Activated sludge mixed liquor heterotrophic active biomass

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Abstract

In the current steady state design and kinetic simulation models for activated sludge systems, the heterotrophic active biomass is a key parameter. However, this parameter remains hypothetical within the structure of the models; it has not been measured directly, primarily due to the lack of suitable simple experimental techniques. In this paper a simple batch test procedure is used to quantify the heterotrophic active biomass concentrations of mixed liquor samples drawn from a well-defined anoxic/aerobic activated sludge system. The measured heterotrophic active biomass concentrations are in close agreement with those calculated theoretically using the steady state design and kinetic simulation models. This agreement provides substantive direct evidence supporting both the models and the experimental method.

List of abbreviations

ATP	=	adenosine triphosphate
BEPR	=	biological excess phosphorus removal
COD	=	chemical oxygen demand
DNA	=	deoxyribonucleic acid
DO	=	dissolved oxygen
(ML)	=	mixed liquor
(ML+WW)	=	mixed liquor and waste water
N	=	nitrogen
OUR	=	oxygen utilisation rate
TKN	=	total Kjeldahl nitrogen
TSS	=	total suspended solids
VSS	=	volatile suspended solids
(WW)	=	waste water

Introduction

To optimise 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; Wentzel et al., 1992; Gujer et al., 1995; Henze et al., 1995) have been developed, to progressively include aerobic COD removal and nitrification, anoxic denitrification and anaerobic/anoxic/aerobic BEPR.

In terms of these design procedures and kinetic models, in the bioreactor of the non-nitrifying aerobic activated sludge system the mixed liquor organic suspended solids is made up of three components: heterotrophic active biomass; endogenous residue; and inert material. In the nitrifying aerobic and anoxic/aerobic activated sludge systems, a fourth mixed liquor organic suspended solids component is included: autotrophic active biomass. The heterotrophic active biomass arises from synthesis of living heterotrophic organisms on biodegradable organic substrates and is "lost" via endogenous respiration/death processes; in the activated sludge system this mixed liquor component performs the biodegradation processes of COD removal and denitrification. The autotrophic active biomass arises from synthesis of autotrophic organisms in the nitrification of ammonia to nitrate under aerobic conditions and is "lost" via endogenous respiration/death processes. The endogenous residue is generated from the unbiodegradable portion of the heterotrophic and autotrophic active biomasses that are lost in the endogenous respiration/death processes. The inert material arises from the influent wastewater unbiodegradable particulate organics which, on entry into the bioreactor, are enmeshed in the mixed liquor organic suspended solids. All four mixed liquor organic suspended 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.

If an anaerobic reactor is included in the system to stimulate BEPR, additionally the organisms mediating the BEPR [variously termed polyP organisms (Wentzel et al., 1986), bioP organisms (Comeau et al., 1986), phosphate accumulating organisms, PAO (Henze et al., 1995)] will contribute to the mixed liquor organic suspended solids - to avoid this complication, only the aerobic and anoxic/aerobic systems will be considered in this paper.

Historically the mixed liquor organic suspended solids has been measured as a lumped parameter, via the VSS test (*Standard Methods*, 1985), or, more recently, the COD test. However, from the description above, in the bioreactor of the aerobic and anoxic/aerobic activated sludge systems only a part of the mixed liquor organic suspended solids is heterotrophic active biomass, the active fraction, and only this part mediates the biological processes of COD removal and denitrification. Currently, the heterotrophic active biomass exists only as a hypothetical parameter within the structure of the design procedures and kinetic models. Although indirect evidence provides support for this parameter (by consistency between observations and predictions over a wide range of conditions, e.g. Dold et al., 1980, 1991; Van Haandel et al., 1981; Warner et al., 1986), it has not been directly measured experimentally and compared to the theoretical values. The problem in measurement of this parameter has been the lack of suitable experimental techniques. In the literature, principally microbiological techniques have been proposed; for example, pour plate or other culturing techniques (e.g. Gaudy and Gaudy, 1980), ATP analysis (Nelson and Lawrence, 1980), DNA analysis (Liebeskind and Dohmann, 1994), using fluorescent probes for ribosomal RNA (Wagner et al., 1994), sequencing of ribosomal

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DNA (Blackall, 1994). However, these techniques have not yet been adequately integrated with the design and kinetic modelling theory; the culturing techniques have been widely criticised for their unreliability (e.g. Cloete and Steyn, 1988); the RNA and the two DNA methods are still in their infancy; and, the last-named four methods require sophisticated equipment and experimental techniques that are not widely available.

In contrast, recently a simple batch test procedure has been developed to quantify heterotrophic active biomass (Kappeler and Gujer, 1992; Wentzel et al., 1995; Mbewe et al., 1995). In this paper, this batch test method will be used to quantify the heterotrophic active biomass of mixed liquor samples drawn from a well-defined laboratory-scale activated sludge system. These measured values will be compared to those calculated theoretically with the steady state design and kinetic simulation models: If agreement between measured and predicted heterotrophic active biomass can be obtained, this will provide powerful evidence validating both the models and the experimental method.

Experimental methods

Experimental approach

A well-defined and controlled parent laboratory-scale anoxic/aerobic nitrification/denitrification system was operated and monitored. Mixed liquor samples were harvested from the parent system and various quantities combined with unsettled municipal waste water in batch reactors under aerobic conditions. The OUR response in the batch tests was monitored with time and used to derive estimates for heterotrophic active biomass. Parallel batch tests were run without addition of mixed liquor to quantify the heterotrophic active biomass present in the waste water. The difference in heterotrophic active biomass between the batch tests with and without mixed liquor addition gives the heterotrophic active biomass due to the mixed liquor addition. This will be compared to the theoretically calculated values for the parent laboratory-scale system.

Parent system

The parent laboratory-scale system was operated in the Modified Ludzack Ettinger (MLE) configuration; system layout is shown in Fig. 1. Sludge age was 12 d 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 15 ℓ/d. Influent feed was raw (unsettled) municipal waste water from Mitchells Plain (Cape Town, South Africa); this waste water is principally domestic, with a minor industrial contribution (<15%). The waste water 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 to 2 weeks. For the parent system, daily a sample of the waste water 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, OUR; effluent COD, TKN, nitrate + nitrite (*Standard Methods*, 1985). [Individual nitrite measurements indicated that nitrite concentrations were very low compared to nitrate concentrations (<2%) and consequently could be neglected for this investigation]. To ensure steady state, the parent system was run 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. The reliability of the experimental measurements was checked by means of mass balances on COD and N (Ekama et al., 1986).

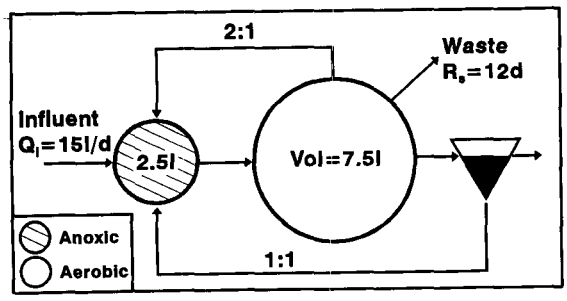

Figure 1
Schematic layout and operational data for parent laboratory-scale anoxic/aerobic activated sludge system

Batch tests

Two variations of the batch tests procedure detailed by Wentzel et al. (1995) and Mbewe et al. (1995) were run. In one type, only unsettled municipal waste water was added to the batch test and in the second, a mixture of waste water and mixed liquor was added. For both types of batch tests, a sample of the waste water 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 waste water only batch tests, a 3 ℓ volume of the diluted waste water 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. In operating the batch test, the surface of the waste water was covered by small plastic balls to limit surface exchange of oxygen. The 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 vice versa). The pH of the reactor was monitored continually and controlled to pH 7.5 (±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 (0.45 μm) 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 24 h. 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 mixture of waste water 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

TABLE 1
STEADY STATE RESULTS FOR PARENT LABORATORY-SCALE ANOXIC/AEROBIC ACTIVATED SLUDGE SYSTEM (FIG. 1). FOR EACH OF THE FOUR WASTE-WATER BATCHES TESTED, THE DAILY RESULTS HAVE BEEN AVERAGED AND THE AVERAGES ARE LISTED WITH 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	
1	12	554 (69)	59 (12)	45.7 (3.3)	4.2 (0.6)	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)
2	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)
3	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)
4	8	496 (20)	50 (8)	48.5 (3.6)	4.0 (0.8)	1.9 (1.3)	10.4 (0.9)	10.4 (1.4)	23.6* (0.8)	2182 (59)	3055 (185)	211 (18)	7.67 (0.08)

TABLE 2
STEADY STATE COD AND N MASS BALANCES, WASTE-WATER FRACTIONS AND MIXED LIQUOR PARAMETERS FOR PARENT LABORATORY-SCALE ANOXIC/AEROBIC ACTIVATED SLUDGE SYSTEM (FIG. 1). DATA CALCULATED FROM DATA IN TABLE 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 (%)		Waste-water 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 praction (f_{av})	
					SS design	Kinetic sim.			SS design	Kinetic sim.
1	12	92	99	0.106	0.125	0.115	1.52	0.104	0.400	0.395
2	13	97	100	0.087	0.165	0.153	1.50	0.106	0.351	0.351
3	11	94	90	0.095	0.167	0.155	1.50	0.111	0.347	0.348
4	8	84	102	0.101	0.150	0.116	1.40	0.097	0.356	0.393

up to 3 ℓ with the same diluted unsettled municipal waste water used in the waste water only batch tests, also preheated to 20°C (see above). The batch test procedure detailed above was then followed.

Results and data interpretation

Parent system

For the four batches of waste water fed to the parent system, the daily results were averaged and the averages are shown listed in Table 1 together with standard deviations. Following the procedures set out by Ekama et al. (1986) the following were determined: The influent waste-water 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 2. Referring to Table 2, acceptable COD and N mass balances were obtained, except for the COD mass balance on waste water Batch No. 4; here the error was traced to problems in measuring the OUR, an error that does not influence the subsequent calculations. Thus, the steady state data can be considered acceptable.

From the steady state data, the theoretical heterotrophic active biomass of the mixed liquor drawn from the parent system and added to the batch tests was calculated. 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 3 d, virtually all the influent biodegradable COD is depleted. Certainly this will be the case for the parent system at 12 d sludge age with the small anoxic mass fraction (25%) present (Fig. 1). Under these conditions the steady design equations

(WRC, 1984) and the kinetic simulation models will give near identical values for heterotrophic active biomass, provided equivalent values for the constants are used. However, the steady state design equations do have the advantage over the kinetic simulation models in that they allow an analytical solution without the use of sophisticated computer programs. Accordingly, the steady state design model in WRC (1984) was used to calculate the theoretical heterotrophic active biomass. However, kinetic simulation results will be shown, to illustrate that the two approaches are nearly identical.

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

$$f_{av} = MX_{BH}/MX_V = MX_{BH}/(MX_{BH} + MX_E + MX_I + MX_{BA}) \quad (1)$$

where:

MX_{BH} = mass of heterotrophic 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) = $V \cdot X_{BA}$

V = system volume (ℓ)

X_{BH} = heterotrophic 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. (1), for activated sludge systems receiving "normal" municipal waste waters (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. (1) for MX_{BH} and $MX_V = (MX_{BH} + MX_E + MX_I)$:

$$\frac{1}{f_{av}} = 1 + f_E^* b_{HT}^* R_S + \frac{f_{S,up}(1 + b_{HT}^* R_S)}{f_{cv} Y_H^* (1 - f_{S,us} - f_{S,up})} \quad (2)$$

where:

f_E^* = fraction of heterotrophic 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^* = heterotrophic active biomass yield, VSS units (mgVSS/mgCOD) = 0.45 mgVSS/mgCOD (WRC, 1984)

Using Eq. (2) and the data in Table 2, values for f_{av} were calculated and are listed in Table 2. Also, using the UCTOLD kinetic simulation computer program (Dold et al., 1991), values for f_{av} were determined from simulations of the four 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 identical and the f_{av} calculated from the simulated results. These values for $f_{S,up}$ and f_{av} are also listed in Table 2. Comparing the values for f_{av} from the steady state design procedure and the kinetic simulations, near identical values were obtained for the four waste-water batches tested.

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 1), the theoretical heterotrophic active biomass concentration in the batch reactor due to the added mixed liquor [Z_{BH} $(theo)_{BT}$, COD units] is given by:

$$Z_{BH}(theo)_{BT} = [X_V(PS) \cdot f_{av} \cdot f_{cv} \cdot V_{ML}]/(V_{ML} + V_{WW}) \quad (3)$$

where:

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

$X_v(PS)$:= mixed liquor VSS concentration measured in parent system, Table 1 (mgVSS/ℓ)

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

V_{WW} := volume of waste water added to batch test (ℓ).

Theoretical values for the heterotrophic active biomass concentration in the batch reactor due to the added mixed liquor are listed in Table 4.

Note that in Eq (3) the parent system mixed liquor organic suspended solids are expressed in VSS units [X_V(PS)], whereas the heterotrophic 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 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 3
BATCH TESTS WITH WASTE WATER (WW) ONLY: COD RECOVERY, REGRESSION DATA FROM LN OUR VS. TIME PLOTS AND HETEROTROPHIC ACTIVE BIOMASS AT THE START OF THE BATCH TEST [$Z_{BH(0)}$ (WW)].

Batch test: waste water (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 (%)
3	W1	92	1.344	0.287	0.992	25	5
	W2	98	1.0588	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)	
4	W5	97	0.537	0.342	0.988	9	2.1

TABLE 4
BATCH TESTS WITH MIXTURE OF WASTE WATER (WW) AND MIXED LIQUOR (ML) DRAWN FROM PARENT LABORATORY-SCALE SYSTEM: VOLUMES ADDED, COD RECOVERIES, REGRESSION DATA FROM LN OUR_H VS. TIME PLOTS AND HETEROTROPHIC ACTIVE BIOMASS PRESENT IN THE BATCH TEST ($Z_{BH(0)}$) DUE TO ML + WW, WW_D (TAKING DUE ACCOUNT OF DILUTION) AND ML. ALSO SHOWN ARE THE THEORETICAL HETEROTROPHIC ACTIVE BIOMASSES DUE TO THE ML ADDITION.

Batch test: waste water (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	
3	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	106	17.5	89	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	184	16.3	168	159
	M8	0.4	2.6	96	2.262	0.076	0.993	188	16.3	172	159
4	M9	0.1	2.9	97	1.696	0.235	0.990	42	8.7	33	36
	M10	0.2	2.8	74	1.893	0.220	0.947	55	8.4	47	73
	M11	0.3	2.7	81	2.104	0.145	0.949	96	8.1	88	109
	M12	0.4	2.6	83	2.207	0.116	0.941	128	7.4	120	145

Batch tests

Batch tests were conducted using two batches of waste water, Batch Nos. 3 and 4, Table 1. As noted above, two types of batch tests were conducted, one type with waste water only and the other type with waste water and mixed liquor.

Waste water only

Four batch tests on waste water Batch No. 3 and one on waste water Batch No. 4 were conducted, see Table 3. As an example, the OUR (mgO/ℓ·h) vs. time (h) response for a batch test with waste water only (Batch test No. W3, Table 3) is shown plotted in Fig. 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 waste water. (Should nitrification take place, it can be taken into account by following the procedures set out below).

Referring to the OUR time plot (Fig. 2), the profile conforms to that described by Wentzel et al. (1995): During the first period of the batch test (<8.5 h) the OUR exhibits an exponential increase due to heterotrophic active biomass growth. After

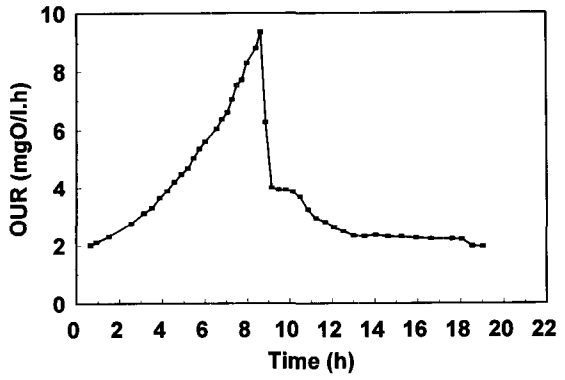

Figure 2
OUR time response for aerobic batch test on raw municipal waste water from Mitchells Plain (Cape Town, South Africa) (Batch test No. W3, Table 3)

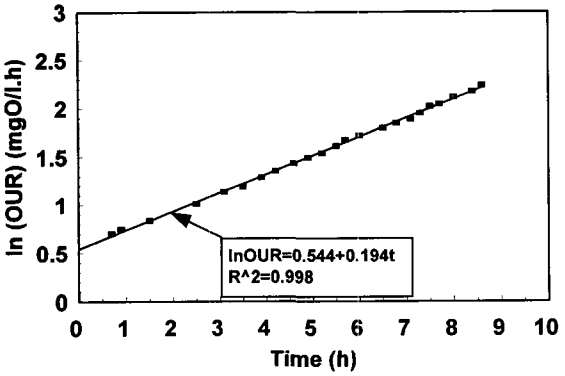

Figure 3
ln OUR vs. time for the OUR data in Fig. 2, up to the precipitous drop in OUR

±8.5 h, the OUR drops precipitously due to depletion of the waste water readily biodegradable COD. For the remainder of the batch test, the OUR exhibits an inverted S pattern typical of saturation kinetics, due to slowly biodegradable COD utilisation.

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

- COD recovery (%)
- waste-water heterotrophic active biomass, $Z_{BH(o)}$ (mgCOD/ℓ).

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

$$\% \text{ COD recovery} = \frac{COD_{t=T} + \int_{t=0}^{t=T} OUR_{H(t)} \cdot dt}{COD_{t=0}} \cdot 100 \qquad (4)$$

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)
- $OUR_{H(t)}$ = heterotrophic active biomass at time t (mgO/ℓ·h)
- $\int_{t=0}^{t=T} OUR_{H(t)} \cdot dt$ = integral (area) under the heterotrophic OUR vs. time plot between start and end of test (mgO/ℓ)
- = oxygen consumed over the test by heterotrophic active biomass.

In Eq. (4), since in this series of batch tests no nitrification was observed, the measured OUR at time t ($OUR_{M(t)}$) is due to heterotrophic growth only and equals $OUR_{H(t)}$. Integrating the area under the measured OUR time profile and substituting into Eq. (4), the COD recoveries for the different batch tests with waste water only were calculated and are listed in Table 3; all COD recoveries were >90%, indicating that the data were acceptable.

To determine heterotrophic 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 in OUR vs. time (h) (for example, the OUR data in Fig. 2 are shown plotted in Fig. 3), and linear regression applied to determine the y-intercept, slope and correlation coefficient; these are listed in Table 3 for the different batch tests on waste water only. From the slopes and y-intercepts, $Z_{BH(o)}$ can be determined (Wentzel et al., 1995):

$$Z_{BH(0)} = \frac{e^{y\text{-intercept}} \cdot 24}{\frac{1 - Y_{ZH}}{Y_{ZH}} \cdot (\text{slope} \cdot 24 + b_{HT})} \qquad (5)$$

where:

- $Z_{BH(0)}$ = heterotrophic active biomass concentration at the start of the batch test (mgCOD/ℓ batch reactor)
- Y_{ZH} = heterotrophic active biomass yield, COD units (mgCOD/mgCOD)
- = 0.666 mgCOD/mgCOD (Dold et al., 1980, 1991; Wentzel et al., 1995)
- b_{HT} = heterotroph specific death rate at temperature T (/d)
- = $b_{H20}\ 1.029^{(T-20)}$
- b_{H20} = heterotrophic specific death rate at 20°C
- = 0.62/d (death/regeneration theory, Dold et al., 1980; Wentzel et al., 1995).

Note that in Eq. (5) for the batch tests, the death regeneration theory (Dold et al., 1980) is used (with b_{H20} = 0.62/d), whereas in Eq. (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 heterotrophic 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 utilised for heterotrophic active biomass synthesis with associated oxygen utilisation [i.e. the oxygen demand arises, in fact, from the energy requirements for resynthesis of heterotrophic 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 heterotrophic active biomass dies at a certain rate; of the biomass lost, the biodegradable portion gives rise directly to oxygen utilisation (there is no slowly biodegradable substrate intermediate) and the unbiodegradable portion 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 heterotrophic active biomass, utilisation 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

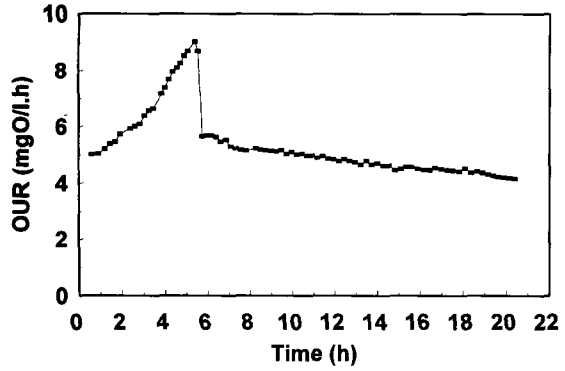

Figure 4

OUR response with time for aerobic batch test on a mixture of raw municipal waste water (2.9ℓ) from Mitchells Plain (Cape Town, South Africa) and mixed liquor (0.1ℓ) drawn from the aerobic reactor of the parent laboratory-scale system (Fig. 1) (Batch test No. M1, Table 4)

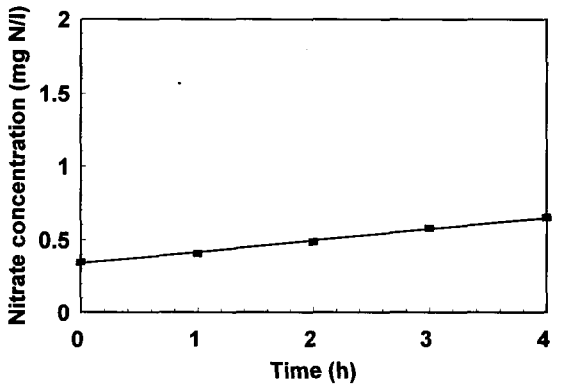

Figure 5

Nitrate concentration with time for aerobic batch test in Fig. 4

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 waste-water biodegradable COD has not been depleted, and is utilised over the course of the batch tests). Thus, to interpret the batch test results, a full kinetic model must 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 also is followed here for analysis of the batch tests.

Accepting Eq. (5) and substituting into the equation the regression data listed in Table 3 for the batch tests, values for $Z_{BH(0)}$ for the various batch tests with waste water (WW) only were calculated and are listed in Table 3 as concentrations and percentages of the total waste water COD: As reported by Mbewe et al. (1995) and Wentzel et al. (1995), for Mitchells Plain waste water, heterotrophic active biomass was found to be present at low concentrations, 2 to 5% of total COD.

Waste water + mixed liquor

Twelve batch tests were conducted on mixtures of various quantities of mixed liquor and waste water, eight with waste-water Batch No. 3 and four with waste water Batch No. 4, see Table 4. As an example, the OUR (mgO/ℓ · h) vs. time (h) response for a batch test with 100 mℓ mixed liquor added to 2.9 ℓ diluted waste water (Batch test No. M1, Table 4) is shown plotted in Fig. 4. Referring to Fig. 4, the general shape of the OUR time profile is the same as that for waste water only (see Fig. 2). However, due to the larger concentration of heterotrophic 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. 5. The OUR due to this nitrification must be taken into account in deriving estimates for % COD recovery and $Z_{BH(0)}$, since both these parameters are determined from the OUR for heterotrophs 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 heterotrophic growth ($OUR_{H(t)}$) and due to nitrification ($OUR_{N(t)}$), i.e.:

$$OUR_{M(t)} = OUR_{H(t)} + OUR_{N(t)} \qquad (5a)$$

[Note that for the batch tests with waste water only, no nitrification was observed, i.e. in Eq. (5a) $OUR_{N(t)} = 0$ and $OUR_{M(t)} = OUR_{H(t)}$].

Rearranging Eq. (5a):

$$OUR_{H(t)} = OUR_{M(t)} - OUR_{N(t)} \qquad (5b)$$

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 (e.g. see Fig. 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:

$$OUR_N = 4.57 \cdot \Delta NO_3^-/\Delta t \qquad (mgO/\ell \cdot h) \qquad (6)$$

Linear regression was applied to the batch tests nitrate time profiles to determine the y-intercept, slope and correlation coefficient, see Table 5. From the slopes, the OUR_Ns for the different batch tests were calculated using Eq. (6), and also are listed in Table 5. 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. (5b), i.e. from each measured OUR value ($OUR_{M(t)}$), a constant nitrification OUR value (OUR_N, Table 5) is subtracted to give the OUR due to the heterotrophs only ($OUR_{H(t)}$).

Having determined the $OUR_{H(t)}$ for the batch tests, the % COD recoveries were calculated using Eq. (4) and are listed in Table 4. Referring to Table 4, for batch tests M1 to M9 COD recoveries range from 95 to 102% indicating the acceptability of the data; however, for batch tests M10 to M12 COD recoveries were poor (< 85%) - these data should be rejected, but are included to illustrate problems that can arise in the test. It is interesting to note that for this batch of waste water (Batch No. 4), the COD recovery for the steady state system also was poor (84%, Table 2).

To determine the heterotrophic active biomass present at the start of the batch tests $Z_{BH(0)}$, the $OUR_{H(t)}$ (i.e. $OUR_{M(t)} - OUR_N$) up

TABLE 5
BATCH TESTS WITH MIXTURE OF WASTE WATER AND MIXED LIQUOR DRAWN FROM PARENT LABORATORY-SCALE SYSTEM: REGRESSION DATA FROM NITRATE VS. TIME PLOTS, NITRIFICATION RATES ($\Delta NO_3^-/\Delta T$) AND OUR FOR NITRIFICATION (OUR_N)

Batch test: waste water (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)
3	M1	0.343	0.075	0.989	0.075	0.343
	M2	0.547	0.129	0.983	0.129	0.590
	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	1.490
4	M9	0.452	0.029	0.937	0.029	0.133
	M10	0.816	0.128	0.923	0.128	0.585
	M11	0.929	0.104	0.935	0.104	0.475
	M12	1.486	0.170	0.957	0.170	0.777

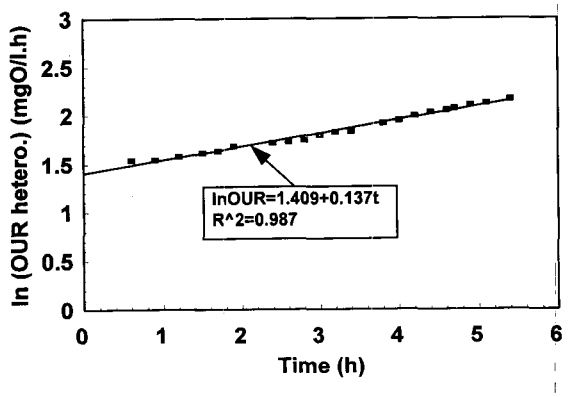

Figure 6
ln OUR due to heterotrophic active biomass vs. time for the OUR data in Fig. 4 up to the precipitous drop in OUR [i.e. OUR due to nitrification (0.343 mgO/ℓ, Table 5, Batch test No. M1) subtracted from measured OUR data in Fig. 4 and then plotted]

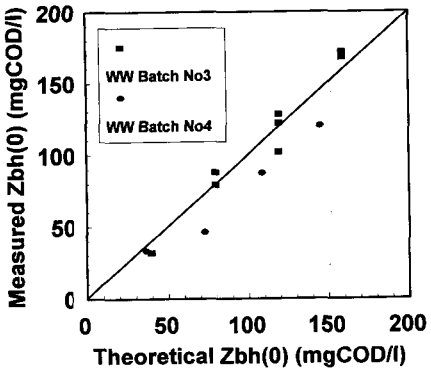

Figure 7
Measured vs. theoretical heterotrophic active biomass concentration [$Z_{BH(0)}$(ML)] in the batch test due to addition of mixed liquor drawn from the parent laboratory-scale activated sludge system (Fig. 1)

to the precipitous drop in OUR were plotted ℓn $OUR_{H(t)}$ vs. time (h), for example see Fig. 6. Linear regression was used to determine the y-intercept, slope and correlation coefficients of the ℓn $OUR_{H(t)}$ vs. time plots, see Table 4. From the y-intercept and slope, values for $Z_{BH(0)}$ were calculated using Eq. (5) and also are listed in Table 4. These values represent the heterotrophic active biomass due to that added with the mixed liquor and that present in the original waste water, i.e. $Z_{BH(0)}$ (WW+ML).

Comparison

In the batch tests with both waste water and mixed liquor present, to determine $Z_{BH(0)}$ due to the mixed liquor only [$Z_{BH(0)}$ (ML)], the $Z_{BH(0)}$ due to the waste water [$Z_{BH(0)}$ (WW)] must be subtracted from the $Z_{BH(0)}$ due to both the waste water and mixed liquor [($Z_{BH(0)}$ (WW+ML)]. Now, $Z_{BH(0)}$ (WW) is available from the batch tests with waste water only; for a particular waste-water batch these were averaged (see Table 3). The dilution in $Z_{BH(0)}$ (WW) caused by the addition of mixed liquor to the batch tests with waste water and mixed liquor was taken into account, as follows:

$$Z_{BH(0)}\,(WW)_D = Z_{BH(0)}\,(WW) \cdot V_{WW}/(V_{ML} + V_{WW}) \qquad (7)$$

where:

$Z_{BH(0)}\,(WW)_D$ = heterotrophic active biomass due to waste water at start of batch test with WW + ML, taking due account of dilution (mgCOD/ℓ)

$Z_{BH(0)}(WW)$ = heterotrophic active biomass present in the original waste water, Table 3 (mgCOD/ℓ)

V_{WW} = volume of waste water added to batch test (ℓ)

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

From the data in Table 3, values for $Z_{BH(0)}(WW)_D$ were calculated using Eq. (7) and are listed in Table 4.

Now:

$$Z_{BH(0)}(ML) = Z_{BH(0)}(ML + WW) - Z_{BH(0)}(WW)_D \quad (8)$$

From the data in Table 4, using Eq. (8) values for $Z_{BH(0)}(ML)$ were calculated and are also listed in Table 4. These values represent the measured mixed liquor heterotrophic active biomass concentration in the total batch test volume. The theoretical values were calculated using Eq. (3) and are listed in Table 4 also.

To compare the measured and theoretical $Z_{BH(0)}(ML)$, the values are plotted against each other in Fig. 7. For waste-water Batch No. 3, very good agreement was obtained between the theoretical and measured heterotrophic active mass. For waste-water Batch No. 4, the correlation between the theoretical and measured values is not as good. Referring to Table 4, as noted earlier the % COD recoveries for Batch tests M10, M11 and M12 on waste-water Batch No. 4 were poor; on this basis these data should have been excluded but have been retained to illustrate problems in the batch test method.

Closure

In the current steady state design and kinetic simulation models for activated sludge systems, the mixed liquor organic suspended solids is made up of a number of components. One key component is the heterotrophic active biomass, as this component mediates the biodegradation processes of COD removal and denitrification. Thus, the rates for these processes are directly related to the heterotrophic active biomass present, and the specific rates should be expressed in terms of this parameter (e.g. Dold et al., 1980; Van Haandel et al., 1981; WRC, 1984; Ekama et al., 1986) to allow a meaningful comparison of the rates measured in different systems (e.g. Clayton et al., 1991). However, the heterotrophic active biomass parameter has been only hypothetical within the structure of these models; it has not been measured directly, primarily due to the lack of suitable simple measurement techniques. This deficiency has cast some measure of doubt on the entire framework within which the steady state design and kinetic simulation models have been developed. In this paper a simple batch test procedure has been used to quantify the heterotrophic active biomass concentration of mixed liquor drawn from a well-defined anoxic/aerobic activated sludge system. The measured heterotrophic active biomass concentrations are in close agreement with those calculated theoretically. This agreement provides substantive evidence supporting both the models and the experimental method. This should promote confidence in application of the models for design, operation and control of activated sludge systems. The study will be extended to a more detailed investigation, including varying the parent system sludge age to change the heterotrophic active biomass component's proportion in the mixed liquor organic suspended solids.

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