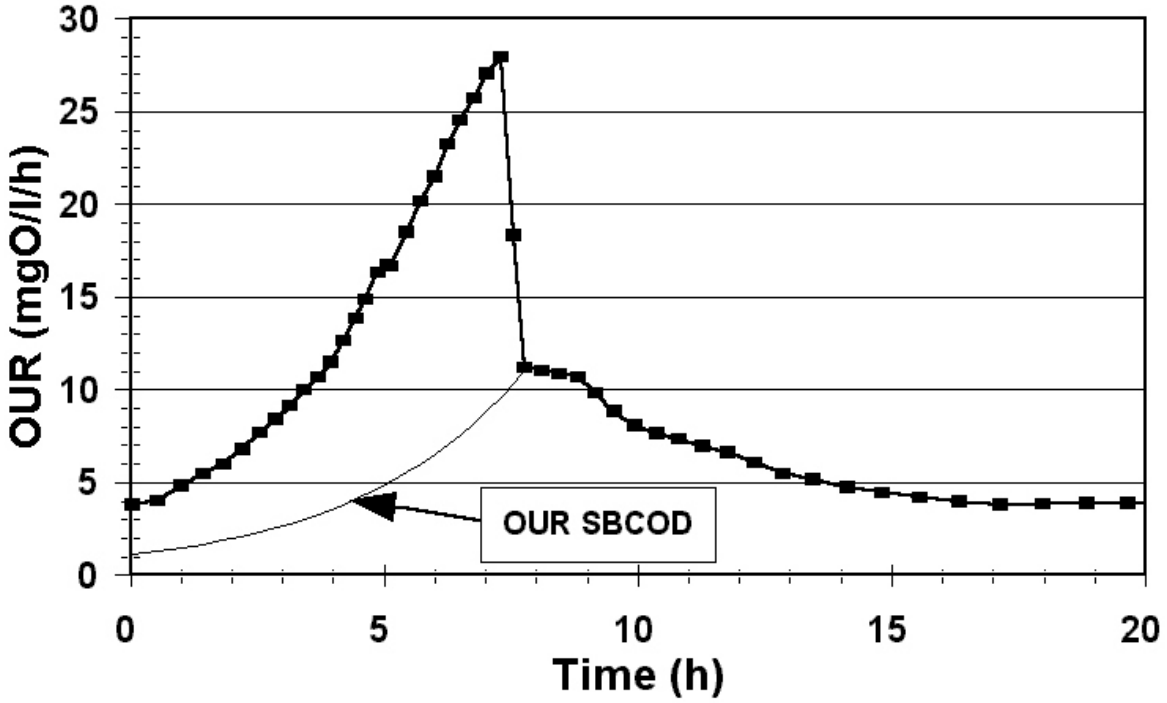

Figure 3.1: Oxygen utilisation rate (OUR) response with time for aerobic batch test on raw municipal wastewater from Mitchells Plain (Cape Town, South Africa) (after Wentzel *et al.*, 1995).

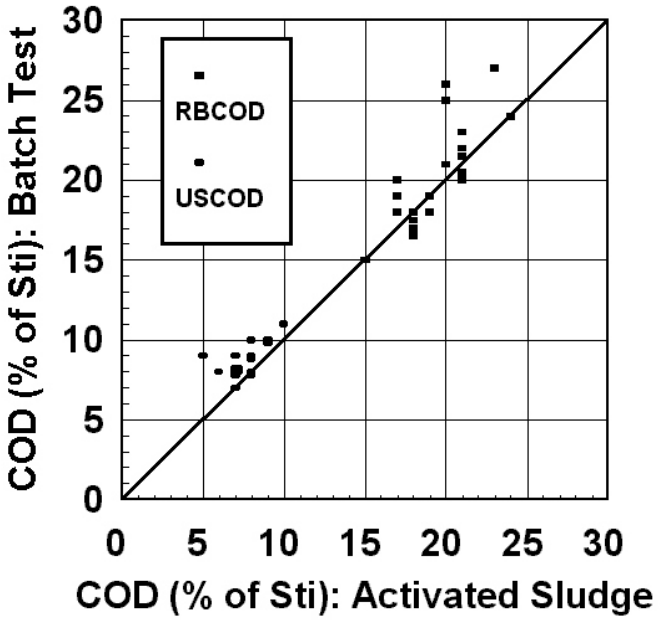

Figure 3.3: Comparison between readily biodegradable (RB)COD and unbiodegradable soluble (US)COD measured in conventional activated sludge system type tests (Ekama *et al.*, 1986) and batch tests. Each data is the mean of a number of tests (Mbewe *et al.*, 1995).

Wentzel *et al*. (1998) evaluated this batch test method by drawing mixed liquor samples from a well defined laboratory-scale anoxic/aerobic activated sludge system operated at 12 and 20 days 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. 1.1. From the comparison they concluded that the results obtained were both encouraging and perplexing. With the parent system at 12d sludge age, the agreement between measured and theoretical values was remarkably good. However, with the parent system at 20d 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.

In this research project, the batch test method of Ubisi *et al*. (1997a,b) will be further investigated, to attempt to identify the cause(s) for the inconsistency noted by Wentzel *et al*. (1998) and propose modifications to the procedure to overcome these.

Summary

The experimental procedure for the batch test is relatively simple and does not require sophisticated equipment. It would appear that the method can be readily adapted to quantify the OHO active biomass in activated sludge mixed liquor samples. Such an application will, however, have to be extensively evaluated.

3.3 CLOSURE

In this Chapter a number of experimental methods to quantify OHO active biomass have been reviewed. The vast majority of these methods find their origin in the microbiological and biochemical sciences, in the detailed study of pure cultures. For most of the tests, their application to activated sludge mixed liquor has been limited. For those that have been applied to activated sludge mixed liquor, or have potential for application, some possible deficiencies have been identified; in general, for the simpler tests, these give estimates that are too approximate to provide meaningful results and for the more rigorous tests, these may be too elaborate for routine use requiring sophisticated equipment and experimental techniques. Of this group of analytical methods, probably epiflourescent microscopy/flow cytometry, ATP analysis and the new molecular techniques appear to be the most suitable for measurement of OHO active biomass. However, these methods provide estimates for active (viable) biomass that are not directly related to the OHO active biomass parameter in the steady state design and kinetic simulation models; integration of the estimates from these tests with the design and modelling theory is an area that requires investigation, and forms part of the joint research project with the collaborators.

Within the engineering and technology paradigm, the batch test method for quantifying OHO active biomass of Kappelar and Gujer (1992) as modified and extended by Wentzel *et al*. (1995) and Mbewe *et al*. (1995) is relatively simple and does not require sophisticated equipment. From this method, estimates for OHO active biomass are obtained that are directly related to this parameter in the modelling theory. Consequently, this test appears to hold promise for possible

application - in this research project this test method will be further evaluated and developed. Should the method provide active biomass estimations that correlate closely with the activated sludge theory, this will provide users of the activated sludge simulation models with greater surety in application. Further, the method will provide an opportunity to integrate results from the microbiological/biochemical analytical methods with the modelling theory, by comparison of the data obtained from the different test methods.

CHAPTER 4

BATCH TEST PROCEDURE TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

4.1 INTRODUCTION

The batch test procedure developed by Kappeler and Gujer (1992) presented a means of quantifying the ordinary heterotrophic organism (OHO) active biomass concentration through monitoring the organisms' OUR response with time in a batch reactor. This procedure was extended by Wentzel *et al*. (1995) and Mbewe *et al*. (1995) to quantify the RBCOD concentration and the OHO active biomass concentration in municipal wastewater. Ubisi *et al*. (1997a,b) modified this method to develop a batch test procedure to determine the OHO active biomass concentration in aerobic and nitrification/denitrification (ND) activated sludge systems, and evaluated the method for mixed liquor drawn from a laboratory-scale ND activated sludge systems operated at sludge ages of 12 and 20 days respectively. The theoretical OHO active biomass concentrations predicted by the current steady state design and kinetic simulation models for the two activated sludge systems were calculated and compared to the measured values obtained through the batch tests. The comparisons showed that for the 12 day sludge age system, good agreement existed between the measured and theoretical values. For the 20 days sludge age system, however, the values compared poorly, with the theoretical values being approximately twice those measured. These contrasting results raised uncertainty regarding the reliability of the results obtained from the batch test. Accordingly, one of the objectives of this investigation was to evaluate the batch test procedure of Ubisi *et al*. (1997a,b), to identify possible modifications to improve it. In this Chapter the batch test experimental procedure and the interpretation of data obtained from the test will be described, using some data obtained by Ubisi *et al*. for illustration purposes. Appropriate modifications will be proposed and evaluated in Chapter 5.

4.2 EXPERIMENTAL PROCEDURE

4.2.1 Batch test principle

A mixed liquor sample is drawn from a parent activated sludge system and mixed with a much larger volume of wastewater in an aerobic batch reactor. In the aerobic batch test environment the active organisms present are thus surrounded by a high concentration of substrate in the form of readily biodegradable COD (RBCOD) and slowly biodegradable COD (SBCOD) from the wastewater. Under these conditions the organisms utilize the substrate at a maximum rate for the synthesis of new cell mass with an associated consumption of oxygen. Within the framework of current kinetic simulation models (e.g. UCTOLD, Dold *et al*., 1991; IAWQ ASM No. 1, Henze *et al*., 1987), with growth/substrate utilisation rates at maxima the oxygen utilization rate (OUR) is independent of the substrate concentration and directly related, *inter alia*, to the OHO active biomass concentration. Therefore, through monitoring the OUR for the duration of the batch test, and interpreting the data in terms of the kinetic simulation models, the OUR response can serve

as a means to quantify the OHO active biomass concentration present at the start of the test. In analysis of the batch test data, due consideration must be taken of the OUR for nitrification and this must be subtracted from the total OUR. Ordinary heterotrophic organisms (OHO) are also present in the wastewater; these are quantified in a separate batch test on wastewater only, performed in parallel with the mixed liquor plus wastewater batch test. The difference in OHO active biomass concentrations between the batch tests with and those without mixed liquor addition gives the OHO active biomass due to the mixed liquor addition. Ubisi *et al*. (1997a,b) describe the batch test procedures in detail, but this is repeated below for elucidation purposes using some data derived by them to illustrate the calculation procedures.

4.2.2 Parent system

The parent laboratory-scale activated sludge system operated by Ubisi *et al*. (1997a,b) as the source of mixed liquor for their batch tests was in the Modified Ludzack Ettinger (MLE) configuration; system layout is shown in Fig. 4.1. Sludge age was 12d, maintained by wasting mixed liquor from the aerobic reactor (hydraulic control) taking due account of any samples drawn from the reactors for analysis; temperature was controlled at 20°C, pH at 7.6 ($\pm$0.2); influent flow rate was constant, set at 15ℓ/d. Influent feed was raw (unsettled) municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa); the sewer retention time for this treatment plant is relatively short (± 4 hours) and the conditions are anaerobic and therefore it was expected that the OHO active biomass concentration in this wastewater would be low. The wastewater was collected in batches from the treatment works, stored in stainless steel tanks at 4°C and served as feed for both the parent system and batch tests for a period of 1-2 weeks (see Ubisi *et al*., 1997a,b for details). For the parent system, a sample of the wastewater was drawn from the storage tanks after thorough mixing and diluted with tap water to give influent feed total COD ~ 500 mgCOD/ℓ. System operational procedures detailed by Ekama *et al*. (1986) were followed. Daily monitoring included influent COD, TKN; all reactors nitrate + nitrite; aerobic reactor VSS, TSS, COD and TKN of the VSS, oxygen utilization rate (OUR); effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985). (Individual nitrite measurements indicated that nitrite concentrations were very low compared to nitrate concentrations and consequently could be neglected for this investigation). To ensure steady state, the parent system was operated for more than three sludge ages before monitoring commenced and for a further two sludge ages before mixed liquor was harvested for the batch tests. Detailed data on the parent system is described by Ubisi *et al*. (1997a,b); here, to illustrate the batch test procedure, the data from the period with wastewater (WW) Batch No. 12 as influent will be used. The reliability of the experimental measurements were checked by means of mass balances on COD and N (Ekama *et al*., 1986).

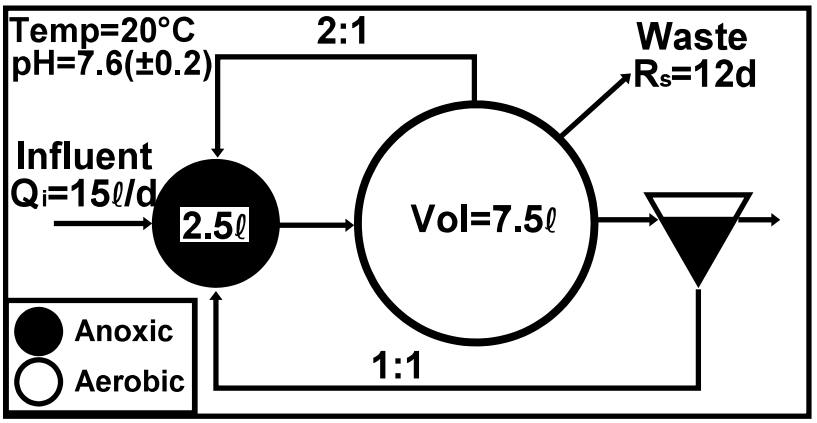

Figure 4.1: Schematic layout and operational data for parent laboratory-scale system of Ubisi *et al.* (1997a,b).

4.2.3 Batch Tests

Two variations of the batch test procedure detailed by Wentzel *et al.* (1995) and Mbewe *et al.* (1995) were run by Ubisi *et al.* (1997a,b): In one type, only unsettled municipal wastewater was added to the batch test and in the second, a mixture of wastewater and mixed liquor was added. For both types of batch tests, a sample of the wastewater was drawn from the storage tanks after thorough mixing and diluted to approximately the same COD concentration as that fed to the parent system (~ 500 mg COD/ℓ).

For the wastewater only batch tests, a 3ℓ volume of the diluted wastewater was preheated to 20°C and then placed in a continually stirred batch reactor maintained at a constant temperature of 20°C. A sample was drawn to obtain the initial total COD concentration (Standard Methods, 1985). In operating the batch test, the surface of the wastewater was covered by small plastic balls to limit surface exchange of oxygen. The oxygen utilization rate (OUR) was monitored continually using an automated technique (Randall *et al.*, 1991) - the DO was raised to ~ 6 mgO/ℓ, the air switched off and the decrease in DO monitored, the rate of decrease giving the OUR; when the DO reached ~ 4 mgO/ℓ, the air was switched on again and the cycle repeated. (The exact values for the high and low DO set points were varied depending on the OUR - if the OUR was low the high and low DO set points were moved closer together and *visa versa*). The pH of the reactor was monitored continually and controlled to pH 7.5 ($\pm$ 0.2). Because of the low OUR values, the walls of the reactor were thoroughly brushed (regularly during an aeration cycle) to prevent particulate matter adhering to them. At intervals, samples were drawn from the reactor, filtered (0.45µm) and analysed for nitrate + nitrite and nitrite (for the purpose of the batch test, nitrite concentrations were found to be negligible compared to nitrate concentrations, <1%). The batch tests were conducted for approximately 24h. At the end of the batch tests, the contents of the batch reactor were homogenised in a liquidiser, a sample drawn and total COD concentration measured.

For the batch tests with a mixture of wastewater and mixed liquor, a sample of mixed liquor was harvested from the aerobic reactor of the parent system and a defined volume (variously 100, 200, 300 or 400 mℓ) placed in the batch reactor. The batch reactor volume was made up to 3ℓ with the same diluted unsettled municipal wastewater used in the wastewater only batch tests, also preheated to 20°C (see above). The batch test procedure detailed above was then followed.

To illustrate the batch test procedure, batch tests conducted by Ubisi *et al*. with their WW Batch No. 12 will be used.

4.3 BATCH TEST DATA INTERPRETATION

4.3.1 Parent system and theoretical OHO active biomass

4.3.1.1 Parent system steady state data

For the batches of wastewater fed to the parent system, detailed results are presented by Ubisi *et al*. (1997a,b); for illustrative purposes the averaged data for WW Batch No. 12 are listed in Table 4.1, together with the averaged data for the preceding two wastewater batches (No. 10 and 11). Following the procedures set out by Ekama *et al*. (1986), Ubisi *et al*. determined the following: The influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively); system COD and N mass balances; the COD and TKN to VSS ratios of the mixed liquor (f_{cv} and f_N respectively). These are listed in Table 4.2. Referring to Table 4.2, acceptable COD (91 - 96%) and N (95 - 103%) mass balances were obtained; thus, the steady state data for the parent system can be considered acceptable.

Table 4.1: Steady state results for parent laboratory-scale anoxic/aerobic sludge system (Fig. 4.1). For each of the three wastewater batches tested, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

ANOXIC/AEROBIC STEADY STATE SYSTEM													
WW Batch	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite (mgN/ℓ)			Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			Aerobic pH
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
10	12	554 (69)	59 (12)	45.7 (3.3)	4.3 (0.7)	0.5 (0.3)	7.5 (0.9)	7.2 (0.8)	27.6 (2.1)	2222 (167)	3372 (330)	231 (18)	7.69 (0.25)
11	13	526 (27)	46 (13)	44.0 (1.5)	3.5 (0.3)	0.4 (0.2)	7.0 (0.3)	7.1 (0.4)	27.9 (1.9)	2339 (169)	3499 (195)	248 (16)	7.73 (0.14)
12	15	517 (30)	49 (15)	41.5 (2.5)	3.6 (0.6)	0.4 (0.1)	5.3 (0.6)	4.9 (0.7)	24.6 (1.7)	2293 (149)	3433 (341)	255 (13)	7.68 (0.17)

Table 4.2: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic activated sludge system (Fig. 4.1). Data calculated from data Table 4.1, either directly or using the steady state (SS) design (WRC, 1984) or kinetic simulation (sim.) models (Dold *et al*., 1991)

ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No of tests	Mass Balance (%)		Wastewater fractions			Mixed liquor			
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)		COD/VSS ratio (mgCOD/ mgVSS) (f_{cv})	TKN/VSS ratio (mgN/ mgVSS) (f_n)	Active Fraction (f_{av})	
					SS Design	Kinetic Sim.			SS Design	Kinetic Sim.
10	12	91	102	0.106	0.125	0.115	1.52	0.104	0.400	0.395
11	13	96	103	0.087	0.165	0.153	1.50	0.106	0.351	0.351
12	11	94	95	0.094	0.167	0.154	1.50	0.111	0.348	0.349

4.3.1.2 Parent system theoretical OHO active biomass

From the parent system steady state data, the theoretical OHO active biomass concentration of the mixed liquor drawn from the parent system and added to the batch tests was calculated by Ubisi *et al*. To do this two approaches could be followed - either the steady state design or the kinetic simulation models could be used. In the kinetic simulation models (Dold *et al*., 1980, 1991; Henze *et al*., 1987), at 20°C (the temperature for all experiments) for sludge ages > about 3d, virtually all the influent biodegradable COD is depleted. Certainly this was the case for the parent system at 12d sludge age with the small anoxic mass fraction (25%) present (Fig. 4.1). Under these conditions the steady design equations (WRC, 1984) and the kinetic simulation models should give near identical values for OHO active biomass, provided equivalent values for the respective constants are used; to verify this, Ubisi *et al*. used both the steady state and kinetic approaches.

Steady state model

From WRC (1984), the OHO active biomass fraction of the mixed liquor volatile suspended solids (VSS) (f_{av}) can be determined from:

$$f_{av} = MX_{BH}/MX_V = MX_{BH}/(MX_{BH} + MX_E + MX_I + MX_{BA}) \quad (4.1)$$

where

MX_{BH} = mass of OHO active biomass, VSS units (mgVSS)
$= V \cdot X_{BH}$

MX_E = mass of endogenous material, VSS units (mgVSS)
$= V \cdot X_E$

MX_I = mass of inert material, VSS units (mgVSS)
= $V \cdot X_I$
MX_{BA} = mass of autotrophic organisms (nitrifiers), VSS units (mgVSS)
= $V \cdot X_{BA}$
V = system volume (ℓ)
X_{BH} = OHO active biomass concentration, VSS units (mgVSS/ℓ)
X_E = endogenous material concentration, VSS units (mgVSS/ℓ)
X_I = inert material concentration, VSS units (mgVSS/ℓ)
X_{BA} = autotrophic active biomass concentration, VSS units (mgVSS/ℓ)
MX_V = mass of volatile suspended solids, VSS units (mgVSS)
= $V \cdot X_V$
X_V = volatile suspended solids concentration, VSS units (mgVSS/ℓ)

In Eq. (4.1), for activated sludge systems receiving "normal" municipal wastewaters (influent TKN/COD ratio < 0.12 mgN/mgCOD) the autotrophic active biomass (MX_{BA}) component of the mixed liquor organic suspended solids is very small compared to the other three components (< 2% of the total for the parent system here). Thus, with very little error, the autotrophic active biomass can be neglected when calculating the mixed liquor VSS. Accordingly, from WRC (1984), substituting in Eq. (4.1) for MX_{BH} and $MX_V = (MX_{BH} + MX_E + MX_I)$:

$$\frac{1}{f_{av}} = 1 + f_E^* b_{HT}^* R_S + \frac{f_{S,up}\,(1 + b_{HT}^* R_S)}{f_{cv}\,Y_H^*\,(1 - f_{S,us} - f_{S,up})} \qquad (4.2)$$

where

f_E^* = fraction of OHO active biomass that is endogenous residue
= 0.2 (endogenous respiration theory, Dold *et al.*, 1980).
b_{HT}^* = specific endogenous mass loss rate at temperature T (/d)
= $b_{H20}^* \, 1.029^{(T-20)}$
b_{H20}^* = specific endogenous mass loss rate at 20°C
= 0.24/d @ 20°C (endogenous respiration theory, Dold *et al.*, 1980)
R_S = system sludge age (d)
= 12 d
$f_{S,up}$ = fraction of influent substrate that is unbiodegradable particulate
$f_{S,us}$ = fraction of influent substrate that is unbiodegradable soluble
f_{cv} = COD to VSS ratio of mixed liquor organic suspended solids (mgCOD/mgVSS)
Y_H^* = OHO active biomass yield, VSS units (mgVSS/mgCOD)
= 0.45 mgVSS/mgCOD (WRC, 1984)

Using Eq. (4.2) and the data in Table 4.1, Ubisi *et al.* calculated values for f_{av}, see Table 4.2.

Kinetic simulation

For the kinetic simulation models, using the UCTOLD computer programme (Dold *et al.*, 1991), Ubisi *et al.* determined values for f_{av} from simulations of the parent system steady state periods - for each steady state period the values for the influent wastewater unbiodegradable particulate

COD fraction ($f_{S,up}$) were adjusted until the simulated and measured reactor mixed liquor organic suspended solids concentrations (COD units) were near equal and the f_{av} calculated from the simulated results: These values for $f_{S,up}$ and f_{av} are also listed in Table 4.2. Comparing the values for f_{av} from the steady state design procedure and the kinetic simulations, near identical values were obtained for the WW batches 10, 11 and 12. The small differences arise principally because (i) with the steady state design models the $f_{S,up}$ and f_{av} were determined from the measured mixed liquor organic suspended solids (MLOSS) VSS concentration whereas with the kinetic simulation models these were determined from the MLOSS COD concentration, and (ii) the kinetic simulation models include autotrophic active biomass and non-utilised slowly biodegradable COD in the MLOSS whereas these are ignored in the steady state design models. Due to their near equality, the f_{av} from either the steady state design or the kinetic simulation models could be used, but the steady state design model is probably preferable for the simpler more direct analytical calculation procedure.

Now, knowing f_{av} and the concentration of the mixed liquor VSS that was drawn from the parent system [X_V (PS)] to be added to the batch tests (available from the averaged steady state VSS concentration measured in the parent system, Table 4.1), the theoretical OHO active biomass *concentration in the batch reactor* due to the added mixed liquor [Z_{BH} (theo)$_{BT}$, COD units] is given by

$$Z_{BH}\,(\text{theo})_{BT} = [X_V\,(\text{PS}) \cdot f_{av} \cdot f_{cv} \cdot V_{ML}]/(V_{ML} + V_{WW}) \qquad (4.3)$$

where

Z_{BH} (theo)$_{BT}$
= theoretical OHO active biomass concentration in batch test reactor due to added mixed liquor, COD units (mgCOD/ℓ batch reactor)

X_v(PS)= mixed liquor VSS concentration measured in parent system, Table 4.1 (mgVSS/ℓ)

V_{ML} = volume of mixed liquor from parent system added to batch test (ℓ)

V_{WW} = volume of wastewater added to batch test (ℓ).

Theoretical values for the OHO active biomass concentration in the batch reactor due to the added mixed liquor are listed in Table 4.4.

Note that in Eq. (4.3) the parent system mixed liquor organic suspended solids are expressed in VSS units [X_V(PS)], whereas the OHO active biomass is expressed in COD units. This is done because conventionally the mixed liquor organic suspended solids in activated sludge systems are measured via the VSS test, whereas the kinetic models used to develop the batch test are in terms of the COD parameter (see later). However, the two units of measure are directly related through the COD/VSS ratio of the mixed liquor organic suspended solids (f_{cv}) which was measured, see Table 4.2. If a value for f_{cv} is not available from measurement, the standard value of 1.48 mgCOD/mgVSS (WRC, 1984) can be accepted.

Table 4.3: Batch tests with wastewater (WW) only: COD recovery, regression data from ℓn OUR versus time plots and OHO active biomass at the start of the batch test [$Z_{BH(0)}$ (WW)].

BATCH TEST: WASTEWATER (WW) ONLY							
WW Batch	Batch Test No	COD Recovery (%)	Regression			$Z_{BH(0)}$WW	
			Y-intcpt	Slope	R^2	Concentration (mgCOD/l)	Fraction of Total COD (%)
12	W1	92	1.344	0.287	0.992	25	5
	W2	98	1.059	0.276	0.961	19	4.6
	W3	91	0.544	0.194	0.998	16	3.9
	W4	94	0.521	0.198	0.999	15	3.6
	Ave.	93.8				18.8 (SD=4.5)	

Table 4.4: Batch tests with mixture of wastewater (WW) and mixed liquor (ML) drawn from parent laboratory-scale system: Volumes added, COD recoveries, regression data from ℓn OUR_H versus time plots and OHO active biomass present in the batch test ($Z_{BH(0)}$) due to mixed liquor+wastewater (ML+WW), wastewater (WW_D, taking due account of dilution) and mixed liquor (ML). Also shown are the theoretical OHO active biomasses due to the mixed liquor addition.

BATCH TEST: WASTEWATER (WW) + MIXED LIQUOR (ML)											
WW Batch	Batch Test No	Volume (ℓ)		COD Recov. (%)	Regression			$Z_{BH(0)}$ (mgCOD/ℓ)			
								Measured			Theoretical ML
		ML	WW		Y-intcpt	Slope	R^2	ML+WW	WW_D	ML	
12	M1	0.1	2.9	95	1.409	0.137	0.987	50	18.0	32	40
	M2	0.2	2.8	102	2.209	0.143	0.972	108	17.5	90	80
	M3	0.2	2.8	99	2.199	0.160	0.990	97	17.5	80	80
	M4	0.3	2.7	100	2.267	0.113	0.907	139	16.9	122	119
	M5	0.3	2.7	101	2.310	0.113	0.962	145	16.9	128	119
	M6	0.3	2.7	97	2.266	0.136	0.969	119	16.9	102	119
	M7	0.4	2.6	96	2.399	0.092	0.960	185	16.3	168	159
	M8	0.4	2.6	96	2.262	0.076	0.993	188	16.3	172	159

4.3.2 Batch tests

Batch tests conducted using WW Batch No. 12 by Ubisi *et al.* are used here to illustrate the calculation procedures. As noted above, two types of batch tests were conducted, one type with wastewater only and the other type with wastewater and mixed liquor.

4.3.2.1 Wastewater only batch tests

Four batch tests on WW Batch No. 12 were conducted by Ubisi *et al.* with wastewater only, see Table 4.3. For detailed data on the batch tests, see Ubisi *et al*. As an example, the OUR (mgO/ℓ·h) versus time (h) responses for a batch test with wastewater only (Batch test No. W3, Table 4.3) is shown plotted in Fig. 4.2. No nitrate or nitrite was detected in this series of batch tests indicating the absence of nitrification, that is, no autotrophic biomass was present in the wastewater. (Should nitrification take place, it can be taken into account by following the procedures set out below).

Referring to the OUR time plot (Fig. 4.2), the profile conforms to that described by Wentzel *et al.* (1995): During the first period of the batch test (<8.5h) the OUR exhibits an exponential increase due to OHO active biomass growth. After ± 8.5h, the OUR drops precipitously due to depletion of the wastewater readily biodegradable COD (RBCOD). For the remainder of the batch test, the OUR exhibits an inverted S pattern typical of saturation kinetics, due to slowly biodegradable COD (SBCOD) utilization.

Following the procedures set out by Wentzel *et al*. (1995) and Mbewe *et al*. (1995), the batch tests were analysed to determine:

- COD recovery (%)
- wastewater OHO active biomass, $Z_{BH(0)}$ (mgCOD/ℓ)

Additionally, if required the maximum specific growth rates as formulated in the UCT model can be determined from the batch test data, for growth of OHOs on:

- RBCOD, μ_H (/d), and
- SBCOD, K_{MP} (/d)

Wentzel *et al*. (1995) and Mbewe *et al*. (1995) describe the derivation of equations to quantify the above parameters in terms of the UCT model. The derivations provide a logical insight into the mathematical modelling of the processes involved in the batch test and thus are repeated here to clarify interpretation of the OUR-time response. (Wentzel *et al*.,1995 and Mbewe *et al*., 1995 also derive the above in terms of the IWA ASM No1, but for brevity this is not repeated here).

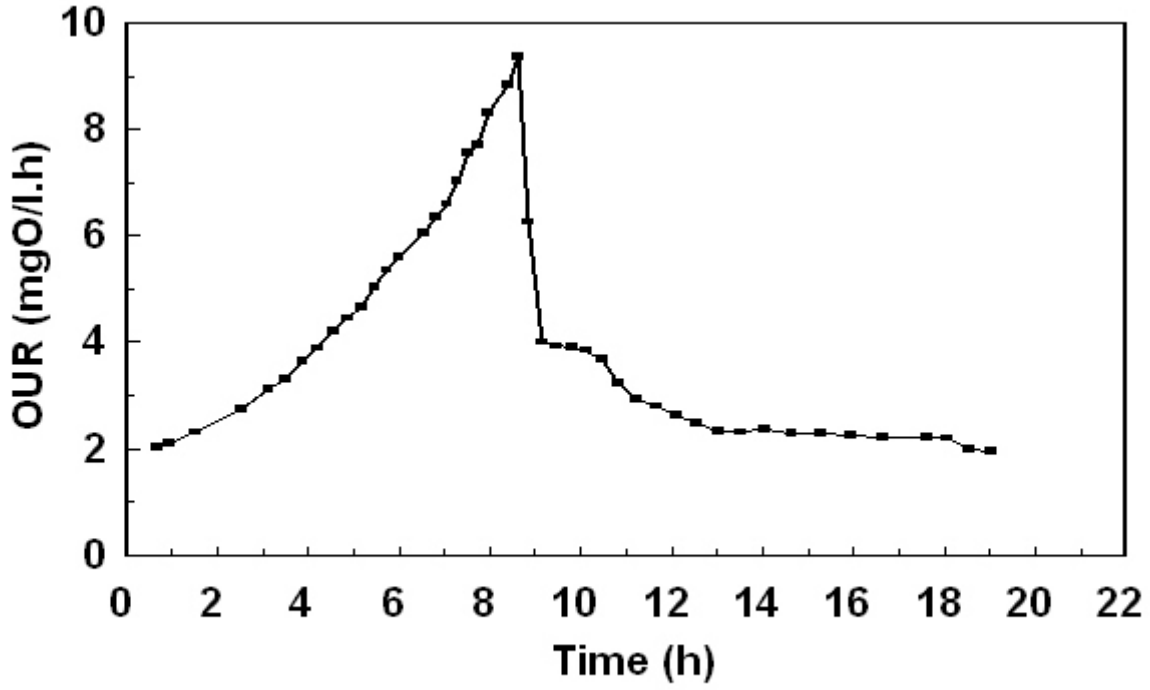

Figure 4.2: Oxygen utilisation rate (OUR) response with time for aerobic bath test on raw municipal wastewater from Mitchell's Plain (Cape Town, South Africa). (Batch Test No. W3, Table 4.3).

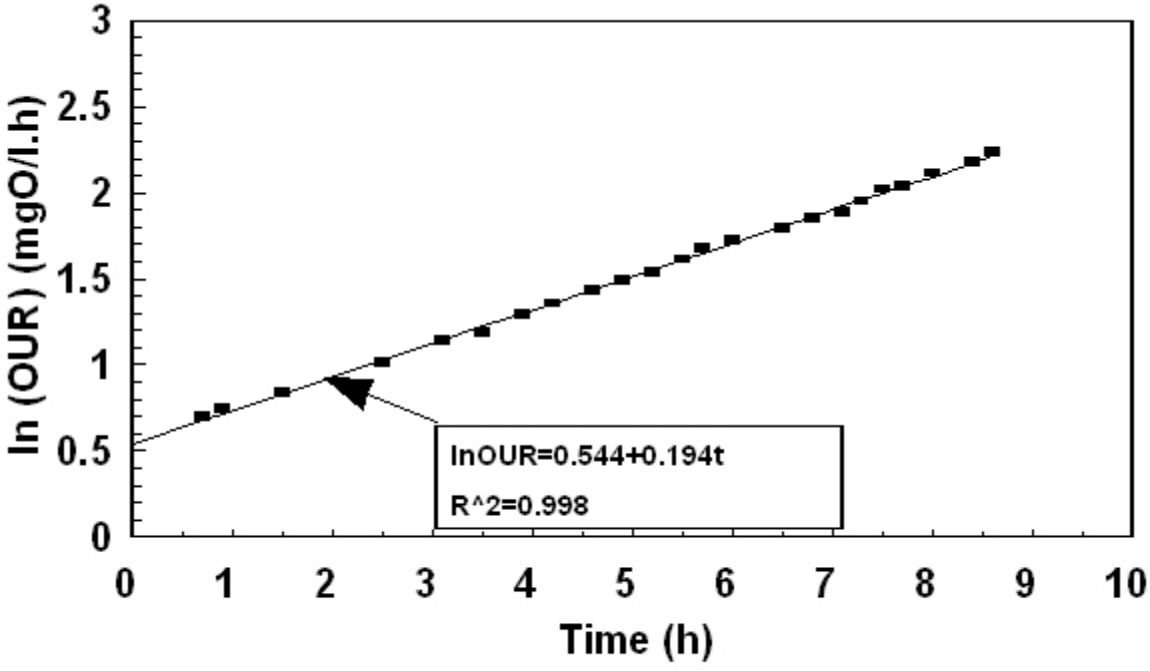

Figure 4.3: ℓn oxygen utilisation rate (OUR) versus time for the OUR data in Fig. 4.2, up to the precipitous drop in OUR.

COD recovery

For COD recovery (Wentzel *et al.*, 1995)

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR_{H(t)} \cdot dt}{COD_{t=0}} \cdot 100 \qquad (4.4)$$

where

t	= time (h)
$COD_{t=0}$	= total unfiltered COD concentration at start of test (t = 0) (mgCOD/ℓ).
$COD_{t=T}$	= total unfiltered COD concentration at end of test (t = T) (mgCOD/ℓ).
$OUR_{H(t)}$	= OHO active biomass oxygen utilization rate at time t (mgO/ℓ/h).
$\int_{t=0}^{t=T} OUR_{H(t)} \cdot dt$	= integral (area) under the OHO OUR versus time plot between start and end of test (mgO/ℓ).
	= oxygen consumed over the test by OHO active biomass.

In Eq. (4.4), since in this series of batch tests no nitrification was observed, the measured OUR at time t ($OUR_{M(t)}$) is due to OHO growth only and equals $OUR_{H(t)}$. Integrating the area under measured OUR time profile and substituting into Eq. (4.4), the COD recoveries for the different batch tests with wastewater only were calculated and are listed in Table 4.3; all COD recoveries were > 90%, providing support for the reliability of the measurements.

Wastewater OHO active biomass, $Z_{BH(O)}$

In applying the UCT model (Dold *et al.*, 1991) to the wastewater only batch test, Wentzel *et al.* (1995) noted that the model can be simplified by recognising that specific conditions prevail in the batch test, namely:

- Aerobic conditions - denitrification processes need not be included
- No nitrification - nitrification processes need not be included
- Excess ammonia present
  - nitrate as an N-source for growth need not be considered
  - transformations from organic to ammonia N need not be included.

Accepting the conditions above, a number of the processes in the full UCT model can be eliminated, to develop a simplified model that is applicable to the batch test. This simplified UCT model is presented in Table 1 of Wentzel *et al.* (1995), and duplicated in Table 4.5.

Table 4.5: Matrix representation of the UCT model (Dold *et al.*, 1991), simplified for the conditions present in the batch test.

COMPOUND i → j ↓ PROCESS	1 Z_{BH}	2 Z_E	3 Z_I	4 S_{ads}	5 S_{enm}	6 S_{bs}	7 S_{us}	8 O	PROCESS RATE, ρ_j
1 Aerobic growth of Z_{BH} on S_{bs}	1					-1/Y_{ZH}		$-\frac{1-Y_{ZH}}{Y_{ZH}}$	$\mu_H\left[\frac{S_{bs}}{K_{SH}+S_{bs}}\right]Z_{BH}$
2 Aerobic growth of Z_{BH} on S_{ads}	1			-1/Y_{ZH}				$-\frac{1-Y_{ZH}}{Y_{ZH}}$	$K_{MP}\left[\frac{(S_{ads}/Z_{BH})}{K_{SP}+(S_{ads}/Z_{BH})}\right]Z_{BH}$
3 Death of Z_{BH}	-1	f_E			1-f_E				$b_H Z_{BH}$
4 Adsorption of S_{enm}				1	-1				$K_A S_{enm} Z_{BH}(f_{MA} - S_{ads}/Z_{BH})$
Stoichiometric constants Y_{ZH} = Heterotroph yield f_E = Endogenous residue f_{MA} = Max. ratio S_{ads}/Z_{BH}	Biological (active) heterotrophic mass M(COD) L^{-3}	Endogenous mass M(COD) L^{-3}	Inert Mass M(COD) L^{-3}	Adsorbed biodegradable substrate M(COD) L^{-3}	Enmeshed (trapped) biodegradable substrate M(COD) L^{-3}	Readily biodegradable (soluble) substrate M(COD) L^{-3}	Unbiodegradable soluble substrate M(COD) L^{-3}	Oxygen M(-COD) L^{-3}	Kinetic constants $\hat{\mu}_H$ = Heterotroph max. specific growth rate on S_{bs} K_{SH} = Heterotroph 1/2 saturation on S_{bs} K_{MP} = Heterotroph max. specific growth rate on S_{ads} K_{SP} = Heterotroph 1/2 sat. on S_{ads} b_H = Heterotroph specific death rate K_A = S_{enm} specific adsorption rate

From the simplified UCT model (Fig. 4.5), the rate of growth of OHO active biomass (dZ_{BH}/dt) is given by:

dZ_{BH}/dt = growth on RBCOD + growth on SBCOD - death

$$\frac{dZ_{BH}}{dt} = \mu_H \frac{S_{bs}}{K_{SH} + S_{bs}} \cdot Z_{BH} + K_{MP} \frac{S_{ads}/Z_{BH}}{K_{SP} + S_{ads}/Z_{BH}} \cdot Z_{BH} - b_H \cdot Z_{BH} \quad (4.5)$$

where

Z_{BH}	=	OHO active biomass concentration (mgCOD/ℓ)
S_{bs}	=	RBCOD concentration (mgCOD/ℓ)
K_{SH}	=	half saturation constant for RBCOD
	=	5 mgCOD/ℓ (Dold *et al.*, 1991)
S_{ads}	=	absorbed SBCOD concentration (mgCOD/ℓ)
K_{SP}	=	half saturation constant for SBCOD
	=	0.027 mgCOD/mgCOD (Dold *et al.*, 1991)

It can be accepted that during the initial stages of the batch test (before RBCOD is depleted and the OUR drops precipitously) $S_{bs} >> K_{SH}$ and $S_{ads} / Z_{BH} >> K_{SP}$, and therefore,

$$\frac{dZ_{BH}}{dt} = (\mu_H + K_{MP} - b_H) \cdot Z_{BH} \quad (4.6)$$

Integrating Eq. (4.6) and solving yields the OHO active biomass concentration at time t [$Z_{BH(t)}$, mgCOD/ℓ] in terms of the initial OHO active biomass concentration [$Z_{BH(0)}$, mgCOD/ℓ], time (t, in h) and the net specific growth rate ($\mu_H + K_{MP} - b_H$) viz;

$$Z_{BH(t)} = Z_{BH(0)} \, e^{(\mu_H + K_{MP} - b_H)t/24} \quad (4.7)$$

The OUR at time t (OUR_t, mgO/ℓ) is a function of $Z_{BH(t)}$, the net specific growth rate and OHO yield coefficient, Y_{ZH} = 0.666 mgCOD/mgCOD:

$$OUR_{(t)} = \frac{1 - Y_{ZH}}{Y_{ZH}} (\mu_H + K_{MP}) \, Z_{BH(t)}/24 \quad (4.8)$$

Substituting Eq. (4.7) for $Z_{BH(t)}$ in Eq. (4.8) and taking natural logs yields

$$\ell n OUR_{(t)} = \ell n \left[\frac{1 - Y_{ZH}}{Y_{ZH}} (\mu_H + K_{MP}) \, Z_{BH(0)}/24 \right] + (\mu_H + K_{MP} - b_H)t/24 \quad (4.9)$$

which is a straight line with,

slope = $(\mu_H + K_{MP} - b_H) /24$ (4.10)

$$\text{y-intercept} = \ell n OUR_{(t=0)} = \ell n\left[\frac{1-Y_{ZH}}{Y_{ZH}}\,(\mu_H + K_{MP})\,Z_{BH(0)}/24\right] \qquad (4.11)$$

To determine the OHO active biomass at the start of the batch test ($Z_{BH(0)}$), the OUR values for the data up to the precipitous drop in OUR were plotted ℓn OUR versus time (h) (for example, the OUR data in Fig. 4.2 are shown plotted in Fig. 4.3; data for the other batch tests are listed in Ubisi *et al*., 1997b), and linear regression applied to determine the y-intercept, slope and correlation coefficient; these are listed in Table 4.3 for the different batch tests conducted during WW Batch No. 12 on wastewater only. From the slopes and y-intercepts, $Z_{BH(0)}$ can be determined (Wentzel *et al*., 1995):

$$Z_{BH(0)} = \frac{e^{\text{y-intercept}} \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_{HT})} \qquad (4.12)$$

where

$Z_{BH(0)}$ = OHO active biomass concentration at the start of the batch test (mgCOD/ℓ batch reactor)

Y_{ZH} = OHO active biomass yield, COD units (mgCOD/mgCOD)
= 0.666 mgCOD/mgCOD (Dold *et al*., 1980, 1991; Wentzel *et al*., 1995)

b_{HT} = OHO specific death rate at temperature T (/d)
= $b_{H20}\ 1.029^{(T-20)}$

b_{H20} = OHO specific death rate at 20°C
= 0.62/d (death/regeneration theory, Dold *et al*., 1980; Wentzel *et al*., 1995).

Note that in Eq. (4.12) for the batch tests, the death regeneration theory (Dold *et al*., 1980) is used (with b_{H20} = 0.62/d), whereas in Eq. (4.2) for the steady state system, the endogenous respiration theory (Dold *et al*., 1980; WRC, 1984) is used (with b_{H20}^{*} = 0.24/d). In the death regeneration theory, the OHO active biomass dies at a certain rate; of the biomass lost, the biodegradable portion adds to the slowly biodegradable COD which passes through the various stages to be utilized for OHO active biomass synthesis with associated oxygen utilization [i.e. the oxygen demand arises, in fact, from the energy requirements for resynthesis of OHO active biomass (regeneration) from the slowly biodegradable substrate liberated from organism death]; the unbiodegradable portion adds to the endogenous residue. In the endogenous respiration theory, the OHO active biomass dies at a certain rate; of the biomass lost, the biodegradable portion gives rise *directly* to oxygen utilization (there is no slowly biodegradable substrate intermediate) and the unbiodegradable portion adds to endogenous residue. For the steady state parent system where all the biodegradable COD has been depleted, with the appropriate selection of constants the two approaches give the same nett result, i.e. same loss of OHO active biomass, utilization of oxygen and generation of endogenous residue (Dold *et al*., 1980). However, the endogenous respiration approach allows a simple analytical procedure to be developed to provide a solution for the steady state system (WRC, 1984); accordingly, the endogenous respiration

approach is followed here for the steady state parent system. For the batch tests, the conditions are transient (e.g. the wastewater biodegradable COD has not been depleted, and is utilized over the course of the batch tests). Thus, to interpret the batch test results, a full kinetic model should be used (e.g. UCTOLD, Dold *et al*., 1991). In the kinetic models the death regeneration approach has been adopted (for reasons see Dold *et al*., 1980), and thus this approach is followed here for analysis of the batch tests. However, the endogenous respiration theory can be used to derive an equation that is numerically identical to Eq. (4.12). Thus, the choice of using the death regeneration theory over the endogenous respiration theory does not influence the results.

Accepting Eq. (4.12) and substituting into the equation the regression data listed in Table 4.3 for the batch tests, values for $Z_{BH(0)}$ for the various batch tests with wastewater (WW) only were calculated and are listed in Table 4.3 as concentrations and percentages of the total wastewater COD: As reported by Mbewe *et al*. (1995) and Wentzel *et al*. (1995), for Mitchell's Plain wastewater, OHO active biomass was found to be present at low concentrations, 3.5 - 5% of total COD.

Determining maximum specific growth rates

From the analysis of an $OUR_{H(t)}$ (mgO/ℓ/h) versus time (h) response obtained in the batch test, valuable information pertaining to the OHOs growth rates can also be determined. Wentzel *et al*. (1995) and Mbewe *et al*. (1995) concluded that the $OUR_{H(t)}$ before the precipitous drop could be separated into the OUR contribution due to RBCOD degradation and the OUR contribution due to SBCOD degradation (see Fig. 4.2). This is equivalent to separating the overall growth rate ($\mu_H + K_{MP}$) into its μ_H and K_{MP} components. The derived equation for K_{MP} (OHO maximum specific growth rate on SBCOD), and μ_H (OHO maximum specific growth rate on RBCOD) will be repeated here to illustrate the calculation procedures.

In terms of the UCT model, growth of OHOs on RBCOD and SBCOD is independent (see Table 4.5). The OUR's (mgO/ℓ/h) associated with these two growth processes are given by Eq. (4.8) which can be separated to give:

$$(1)\quad OUR_{RBCOD(t)} \cdot 24 = \frac{1-Y_{ZH}}{Y_{ZH}} \cdot \mu_H \cdot Z_{BH(0)} \cdot e^{(\mu_H + K_{MP} - b_H)t/24} \qquad (4.13)$$

$$(2)\quad OUR_{SBCOD(t)} \cdot 24 = \frac{1-Y_{ZH}}{Y_{ZH}} \cdot K_{MP} \cdot Z_{BH(0)} e^{(\mu_H + K_{MP} - b_H)t/24} \qquad (4.14)$$

OHO maximum specific growth rate on SBCOD, K_{MP}

Up to the precipitous decrease, the OUR is the sum of both the RBCOD and SBCOD utilization [Eq. (4.13) + Eq. (4.14)]. Once the RBCOD is depleted, which causes the precipitous OUR decrease, the OUR is that for SBCOD only [Eq. (4.14)]. If the precipitous decrease occurs at t=s hours at which time the OUR is $OUR_{SBCOD(t)}$, then from Eq. (4.14) K_{MP} is given by:

$$K_{MP} = \frac{OUR_{SBCOD(t=s)} \cdot 24}{\frac{1-Y_{ZH}}{Y_{ZH}} \cdot Z_{BH(0)} \; e^{(\mu_H + K_{MP} - b_H)\cdot(t=s)/24}} \qquad (4.15)$$

where

$OUR_{SBCOD(t=s)}$	=	OUR due to SBCOD only, i.e. observed OUR immediately following the precipitous drop in OUR (mgO/ℓ/h).
(t=s)	=	time immediately following the precipitous drop in OUR (h)
$(\mu_H+K_{MP}-b_H)/24$	=	slope of ℓn $OUR_{(t)}$ versus time (h) plot.

Using the data in Figs. 4.2 and 4.3 as an example, time s = 9.1h; $OUR_{SBCOD(t=7,8h)}$ = 4.0 mgO/ℓ/h; slope ℓn $OUR_{(t)}$ vs time plot = 0.194 (Fig. 4.3); $Z_{BH(0)}$ = 16 mgCOD/ℓ:

$$K_{MP} = \frac{4.0 \cdot 24}{\frac{1-0.666}{0.666} \cdot 16 \cdot e^{0.194 \cdot 9.1}} = 2.05/d$$

OHO maximum specific growth rate on RBCOD, μ_H

For the UCT model, the value for μ_H can be calculated from the value for K_{MP} derived above and the slope of the ℓn OUR versus time plot, as follows

$$\mu_H = Slope \cdot 24 - K_{MP} + b_H \qquad (4.16)$$

For the example above,

$$\mu_H = 0.194 \cdot 24 - 2.05 + 0.62 = 3.2/d$$

4.3.2.2 Wastewater + mixed liquor batch tests

Ubisi *et al*. conducted eight batch tests on mixtures of various quantities of mixed liquor and wastewater for WW Batch No. 12, see Table 4.4. The data for the batch tests are set out in detail in Ubisi *et al*. (1997b). As an example, the OUR (mgO/ℓ · h) versus time (h) response for a batch test with 100 mℓ mixed liquor added to 2.9 ℓ diluted wastewater (Batch test No. M1, Table 4.4) is shown plotted in Fig. 4.4. Referring to Fig. 4.4, the general shape of the OUR time profile is the same as that for wastewater only (see Fig. 4.2). However, due to the larger concentration of OHO active biomass present (added with the mixed liquor) the OURs are higher and the time to the precipitous drop in OUR shorter. Further, since mixed liquor was drawn from a nitrifying activated sludge system and added to the batch test, nitrification can be expected and indeed was observed, see Fig. 4.5. The OUR due to this nitrification must be taken into account in deriving estimates for % COD recovery and $Z_{BH(0)}$ via the procedures set out above, since both these parameters are determined from the OUR for OHOs only. This can be done by noting that the measured OUR at any time t ($OUR_{M(t)}$) is made up of the OUR due to OHO growth ($OUR_{H(t)}$) and due to nitrification ($OUR_{N(t)}$), i.e.

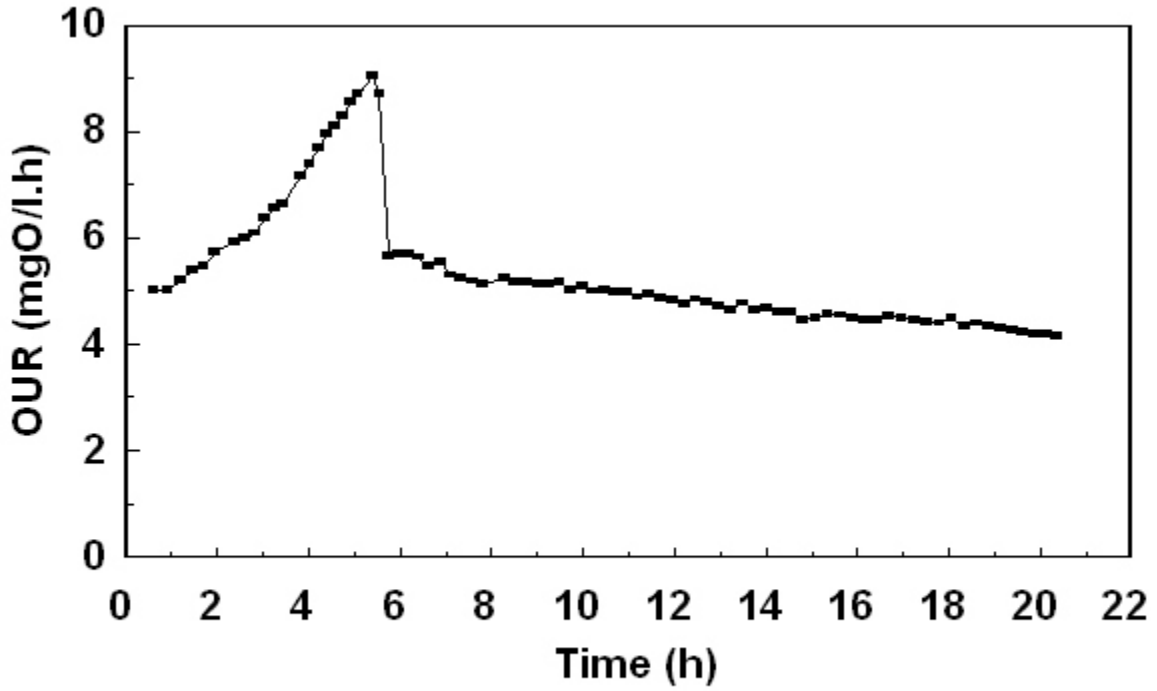

Figure 4.4: Oxygen utilisation rate (OUR) response with time for aerobic batch test on a mixture of raw municipal wastewater (2.9ℓ) from Mitchell's Plain (Cape Town, South Africa) and mixed liquor drawn from the aerobic reactor of the parent laboratory-scale system (Fig. 4.1). (Batch test No. M1, Table 4.4).

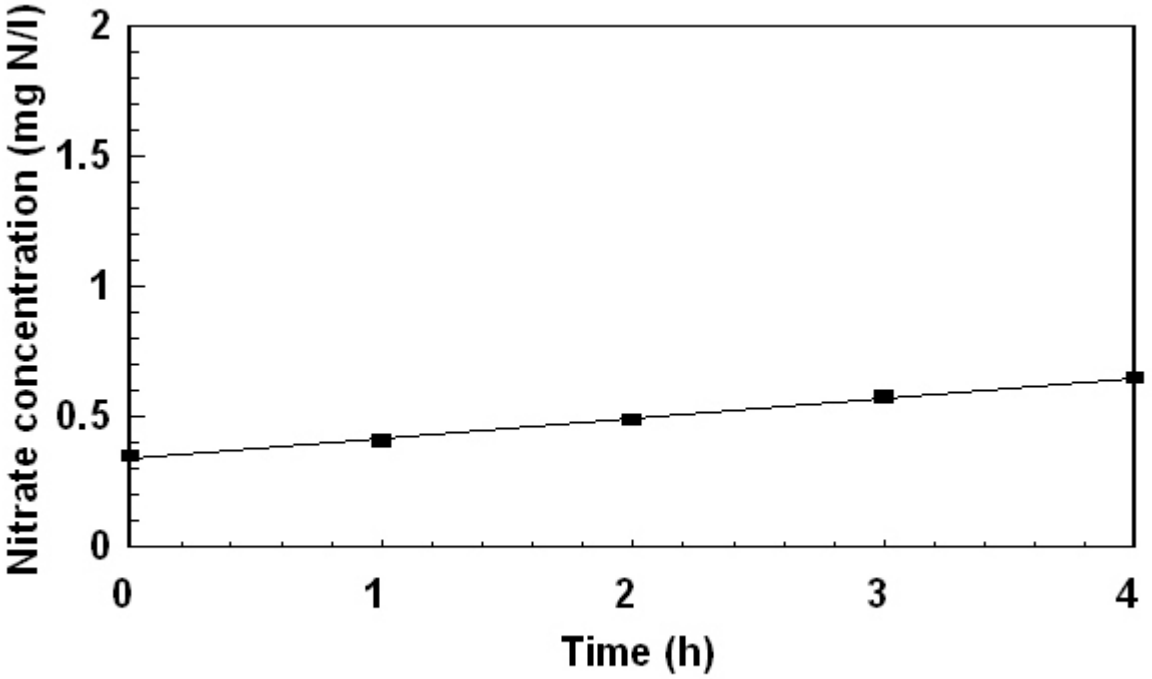

Figure 4.5: Nitrate concentration with time for aerobic batch test in Fig. 4.4.

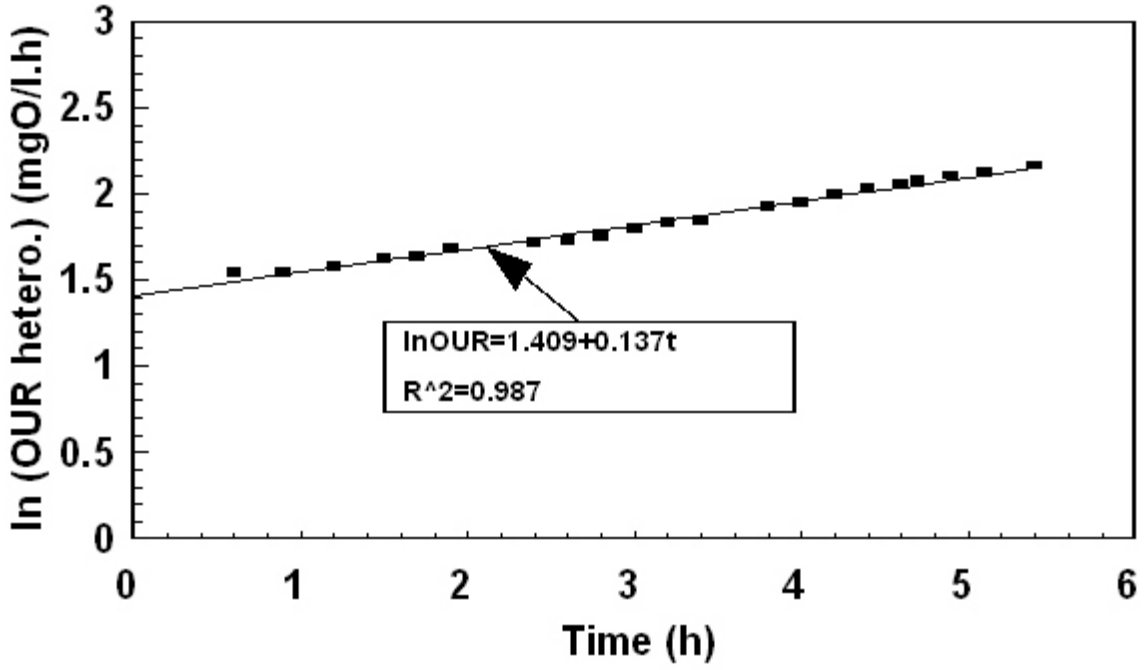

Figure 4.6: ℓn oxygen utilization rate (OUR) due to OHO active biomass versus time for the OUR data in Fig. 4.4 up to the precipitous drop in OUR [i.e. OUR due to nitrification (0.343mgO/ℓ/h, Table 4.6, Batch Test No. M1) subtracted from measured OUR data in Fig. 4.4 and then plotted].

$$OUR_{M(t)} = OUR_{H(t)} + OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (4.17a)$$

[Note that for the batch tests with wastewater only, no nitrification was observed, i.e. in Eq. (4.17a) $OUR_{N(t)} = 0$ and $OUR_{M(t)} = OUR_{H(t)}$.]

Rearranging Eq. (4.17a)

$$OUR_{H(t)} = OUR_{M(t)} - OUR_{N(t)} \qquad (mgO/\ell/h) \qquad (4.17b)$$

Accordingly, to determine $OUR_{H(t)}$, an estimate for $OUR_{N(t)}$ is essential. The $OUR_{N(t)}$ can be readily determined from the nitrate concentration time profile (for example for Batch test No. M1, see Fig. 4.5). For the batch tests with nitrification, ammonia - N is available in excess and nitrification proceeds at the maximum rate. Further, since the yield and maximum specific growth rate of the autotrophs are relatively low, the nitrification rate can be assumed constant within the time scale of the batch test - this assumption is confirmed by the linearity of the nitrate concentration-time profiles. Accepting a constant nitrification rate, the slope of a "best-fit" linear line to the nitrate (mgN/ℓ) time (h) profile is the nitrification rate ($\Delta NO_3^-/\Delta t$, mgN/ℓ/h), and the OUR_N is given by:

$$OUR_{N(t)} = 4.57 \cdot \Delta NO_3^- / \Delta t \qquad (mgO/\ell/h) \qquad (4.18)$$

Linear regression was applied to the batch tests nitrate time profiles to determine the y-intercept, slope and correlation co-efficient, see Table 4.6. From the slopes, the OUR_N's for the different batch tests were calculated using Eq. (4.18), and also are listed in Table 4.6. The OUR_N is assumed constant over the batch test (see above) so that $OUR_N = OUR_{N(t)}$, and hence $OUR_{H(t)}$ can be calculated via Eq. (4.17b), i.e. from each measured OUR value ($OUR_{M(t)}$), a constant nitrification OUR value (OUR_N, Table 4.6) is subtracted to give the OUR due to the OHOs only ($OUR_{H(t)}$).

Having determined the $OUR_{H(t)}$ for the batch tests, the % COD recoveries were calculated using Eq. (4.4) and are listed in Table 4.4. Referring to Table 4.4, for batch tests M1-M8 COD recoveries range from 95-102%. The good mass balances provide support for the reliability of the measurements.

To determine the OHO active biomass present at the start of the batch tests, the $OUR_{H(t)}$ (i.e. $OUR_{M(t)}$ - OUR_N) up to the precipitous drop in OUR were plotted $\ell n\ OUR_{H(t)}$ versus time (h), for example see Fig. 4.6. Linear regression was used to determine the y-intercept, slope and correlation coefficients of the $\ell n\ OUR_{H(t)}$ versus time plots, see Table 4.4. From the y-intercept and slope data, values for $Z_{BH(0)}$ (ML+WW) [the OHO active biomass present at the start of the batch tests, due to that added with the mixed liquor (ML) and that present in the original wastewater (WW)] were calculated using Eq. (4.12) and also are listed in Table 4.4.

Table 4.6: Batch tests with mixture of wastewater (WW) and mixed liquor drawn from parent laboratory-scale system: Regression data from nitrate versus time plots, nitrification rates ($\Delta NO_3^-/\Delta t$) and OUR for nitrification (OUR_N).

BATCH TEST: WASTEWATER (WW) + MIXED LIQUOR (ML)						
WW Batch	Batch Test No	Regression			$\Delta NO_3^-/\Delta t$	OUR_N
		Y-intcpt	Slope	R^2	(mgN/ℓ.h)	(mgO/ℓ.h)
12	M1	0.343	0.075	0.989	0.075	0.343
	M2	0.547	0.129	0.983	0.129	0.59
	M3	0.557	0.211	0.882	0.211	0.964
	M4	0.426	0.506	0.923	0.506	2.312
	M5	0.541	0.296	0.987	0.296	1.352
	M6	0.645	0.191	0.913	0.191	0.873
	M7	0.572	0.257	0.962	0.257	1.174
	M8	0.739	0.326	0.966	0.326	1.49

4.3.3 Determination of mixed liquor OHO active biomass concentration

In the batch tests with both wastewater (WW) and mixed liquor (ML) present, OHO active biomass at the start of the test [$Z_{BH(0)}$] is due to the OHO active biomass present in the original wastewater (WW) and that added with the mixed liquor (ML); therefore, the $Z_{BH(0)}$ due to the wastewater [$Z_{BH(0)}$ (WW)] must be subtracted from the $Z_{BH(0)}$ due to both the wastewater and mixed liquor [($Z_{BH(0)}$ (WW+ML)] to determine $Z_{BH(0)}$ due to the mixed liquor only [$Z_{BH(0)}$ (ML)]. Now, $Z_{BH(0)}$ (WW) is available from the batch tests with wastewater only; for a particular wastewater batch these were averaged (see Table 4.3) and then the dilution in $Z_{BH(0)}$ (WW) caused by the addition of mixed liquor to the batch tests with wastewater and mixed liquor was taken into account, as follows:

$$Z_{BH(0)}\,(WW)_D = Z_{BH(0)}\,(WW) \cdot V_{WW}/(V_{ML} + V_{ww}) \qquad (4.19)$$

where

$Z_{BH(0)}\,(WW)_D$	=	OHO active biomass due to wastewater at start of batch test with WW + ML, taking due account of dilution (mgCOD/ℓ)
$Z_{BH(0)}\,(WW)$	=	OHO active biomass present in the original wastewater, Table 4.3 (mgCOD/ℓ)
V_{WW}	=	volume of wastewater added to batch test (ℓ)
V_{ML}	=	volume of mixed liquor added to batch test (ℓ)

From the data in Table 4.3, the OHO active biomass *concentration* in the batch tests due to the added wastewater [$Z_{BH(0)}\,(WW)_D$] were calculated using Eq. (4.19); values are listed in Table 4.4. Now,

$$Z_{BH(0)}\,(ML) = Z_{BH0o)}\,(ML + WW) - Z_{BH(0)}\,(WW)_D \qquad (4.20)$$

Using Eq. (4.20), the OHO active biomass *concentrations* in the total batch test volumes (WW + ML) due to the added mixed liquor [$Z_{BH(0)}$ (ML)] were calculated; values are listed in Table 4.4. These values represent the measured mixed liquor OHO active biomass concentration in the total batch test volume. The theoretical values were calculated using Eq. (4.3) and are listed in Table 4.4 also.

4.3.4 Measured verus theoretical OHO active biomass concentrations

To compare the measured and theoretical mixed liquor $Z_{BH(0)}$ (ML), the values are plotted against each other in Fig. 4.7: For WW Batch No. 12, very good agreement was obtained between the theoretical and measured OHO active mass. However, as noted earlier, the above batch test procedure was applied by Ubisi *et al.* (1997a,b) to mixed liquor drawn from laboratory-scale ND activated sludge systems operated at sludge ages of 12 and 20 days respectively and the theoretical values compared to those measured (see Fig. 1.1). This comparison showed that for the 12 day sludge age system, good agreement existed between the measured and theoretical values. For the 20 days sludge age system, however, the values compared poorly, with the theoretical values being approximately twice those measured. These contrasting results raised uncertainty regarding the reliability of the results obtained from the batch test. Accordingly, one of the objectives of this investigation was to evaluate the batch test procedure of Ubisi *et al.*

(1997a,b), to identify possible modifications to improve it. Appropriate modifications will be proposed and evaluated in Chapter 5.

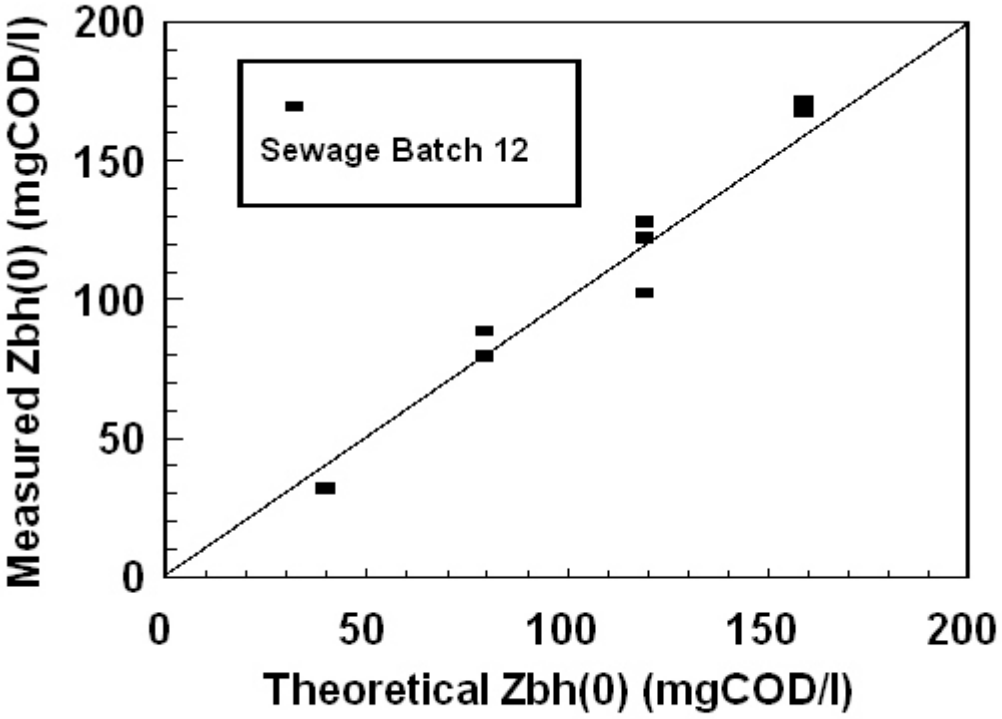

Figure 4.7: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) in the batch test due to addition of mixed liquor drawn from the parent laboratory-scale activated sludge system (Fig. 4.1).

4.4 CLOSURE

In this Chapter the batch test method developed by Ubisi *et al*. (1997a,b) to quantify the OHO active biomass concentration present in mixed liquor from an aerobic and a nitrification/ denitrification activated sludge system has been reviewed. The experimental procedures together with the interpretation of experimental data with respect to the concepts of the UCT kinetic simulation model have been described in detail. In application of the batch test procedure, Ubisi *et al*. observed that in some instances the experimental results showed remarkable agreement with theoretically predicted responses. Thus, the batch test method has shown promise as a means to substantiate the hypothesized concentration of OHO active biomass present in the above-mentioned activated sludge system and would therefore appear to validate the mathematical formulations contained within the simulation model.

However, the batch test method has also produced results which are inconsistent with the theoretical models. One of the objectives of this investigation was to establish whether the inconsistencies were related to the batch test procedure itself or, more perplexing, whether the inconsistencies were the result of incorrect mathematical formulations within the framework of the simulation model. These aspects will be dealt with in more detail in Chapter 5.

CHAPTER 5

EVALUATION AND MODIFICATION OF THE BATCH TEST PROCEDURE TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

5.1 INTRODUCTION

In Chapter 4 a detailed description is given of the batch test procedure developed by Ubisi *et al*. (1997a,b) to quantify the ordinary heterotrophic organism (OHO) active biomass concentration present in aerobic and anoxic/aerobic nitrification/ denitrification (ND) activated sludge systems. Ubisi et al. evaluated the batch test procedure by comparing theoretical values for OHO active biomass predicted by the UCT steady state design and kinetic simulation models with values measured on two separate laboratory-scale ND systems operated at 12 and 20 day sludge ages respectively. For the batch tests performed on mixed liquor drawn from the system operated at a 12 day sludge age, good agreement was obtained between the theoretical values and the values measured in the batch tests. In contrast, the results from the batch tests performed on mixed liquor drawn from the system operated at 20 day sludge age exhibited poor agreement between the theoretical and measured values. Ubisi *et al*. were unable to identify any specific cause for these contradictory results and the uncertainty in the OHO active biomass parameter largely remained.

The promise that the batch test method holds as a means of quantifying the OHO active biomass prompted an investigation to establish possible causes for the contradictory results. This Chapter summarises the outcome of this investigation; details are set out by Cronje *et al*. (2000).

5.2 RESEARCH APPROACH

The point of departure for the investigation was to repeat a series of batch tests in accordance with the procedures followed by Ubisi *et al*., as detailed in Chapter 4. This would establish whether the results of Ubisi *et al*. are reproducible, and whether their good correlation at 12 days sludge age was fortuitous. Thereafter, the batch test procedure would be examined to identify possible procedural or conceptual deficiencies, and modifications evaluated to improve the procedure and the results it provides.

Accordingly, a well defined and controlled parent laboratory-scale nitrification/denitrification activated sludge system was operated at 10 days sludge age and continually monitored. Mixed liquor samples were harvested regularly from the parent system in various quantities and combined with unsettled municipal wastewater in a batch reactor under aerobic conditions. The oxygen utilization rate (OUR) response in each batch test was monitored with time and used to obtain estimates for OHO active biomass concentration, derived from both the mixed liquor and wastewater. A parallel separate batch test was conducted on the wastewater, without mixed liquor addition, to quantify the OHO active biomass concentration present in the wastewater itself.

The difference in OHO active biomass concentration between the batch tests with and without mixed liquor addition gives the OHO active biomass concentration due to the mixed liquor addition only. The OHO active biomass measurements were evaluated by comparing the results from the batch tests with the results calculated using the steady state design model (WRC, 1984). From this evaluation, and a detailed analysis of the batch test responses, modifications to the batch test procedure were proposed and evaluated.

5.3 PARENT SYSTEM

5.3.1 System operation

The parent laboratory-scale activated sludge system operated as the source of mixed liquor for the batch tests was in the Modified Ludzack Ettinger (MLE) configuration; system layout is shown in Fig. 5.1. Sludge age was 10d, maintained by wasting mixed liquor from the aerobic reactor (hydraulic control) taking due account of any samples drawn from the reactors for analysis; temperature was controlled at 20°C, pH at 7.6 (±0.2); influent flow rate was constant, set at 20ℓ/d. Influent feed was raw (unsettled) municipal wastewater from Mitchell's Plain Treatment Plant (Cape Town, South Africa); the sewer retention time for this treatment plant is relatively short (± 4 hours) and the conditions are anaerobic and therefore it was expected that the OHO active biomass concentration in this wastewater would be low. The wastewater was collected in batches from the treatment works, stored in stainless steel tanks at 4°C and served as feed for both the parent system and batch tests for a period of 1-2 weeks (see Cronje *et al*., 2000 for details). For the parent system, a sample of the wastewater was drawn from the storage tanks after thorough mixing and diluted with tap water to give influent feed total COD ~ 500 mgCOD/ℓ. System operational procedures detailed by Ekama *et al*. (1986) were followed. Daily monitoring included influent COD, TKN; all reactors nitrate + nitrite; aerobic reactor VSS, TSS, COD and TKN of the VSS, oxygen utilization rate (OUR); effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985). (Individual nitrite measurements indicated that nitrite concentrations were very low compared to nitrate concentrations and consequently could be neglected for this investigation). To ensure steady state, the parent system was operated for more than ten sludge ages before mixed liquor was harvested for the batch tests. Detailed data on the parent system are described by Cronje *et al*. (2000). The reliability of the experimental measurements were checked by means of mass balances on COD and N (Ekama *et al*., 1986).

5.3.2 System results

The parent system was operated for 323 days and received 23 batches of wastewater as influent. Each wastewater batch was accepted as a steady state period, and the data for each batch were averaged (after statistical analysis for outliers). Batch tests commenced when the system was receiving Wastewater (WW) Batch No. 11 as influent, and the averaged data for the wastewater batches during which batch tests were conducted are listed in Table 5.1 (detailed data are listed by Cronje *et al*., 2000 as well as averages for the wastewater batches where no batch test were conducted).

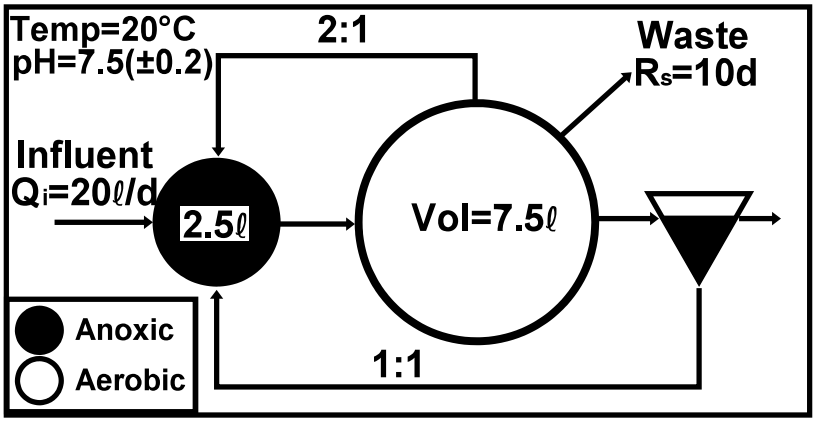

Figure 5.1: Schematic layout and operational data for parent laboratory-scale system of Cronje *et al.* (2000).

Table 5.1: Steady state results for parent laboratory-scale anoxic/aerobic activated sludge system (Fig. 5.1). For each of the wastewater batches tested, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

ANOXIC/AEROBIC STEADY STATE SYSTEM													
WW Batch	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite (mgN/ℓ)			Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			Aerobic pH
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
11	9	488	52	46	3.7	1.1	6.7	8.1	33.5	2419	3471	248	7.59
		(29)	(12)	(1)	(0.2)	(0.7)	(1.3)	(1.5)	(0.7)	(111)	(230)	(9)	(0.09)
12	6	500	43	47	4.0	2.2	7.8	10.2	33.5	2293	3372	248	7.53
		(42)	(9)	(2)	(0.9)	(1.5)	(2.9)	(2.4)	(1.9)	(70)	(121)	(33)	(0.03)
13	8	540	59	47	4.1	3.7	9.8	11.3	34.8	2469	3681	243	7.57
		(25)	(14)	(1)	(0.3)	(0.5)	(0.8)	(0.9)	(0.7)	(83)	(148)	(6)	(0.03)
14	4	517	61	48	3.7	5.5	11.7	13.2	37.4	2227	3403	226	7.61
		(25)	(12)	(1)	(0.4)	(1.2)	(0.8)	(0.5)	(2.1)	(162)	(185)	(7)	(0.06)
18	9	526	73	44	5.3	4.1	10.6	11.5	36.1	2387	3502	230	7.52
		(37)	(10)	(4)	(0.5)	(0.9)	(1.7)	(1.2)	(1.7)	(59)	(118)	(7)	(0.06)
19	9	498	60	46	4.3	5.5	13.8	15.4	35.4	2400	3513	231	7.48
		(22)	(7)	(1)	(0.4)	(2.6)	(2.8)	(2.6)	(1.2)	(90)	(103)	(7)	(0.05)
20	9	500	83	39	5.2	1.8	7.1	9.2	30.3	2202	3418	219	7.64
		(10)	(8)	(1)	(0.5)	(2.1)	(3.9)	(2.4)	(1.4)	(84)	(109)	(4)	(0.13)
21	9	510	69	50	4.0	10.3	20.6	22.9	39.4	2457	3664	250	7.53
		(39)	(7)	(3)	(0.9)	(2.1)	(2.0)	(2.4)	(1.6)	(74)	(92)	(8)	(0.08)
22	7	493	79	45	5.0	8.1	15.7	18.1	35.2	2138	3427	219	7.46
		(28)	(17)	(2)	(0.6)	(1.1)	(1.2)	(0.8)	(2.5)	(135)	(170)	(11)	(0.04)
23	7	530	80	51	6.2	7.8	14.9	16.7	34.1	2043	3116	216	7.52
		(30)	(15)	(5)	(1.9)	(1.1)	(0.9)	(1.1)	(1.3)	(145)	(263)	(26)	(0.08)

Following the procedures set out in Chapter 4, from the averaged data 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 both the steady state design procedure (WRC, 1984) and UCTOLD kinetic simulation model (Dold *et al*., 1991), see Chapter 4.

- 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 both the steady state design procedure (WRC, 1984) and UCTOLD kinetic simulation model (Dold *et al*., 1991), see Chapter 4.

- The theoretical OHO active biomass concentration in the steady state system bioreactor.

These values are listed in Table 5.2.

Table 5.2: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic activated sludge system (Fig. 5.1). Values calculated from data in Table 5.1, either directly or using the steady state (SS) design (WRC, 1984) or kinetic simulation (sim.) models (Dold *et al*., 1991)

ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No of tests	Mass Balance (%)		Wastewater fractions			Mixed liquor			
		COD	N	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)		COD/VSS ratio (mgCOD/ mgVSS) (f_{cv})	TKN/VSS ratio (mgN/ mgVSS) (f_n)	Active Fraction (f_{av})	
					SS Design	Kinetic Sim.			SS Design	Kinetic Sim.
11	9	94	90	0.086	0.143	0.125	1.43	0.103	0.424	0.434
12	6	87	92	0.074	0.100	0.079	1.47	0.108	0.480	0.491
13	8	88	93	0.091	0.105	0.086	1.49	0.098	0.462	0.472
14	4	93	90	0.091	0.078	0.056	1.53	0.100	0.495	0.506
18	9	93	101	0.093	0.103	0.082	1.47	0.096	0.473	0.484
19	9	90	113[1]	0.088	0.130	0.111	1.46	0.096	0.434	0.444
20	9	90	106	0.092	0.088	0.070	1.55	0.099	0.470	0.479
21	9	90	124[2]	0.096	0.133	0.113	1.49	0.102	0.420	0.431
22	7	92	113[1]	0.122	0.093	0.074	1.6	0.102	0.442	0.453
23	7	83	96	0.093	0.031	0.008	1.53	0.106	0.584	0.598

[1]Source of poor N mass balance traced to error in influent TKN measurement; influent TKN values from parallel investigations on the same wastewater gave mass balances 104 and 108% for batches 19 and 22 respectively.
[2]Source of poor N mass balance could not be identified; batch should be rejected for analysis.

From the results on the parent system listed in Tables 5.1 and 5.2:

- Generally COD mass balances were reasonable, with only 3 out of 10 wastewater batches giving mass balances < 90%. The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements for the wastewater batches during which batch tests were conducted gave COD/VSS = 1.50mCOD/mgVSS (sample standard deviation = 0.05) and TKN/VSS = 0.101mgN/mgVSS (sample standard deviation = 0.004). These values are very close to the standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). Consequently, it was accepted that the error in the COD mass balance did not lie in the measurement of the mixed liquor organic solids, the parameter of importance in the measurement of OHO active biomass. Accordingly, the lower limit for the COD mass balance was set at 80%. On this basis, no wastewater batches were rejected for further analysis.

- N mass balances were variable, but generally fell in the range 90 to 110%. Wastewater batches that gave mass balances falling outside this range should be rejected for further analysis, i.e. WW Batch Nos. 19, 21 and 22. However, parallel investigations on the same wastewater indicated that the influent TKN measurement was in error for WW Batch Nos. 19 and 22; correcting the influent TKN to take account of this error gave N mass balances of 104 and 108% respectively. On this basis, the only wastewater batch during which batch tests were conducted considered for rejection was WW Batch No. 21. However, as noted above for the COD mass balances, the parameter of importance for this investigation is the mixed liquor solids – the three independent tests on this parameter during WW Batch No. 21 gave COD/VSS = 1.49 and TKN/VSS = 0.102; since these values are near identical to the standard values (see above), this wastewater batch was retained for further analysis.

- For the wastewater batches during which batch tests were conducted, from the influent and filtered effluent COD measurements, the wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.093 (sample standard deviation = 0.012). The mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined via the steady state design procedure (WRC, 1984; see Ekama *et al.*, 1986) to be 0.100 (sample standard deviation = 0.032). These values conform closely to those observed by Ubisi *et al.* (1997a,b) for the same Mitchell's Plain wastewater, $f_{S,us}$ = 0.09 and $f_{S,up}$ = 0.12 respectively.

- Following the procedure in Chapter 4, the OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each wastewater batch using the steady state design model (Eq. 4.2) and the UCTOLD kinetic simulation computer programme, see Table 5.2. Reasonably close agreement was obtained between the steady state and kinetically simulated predictions for f_{av} obtained for each wastewater batch tested where batch tests were conducted. The kinetically simulated predictions were slightly higher than the values for f_{av} estimated via the steady state approach (average difference = 2.3%). As noted in Chapter 4, the small differences arise principally because (i) with the steady state design models, $f_{S,up}$ and f_{av} were determined from the measured mixed liquor VSS concentration, whereas with the kinetic simulation model, these were

determined from the measured mixed liquor COD concentration, and (ii) the kinetic simulation models include autotrophic active biomass and non-utilized slowly biodegradable COD in the mixed liquor whereas these are not included in the steady state design model. Due to their near equality, the f_{av} from either the steady state design or kinetic simulation models could be used, but because of the simpler more direct analytical calculation procedure, the f_{av} values from the steady state design model were used.

5.4 EVALUATION OF THE UBISI *et al.* BATCH TEST PROCEDURE

5.4.1 Batch test procedure

The batch test procedure of Ubisi *et al*. (1997a,b) as set out in Chapter 4 was followed (i.e. parallel batch tests, one with wastewater only added and the other with wastewater + mixed liquor drawn from the parent system added) while the parent system received WW Batch Nos. 11 to 14 and 18 to 20; a total of 23 parallel batch tests were conducted, see Tables 5.3 and 5.4 for the wastewater only and wastewater + mixed liquor batch tests respectively. During this period the following minor modifications were made to the batch test procedure:

- In the batch test method of Ubisi *et al*. (1997a,b) a 3ℓ volume of wastewater was used in the wastewater (WW) only batch test, while in the wastewater + mixed liquor (WW + ML) batch test, the volume of wastewater was decreased by the volume of mixed liquor added, to give a total batch volume of 3ℓ. This procedure was initially adopted for this investigation and was applied to the first four WW only batch tests (see batch tests W1–W4 in Table 5.3). To simplify the procedure, it was decided to use the same volume of wastewater in the parallel batch reactors; thus it could be accepted that each batch reactor contained the same mass of OHO active organisms originating from the wastewater itself. This modification enabled a direct comparison between the WW only OUR response and the WW + ML OUR response. Hence, from batch test W5 (Wastewater Batch No. 13) onwards, the same volume of wastewater required in the respective parallel WW+ML batch test (Table 5.4), was used in the WW only batch test. The remainder of the 3ℓ volume was supplemented by adding a volume of filtered effluent from the parent system equal in volume to that of the mixed liquor added to the parallel batch test.

- To establish an estimate for the *wastewater* OHO active biomass [$Z_{BH(0)}$(WW)] present at the start of each WW+ML batch test, Ubisi *et al*. used an *average* value calculated from a number of individual WW only batch tests conducted during a particular wastewater batch period. When calculating the OHO active biomass due to the mixed liquor addition only [$Z_{BH(0)}$(ML)], this *average* $Z_{BH(0)}$(WW) was subtracted from the total OHO active biomass $Z_{BH(0)}$(WW+ML) measured in each WW+ML batch test conducted during that particular wastewater batch. During this investigation it was observed that the $Z_{BH(0)}$(WW) value, measured in successive WW only batch tests conducted during a particular wastewater batch period, appeared to increase towards the end of the ~2 week period the particular wastewater batch served as influent. This increase would indicate some growth of wastewater OHO organisms during storage in the cold room at 4˚C. To use an average $Z_{BH(0)}$(WW) value in the calculation of the $Z_{BH(0)}$(ML) value from each

individual WW+ML batch test, would most likely overestimate the value of $Z_{BH(0)}$(WW) during the earlier part of a particular wastewater batch and underestimate the value towards the latter part of the same wastewater batch. To avoid distorted values for $Z_{BH(0)}$(ML), for this investigation the $Z_{BH(0)}$(WW) measured in each WW only batch test was applied directly in the calculation of $Z_{BH(0)}$(ML) measured in the parallel WW+ML batch test, conducted on the same day.

5.4.2 Batch test results

From the batch test data using the procedures set out in Chapter 4, but taking due account of the modifications above (i.e. change in the volume of wastewater added, and the filtered effluent added) the following was calculated:

- From the mass of oxygen consumed by the OHOs and the initial and final COD concentration, COD recovery (%) via Eq. (4.4).

- From the slope and y-intercept of a linear regression of a plot of natural log of OHO OUR ($OUR_{H(t)}$) versus time, OHO active biomass [$Z_{BH(0)}$ in COD units (mgCOD/ℓ)] at the start of the batch test via Eq. (4.12): For the WW only batch tests, this gives Z_{BH} in the wastewater; for the WW + ML batch tests, this gives the Z_{BH} due to both the wastewater and mixed liquor.

- The difference between Z_{BH} in the two types of batch tests (taking due account of dilution), gives the Z_{BH} due to the addition of mixed liquor – this represents the measured Z_{BH} in the parent system mixed liquor.

- Maximum specific growth rate of OHOs on both RBCOD (μ_H) and SBCOD (K_{MP}).

In total, 23 parallel batch tests were conducted and the results are summarised in Table 5.3 for the WW only batch tests and in Table 5.4 for the WW + ML batch tests (detailed results are given by Cronje *et al.*, 2000). From the batch tests, the following was concluded:

- For the wastewater (WW) only batch tests, the correlation coefficients (R^2) in linear regression of the $\ell nOUR_{H(t)}$-time plots were generally very good. For the vast majority of the batch tests $R^2 > 0.95$, which indicates that the observed responses conform to the hypothesized behaviour.

- For the WW only batch tests, the mean %COD recovery of 93% is relatively good (see Fig. 5.2). However, 9 out of 23 batch tests yielded %COD recoveries which were marginally less than 90%. The cause for these low % COD recoveries was traced to inadequate sampling for measurement of the final total COD concentration in the batch test. When this deficiency was rectified, acceptable COD mass balances > 90% were obtained. Since the source of the error lay in the measurement of COD and not OUR, the low % COD recoveries do not impact the analysis as this relies on OUR measurements.

- In the batch tests with wastewater only, no nitrification was observed indicating the inactivity of autotrophic organisms in the wastewater.

Table 5.3: Summary of batch test results with wastewater (WW) only: Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, OHO active biomass present in the batch test ($Z_{BH(0)}$) due to the WW expressed as a concentration and % of the WW total COD concentration at the start of the test.

BATCH TESTS: WASTEWATER (WW) ONLY								
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		Measured $Z_{BH(0)}$	
		FE	WW		K_{MP}	μ_H	Concentration (mgCOD/ℓ)	Fraction of Total COD (%)
11	W1	-	3.00	92	3.0	4.8	19	3.7
	W2	-	3.00	91	3.5	5.3	14	2.8
	W3	-	3.00	88	3.6	4.3	28	6.0
12	W4	-	3.00	89	3.2	5.8	9	2.1
13	W5	0.20	2.80	109	3.9	4.3	30	7.4
	W6	0.10	2.90	101	4.5	4.6	27	6.0
	W7	0.25	2.75	99	4.4	4.7	31	7.5
14	W8	0.20	2.80	101	4.2	4.7	15	3.3
18	W9	0.20	2.80	84	3.4	4.1	16	3.4
	W10	0.25	2.75	86	2.1	7.0	6	1.4
	W11	0.30	2.70	83	3.1	4.0	18	4.0
	W12	0.15	2.85	75	3.3	3.2	30	7.1
19	W13	0.30	2.70	86	4.3	5.3	6	1.5
	W14	0.35	2.65	87	4.4	5.4	7	1.6
	W15	0.40	2.60	88	3.2	4.3	14	3.2
	W16	0.30	2.70	102	3.5	5.6	11	2.4
	W17	0.35	2.65	98	3.2	6.9	17	3.9
20	W18	0.25	2.75	93	3.9	5.8	6	1.3
	W19	0.20	2.80	96	3.5	6.3	6	1.3
	W20	0.15	2.85	100	3.6	6.1	6	1.4
	W21	0.30	2.70	96	2.7	4.9	13	2.9
	W22	0.35	2.65	97	2.2	5.5	19	4.6
	W23	0.40	2.60	102	2.0	6.6	17	4.1

- For the WW only batch tests, the wastewater OHO active biomass ranged from 1.3 to 7.4 % of the total wastewater COD, with a mean of 3.6 % and a sample standard deviation of 2.0 % (see Fig. 5.3). This mean compares favourably with the values measured by Ubisi *et al.* (1997a,b) and Mbewe *et al.* (1995) of 4.5 % and 6.1 % respectively, for the same wastewater type.

- For the batch tests conducted with a mixture of wastewater and mixed liquor (WW+ML), the correlation coefficients (R^2) in linear regression of the $\ell nOUR_{H(t)}$-time plots were generally lower than the R^2 values obtained in the WW only batch tests. However the R^2 values were generally still acceptable, with the vast majority of the R^2 values > 0.90.

Table 5.4: Summary of results from batch tests with a mixture of wastewater (WW) and mixed liquor (ML) drawn from parent lab-scale system (Fig. 5.1): Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, OHO active biomass present in the batch test ($Z_{BH(0)}$) due to the ML+WW, WW only and ML only. Also shown are the theoretical $Z_{BH(0)}$ values due to ML.

BATCH TESTS: WASTEWATER (WW) + MIXED LIQUOR (ML)										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured			Theo. ML
		ML	WW		K_{MP}	μ_H	WW+ML	WW	ML	
11	M1	0.1	2.90	97	3.2	4.6	26	18	8	49
	M2	0.15	2.85	99	3.7	4.1	29	13	16	74
	M3	0.30	2.70	95	3.3	1.6	101	25	76	147
12	M4	0.30	2.70	100	2.8	1.8	83	8	75	162
13	M5	0.20	2.80	102	3.4	2.0	90	30	60	113
	M6	0.10	2.90	90	5.1	4.2	33	27	7	57
	M7	0.25	2.75	91	3.7	3.0	72	31	41	142
14	M8	0.20	2.80	93	3.9	2.9	37	15	22	113
18	M9	0.20	2.80	103	4.1	3.6	30	16	14	110
	M10	0.25	2.75	106	3.2	2.2	53	6	46	138
	M11	0.30	2.70	101	3.6	2.6	57	18	38	166
	M12	0.15	2.85	100	3.7	3.1	43	30	12	83
19	M13	0.30	2.70	103	3.4	3.0	37	6	31	153
	M14	0.35	2.65	95	2.9	1.8	85	7	79	178
	M15	0.40	2.60	99	3.0	1.5	102	14	89	203
	M16	0.30	2.70	94	2.7	2.0	71	11	61	153
	M17	0.35	2.65	99	2.7	2.5	76	17	59	178
20	M18	0.25	2.75	94	3.1	2.7	38	6	33	134
	M19	0.20	2.80	97	3.2	3.5	28	6	22	107
	M20	0.15	2.85	91	2.4	3.4	29	6	23	80
	M21	0.30	2.70	97	1.4	1.6	132	13	119	161
	M22	0.35	2.65	100	2.4	3.1	79	19	60	187
	M23	0.40	2.60	108	2.2	2.2	129	17	112	214

- All the WW+ML batch tests yielded %COD recoveries in the range 90 – 110 % with a mean of 98.0 % and a sample standard deviation of 4.6 % (see Fig. 5.4). The good %COD recoveries lend credibility to the reliability of the results.

- For the WW+ML batch tests, nitrification was observed since the mixed liquor added to the batch tests was drawn from a nitrifying activated sludge system.

- The maximum specific growth rates for the OHOs on RBCOD (μ_H) were higher in the WW only batch tests than in the WW + ML batch tests, Tables 5.3 and 5.4.

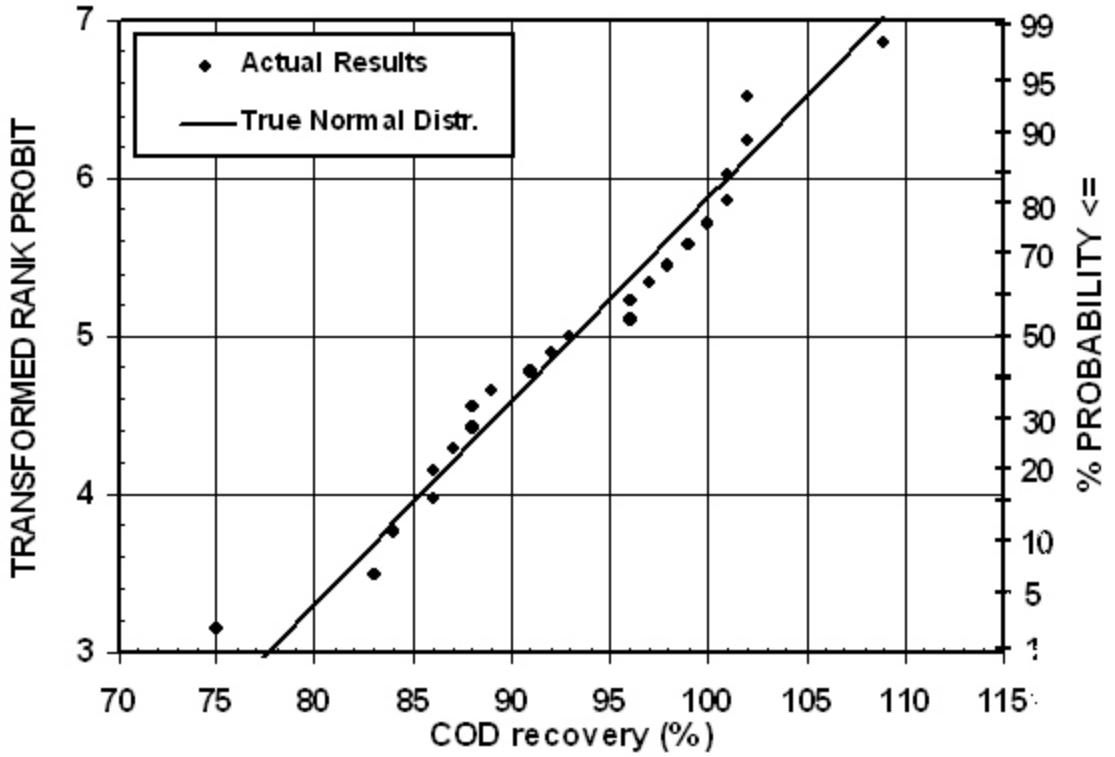

Figure 5.2: Statistical plot of % COD recovery for all the wastewater only batch tests.

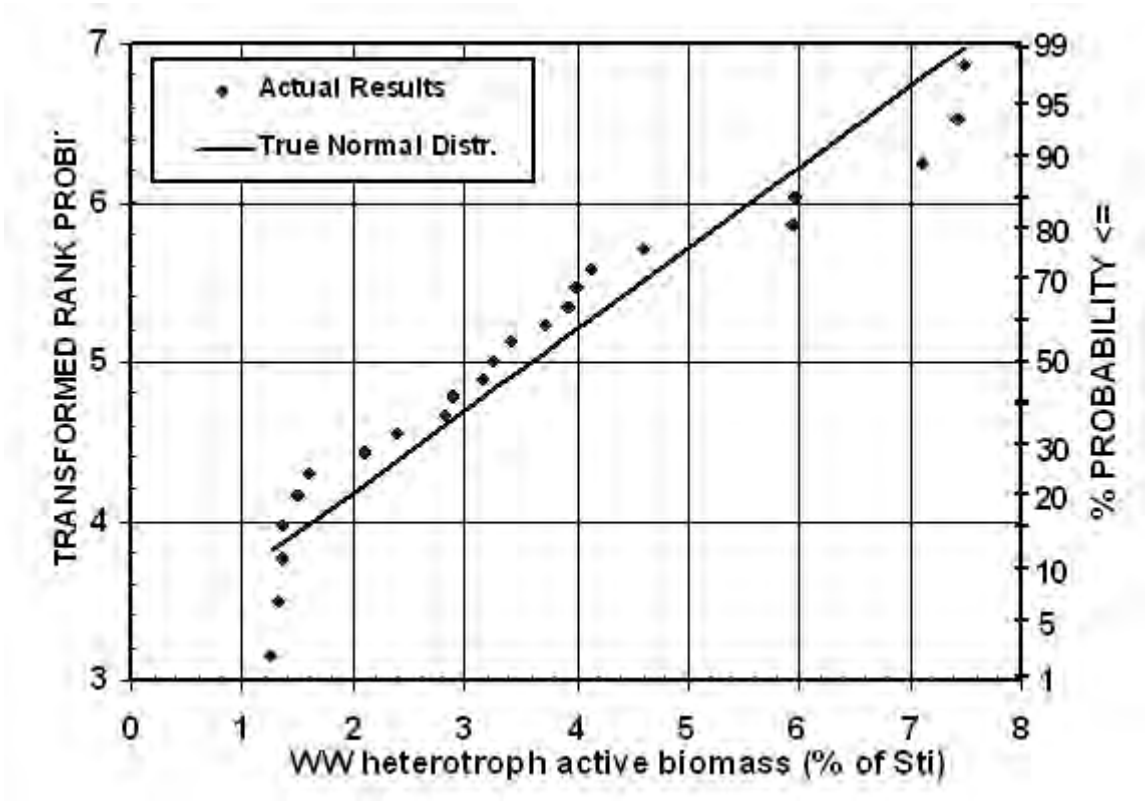

Figure 5.3: Statistical plot of measured OHO active biomass for all the wastewater only batch tests.

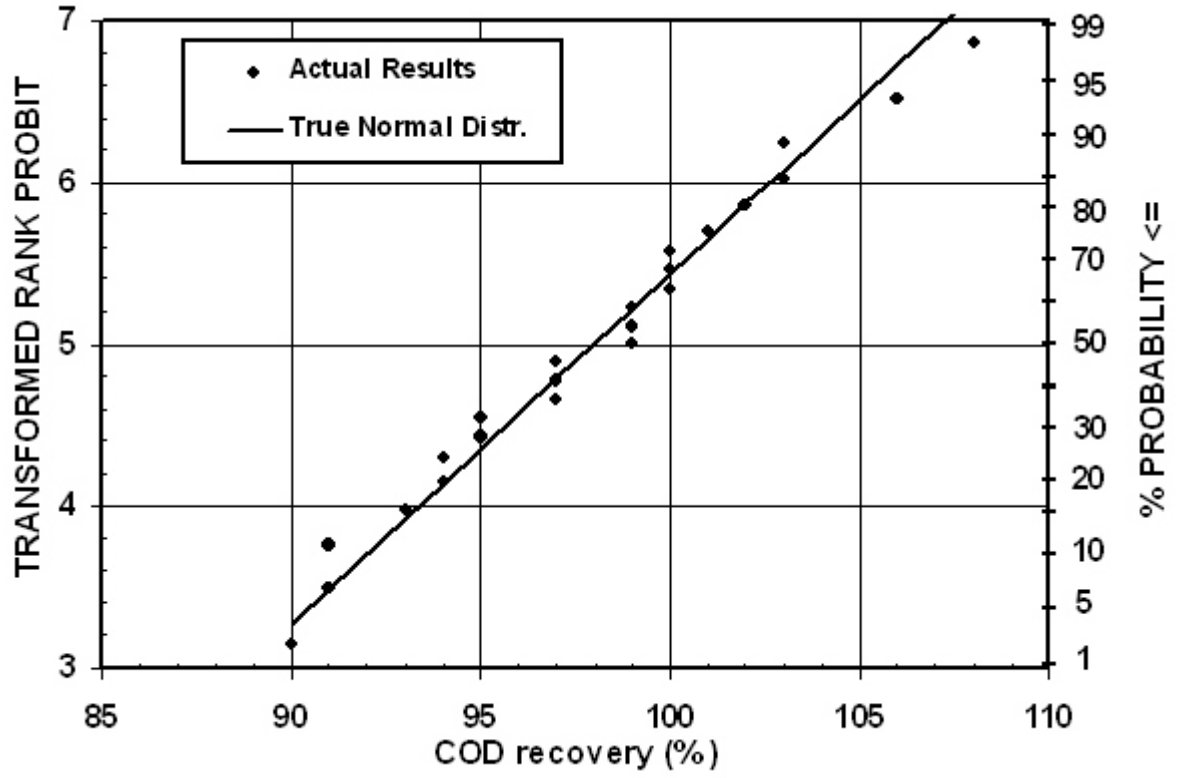

Figure 5.4: Statistical plot of % COD recovery for all the wastewater + mixed liquor batch tests.

5.4.3 Comparison between measured and theoretical OHO active biomass

Table 5.4 lists the *measured* OHO active biomass concentration at the start of each WW+ML batch test and the *theoretical* OHO active biomass concentration contained in the appropriate mixed liquor sample drawn from the parent system, predicted via the steady state design model. In Fig. 5.5 these data are shown plotted.

From Table 5.4 and Fig. 5.5 it is evident that very poor correspondence between the measured and theoretical mixed liquor OHO active biomass was obtained. The measured values were consistently lower and the comparison shows remarkable resemblance to that obtained by Ubisi *et al* (1997a,b) for batch tests conducted on a parent system operated at 20d sludge age (see Fig. 1.1b). Furthermore, the comparison is almost identical to that obtained by Ubisi *et al.* for batch tests conducted on their parent system operated at 12d sludge age, during the period when the parent system received WW Batch No. 19 (see Fig. 1.1a).

This similarity in results obtained from batch tests conducted on independent parent systems by independent investigators, clearly demonstrate that the batch test method (in its present format) cannot serve as a reliable means of measuring the OHO active biomass concentration present in activated sludge systems. However, the undoubted promise that the batch test concept holds, *stimulated a thorough scrutiny of the present batch test method and the mathematical formulations applied in the analysis of recorded data.*

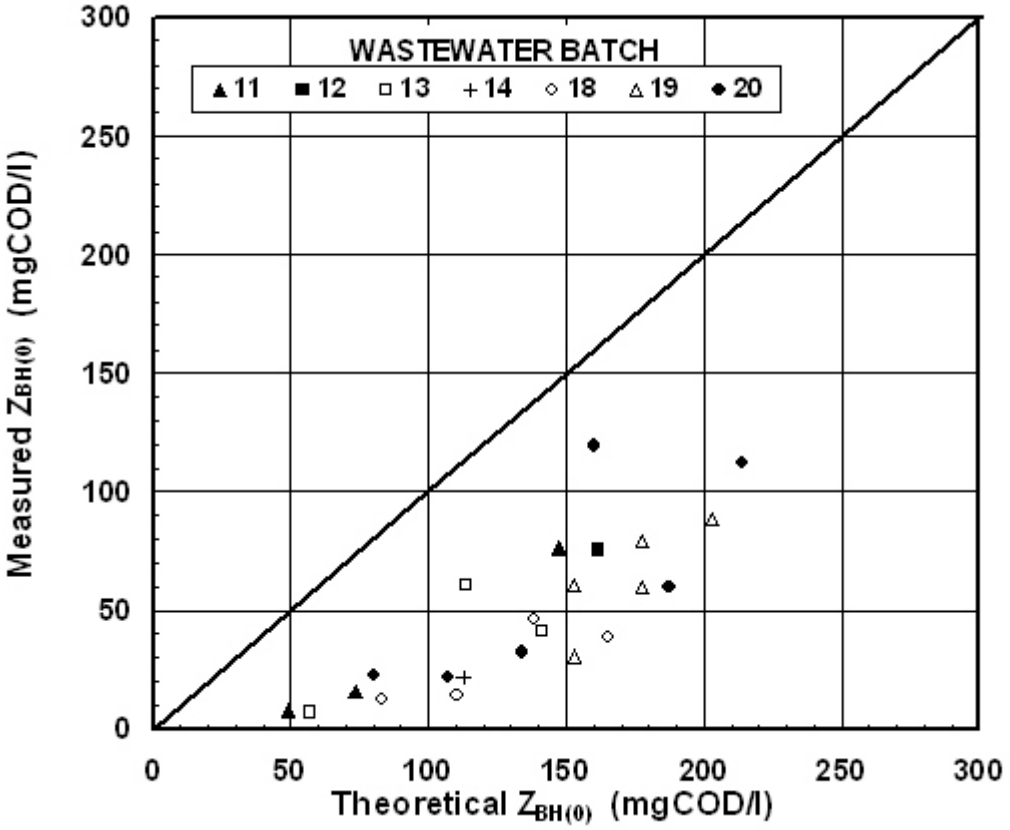

Figure 5.5: Measured versus theoretical OHO active biomass ($Z_{BH(0)}$) for the various wastewater batches with the parent laboratory-scale system operated at 10d sludge age.

5.5 EVALUATION OF BATCH TEST METHOD

The poor correlation between the theoretically predicted and the batch test measured OHO active biomass concentration values obtained in this investigation substantiated the perception from the work by Ubisi *et al*. (1997a,b), that the batch test method in its present format was unable to yield reliable estimations of the OHO active biomass concentration present in activated sludge systems. The poor correlations obtained in the various investigations could be due to deficiencies in either the concepts on which the theoretical models are based, or the batch test method itself. With regard to the theoretical models, these were used to calculate the theoretical values for OHO active biomass concentrations. In evaluating whether the models give theoretical OHO active biomass concentrations that are in error, it was noted that:

- On examining the measured versus theoretical values for the three independent systems operated at 10, 12 and 20 d sludge ages, it is clear that a remarkable similarity exists between the measured and theoretical results; although the measured values are lower, they are consistently only about half the theoretical values.

- The theoretical models have been applied to successfully predict the behaviour of a wide variety of system configurations operated under a range of conditions (e.g. Dold *et al.*, 1991).

The consistent (though erroneous) correlation between the batch test measured and theoretical OHO active biomass concentrations obtained in the different investigations, and the reliable application of the models indicated that most likely the deficiency lay in the batch test method, either in the method itself or in the techniques applied in analysing the batch test data. Thus, it was concluded that the batch test method should be evaluated in more detail, to investigate possible causes for the deviation of measured OHO active biomass from the theoretically predicted values.

From this evaluation it was concluded that the poor correlation between the OHO active biomass measured in the batch tests and the theoretical OHO active biomass predicted via the steady state design model probably could be attributed to mainly two factors (for details of this evaluation see Cronje *et al.*, 2000):

(i) Since the same volume of wastewater was added to the parallel batch tests (WW only and WW + ML), it was possible to directly compare the OUR responses from the separate batch tests: It was observed that the OHOs present in the wastewater exhibited a growth rate that was much faster than the growth rate exhibited by the OHOs present in the mixed liquor of the parent laboratory-scale activated sludge system. In particular, the OHO maximum specific growth rate on RBCOD (μ_H) was significantly higher for the wastewater OHO active biomass than for the combination of wastewater + mixed liquor OHO active biomass, see Fig. 5.6. This is not unexpected - the wastewater OHO active biomass has developed under conditions of high COD concentrations whereas the mixed liquor OHO active biomass has developed under conditions of COD limitation. The higher μ_H of the wastewater OHO active biomass caused that the OUR response of these organisms was significantly larger than the OUR response of the mixed liquor OHO active biomass, and thus the wastewater OHO active biomass active mass OUR masked the OUR response of the OHO active biomass from the mixed liquor (for example, see Fig. 5.7 where the OUR responses from two parallel batch tests are shown plotted). Accordingly, a potential source of error in the batch test arose when subtracting the wastewater OHO active biomass concentration (determined from the WW only batch test OUR) from that for the mixed liquor and wastewater OHO active biomass concentration, to derive the mixed liquor OHO active biomass concentration.

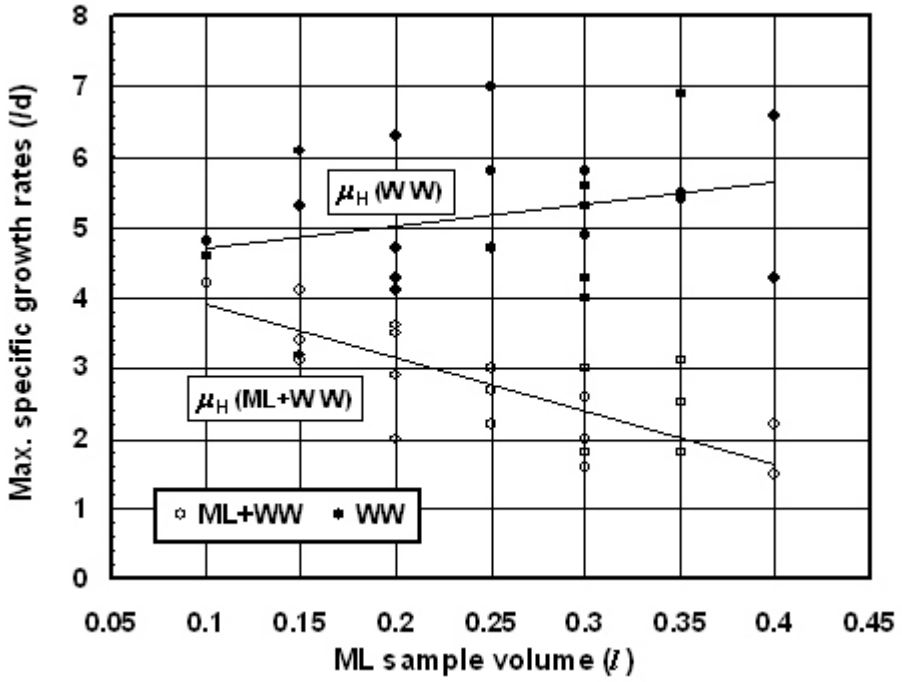

Figure 5.6: Measured OHO maximum (max.) specific growth rate on RBCOD (μ_H) versus mixed liquor volume added to batch tests, for wastewater only and wastewater + mixed liquor batch tests.

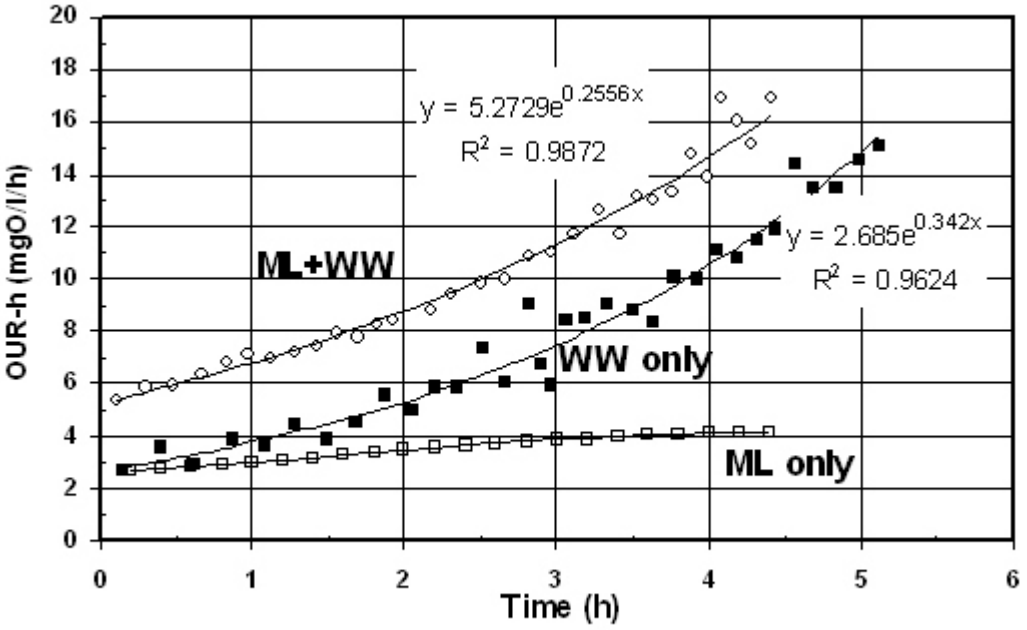

Figure 5.7: $OUR_{H(t)}$-time response for wastewater only (WW) batch test W8 and wastewater + mixed liquor (WW + ML) batch test M8. Approximated OUR response due to mixed liquor addition only (ML only) obtained through analytically separating the "best fit" WW+ML and WW only responses.

(ii) The premise that nitrification in the WW + ML batch test with exhibited a linear increase in the nitrate concentration with time (see Chapter 4), proved to be unduly simplified. In agreement with the observations of Antoniou *et al.* (1990) and Sözen *et al.* (1996), it was observed that the generation of nitrate in the batch reactor was better represented by an exponential increase than the linear increase with time followed by Ubisi *et al*. (1997a,b), for example see Fig. 5.8. Thus, in the present batch test concept when the oxygen demand due to nitrification (OUR_N), with a constant value in terms of the linear approach, is subtracted from the measured OUR response (OUR_M) to obtain the OUR response due to the OHO active biomass (OUR_H), another potential source of error is introduced.

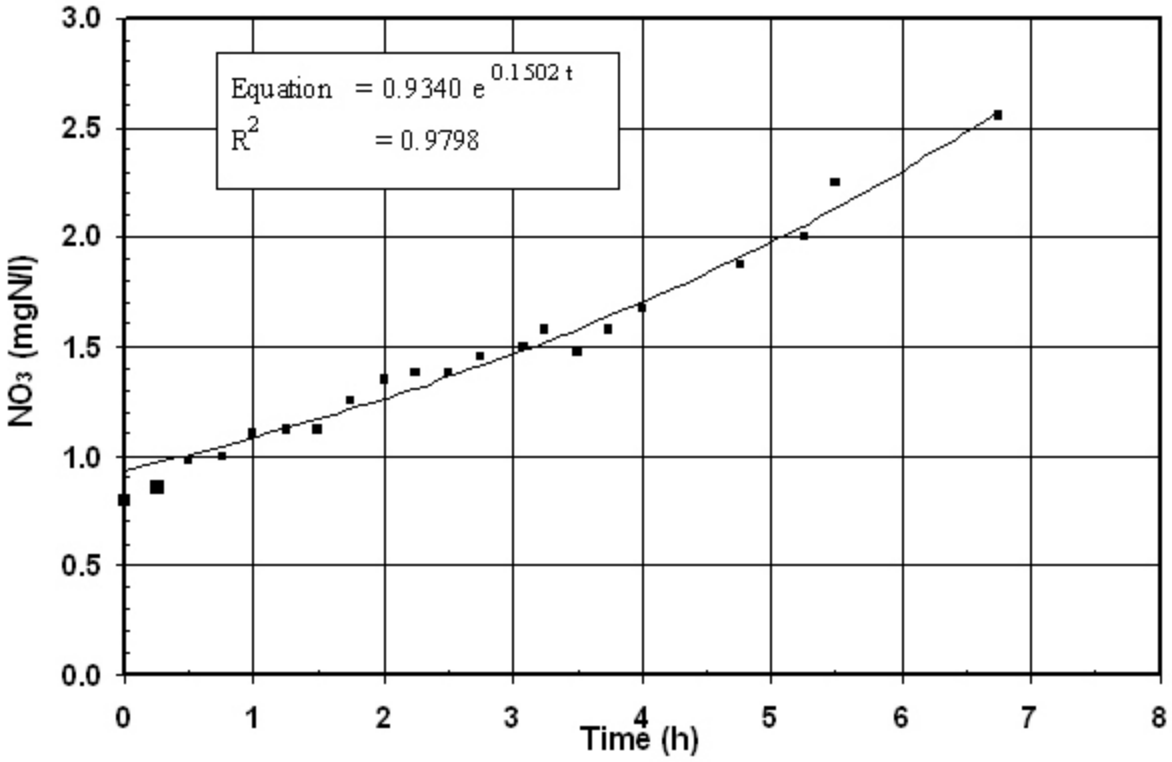

Figure 5.8: Measured nitrate (NO_3) concentration versus time plot for wastewater (2.75ℓ) plus mixed liquor (0.25ℓ) batch test M18 showing "best fit" exponential expression.

5.6 MODIFICATIONS TO THE BATCH TEST METHOD AND EVALUATION

5.6.1 Modifications to batch test procedure

To eliminate the possible sources of error in the batch test method identified above, the following modifications were proposed:

- The physical removal of the OHO active biomass from the wastewater through pre-filtration. This was done by flocculating and filtering the diluted wastewater; in a settling cylinder, 10 mℓ of stock aluminium sulfate [$A\ell(SO_4) \cdot 15\ H_2O$, stock at 50g/ℓ] were added per ℓ wastewater, the mixture was stirred rapidly (~200rpm) for 2 minutes (rapid mix phase) and then slowly (~1rpm) for 30 minutes (flocculation and settling phase). The clear supernatant that developed in the settling cylinder was drawn off and filtered through a glass fibre filter (Whatman's GF/C).

- The adoption of the exponential approach towards nitrification (Antoniou *et al.*, 1990; Sözen *et al.*, 1996), where it was accepted that the nitrate concentration – time profile follows an exponentially increasing trend, as opposed to a linear increase.

The experimental approach is similar to the approach described for the batch test method developed by Ubisi *et al*. (1997a,b) (see Chapter 4), with the exception that flocculated filtered wastewater is used as substrate source. Having removed the OHO active biomass from the wastewater through flocculation and filtration (batch test with the flocculated wastewater only gave no measurable OUR indicating that the OHO active biomass had been successfully removed, see below), the need to operate a WW only batch test in parallel to the WW + ML batch test no longer was necessary. The oxygen utilization rate (OUR) response observed in a single flocculated filtered WW + ML batch test can be considered as the OUR response emanating from the mixed liquor active biomass addition only, requiring no correction for the OUR contribution from the OHO active biomass in the wastewater. This clearly will enhance the accuracy of the OHO active biomass measurement since an estimate of the wastewater OHO active biomass is no longer required. Further, the fact that direct results for the measurement of the mixed liquor OHO active biomass at the start of the batch test can be obtained in a single batch reactor, considerably simplified the batch test procedure.

5.6.2 Evaluation of modified batch test procedure

5.6.2.1 Parent system

The modified batch test was applied when the parent system above received Wastewater Batch Nos. 21 to 23. Averaged wastewater batch data for the parent system during this period are summarised in Table 5.1. From the averaged data, for each wastewater batch using the procedures detailed above, the following were determined:

- Influent wastewater unbiodegradable soluble and particulate COD fractions ($f_{S,us}$ and $f_{S,up}$, respectively); system COD and N mass balances (Ekama *et al.* 1986) and the COD and TKN to VSS ratios for the mixed liquor (f_{CV} and f_N respectively). The calculation procedures are set out in detail in Cronje *et al*. (2000) and the results for each wastewater

batch are listed in Table 5.3.

- The OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each wastewater batch using the steady state design model (Eq. 4.2) and the UCTOLD kinetic simulation computer programme, see Chapter 4. The results are listed in Table 5.3; close agreement was obtained between the steady state and kinetical simulation predictions for f_{av}. For the reasons set out in Chapter 4 and above, the kinetically simulated values for f_{av} were slightly higher (for Wastewater Batch Nos. 21 to 23, mean difference = 2.3%). Because of the simpler, more direct analytical calculation procedure, the f_{av} values from the steady state design model were used.

In examining the results for f_{av} for Wastewater Batch No. 23, it was noted that the value for this wastewater batch was higher than for the others (see Table 5.3). This unusually high value was traced to the low VSS concentration measured in the parent system during this wastewater batch, which reflected in a high $f_{S,up}$ and low f_{av}. Accordingly, to analyse data from this sewage batch it was proposed to use both the calculated f_{av} (Table 5.3) and the average value from the previous two wastewater batches.

5.6.2.2 Batch test results

To evaluate the modified batch test method, a total of 23 batch tests were conducted, see Table 5.5. As an example the OUR-time and nitrate-time profiles for one batch test (F1, Table 5.5) are shown plotted in Figs. 5.9 and 5.10 respectively. Following the procedure set out in Chapter 4, the following were calculated:

- %COD recovery using Eq. (4.4).

- Nitrification OUR ($OUR_{N(t)}$), from a non-linear regression of the nitrate-time concentration profiles (for example see Fig. 5.10) and using the fitted equation to determine the change in nitrate concentration over a discrete time interval, thereby to give ΔNO_3^- and Δt in Eq. (4.18).

- OHO OUR ($OUR_{H(t)}$), by subtracting the $OUR_{N(t)}$ above from the measured $OUR_{M(t)}$ as in Eq. (4.17b), for example see Fig. 5.11.

- The OHO active biomass concentration at the start of the batch test, from linear regression data of a $\ell nOUR_{H(t)}$ versus time plot (for example see Fig. 5.12), using Eq. (4.12).

- The OHO maximum specific growth rates on SBCOD (K_{MP}) and RBCOD (μ_H) from Eqs. (4.15) and (4.16) respectively.

The results of the above calculations are summarised in Table 5.5. From an analysis of the modified batch test results, the following was concluded (Cronje *et al.*, 2000):

- The flocculation filtration procedure (using alum flocculation and filtration through a glass fibre filter) 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.

- In general, the modified batch tests yielded good %COD recoveries with only 2 out of 20 batch tests rejected on the basis of %COD recovery > 110% (batch tests F2 and F4, Table 5.5). From a statistical plot of the %COD recoveries (Fig. 5.13), 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 (see Table 5.5) resulted in extremely flat slopes measured from the $\ell n(OUR_{H(t)})$ plots (for example see Fig. 5.12). The low slope values made the mathematical formulation in Eq. (4.12) very sensitive to even the smallest changes in the measured slope. This requires that the OURs in the batch test are measured accurately. To demonstrate the low growth rates, statistical plots of the OHO maximum specific growth rates on RBCOD and SBCOD (μ_H and K_{MP} respectively) are shown in Figs. 5.14 and 5.15 respectively; mean μ_H and K_{MP} were both 0.84/d.

- Excluding the batch test results rejected on the COD recovery above, the measured OHO active biomass concentrations were plotted against the theoretical values in Fig. 5.16; good agreement existed between the theoretical OHO active biomass concentration (Z_{BH}) and the Z_{BH} values measured in the modified batch tests. However, individual batch tests do not provide a good estimate of the OHO active biomass concentration. Clearly, a number of tests are required for a reliable estimate.

- 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, and are shown plotted statistically in Fig. 5.17 From this plot the **mean** Z_{BH} value in the parent system measured in the batch test was 1 587 mgCOD/ℓ which compares remarkably closely with the theoretical steady state design value of 1 567 mgCOD/ℓ calculated for the parent system.

- Despite the remarkable agreement between the theoretical Z_{BH} and the mean of the measured Z_{BH} values, the individually measured Z_{BH} values were variable. This was attributed mainly to the sensitivity of the measured OHO active biomass values to the low values measured for the slopes of the $\ell n(OUR_H)$-time plots. Even a small change in the slope of the $\ell n(OUR_H)$-time plot resulted in a marked change in the measured Z_{BH} values. The slope of the $\ell n(OUR_H)$-time plot was low because the maximum specific growth rates for the OHOs were low (mean K_{MP} and μ_H = 0.84/d for both parameters, see Figs. 5.14 and 5.15 respectively).

Table 5.5: Results from modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML): Batch test numbers, volumes added, COD recoveries, max. specific growth rates, measured and theoretical OHO active biomass ($Z_{BH(0)}$). Also shown are the measured and theoretical $Z_{BH(0)}$ concentrations in the parent system, taking due account of dilution.

MODIFIED BATCH TESTS: WASTEWATER (WW) + MIXED LIQUOR (ML)										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_H	Batch test	ML	ML	Batch test
21	F1	0.20	2.80	105	1.15	0.74	121	1821	1540	103
	F2	0.10	2.90	114	1.06	0.74	81	2418	1540	51
	F3	0.15	2.85	103	0.84	1.28	50	1009	1540	77
	F4	0.35	2.65	115	0.67	0.47	276	2369	1540	180
	F5	0.40	2.60	95	0.90	0.91	184	1386	1540	205
	F6	0.25	2.75	94	1.50	0.89	90	1077	1540	128
	F7	0.30	2.70	100	0.94	0.70	161	1613	1540	154
13	F8	0.35	2.865	94	0.68	0.67	210	1800	1516	177
	F9	0.30	2.70	95	0.65	0.82	170	1700	1516	152
	F10	0.40	2.60	107	0.66	0.60	239	1791	1516	202
	F11	0.20	2.80	96	0.41	0.66	156	2345	1516	101
	F12	0.25	2.75	100	0.57	0.77	148	1780	1516	126
	F13	0.10	2.90	101	1.03	1.07	29	857	1516	51
	F14	0.15	2.85	106	0.74	0.89	62	1248	1516	76
18	F15	0.20	2.80	90	0.83	1.10	86	1292	1820	121(102)
	F16	0.25	2.75	94	0.79	0.73	116	1396	1820	152(127)
	F17	0.10	2.90	90	0.72	1.03	52	1549	1820	61(51)
	F18	0.15	2.85	108	0.91	0.86	69	1370	1820	91(76)
	F19	0.30	2.70	100	1.14	0.98	111	1106	1820	182(153)
	F20	0.35	2.65	104	0.54	0.79	212	1815	1820	212(178)

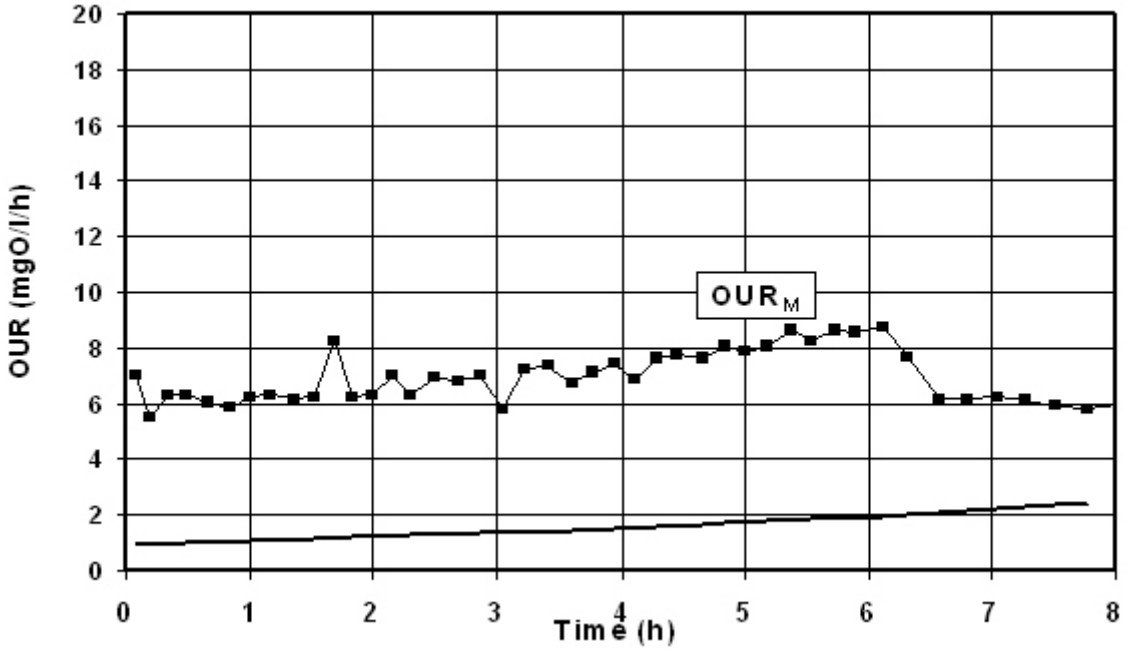

Figure 5.9: Oxygen utilisation rate (OUR) versus time for a modified batch test on a mixture of flocculated filtered wastewater (2.8ℓ) and mixed liquor (0.2ℓ) drawn from the aerobic reactor of the parent system; batch test F1 (Table 5.5).

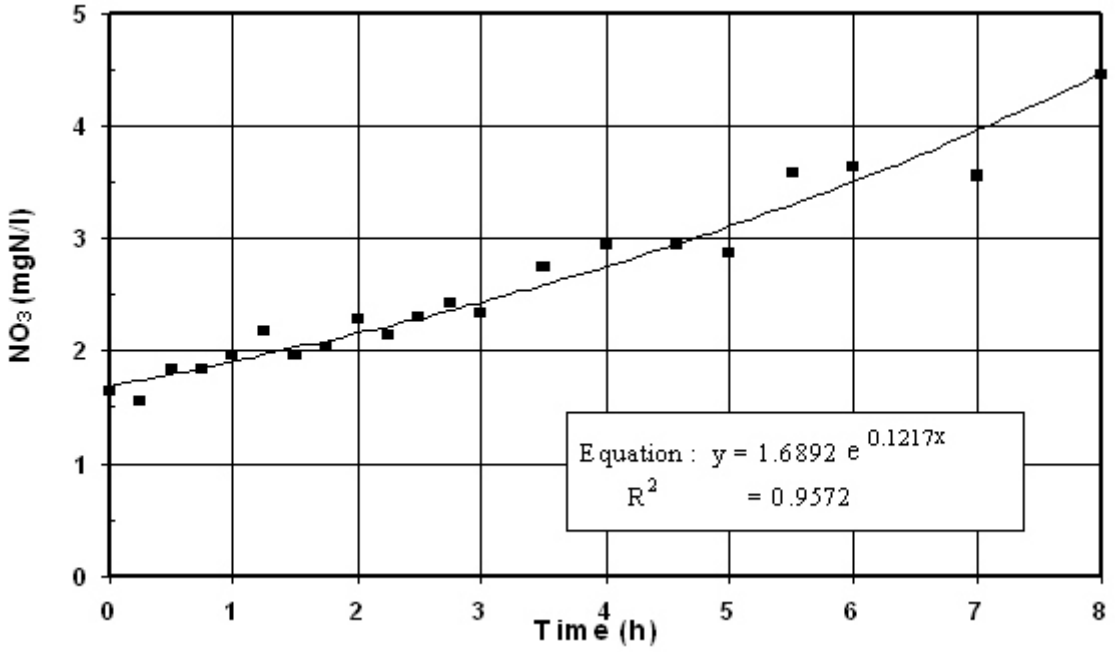

Figure 5.10: Nitrate (NO_3) concentration with time (t) for the modified batch test in Fig. 5.9. Also shown is the exponential expression and correlation coefficient (R^2), obtained through regression. The nitrification OUR, from $OUR_N = 4.57(\Delta NO_3/\Delta t)$ is shown in Fig. 5.9.

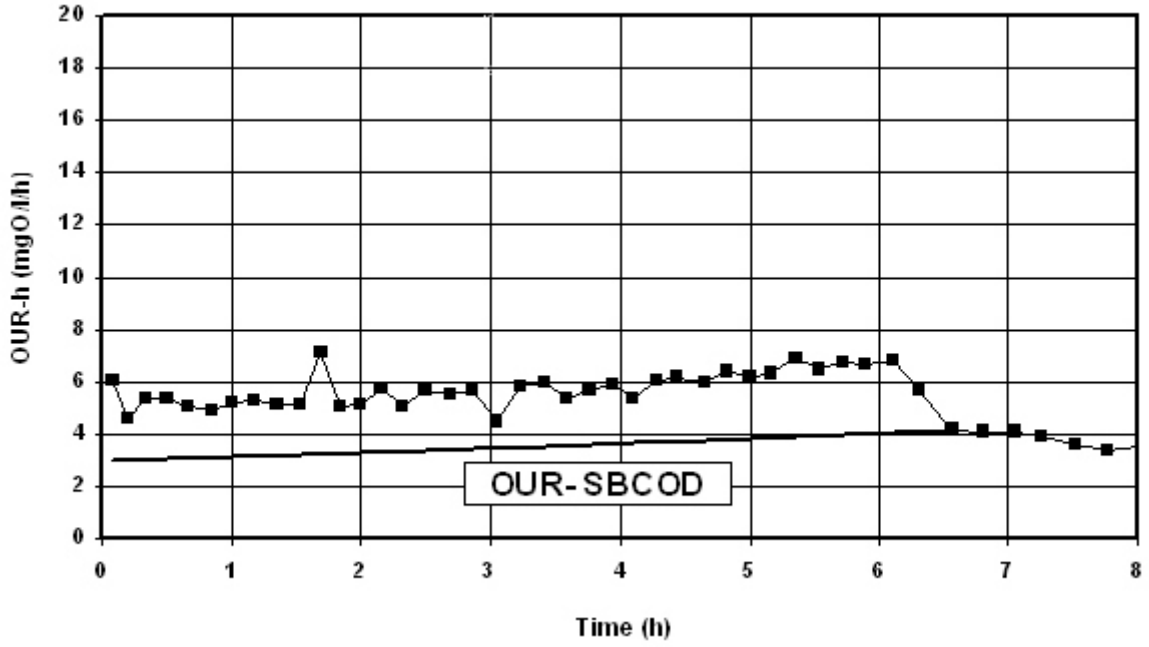

Figure 5.11: Oxygen utilisation rate due to OHO active biomass (OUR-h) versus time for modified batch test F1. The OUR due to nitrification (OUR_N) was subtracted from the measured OUR data in Fig. 5.9.

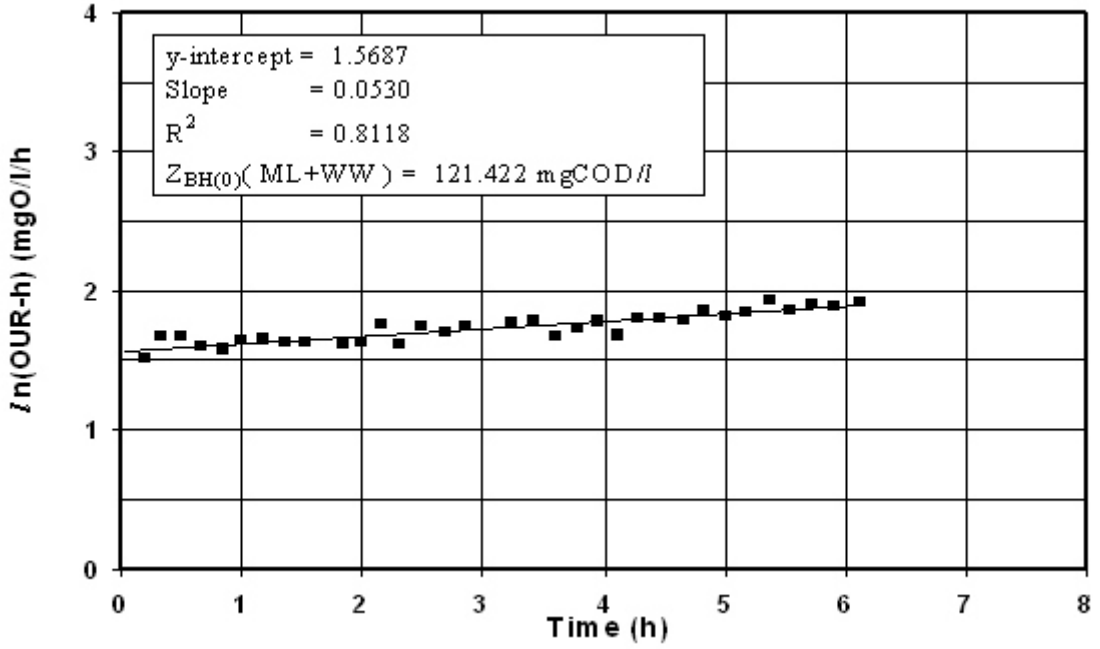

Figure 5.12: ℓn oxygen utilisation rate due to OHO active biomass (ℓn OUR-h) versus time for the OUR-h data in Fig. 5.11, up to the precipitous drop in OUR; batch test F1. Also shown are the regression data for the best fit linear line.

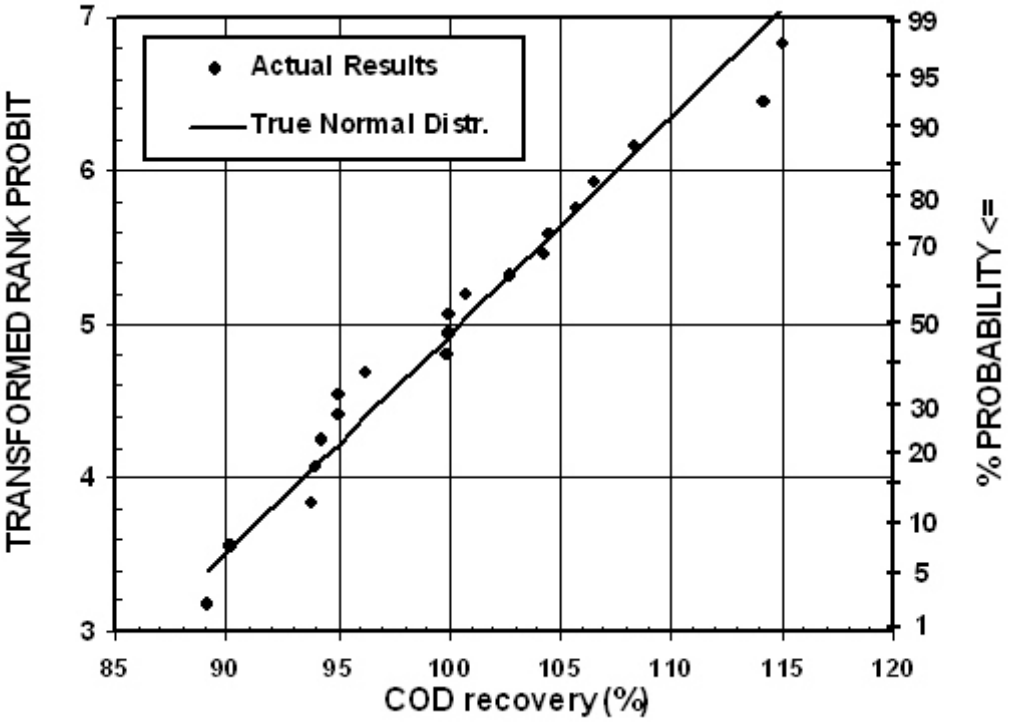

Figure 5.13: Statistical plot of % COD recovery for all the modified batch tests conducted on flocculated filtered wastewater and mixed liquor.

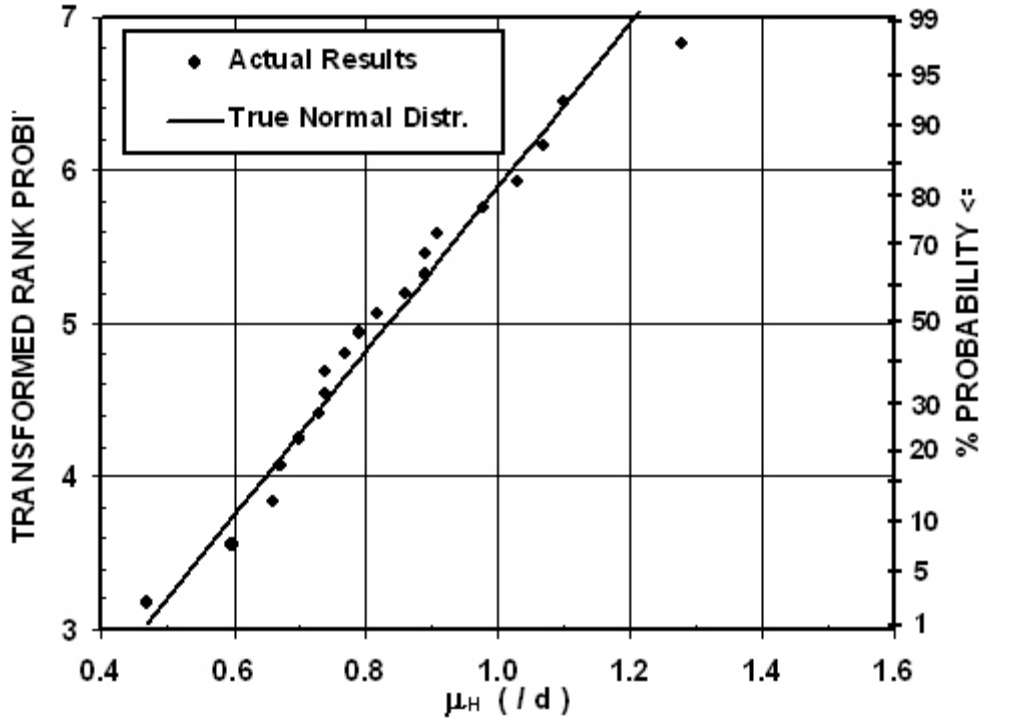

Figure 5.14: Statistical plot of OHO maximum specific growth rate (μ_H) values measured for all the modified batch tests.

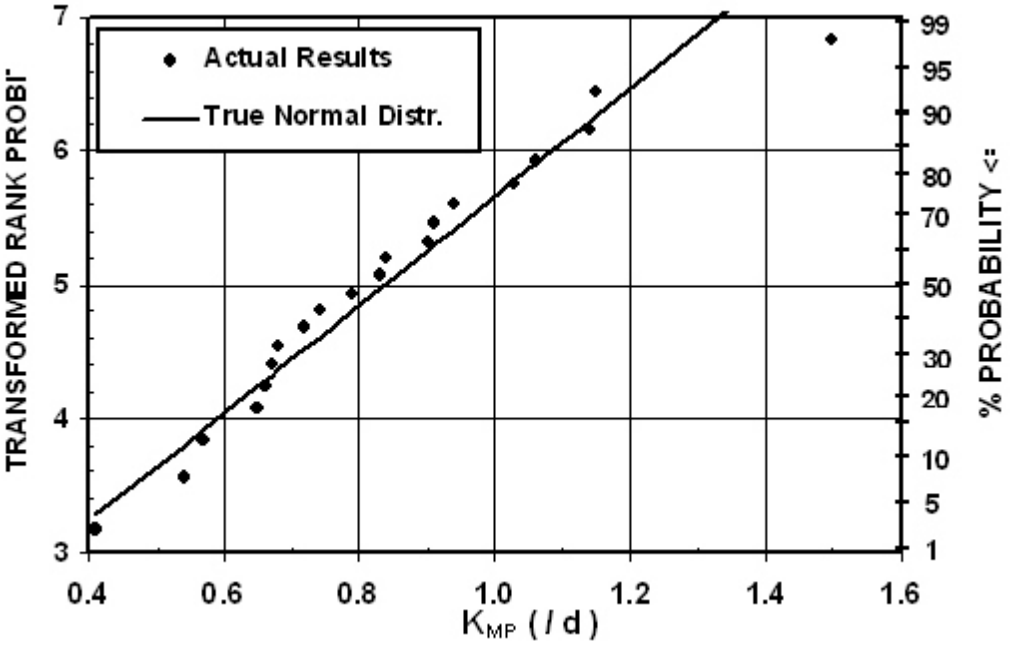

Figure 5.15: Statistical plot of OHO maximum specific growth rates on SBCOD (K_{MP}) values measured for all modified batch tests.

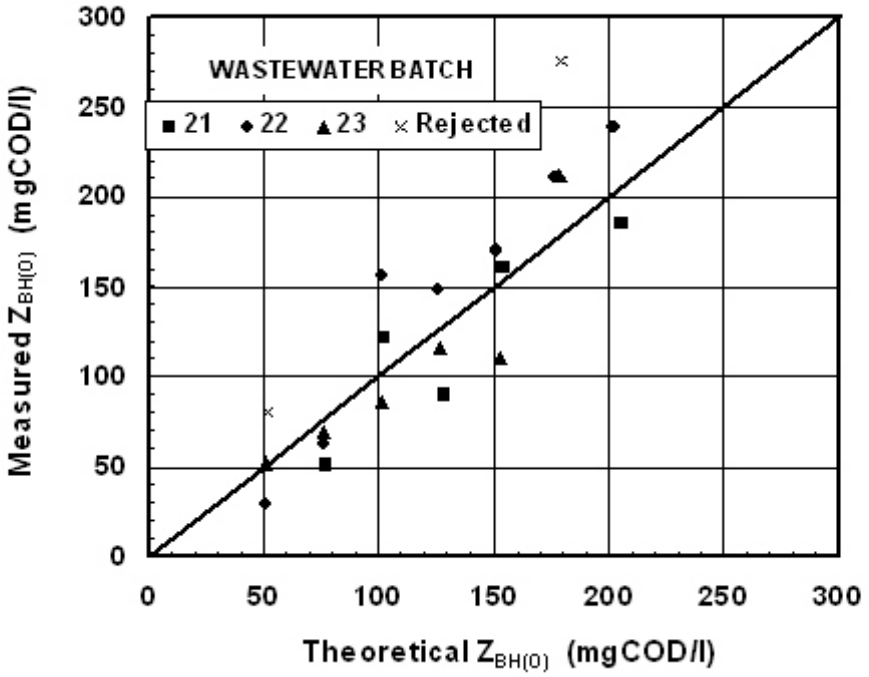

Figure 5.16: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch test data; theoretical values for WW Batch No. 23 using average active fraction from WW Batch Nos. 21 and 22.

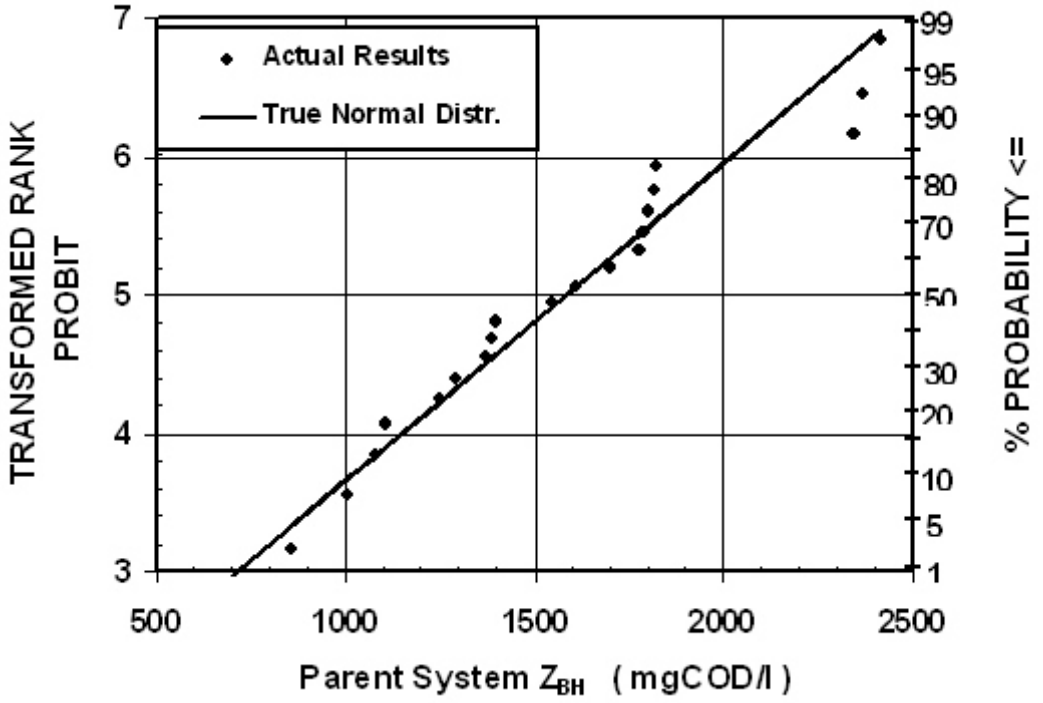

Figure 5.17: Statistical plot of measured OHO active biomass concentrations (Z_{BH}) projected to the concentrations in the parent system operated at 10d sludge age.

5.7 INCREASING OHO MAXIMUM SPECIFIC GROWTH RATES

In evaluating the modified batch test procedure above, it was found that the low values for the maximum specific growth rateS of the OHOs drawn from the continuously fed parent system caused undue sensitivity in the analytical procedure. If the maximum specific growth rate of the OHOs can be increased, this sensitivity can be correspondingly decreased. Accordingly, the effect of increased OHO maximum specific growth rate was investigated. From the literature, operating activated sludge systems in an intermittently fed fill and draw (IFFD) mode induces high heterotroph maximum specific growth rates (Ekama *et al.*, 1986). Thus, it was decided to change operation of the continuously fed system above to IFFD. This would also have provided the opportunity to evaluate the capacity of the modified batch test method to provide a reliable estimate for OHO active biomass concentrations in activated sludge systems operated under significantly different conditions. Accordingly, a well-defined and controlled laboratory-scale intermittently fed fill and draw (IFFD) parent system was operated at the same sludge age of 10 days and bioreactor volume of 10 ℓ as the continuously fed system and received the same COD mass load per day. This parent system provided the mixed liquor samples required for measuring OHO active biomass. However, the parent IFFD system response exhibited the following deviations from the expected steady state behaviour (for details, see Cronje *et al.*, 2000):

- Because the parent IFFD system received the same daily mass of COD and was operated at the same sludge age and bioreactor volume as the continuously fed system, it was expected that the two systems would produce equal concentrations of volatile suspended solids (VSS). However, the IFFD system yielded a consistently higher VSS concentration. In the steady state design model the high VSS concentration is ascribed to a concomitantly high wastewater unbiodegradable particulate fraction ($f_{S,up}$), which causes the theoretical heterotrophic active biomass fraction (f_{av}) to be low. Previous research indicates that it is uncharacteristic of the Mitchell's Plain wastewater to consistently sustain such an unusually high $f_{S,up}$ fraction. In addition, the comparison between the theoretical and measured OHO active biomass concentration in the modified batch tests (see below) suggested an under-prediction of the "true" theoretical OHO active biomass.

- The observed daily OUR response in the parent IFFD system deviated from the typical IFFD system OUR response pattern. The expected OUR plateau associated with RBCOD utilization, followed by a well-defined precipitous drop in OUR, was not evident from the observed OUR responses. Instead a high initial OUR with a subsequent progressive decrease in OUR was observed. It was concluded that the deviation in OUR response was mainly the result of continually decreasing growth rates of the organisms when subjected to cyclic feed-starve conditions. The nitrate generation profile, recorded during the period when the system was feeding, indicated that the nitrification rate gradually leveled off after an initial high rate of nitrification. This decreasing nitrification rate, in particular, had a significant impact on the shape of the OUR profile. Previous research projects on similar systems subjected to high COD loading rates (Dold *et al.*,1980 and Dold *et al.*, 1991), indicate that the OHOs may exhibit a similar decreasing rate of RBCOD utilization.

- It was thus concluded that the IFFD system induced a behavioural response that could not be accurately interpreted in terms of the current activated sludge modelling theory.

In total 18 modified batch tests were conducted on mixed liquor drawn from the IFFD parent system. From these modified batch tests, the following was concluded (see Cronje *et al.*, 2000):

- The shape of OUR response curve recorded during each of the modified batch tests, did not conform to the expected OUR profile described by Wentzel *et al.* (1995) and that observed for the batch tests conducted on the continuously fed parent system mixed liquor. In contrast to the expected exponential increase in OUR, a relatively high initial OUR was established, followed by a progressive decrease in OUR during the first period of the batch test, see Fig. 5.18. To a large extent the shape of OUR response recorded during the first period of each batch test correlated well with the unexpected daily OUR profiles observed for the parent IFFD system after feeding. The consistency of occurrence indicated that the deviation of the observed response from the expected behaviour was replicated in the batch tests.

- In contrast to the parent system observations, where the deviation in OUR response could be attributed principally to a continually decreasing nitrification rate, the nitrate generation profiles recorded during the batch tests revealed only a moderate impact of the nitrification oxygen demand on the observed OUR response.

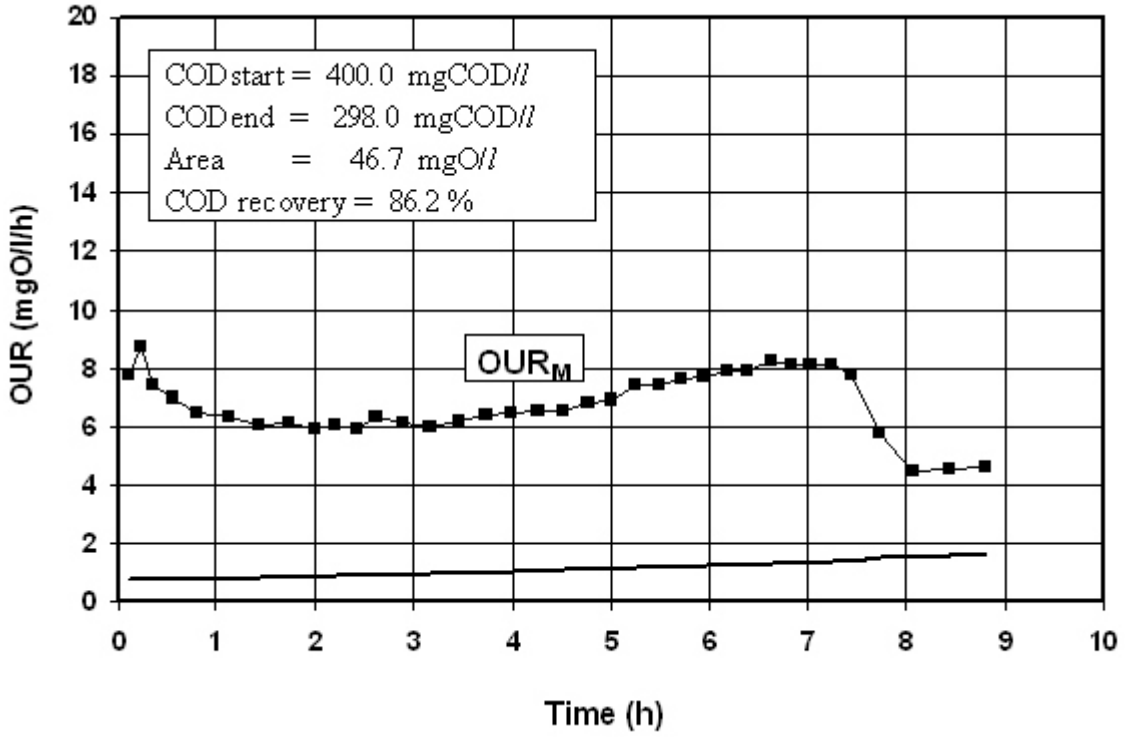

Figure 5.18: Oxygen utilisation rate (OUR) response with time for modified batch test on a mixture of filtered wastewater (2.9ℓ) and mixed liquor (0.1ℓ) drawn from the parent IFFD system.

- Batch tests conducted with filtered effluent demonstrated that the high initial OUR levels observed in the batch tests were not related to intracellular COD storage in the parent IFFD system, and subsequent utilization of this stored COD in the batch tests.

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

- When disregarding the initial batch test OUR data, the analysis of the subsequent exponentially increasing OUR data revealed that the IFFD mixed liquor OHO maximum specific growth rate (μ_H) values (mean = 1.35/d, see Fig. 5.19) were only slightly higher than the values μ_H calculated for the continuously fed system (mean = 0.84/d). However, the mean μ_H value of 1.35/d was significantly lower than the 4.3 /d obtained via an earlier investigation by Dold *et al.* (1991) for mixed liquor samples harvested from a similar IFFD system. That the μ_H for the OHOs was not significantly increased by the IFFD operation negated one of the principle objectives for this part of the investigation, namely to investigate the effect of increased μ_H on the batch test procedure.

- In general, the variable %COD recoveries (see Fig. 5.20) achieved in the batch tests appear to reflect the uncertainty surrounding the OUR responses observed in the modified batch tests conducted on the IFFD system mixed liquor.

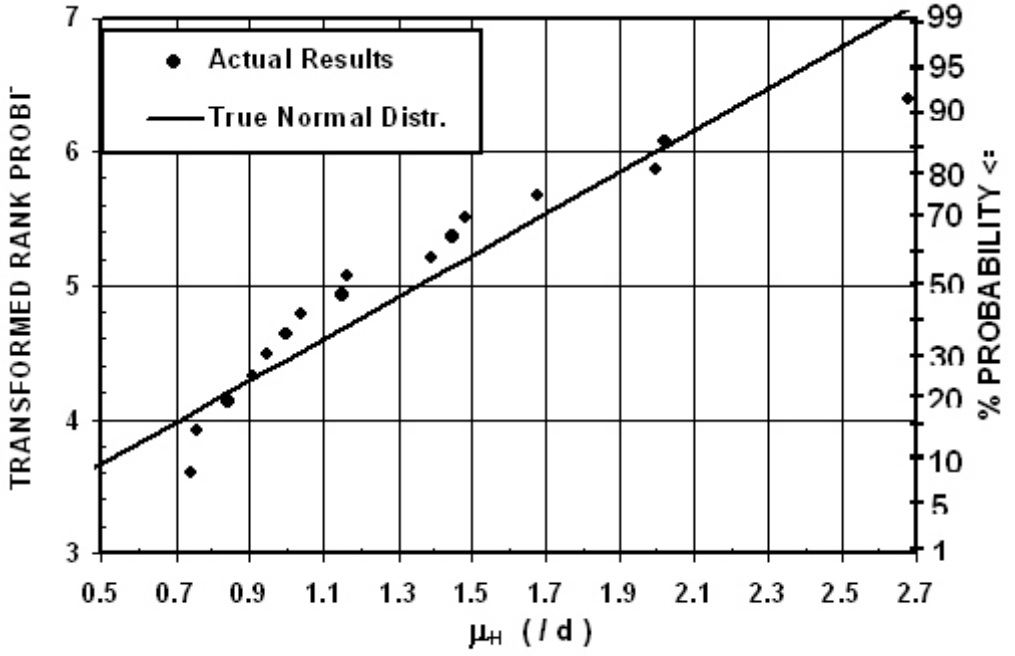

Figure 5.19: Statistical plot of μ_H values measured for all the modified batch tests conducted with IFFD parent system mixed liquor.

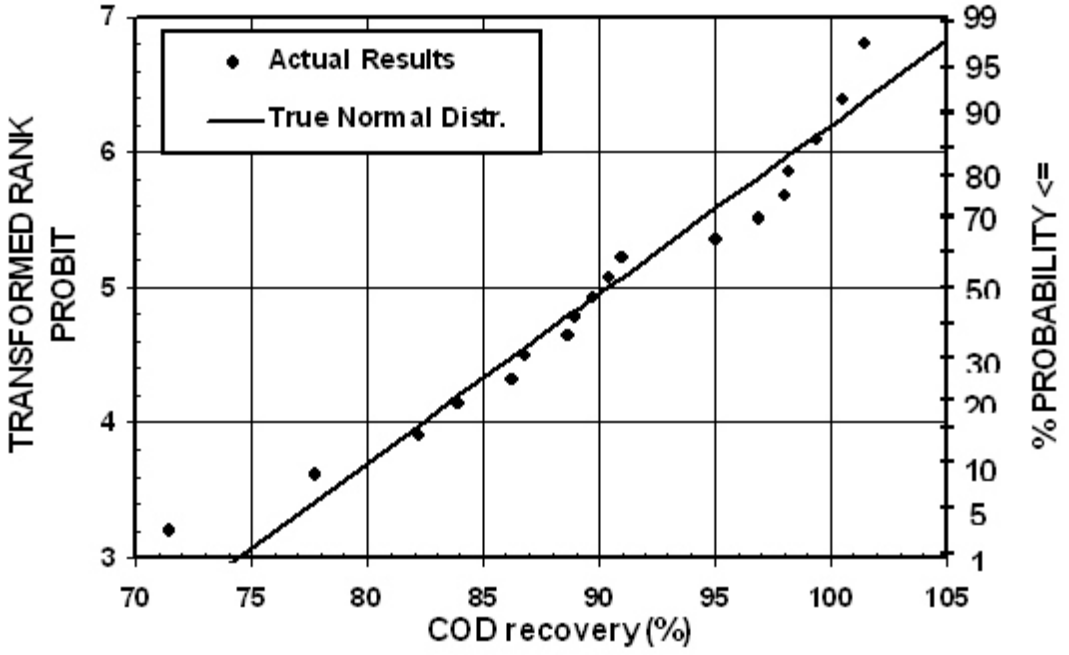

Figure 5.20: Statistical plot of % COD recovery for the modified batch tests conducted with flocculated filtered wastewater and mixed liquor drawn from parent IFFD system.

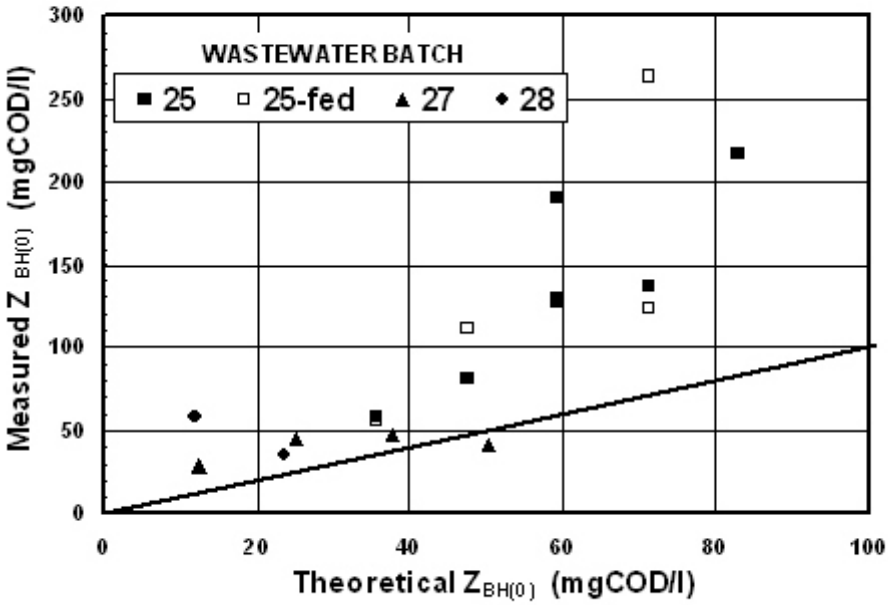

Figure 5.21: Measured versus theoretical OHO active biomass concentration in the batch test ($Z_{BH(0)}$) for modified batch tests on mixed liquor drawn from the IFFD parent system operated at 10d sludge age.

- Also, due to the uncertainty surrounding the OUR responses, the measured OHO active biomass concentrations showed considerable variability, see Fig. 5.21, with the measured values being consistently higher than the theoretical values.

It is 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 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.

5.8 CLOSURE

In this Chapter the batch test method of Ubisi *et al*. (1997a,b) to quantify OHO active biomass concentration has been evaluated by applying the method to mixed liquor drawn from a well defined and controlled parent laboratory-scale MLE activated sludge system operated at 10d sludge age. From this evaluation, it became evident that the correlation between measured and theoretical OHO active biomass concentrations was poor, and remarkably similar to that obtained by Ubisi *et al*. on mixed liquor samples drawn from their system operated at 20d sludge age. This prompted a detailed evaluation of the batch test method. Two sources of potential error in the method were identified:

- 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 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 the constant nitrification OUR which was subtracted from the measured OUR to give the OHO 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. Batch tests demonstrated no observable biological activity in the flocculated-filtered wastewater, indicating that all OHO active biomass had been successfully removed.

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

The modifications proposed above greatly simplify the batch test procedure - since the flocculated-filtered wastewater does not contain OHO active biomass, a parallel batch test no longer needs 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 the correlation between measured and theoretical OHO active biomass concentrations was good. This indicates 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 does require more extensive evaluation; this will be addressed in Chapter 6.

CHAPTER 6

EVALUATION OF THE MODIFIED BATCH TEST PROCEDURE TO QUANTIFY HETEROTROPHIC ACTIVE BIOMASS

6.1 INTRODUCTION

In Chapter 5 (and reported in detail by Cronje *et al*., 2000), the batch test procedure of Ubisi *et al*. to measure OHO active biomass in activated sludge mixed liquor has been modified by flocculating and filtering the wastewater prior to the batch test, to physically remove the OHO active biomass from the wastewater. This modification greatly simplifies the batch test procedure - since the flocculated filtered wastewater does not contain OHO active biomass, a parallel batch test on wastewater only no longer needs to be conducted to quantify the wastewater OHO active biomass, which in the Ubisi *et al*. procedure was subtracted from the mixed liquor + wastewater OHO active biomass, to give the mixed liquor OHO active biomass. The results from the modified batch test have been preliminarily evaluated by comparing them with theoretical values for OHO active biomass concentrations from the steady state design model (WRC, 1984). From this comparison, the results obtained showed good agreement. Also, there was remarkable agreement between the theoretical OHO active biomass concentration in the parent system and the mean of the measured OHO active biomass values *projected* to the parent system. This appears to substantiate the modified batch test procedure as a means of quantifying the OHO active biomass. However, the evaluation of the modified batch test procedure was not exhaustive, and hence it does require more thorough evaluation. This Chapter summarises a more extensive evaluation of the modified batch test method of Cronje *et al.* (2000), as described in Chapter 5; details are set out by Beeharry *et al*. (2001).

6.2 RESEARCH OBJECTIVES

To evaluate the modified batch test procedure, two primary objectives were identified:

(1) Evaluate the modified batch test method, by comparing the OHO active biomass concentrations measured in the batch test on samples drawn from a well-defined parent anoxic/aerobic activated sludge system (termed the ***control***), with the theoretical values predicted by the steady state design model. Good correspondence between the theoretical and measured values would provide substantive direct evidence supporting both the steady state design model and the modified experimental method.

(2) In addressing the main aim above, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system (termed the ***experimental***) having a ***different*** OHO active biomass fraction of the mixed liquor. To change the OHO active biomass fraction of the mixed liquor, a known concentration of macerated toilet paper

solution was dosed to this system. Toilet paper mainly is constituted of wood pulp, which is composed of 75% cellulose and 25% lignin. These two organic components are difficult to biodegrade (e.g. Whitehouse, 1990; Andrady *et al*., 1992), and hence are believed to be largely unbiodegradable in the activated sludge system. Accordingly, toilet paper should contribute significantly to the inert sludge mass in the laboratory-scale anoxic/aerobic activated sludge system, thereby significantly increasing the MLOSS concentration in the system, and ***reducing*** the OHO active biomass fraction of the MLOSS. This would provide the opportunity to evaluate the ability of the modified batch test procedure to detect the ***decreased*** OHO active biomass fraction.

6.3 RESEARCH APPROACH

The research approach adopted was to operate and monitor two well-defined and controlled continuously fed parent activated sludge systems in parallel. The ***control*** activated sludge system provided the mixed liquor samples for measuring the OHO active biomass to address objective (1) above. To address objective (2), the ***experimental*** activated sludge system provided the mixed liquor samples for measuring the OHO active biomass.

6.4 PARENT SYSTEMS

The modified batch test method was evaluated by conducting batch tests on mixed liquor drawn from three laboratory-scale parent activated sludge systems:

- Control anoxic/aerobic parent system
- Control fully aerobic parent system
- Experimental anoxic aerobic parent system

6.4.1 Control anoxic/aerobic and aerobic parent systems

To evaluate the modified batch test procedure, the first objective was to operate and maintain a ***control*** parent laboratory-scale nitrification / denitrification activated sludge system identical to that of Cronje *et al.* (2000) (see Chapter 5) which would have the same mixed liquor characteristics. This system would serve as a source of mixed liquor for the batch tests.

6.4.1.1 System operation

Initially the control parent laboratory-scale activated sludge system operated as the source of mixed liquor for the batch tests was in the Modified Ludzack Ettinger (MLE) configuration; system layout was exactly the same as for the Cronje *et al*. (2000) system, as shown in Fig. 5.1. System operation was also the same as for the Cronje *et al*. (2000) system (see Chapter 5, Section 5.3.1): Sludge age was 10d, maintained by wasting mixed liquor from the aerobic reactor (hydraulic control) taking due account of any samples drawn from the reactors for analysis; temperature was controlled at 20°C, pH at 7.6 (±0.2); influent flow rate was constant, set at 20ℓ/d. Influent feed was raw (unsettled) municipal wastewater from the Mitchell's Plain Treatment Plant (Cape Town, South Africa). The wastewater was collected in batches from the

treatment works, stored in stainless steel tanks at 4°C and served as feed for both the parent system and the batch tests for a period of 1-2 weeks (see Beeharry *et al*., 2001 for details). For the parent system, a sample of the wastewater was drawn from the storage tanks after thorough mixing and diluted with tap water to give influent feed total COD ~ 500 mgCOD/ℓ. System operational procedures detailed by Ekama *et al*. (1986) were followed.

As the experimental investigation proceeded, denitrification performance in the system deteriorated. This caused increased nitrate concentrations to be present in the anoxic reactor, which resulted in increased DSVIs, in conformity with the AA filament bulking hypothesis of Casey *et al*. (1994a,b). Thus, on day 107 it was decided to increase the denitrification potential in the system by modifying the original MLE configuration, by increasing the anoxic mass fraction and correspondingly decreasing the aerobic mass fraction; new system layout is shown in Fig. 6.1. System operation and feeding were as described above, except that the target influent COD concentration was increased to a total COD ~ 750 mgCOD/ℓ, and the influent feed rate correspondingly decreased to 13.3ℓ/d, to keep the COD load on the system constant.

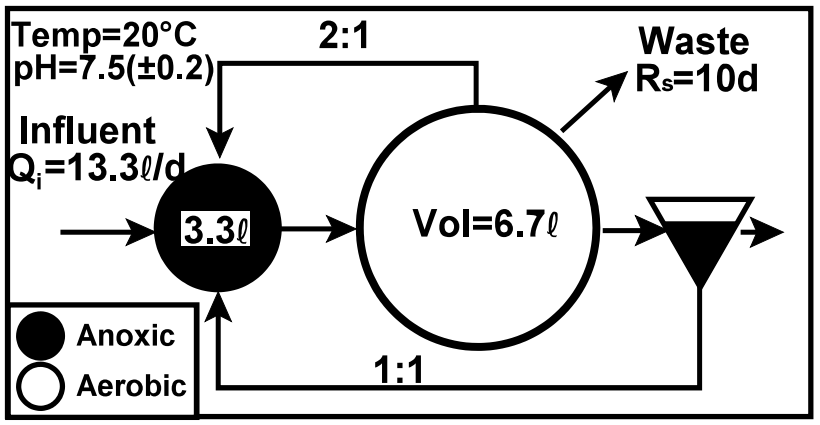

Figure 6.1: Schematic layout and operational data for control MLE parent laboratory-scale system of Beeharry *et al*. (2001).

After 304 days of operation, the system experienced severe bulking problems; the DSVI value increased steadily from 107 to 333 mℓ/g in a few weeks. Filamentous organism identification indicated proliferation of the filament *Microthrix parvicella*, at levels very common to abundantly present. A short-term remedy was to dose aluminium sulphate [$A\ell(SO_4) \cdot 15H_2O$, stock at 133g/ℓ] to prevent secondary settling tank overflow, and hence contain the mixed liquor in the system; mixed liquor loss to the effluent would reduce the system effective sludge age and hence change the OHO active fraction in an uncontrolled fashion. A starting dose of 50 mℓ was added on the first day to the aerobic reactor of the activated sludge system, thereafter 5 mℓ of aluminium sulphate was dosed daily for the next 47 days, until the DSVI decreased to 177 mℓ/g. While successful, the problem with non-specific control methods is that they treat temporarily the symptoms of bulking, but do not constitute a permanent cure (Casey *et al*., 1994a,b) – immediately after dosing of aluminium sulphate ceased, the DSVI increased steadily to 208 mℓ/g in 2 days. The dominant species were again identified as *M. parvicella*.

With specific bulking control, the causes for proliferation of the filaments are sought. Casey *et al*. (1994a,b) concluded that by eliminating these through wastewater characteristic or system

modification, the bulking problems caused by specific filamentous organism types are cured permanently. They also concluded that AA filament bulking sludges, containing amongst others the filamentous organism *M. parvicella*, from full-scale or laboratory-scale activated sludge systems invariably ceased bulking within a short space of time under fully aerobic conditions. Hence, to improve the sludge settleability, on day 377 it was decided to change the laboratory-scale *Modified Ludzack-Ettinger* (MLE) system to a completely mixed *fully aerobic* system; system layout is shown in Fig. 6.2. System operation and feeding were as described above.

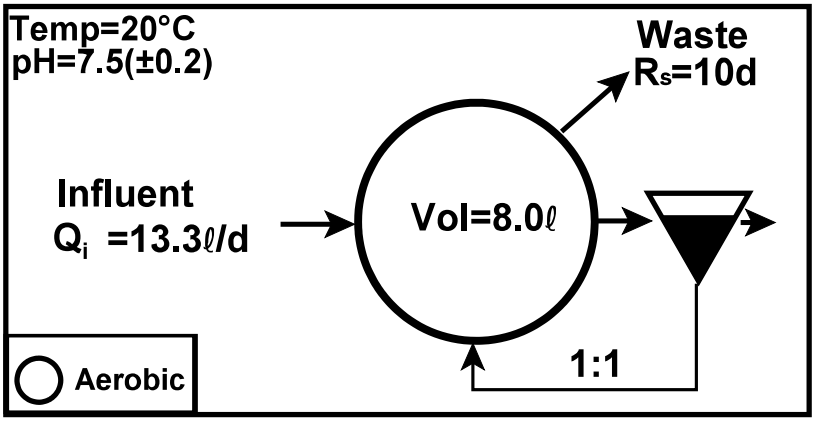

Figure 6.2: Schematic layout and operational data for control aerobic parent laboratory-scale system of Beeharry *et al*. (2001).

For all system configurations, daily monitoring included influent COD, TKN; all reactors nitrate + nitrite; aerobic reactor VSS, TSS, COD and TKN of the VSS, oxygen utilization rate (OUR); effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985). (Individual nitrite measurements indicated that in the parent system, nitrite concentrations were very low compared to nitrate concentrations and consequently could be neglected for this investigation).

To ensure steady state, the parent system was operated for more than twenty sludge ages before mixed liquor was harvested for the batch tests. Detailed data on the parent system are described by Beeharry *et al*. (2001). The reliability of the experimental measurements were checked by means of mass balances on COD and N (Ekama *et al*., 1986).

6.4.1.2 Control parent system results

The control parent system was operated for 417 days in total and received 26 batches of wastewater as influent, see Table 6.1.

Table 6.1: Details of the parent ***control*** laboratory-scale activated sludge system; system configuration, batch tests conducted, days of operation and wastewater batch number.

Configuration	Batch tests	Days of Operation	Wastewater Batch No.
MLE (Fig. 5.1) 25% Anoxic 75% Aerobic	No	Day 1 - 106	1 - 8A
MLE (Fig. 6.1) 33% Anoxic 67% Aerobic	No	Day 107 - 294	8B - 17
	Yes	Day 295 - 376	18 - 23A
Aerobic (Fig. 6.2) 100% Aerobic	Yes	Day 377 - 417	23B - 26

Each wastewater batch was accepted as a steady state period, and the data for each batch were averaged (after statistical analysis for outliers). Batch tests commenced when the system was receiving Wastewater Batch No. 18 as influent, and the averaged data for the wastewater batches during which batch tests were conducted are listed in Table 6.2 (detailed data are listed by Beeharry *et al*., 2001, as well as averages for the wastewater batches where no batch tests were conducted).

Table 6.2: Steady state results for ***control*** parent laboratory-scale anoxic/aerobic (Fig. 6.1) and aerobic (Fig. 6.2) activated sludge systems. For each wastewater batch tested when samples were drawn for the batch tests, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

CONTROL ANOXIC/AEROBIC AND AEROBIC STEADY STATE SYSTEMS													
WW Batch	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite[1] (mgN/ℓ)			Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			DSVI (mℓ/g)
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
18	8	655 (56)	36 (13)	63 (3)	8.1 (0.7)	1.6 (0.4)	9.2 (1.1)	8.6 (0.9)	37.5 (1.3)	2409 (138)	3119 (282)	208 (14)	108 (8)
19	8	728 (35)	52 (9)	85 (4)	6.2 (1.8)	4.8 (1.9)	17.3 (1.5)	15.2 (1.2)	41.6 (0.9)	3042 (80)	4073 (243)	243 (14)	236 (25)
20	8	741 (52)	65 (13)	70 (4)	5.1 (0.9)	5.4 (3.5)	14.6 (5.2)	12.6 (3.0)	40.9 (2.2)	3760 (106)	3936 (148)	239 (7)	272 (37)
21	9	774 (28)	40 (9)	70 (3)	5.6 (1.3)	5.7 (1.4)	14.7 (1.4)	11.5 (1.8)	36.4 (1.3)	2890 (164)	3908 (86)	241 (11)	232 (15)
22	10	749 (35)	46 (17)	73 (2)	8.3 (0.6)	3.3 (1.5)	11.5 (2.1)	9.1 (1.3)	37.1 (2.4)	2736 (143)	3862 (205)	224 (16)	198 (12)
23A	3	795 (19)	57 (6)	86 (5)	5.5 (1.4)	24.8 (0.3)	36.4 (0.3)	35.6 (1.1)	41.4 (0.7)	3111 (88)	4110 (105)	252 (11)	180 (8)
23B	5	785 (26)	66 (24	80 (3)	6.1 (1.4)		60.4 (3.1)	58.8 (4.7)	47.9 (1.1)	3220 (69)	4429 (99)	257 (11)	157 (6)
24	6	798 (14)	52 (19)	57 (6)	7.1 (1.6)		28.8 (3.9)	30.0 (6.0)	43.0 (1.9)	3625 (160)	5083 (114)	258 (21)	145 (4)
25	5	815 (22)	54 (11)	104 (2)	4.3 (0.4)		69.3 (3.0)	73.1 (1.6)	43.7 (0.8)	3726 (128)	5313 (57)	277 (7)	108 (12)
26	5	787 (26)	31 (11)	72 (5)	4.5 (0.5)		50.0 (1.0)	52.6 (0.3)	45.9 (1.5)	3526 (72)	5112 (156)	250 (6)	85 (8)

[1]From measurements, nitrite concentrations were found to be negligible.

Following the procedures set out in Chapter 4, from the averaged data in Table 6.2, 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 Chapter 4 - in Chapter 5 it was shown 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 Chapter 4.
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

These values are listed in Table 6.3.

Table 6.3: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for ***control*** parent laboratory-scale anoxic/aerobic (Fig. 6.1) and aerobic (Fig. 6.2) activated sludge systems, for wastewater (WW) batches during which batch tests were conducted. Values calculated from data in Table 6.2, either directly or using the steady state (SS) design model (WRC, 1984).

CONTROL ANOXIC/AEROBIC AND AEROBIC STEADY STATE SYSTEMS								
WW Batch	No of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbiod. Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)	COD/VSS ratio (mgCOD/mgVSS) (f_{cv})	TKN/VSS ratio (mgN/mgVSS) (f_n)	Active Fraction (f_{av})
18	8	98	93	0.043	0.171	1.31	0.087	0.3323
19	8	99	94	0.066	0.249	1.35	0.080	0.2659
20	8	100	96	0.081	0.198	1.44	0.087	0.3407
21	9	86	92	0.040	0.170	1.38	0.085	0.3725
22	10	93	86[1]	0.038	0.168	1.45	0.084	0.3826
23A	3	86	98	0.044	0.210	1.42	0.081	0.3354
23B	5	96	101	0.041	0.111	1.38	0.080	0.4551
24	6	105	91	0.026	0.172	1.40	0.071	0.3583
25	5	100	90	0.022	0.165	1.43	0.074	0.3914
26	5	96	101	0.026	0.159	1.45	0.071	0.4009

[1]Source of poor N mass balance could not be identified; batch should be rejected for analysis.

From the results on the control parent system listed in Tables 6.2 and 6.3:

- Generally COD mass balances were reasonable, with an average mass balance of 96% (sample standard deviation, SSD = 6.1%) and only 2 out of 10 wastewater batches giving mass balances < 90%. Recognising that for Wastewater (WW) Batch No. 23A only 3 measurements were taken, then only WW Batch No. 21 is of importance. The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements for the wastewater batches during which batch tests were conducted gave COD/VSS = 1.40mCOD/mgVSS (SSD = 0.05) and TKN/VSS = 0.080mgN/mgVSS (SSD = 0.006). These values are reasonably close to the standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984), and the values for WW Batch No. 21 are close to the average. Consequently, it was accepted that the error in the COD mass balance did not lie in the measurement of the mixed liquor organic solids, the parameter of importance in the measurement of OHO active biomass. Accordingly, the lower limit for the COD mass balance was set at 85%. On this basis, no wastewater batches were rejected for further analysis.

- N mass balances were excellent, with average 94% (SSD = 4.8%) and only WW Batch No. 22 < 90%. No source for this low mass balance could be found. The batch test data collected during this sewage batch was included where appropriate, and analysed, but it was noted that the data should be interpreted with caution.

- For the wastewater batches during which batch tests were conducted, from the influent and filtered effluent COD measurements, the wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.043 (SSD = 0.018). This value is 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. Of interest is the fact that both Ubisi *et al*. (1997a,b) and Cronje *et al*. (2000) were feeding an influent COD concentration of 500 ± 50 mgCOD/ℓ to their parent systems. In this part of this experimental investigation, the average influent COD concentration was 763 (SSD = 47) mgCOD/ℓ. 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, the higher COD concentration gave a lower $f_{S,us}$. The lower $f_{S,us}$ value is however, in the range of accepted values of 0.04 – 0.10 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984).

- The mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined via the steady state design procedure (WRC, 1984; see Ekama *et al*., 1986) to be 0.177 (sample standard deviation = 0.036). This value is slightly higher than that observed by Ubisi *et al*. (1997a,b) ($f_{S,up}$ = 0.120) and Cronje *et al*. (2000) ($f_{S,up}$ = 0.103) for the same Mitchell's Plain raw wastewater, but conforms to the accepted range of 0.07 – 0.20 mgCOD/mgCOD for municipal raw wastewaters in South Africa (WRC, 1984). This indicates that the value obtained for $f_{S,up}$ is acceptable.

- Following the procedure in Chapter 4, the OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each wastewater batch using the steady state design model (Eq. 4.2), see Table 6.32. The steady state model was used due to the near equality in f_{av} values between the steady state and kinetic simulation models

(see Chapter 5), and the simpler more direct analytical calculation procedure of the steady state design model.

6.4.2 Experimental anoxic/aerobic parent system

As noted above, it was decided to run and operate a parallel parent laboratory-scale anoxic/aerobic activated sludge system, termed the ***experimental*** system, having a different OHO active biomass fraction of the mixed liquor. Both the control and experimental parent systems were set-up and operated identically, but the experimental system additionally received a known mass of toilet paper as influent. It was envisaged that the toilet paper would be largely unbiodegradable particulate, and hence the experimental system would have a mixed liquor OHO active biomass fraction that would deviate significantly from the parallel control system. The ability of the batch test procedure of Cronje *et al*. (2000) (described in Chapter 5) to correctly detect this difference in OHO active biomass would be evaluated.

6.4.2.1 System operation and monitoring

The experimental parent laboratory-scale was identical in set-up to the control system. The main changes to system configuration and operation were identical to those made to the control system, described above. The procedures followed for the wastewater collection and storage were as for the control system, as above. For the wastewater feed, the procedures followed for the feed preparation were identical to those for the control system, described above; however, additionally toilet paper was dosed to the experimental system. Prior to the addition of toilet paper, the system was run for 3 sludge ages (days 1-35) to ensure steady state conditions in the experimental system, and that the response of the control and experimental systems were near identical.

From a literature review on the composition and biodegradation of toilet paper (see Beeharry *et al*., 2001), it was initially thought that the toilet paper would contain a high unbiodegradable particulate fraction ($f_{S,up}$) which would contribute significantly to the mixed liquor inert component in the experimental activated sludge system. Thus, it was decided to add a dose of ~ 2 000 mgCOD/d as toilet paper solution to the influent wastewater feed of ~ 10 000 mgCOD/d. A stock solution of toilet paper of 20g/ℓ was made by macerating 20g of toilet paper into a litre of distilled water. A known volume of the stock toilet paper solution was macerated in a liquidizer with some diluted raw influent sewage and was added to the total feed volume, from day 36. The total COD load per day on the *experimental* activated sludge system was ~ 12 000 mgCOD/d.

System operation and monitoring procedures detailed above for the control system were followed. Details of system configuration and wastewater batches as set out in Table 6.1 for the control system applied to the experimental system also, see Table 6.3. From WW Batch No. 21 the daily toilet paper dose was doubled, because it was found that the toilet paper was more biodegradable than expected and was, therefore, not achieving the desired objective of a significant increase in the unbiodegradable inert fraction of the mixed liquor. However, when the toilet paper dose was increased, the experimental system became prone to blockages of the pipes connecting the reactors, resulting in mixed liquor overflows and losses from the system. This caused that, from WW Batch No. 22 batch tests could not be conducted on mixed liquor drawn from the system, and that the steady state response of the parent system could not be monitored.

Table 6.3: Details of the parent ***experimental*** laboratory-scale activated sludge system; system configuration, batch test conducted, days of operation, wastewater batch number and toilet paper dose.

Configuration	Batch tests	Days of Operation	Wastewater Batch No.	Toilet Paper Dose (mgCOD/d)
MLE (Fig. 5.1) 25% Anoxic 75% Aerobic	No	Day 1 - 35[1]	1 - 2	0
	No	Day 36 - 106	1 - 8A	2 000
MLE (Fig. 6.1) 33% Anoxic 67% Aerobic	No	Day 107 - 294	8B - 17	0
	Yes	Day 295 - 346	18 - 20	2 000
	No	Day 347 - 376[2]	21 - 22	4 000
Aerobic (Fig. 6.2) 100% Aerobic	No	Day 377 - 382[2]	23 - 24	2 000 - 3 000

[1]No testing on parent system, to allow initial steady state.
[2]No testing on parent system, due to pipe blockages and mixed liquor losses

Similarly to the control system, the experimental system experienced severe sludge bulking problems starting after day 304, and therefore aluminium sulphate was dosed to the system. A starting dose of 50 mℓ from the stock solution was added on the first day to the aerobic reactor of the activated sludge system, thereafter 5 mℓ of aluminium sulphate was dosed daily for the next 24 days, until the DSVI decreased to 119 mℓ/g when the dose was terminated.

To ensure steady state, the parent system was operated for more than twenty sludge ages before mixed liquor was harvested for the batch tests. Detailed data on the experimental parent system are described by Beeharry *et al*. (2001). The reliability of the experimental measurements were checked by means of mass balances on COD and N (Ekama *et al*., 1986).

6.4.2.2 Toilet paper characteristics

To chemically characterize the toilet paper, analytical tests were done on the toilet paper stock solution [stock at 20 g/ℓ], and included COD, TKN, FSA, Total Phosphate, TSS, VSS and ISS. Each of these tests were repeated three times to confirm that the results were reproducible; this was done because the various samples that were used for the tests were not homogeneous since they contained lumps of toilet paper (these could not be dissolved into solution), making the sampling procedure (such as pipetting an exact volume into a flask) difficult. All the independent test results were reasonably close (within 10%), so mean values for the COD, TKN, Total Phosphates, TSS, VSS and ISS of the toilet paper were determined; these values are listed in Table 6.4 below.

Table 6.4: Mean values for the characterisation of toilet paper stock solution (stock at 20g toilet paper/ℓ) used to provide daily dose to the parent ***experimental*** laboratory-scale activated sludge system.

Parameter	Mean value
COD	20 000 mgCOD/ℓ
TKN	6 mgN/ℓ
FSA	5 mgN/ℓ
Total Phosphate	2 mgP/ℓ
TSS	17 000 mgTSS/ℓ
VSS	16 800 mgVSS/ℓ
ISS	200 mgISS/ℓ

From the above table it can be seen that the TKN, FSA and Total Phosphate concentrations of toilet paper are very low and consequently can be neglected. The toilet paper does however contain significant COD, TSS, VSS and ISS which will contribute to the mixed liquor in the *experimental* activated sludge system.

6.4.2.3 Experimental parent system results

The *experimental* system was operated for 382 days in total and received 24 batches of wastewater as influent, see Table 6.3.

Each wastewater batch was accepted as a steady state period, and the data for each batch were averaged (after statistical analysis for outliers). Batch tests commenced when the system was receiving Wastewater Batch No. 18 as influent, and the averaged data for the wastewater batches during which batch tests were conducted are listed in Table 6.5 (detailed data are listed by Beeharry *et al*., 2001, as well as averages for the wastewater batches where no batch tests were conducted).

Table 6.5: Steady state results for ***experimental*** parent laboratory-scale anoxic/aerobic (Fig. 6.1) activated sludge system. For each of the wastewater batches tested, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

EXPERIMENTAL ANOXIC/AEROBIC AND AEROBIC STEADY STATE SYSTEMS													
WW Batch	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite (mgN/ℓ)			Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			DSVI (mℓ/g)
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
18	8	799 (58)	66 (24)	63 (3)	9.2 (1.7)	0.7 (0.1)	7.7 (1.6)	8 (0.7)	41.8 (2.2)	3103 (178)	4011 (235)	227 (12)	105 (7)
19	8	807 (32)	66 (12)	83 (5)	7.8 (0.8)	2.3 (1.1)	15.8 (1.5)	14.8 (1.5)	49 (4.1)	3482 (72)	4720 (251)	266 (14)	202 (44)
20	8	904 (22)	81 (18)	71 (2)	6.6 (1.3)	3.2 (1.1)	11.3 (2.4)	15.5 (2.3)	42.6 (1)	3359 (92)	4778 (271)	247 (12)	113 (12)

Following the procedures set out in Chapter 4, from the averaged data in Table 6.5, the following were calculated:

- System COD and TKN 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 Chapter 4 - in Chapter 5 it was shown that the steady state design procedure and the kinetic simulation models gave near identical results, 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 Chapter 4.
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

These values are listed in Table 6.6. Additionally, from a comparison of the steady state performance of the experimental system with that of the control systems for all WW Batches tested (data not shown, see Beeharry *et al.*, 2001), the following were determined:

- Toilet paper unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$ respectively), see Table 6.6 for WW Batch No. 18 - 20.

Table 6.6: Steady state COD and N mass balances, wastewater fractions, toilet paper fractions and mixed liquor parameters for ***experimental*** parent laboratory-scale anoxic/aerobic (Fig. 6.1) activated sludge system. Data calculated from data in Tables 6.2 and 6.5, either directly or using the steady state (SS) design model (WRC, 1984)

EXPERIMENTAL ANOXIC/AEROBIC STEADY STATE SYSTEM										
WW Batch	No of tests	Mass Balance (%)		WW + TP fractions		TP fractions		Mixed liquor		
		COD	N	Unbiod. Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)	Unbiod Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)	COD/VSS (mgCOD/ mgVSS) (f_{cv})	TKN/VSS (mgN/ mgVSS) (f_n)	Active Fraction (f_{av})
18	8	99	94	0.047	0.198	0.064	0.325	1.29	0.073	0.3027
19	8	107	107	0.076	0.275	0.169	0.529	1.36	0.076	0.2443
20	8	90	110	0.076	0.194	0.058	0.185	1.42	0.074	0.3453

From the results on the control parent system listed in Tables 6.5 and 6.6:

- COD mass balances all fell within the acceptable range of 90 - 110%.

- N mass balances all fell within the acceptable range of 90 - 110%.

- The mixed liquor organic solids were determined by three independent tests – VSS, COD and TKN. Mean ratios for these measurements for the wastewater batches during which batch tests were conducted gave COD/VSS = 1.36mgCOD/mgVSS (SSD = 0.07) and TKN/VSS = 0.074mgN/mgVSS (sample standard deviation = 0.002). These values are lower than the values measured for the *control* parent system (1.40 mgCOD/mgVSS and 0.080 mgN/mgVSS respectively) and the accepted standard values of 1.48 mgCOD/mgVSS and 0.10 mgN/mgVSS respectively (WRC, 1984). More than likely, the lower values were caused by the toilet paper dose.

- For the wastewater batches during which batch tests were conducted, from the influent and filtered effluent COD measurements, the wastewater mean unbiodegradable soluble COD fraction ($f_{S,us}$) was determined to be 0.066 (SSD = 0.017). This value is higher than the $f_{S,us}$ value obtained for the control system ($f_{S,us}$ = 0.043), most likely due to the added toilet paper.

- The mean unbiodegradable particulate COD fraction ($f_{S,up}$) was determined via the steady state design procedure (WRC, 1984; see Ekama *et al.*, 1986) to be 0.222 SSD = 0.046). This value is higher than the value calculated for the control system ($f_{S,up}$ = 0.177), due to the added toilet paper.

- Following the procedure in Chapter 4, the OHO active biomass fractions of the mixed liquor organic suspended solids (f_{av}) were determined for each wastewater batch using the steady state design model (Eq. 4.2), see Table 6.6. The steady state model was used due to the near equality in f_{av} values between the steady state and kinetic simulation models (see Chapter 5), and the simpler more direct analytical calculation procedure of the steady state design model.

- From a comparison of the complete data sets for the control and experimental systems (data not shown, see Beeharry *et al.*, 2001), the $f_{S,us}$ and $f_{S,up}$ for the toilet paper were determined to be 0.035 (SSD = 0.015) and 0.309 (SSD = 0.141) mgCOD/mgCOD respectively. These values are reasonably close to the values determined for the wastewater itself, 0.050 (SSD = 0.014) and 0.161 (SSD = 0.03) mgCOD/mgCOD respectively (for the complete data set). This implies that the toilet paper was 65.6 % biodegradable, significantly higher than expected (wastewater biodegradable fraction = 78.9%). Hence, the toilet paper dose did not increase the inert fraction of the mixed liquor to the extent expected, and thus the concentration of OHO active biomass fraction of the mixed liquor did not decrease as much as expected. Larger doses of toilet paper could not be used, as these led to blockages of pipes between reactors, resulting in reactor overflows.

6.5 EVALUATION OF BATCH TEST PROCEDURE

6.5.1 Batch test procedure

For the batch tests, the procedure developed by Cronje *et al*. (2000) and described in Chapter 5 was followed. As an example the OUR-time and nitrate-time and nitrite-time profiles for one batch test are shown plotted in Figs. 6.3 and 6.4 respectively. From the measured OUR, nitrate and nitrite time data, the following were calculated:

- %COD recovery, using Eq. (4.4).

- Nitrification OUR ($OUR_{N(t)}$), from a regression (both linear and exponential were used, see below) of the nitrate-time concentration profiles (for example, see Fig. 6.4) and using the fitted equation to determine the change in nitrate concentration over a discrete time interval, thereby to give ΔNO_3^- and Δt in Eq. (4.18).

- OHO OUR ($OUR_{H(t)}$), by subtracting the $OUR_{N(t)}$ above from the measured $OUR_{M(t)}$ as in Eq. (4.17b), for example see Fig. 6.5.

- The OHO active biomass concentration at the start of the batch test, from linear regression data of a $\ell nOUR_{H(t)}$ versus time plot (for example see Fig. 6.6), using Eq. (4.12).

- The OHO maximum specific growth rates on SBCOD (K_{MP}) and RBCOD (μ_{HM}) from Eqs. (4.15) and (4.16) respectively.

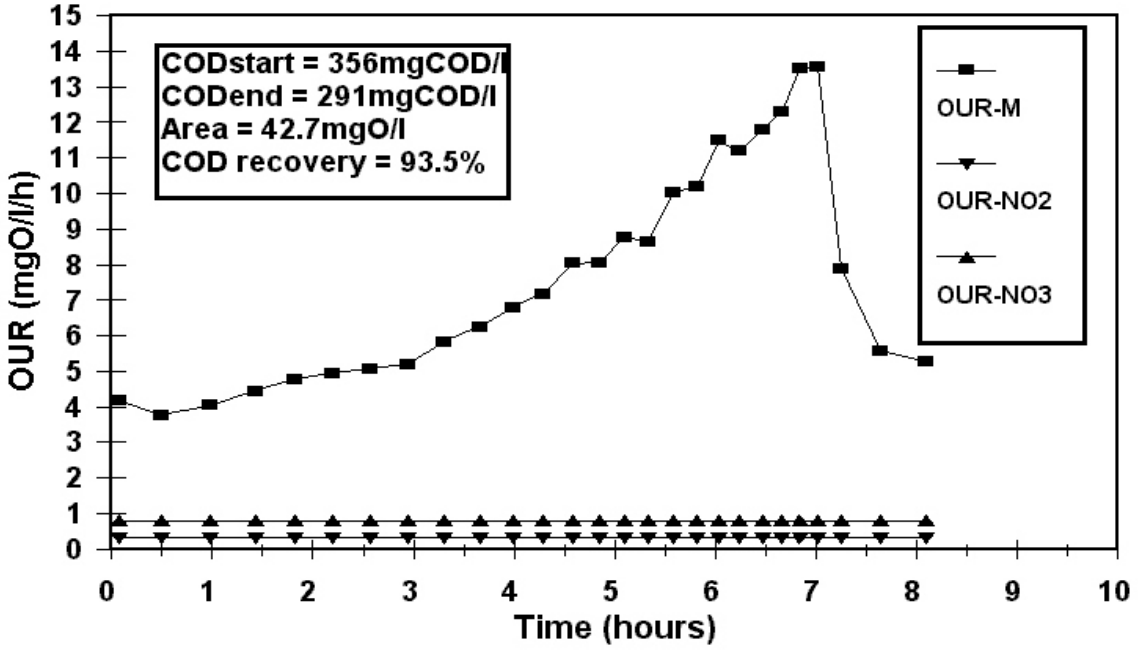

Figure 6.3: Oxygen utilisation rate (OUR) response with time for modified batch test on a mixture of flocculated filtered wastewater (2.85ℓ) and mixed liquor (0.15ℓ) drawn from the aerobic reactor of the experimental anoxic/aerobic parent system (Section 6.4.1).

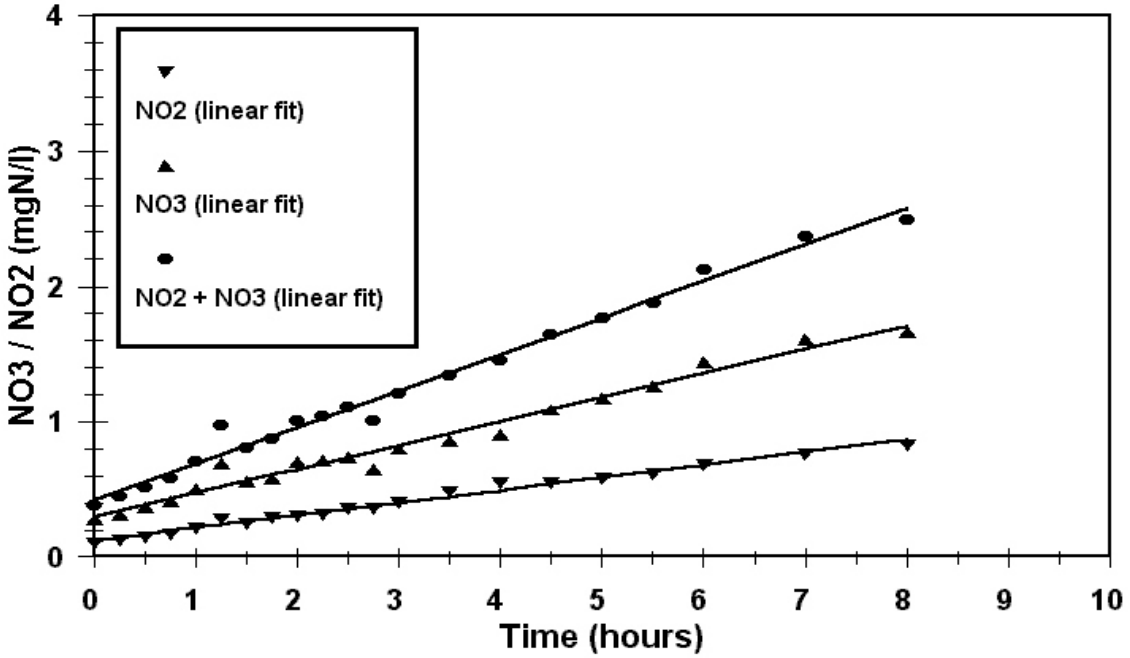

Figure 6.4: Nitrate (NO_3) and nitrite (NO_2) concentrations with time for the modified batch test in Fig. 6.3. Also shown are the linear regression fits to the experimental data. The nitrate and nitrite nitrification OURs are shown in Fig. 6.3.

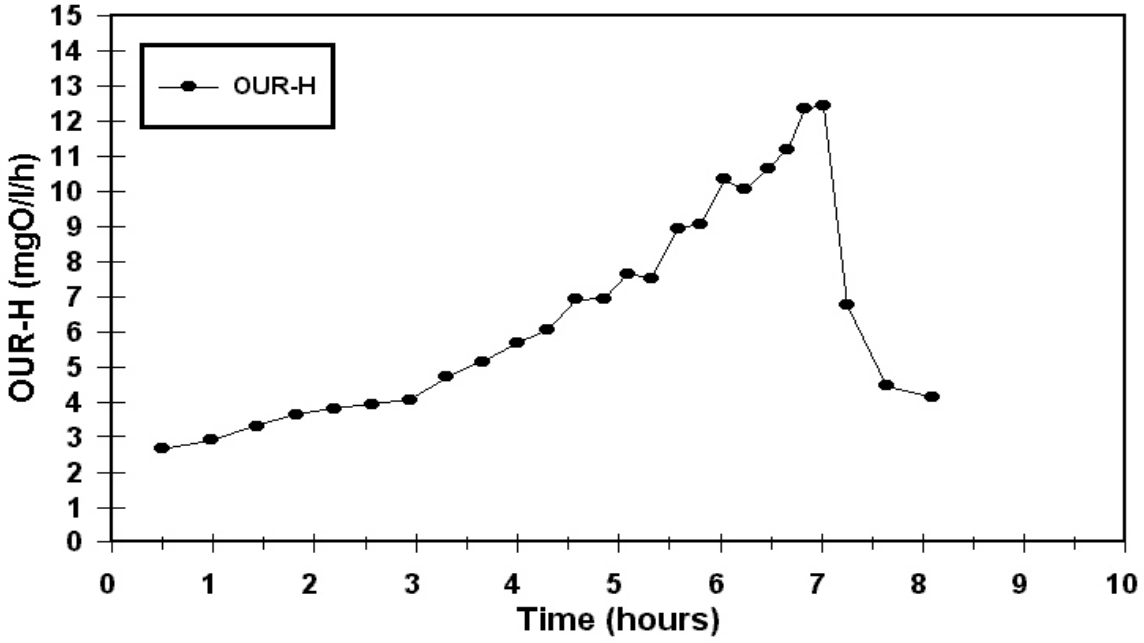

Figure 6.5: Oxygen utilisation rate (OUR) due to OHO active biomass (OUR_H) versus time for modified batch test in Fig. 6.3. The OUR due to nitrate and nitrite nitrification was subtracted from the measured OUR data in Fig. 6.3.

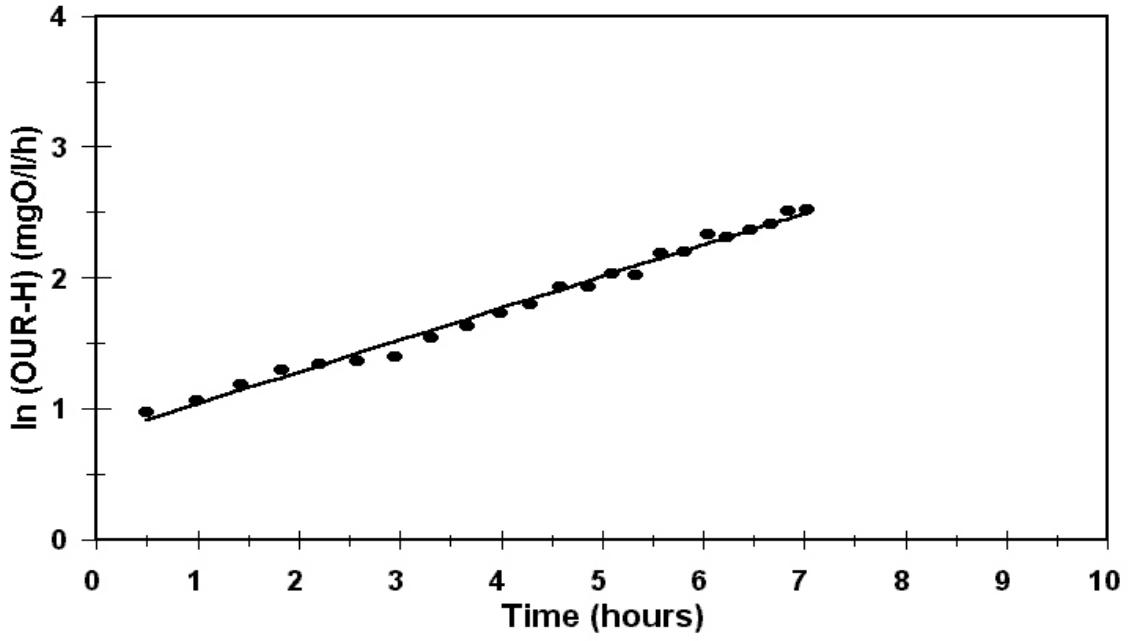

Figure 6.6: ℓn oxygen utilisation rate (OUR) due to OHO active biomass ($\ell nOUR_H$) versus time for the OUR data in Fig. 6.5 up to the precipitous drop in OUR.

6.5.2 Batch tests on control parent system

A total of 18 modified batch tests were conducted on mixed liquor drawn from the ***control*** parent MLE system, see Table 6.7.

Table 6.7: Results from modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML) drawn from the ***control*** parent system: Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass ($Z_{BH(o)}$). Also shown are the measured and theoretical $Z_{BH(o)}$ concentrations in the parent system (PS), taking due account of dilution.

MODIFIED BATCH TESTS: CONTROL PARENT SYSTEM										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_{HM}	Batch test	PS	PS	Batch test
18	1	0.25	2.75	104.3	3.56	1.81	35	415	1036	86
	3	0.30	2.70	86.7[1]	4.71	4.83	7	71	1036	104
	5	0.20	2.80	102.4	2.36	4.33	27	400	1036	69
	7	0.15	2.85	84.1[1]	2.06	4.73	23	453	1036	52
19	9	0.40	2.60	109.6	1.91	1.64	120	900	1083	144
	11	0.35	2.65	105.0	1.55	1.62	92	790	1083	126
	13	0.30	2.70	106.3	1.91	2.44	62	619	1083	108
	15	0.25	2.75	100.6	1.20	2.08	65	782	1083	90
	17	0.20	2.80	102.9	2.29	1.71	57	704	1083	72
	19	0.15	2.85	90.7	0.87	2.87	33	663	1083	54
	21	0.10	2.90	92.7	2.03	4.33	9	264	1083	36
20	23	0.10	2.90	91.7	0.40	1.70	49	1480	1341	45
	25	0.15	2.85	98.0	1.45	3.18	29	576	1341	67
	27	0.20	2.80	92.4	1.55	1.54	62	936	1341	89
	29	0.25	2.75	92.5	ND	ND	138	1651	1341	112
	31	0.30	2.70	101.5	ND	ND	165	1650	1341	134
	33	0.35	2.65	99.3	ND	ND	147	1259	1341	156
	35	0.40	2.60	100.4	ND	ND	166	1247	1341	179

[1]Poor COD mass balance
ND = not determined

From analysis of the batch test results, the following were concluded:

- In interpreting the nitrate and nitrite concentrations with time 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 compared to nitrate concentrations, and hence had to be taken into account in determining OUR_N. This arises because the oxygen requirement to nitrify ammonia-N to nitrite (3.43 mgO/mgN) is lower than that for nitrification of ammonia-N to nitrate (4.57 mgO/mgN).

- In examining the nitrate concentration time profiles, it was found that when the dilution of the later samples was increased to ensure the concentrations fell within the range of the nitrate standards used in the analytical method (Technicon Auto Analyzer Method No. 33.68 and 35.67w), there was a step increase in nitrate concentration. This implied that the sample background matrix (the flocculated-filtered wastewater) caused interference with the analytical method - as the dilution with distilled water increased, the interference diminished. This aspect was investigated further, by making nitrate and nitrite standards in the flocculated-filtered wastewater, at various dilutions. From the results obtained, the *flocculated-filtered* wastewater matrix clearly interfered with the ***nitrate*** measurement. The more the samples were diluted, the less the interference on nitrate determination. The wastewater matrix had a very small impact on ***nitrite*** determination. All batch test nitrate/nitrite data were corrected using appropriate nitrate/nitrite standard curve respectively for the specific dilution; that is, nitrate and nitrite standards were made up in the flocculated filtered wastewater, and these were diluted with distilled de-ionised water in the same ratio as the samples were diluted.

- In the batch tests with wastewater and mixed liquor conducted by Ubisi *et al*. (1997a,b), they observed that nitrification in these batch tests caused a linear increase in the nitrate concentration with time. Cronje *et al*. (2000) (Chapter 5) 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. To select the best type of fit for a particular batch test, this was done by doing both linear and exponential regression analysis and visually checking which of the regression lines best fitted the data. The selected fit was confirmed by the correlation coefficient; a reasonable correlation coefficient ($R^2 > 0.90$) implies that the selected best-fit line gives a good approximation of the experimental data. For the various batch tests, both linear and exponential fits were used. 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 *MLE control* activated sludge system yielded good %COD recoveries, with only 2 out of 18 batch tests (No. 3 and 7) yielding %COD recoveries < 90 %, see Table 6.7. Statistical analysis (Fig. 6.7) indicated that these poor COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected. The mean %COD recovery was 97.8 % with sample standard deviation of 6.9 %. 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}) are shown plotted in Figs. 6.8 and 6.9 respectively; average values are K_{MP} = 1.78 /d (SSD = 0.74) and μ_{HM} = 2.8 /d (SSD = 1.22). These average values are higher than those measured by Cronje *et al*. (2000, Chapter 5) (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. 6.10), it would appear that there is reasonably close correspondence between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor give 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 is zero, the theoretical OHO active biomass concentration in the batch test is approximately 25 mgCOD/ℓ. 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, for some wastewater batches (e.g. WW Batch No. 20, Fig. 6.10) individual data points tend to exhibit some variation from the appropriate correlation line. 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. Even a small change in the slope (magnitude ~ 0.05) determined from the linear regression of the $\ell nOUR_{H(t)}$ - time plot can result in marked variations in the OHO active biomass concentration values. This would suggest that a number of batch tests need to be conducted to establish a reasonable estimate for OHO active biomass concentration.

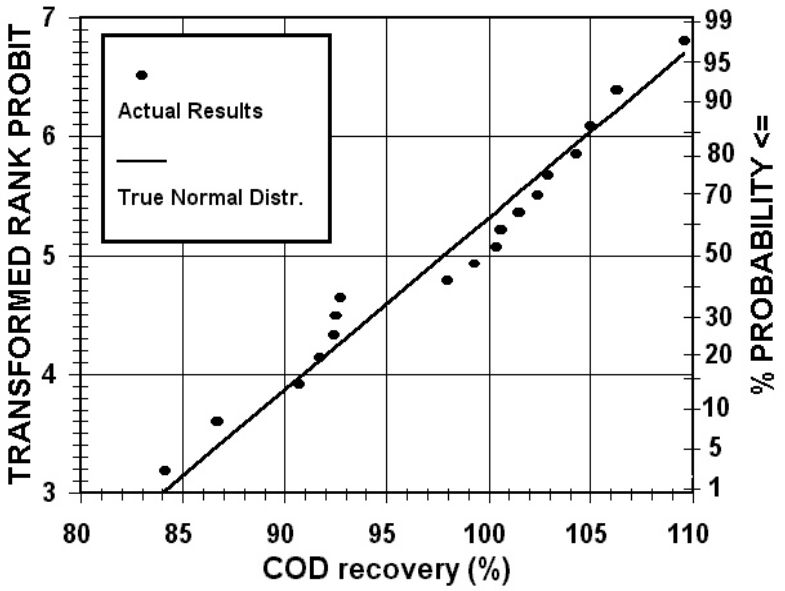

Figure 6.7: Statistical plot of COD recovery (%) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

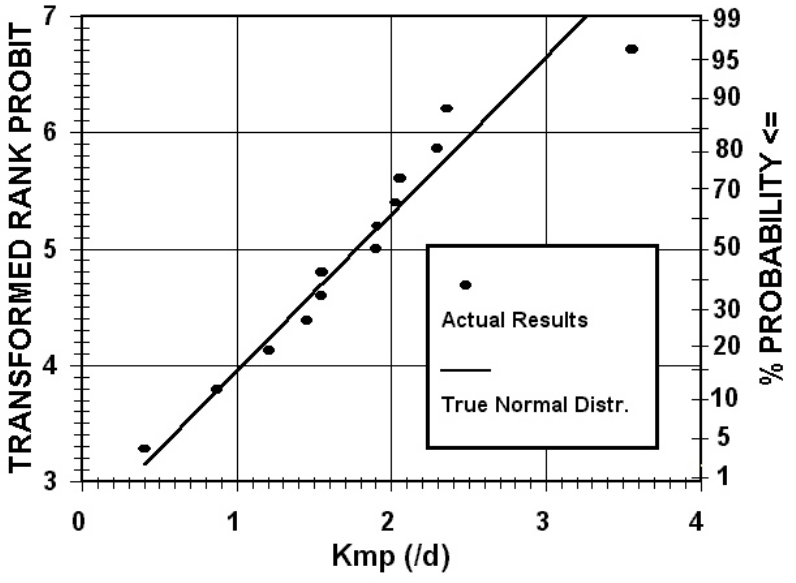

Figure 6.8: Statistical plot of OHO maximum specific growth rate on SBCOD (K_{MP}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

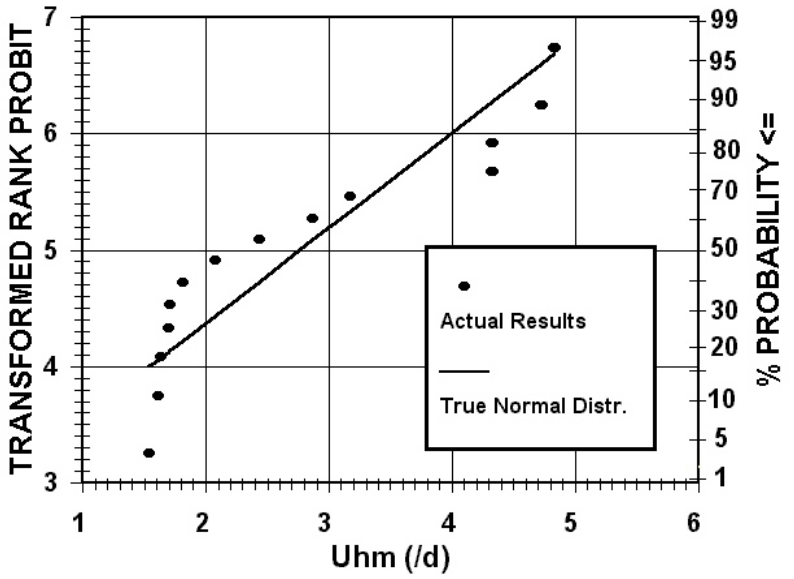

Figure 6.9: Statistical plot of OHO maximum specific growth rate on RBCOD (μ_{HM}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

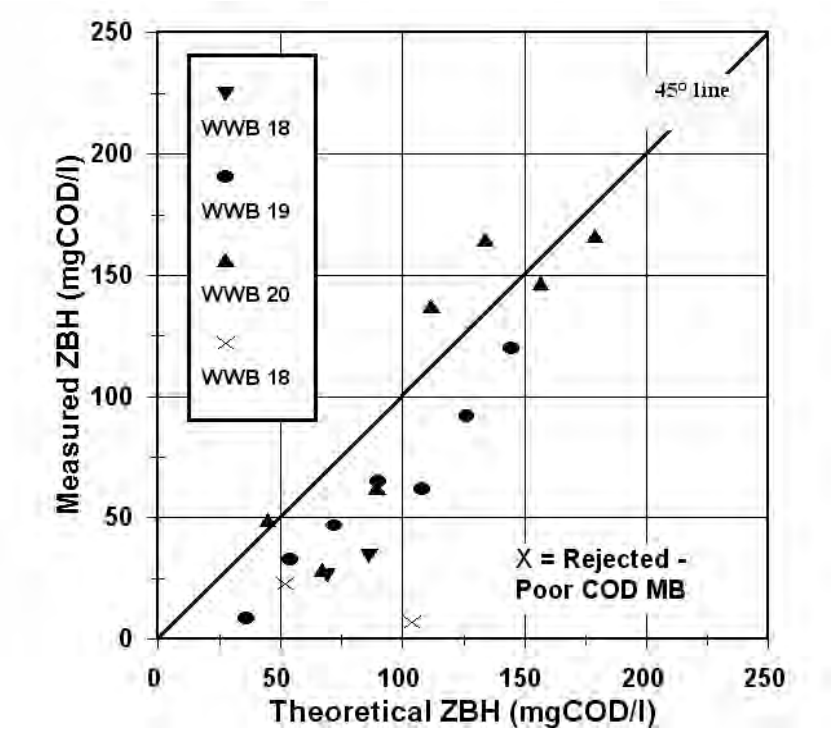

Figure 6.10: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

6.5.3 Batch tests on experimental parent system

To further evaluate the reliability of the modified batch test procedure and its application to anoxic/aerobic activated sludge systems, subjected to decreased OHO active biomass fractions, a total number of 18 modified batch tests were conducted using mixed liquor drawn from the MLE ***experimental*** activated sludge system and were done in parallel to the batch tests on the *control* system, see Table 6.8.

Table 6.8: Results from modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML) drawn from the ***experimental*** parent system: Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass ($Z_{BH(0)}$). Also shown are the measured and theoretical $Z_{BH(0)}$ concentrations in the parent system(PS), taking due account of dilution.

MODIFIED BATCH TESTS: EXPERIMENTAL PARENT SYSTEM										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_{HM}	Batch test	PS	PS	Batch test
18	2	0.25	2.75	105.4	2.51	2.91	27	322	1214	101
	4	0.3	2.7	90.7	1.40	2.67	44	444	1214	121
	6	0.2	2.8	106.0	1.61	3.54	36	545	1214	81
	8	0.15	2.85	93.5	2.24	4.54	16	325	1214	61
19	10	0.4	2.6	98.4	1.74	1.21	158	1183	1153	154
	12	0.35	2.65	106.2	1.74	1.40	100	856	1153	134
	14	0.3	2.7	91.0	1.68	1.89	77	769	1153	115
	16	0.25	2.75	91.1	0.91	2.63	64	770	1153	96
	18	0.2	2.8	97.9	1.92	2.78	36	541	1153	77
	20	0.15	2.85	99.3	1.39	3.15	27	532	1153	58
	22	0.1	2.9	95.9	1.26	3.95	15	463	1153	38
20	24	0.1	2.9	92.2	1.15	2.50	16	480	1650	55
	26	0.15	2.85	93.3	1.71	2.29	38	765	1650	82
	28	0.2	2.8	94.3	1.32	0.98	99	1480	1650	110
	30	0.25	2.75	93.5	1.56	1.20	81	976	1650	137
	32	0.3	2.7	89.0[1]	1.26	0.73	136	1360	1650	165
	34	0.35	2.65	96.8	1.03	5.09	53	455	1650	192
	36	0.4	2.6	92.0	0.37	0.88	326	2448	1650	220

[1]Poor COD mass balance

From analysis of the batch test results, the following were concluded:

- Similarly to the batch tests on the mixed liquor drawn from the control parent system, nitrite measurements needed to be taken into account in calculating $OUR_{H(t)}$, both linear and exponential regression were used to fit the nitrate and nitrite time profiles, and interference in the nitrate and nitrite analysis had to be compensated for.

- In general, good %COD recoveries were achieved with only one batch test (No. 32) yielding %COD recovery < 90 %, see Table 6.8. The %COD recovery for Batch Test No. 32 was marginally < 90 % (89.0 %); however, statistical analysis (Fig. 6.11) indicated that this COD mass balance arose from random effects and accordingly this batch test data was not rejected. The mean %COD recovery was 95.9 % with sample standard deviation 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}) are shown plotted in Figs. 6.12 and 6.13 respectively; average values are 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 from the values measured in the batch tests on mixed liquor drawn from the control parent system. Again, the average values are higher than those measured by Cronje *et al*. (2000, Chapter 5) (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. 6.14), the correlations show remarkable similarity to those obtained for the *control* system – there is a close correlation, but the values plot parallel to the 45º line. Again, this implies that there is a constant difference between measured and theoretical OHO active biomass concentrations; as for the *control* system, this difference is approximately 25 mgCOD/ℓ.

- 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, for some wastewater batches individual data points tend to exhibit some variation from the appropriate correlation line. This variation can be attributed to the sensitivity of the measured OHO active biomass concentration (as explained above).

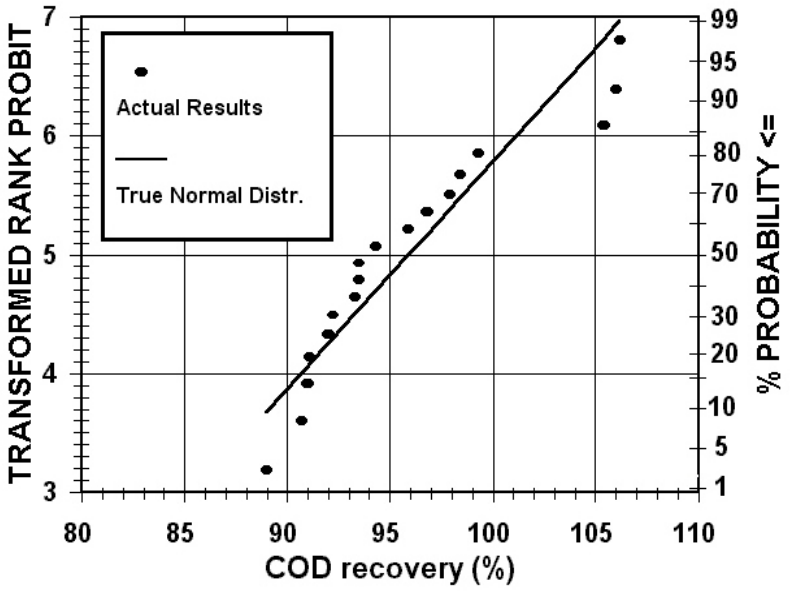

Figure 6.11: Statistical plot of COD recovery (%) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the experimental parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

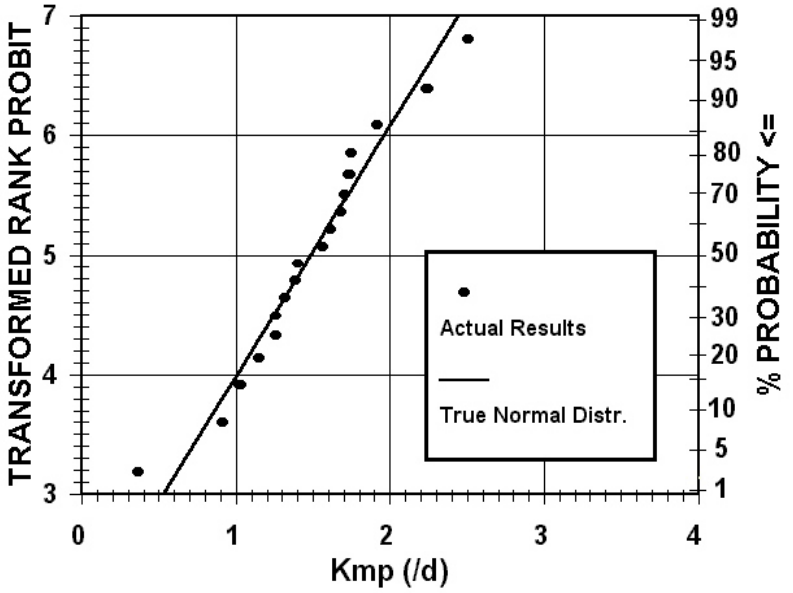

Figure 6.12: Statistical plot of OHO maximum specific growth rate on SBCOD (K_{MP}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the experimental parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

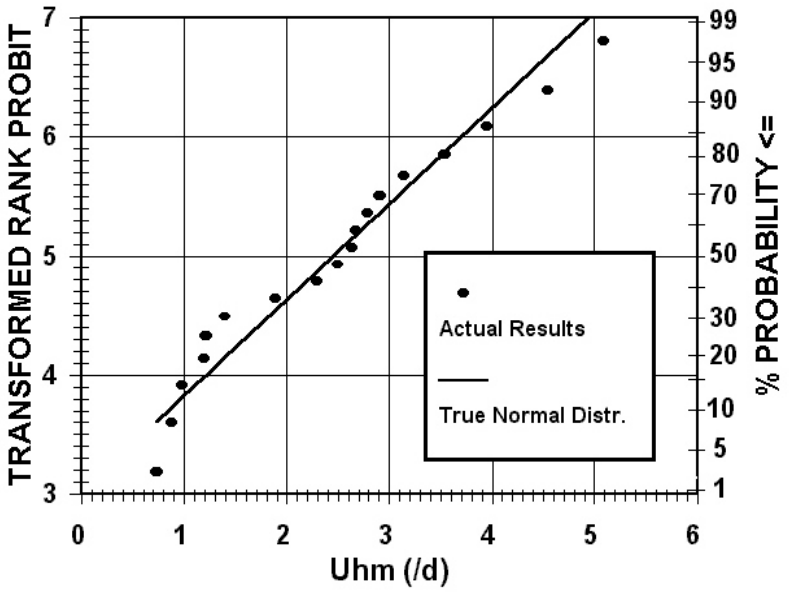

Figure 6.13: Statistical plot of OHO maximum specific growth rate on RBCOD (μ_{HM}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the experimental parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

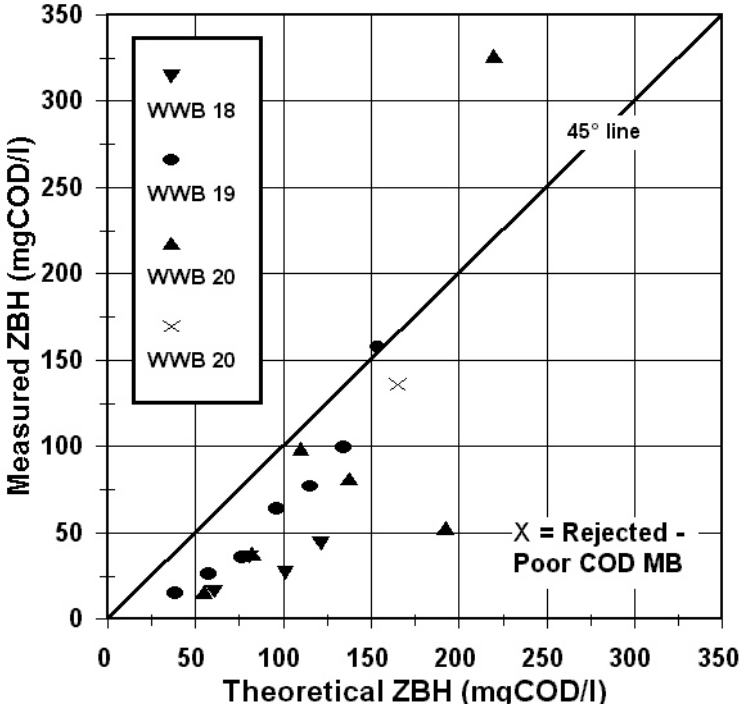

Figure 6.14: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the experimental parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1).

6.5.4 Comparison between OHO active biomass in the control and experimental systems

One of the common tasks was to perform batch tests, identical in procedure to those conducted by Cronje *et al.* (2000, Chapter 5), using mixed liquor samples drawn from both the control and experimental parent laboratory-scale anoxic/aerobic activated sludge systems. From a comparison of the OHO active biomasses between the two systems, it would be possible to evaluate whether the batch test successfully detects any change in OHO active biomass fraction of the mixed liquor in the parent systems. From a comparison of the results, it is apparent that the data for the control and experimental systems are remarkably similar, see Fig. 6.15: Both data sets plot on a line parallel to the 1:1 correspondence (45°) 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.

Thus, the original objectives of this investigation were achieved. However, since the toilet paper proved largely biodegradable, its effect was not as marked as was hoped. Hence, it was decided to increase the dosage of toilet paper to the experimental activated sludge system (to further increase the contribution of inert sludge mass in the system, thereby achieving a larger increase in the MLOSS concentration in the system, and a more significant reduction of the OHO active biomass fraction of the MLOSS). Unfortunately, in practice it proved not possible to operate the laboratory-scale experimental activated sludge system with the higher toilet paper dose; the toilet paper caused frequent blockages of pipes between reactors which caused reactor overflows.

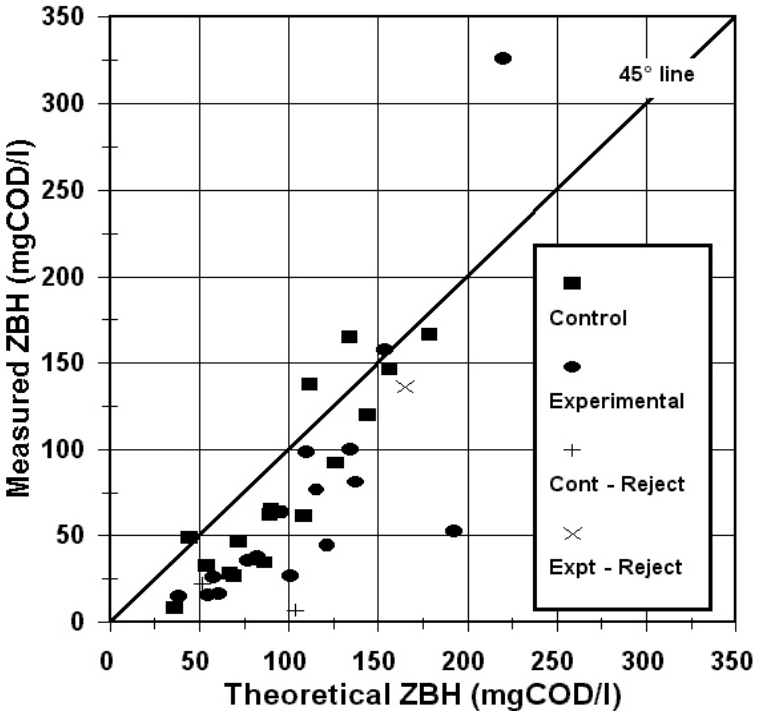

Figure 6.15: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for both control and experimental parent anoxic/aerobic (MLE) activated sludge systems.

6.5.5 Effect of aluminium sulphate on batch test results

The preparation of the wastewater for the modified batch tests incorporated flocculating and filtering the raw wastewater to remove all the particulate material: Aluminium sulphate was selected as flocculent. During the course of the experimental investigation, it was thought that possibly the aluminium sulphate flocculent removed a large fraction of the available phosphorus 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 phosphorus. It was thought that this possibly caused the deviation in correlation between theoretical and measured values from the 1:1 line (Fig. 6.15).

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, phosphorus 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 for WW Batch Nos. 21, 22 and 26; to the one batch test *flocculated-filtered* wastewater plus mixed liquor were added and to the other, *flocculated-filtered* wastewater plus mixed liquor plus 5 mℓ of stock di-potassium hydrogen phosphate (K_2HPO_4, stock at 33.68 g/ℓ) were added per ℓ of wastewater (10 mgP/ℓ *batch reactor*). It must be emphasized that 6 batch tests were conducted during WW Batch Nos. 21 and 22 using mixed liquor drawn from the ***MLE control*** activated sludge system and another 6 batch tests were conducted during Sewage Batch No. 26 using mixed liquor drawn from the ***fully aerobic control*** activated sludge system. Data are shown in Table 6.9.

Table 6.9: Results from modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML)drawn from the ***control*** parent system, with (+ P) and without phosphorus (- P) added: Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass ($Z_{BH(o)}$). Also shown are the measured and theoretical $Z_{BH(o)}$ concentrations in the parent system (PS), taking due account of dilution.

MODIFIED BATCH TESTS: CONTROL PARENT SYSTEM										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_{HM}	Batch test	PS	PS	Batch test
21	37(-P)	0.35	2.65	92.7	2.20	3.22	12	102	1456	170
	38(+P)	0.35	2.65	90.1	1.66	2.39	37	319	1456	170
	39(-P)	0.35	2.65	101.5	1.10	1.08	137	1176	1456	170
	40(+P)	0.35	2.65	102.8	0.57	1.39	169	1446	1456	170
22	41(-P)	0.35	2.65	95.2	1.36	1.01	142	1218	1478	172
	42(+P)	0.35	2.65	95.4	0.98	0.63	225	1929	1478	172
26 Fully aerobic system	61(-P)	0.08	2.92	87.5[1]	1.04	2.62	18	659	2049	55
	62(+P)	0.08	2.92	94.7	1.06	2.53	17	630	2049	55
	63(-P)	0.16	2.84	86.0[1]	0.98	2.47	29	5520	2049	109
	64(+P)	0.16	2.84	97.8	1.32	2.33	29	552	2049	109
	65(-P)	0.24	2.76	85.1[1]	1.75	1.78	61	759	2049	164
	66(+P)	0.24	2.76	97.4	1.54	2.56	40	497	2049	164

[1]Poor COD mass balance

From the data in Table 6.9:

- The N mass balance for the *parent* system for Sewage Batch No. 22 was < 90 % (see Table 6.9). Hence, the batch test results conducted during this sewage batch should be rejected for further analysis, but were included where appropriate, and analysed.

- In general, good %COD recoveries were achieved with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis (Fig. 6.16) indicated that these COD mass balance may have arisen from random effects and accordingly these batch tests data were not rejected. 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 (not shown), 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 system. Again, the average values are higher than those measured by Cronje *et al*. (2000, Chapter 5) (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).

- Measured OHO active biomass with and without P addition versus theoretical values are shown plotted in Fig. 6.17. For WW Batch No. 21, in the two sets of parallel batch tests conducted, the addition of P caused the measured OHO active biomass concentration to increase. For WW Batch No. 22, in the single set of parallel batch tests again addition of P caused a significant increase in the measured OHO active biomass concentration. However, for WW Batch No. 26, either no significant change was observed (2 sets of batch tests), or a slight decrease in OHO active biomass concentration with P addition (1 set of batch tests).

From the results above, it was noted that 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 an increase or decrease in the OHO active biomass concentration. 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. Clearly, this aspect deserves further attention. However, from the results for WW Batch No. 26, it is evident that P limitation was not the cause for the significant deviation between measured and theoretical values observed for the ***fully aerobic*** parent system below.

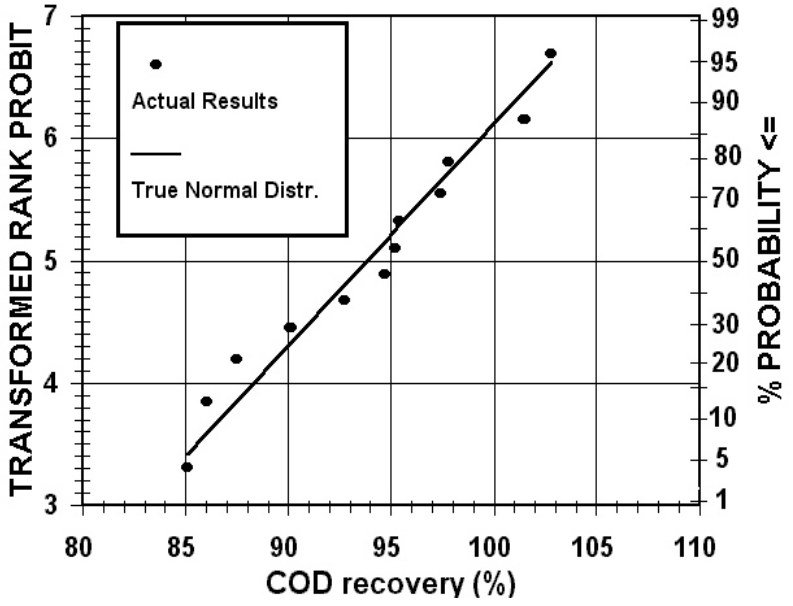

Figure 6.16: Statistical plot of COD recovery (%) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the experimental parent anoxic/aerobic (MLE) activated sludge system (Fig. 6.1), with and without P addition.

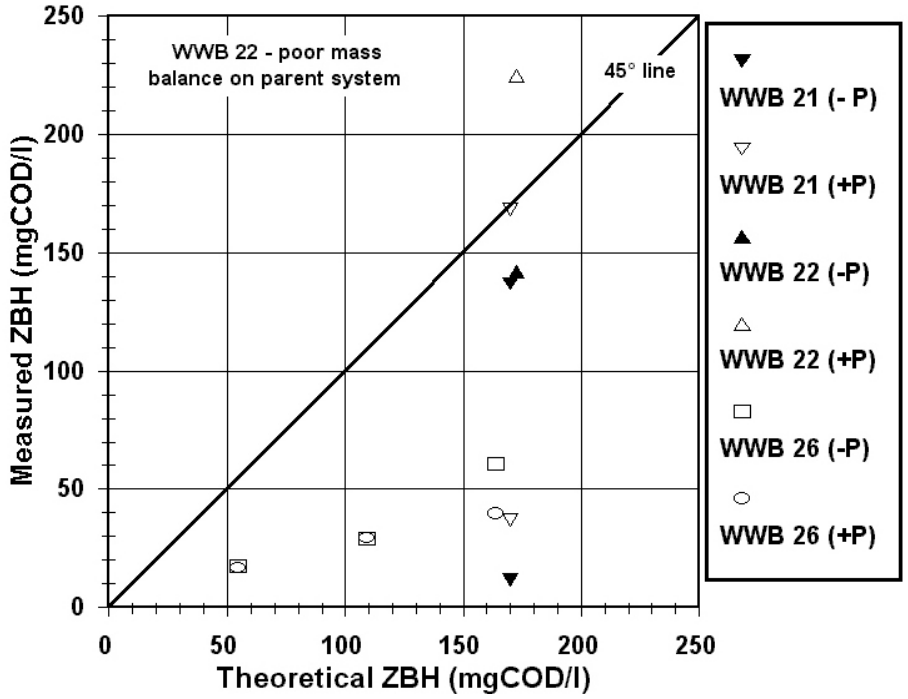

Figure 6.17: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for modified batch tests with (+P) and without (-P) P addition; mixed liquor drawn from both control anoxic/aerobic (MLE, Fig.6.1) and aerobic (Fig. 6.2) activated sludge systems.

6.5.6 Control fully aerobic system

Throughout the experimental investigation, bulking in the parent systems was a continual problem. Whenever bulking manifested itself, a short-term remedy to mitigate its effects was to dose aluminium sulphate to the aerobic reactor of the MLE activated sludge system. However, during the final stages of the experimental investigation, to try to permanently cure bulking, it was decided to modify the ***MLE*** activated sludge system to a ***fully aerobic*** system (single aerobic reactor and secondary settling tank, i.e. the anoxic reactor was removed), see Table 6.1.

A total number of 24 modified batch tests (including 6 batch test where the effect of phosphate addition was monitored, see Section 6.5.5 above) were conducted using mixed liquor drawn from the ***fully aerobic control*** activated sludge system. The wastewater batches during which batch tests were conducted on the fully aerobic control system were WW Batch Nos. 23A, 23B, 24, 25 and 26. WW Batch No. 23 is divided into 23A and 23B, because the system configuration was changed from MLE to fully aerobic in the middle of WW Batch No. 23. Data are shown in Table 6.10.

Table 6.10: Results from modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML) drawn from the ***control*** parent system: Batch test numbers, volumes added, COD recoveries, maximum specific growth rates, measured and theoretical OHO active biomass ($Z_{BH(o)}$). Also shown are the measured and theoretical $Z_{BH(o)}$ concentrations in the parent system (PS), taking due account of dilution.

MODIFIED BATCH TESTS: CONTROL PARENT SYSTEM										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_{HM}	Batch test	PS	PS	Batch test
23A MLE	43	0.08	2.92	95.5	1.16	2.68	44	1667	1479	369
	44	0.32	2.68	95.6	0.62	0.96	234	2192	1479	158
23B aerobic	45	0.16	2.84	90.9	0.78	1.71	61	1137	2016	107
	46	0.24	2.76	94.1	0.88	1.47	88	1105	2016	161
	47	0.12	2.88	93	1.33	4.21	20	491	2016	81
	48	0.28	2.72	94.7	0.80	2.51	58	626	2016	188
24 aerobic	49	0.08	2.92	97.4	0.74	2.97	14	537	1821	49
	50	0.12	2.88	95.9	0.83	2.60	20	496	1821	73
	51	0.16	2.84	92.4	1.45	2.12	22	417	1821	97
	52	0.2	2.8	92.9	1.31	1.95	30	453	1821	121
	53	0.24	2.76	96.6	2.10	2.43	27	343	1821	146
	54	0.28	2.72	101.1	1.84	1.85	44	473	1821	170
25 aerobic	55	0.08	2.92	90.1	1.77	5.43	16	613	2080	55
	56	0.16	2.84	94.9	1.76	4.98	26	485	2080	111
	57	0.24	2.76	95	2.09	2.03	52	650	2080	166
	58	0.28	2.72	94.7	2.13	1.44	74	792	2080	194
	59	0.12	2.88	90.9	1.84	3.34	15	385	2080	83
	60	0.2	2.8	98.4	1.90	2.54	27	398	2080	139
26 aerobic	61	0.08	2.92	87.51	1.04	2.62	18	659	2049	55
	62(+P)	0.08	2.92	94.7	1.02	2.58	17	630	2049	55
	63	0.16	2.84	86.01	0.90	2.50	29	552	2049	109
	64(+P)	0.16	2.84	97.8	1.33	2.31	29	552	2049	109
	65	0.24	2.76	85.11	1.77	1.75	61	759	2049	164
	66(+P)	0.24	2.76	97.4	1.61	2.49	40	497	2049	164

[1]Poor COD mass balance

(+P) indicates P added to batch test; ------ indicates change from MLE to fully aerobic

From Table 6.10:

- The fact that the COD and N mass balances for the parent system (Table 6.3) were good (86 - 105 and 90 - 101% respectively) during these sewage batches lends credibility to the measurements done on the parent system.

- In general, good %COD recoveries were achieved, with only three batch tests (No. 61, 63 and 65) yielding %COD recoveries < 90 %. Statistical analysis (Fig. 6.18) indicated that these low COD mass balances may have arisen from random effects and accordingly these batch tests data were not rejected for further analysis. 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}) are shown plotted in Figs. 6.19 and 6.20 respectively; average values are 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. Again, the average values are higher than those measured by Cronje *et al*. (2000, Chapter 5) (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).

- In Fig. 6.21, the measured OHO active biomass concentrations are compared to the theoretical values. The batch tests done using mixed liquor drawn from the ***MLE*** activated sludge system during WW Batch No. 23A show a reasonable agreement between the measured and the theoretical OHO active biomass concentrations. However, the batch tests performed using mixed liquor drawn from the ***fully aerobic*** activated sludge system during all other wastewater batches show a very poor agreement between the measured and the theoretical OHO active biomass concentrations: There is a close correlation between the theoretical and measured values, but the theoretical values are approximately 3 to 4 times those measured. The fact that the COD and N mass balances both on the parent system and for the batch tests were good during all these wastewater batches lends credibility to the measurements.

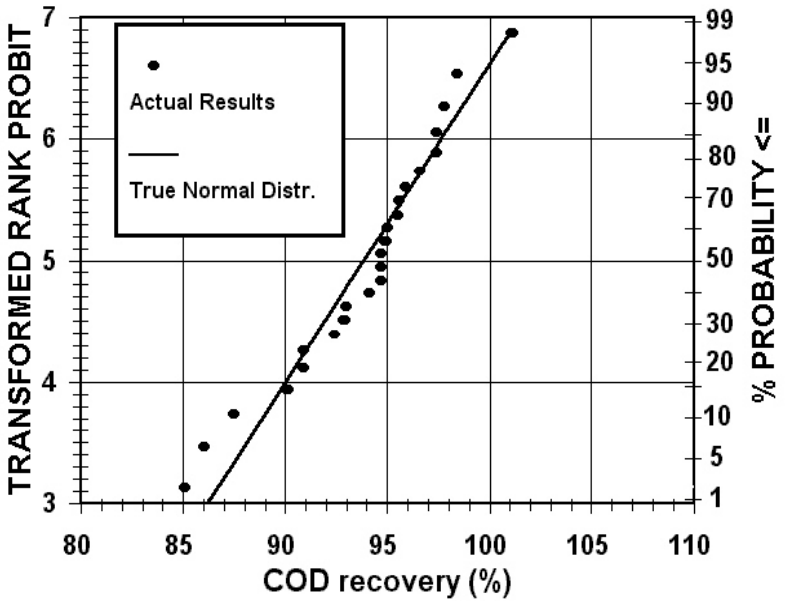

Figure 6.18: Statistical plot of COD recovery (%) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system (Fig. 6.2).

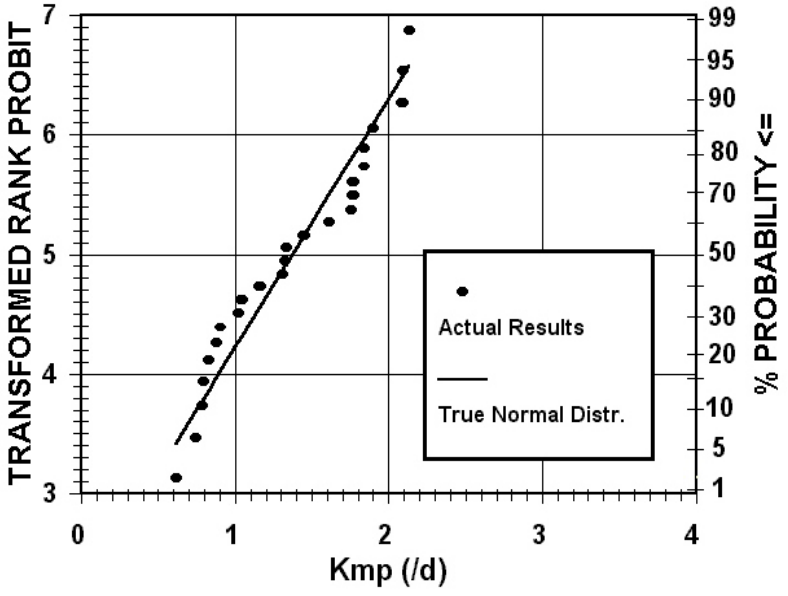

Figure 6.19: Statistical plot of OHO maximum specific growth rate on SBCOD (K_{MP}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system (Fig. 6.2).

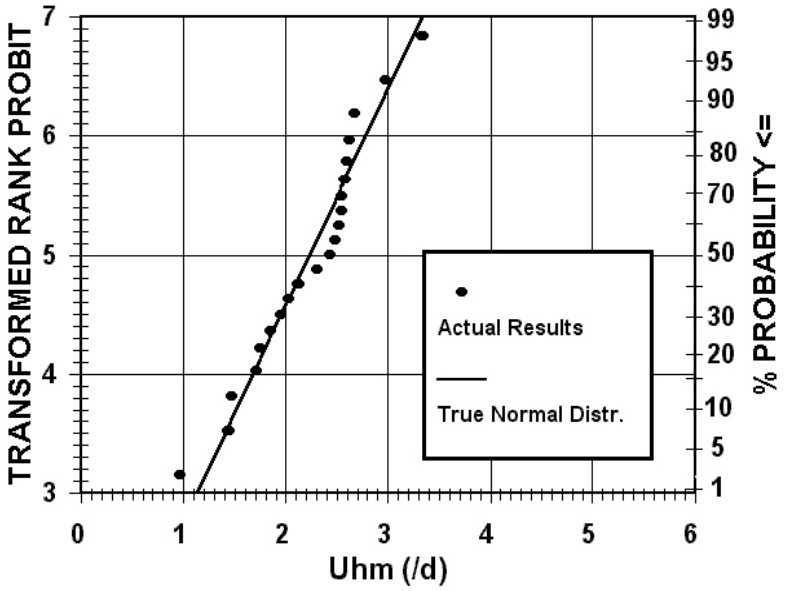

Figure 6.20: Statistical plot of OHO maximum specific growth rate on RBCOD (μ_{HM}) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system (Fig. 6.2).

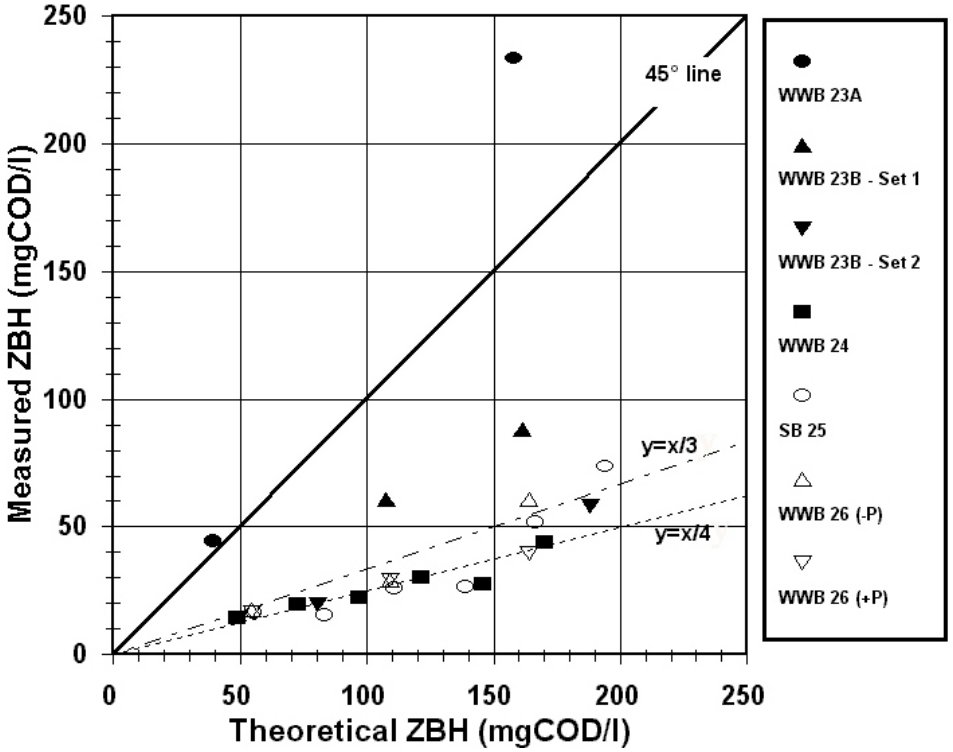

Figure 6.21: Measured versus theoretical OHO active biomass concentration ($Z_{BH(0)}$) for all modified batch tests conducted on a mixture of flocculated-filtered wastewater and mixed liquor drawn from the control parent aerobic activated sludge system (Fig. 6.2).

Comparing the data obtained with the mixed liquor drawn from the fully aerobic system with that from the *MLE anoxic/aerobic* system, 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: For the batch test conducted during WW Batch No. 23A, the system was operated as an ***MLE*** and the batch test data falls 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, 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. 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.

6.6 CLOSURE

In closure, (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.

CHAPTER 7

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

7.1 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). 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 (K5/1191) and the Centre for Water and Wastewater Research at the Technikon Natal (K5/1178). These two research organisations applied the microbiological/biochemical analytical methods (Chapter 2) to 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 to be calculated. Also, the batch test method to quantify OHO active biomass (Chapters 5 and 6) was run in parallel to the microbiological/biochemical methods. This allowed the engineering and technology defined theoretical and measured OHO active biomass to be compared to the microbiological/biochemical quantified OHO active biomass. This Chapter describes these comparisons.

7.2 UNIVERSITY OF PRETORIA

The University of Pretoria (UP) research group measured ATP both *in situ* in the laboratory-scale activated sludge systems, and during the course of the modified batch tests as described in Chapters 5 and 6. ATP was selected as a measurement parameter as it is a non-conservative constituent of all living cells, and sensitive analytical methods are available to quantify it (Cloete and Thantsha, 2002, see Chapter 2).

7.2.1 Laboratory-scale activated sludge systems

Two laboratory-scale activated sludge systems were operated in parallel, one system at sludge age of 10d and the other at 20d. System configurations and operating parameters are summarised in Fig. 7.1. System operation was as described in Chapter 6, Section 6.4. System behaviour and performance are summarised in Table 7.1.

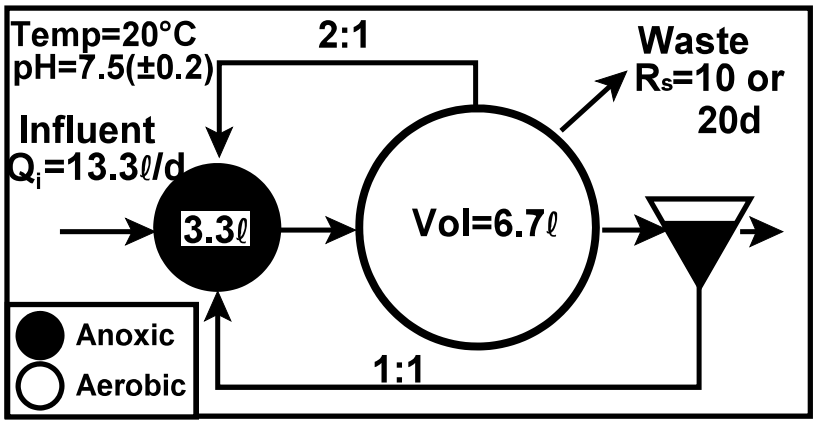

Figure 7.1: Schematic layout and operational data for parent laboratory-scale systems.

Table 7.1: Steady state results for parent laboratory-scale anoxic/aerobic (Fig. 7.1) activated sludge systems operated at 10 and 20d sludge age. For the wastewater batch during which the ATP analysis and parallel batch tests were done, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

ANOXIC/AEROBIC STEADY STATE SYSTEMS (10 AND 20d SLUDGE AGE)

Sludge Age (d)	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite[1] (mgN/ℓ)			Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			DSVI (mℓ/g)
		Inf	Eff	Inf	Eff	Anoxic	Aerobic	Eff		VSS	COD	TKN	
10	7	757 (15)	27 (7)	62 (2)	3.4 (0.3)	3.2 (3.7)	14.5 (3.6)	14.6 (3.1)	32.1 (1.5)	2784 (115)	3923 (96)	222 (8)	117 (3)
20	7	757 (15)	27 (10)	62 (2)	3.0 (0.6)	1.4 (1.4)	12.7 (2.0)	? (?)	38.0 (1.5)	4209 (190)	5703 (235)	314 (9)	105 (4)

[1]From measurements, nitrite concentrations were found to be low.

Following the procedures set out in Chapter 4, from the averaged data in Table 7.1, 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 Chapter 4 - in Chapter 5 it was shown 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 Chapter 4.
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

These values are listed in Table 7.2.

Table 7.2: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale anoxic/aerobic (Fig. 7.1) activated sludge systems, for the wastewater (WW) batch during which samples were drawn for ATP analysis and batch tests. Values calculated from data in Table 7.1, either directly or using the steady state (SS) design model (WRC, 1984).

ANOXIC/AEROBIC STEADY STATE SYSTEMS (10 AND 20d SLUDGE AGE)								
Sludge Age (d)	No of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbiod. Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)	COD/VSS ratio (mgCOD/mgVSS) (f_{cv})	TKN/VSS ratio (mgN/mgVSS) (f_n)	Active Fraction (f_{av})
10	7	77	104	0.036	0.171	1.41	0.081	0.3795
20	7	74	?	0.036	0.105	1.36	0.075	0.2732

Accepting the parameters in Tables 7.1 and 7.2, the theoretical OHO active biomass concentrations in the parent laboratory-scale systems were calculated with the steady state theory (WRC, 1984) to be 1 489 and 1 558 mgCOD/ℓ for the 10 and 20d sludge age systems respectively.

7.2.2 Batch tests

The batch tests were done on mixed liquor drawn from the parent systems, one on mixed liquor from the 10d sludge age system and one on mixed liquor from the 20d sludge age system. In the batch tests, 300mℓ mixed liquor was mixed with 2 700mℓ flocculated filtered wastewater, and the method described in Chapters 5 and 6 followed. For the mixed liquor drawn from the system at 10d sludge age, measured OUR with time and nitrate and nitrite concentration time profiles are shown in Figs 7.2 and 7.3 respectively. Similarly for the mixed liquor drawn from the system at 20d sludge age, in Figs. 7.4 and 7.5 respectively. From the measured OUR, nitrate and nitrite time data, the following were calculated (see Chapter 6):

- %COD recovery, using Eq. (4.4).

- 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 (for the 10d sludge age mixed liquor these were exponential, while for the 20d sludge age mixed liquor these were linear, see Figs. 7.3 and 7.5 respectively) and using the differential of the fitted equation to determine the rate of nitrification, and hence the associated OUR, as 4.57 and 3.43 times the nitrification rate for nitrate and nitrite nitrification respectively, see Figs. 7.2 and 7.4 respectively.

- OHO OUR ($OUR_{H(t)}$), by subtracting the $OUR_{N(t)}$ (for both nitrate and nitrite nitrification) above from the measured $OUR_{M(t)}$ as in Eq. (4.17b), see Figs. 7.2 and 7.4.

- 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 (see Figs. 7.6 and 7.7), using Eq (4.12).

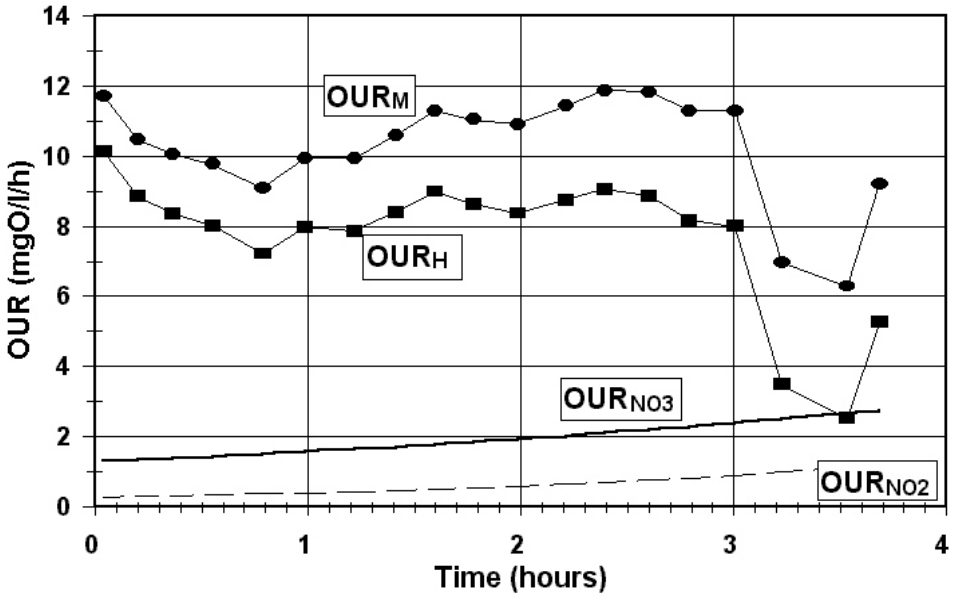

Figure 7.2: Measured oxygen utilisation rate (OUR_M), OUR for nitrate and nitrite nitrification (OUR_{NO3} and OUR_{NO2} respectively) and OHO OUR (OUR_H) for batch test on mixture of mixed liquor drawn from parent activated sludge system at 10d sludge age (300mℓ) and flocculated filtered wastewater (2 700mℓ) from Mitchells Plain Wastewater Treatment Plant (Cape Town, South Africa).

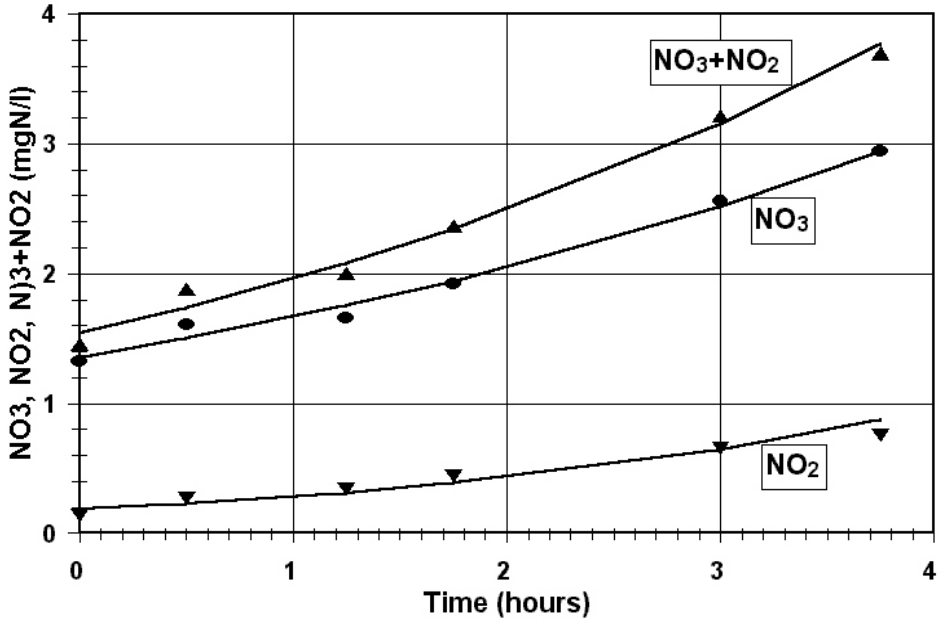

Figure 7.3: Measured and regression fitted nitrate (NO_3) and nitrite (NO_2) concentrations with time for batch test in Fig. 7.2 above.

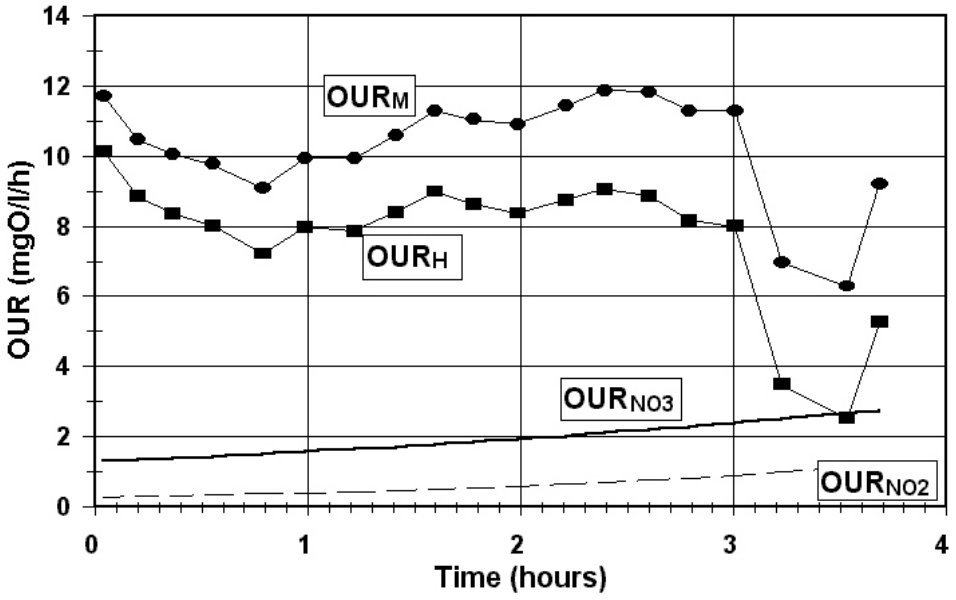

Figure 7.4: Measured oxygen utilisation rate (OUR_M), OUR for nitrate and nitrite nitrification (OUR_{NO3} and OUR_{NO2} respectively) and OHO OUR (OUR_H) for batch test on mixture of mixed liquor drawn from parent activated sludge system at 20d sludge age (300mℓ) and flocculated filtered wastewater (2 700mℓ) from Mitchells Plain Wastewater Treatment Plant (Cape Town, South Africa).

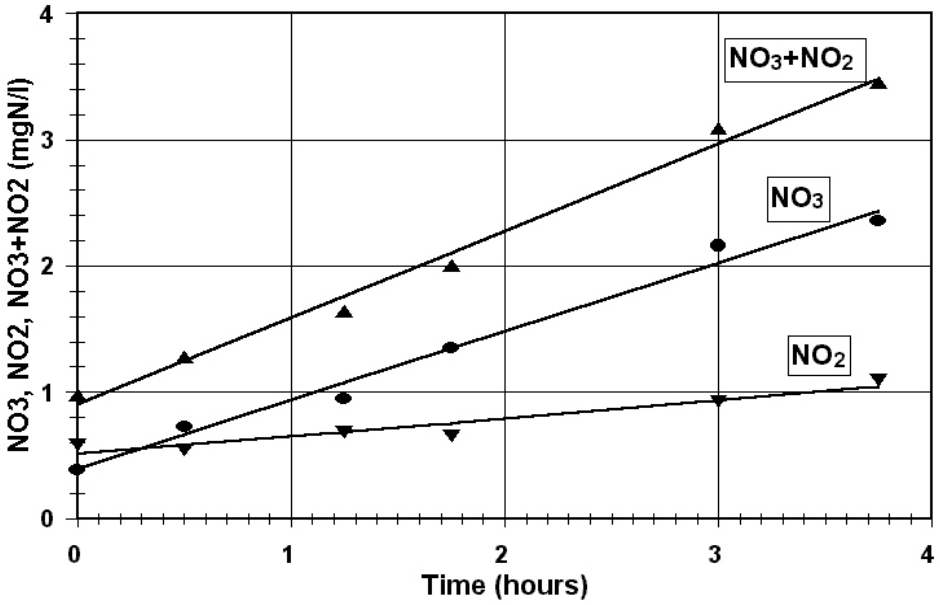

Figure 7.5: Measured and regression fitted nitrate (NO_3) and nitrite (NO_2) concentrations with time for batch test in Fig. 7.4 above.

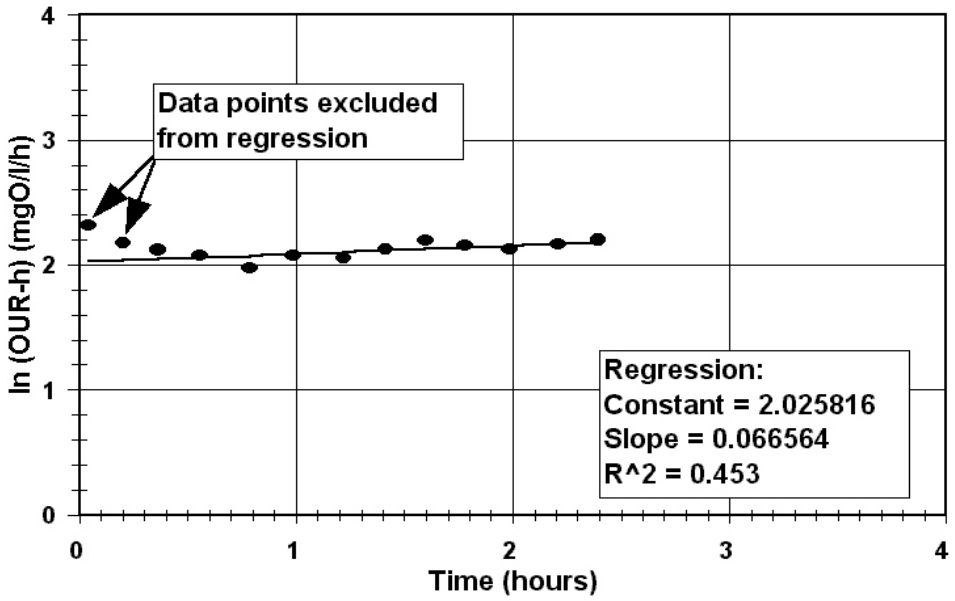

Figure 7.6: ℓn oxygen utilisation rate due to OHO active biomass (OUR_H) versus time for the OUR data in Fig. 7.2, up to the precipitous drop in OUR.

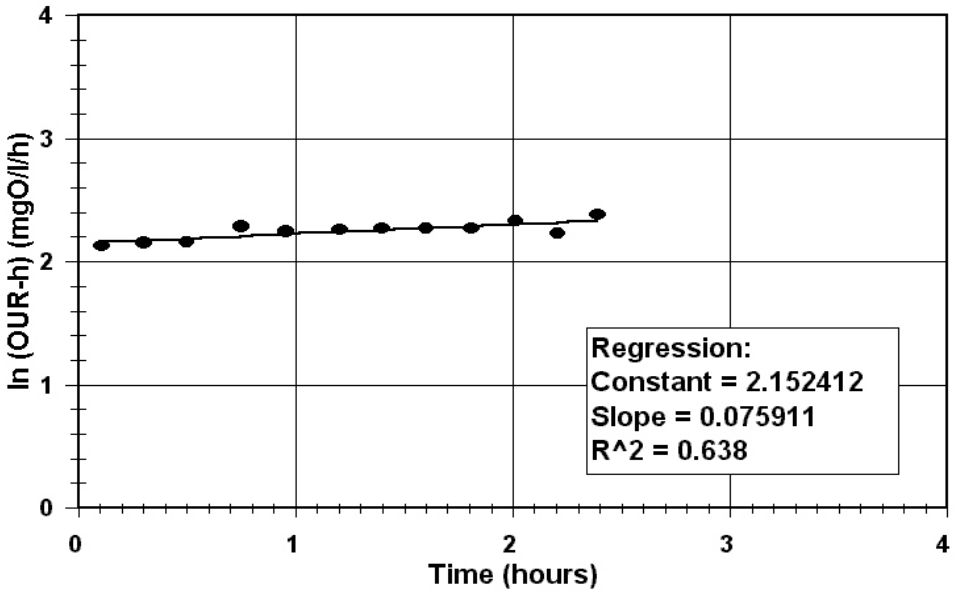

Figure 7.6: ℓn oxygen utilisation rate due to OHO active biomass (OUR_H) versus time for the OUR data in Fig. 7.4, up to the precipitous drop in OUR.

Following the procedure above, the OHO active biomass concentrations in the batch tests were determined to be 164 and 169 mgCOD/ℓ batch reactor for the 10d and 20d sludge age mixed liquors respectively. Taking into account the dilution in the batch test (300mℓ mixed liquor into 3ℓ), this gives 1 636 and 1 687 mgCOD/ℓ respectively in the steady state systems.

7.2.3 ATP measurements

ATP was measured on samples drawn both directly from the two steady state systems, and from the two batch tests above. Samples were sonicated for 5 min using an ultrasonic homogeniser (Cole-Parmer) at 50% output, and thereafter ATP measured with a ATP Bioprobe (Hughes Whitlock), for details see Cloete and Thantsha (2002).

7.2.3.1 Steady state systems

Samples were drawn from the aerobic reactors of the two steady state systems above, hourly for a period of three hours. Readings on each sample were done in triplicate. The ATP concentrations were converted to cell counts, by accepting a constant cell ATP content of 1 phentogram of ATP/cell. Results are listed in Table 7.3.

Table 7.3: Cell concentrations determined from ATP readings on samples drawn from the aerobic reactors of the two steady state activated sludge systems (Fig. 7.1 and Table 7.1); samples drawn hourly for three hours and ATP readings done in triplicate.

Sample	Reading	ATP Value (cells/mℓ)	
		10d Sludge Age	20d Sludge Age
1	1	28525	14517
	2	29308	18281
	3	26024	10841
	Ave	27952	14546
2	1	37564	12880
	2	28017	15154
	3	32625	10985
	Ave	32735	13006
3	1	36010	14004
	2	33711	13444
	3	28887	13671
	Ave	32869	13706
Overall Average		31186	13753
Sample Standard Deviation		3953	2239

Taking the overall average cell count, and accepting a VSS/cell of 8.51×10^{-11} mgVSS/cell (Mudaly, pers. comment), then the ATP determined VSS concentrations of OHO active biomass for the steady state systems are 0.002654 and 0.001170 mgVSS/ℓ for the 10 and 20 d sludge age systems respectively. These, with the measured mixed liquor COD/VSS ratio (Table 7.1) give 0.00374 and 0.001595 mgCOD OHO active biomass/ℓ respectively.

7.2.3.2 Batch tests

Parallel to the OUR and nitrate and nitrite concentration measurements on the batch tests above, samples were drawn from the batch test immediately after the flocculated filtered wastewater was added to the mixed liquor in the batch reactors, and thereafter at regular intervals. Samples were analysed for ATP as described above. The ATP concentrations were converted to cell counts, by accepting a constant cell ATP content of 1 phentogram of ATP/cell. Results are listed in Table 7.4 for mixed liquors drawn from the steady state systems at 10 and 20d sludge ages. The ATP cell counts were converted to OHO active biomass concentrations in units mgVSS/ℓ and mgCOD/ℓ as described above for the steady state systems; these data are also listed in Table 7.4.

Table 7.4: ATP cell counts, and derived OHO active biomass concentrations with time in the two batch tests on mixed liquor drawn from the 10 and 20d sludge age steady state systems, as described above.

Time (hours)	10d Sludge Age			20d Sludge Age		
	ATP (cells/mℓ)	OHO Active Biomass Concs.		ATP (cells/mℓ)	OHO Active Biomass Concs.	
		VSS (mgVSS/ℓ)	COD (mgCOD/ℓ)		VSS (mgVSS/ℓ)	COD (mgCOD/ℓ)
0	830335	0.070662	0.099571	523262	0.04453	0.060667
0.5	1039764	0.088484	0.124685	538038	0.045787	0.062380
1.0	1196646	0.101835	0.143497	509671	0.043373	0.059091
2.0	1270674	0.108134	0.152375	513561	0.043704	0.059542
3.0	2128712	0.181153	0.255268	576620	0.049070	0.066853
4.0	591469	0.050334	0.070927	437868	0.037263	0.050766

7.2.4 Comparison between ATP, batch test and theoretical OHO active biomass data

7.2.4.1 Steady state systems

The OHO active biomass concentrations in the two steady state systems derived from the ATP and batch test measurements are listed in Table 7.5, together with the theoretically calculated values; the measured versus theoretical values are also shown plotted in Fig. 7.8.

From Table 7.5 and Fig. 7.8, the batch test measured values agree closely with the theoretical values. However, the ATP derived values are between 5 and 6 orders of magnitude smaller, possibly due to the solids concentrations interfering in the ATP test procedure, see below.

Table 7.5: OHO active biomass concentrations in the two steady state systems (Fig. 7.1), derived from the ATP and batch test measurements, and from steady state theory (WRC, 1984).

Sludge Age (d)	OHO Active Biomass Concentration (mgCOD/ℓ)		
	ATP	Batch Test	Theoretical
10	0.00374	1 636	1 489
20	0.00160	1 687	1 558

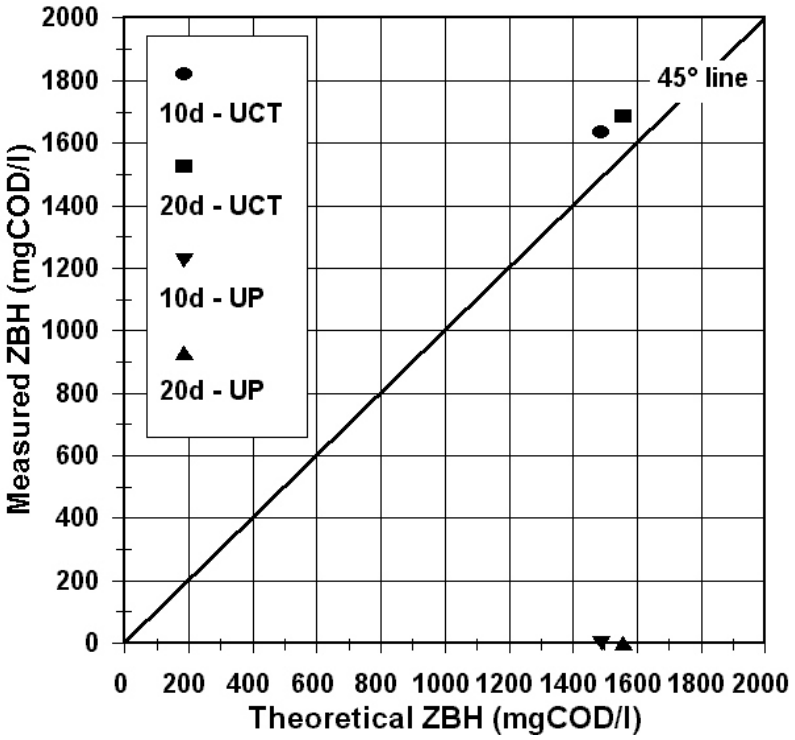

Figure 7.8: Measured versus theoretical OHO active biomass concentrations in the 10 and 20d sludge age parent laboratory-scale activated sludge systems (Fig. 7.1); measured values from batch test method (UCT) and ATP measurements (UP).

7.2.4.2 Batch tests

For the two batch tests, the measured OUR and the OUR due to OHOs only are shown plotted with time in Figs. 7.9 and 7.10 for the 10 and 20d sludge age mixed liquors respectively. Also shown plotted with time are the ATP derived cell counts. The OHO OURs and ATP derived cell counts appear to follow similar trends - both show increase with time during OHO growth with readily biodegradable COD. Using the procedures above, the ATP cell counts were converted to OHO active biomass concentrations. Also, from the batch test determined initial OHO active biomass concentration, and the slope of the ℓnOUR-time plot, the OHO active biomass concentration with time in the batch test was calculated with Eq. (4.7). OHO active biomass concentrations with time in the batch test calculated with both methods are shown plotted in Figs. 7.11 and 7.12 for the 10 and 20d sludge age mixed liquors respectively. Although both methods to determine OHO active biomass show small increase with time in the batch tests, the ATP derived concentrations are approximately 2 000 to 4 000 times smaller than the batch test derived values.

7.2.5 Closure

The OHO active biomass concentrations derived from the batch test method are in reasonable agreement with the theoretical values. In contrast, the OHO active biomass concentrations derived from the ATP measurements are 3 and 5 to 6 orders of magnitude smaller than the theoretical and batch test measured values for the batch test and steady state systems respectively. Further, the ATP derived values are higher in the batch test than in the steady state system, despite the mixed liquor samples drawn from the steady state systems being diluted in the batch test with flocculated filtered wastewater (that exhibits no biological activity, see Chapter 5 and 6). This would suggest that the ATP method as applied is not a reliable estimate for OHO active biomass concentrations. It is possible that the high VSS concentrations in the batch and steady state systems interfered in some manner with the ATP measurement - 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.

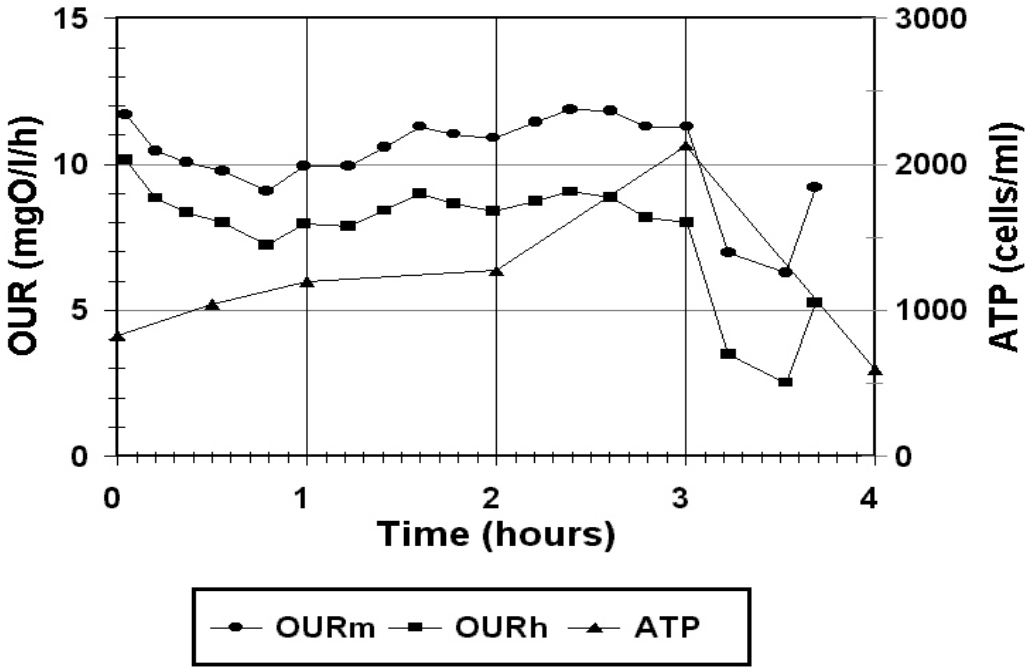

Figure 7.9: Measured oxygen utilisation rate (OUR_M), OUR due to OHO activity (OUR_h) and ATP cell counts with time in aerobic batch test on mixture of mixed liquor drawn from parent laboratory-scale activated sludge system at 10d sludge age (Fig. 7.1) and flocculated filtered wastewater.

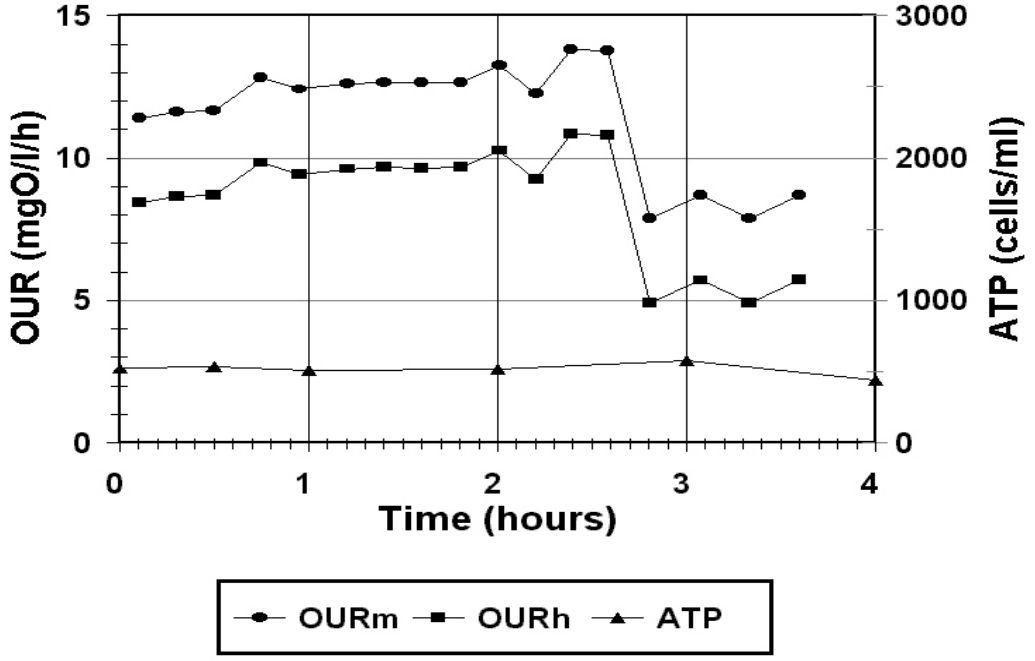

Figure 7.10: Measured oxygen utilisation rate (OUR_M), OUR due to OHO activity (OUR_h) and ATP cell counts with time in aerobic batch test on mixture of mixed liquor drawn from parent laboratory-scale activated sludge system at 20d sludge age (Fig. 7.1) and flocculated filtered wastewater.

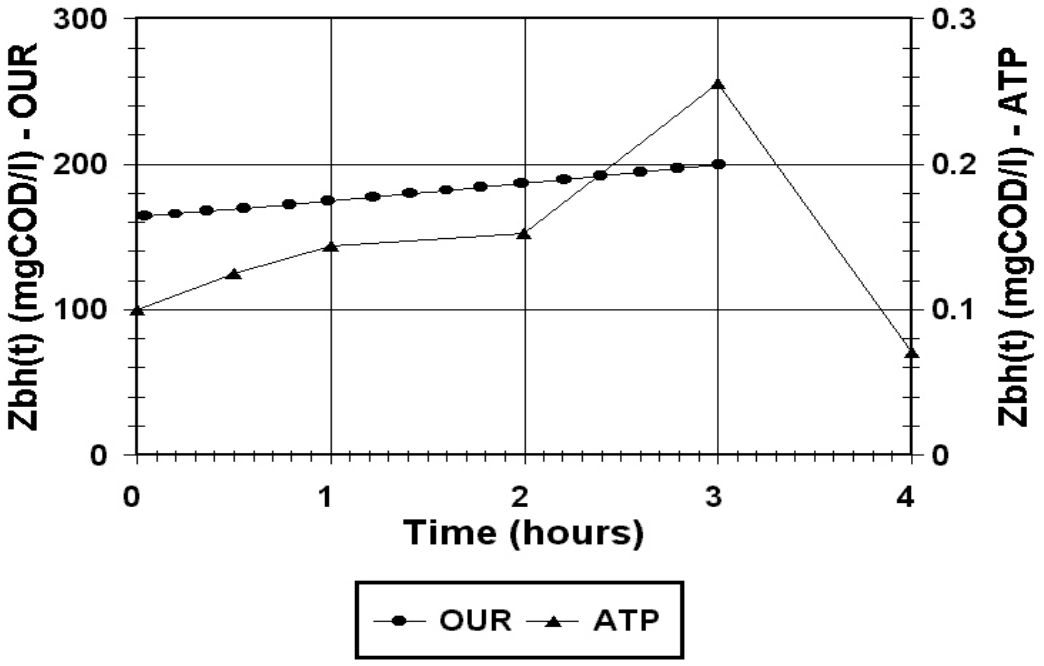

Figure 7.11: OHO active biomass concentrations ($Z_{BH(t)}$) with time in aerobic batch test on mixture of mixed liquor drawn from parent laboratory-scale activated sludge system at 10d sludge age (Fig. 7.1) and flocculated filtered wastewater; OHO active biomass concentrations determined from ATP and OUR measurements.

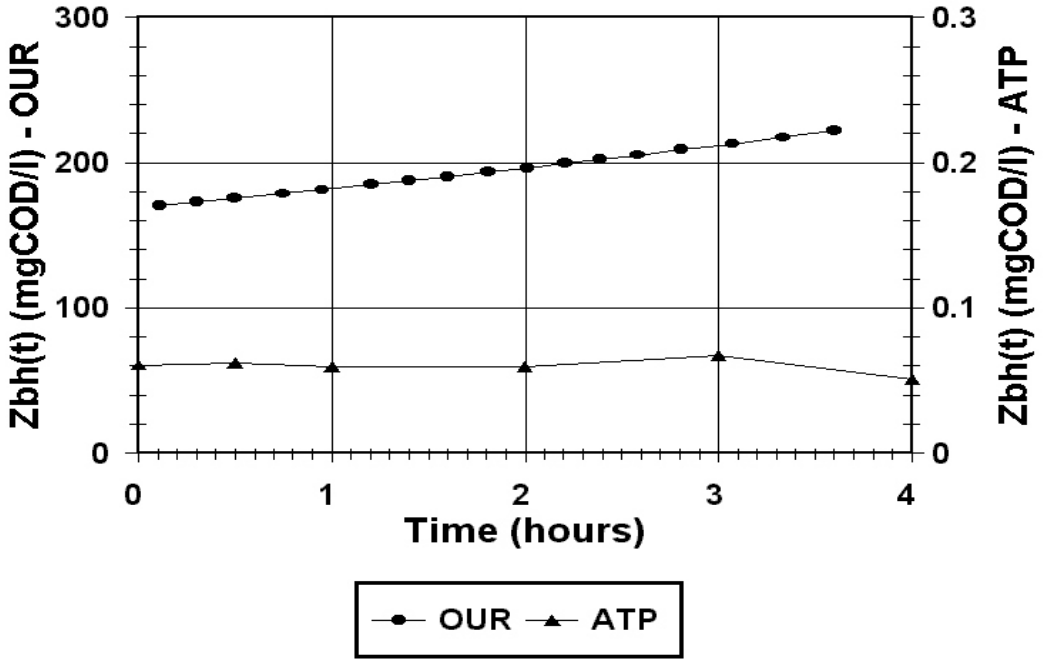

Figure 7.12: OHO active biomass concentrations ($Z_{BH(t)}$) with time in aerobic batch test on mixture of mixed liquor drawn from parent laboratory-scale activated sludge system at 20d sludge age (Fig. 7.1) and flocculated filtered wastewater; OHO active biomass concentrations determined from ATP and OUR measurements.

7.3 TECHNIKON NATAL

The Centre for Water and Wastewater Research at the Technikon Natal used a combination of DAPI staining and Flourescent *in situ* Hybridisation (FISH) to determine both OHO and autotroph active biomass concentrations in samples drawn from the laboratory-scale activated sludge systems operated at UCT. These techniques were selected as a number of studies have shown them to have potential for quantitative measurement of active bacteria (see Chapter 2). In parallel, the modified batch test method described in Chapter 6 was applied to samples drawn from the parent system.

7.3.1 Laboratory-scale activated sludge systems

The laboratory-scale system operated when samples were drawn for DAPI and FISH analysis was the completely aerobic activated sludge system described in Chapter 6, Section 6.4. The system configuration and operating parameters are summarised in Fig. 7.13. System behaviour and performance are summarised in Table 7.3.

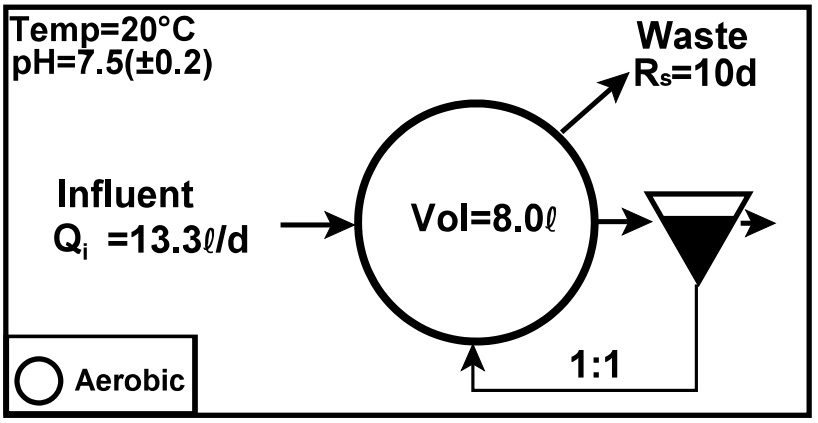

Figure 7.13: Schematic layout and operational data for control aerobic parent laboratory-scale system of Beeharry *et al*. (2001).

Table 7.3: Steady state results for parent laboratory-scale aerobic activated sludge system (Fig. 7.13). For each of the wastewater (WW) batches tested when samples were drawn for DAPI an FISH analysis and parallel batch tests, the daily results have been averaged and the averages are listed with sample standard deviations in brackets.

AEROBIC STEADY STATE SYSTEM												
WW Batch	No of tests	COD (mg/ℓ)		TKN (mg/ℓ)		Nitrate+Nitrite[1] (mgN/ℓ)		Aerobic OUR (mgO/ℓ/h)	Mixed liquor (mg/ℓ)			DSVI (mℓ/g)
		Inf	Eff	Inf	Eff	Aerobic	Eff		VSS	COD	TKN	
24	6	798	52	57	7.1	28.8	30.0	43.0	3625	5083	258	145
		(14)	(19)	(6)	(1.6)	(3.9)	(6.0)	(1.9)	(160)	(114)	(21)	(4)
25	5	815	54	104	4.3	69.3	73.1	43.7	3726	5313	277	108
		(22)	(11)	(2)	(0.4)	(3.0)	(1.6)	(0.8)	(128)	(57)	(7)	(12)
26	5	787	31	72	4.5	50.0	52.6	45.9	3526	5112	250	85
		(26)	(11)	(5)	(0.5)	(1.0)	(0.3)	(1.5)	(72)	(156)	(6)	(8)

[1]From measurements, nitrite concentrations were found to be negligible.

Following the procedures set out in Chapter 4, from the averaged data in Table 7.3, 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 Chapter 4 - in Chapter 5 it was shown 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 Chapter 4.
- The theoretical OHO active biomass concentration in the steady state system bioreactor.

These values are listed in Table 7.4.

Table 7.4: Steady state COD and N mass balances, wastewater fractions and mixed liquor parameters for parent laboratory-scale aerobic activated sludge system (Fig. 7.13) for wastewater (WW) batches during which samples were drawn for DAPI and FISH analysis and parallel batch tests. Values calculated from data in Table 7.3, either directly or using the steady state (SS) design model (WRC, 1984).

AEROBIC STEADY STATE SYSTEM								
WW Batch	No of tests	Mass Balance (%)		Wastewater fractions		Mixed liquor		
		COD	N	Unbiod. Soluble COD ($f_{S,us}$)	Unbio. Particulate COD ($f_{S,up}$)	COD/VSS ratio (mgCOD/mgVSS) (f_{cv})	TKN/VSS ratio (mgN/mgVSS) (f_n)	Active Fraction (f_{av})
24	6	105	91	0.026	0.172	1.40	0.071	0.3583
25	5	100	90	0.022	0.165	1.43	0.074	0.3914
26	5	96	101	0.026	0.159	1.45	0.071	0.4009

Accepting the parameters in Tables 7.3 and 7.4, the theoretical OHO active biomass concentrations in the parent system were calculated with the steady state theory (WRC, 1984) to be 1 821, 2 080 and 2 049 mgCOD/ℓ for wastewater batches 24, 25 and 26 respectively.

7.3.2 Batch tests

The modified batch test procedure was applied to samples drawn from the parent laboratory-scale system. In total 18 batch tests were done during the period when samples were harvested and sent to Technikon Natal for analysis, six for each wastewater batch, see Table 7.5.

Table 7.5: Results of modified batch tests with a mixture of flocculated filtered wastewater (WW) and mixed liquor (ML) drawn from the aerobic parent system during the period when samples were sent to Technikon Natal for DAPI and FISH analysis: Batch test numbers, 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: CONTROL PARENT SYSTEM										
WW Batch	Batch Test No.	Volume Added (ℓ)		COD Recovery (%)	Max. Specific Growth Rates (/d)		$Z_{BH(0)}$ (mgCOD/ℓ)			
							Measured		Theoretical	
		ML	WW		K_{MP}	μ_{HM}	Batch test	PS	Batch test	PS
24 aerobic	49	0.08	2.92	97.4	0.74	2.97	14	537	49	1821
	50	0.12	2.88	95.9	0.83	2.60	20	496	73	1821
	51	0.16	2.84	92.4	1.45	2.12	22	417	97	1821
	52	0.2	2.8	92.9	1.31	1.95	30	453	121	1821
	53	0.24	2.76	96.6	2.10	2.43	27	343	146	1821
	54	0.28	2.72	101.1	1.84	1.85	44	473	170	1821
25 aerobic	55	0.08	2.92	90.1	1.77	5.43	16	613	55	2080
	56	0.16	2.84	94.9	1.76	4.98	26	485	111	2080
	57	0.24	2.76	95	2.09	2.03	52	650	166	2080
	58	0.28	2.72	94.7	2.13	1.44	74	792	194	2080
	59	0.12	2.88	90.9	1.84	3.34	15	385	83	2080
	60	0.2	2.8	98.4	1.90	2.54	27	398	139	2080
26 aerobic	61	0.08	2.92	87.5[1]	1.04	2.62	18	659	55	2049
	62(+P)	0.08	2.92	94.7	1.02	2.58	17	630	55	2049
	63	0.16	2.84	86.0[1]	0.90	2.50	29	552	109	2049
	64(+P)	0.16	2.84	97.8	1.33	2.31	29	552	109	2049
	65	0.24	2.76	85.1[1]	1.77	1.75	61	759	164	2049
	66(+P)	0.24	2.76	97.4	1.61	2.49	40	497	164	2049

[1]Poor COD mass balance
(+P) indicates P added to batch test (see Chapter 6, Section 6.5.5)

From the measured OUR, nitrate and nitrite time data, the following were calculated (see Chapter 6):

- %COD recovery, using Eq. (4.4).

- 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, as described in Section 7.2.2 above.

- OHO OUR ($OUR_{H(t)}$), by subtracting the $OUR_{N(t)}$ (for both nitrate and nitrite nitrification) above from the measured $OUR_{M(t)}$ as in Eq. (4.17b).

- 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, using Eq. (4.12).

Following the procedure above, the OHO active biomass concentrations in the batch tests were determined and are listed in Table 7.5. 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 Table 7.5.

7.3.3 DAPI and FISH measurements

DAPI and FISH measurements were done by Technikon Natal on samples harvested from the parent activated sludge system (Fig. 7.13). Samples were harvested from the aerobic reactor of the parent system on the same day samples were harvested for the batch tests above. This gave three samples per wastewater batch. Samples were mixed with 98% ethanol in a 1:1 ratio (25 or 20mℓ sample to 25 or 20mℓ ethanol) and stored at 4°C. The samples were couriered to Technikon Natal as a single batch in a cooler box filled with dry ice. Sample cells were first fixed and spotted onto slides. Then, the fixed samples were subject to whole cell hybridisation and total cell counts by membrane filtration and staining with DAPI.

7.3.3.1 Cell fixation

During hybridization the cells are exposed to elevated temperatures, detergents and osmotic gradients. Thus, fixation is essential to maintain the morphological integrity of the cells. 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 0.2µm 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 were added to one volume of sample, and the mixture held for 1 to 3 h at 4 °C.
3. The solution was pelleted by centrifugation (5 000g) and the fixative removed.

4. The cells were washed in 1x PBS, and then resuspended in 1x PBS to give a final concentration of 10^8 to 10^9 cells/mℓ (i.e. to the original sample volume).
5. Ice-cold 98% ethanol was added in a 1:1 ratio to the above, and mixed
6. The fixed cells were then spotted onto glass slides, as described below.

7.3.3.2 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. Hybridization and enumeration were achieved 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; for the EUB probe, 0%)
   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 hybridization buffer 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 cell count for each probe was determined by using the following equation:

$$\frac{n(\text{probe})}{n(\text{DAPI})} \times n(\text{MF}) \qquad (7.1)$$

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 below

In the procedure above, two flourescent probes were used, EUB for bacteria in general, and NIT for nitrifiers.

7.3.3.3 Total cell counts by membrane filtration and staining with DAPI

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

Cellulose acetate filters (pore size, 0.22 µm, Micron Seperations Inc.) were counter stained with Sudan Black solution for 12 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 50 W 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:

$$TCC = MTCC \times DF \times MF \qquad (7.2)$$

Where:

TCC = total cell count (=n(MF) in Eq. (7.1) above)
MTCC = mean total cell count for twenty fields
DF = dilution factor
MF = total number of microscope fields on filter (55703 under 1000x magnification)

Following the procedures above, the cells counts for OHO and autotrophic active biomass concentrations are shown in Table 7.6.

Table 7.6: Results from DAPI and FISH enumeration of OHO and autotrophic active biomass concentrations on mixed liquor (ML) drawn from the aerobic parent system (Fig. 7.13): Wastewater (WW) batch numbers, volume of original sample and volume when analysis done, measured cell counts for OHO (Z_{BH}) and nitrifier (Z_{BA}) active biomasses, and measured and theoretical OHO active biomass (Z_{BH}) in the parent system (PS), taking due account of dilution.

AEROBIC PARENT SYSTEM						
WW Batch	Volume of Sample (mℓ)		Cell Counts (cells/mℓ)		PS Z_{BH} (mgCOD/ℓ)	
	Original	Final	Z_{BH}	Z_{BA}	Measured	Theoretical
24 aerobic	50	48	2.261E+08	0	56	1821
	40	37	1.933E+08	0	50	1821
	40	37	2.576E+08	0	66	1821
25 aerobic	40	38	1.941E+08	0	50	2080
	40	42	3.797E+08	0.22	69	2080
	40	38	5.324E+08	0.11	121	2080
26 aerobic	40	37	4.466E+08	0.08	110	2049
	40	37	2.418E+08	0.11	57	2049
	40	37	2.462E+08	0	66	2049

7.3.3.3 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 of 8.51×10^{-11} mgVSS/cell (Mudaly, pers. comment) and taking into account the change in sample volume with storage (original to final volume, Table 7.6) and the 1:1 dilution of the original sample with ethanol (i.e. 2x dilution). Thereafter the measured COD/VSS ratios (Table 7.4) were used to convert from VSS to COD concentration units. The COD concentration units for OHO active biomass concentrations are listed in Table 7.6.

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

The batch test derived OHO active biomass concentrations are listed in Table7.5, the DAPI/FISH derived values are listed in Table 7.6 and the theoretical values are listed in both tables. In Fig. 7.14 the OHO active biomass concentrations in the parent aerobic activated sludge system as measured by means of the two techniques are shown plotted against the corresponding theoretical values. From Tables 7.5 and 7.6 and Fig. 7.14, it is evident that:

(i) As noted in Chapter 6, the batch test measured OHO active biomass concentration values for the parent aerobic activated sludge system are approximately 1/4 the theoretical values.

(ii) The DAPI/FISH determined OHO active biomass concentration values are more than an order of magnitude smaller than the corresponding theoretical values (about 3% of the theoretical values), and approximately 1/10 of the batch test measured values.

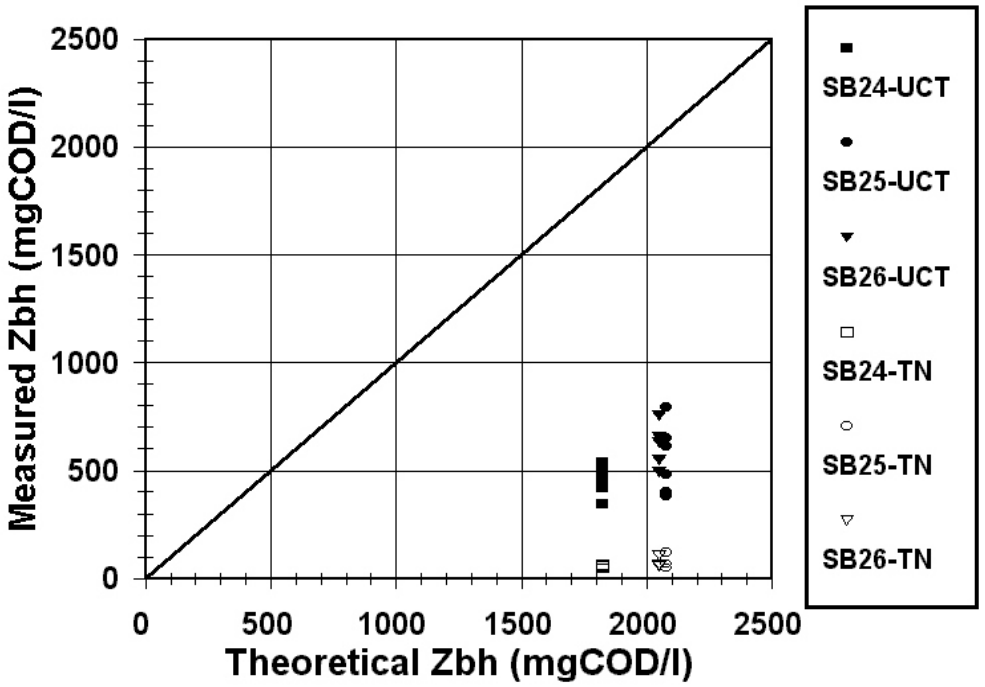

Figure 7.14: Measured versus theoretical OHO active biomass concentrations (Z_{BH}) for mixed liquor from parent laboratory-scale aerobic activated sludge system (Fig. 7.13); measured values from batch test method taking due account of dilution (UCT) and DAPI/FISH enumeration (TN).

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 has been 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 is being investigated further.

7.4 CLOSURE

In this Chapter a first attempt has been made to create cross-links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical methods. This was achieved through collaborative WRC sponsored research projects, with the Water Research Group at the University of Cape Town (UCT), the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) and the Centre for Water and Wastewater at the Technikon Natal (TN). In these projects, the 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 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 (Chapters 5 and 6) was run

on mixed liquor samples drawn from the parent activated sludge systems. The research group at UP measured ATP both *in situ* in the laboratory-scale activated sludge systems, and during the course of the modified batch tests as described in Chapters 5 and 6. And the research group at TN used 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 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 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.
  - For the parent aerobic laboratory-scale activated sludge system, the batch test measured OHO active biomass concentrations are approximately 1/4 the theoretical values.

  These observations are in agreement with those in Chapter 6, 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.

- The ATP method applied by UP gave:
  - OHO active biomass concentrations that are 3 and 5 to 6 orders of magnitude smaller than both the theoretical and batch test measured values, for the batch test and parent activated sludge systems respectively.
  - OHO active biomass concentrations that are higher in the batch test than in the parent activated sludge systems, 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, than in the 20d sludge age parent activated sludge system, despite the theoretical and batch test measured near equivalence of 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 applied by TN gave:
  - 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 batch test 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 has been 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 is being investigated further.

CHAPTER 8

DISCUSSION AND FUTURE WORK

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 C, N and/or P. This implementation has been aided by the development of a suite of steady state and kinetic simulation models which have facilitated optimization of design and operation. Parallel to this development, 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 and 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 phosphorus accumulating organism, PAO), yet these parameters remain hypothetical as they have not been measured and favourably compared to theoretical values. Recently, new batch test methods have been developed to quantify OHO active biomass concentrations (Kappeler and Gujer, 1992; Wentzel *et al*., 1995; Mbewe *et al*., 1995; Ubisi *et al*., 1997a,b; Wentzel *et al.*, 1998). However, the interpretation and analysis of the data from these tests remains firmly rooted within the engineering and technology paradigm. No cross-linking exists with the microbiological and biochemical paradigms.

In this research project, quantification of the OHO active biomass concentration within the engineering and technology paradigm has been investigated. The batch test method of Ubisi *et al*. (1997a,b) above has been evaluated. In this batch test procedure, 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 this evaluation it became apparent that:

- In the batch test with mixed liquor + wastewater, the OHO active biomass from the wastewater 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.

To overcome this difficulty, the batch test procedure was modified, 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 simplifies the batch test procedure - since the flocculated-filtered wastewater does not contain OHO active biomass, a parallel batch test no longer needs 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 has been 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 have been compared to the corresponding theoretical values predicted from the activated sludge models. Results from these comparisons indicate that the some correspondence does exist between theoretical and measured values, but that this correspondence is by no means perfect:

(1) For mixed liquor drawn from a parent anoxic/aerobic activated sludge system at 10d sludge age, good correspondence between batch test measured and theoretical OHO active biomass concentrations exists (Chapter 5).

(2) For mixed drawn from two parallel parent anoxic/aerobic activated sludge system at 10d sludge age, one with toilet paper dosed to the influent and the other without, there is reasonably close correspondence between theoretical and measured OHO active biomass concentrations; the "serial dilutions" of mixed liquor give an almost linear decrease in OHO active biomass concentration (Chapter 6). However, there is 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.

(3) For mixed drawn from a parent aerobic activated sludge system at 10d sludge age there is a parallel correlation between the theoretical and measured OHO active biomass concentration values, but the theoretical values are approximately 3 to 4 times those measured (Chapter 6).

(4) In changing from the parent anoxic/aerobic to aerobic activated sludge system at 10d sludge age above, there was a progressive change in the batch test measured OHO active biomass concentrations, from (2) to (3) above (Chapter 6). 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 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 is not clear: It would be expected that with time the data should return to a 1:1 correlation – this did not happen.

(5) 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 (Chapter 6). For some wastewater batches, the effect was negligible, while for others adding P caused an increase or decrease in the OHO active biomass concentration. 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. **Clearly, this aspect deserves 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 single parent anoxic/aerobic (MLE) activated sludge system, (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, (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 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.

The concepts developed in this research project 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 have been 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 PAO contribution to the measured OUR can not be isolated because the PAOs do not have a unique compound associated with their growth - the PAO mediated P release and uptake are not growth associated processes.

As noted earlier, 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 substantiate the active biomass concept. However, little cross-linking exists between the microbiological and biochemical and the engineering and technology paradigms. To overcome this shortfall, in this research project a first attempt has been made to create cross-links between the engineering and technology of activated sludge systems and the microbiological and biochemical analytical

methods (Chapter 7). This was achieved through collaborative WRC sponsored research projects, with the Water Research Group at the University of Cape Town (UCT), the Department of Microbiology and Plant Pathology at the University of Pretoria (UP) and the Centre for Water and Wastewater at the Technikon Natal (TN). In these 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 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 (Chapters 5 and 6) 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 TN used the microbiological 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 activated sludge systems operated at UCT. From a comparison of the results for OHO active biomass concentrations from the various research groups, it is apparent that:

- The microbiological and biochemical test methods gave OHO active biomass concentrations that are 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 have been 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). **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.

- For the DAPI/FISH method applied by TN, in subsequent investigations it has been 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 is being investigated further.**

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 has not provided even near a close correspondence, it has, for the first time, placed the magnitudes of the microbiological and biochemical measurements

within the context of the engineering and technology paradigm. This will 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. 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.

The research in this contract has initiated the development of cross-linkages between the engineering and technology concepts for activated sludge systems and the newly developed microbiological and biochemical analytical techniques. This research should be continued under future WRC guided projects.

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