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Measurement of Ordinary Heterotrophic Organism Active Biomass in Activated Sludge Mixed Liquor: Evaluation and Comparison of the Quantifying Techniques

Byung Joon Lee[1], Mark Wentzel[2], George Ekama[2], Yun Young Choi[1†], Jung Woo Choi[1]

[1]*School of Construction and Environmental Engineering, Kyungpook National University, Sangju 742-711, Korea*
[2]*Department of Civil Engineering, University of Cape Town, Rondebosch 7701, South Africa*

Abstract

Ordinary heterotrophic organism (OHO) active biomass plays key roles in biological wastewater treatment processes. However, due to the lack of measurement techniques, the OHO active biomass exists hypothetically within the design and simulation of biological wastewater treatment processes. This research was purposed to develop a quick and easy quantifying technique for the OHO active biomass applying a modified batch aerobic growth test. Two nitrification-denitrification activated sludge systems, with 10- and 20-day sludge ages, were operated to provide well-cultured mixed liquor to the batch tests. A steady state design model was firstly applied to quantify the "theoretical" OHO active biomass concentration of the two parent systems. The mixed liquor from the parent systems was then inoculated to a batch growth test and a batch digestion test to estimate the "measured" OHO active biomass concentration in the mixed liquor. The measured OHO active biomass concentrations with the batch growth test and the batch digestion test were compared to the theoretical concentrations of the parent system. The measured concentrations with the batch growth test were generally smaller than the theoretical concentrations. However, the measured concentrations with the batch aerobic digestion tests showed a good correlation to the theoretical concentrations. Thus, a different microbial growth condition (i.e., a higher food/biomass ratio) in the batch growth test, compared to the parent system or the batch digestion test, was found to cause underestimation of the OHO active biomass concentrations.

Keywords: Active biomass, Activated sludge system, Endogenous respiration, Growth, Heterotrophic, Models

1. Introduction

To aid the design and operation of the activated sludge system, a suite of steady state design models [1-3] and kinetic simulation models [4-6] have been developed over the past four decades. In the development of these models, it was recognised that it would not be possible to incorporate the behaviour of specific microorganism species, as the mixed liquor in the activated sludge system contains a wide diversity of different microorganism species, for which identification techniques have only recently started becoming available. Instead, microorganisms that fulfil a particular function in the activated sludge system (e.g., aerobic degradation of organics) are grouped together as a single entity, which has been called a "surrogate" organism. This surrogate organism is assigned a set of unique characteristics that reflect the behaviour of the group, but which may not reflect the characteristics of any individual organism or species of organisms in the group. A similar approach has been adopted for the "non-organism" components of the activated sludge mixed liquor, e.g., inert organics. Together, the surrogate organism and non-organism groups make up the activated sludge mixed liquor organic (volatile) suspended solids (MLOSS). In terms of the current models, the MLOSS is normally made up of the following components: 1) ordinary heterotrophic organism (OHO) active biomass, 2) endogenous residue, and 3) inert material, 4) autotrophic organism (AO) active biomass, 5) phosphate accumulating organism (PAO) active biomass, and 6) this organism group's endogenous residue that contributes to the MLOSS [7]. In particular, the active biomass components (surrogate organism groups) of the MLOSS above mediate the relevant biological processes deemed to be of importance: the OHO's chemical oxygen demand (COD) removal and denitrification, AO's nitrification, and PAO's biological excess phosphorus removal and COD removal. Except P-removal systems, which might be subjected to later investigation, the parameter of OHO active biomass is fundamental. This MLOSS mediates the biodegradation processes

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†Corresponding Author
E-mail: yunchoi@knu.ac.kr
Tel: +82-54-530-1443 Fax: +82-54-530-1449

of COD removal and denitrification (and associated processes). In addition, all the relevant specific process rates in the models are expressed in terms of it. However, due to the lack of suitable experimental measurement techniques, the OHO active biomass only exists hypothetically within the structure of the steady state design and dynamic simulation models. Historically, the MLOSS has been measured as a lumped parameter via the volatile suspended solids (VSS) or COD test [8]; this casts a measure of uncertainty on the entire framework within which the models have been developed and is a weakness in the models. To promote further confidence in the application of the models, and indeed, in the models themselves, the OHO active biomass concept needs to be validated by experimental measurement.

For validation, independent quantification of active biomass, and close correspondence to the theoretical values calculated by the steady state design or dynamic simulation models, is required. In this regard, the (modified) batch growth test procedure has shown considerable promise to quantify the OHO active biomass. However, results from the batch test are variable ranging from close correspondence with the theoretical values to poor. In comparing the theoretical OHO active biomass concentration with those measured in the batch tests and modified batch tests, a variety of correlations have been found ranging from remarkably close to 1:1 (with 12 sludge age mixed liquor) [9], through reasonable close to 1:1 (with 10 sludge age mixed liquor) [10] to poor (with 20 days sludge age mixed liquor [9] and with 10 days sludge age mixed liquor [11]). To explain this variability in results, this research adopted the following approaches: 1) re-evaluation of the batch growth test method [10, 11] by repeating the modified batch test procedure and comparing the measured OHO active biomass concentrations with the theoretical values predicted by the steady state model; and 2) application of the more laborious batch aerobic digestion method of Marais and Ekama [12] to quantify OHO active biomass, to establish whether discrepancies between measured and theoretical OHO active biomass concentrations arise from the activated sludge theory (i.e., the theoretical) or from the batch test procedure itself (i.e., the measured).

2. Material and Methods

2.1. Parent System (Steady State Design Model)

The two parent systems, 10- and 20-day sludge age systems, served as a source of mixed liquor for the batch growth tests developed by Cronje et al. [10], but had different OHO active biomass fractions. According to Ekama et al. [13] and Water Research Commission (WRC) [1], the mixed liquor OHO active biomass fraction decreases as the sludge age increases. The capability of the modified batch test to correctly detect this difference in OHO active biomass fraction was evaluated. Basically, the system layout of both the 10- and 20-day sludge age parent activated sludge systems constituted a Modified Ludzack-Ettinger (MLE) configuration and consisted of an anoxic reactor of 33% of the total system volume, an aerobic reactor of 67% of the total system volume, and a secondary settling tank, all in series with an underflow recycle (s-recycle) from the settling tank to the anoxic reactor of 1:1 and from the aerobic reactor to the anoxic reactor (a-recycle) of 2:1 during periods 1 and 2, or 1:1 during periods 3 and 4. The system configuration was similar to that described by Beeharry et al. [11] and Marais and Ekama [12]. In their investigations, they found that the inclusion of an unaerated zone (anoxic reactor) was essential to prevent the proliferation of the filamentous organism *Sphaerotilus natans,* which caused severe sludge bulking in completely aerobic lab-scale activated sludge systems. During the experimental investigation, both systems were kept in the basic MLE system configuration as described above. However, the system layout and operation of both the 10- and 20-day sludge age systems were slightly modified several times to cope with operational problems. Table 1 lists these modifications to the two parent systems. The influent for the parent lab-scale activated sludge systems was raw (unsettled) sewage from the Mitchell's Plain Treatment Plant, in Cape Town, South Africa. This sewage is primarily domestic with a small (<25%) industrial component. The sewage batch was brought to the laboratory and stored in 400-L stainless steel tanks in a cold room at 4°C for 10 to 14 days. The total COD concentration which served as feed to both the parent lab-scale activated sludge systems was targeted at 750 ± 50 mgCOD/L with influent flow rate of 13.3 L/day (periods 1, 2, and 3) or 10 L/day (period 4). To maintain the pH in the aerobic reactor at approximately 7.5, the alkalinity of the influent was increased by 200 mg/L (as $CaCO_3$). Daily monitoring included influent COD, total Kjeldahl nitrogen (TKN); all reactors nitrite + nitrate; aerobic reactor total suspended solids (TSS), VSS, COD, and TKN; and effluent COD, TKN, nitrate + nitrite [8].

Both the 10- and 20-day sludge age parent systems were operated for 294 days and received 17 batches of unsettled municipal wastewater from Mitchell's Plain, Cape Town. Each wastewater batch was accepted as a steady state period and the results for each batch were averaged (after statistical analysis for outliers). From the averaged data, the following were calculated according to WRC [1]: 1) system COD and N mass balances, 2) influent wastewater unbiodegradable soluble and unbiodegradable particulate COD fractions ($f_{S,us}$ and $f_{S,up}$, respectively), 3) mixed

Table 1. Details of changes in the parent lab-scale system operating parameters

Period	Date	Day	Sewage batch no.	Inflow rate (Qi, L/day)	Recycle ratio (with respect to Qi)		System volume (L) (33% AX, 67% AE)	
					s-recycle	a-recycle	10-day	20-day
1	Jul 7, 2001 – Aug 19, 2001	1st – 42nd	1 – 3	13.3	1:1	2:1	10	10
2	Aug 20, 2001 – Sep 1, 2001	43rd – 55th	4	13.3	1:1	2:1	7.8	13.2
3	Sep 2, 2001– Feb 13, 2002	56th – 220th	5 – 13	13.3	1:1	1:1	7.8	13.2
4	Feb 14, 2002 – Apr 28, 2002	221st – 294th	14 – 17	10	1:1	1:1	7.8	13.2

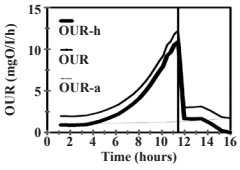
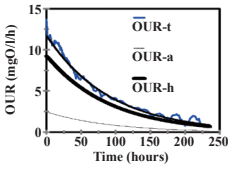
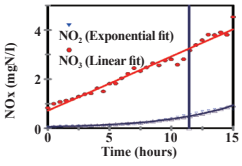
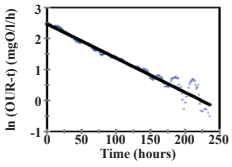
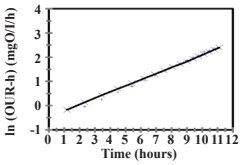
AX: anoxic, AE: aerobic.

liquor COD/VSS and TKN/VSS ratios (f_{CV} and f_N, respectively), 4) the OHO active biomass fraction of the mixed liquor organic suspended solids (f_{av}), and 5) the theoretical OHO active biomass concentration in the steady state system bioreator (the first row in Table 2).

2.2. Batch Aerobic Growth Tests

The batch growth test procedure of Cronje et al. [10] was used in this research to quantify the OHO active biomass concentration in mixed liquor drawn from aerobic and anoxic/aerobic

Table 2. Summary of the quantifying techniques: steady state design model, batch aerobic growth test, and batch aerobic digestion test [18]

Steady state design model	Batch aerobic growth test	Batch aerobic digestion test
Water Research Commission [1]	Cronje et al. [10]	Marais and Ekama [12]
OHO	OHO	OHO
Aerobic growth	Aerobic growth	Endogenous respiration
Anoxic growth	Death regeneration	
Endogenous respiration		
AO	AO	AO
Nitrification	Nitrification	Nitrification
	 <OUR profiles> OUR_{OHO} $=OUR_{Total}-OUR_{AO}$	 <OUR profiles> OUR_{OHO} $=OUR_{Total}-OUR_{AO}$
	 <NO_3 & NO_2 profile>	 <$logOUR_{OHO}$ profile> Y-intercept & slope
	 <$logOUR_{OHO}$ profile> Y-intercept & slope	
$\frac{1}{f_{av}} = 1 + f_E^* b_{HT}^* R_S + \frac{f_{s,up}(1+b_{HT}^* R_S)}{f_{cv} Y_H^* (1 - f_{s,us} - f_{s,up})}$	$Z_{BH(0)} = \frac{e^{Y-\text{int}\,ercept}\,24}{\frac{1-Y_{ZH}}{Y_{ZH}}(slope\,24 + b_{HT})}$ (mgCOD/L batch reactor)	$X_{BHi} = \frac{e^{Y-\text{int}\,ercept}}{(f_{cv} + 4.57 f_n)(1 - f_E^*)(-slope)}$ (mgVSS/L batch reactor)

f_E^* = fraction of OHO active biomass that is endogenous residue
= 0.2 (endogenous respiration theory, Dold et al. [4]).
b_{HT}^* = specific endogenous mass loss rate at temperature T (day^{-1})
= $b_{H20}^* 1.029^{(T-20)}$
b_{H20}^* = specific endogenous mass loss rate at 20°C
= 0.24 day^{-1} at 20°C (endogenous respiration theory, Dold et al. [4])
b_{HT} = OHO specific death rate at temperature T (day^{-1})
= b_{H20} $1.029^{(T-20)}$
b_{H20} = heterotrophic specific death rate at 20°C
= 0.62 day^{-1} (death/regeneration theory, Dold et al. [4]; Wentzel et al. [9]).
R_S = system sludge age (day)
$f_{S,up}$ = 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)
f_N = TKN to VSS ratio of mixed liquor organic suspended solids (mgTKN/mgVSS)
Y_H^* = OHO active biomass yield, VSS units (mgVSS/mgCOD)
= 0.45 mgVSS/mgCOD (Water Research Commission [1])
Y_{ZH} = OHO active biomass yield, COD units (mgCOD/mgCOD)
= 0.666 mgCOD/mgCOD (Dold et al. [4, 15] and Wentzel et al. [9])

OHO: ordinary heterotrophic organism, AO: autotrophic organism, OUR: oxygen utilization rate, COD: chemical oxygen demand, VSS: volatile suspended solids, TKN: total Kjeldahl nitrogen.

activated sludge systems. The batch growth test was done by flocculating and filtering the wastewater to remove the OHO active biomass present in the raw wastewater itself, thereby simplifying the procedure. For flocculating and filtering, the raw wastewater (10 mL of stock aluminium sulphate [$Al(SO_4)\cdot 15H_2O$, stock at 50 g/L]) were added per liter wastewater; the mixture was stirred rapidly (200 rpm) for 2 min (rapid mix phase) and then slowly (1 rpm) for 30 min (flocculation and settling phase); and was then allowed to settle (without stirring) for a further 30 min period. The clear supernatant developed in the settling cylinders was drawn off and filtered through a glass fiber filter (GF/C; Whatman, Maidstone, Kent, UK). The batch growth tests were conducted using a mixture of flocculated and filtered wastewater and mixed liquor. The required volume of mixed liquor was harvested from the aerobic reactor of the parent system and added to the flocculated-filtered wastewater giving a combined volume of 3 L for the mixture in the batch reactor. A sample was drawn to obtain the initial total COD concentration. The oxygen supply and oxygen utilization rate (OUR) response in the batch test were measured using an automated technique [14]. At regular intervals, samples were drawn from the batch reactor immediately filtered through 0.45 µm filter paper, and 2–3 drops of $HgCl_2$ were added to the filtrate, which was stored for subsequent nitrate and nitrite analysis. At the end of the batch tests, the final sample was drawn from the batch reactor, macerated, and the final total COD concentration measured. The batch growth test data were analyzed and interpreted using the procedure detailed by Cronje et al. [10] and Beeharry et al. [11], which is based on a simplified UCT model [9], which consists of a single OHO population. From the batch test data, the following was calculated: 1) the %COD recovery, 2) OHO maximum specific growth rates on RBCOD (μ_H) and SBCOD (K_{MP}), and 3) OHO active biomass concentration at the start of the batch test, $Z_{BH(0)}$ (the second row in Table 2).

2.3. Analytical Methods

The chemical analyses were daily performed on the samples throughout the parent systems. COD and TKN were measured with the open reflux method and the digestion/distillation method, respectively [8]. The COD and TKN analyses were done on the unfiltered influent, the unfiltered effluent sample, filtered effluent sample (filtered through 0.45 µm filter paper), and the aerobic reactor mixed liquor unfiltered samples. Nitrate and nitrite concentrations were automatically measured with Technicon AutoAnalyser (Seal Analytical Ltd., Mequon, WI, USA) on the filtered samples from the effluent, aerobic, and anoxic reactors. TSS concentration of the aerobic reactor mixed liquor was measured with the dried weight at 105°C and organic/volatile suspended solids (VSS) concentration was measured with the residual weight after burning the dried sample at 550°C [8]. Diluted sludge volume index (DSVI) [8] were also monitored daily on the aerobic reactor mixed liquor of the parent system. The OUR was continuously measured with an automated technique, which was equipped with a dissolved oxygen (DO) probe (YSI Inc., Yellow Springs, OH, USA) and an automated DO meter/OUR logger (Hi Tech Microsystems, Cape Town, South Africa) [14].

2.4. Batch Aerobic Digestion Test

To establish whether the causes for the deviations between theoretical and measured OHO active biomass lay in the activated sludge theory, especially in the OHO growth process or in the batch growth test procedure, an alternative method to quantify OHO active biomass was investigated. As an alternative, the aerobic batch digestion method of Marais and Ekama [12] was applied to quantify the OHO active biomass concentration of mixed liquor drawn from both the 10- and 20-day sludge age parent activated sludge systems. The 1.56 and 1.32 L of mixed liquor, which are equivalent to the volumes of sludge wastage for 2 days, were drawn from the 10- and 20-day sludge age parent MLE systems, respectively. The mixed liquor drawn from the parent system was placed into the batch reactor and immediately the batch aerobic digestion test was started. The OUR response in the batch test was measured using an automated technique [14] for approximately 10 days. At the beginning of the test and at the same time on each of the following days, pH was measured to ensure the pH was sufficiently high for complete nitrification (pH > 7), and samples were drawn from the batch reactor immediately filtered through 0.45 µm filter paper and 2–3 drops of $HgCl_2$ were added to the filtrate, which was stored for subsequent nitrate, nitrite, and free and saline ammonia (FSA) analysis. For all the batch aerobic digestion tests, the pH was sufficiently high to ensure complete nitrification indicated by the fact that the FSA was constantly kept at a low concentration, i.e., FSA did not accumulate during the test. The batch aerobic digestion test data were analysed and interpreted using the procedure based on Marais and Ekama [12]. From the batch test data, the following were calculated: 1) specific endogenous mass loss rate (b_{H20T}) at temperature 20°C (day^{-1}), 2) initial OHO active biomass concentration ($X_{BH(0)}$) (mg VSS/L), and 3) theoretical nitrate concentrations ($NO_{3(t)}$) with time (the third row in Table 2).

3. Results and Discussion

3.1. Parent System (Steady State Design Model)

N mass balances were consistent and generally in the range 90% to 110%. Generally, COD mass balances were not as good as the N mass balances with 5 of 18 sewage batches for the 10-day sludge age system and 6 of 17 sewage batches for the 20-day sludge age system giving mass balances outside the range 90% to 110% (Tables 2 and 3).

The influent wastewater mean unbiodegradable soluble COD fractions ($f_{S,us}$) were determined to be 0.043 (sample standard deviation = 0.0066) and 0.040 (sample standard deviation = 0.0068) for the 10- and 20-day sludge age systems, respectively. The influent wastewater mean unbiodegradable particulate COD fractions ($f_{S,up}$) were determined to be 0.165 with sample standard deviation of 0.0295 for the 10-day system and 0.148 with sample standard deviation of 0.0254 for the 20-day system. The $f_{S,us}$ and $f_{S,up}$ values of the 10-day system are slightly higher than the values of the 20-day system. However, the differences between the two $f_{S,us}$ values and between the two $f_{S,up}$ values are not statistically significant at the 95% confidence interval (*t*-test). The $f_{S,us}$ and $f_{S,up}$ values in this experiment are in the range of accepted values of 0.04–0.10 and 0.07–0.20 mgCOD/mgCOD, respectively, for municipal raw wastewaters in South Africa [1].

The means for COD/VSS were 1.40 and 1.37 mgCOD/mgVSS with sample standard deviation 0.01 and 0.03 mgCOD/mgVSS for the 10- and 20-day sludge age systems, respectively. The means for TKN/VSS were 0.083 and 0.079 mgTKN/mgVSS with sample standard deviation 0.003 and 0.003 mgTKN/mgVSS for the 10- and 20-days sludge age systems, respectively.

Table 3. Experimental results of the steady state design model, batch aerobic growth test, and batch aerobic digestion test for the 10-day sludge age parent lab-scale anoxic/aerobic activated sludge system

Steady state design model											Batch aerobic growth test										Batch aerobic digestion test						
Sew. batch No.	No. of Test	Mass balance (%)		WW fraction		Mixed liquor			Theoretical OHO ACTI		Test no.	Volume (L)		%COD recovery	Regression			Growth rate (day^{-1})		$Z_{BH(0)}$	Test no.	Regression			b_{H20} (day^{-1})	X_{BHi}
		N	COD	$f_{S,us}$	$f_{S,up}$	f_{CV}	f_N	f_{av}	$Z_{BH(0)}$	X_{BHi}		WW	ML		Y-int.	Slope	R^2	K_{MP}	μ_H			Y-int.	Slope	R^2		
1	96	96	93	0.03	0.1931	1.33	0.075[a]	0.346																			
2	7	99	92	0.0345	0.2046	1.34	0.08	0.334																			
3	7	104	79	0.0361	0.1654	1.40	0.081	0.385																			
4	7	102	78	0.0421	0.2037	1.44	0.083	0.345																			
5	3	98	89	0.0387	0.1703	1.37	0.088	0.375	172.7		A1	2.70	0.30	92.8	1.542	0.058	0.146	1.18	0.46	108.1							
6	3	104	84	0.0346	0.1739	1.44	0.086	0.375																			
7	7	99	97	0.0382	0.1345	1.42	0.085	0.427	196.5		A2	2.70	0.30	90.3	2.162	0.083	0.761	1.30	1.30	154.4							
									131.0		A3	2.80	0.20	104.3	1.714	0.106	0.891	1.51	1.65	81.9							
									65.5		A4	2.90	0.10	93.6	1.263	0.215	0.923	1.23	4.55	28.5							
8	3	115	103	0.0060[a]	0.2147	1.45	0.087	0.343	166.3		A5	2.70	0.30	96.5	1.987	0.125	0.979	1.93	1.69	93.9							
									110.9		A6	2.80	0.20	100.7	1.854	0.080	0.925	1.18	1.35	117.5							
									55.4		A7	2.90	0.10	95.7	0.861	0.235	0.962	1.58	4.67	17.6							
9	9	96	113	0.0390	0.1794	1.38	0.086	0.370	159.2		A8	2.70	0.30	93.0	2.482	0.133	0.964	1.76	2.04	146.4							
									106.1		A9	2.80	0.20	96.5	2.190	0.167	0.928	1.68	2.95	89.8							
									53.1		A10	2.90	0.10	100.6	1.769	0.218	0.988	1.77	4.08	46.7							
10	9	94	92	0.0488	0.1113	1.39	0.084	0.454	191.2		A11	2.70	0.30	95.8	2.227	0.022	0.379	0.54	0.60	377.6							
									127.5		A12	2.80	0.20	104.9	1.733	0.155	0.954	1.65	2.67	60.9							
									63.7		A13	2.90	0.10	100.8	1.031	0.266	0.993	1.65	5.35	18.7							
									31.9		A14	2.95	0.05	96.5	0.807	0.266	0.999	1.10	5.91	14.9							
11	11	95	104	0.0513	0.1794	1.39	0.083	0.368	230.4		A15	2.60	0.40	100.2	2.492	0.022	0.581	0.67	0.48	489.4							
									172.8		A16	2.70	0.30	98.4	1.974	0.077	0.877	1.26	1.21	136.3							
									115.2		A17	2.80	0.20	93.3	1.381	0.146	0.926	1.51	2.61	45.0							
									57.6		A18	2.90	0.10	90.5	0.459	0.225	0.967	1.86	4.16	12.2							
									28.8		A19	2.95	0.05	85.6	0.878	0.315	0.997	0.90	7.28	13.7							
12	11	99	110	0.0490	0.1717	1.40	0.082	0.374	233.4		A20	2.60	0.40	101.8	2.476	0.029	0.594	0.80	0.51	421.6							
									175.1		A21	2.70	0.30	92.9	2.203	0.044	0.852	0.84	0.84	252.0							
									116.7		A22	2.80	0.20	93.3	1.538	0.152	0.982	1.90	2.38	50.7							
									58.4		A23	2.90	0.10	92.1	0.759	0.173	0.950	1.58	3.18	20.9							
									29.2		A24	2.95	0.05	83.2	0.306	0.221	0.992	1.65	4.28	10.7							
13	8	97	85	0.0450	0.1083	1.35	0.081	0.456	32.9		A25	2.95	0.05	80.2[b]	-0.536	0.252	0.992	0.64	6.03	4.1							
									26.3		A26	2.96	0.04	85.3	0.197	0.189	0.951	1.59	3.56	11.0							
14	9	115	107	0.0482	0.1625	1.39	0.084	0.385	265.2		A27	2.40	0.60	96.4	2.688	0*	0*	0.42	0.20	1,093.8							
									176.8		A28	2.60	0.40	96.6	2.085	0.078	0.985	1.35	1.14	150.8							
									88.4		A29	2.80	0.20	96.1	0.896	0.178	0.962	1.90	3.00	23.3							
									44.2		A30	2.90	0.10	86.2	0.301	0.275	0.978	1.79	5.42	8.7							
									30.9		A31	2.93	0.07	78.5[b]	0.438	0.261	0.994	0.97	5.91	10.5							
									22.1		A32	2.95	0.05	76.3[b]	1.085	0.172	0.995	0.60	4.14	29.0							
									13.3		A33	2.97	0.03	74.0[b]	1.362	0.161	0.989	0.62	3.87	40.5							
15	6	115	105	0.0477	0.1442	1.41	0.080	0.410		993												C1	2.9029	-0.01	0.971	0.31	996
16	11	99	106	0.0519	0.1453	1.40	0.083	0.408		983												C2	2.9768	-0.01	0.987	0.33	1,008
17	8	97	99	0.0474	0.1515	1.39	0.081	0.399	95.2		A34	2.80	0.20	97.5	0.967	0.128	0.958	1.38	2.31	33.2							
									47.6		A35	2.90	0.10	100.3	-0.837	0.283	0.978	1.14	6.27	2.7							

WW: wastewater, OHO: ordinary heterotrophic organism, ML: mixed liquor, N: nitrogen, COD: chemical oxygen demand.

[a] Rejected as outliers at 95% confidence interval to calculate mean values. [b] Rejected on poor COD mass balance for further analysis. * Approached almost zero.

Table 4. Experimental results of the steady state design model, batch aerobic growth test, and batch aerobic digestion test for the 20-day sludge age parent lab-scale anoxic/aerobic activated sludge system

Steady state design model											Batch aerobic growth test										Batch aerobic digestion test						
Sew. batch No.	No. of Test	Mass balance (%)		WW fraction		Mixed liquor			Theoretical OHO ACTI		Test no.	Volume (L)		%COD recovery	Regression			Growth rate (day^{-1})		$Z_{BH(0)}$	Test no.	Regression			b_{H20} (day^{-1})	X_{BHi}
		N	COD	$f_{S,us}$	$f_{S,up}$	f_{CV}	f_N	f_{av}	$Z_{BH(0)}$	X_{BHi}		WW	ML		Y-int.	Slope	R^2	K_{MP}	μ_H			Y-int.	Slope	R^2		
1	96	99	81	0.0280	0.1123	1.36	0.072[a]	0.313																			
2	7	102	86	0.0292	0.1280	1.35	0.078	0.293																			
3	7	103	79	0.0363	0.1056	1.35	0.081	0.319																			
4	7	106	85	0.0401	0.1870	1.37	0.079	0.236																			
5	3	110	83	0.0425	0.1716	1.37	0.080	0.249	172.7		B1	2.70	0.30	96.8	1.573	0.027	0.131	0.51	0.77	175.5							
6	3	113	94	0.0390	0.1702	1.39	0.083	0.249																			
7	7	96	108	0.0390	0.1707	1.39	0.081	0.253	196.5		B2	2.70	0.30	92.9	1.641	0.129	0.908	1.89	1.84	64.6							
									131.0		B3	2.80	0.20	97.5	1.321	0.141	0.957	1.11	2.89	43.7							
									65.5		B4	2.90	0.10	91.3	0.916	0.217	0.965	0.56	5.26	20.0							
8	3	98	98	-0.019[a]	0.1896	1.37	0.079	0.236	166.3		B5	2.60	0.40	93.7	2.093	0.108	0.972	1.83	1.38	117.7							
									110.9		B6	2.70	0.30	93.9	1.797	0.089	0.906	1.65	1.11	101.8							
									55.4		B7	2.80	0.20	91.7	1.489	0.182	0.971	1.80	3.18	41.4							
9	9	103	101	0.0331	0.1560	1.39	0.084	0.263	159.2		B8	2.60	0.40	93.8	2.437	0.112	0.979	1.58	1.72	161.6							
									106.1		B9	2.70	0.30	96.7	2.112	0.172	0.996	1.77	2.99	81.0							
									53.1		B10	2.80	0.20	97.3	1.970	0.079	0.654	0.93	1.59	132.7							
10	9	93	110	0.0523	0.1713	1.38	0.080	0.247	191.2		B11	2.60	0.40	100.2	2.379	0.024	0.674	0.74	0.44	424.5							
									127.5		B12	2.70	0.30	105.1	2.099	0.104	0.958	1.44	1.68	121.6							
									63.7		B13	2.80	0.20	95.6	1.647	0.174	0.992	1.62	3.18	50.3							
									31.9		B14	2.90	0.10	93.6	1.136	0.217	0.996	1.45	4.38	24.9							
11	11	95	101	0.0513	0.1448	1.38	0.079	0.275	230.4		B15	2.50	0.50	97.9	2.209	0.043	0.860	0.92	0.72	258.4							
									172.8		B16	2.60	0.40	96.9	1.948	0.128	0.968	1.83	1.85	88.7							
									115.2		B17	2.70	0.30	93.2	1.530	0.148	0.957	1.92	2.26	51.4							
									57.6		B18	2.80	0.20	102.1	0.962	0.178	0.962	1.86	3.02	25.0							
									28.8		B19	2.90	0.10	90.6	1.043	0.282	0.995	1.55	5.82	17.9							
12	11	106	97	0.0471	0.1405	1.38	0.082	0.279	233.4		B20	2.50	0.50	90.6	2.268	0.050	0.941	1.22	0.6	247.7							
									175.1		B21	2.60	0.40	90.8	2.196	0.045	0.867	1.04	0.66	247.5							
									116.7		B22	2.70	0.30	95.9	1.637	0.113	0.971	1.80	1.54	71.8							
									58.4		B23	2.80	0.20	94.8	1.252	0.114	0.916	1.37	1.99	48.5							
									29.2		B24	2.90	0.10	86.6	0.545	0.213	0.967	1.61	4.13	14.0							
13	8	102	87	0.0332	0.1149	1.38	0.084	0.310	32.9		B25	2.93	0.07	85.4	-0.752	0.276	0.976	0.67	6.57	3.0							
									26.3		B26	2.95	0.05	80.2[b]	0.027	0.209	0.962	1.58	4.05	8.5							
14	9	116	103	0.0427	0.1353	1.39	0.082	0.286	265.2		B27	2.34	0.66	95.4	2.247	0.083	0.984	1.44	1.16	169.2							
									176.8		B28	2.50	0.50	95.2	1.945	0.064	0.941	0.92	1.24	150.7							
									88.4		B29	2.70	0.30	96.3	1.000	0.157	0.945	1.69	2.71	28.8							
									44.2		B30	2.80	0.20	89.7	0.591	0.238	0.974	1.84	4.51	13.3							
									30.9		B31	2.90	0.10	87.9	-0.320	0.307	0.994	2.06	5.92	4.2							
									22.1		B32	2.93	0.07	75.9[b]	0.115	0.265	0.971	0.99	5.98	7.5							
									13.3		B33	2.95	0.05	72.5[b]	0.929	0.237	0.990	0.81	5.49	18.7							
15	6	116	100	0.0415	0.1214	1.36	0.080	0.300		758												D1	2.3453	0	0.979	0.22	834
16	11	106	113	0.0454	0.1453	1.38	0.083	0.275		718												D2	2.4741	-0.01	0.987	0.26	774
17	8	102	92	0.0382	0.1429	1.38	0.081	0.279	95.2		B34	2.70	0.30	101.0	1.165	0.118	0.945	1.34	2.13	43.1							
									47.6		B35	2.80	0.20	102.8	0.167	0.200	0.953	1.34	4.07	10.2							

WW: wastewater, OHO: ordinary heterotrophic organism, ML: mixed liquor, N: nitrogen, COD: chemical oxygen demand.
[a]Rejected as outliers at 95% confidence interval to calculate mean values. [b] Rejected on poor COD mass balance for further analysis.

3.2. Batch Aerobic Growth Tests

In total, 35 batch growth tests were conducted on mixed liquor drawn from the 10-day sludge age parent system, and 35 on that drawn from the 20-day sludge age parent system. Nitrite concentrations in the batch growth tests were significant, and hence were taken into account in calculating the nitrification OUR.

In general, %COD recoveries were good with only 8 out of 35 batch tests on 10-day sludge age mixed liquor yielding %COD recoveries <90%, and only 7 out of 35 for the 20-day sludge age mixed liquor. Excluding data for those batch tests that deviated from the "true" normal probability line (4 for 10-day; 3 for 20-day), the mean %COD recoveries were 95.2% and 94.8% for the batch tests on 10- and 20-day sludge age mixed liquors, respectively, with sample standard deviations of 5.3% and 4.5%, respectively. The %COD recoveries are somewhat lower than that obtained (100.5%) by Cronje et al. [10], but are similar to those (97.8% and 95.9%) of Beeharry et al. [11].

For the OHO maximum specific growth rate on SBCOD (K_{MP}), excluding the batch tests with low %COD recoveries, statistical analysis gave mean K_{MP} of 1.37 and 1.42 day^{-1} for the 10- and 20-day sludge age mixed liquors, respectively, with sample standard deviations of 0.412 and 0.428 day^{-1}, respectively. The close means (difference 3.5%, not statistically significant at 95% confidence interval, *t*-test) indicates that the sludge age did not have a significant influence on the value for this parameter. The values are larger than that measured (0.84 day^{-1}) by Cronje et al. [10], but are close to the values measured (1.49 and 1.38 day^{-1}) by Beeharry et al. [11]. Further, the values are close to the default value for K_{MP} in the UCT kinetic simulation model of 1.35 day^{-1} [15]. It should be noted that there was some measure of uncertainty in the determination of K_{MP} at the relatively large and small mixed liquor volumes. However, it was concluded that the uncertainty was not large.

For the OHO maximum specific growth rate on RBCOD (μ_H) for batch tests on mixed liquors from both parent systems, a clearly discernable trend was noted: as the volume of mixed liquor added to the batch test increased, the value for μ_H decreased (Fig. 1). This indicates that one (or more) factor has a dominating influence, which precluded statistical analysis of the data. Re-examination of the data of Beeharry et al. [11] indicated that they obtained, but did not note a similar trend.

3.3. Batch Aerobic Digestion Test

In total, 2 batch aerobic digestion tests were conducted on mixed liquor drawn from the 10-day sludge age parent system, and 2 on that drawn from the 20-day sludge age parent system. Filtered samples taken at regular intervals during the test showed FSA concentrations were constant at low values. This implies nitrification was complete a pre-requisite for analysis of the data. The high linear regression correlation coefficients (R^2 > 0.97) in the fit to the $lnOUR_t$ - time plots lends credibility to the experimental data.

For the values for the OHO specific endogenous respiration rate (b_{H20}) values for the 20-day sludge age system mixed liquor, these were estimated as 0.22 and 0.26 day^{-1}; for the 10-days sludge age system mixed liquor, these were estimated as 0.31 and 0.33 day^{-1}. The values for the 10-day sludge age system are significantly higher than the default value of 0.24 day^{-1} [1, 12]. This would imply that sludge age influenced b_{H20}, which is contrary to current activated sludge theory. This warrants further investigation.

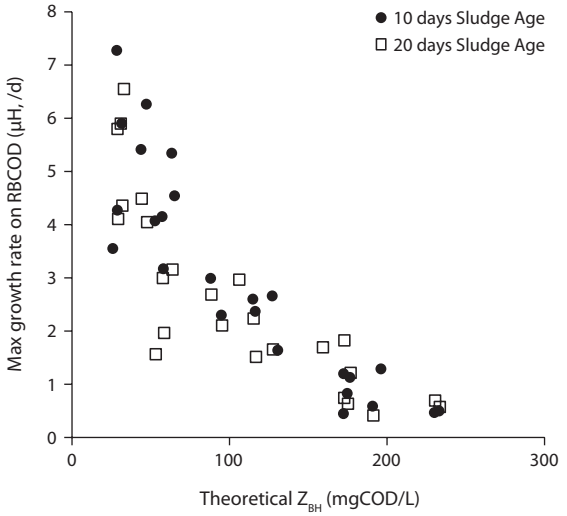


Fig. 1. Maximum specific growth rate on RBCOD (μ_H, day^{-1}) versus theoretical active biomass concentration at the start of the batch tests ($Z_{BH(0)}$; mgCOD/L) for batch aerobic growth tests on mixed liquor drawn from 10- and 20-day sludge age Modified Ludzack-Ettinger activated sludge systems (adapted from Lee et al. [16]).

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

3.4. Comparison between the Parent System (Steady State Design Model) and Batch Growth Test

From the analysis of the N and COD mass balances, sewage batches which yielded poor mass balances were rejected for further analysis. The batch growth test data collected on these rejected sewage batches were included, where appropriate, and were analyzed; but it was noted that the data should be rejected and the data should not be used to draw conclusions. In addition, from the statistical plot, the several %COD recovery data points of batch growth tests deviate significantly from the "true" normal distribution line of the statistical plot. Most likely, the dominating cause was the difficulty in accurately measuring the low OUR values arising from the small mixed liquor volume additions. Accordingly, these data points were also rejected for further analysis.

With the batch test method, Wentzel et al. [9] measured the OHO active biomass in mixed liquor from parent systems at sludge ages 12 and 20 days. With the parent system at 12-day sludge age, the agreement between measured values from batch growth test and theoretical values from steady state design model was remarkably good. However, with the parent system at 20-day sludge age, the agreement was poor with the theoretical values being about 2 times those measured. No explanation could be found for this inconsistency. In this research, a similar experimental approach was followed to attempt to provide an explanation for the above inconsistency. Two parallel parent MLE sys-

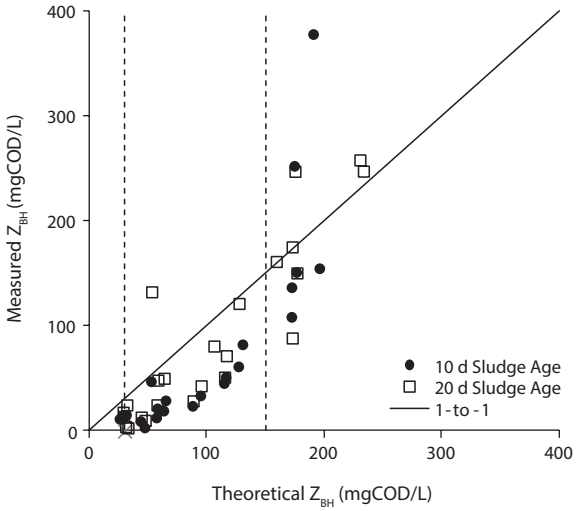


Fig. 2. Batch aerobic growth tests results; graph of measured versus theoretical ordinary heterotrophic organism active biomass concentration at the start of the batch test [$Z_{BH(0)}$] for the various sewage batches, for the 10- and 20-day Modified Ludzack-Ettinger activated sludge system (adapted from Lee et al. [16]).

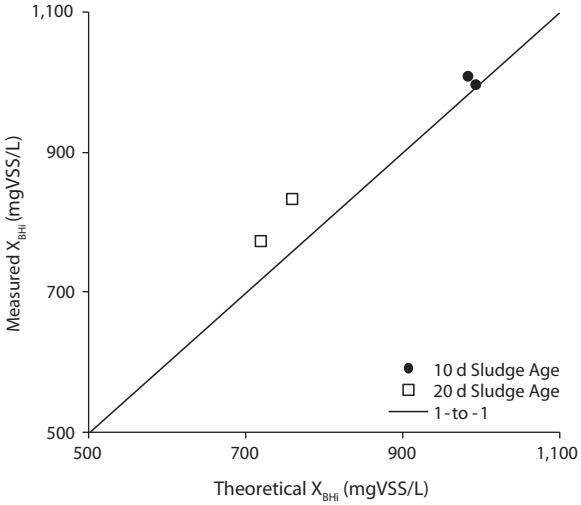


Fig. 3. Measured versus theoretical ordinary heterotrophic organism active biomass concentration at the start of the batch aerobic digestion tests (X_{BHi}) for the 10- and 20-day sludge age parent Modified Ludzack-Ettinger activated sludge systems.

tems at 10- and 20-day sludge age were run and the batch growth tests were conducted on mixed liquor drawn from the two parent systems. A combined comparison between the 10- and 20-day sludge age system mixed liquor is shown in Fig. 2(a) and (b). In comparing the measured OHO active biomass concentration at the start of the batch test $Z_{BH(0)}$ with the theoretical value, the data for both the 10- and 20-day sludge age mixed liquor showed very similar trends. Also, superficially, both comparisons bear a strong resemblance to the data of Beeharry et al. [11], namely that there is a correspondence; but the values plot parallel to the 1:1 correspondence line. However, if the data is examined more closely for both the 10- and 20-day mixed liquors, three regions were identified [16, 17]:

- Theoretical $Z_{BH(0)}$ < 30 mgCOD/L : As the theoretical $Z_{BH(0)}$ increases, the measured values decrease to approach near zero. Beeharry et al. [11] did not collect data in this region so a comparison with their data is not possible.
- 30 mgCOD/L < theoretical $Z_{BH(0)}$ < 150 mgCOD/L: As the theoretical $Z_{BH(0)}$ increases, the measured values correspondingly increase parallel to a 1:1 correspondence line, but below it. This trend is near identical to that observed by Beeharry et al. [11], whose data fall primarily in this region.
- Theoretical $Z_{BH(0)}$ > 150 mgCOD/L : As the theoretical $Z_{BH(0)}$ increases, the measured values increase sharply to cross the 1:1 correspondence line. Beeharry et al. [11] collected limited data in this region, but the data available indicates it is consistent with the observation here.

From Fig. 2(a) and (b), the general trends for the 10- and 20-day sludge age mixed liquors show remarkable similarity. Further, as noted above, there is close similarity between the data obtained in this investigation and that of Beeharry et al. [11]. This similarity in the OHO active organism's behaviour in the 10- and 20-day sludge age mixed liquors and in the investigation of Beeharry et al. [11] would suggest that the difference in the correspondence between theoretical and measured OHO biomass concentration observed by Wentzel et al. [9] at the different sludge ages, is not caused by the sludge age itself. The poor agreement between the measured and theoretical OHO active biomass for the 20-day sludge age mixed liquor of [9] appears to be caused by some factor(s), which does not depend on the sludge age.

3.5. Comparison between Parent System vs. Batch Aerobic Digestion Test

The measured OHO active biomass concentration at the start of each batch test is compared to the theoretical OHO active biomass concentration estimated by the steady state design model [1]. To illustrate the comparison, the measured vs. theoretical mixed liquor OHO active biomass data for all the batch tests are shown in Fig. 3. The correlation between the measured and theoretical OHO active biomass concentrations is remarkably good. The differences between the measured and theoretical values were estimated as 1.4% and 8.2% for the 10- and 20-day sludge age parent systems, respectively.

This close correlation provides substantive support for the OHO active biomass concept incorporated in activated sludge theory. In the previous batch growth tests, which are based on the growth process of OHO active biomass, the differences between the measured and theoretical values were large. However, in the batch aerobic digestion tests, which are based on the endogenous respiration process, the results show remarkably good correlation between the measured and theoretical values. This observation indicates that the major cause for the difference between the measured and theoretical OHO active biomass concentrations in the batch growth tests lies in the OHO growth process. Further investigations on the growth process in the batch growth test may need to be investigated.

4. Conclusions

The batch growth test, as a quick and easy measurement technique, was originally supposed to provide the measured OHO active biomass concentrations that would compare favourably with the theoretical values predicted by the steady state design and kinetic simulation models. This would substantiate the active biomass concept incorporated in these models, and thereby promote confidence in their application. However, similarly to that previously of Beeharry et al. [11], this proved to not be true. The OHO active biomass concentrations for both the 10- and 20-day sludge age mixed liquor were away from the theoretical concentrations. Despite the dissimilarity, in comparing the measured OHO active biomass concentration with the theoretical value, the data for both the 10- and 20-day sludge age mixed liquor were similar to each other. Also, if the data is examined more closely for both the 10- and 20-day mixed liquors, three regions could be identified. This general trend, irrespective of sludge age, requires further investigation for the microbiological behaviour. In the batch growth tests, which are largely based on the growth process of OHO active biomass, the differences between the measured and theoretical values were large. However, in the batch aerobic digestion tests, which are based on the endogenous respiration process, remarkably good correlation between the measured and theoretical values was obtained. From this observation, it is clear that the major cause for the difference between the measured and theoretical OHO active biomass concentrations in the batch growth tests, lies in the description and interpretation of the OHO growth process within the batch growth test itself.

Acknowledgments

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