Measurement and modelling of ordinary heterotrophic organism active biomass concentrations in anoxic/aerobic activated sludge mixed liquor

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Abstract Ordinary heterotrophic organism (OHO) active biomass \( Z_{BH} \) is a key parameter in models for activated sludge systems, which defines quantitatively the kinetic rates of relevant processes. However, \( Z_{BH} \) has not been measured directly with consistent success: a simple respirometric batch test has provided varying correspondence between measured and theoretical concentrations. In this paper, the batch test is applied to mixed liquors drawn from well defined anoxic/aerobic parent systems at 10 and 20 d sludge ages, with consistent but poor correspondence between measured and theoretical values. In contrast, aerobic digestion batch tests on the same mixed liquors give good correspondences. It is concluded that the differences between theoretical and batch test measured values are due to the batch test method itself and its interpretation. It is found that the batch test conditions (particularly the substrate/\( Z_{BH} \) ratio) influence the kinetic constants derived from the data, and hence the \( Z_{BH} \) estimate. Two kinetic models with two competing OHO populations, a fast and a slow grower, are developed and applied to the batch tests and parent systems. The first model is based on kinetic selection only, while the second includes additional metabolic selection. Both models can account for the observations in the batch tests, but the second provides greater consistency between simulations of the parent systems and batch tests.

Keywords Activated sludge system; active biomass; batch test; heterotroph; kinetic models; model constants

Introduction
In the steady-state design (e.g. WRC, 1984) and dynamic simulation (e.g. Dold et al., 1980, 1991; Henze et al., 1987) models for aerobic and anoxic/aerobic activated sludge systems, the ordinary heterotrophic organism (OHO) active biomass is a fundamental parameter (Ubisi et al., 1997; Wentzel et al., 1998). This mixed liquor (ML) organic component mediates the biological processes of COD removal and denitrification. Accordingly, in the models the relevant kinetic rates for these processes are expressed in terms of the active biomass concentration. However, due to the lack of suitable experimental measurement techniques, the OHO active biomass exists only hypothetically within the structure of the models (Wentzel et al., 1998). To promote further confidence in model application, the active biomass concept needs to be validated by experimental measurement. To quantify the OHO active biomass concentration, a simple respirometric batch test has been developed (Kappeler and Gujer, 1992; Wentzel et al., 1995). This batch test method was extended by Ubisi et al. (1997) and Wentzel et al. (1998) and modified by Cronje et al. (2002) to quantify the OHO active biomass concentration of ML drawn from aerobic and anoxic/aerobic activated sludge systems. The batch test and its modifications have been extensively evaluated, by comparing batch tests measured with theoretical OHO active biomass concentrations for MLs from a variety of well defined lab-scale aerobic and anoxic/aerobic activated sludge systems. Results have been variable, with correspondences between theoretical and measured values ranging from remarkably close (Wentzel et al., 1998; 12 d sludge age ML), through reasonable
(Cronje et al., 2002; 10d sludge age ML) to poor (Wentzel et al., 1998; 20d sludge age ML; Beeharry et al., 2001; 10d sludge age ML).

In this paper this variability is investigated by: (1) re-evaluation of the modified batch test method; (2) application of batch aerobic digestion to quantify OHO active biomass; and (3) development of kinetic models and application of these to the parent and batch tests responses (see Lee et al. (2003)).

Experimental investigation

Parent systems

Two lab-scale parent systems at 10 and 20 d sludge age and at 20°C served as ML source for batch tests. Both systems were MLE configurations, see Table 1. Influent was raw municipal WW from Mitchells Plain (Cape Town, South Africa). The WW was collected in batches, stored in stainless steel tanks at 4°C and served as feed for both the parent systems and batch tests for 10–14 d. For the parent systems, daily WW was drawn from the storage tanks and diluted with tap water to give influent feed total COD − 750 mg COD/P. 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, TKN and OUR; effluent COD, TKN, nitrate + nitrite (Standard Methods, 1985). Both parent systems were operated for 294 d and received 17 WW batches. For each WW batch daily results were averaged (after statistical analysis for outliers). With the averaged data, from Ekama et al. (1986) and Ubisi et al. (1997) the following were calculated: COD and N mass balances; influent WW unbiodegradable soluble and particulate COD fractions (fS,us and fS,up); ML COD/VSS and TKN/VSS ratios (fCV and fN); OHO active biomass fraction of the ML (fav); theoretical OHO active biomass concentration in batch reactor (Lee et al., 2003). To ensure steady state, parent systems were run for >2 sludge ages before doing batch tests on the ML.

Modified batch tests

To quantify the OHO active biomass concentration in ML from the parent systems, the modified batch test procedure of Cronje et al. (2002) was followed: WW was drawn from the storage tanks and diluted to about the same COD concentration fed to the parent systems (~750 mg COD/P). This raw WW was pre-heated to 20°C, then flocculated with alum and filtered (Whatman’s GF/C). The required volume of ML was harvested from the aerobic reactor of the relevant parent system and added to the flocculated-filtered WW in the batch reactor maintained at 20°C, giving a combined volume of 3P. A sample was drawn to obtain the initial total COD concentration. The OUR response in the batch

Table 1 Lab-scale system operating parameters; AX = anoxic, AE = aerobic, WW = wastewater

<table>
<thead>
<tr>
<th>Period</th>
<th>Dates (2001-2002)</th>
<th>Day No.</th>
<th>WW batches</th>
<th>Influent flowrate (Qi, P/d)</th>
<th>Recycle ratios (wrt Qi)</th>
<th>System vol (P) (33% AX, 67% AE)</th>
<th>Batch test?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>7 Jul–19 Aug</td>
<td>1–42</td>
<td>1–3</td>
<td>13.3</td>
<td>1:1</td>
<td>7.8</td>
<td>10, 10</td>
</tr>
<tr>
<td>2</td>
<td>20 Aug–1 Sep</td>
<td>43–55</td>
<td>4</td>
<td>13.3</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
<tr>
<td>3A</td>
<td>2 Sep–13 Sep</td>
<td>56–67</td>
<td>5</td>
<td>13.3</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
<tr>
<td>3B</td>
<td>14 Sep–19 Sep</td>
<td>68–73</td>
<td>6</td>
<td>13.3</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
<tr>
<td>3C</td>
<td>20 Sep–13 Feb</td>
<td>74–220</td>
<td>7–13</td>
<td>13.3</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
<tr>
<td>4A</td>
<td>14 Feb–28 Feb</td>
<td>221–235</td>
<td>14</td>
<td>10</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
<tr>
<td>4B</td>
<td>16 Mar–12 Apr</td>
<td>251–278</td>
<td>15–16</td>
<td>10</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
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<tr>
<td>4C</td>
<td>13 Apr–28 Apr</td>
<td>279–294</td>
<td>17</td>
<td>10</td>
<td>1:1</td>
<td>7.8</td>
<td>13.2</td>
</tr>
</tbody>
</table>
test was continually measured. Samples were regularly drawn from the batch reactor, immediately filtered (0.45 μm) and then analysed for nitrate and nitrite. At the end of the batch test, a final sample was drawn, macerated and final total COD concentration measured. The modified batch test data were analysed and interpreted using the procedure detailed by Wentzel et al. (1995) and Lee et al. (2003), to give: %COD recovery; OHO maximum specific growth rates on RBCOD (Φ_{1\text{Hm}}) and SBCOD (K_{MP}); OHO active biomass concentration at the batch test start, Z_{BH(0)}.

**Batch aerobic digestion test**

To establish whether the causes for the differences between theoretical and measured OHO active biomass concentrations lay in the activated sludge theory or in the modified batch test procedure, the alternative aerobic batch digestion method of Marais and Ekama (1976) was applied to ML drawn from both parent systems (Table 1). ML was drawn from the aerobic reactors of the two systems, placed into batch reactors maintained at 20°C and aerobically digested for ~10d. The OUR response was measured continually, and regularly; (i) pH was measured and maintained >7 to ensure complete nitrification and (ii) samples were drawn from the batch reactor, immediately filtered (0.45 μm) and then analysed for nitrate, nitrite and free and saline ammonia (FSA). The data were analysed to calculate (Marais and Ekama, 1976): specific endogenous mass loss rate at temperature 20°C (b_{120}, /d) and initial OHO active biomass concentration (Z_{BH(0)}).

**Results and discussion**

**Parent systems**

For the WW batches, N mass balances were consistent and generally in the range 90–110%. In contrast, 5 and 7 of the 17 WW batches for the 10 and 20 d sludge age systems respectively gave COD mass balances outside this range; however, batch tests were only conducted during 2 of these WW batches. Batch tests conducted during these WW batches will be included, but appropriately marked. Influent WW mean f_{S,us} were 0.043 (standard deviation, 0.0066) and 0.040 (0.0068) for the 10 and 20 d sludge age systems respectively. The influent WW mean f_{S,up} were 0.165 (0.0295) and 0.148 (0.0254) for the 10 and 20 d sludge age systems respectively. The f_{S,us} and f_{S,up} values of the 10 d system are slightly higher than the values of the 20 d system. However, these differences are not statistically significant at the 95% confidence interval (t-test), and both sets of f_{S,us} and f_{S,up} values fall within the range of values for raw municipal WW in South Africa (0.04–0.10 and 0.07–0.2 respectively, WRC, 1984).

**Modified batch tests**

In total, 35 modified batch tests were conducted on ML drawn from each of the parent systems. OUR–time responses were similar to those of previous investigations (Lee et al., 2003). In general, %COD recoveries were good, with only 8 out of 35 and 7 out of 35 batch tests on 10 and 20 d sludge age MLs respectively yielding %COD recoveries <90%. Rejecting data for those batch tests that deviated from a “true” normal probability plot (4 for 10 d; 3 for 20 d), mean %COD recoveries were 95.2 (5.3) and 94.8 (4.5) % for the 10 and 20 d sludge age MLs respectively. The batch tests rejected will be included, but appropriately marked, so also batch tests with low R² values in the fit to the PnOUR-time plots. Excluding batch tests with low %COD recoveries, mean K_{MP} were 1.37 (0.412) and 1.42 (0.428) /d for the 10 and 20 d sludge age MLs respectively. The means were not significantly different at 95% confidence interval (t-test) which indicates that sludge age did not have a significant influence on this parameter. The values are close to those measured by Beeharry et al. (2001) (1.49 and 1.38/d), and
also to the default value for K_M of Dold et al. (1991), 1.35/d. For \( \Phi_{Hm} \), a clearly discernable trend was noted: as the volume of ML added to the batch test increased, \( \Phi_{Hm} \) decreased. This indicates that one or more factors had a dominating influence, which precluded statistical analysis of the \( \Phi_{Hm} \) data. This aspect is examined in more detail below.

**Batch aerobic digestion test**

In total, 2 batch aerobic digestion tests each were conducted on ML drawn from each of the two parent systems. During all tests FSA concentrations were constant at low values indicating nitrification was complete, a pre-requisite for data analysis. The high linear regression correlation coefficients (\( R^2 > 0.97 \)) in the fit to the Pn OUR–time plots (Marais and Ekama, 1976) lend credibility to the experimental data (see Lee et al., 2003). For the 20 d sludge age ML, \( b_{H2O} \) values were 0.22/d and 0.26/d, and for the 10 d sludge age ML 0.31/d and 0.33/d. The values for the 20 d sludge age ML are close to the default value of 0.24/d (Dold et al., 1980; WRC, 1984), but the 10 d sludge age ML values are higher. This would imply that sludge age influences \( b_{H2O} \), which is contrary to current activated sludge theory. This warrants further investigation.

**Comparison between theoretical and measured OHO active biomass concentrations**

**Modified batch test.** A comparison between the theoretical OHO active biomass concentrations at the start of the batch test (\( Z_{BH(0)} \)) and the batch test measured values is shown in Figure 1. The data for the 10 and 20 d sludge age MLs show remarkable similarity. Three regions can be identified.

(i) Theoretical \( Z_{BH(0)} < -30\) mgCOD/P: as the theoretical \( Z_{BH(0)} \) increases, the measured values decrease, to approach near zero. However, data in this region exhibit poor %COD recoveries.

(ii) \(-30\) mgCOD/P < theoretical \( Z_{BH(0)} < -150\) mgCOD/P: as the theoretical \( Z_{BH(0)} \) increases, the measured values increase virtually parallel to the 1:1 correspondence line, but below it. This trend is near identical to that of Beeharry et al. (2001), whose data falls primarily in this region.

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

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

**Batch aerobic digestion test.** The average differences between measured and theoretical $Z_{BH(0)}$ values were 1.4 and 8.2% for the 10 and 20 d sludge age MLs respectively. This close correlation provides substantive support for the OHO active biomass concept in activated sludge theory.

In the modified batch tests, which are based on OHO active biomass growth processes, the differences between the measured and theoretical values were large (Figure 1). In contrast, in the batch aerobic digestion tests, which are based on endogenous respiration/“death” processes, the correlation is close. Thus, it appears that the cause for the differences between the modified batch test measured and the theoretical concentrations lies in the description and interpretation of the OHO growth processes within the modified batch test itself. Accordingly, this was examined more closely (Lee et al., 2003).

**Evaluation of OHO behaviour in modified batch tests**

OHO behaviour within the batch tests was evaluated through a detailed analysis of the data:

(i) The similarity in the data for both sludge age MLs (Figure 1) substantiates data consistency and the observations made. It also excludes sludge age and related phenomena as the underlying causes for any deviations.

(ii) The $\Phi_{BHm}$ appears to be a function of $Z_{BH(0)}$: as $Z_{BH(0)}$ decreases, $\Phi_{BHm}$ increases, see Figure 2a. $K_{MP}$ values exhibit less variation, but also are apparently linked to $Z_{BH(0)}$, see Figure 2b. Re-examination of the data of Beeharry et al. (2001) indicated that they obtained, but did not note, similar trends.

(iii) Similar, though reversed, trends noted above for $\Phi_{BHm}$ and $K_{MP}$ were evident for the initial substrate ($S_0$) to active biomass ratio ($S_0/Z_{BH(0)}$) (Lee et al., 2003). The influence of $S_0/Z_{BH}$ on batch test behaviour and derived kinetics has been noted previously (e.g. Chudoba et al., 1992; Novak et al., 1994; Grady et al., 1996). With the single OHO population model incorporated in the batch test analytical procedure, it would be expected that $\Phi_{BHm}$ should remain constant.

(iv) In a plot of Pn OURH versus time, the measured data deviated from the fitted linear regression line, showing upward curvature. This strongly suggests that the net OHO maximum specific growth rates ($\Phi_{BHm} + K_{MP} - \beta_H$) change during the course of the batch test, in agreement with the observations of Pollard et al. (1998). This is not accommodated in the simplified model used to develop the analytical procedure for the batch test.

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

(vi) The higher OHO $\Phi_{BHm}$ values were significantly higher than (up to about 7/d, Figure 2a), and outside the range of, values accepted in the kinetic model (1.5–3.5/d, Dold et al., 1991).

(vii) The batch test measured OHO active biomass concentrations [$Z_{BH(0)}$] were consistently lower than the corresponding theoretical values (Figure 1). One possible
explanation is that the OHO maximum specific growth rates increase with time, as noted above. In the batch test procedure, a single constant OHO maximum specific growth is determined and applied to the start of the test to derive a value for $Z_{BH(0)}$. Thus, if the OHO maximum specific growth rate increases with time, then the value at the start of the test is overestimated and hence $Z_{BH(0)}$ would be underestimated.

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

**Competition kinetic models**

To explain observations similar to those above, Novak *et al.* (1994) and Grady *et al.* (1996) proposed substrate competition between different OHO groups as a possible cause. This possibility was investigated, by developing a kinetic model for competition between two OHO populations, one a fast grower and the other a slow grower, based on the concepts of Novak *et al.* (1994). The second OHO group was incorporated into the simplified kinetic model used to analyse the batch test data (Wentzel *et al.*, 1995), as follows (Lee *et al.*, 2003): (1) the single OHO active biomass was subdivided into two,
a fast grower (ZBH1) and a slow grower (ZBH2); (2) all OHO mediated processes were duplicated, with the new processes allocated to the second OHO group; (3) the adsorbed SBCOD was split into two, S_{ads1} and S_{ads2}, utilized by ZBH1 and ZBH2 respectively.

The kinetic model was applied to both the (i) batch tests and (ii) parent systems, using AQUASIM 2.0 (Reichert, 1994). For application to the batch tests, the batch test data with the greatest surety were selected, 30 < Z_{BH(0)} < 150 mgCOD/P and those with good mass balances (Figure 2a). Values for all constants except those related to OHO growth on RBCOD were those of Dold et al. (1991). With regard to $\Phi_{bas}$ and $K_{SH}$ on RBCOD of ZBH1 and ZBH2, these were assumed as 12/d and 3 mgCOD/P and 2/d and 0.1 mgCOD/P respectively, to ensure competition on RBCOD and that the precipitous drop in OUR could be correctly predicted. Parameters estimated with AQUASIM were initial concentrations of $Z_{BH(0)}$ and $Z_{BH2(0)}$, and “substrate”. To reduce the complexity of parameter estimation, the initial substrate was ascribed to RBCOD only. This restricted the parameter estimation to the period up to the OUR precipitous drop. From the application to the batch tests:

(i) The model could accurately simulate OUR_{H–time} observed in batch tests (Lee et al., 2003).

(ii) Parameter estimation of batch test $Z_{BH1(0)}$ and $Z_{BH2(0)}$ concentrations gave exceptionally low $Z_{BH1(0)}/(Z_{BH1(0)} + Z_{BH2(0)})$ values, average 1%. However, with time $Z_{BH1}$ increased its proportion significantly and had a marked influence on the predicted OUR_{H–time} profile (Lee et al., 2003). This indicates that the batch test procedure is very sensitive to the presence of fast growing OHOs.

(iii) The model could simulate the variety of observations made on the batch tests, including the increase in overall OHO maximum specific growth rates with increasing $S_{0}Z_{BH1}$ and with time.

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

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

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

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

To address (ii) above, the competition model was modified by removing the processes for growth of $Z_{BH1}$ on SBCOD (kinetic + metabolic selection). This was considered reasonable, since the original source of $Z_{BH1}$ is seeding with the influent WW to the parent systems – this implies growth in the sewer where RBCOD concentrations are high, which would favour RBCOD utilization. Simulation of the batch tests and parent systems gave results very similar to the competition only model above, except that for influent $Z_{BH1}$ at 3% of total COD, the predicted proportions of $Z_{BH1}$ in the parent system MLs were < 3% compared to $\forall$ 40% for the competition only model above. This former value is very close to those from parameter estimation on the batch test data.
In comparing the various estimates for OHO active biomass concentrations, it was found that the two OHO population kinetic models gave concentrations that were significantly closer to the theoretical values (Figure 3) than the single OHO population model (Figure 1). Thus, the competition hypothesis, in agreement with previous researchers, is one feasible explanation for the observations in the batch tests. However, the kinetic models developed here are largely hypothetical, insufficient information is available to separate the two OHO populations and quantify the individual kinetic processes. Further, alternative hypotheses could possibly explain the observed behaviours, e.g. physiological adaptation (Daigger and Grady, 1982). Clearly, this requires further investigation. This may be facilitated by the microbiologically based analytical techniques, e.g. FISH.

Conclusions
In evaluating the modified batch test to quantify OHO active biomass, it was hoped that the method would provide measured values that would compare favourably with the theoretical values predicted by the activated sludge models. This would provide independent validation in a simple way of the active biomass concept in the models, and thereby promote confidence in their application. However, similarly to previous research, correspondence was not good and the problem more complex than originally thought. In examining causes for this lack of correspondence, it was noted that the modified batch test method relies on a single OHO population with constant kinetics. Observations in this and previous research suggest that this may not be appropriate, and that the batch test conditions may cause the overall OHO behaviour to deviate significantly from that in the parent system. Unfortunately, this renders the batch test unsuitable as a method to directly quantify the OHO active biomass concentration (and kinetic constants) with sufficient accuracy. The test does, however, hold merit as a tool to investigate OHO population dynamics, as shown here.

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

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References
