Evaluation of Simultaneous Storage and Growth Model to explain Aerobic Biodegradation of Acetate

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Abstract
Though mathematical models have been developed to describe the process kinetics of microorganisms in activated sludge during the aerobic biodegradation of carbon sources, there is no commonly agreed model yet. ASM3 is a benchmark model which is based on the assumption that substrate is first stored as internal polymers before being used for growth during famine phase. But experimental observations indicated that the storage and growth occur simultaneously during the feast phase. Hence in this study, the evaluation of simultaneous storage and growth model (SSGM) is performed by comparing this with three established models such as ASM1, ASM3 and accumulation model for acetate biodegradation under aerobic conditions.

Oxygen uptake rate (OUR) measurements of biomass were carried out through batch experimental studies. The model parameters were estimated by fitting the experimentally observed OUR to the model using the algorithms of the optimization toolbox included in MATLAB.

Model calibration reveals that ASM1 model is not suitable to explain the observed experimental OUR during the famine phase implying storage compounds could play important role during that stage. Besides, the model corresponds to accumulation concept is not well fitted though it includes the storage phenomena. While both the ASM3 model and the model for simultaneous storage and growth on substrate can well describe the acetate biodegradation process, the SSGM seems to be sound from statistical point of view. However the OUR data alone is not sufficient to justify the suitability of those models. In this study, the statistically interpreted outcome related to parameter estimation appears to be quite reasonable as compared with previous study.

Keywords: model, oxygen uptake rate, parameter estimation, respirometer, activated sludge

Introduction

Proper understanding of substrate removal mechanisms in activated sludge plants is very essential for modeling purposes. Activated Sludge Model 1 (ASM1) was introduced in 1987[1], which became a benchmark for many researchers. The biomass in ASM1 model was considered to grow solely on the external substrate present and the oxygen consumption after the external substrate depletion was explained with the decay of biomass. Simplification and modification on ASM1 model was made by Gernaey et al.[2] and Vanrolleghem et al.[3] to describe the degradation of readily biodegradable substrate like acetate. However, ASM1 model could not explain the "tail" part of OUR profile that occurs during acetate biodegradation in batch experiments.

Research shows that the internal storage polymers play an important role in substrate removal mechanisms in activated sludge when the biomass is subjected to dynamic feast and famine conditions[4]. This fact was recognized in ASM3 which was formulated with the assumption that the readily biodegradable substrate is first stored as internal storage products and the growth occurs on the internal storage products[5]. However, the respirometric study conducted by Guisasola et al. revealed that while ASM3 model can describe the tail of oxygen uptake profile, it results in unrealistic and non-mechanistic model parameters[6].

This shortcoming in ASM3 was addressed with the formation of simultaneous storage and growth model (SSGM) that better interpreted the experimental data[7-9]. Sin et al. further modified the SSGM by improving the modeling of substrate metabolism under feast conditions, and proposing a second-order type kinetic expression for the degradation of storage products under famine conditions using acetate as the model substrate[10].

However, the study conducted by Beccari et al. revealed that SSGM predicted the growth much higher than estimated from experimentally observed ammonia consumption[11]. So, they proposed a model that assumes a preliminary "internal accumulation" where substrate is transported into the cell and maintained inside as such or slightly metabolized and/or "biosorption" step. Then, the accumulated compound can be used for growth either directly or through previous storage and subsequent use of stored product as described in ASM3. Though

several models have been proposed to explain the biodegradation mechanism of readily biodegradable compound like acetate, there is no commonly agreed model yet.

The main goal of this paper is to evaluate the simultaneous storage and growth model (SSGM) by comparing this with three established models such as ASM1, ASM3 and accumulation model for acetate biodegradation under aerobic conditions. An oxygen uptake rate (OUR) measurement of biomass was carried out during the experimental study and the estimated model parameters are analyzed and compared so as to get a quantitative description of acetate biodegradation process.

Methods

Batch Experiment

A titrimetric respirometer was installed in the laboratory that was equipped with DO and pH sensors along with a reactor having a capacity of 3.5 L (Figure 1). Compressed air was supplied continuously for proper aeration and an overhead stirrer was provided with the reactor in order to mix the contents uniformly. During the batch experiments, both pH and DO profiles were monitored every 5 seconds and pH was controlled at a set point of 7.8 ± 0.05 by automatic addition of base or acid solutions with two 3-way solenoid valves. Data acquisition of the analogue signals from the sensors was processed by a personal computer equipped with the *Labview* software package. The experiments were performed at a temperature of $20 \pm 0.5\ ^0$C. OUR was calculated based on the procedure explained in Gernaey et al.[12] using the experimentally determined value for K_{La}. Sodium acetate with a concentration of 75 mg COD/L was used in order to investigate the biodegradation mechanism. The sludge was collected from Wetalla Water Reclamation Plant (operated by Toowoomba City Council), Australia and acclimatized for at least for 3 days before starting the experiments. Thiourea (8 mg/L) was also added at the beginning of experimental run to inhibit nitrification.

Activated sludge models

The evaluation of SSGM was performed by comparing four different activated sludge models herein named Model 1, Model 2, Model 3 and Model 4 for the aerobic biodegradation of acetate and the simulation and parameter estimation were done by using MATLAB 7.1. Parameter estimation procedure consisted of using non-linear least-squares optimization to minimize the sum of squared errors (SSE) between the numerical solution for the modelled output and experimentally obtained OUR.

Simplified ASM1 model used by Vanrolleghem et al.[3] is presented in this paper as Model 1 where it was assumed that the biomass growth was linked directly with the external substrate consumption (Figure 2). The process matrix is presented in Table 1. The substrate affinity constant, K_S, the growth yield on substrate, $Y_{H,S}$, the first order time constant, τ and combined parameter, $\mu_{MAX,S}X_H$ were estimated by using non-linear estimation technique whereas the biomass growth, X_H was considered constant due to short degradation period of acetate.

The suitability of Model 2 was verified in this study which is based on simplified ASM3 model[5-6]. The basic assumption is that the readily biodegradable compound, acetate is removed only by storage and then growth occurs on internal storage polymer (Refer Figure 3 and Table 2 for concept diagram and the process matrix respectively). With reference to parameter estimation, the maximum storage rate of biomass, k_{STO}, the yield coefficient for storage on substrate, Y_{STO}, the maximum growth rate on storage products, $\mu_{MAX,STO}$, the yield coefficient for growth on storage products, $Y_{H,STO}$ and the parameters K_S as well as τ were estimated.

Simultaneous storage and growth model (SSGM) for aerobic biodegradation of carbon source described by Sin et al.[10] is presented in this paper as Model 3. Figure 4 represents the processes involved in Model 3 during the aerobic biodegradation of acetate. Under feast condition, the metabolic model approach was employed. The yield coefficients of storage, direct growth on substrate and growth on internal storage products respectively were linked to each other through metabolism of the substrate. Besides, the yield coefficients were found correlated with the efficiency of the oxidative phosphorylation (δ). This model eases the way to estimated only one parameter (δ), instead of three yield coefficients[10]. Besides, under famine conditions, a second order model was used to describe the degradation of storage products. The maximum substrate uptake rate (q_{MAX}), the fraction of substrate used for storage (f_{STO}) as well as the parameters K_S, δ, K_1, K_2, and τ were estimated by following the non-linear estimation technique (process matrix is shown in Table 3). The maximum storage rate (k_{STO}) and the maximum growth rate of biomass ($\mu_{MAX,S}$) were calculated from the estimates of the parameters q_{MAX} and f_{STO} based on the procedure explained by Sin et al. where they assumed the parameter $\mu_{MAX,STO}$ to be the same order of magnitude as $\mu_{MAX,S}$[10].

In Model 4, it was assumed that the first step of substrate removal is always a sort of internal accumulation according to Beccari et al.[11]. The accumulation compound then can be used for growth either directly or through previous storage and subsequent use of the stored product (Figure 5). The process matrix is presented in Table 4. With regard to parameter estimation, the parameters k_{STO}, $\mu_{MAX,STO}$, $Y_{H,ACC}$, k_{ACC}, $K_{H,ACC}$, K_S and $K_{STO,ACC}$ were estimated to fit the model with the experimental OUR data. The maximum amount of biosorbed/accumulated compound ($f_{max,acc}$) and the parameter $K_{H,STO}$ were fixed to 0.2 and 1.0 respectively as used by Beccari et al. in his model[11],whereas, the parameter Y_{ACC} was fixed to 0.99 considering low energy requirement in the process. Besides, the reasonable values for the parameters $Y_{H,STO}$ and $Y_{STO,ACC}$ were assumed to fit the experimental profile with the model.

The initial concentration of biomass, $X_H(0)$ was calculated using the baseline endogenous OUR level prior to substrate addition for Models 1, 2 and 4 using $OUR_{end}(0) = b_H.X_H(0)$, whereas it was calculated using $OUR_{end}(0) = (1-f_{XI}).b_H.X_H(0)$ for Model 3. The default values assigned in the ASM3 model for the parameters b_H and b_{STO} (0.2 per day), f_{XI} (0.2) and $K_{H,STO}$ (1) were assumed during the analysis. The empirical factor ($1-e^{-t/\tau}$) was added in the kinetics to describe the so-called "start-up" phase observed in the batch OURs for Models 1, 2 and 3, where as it was not considered in Model 4 (refers to the model by Beccari et al.[11]).

Results and Discussions

OUR profile

Batch experiment was conducted for OUR profile study using acetate with a concentration of 75 mg COD/L. Figure 6 represents the OUR profiles along with model calibration.

Acetate is consumed during the feast period, as a result the OUR increases to a maximum level and remains same until acetate is completely removed for aerobic growth which is described in ASM1 model[1-3], or for the storage followed by growth that refers to ASM3 model[13], or for simultaneous storage and growth[10,14] or even through accumulation or sorption phenomena[15-16].The OUR during the famine phase drops from the maximum level to a level higher than the endogenous OUR level and gradually reaches to the endogenous level (Figure 6) which is assumed due to the consumption of previously stored product[4].

Parameter Estimation Results

Four different models are compared to get a quantitative description of the experimental behaviour on the basis of OUR profile study.

The parameters related to Model 1 is presented in Table 5. The model parameter $Y_{H,S}$ (growth yield) is found 0.81 after fitting the experimental data with ASM1 model (Model 1) which is higher than its default value (0.67). However, a closer value (0.78) for growth yield was observed by Vanrolleghem et al. in his study[3]. Other study conducted by Guisasola et al. found $Y_{H,S}$ varies from 0.76 to 0.79 which is due to the storage phenomena[6]. From current research, the parameters K_S (2.3 mg COD/L) and $\mu_{MAX,S}$ (0.0046 min^{-1}) are found to be within the range observed by Guisasola et al.[6] whereas these values found to be 0.66 mg COD/L and 0.00071 min^{-1} by Vanrolleghem et al.[3] in his study respectively. However, it is clear from the current study that the Model 1 is not suitable to fit the famine part of the experimental data due to exclusion of storage phenomena.

In Model 2, the estimated parameters $\mu_{MAX,STO}$ (27.9 day^{-1}) and $Y_{H,STO}$ (0.83) show higher values than the ASM3 default one (2 day^{-1} and 0.63 respectively) which is presented in Table 6. Such a high value for both the parameters $\mu_{MAX,STO}$ (28-64 day^{-1}) and $Y_{H,STO}$ (0.8-0.96) were also noticed by Guisasola et al. in his study where the possible reason he attributed was due to the overestimation of storage production (X_{STO}) with respect to experimental one[6]. High growth yields were also observed from the study conducted by Beccari et al. and Koch et al.[11,17], which were 0.85 and 0.8 respectively. The estimated yield coefficient for storage, Y_{STO} is 0.85, which meets the default value prescribed by ASM3 model.

The estimated parameter, f_{STO} in Model 3 shows a value of 0.65 (Table 7) which supports the observation made by Sin et al. (0.6-0.65). The calculated storage uptake rate, k_{STO} (3.7 day^{-1}) is found faster than the maximum growth rate, $\mu_{MAX,S}$ (1.6 day^{-1}) which is also observed by Sin et al. and Pratt et al.[10,18]. Besides, the average yield coefficient for storage on substrate, Y_{STO} is found to be higher (0.88) than the average yield coefficient for growth on substrate, $Y_{H,S}$ (0.71) which is similar to the findings by Sin et al.[10]. The substrate affinity constant, K_S estimated by Sin et al. is lower (0.6 -0.67 mg COD/L) than the ASM3 default value (2.0 mg COD/L), and the current study estimated the value as 2.29 mg COD/L. However, the estimated parameter, K_2 shows higher confidence interval though similar problem was noticed by Sin et al. in his study which is

explained due to the correlation between the parameters K_1 and K_2 under feast phase of the biodegradation process[10].

Table 8 shows the estimated parameters related to Model 4. In current study, the parameters k_{ACC}, k_{STO}, $\mu_{MAX,STO}$ and $Y_{H,ACC}$ are found higher than the values optimized by Beccari et al.[11], where he concluded the values 0.03 h^{-1}, 1.8 h^{-1}, 0.3 h^{-1} and 0.77 respectively. Besides, the estimated parameter $K_{STO,ACC}$ shows a value of 0.37 where as the study conducted by Beccari et al. showed the value equal to 0.45. The results for the estimated parameters K_S (1.67 mg COD/L) and $K_{H,ACC}$ (0.12 mg/mg) give lower values than that predicted by Beccari (2 mg COD/L and 0.21 mg/mg respectively). Besides, the calculated confidence intervals particularly for the parameters k_{STO} and $K_{STO,ACC}$ are found to be much higher in this model which is questionable.

Along with parameter values Tables 5-8 represent the confidence interval and the sum of squared errors (SSE) related to four different models in order to evaluate them statistically. The calculated SSE in Model 1 is found 9.784 that is relatively higher as compared with other three models. The lowest SSE was observed for both Model 2 and Model 3. The successful validation of these models requires the measurement of substrate, storage products, accumulated substrate and ammonia along with OUR data. In the absence of such data, an attempt was made to compare the simulated profiles of acetate and storage for the acetate pulse of 75 mg COD/L for the three models (Figure 7). The substrate degradation rate for Models 2, 3 and 4 were almost same as shown in Figure 7 except that for Model 4 as the initial lag phase was excluded in the original model. The simulated storage profile using the model outputs corresponds to Model 2 (representing ASM3) gives higher storage rate, followed by Model 3 (representing SSGM) and Model 4 (accumulation). This reinforces the fact that ASM3 overestimates the formation of storage products as experimentally determined by Krishna and van Loosdrecht and Beccari et al.[8-11]. Besides, in their experimental evaluation of storage products, Beccari et al. concluded that both Model 3 and Model 4 were found to be well fitted with the observed storage phenomenon[11]. In our simulation, Model 4 predicted the storage formation lower than that of Model 3. On the basis of parameter estimation and model simulation, the simultaneous storage and growth mechanism (Model 3) as modified by Sin et al.[10] seems to be reasonable as compared with other three models in order to explain the acetate biodegradation.

Conclusions

Three different established models have been compared with SSGM along with their parameter estimation on the basis of OUR profile study where acetate was used as test substrate. Model 1 (ASM1) is found to be unsuccessful to describe the experimental behaviour particularly the "tail" part of the OUR profile as ASM1 model excluded the storage principle during degradation process. Model 4 that includes "accumulation" phenomena is not well-fitted with the experimental OUR data. Besides, the concept of accumulation is too difficult to quantify using experiments for validation purposes. Though Model 2 (ASM3) and Model 3 (SSGM) give better representation of experimental OUR profile, simulated storage profiles confirm the observations made by previous researchers that ASM3 overestimates the storage product higher than the experimentally detected ones. As a result, among the models investigated, the simultaneous storage and growth mechanism appears better to explain the acetate biodegradation process.

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Tables and Figures

Table 1 Stoichiometric matrix related to Model 1

Process	X_H	S_S	S_O	Kinetics
Aerobic growth on S_S	1	$-1/Y_{H,S}$	$-(1-Y_{H,S})/Y_{H,S}$	$(1-e^{-t/\tau}).\mu_{MAX,S}.M_S.X_H$

Table 2 Stoichiometric matrix related to Model 2

Process	X_H	X_{STO}	S_S	S_O	Kinetics
Formation of X_{STO}		1	$-1/Y_{STO}$	$-(1-Y_{STO})/Y_{STO}$	$(1-e^{-t/\tau}).k_{STO}.M_S.X_H$
Aerobic growth on X_{STO}	1	$-1/Y_{H,STO}$		$-(1-Y_{H,STO})/Y_{H,STO}$	$\mu_{MAX,STO}.M_{X_{STO}/X_H}.X_H$
Endogenous respiration	-1			-1	$b_H.X_H$
X_{STO} respiration		-1		-1	$b_{STO}.X_{STO}$

Table 3 Stoichiometric matrix related to Model 3

Process	X_H	X_{STO}	S_S	S_O	Kinetics
Formation of X_{STO}		1	$-1/Y_{STO}$	$-(1-Y_{STO})/Y_{STO}$	$(1-e^{-t/\tau}).k_{STO}.M_S.X_H$
Aerobic growth on S_S	1		$-1/Y_{H,S}$	$-(1-Y_{H,S})/Y_{H,S}$	$(1-e^{-t/\tau}).\mu_{MAX,S}.M_S.X_H$
Aerobic growth on X_{STO}	1	$-1/Y_{H,STO}$		$-(1-Y_{H,STO})/Y_{H,STO}$	$\mu_{MAXSTO}\left(\frac{(X_{STO}/X_H)^2}{K_2+K_1.(X_{STO}/X_H)}\right)\left(\frac{K_S}{S_S+K_S}\right)X_H$
Endogenous respiration	-1			$-(1-f_{XI})$	$b_H.X_H$
X_{STO} respiration		-1		-1	$b_{STO}.X_{STO}$

Table 4 Stoichiometric matrix related to Model 4

Process	X_H	X_{STO}	X_{ACC}	S_S	S_O	Kinetics
Accumulation of X_{ACC}		1	Y_{ACC}	-1	$-(1-Y_{ACC})$	$k_{ACC}.M_S.\left(1-\frac{X_{ACC}/X_H}{f_{\max,acc}}\right).X_H$
Storage of X_{ACC}		$Y_{STO,ACC}$	-1		$-(1-Y_{STO,ACC})$	$k_{STO}.\left(\frac{X_{ACC}/X_H}{K_{STO,ACC}+X_{ACC}/X_H}\right).X_H$
Aerobic growth on X_{ACC}	1		$-1/Y_{H,ACC}$		$-(1-Y_{H,ACC})/Y_{H,ACC}$	$\mu_{MAX,STO}.M_{X_{ACC}/X_H}.X_H$
Aerobic growth on X_{STO}	1	$-1/Y_{H,STO}$			$-(1-Y_{H,STO})/Y_{H,STO}$	$\mu_{MAX,STO}.M_{X_{STO}/X_H}.X_H$
Endogenous respiration	-1				-1	$b_H.X_H$
X_{STO} respiration		-1			-1	$b_{STO}.X_{STO}$

Table 5 Parameter estimation results related to Model 1

Parameters	Acetate 75 mg COD/L (Confidence interval, %)
Parameters Estimated:	
$\mu_{MAX,S}$ .X_H (mg/L.min)	3.19+/-0.008 (0.25)
K_S (mgCOD/L)	2.3+/-0.041 (1.78)
$Y_{H,S}$ (mg X_H/mg S_S)	0.81+/-2.6x10^{-4} (0.03)
τ (min)	2.89+/-0.025 (0.87)
Parameters Assumed:	
b_H (1/min)	0.000139
Parameters Calculated:	
$\mu_{MAX,S}$ (1/min)	0.0046
X_H (mgCOD/L)	720
SSE	**9.784**

Table 6 Parameter estimation results related to Model 2

Parameters	Acetate 75 mg COD/L (Confidence interval, %)
Parameters Estimated:	
k_{STO} (1/min)	0.0046+/-7.38x10^{-6} (0.16)
$\mu_{MAX,STO}$ (1/min)	0.0194+/-1.6 x10^{-4} (0.83)
K_S (mgCOD/L)	2.16+/-0.041 (1.9)
$Y_{H,STO}$ (mgCOD X_H/mgCOD X_{STO})	0.83+/-7.6 x10^{-4} (0.09)
Y_{STO} (mgCOD X_{STO}/mgCOD S_S)	0.85+/-3.3 x10^{-4} (0.04)
τ (min)	1.88+/-0.012 (0.64)
Parameters Assumed:	
b_H (1/min)	0.000139
b_{STO} (1/min)	0.000139
$K_{H,STO}$ (mgCOD X_{STO}/mgCOD X_H)	1
Parameters Calculated:	
X_H (mgCOD/L)	720
SSE	**0.167**

Table 7 Parameter estimation results related to Model 3

Parameters	Acetate 75 mg COD/L (Confidence interval, %)
Parameters Estimated:	
q_{MAX} (1/min)	$0.004509+/-1.3x10^{-5}$ (0.29)
K_S (mgCOD/L)	2.29+/-0.063 (2.75)
f_{STO} (mgCOD X_{STO}/mgCODS_S)	0.65+/-0.022 (3.38)
K_1 (mgCOD X_{STO}/mgCOD X_H)	0.065+/-0.004 (13.8)
K_2 (mgCOD X_{STO}/mgCOD X_H)	$5.7x10^{-6}+/-1.5x10^{-5}$ (263.2)
δ (mol/mol)	4.16+/-0.098 (2.36)
τ (min)	2.78+/-0.02 (0.72)
Parameters Assumed:	
b_H (1/min)	0.000139
b_{STO} (1/min)	0.000139
f_{XI} (mgCOD /mgCOD)	0.2
Parameters Calculated:	
k_{STO} (1/min)	0.002573
$\mu_{MAX,S}$ (1/min)	0.001118
$\mu_{MAX,STO}$ (1/min)	0.001118
$Y_{H,S}$ (mg X_H/mg S_S)	0.71
$Y_{H,STO}$ (mgCOD X_H/mgCOD X_{STO})	0.78
Y_{STO} (mgCOD X_{STO}/mgCOD S_S)	0.88
X_H (mgCOD/L)	900
SSE	**0.167**

Table 8 Parameter estimation results related to Model 4

Parameters	Acetate 75 mg COD/L (Confidence interval, %)
Parameters Estimated:	
k_{ACC} (mgCOD S_S/mgCOD X_H .min)	$0.005459+/-2.2x10^{-5}$ (0.4)
k_{STO} (1/min)	0.132+/-0.273 (206.82)
$\mu_{MAX,STO}$ (1/min)	$0.01614+/-1.8x10^{-4}$ (1.12)
K_S (mgCOD/L)	1.67+/-0.072 (4.31)
$K_{H,ACC}$ (mgCOD X_{ACC}/mgCOD X_H)	0.12+/-0.0019 (1.58)
$K_{STO,ACC}$ (mgCOD X_{STO}/mgCOD X_{ACC})	0.37+/-0.775 (209.46)
$Y_{H,ACC}$ (mgCOD X_H/mgCOD X_{ACC})	0.84+/-0.0015 (0.18)
Parameters Assumed:	
b_H (1/min)	0.000139
b_{STO} (1/min)	0.000139
$K_{H,STO}$ (mgCOD X_{STO}/mgCOD X_H)	1
Y_{ACC} (mgCOD X_{ACC}/mgCOD S_S)	0.99
$Y_{H,STO}$ (mgCOD X_H/mgCOD X_{STO})	0.75
$Y_{STO,ACC}$ (mgCOD X_{STO} /mgCOD X_{ACC})	0.85
$f_{max,acc}$ (mgCOD X_{ACC}/mgCOD X_H)	0.2
Parameters Calculated:	
X_H (mgCOD/L)	720
SSE	**0.242**

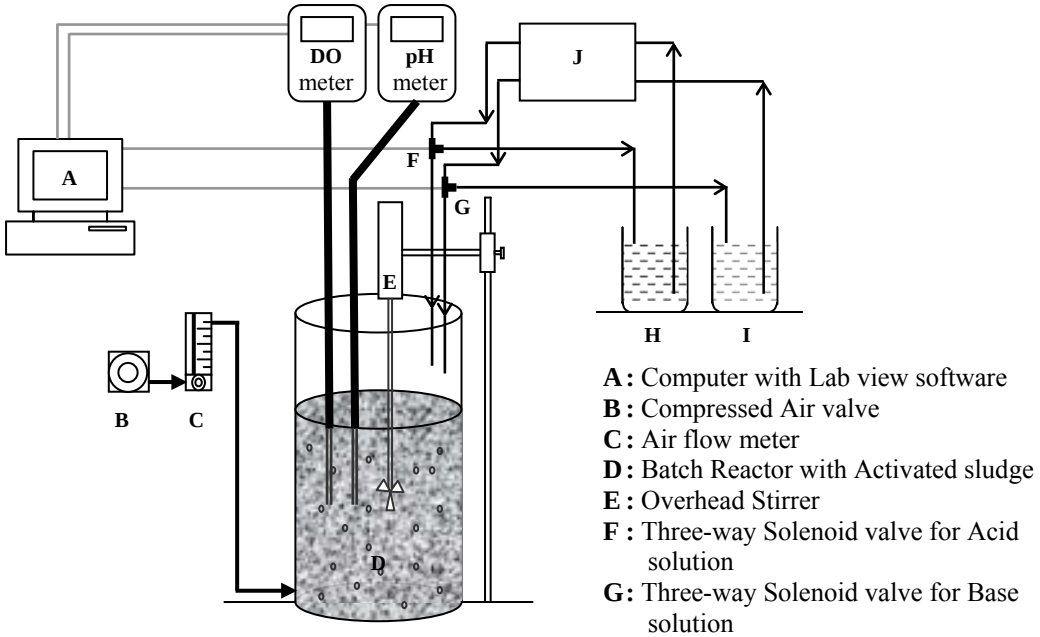

Figure 1 A schematic overview of activated sludge-based respirometer

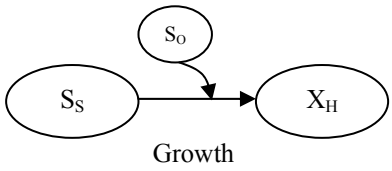

Figure 2 Processes involved in Model 1 during acetate biodegradation

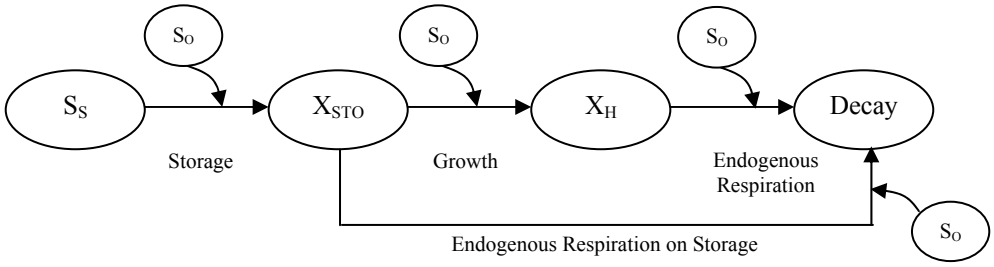

Figure 3 Processes involved in Model 2 during acetate biodegradation

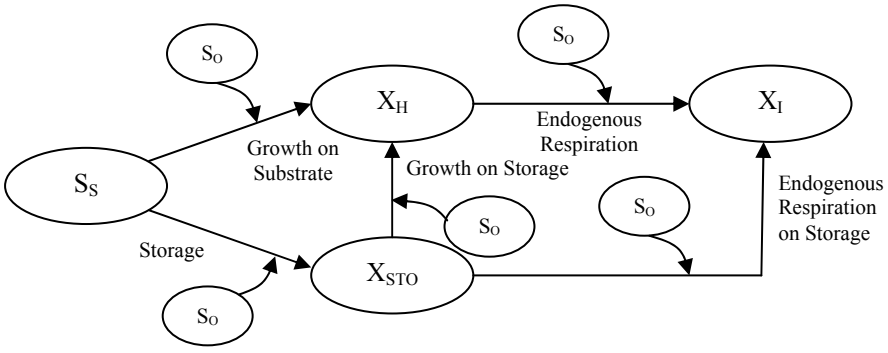

Figure 4 Processes involved in Model 3 during acetate biodegradation

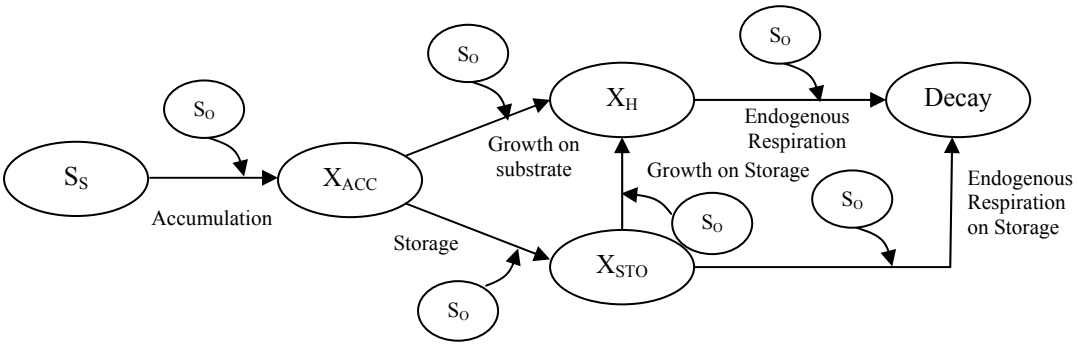

Figure 5 Processes involved in Model 4 during acetate biodegradation

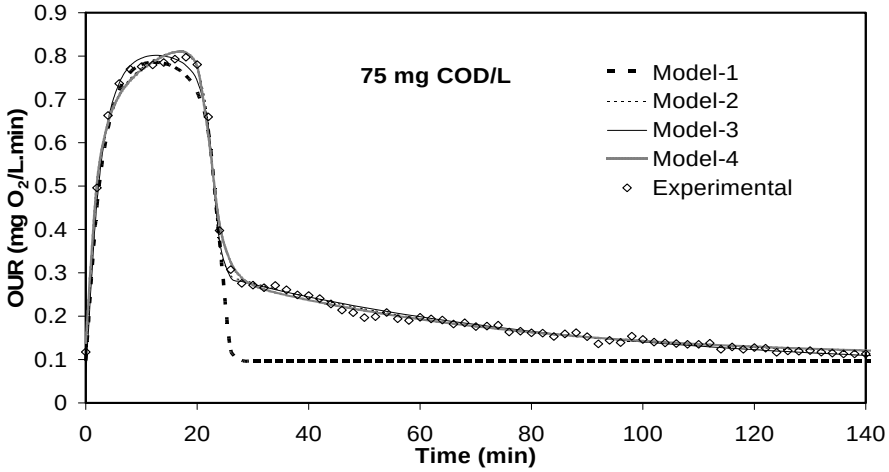

Figure 6 Model comparisons with experimental data (every 24th data point is presented to keep the figure clearer) for the acetate pulse of 75 mg COD/L

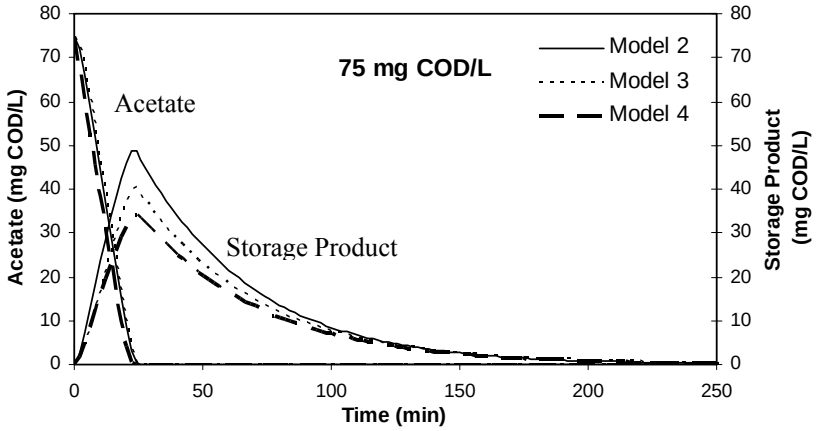

Figure 7 Simulated profiles (acetate and storage product) for the acetate pulse of 75 mg COD/L