A theory of the lifecycle of bacteria

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Abstract: The principal objective of this study was to investigate viability kinetics in aerobic AS (Activated Sludge). A kinetic model was developed in order to predict viable cells, non-degraded dead cells and inert VSS (Volatile Suspended Solids) in a biological reactor – usually conventional models only predict active biomass and inert VSS. The following processes were considered in the derivation of the model: the death rate of viable cells, and the hydrolysis rate of dead cells. Equations development led to a decay coefficient (b) – previously considered constant – function of death rate, hydrolysis rate and solid retention time. Cell growth on soluble COD (Chemical Oxygen Demand) resulting from lysis/hydrolysis has been introduced in the model. Simulation methods of viability in AS, and OUR (Oxygen Uptake Rate) tests of AS in batch under starvation were developed in order to validate the approach, and to estimate model parameters. The OUR test results have been refined introducing the lysis process. The results from the herein study indicate that the proposed models have good explanatory power of experimental data, and provide strong evidence of regulatory processes controlling bacterial death and lysis. [Nature and Science 2010;8(9):121-31]. (ISSN: 1545-0740).

Key words: activated sludge; cryptic growth; death; hydrolysis; lysis; viability.

1. Introduction

The early models for AS were based on the solid retention time parameter, the yield coefficient for biomass growth on available substrate, and the decay coefficient (Lawrence & McCarty, 1970). While investigating AS with high solid retention time, Kountz & Forney (1959), and Symons & McKinney (1958) concluded that cells contained an inert fraction which could not be biodegraded. Some investigators tried to incorporate the viability concept in the bio-oxidation process (Grady & Roper, 1974); however, their model did not take into account inert VSS. In more recent works the death/lysis and cryptic growth of bacteria has been examined (Mason et al., 1986).

The IWA task group (Henze et al., 1987; Henze et al, 2000) developed a series of activated sludge models which constitute the platform for waste water treatment modeling. A quotation from Henze et al. (1987) is that “biomass is lost by decay, which incorporates a large number of mechanisms including endogenous metabolism, death, predation and lysis”.

Loosdrecht & Henze (1999) also defined a mechanism called “maintenance” which is energy consumption under the use of internal stored substrate such as glycogen or PHA.

One of the core hypotheses underlying the herein study is that there are regulatory processes controlling bacterial death and lysis. This idea has been previously proposed by Rice and Bayles (2008) from their statement: "Contemporary study of cell death and lysis in a number of different bacteria has revealed that these processes, once thought of as being passive and unregulated, are actually governed by highly complex regulatory systems."

The current study does not consider biological processes such as nitrification, denitrification, and phosphorous removal, which are beyond the scope of the study - the focus is on viability processes for carbon oxidation of AS.

2 Material and Methods

2.1 Model kinetics

The notations from Corominas et al. (2010) have been used for the mathematical development below, and new notations were introduced when necessary.

Notion of viable cells, active biomass and inert VSS

The definition of viable and active biomass is subject to interpretation. The following definitions

were used in the present study: What is called "active VSS" (Xa) is not linked to the microbial activity, but represents the total amount of non-inert VSS (i.e. all cells dead or alive that are not degraded). What is called "viable VSS " (Xv) represents the amount of living bacteria which are able to grow and breathe, and can be detected with activity methods such as ATP measurements. Hence (Xd) – the "active VSS" minus the "viable VSS" – is the amount of non-degraded dead cells. The inert fraction (Xi) is the amount of material that is not degraded further.

The decay coefficient (b) may be defined as the lysis/hydrolysis rate of dead cells relative to the total active biomass.

Phases in an activated sludge:

$$X_{vss} = X_a + X_i = (X_v + X_d) + X_i$$

Where: Xvss = total VSS (gVSS/L); Xi = refractory inert VSS (gVSS/L); Xa = active biomass (gVSS/L); Xv = viable cells (gVSS/L); Xd = dead cells still not degraded (gVSS/L); [L for litre]

The following processes were considered for model development: the death rate of viable cells; hydrolysis of dead cells; cell growth on influent substrate; cell growth on soluble substrate from lysis/hydrolysis, usually designated the cryptic growth.

The heuristic for the development of the models below is that the death rate, lysis and hydrolysis rates, follow a first order rate kinetics.

For the model development below it is assumed that the use of internal storage products for cellular maintenance may be neglected, or simply aggregated in the lysis process.

Model 1 (model of the present study)

In the model shown in figure 1, the model of this study, for simplicity purpose the lysis process was not taken into account, and has been aggregated with the model hydrolysis rate.

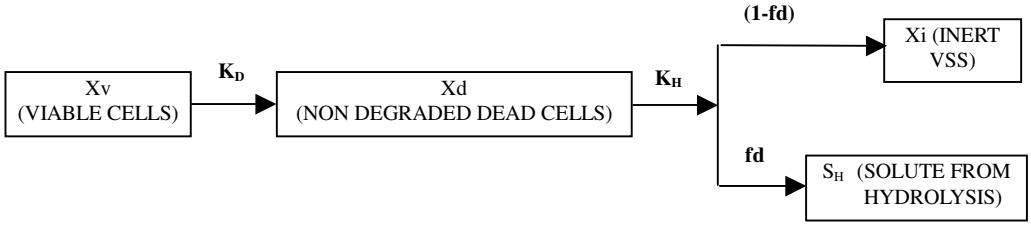

Figure 1. Model of this study: activated sludge kinetics including death and hydrolysis processes, where: K_D = model death rate (M M^{-1} T^{-1}); K_H = model hydrolysis rate (M M^{-1} T^{-1}); fd = degradable fraction of cells; S_H = substrate from hydrolysis, in solute; [M for mass, T for time].

Model 2: Notion of lysis, intrinsic hydrolysis rate and death rate

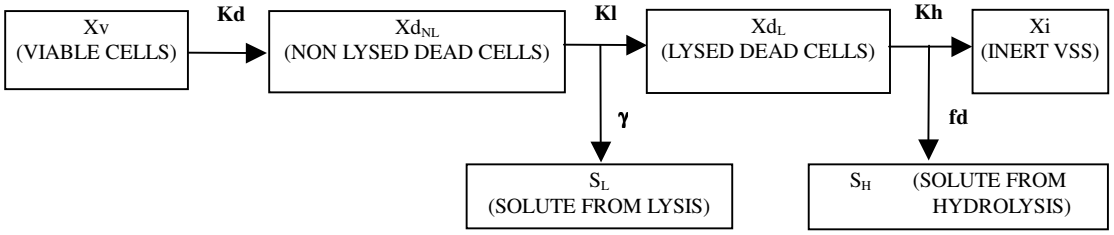

Figure 2. Activated sludge kinetics model including death, lysis and hydrolysis processes (in which: Kd = intrinsic death rate (M M^{-1} T^{-1}); Kl = lysis rate (M M^{-1} T^{-1}); Kh = intrinsic hydrolysis rate (M M^{-1} T^{-1}); γ = the ratio of VSS released during lysis to the total VSS of the cell (gVSS/gVSS); S_L = substrate from lysis, in solute; S_H = substrate from hydrolysis, in solute); [M for mass, T for time].

The model shown in figure 2 includes the lysis process, and therefore, is more detailed than model 1. The equation development for this model are more complex, and the results do not differ significantly from the simplified model 1 (figure 1).

However, it should be emphasized that neglecting the lysis process leads to a model hydrolysis rate (K_H) different from the intrinsic hydrolysis rate (Kh). The rationale is that the model hydrolysis rate (K_H) incorporates the lysis process, and therefore differs from the intrinsic hydrolysis rate (Kh).

2.2 Hypothesis of an aging process controlling bacterial death

A new aspect in this study is the introduction of a viable cell death rate, which can be defined as the number of cells dying per unit of time divided by the total amount of viable cells. Skeptics might criticize this approach, as it is generally perceived that bacterial death is caused by predation, bacteriophage, and viruses. This would raise the thorny question: how can one measure the life duration of a bacterium which performs many cell divisions? The results of the present study show that there is an overall death rate for the bacterial population of interest; although this may be due by many factors.

Let us introduce an approach that may be used to test the hypothesis of an aging process controlling bacterial death. Based upon the analogy between cells in a bioreactor and living organisms in a surrounded space, it can be inferred that in such system, the viable cell population has an expected life duration (τ) and an average age (A_{av}), which are statistical measures. The time needed for such an ideal population to die without growth is (τ-A_{av}). The death rate (Kd) being defined as the number of bacteria that die per unit of time divided by the total amount of alive bacteria, the following expression is obtained:

$$Kd = \frac{1}{X_v}\left(\frac{dX_v}{dt}\right) \approx \frac{1}{X_v}\frac{X_v}{(time\ left)}$$

Hence: $$Kd = \frac{1}{\tau - A_{av}} \quad (1)$$

For high solid retention time (θc), the average age (A_{av}) of a population controlled by aging should be roughly τ/2 (which is true for a flat or nodal distribution of the age population). In this situation, the observed death rate is Kd[θc large] = 2/τ. Let us consider the situation where θc is smaller than τ/2; in this case the expectation is that the average age (A_{av}) of such a population is between 0 and θc. In other words the solid retention time is being used as a way to select young bacteria. For the limit condition, θc tending to zero, the observed death rate should converge towards Kd[θc small] = 1/τ = Kd[θc large]/2. If the hypothesis of an aging process for bacteria holds, then for solid retention time smaller than τ/2 the death rate shall be half its observed value with solid retention time larger than τ. This analysis does not take into consideration the synchronized growth phenomenon which should produce a spike between τ/2 and τ. The author suggests using indirect methods such as decay coefficient measurements to derive the observed death rate under different solid retention times.

2.3 Equation development for model 1

Let κ, the dead over viable cell ratio, be set so that :

$$\kappa = \frac{X_d}{X_v} \quad (2)$$

Mass balance on dead cells (Xd)

We get: $$\frac{dX_d}{dt} = K_D \cdot X_v - K_H \cdot X_d$$

Considering a CSTR (Continuous Stirred-Tank Reactor), and dead cells equal to zero at initial conditions, the following expression is obtained:

$$\frac{X_d}{\theta_c} = \frac{K_D \cdot X_d}{\kappa} - K_H \cdot X_d$$

Hence: $$\kappa = \frac{K_D}{K_H + 1/\theta_c} \quad (3)$$

Mass balance on inert VSS (Xi)

We get: $$\frac{dX_i}{dt} = K_H\ (1 - f_d)\ Xd$$

For a CSTR with initial Xi equal to zero, we get :

$$X_i = K_H\ (1 - fd)\ \theta_c\ \frac{Xa}{(1 + 1/\kappa)}$$

As: $X_i = b\ (1 - f_d)\theta_c \cdot X_a$

The following expression is derived:

$$b = \frac{\kappa \cdot K_H}{1 + \kappa} \quad (4)$$

Combining equations (3) and (4), the following expression is obtained:

$$\kappa = \frac{\theta_c\ (K_D - b)}{1 + b \cdot \theta_c} \quad (5)$$

Mass balance on active biomass (Xa)

(i) As: $X_a = X_v + X_d$
Then: $X_a = (1+\kappa)\ X_v$ (6)

(ii) Given:

$$\frac{dX_a}{dt} = Y \cdot \frac{dS}{dt} - b \cdot X_a + Y^H \cdot f_d \cdot b \cdot 1.42 \cdot X_a$$

And considering a CSTR, and Xa initial equal to zero, the following expression is obtained :

$$X_a = \frac{\theta_c \cdot Y\ (S_o - S)}{\theta\,(1 + b\,(1 - 1.42 \cdot f_d \cdot Y^H))} \quad (7)$$

Where: So = influent substrate concentration (mgBOD/L); S = substrate concentration in reactor (mgBOD/L); Y = growth cell yield on influent substrate (gVSS/gBOD$_5$); Y^H = growth cell yield on substrate from lysis/hydrolysis (gVSS/gCOD); θ = hydraulic retention time (days); θc =solid retention time (days); [BOD for biological oxygen demand, COD for chemical oxygen demand].

With: $X_i = b\,(1 - fd)\ \theta_c \cdot X_a$ (8)

As: $X_{vss} = X_a + X_i$; then:

$$X_{vss} = X_a\ (1 + b\,(1 - f_d)\ \theta_c) \quad (8a)$$

In equation 7, was introduced 1.42 gCOD/gVSS, the conversion factor of gram COD to oxidize one gram cell-VSS, computed as the stoichiometric ratio of gram oxygen over gram VSS; where the average activated sludge composition is $C_5H_7O_2N$ (Symons and McKinney, 1958), and the reaction is $C_5H_7O_2N + 5.O_2 \rightarrow 5.\ CO_2 + 2.\ H_2O + NH_3$ (Hence, the ratio is $160gO_2/113gC_5H_7O_2N$ = 1.42 gCOD/gVSS). (Note 8b)

The limit conditions with equations (3), (4) and (5) for θc large are the followings:

$$\kappa_\infty = \frac{K_D}{K_H} \quad (9)$$

$$K_{D\infty} = \frac{b_\infty \cdot K_H}{K_H - b_\infty} \quad (10)$$

$$b_\infty = \frac{K_D \cdot K_H}{K_D + K_H} \quad (11)$$

Equations (10) and (11) allow us to do the conversion between the decay (b) and the death rate (K_D), as VSS from substrate or biomass cannot be distinguished experimentally. For example, one can determine experimentally a death coefficient (K_D) for acidogenic biomass growing under anaerobic conditions using viability data with the ATP (Adenosine Tri-Phosphate) method. Then the corresponding decay (b) is derived from equation (11).

Viability in CSTR

Prior investigators could determine the viability of an AS with ATP measurements, by assuming that this activity is proportional to the viable biomass. From equations (3), (4), (6), (7) and (8a), we derive (equ. 12):

$$\frac{X_v}{X_{vss}} = \frac{K_H + 1/\theta_c}{K_H + 1/\theta_c + K_D\,(1 + K_H\ (1 - f_d)\ \theta_c)}$$

OUR (Oxygen Uptake Rate) in CSTR and in batch

From the mass balance on oxygen to metabolize the soluble substrate, we get equation (13):

$$OUR = \frac{dS}{dt}(1 - 1.42 \cdot Y) + \frac{dS_H}{dt}(1 - 1.42 \cdot Y^H)$$

With: $$\frac{dS_H}{dt} = 1.42 \cdot f_d \cdot K_H \cdot X_d$$

Where: S_H = substrate concentration from lysis/ hydrolysis (M L^{-3})

For a batch under starvation, dS/dt equal to zero, then:

$$OUR = (1 - 1.42 \cdot Y^H)\ 1.42 \cdot f_d \cdot K_H \cdot X_d \quad (14)$$

(1.42 is the conversion factor of gram COD to oxidize one gram cell-VSS, see note 8b)

In other words, the Oxygen Uptake Rate corresponds to the oxygen consumed during the oxidation of the soluble substrate available for bacteria. The growth yield coefficient (Y) [gVSS/gCOD] or 1.42*Y (converted in units of [gCOD/gCOD]) corresponds to the fraction of substrate used for synthesis. Hence, the remaining fraction (1-1.42*Y) is what is oxidized to CO2 and H20. (note 14 a)

Equations for the batch

To solve the mass balance equations on dead cells and viable cells for a batch, let us consider that a batch is equivalent to an infinite number of small CSTR that follow each other over the time. The approach to solve the equations for a PF (Plug Flow) is similar, considering that a PF is equivalent to an infinite number of small CSTR that follow each other in the

space dimension. Therefore, the following equations are obtained for the batch (under starvation).

Equation (15) is based on mass balance on viable cells:

$$\frac{dX_v}{dt} = Y\frac{dS}{dt} - K_D \cdot X_v + Y^H \cdot K_H \cdot f_d \cdot 1.42 \cdot X_d$$

For a batch under starvation, dS/dt = 0, then equation (15):

$$X_{v,N+1} = \frac{X_{v,N} + \Delta t \cdot Y^H \cdot f_d \cdot K_H \cdot 1.42 \cdot X_{d,N}}{1 + K_D \cdot \Delta t}$$

Equation (16) is based on mass balance on dead cells:

$$\frac{dX_d}{dt} = K_D \cdot X_v - K_H \cdot X_d$$

Leading to:

$$X_{d,N+1} = \frac{X_{d,N} + \Delta t \cdot K_D \cdot X_{v,N}}{1 + \Delta t \cdot K_H} \qquad (16)$$

Where: $Xv_{,N}$ = viable cells at time interval N (gVSS/L); $Xv_{,N+1}$ = viable cells at time interval N+1 (gVSS/L); $Xd_{,N}$ = dead cells at time interval N (gVSS/L); $Xd_{,N+1}$ = dead cells at time interval N+1 (gVSS/L); Δt = time interval; [L for litre].

2.4 Equation development for model 2

To be economical, details for the derivation of the equations of model 2 below were not provided; however, the approach is the same as for model 1. An assumption to derive the equations of model 2 is that the material released by lysis has the same stoichiometric composition than the remaining parts of the cell leading to the 1.42 gCOD/gVSS factor, and that the growth yield on the lysis substrate is the same than on the hydrolysis substrate.

CSTR equations

Initial conditions for the OUR test (figure 4) require the calculation of non lysed dead cells to the total amount of dead cells for the CSTR.

Let's define the dead non-lysed over dead cell ratio:

$$\lambda = \frac{X_d^{NL}}{X_d} \qquad (17)$$

From the mass balance on lysed dead cells (CSTR), and introducing $Xd = Xd^{NL} + Xd^{L}$, the following expression is obtained:

$$\lambda = \frac{K_h + 1/\theta_c}{K_h + K_l(1-\gamma) + 1/\theta_c} \qquad (17a)$$

Equations for the batch under starvation

The following expression for the OUR calculation (equation 18) is obtained for a batch under starvation:

$$OUR = (1 - 1.42 \cdot Y^H)(1.42 \cdot f_d \cdot K_h \cdot X_d^L + 1.42 \cdot \gamma \cdot K_l \cdot X_d^{NL})$$

From the mass balance on viable cells, we get the following discrete equation (19) for a batch under starvation:

$$X_{v,N+1} = \frac{X_{v,N} + \Delta t \left(1.42 \cdot Y^H \cdot f_d \cdot K_h \cdot X_{d,N}^L + 1.42 \cdot Y^H \cdot \gamma \cdot K_l \cdot X_{d,N}^{NL}\right)}{1 + \Delta t \cdot K_d}$$

The discrete equation to compute non lysed dead cells under starvation (model 2) is the following:

$$X_{d,N+1}^{NL} = \frac{X_{d,N}^{NL} + \Delta t \cdot K_d \cdot X_{v,N}}{1 + \Delta t \cdot K_l} \qquad (20)$$

The discrete equation to compute lysed dead cells under starvation (model 2) is the following:

$$X_{d,N+1}^{L} = \frac{X_{d,N}^{L} + \Delta t\ (1-\gamma)\ K_l \cdot X_{d,N}^{NL}}{1 + \Delta t \cdot K_h} \qquad (21)$$

Where: $Xv_{,N}$ = viable cells at time interval N (gVSS/L); Xv_{N+1} = viable cells at time interval N+1 (gVSS/L); $Xd^{NL}{}_{N+1}$ = non lysed dead cells at time interval N+1 (gVSS/L); $Xd^{NL}{}_{N}$ = non lysed dead cells at time interval N (gVSS/L); $Xd^{L}{}_{N+1}$ = lysed dead cells at time interval N+1 (gVSS/L); $Xd^{L}{}_{N}$ = lysed dead cells at time interval N (gVSS/L); Δt = time interval; [L for litre].

2.5 Unification of model 1 with model 2

As experimental data (figure 4) show that (K_H) is sensitive to the time scale, whereas the first order kinetics (Kh) and (Kl) are not, a unification of model 1 with model 2 was necessary. In addition the model unification is required to prove that the death rate is the same for both model 1 and 2, which is being used for the calibration in figure 4.

From the mass balance on dead cells for both models 1 and 2, we get the following expressions:

$$\left(\frac{dX_d}{dt}\right)_{model\ 1} = K_D \cdot X_v - K_H \cdot X_d$$

$$\left(\frac{dX_d}{dt}\right)_{model\ 2} = \frac{dX_d^{NL}}{dt} + \frac{dX_d^{L}}{dt}$$

With: $\frac{dX_d^{NL}}{dt} = K_d \cdot X_v - K_l \cdot X_d^{NL}$

And: $\frac{dX_d^{L}}{dt} = (1-\gamma)\ K_l \cdot X_d^{NL} - K_h\ X_d^{L}$

Hence matching (dXd/dt)model 1 with (dXd/dt)model 2, and introducing ($Xd = Xd^{NL} + Xd^{L}$), we get the following relationship (25):

$$K_D = K_d + \kappa \cdot \lambda\ (K_h - \gamma \cdot K_l) + \kappa\ (K_H - K_h)$$

From the mass balance on the overall decay for both models 1 and 2, we get the following expressions:

$$(b \cdot X_a)_{model\ 1} = K_H \cdot X_d$$

$$(b \cdot X_a)_{model\ 2} = K_l \cdot \gamma \cdot X_d^{NL} + K_h \cdot X_d^{L}$$

Matching these two equations, and introducing ($Xd = Xd^{NL} + Xd^{L}$), we get the following unification relationship for the hydrolysis rate:

$$K_H = K_h + \lambda\ (\gamma \cdot K_l - K_h) \quad (26)$$

Combining equation (25) and (26) we get the unification relationship for the death rate which is:

$$K_D = K_d \quad (27)$$

This is the proof that the death rate is the same for both model 1 and 2.

Table 1. Model from this study and conventional models (CSTR)

Characteristic	This study model (figure 1)	Conventional and prior models
Active biomass (gVSS/L)	$Xa = \frac{\theta c * Y * (So-S)}{\theta * (1+b*(1-1.42*fd*Y^{H})*\theta c)}$	$Xa = \frac{\theta c * Y * (So-S)}{\theta * (1+b*\theta c)}$ *(Lawrence & McCarty, 1970)*
Inert biomass (gVSS/L)	$Xi = b*(1-fd)*\theta c*Xa$	$Xi = b*(1-fd)*\theta c*Xa$
Dead over viable cells ratio	$\kappa = \frac{K_D}{K_H + 1/\theta c}$	
Decay coefficient	$b = \frac{\kappa * K_H}{1+\kappa}$	
Relationship between decay, death and lysis/hydrolysis for θc large	$b_\infty = \frac{K_D * K_H}{K_D + K_H}$	
Total VSS (gVSS/L)	$Xvss = Xa + Xi$	$Xvss = Xa + Xi$
Viable biomass (gVSS/L)	$Xv = \frac{Xa}{1+\kappa}$	
Viability	$Xv/Xvss = \frac{K_H+1/\theta c}{K_H+1/\theta c+K_D*(1+K_H*(1-fd)*\theta c)}$	$Xv/Xvss = \frac{K_H+1/\theta c}{K_H+1/\theta c+K_D}$ *(Grady & Roper, 1974)*
Oxygen Uptake Rate (gO2/L/d)	$OUR = \frac{dS}{dt}*(1-1.42*Y) + \frac{dS_H}{dt}*(1-1.42*Y^{H})$ with : $\frac{dS_H}{dt} = 1.42*b*fd*Xa$	
Oxygen Uptake Rate for a Batch under Starvation (gO_2/L/d)	$OUR = (1-1.42*Y^{H})*1.42*b*fd*Xa$	

Table 1 is a summary of the equations derived from mass balances from the model of this study (figure 1) for a CSTR, and compares the herein equations with conventional models available in the literature.

Some studies have shown that in specific cases, bacteria are substrate selective, and oxidize in priority the most favorable substrate from the energetic point of view, and subsequently adapt their enzymatic system to the metabolic pathway. The classical example of diauxic growth is E. coli with the substrates glucose and lactose, where glucose will be used preferentially. If the growth yield is higher for influent substrate than for lysis/hydrolysis, will the bacteria in the AS selectively oxidize the most favorable substrate? The author believes that diauxic growth is unlikely to happen for an ecological population, rather than single strain culture.

In many lab test studies, the sewage sludge is a filtrate, or a soluble synthetic sewage is used to feed the system, and there is no non-settled organic particulate matter that passes through the primary settler tank. For real waste water treatment plants it is recommended to account for both influent soluble COD and particulate COD remaining after primary sedimentation, introducing the hydrolysis of influent particulate COD. It is expected that the growth yield of bacteria on soluble substrate from hydrolysis of influent organic particulate be in the same order of magnitude than growth yield of bacteria on hydrolysis/lysis products.

3 Results

Parameter estimations from the literature for individual bacterial strains are given below for indication purpose only. It should be emphasized that it is not rigorous to compare kinetic parameters for an ecological bacterial population, with individual bacterial strains parameters; however, this may provide an indication for parameter estimation.

The decay coefficient (b)

As soon as you move to very low or very high sludge ages, the conventional activated sludge model does not work very well. The decay coefficient (b) has been determined with several methods leading to a wide range of values. Adjusting these decays, based upon the model of this study give consistent results. Some of these estimates and adjustments are described below:

For an AS of small solid retention time (θc) (typically θc < 3 days), inert VSS can be neglected; therefore, it is not a bad premise to consider that Xa ≈ Xvss. With this approach, a decay coefficient equal to 0.05 d^{-1} was found, based on experimental data from Metcalf & Eddy (1991). Taking into account the soluble substrate from lysis/hydrolysis of dead cells (equation (7)), the previously computed decay coefficient has to be divided by $(1-1.42*fd*Y^H)$. Hence, the adjusted decay coefficient (b) is 0.07-0.08 d^{-1}, which is consistent with decay obtained for a 2 day AS with the model of this study. Decay determined on basis of biodegradable microbial mass led to the following (b) value 0.18 d^{-1} (Lawrence & McCarty, 1962).

From the approach of this study, the decay coefficient (b) – not anymore a constant – is a function of death, lysis and hydrolysis processes. Assuming that (K_H) is constant, the decay (b) is sensitive to solid retention time (θc). From the practical point of view, it may be acceptable to use a constant value for the decay (b).

Estimation of the death rate (K_D)

The death rate of viable cells in AS under aerobic conditions was determined based on viability data for the CSTR. Equation (12), (Xviable/Xvss versus θc) was fitted with viability data – measured with ATP method – from previous studies. This method appears to be rather reliable, as (K_D) values were stable, despite the wide range of (K_H) values considered: 0.15 d^{-1} to 0.25 d^{-1}. Using this method, the model death rate (K_D) is estimated in the range of 0.55 to 0.7 d^{-1} (figure 3).

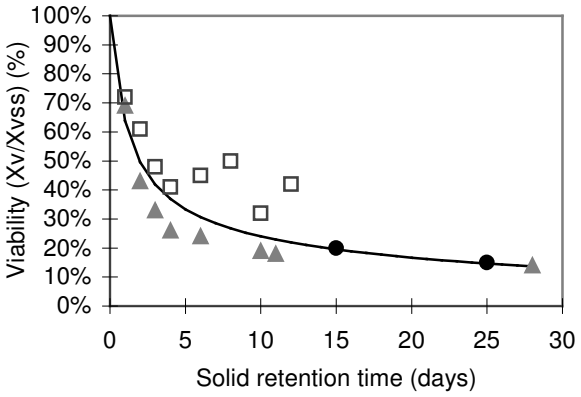

Figure 3. Calibration of (K_D) parameter using viability data based on ATP measurements. Viability in AS: (●), Patterson et al. (1970); (▲), Weddle and Jenkins (1971); (□), Nelson and Lawrence (1980), AS fed with synthetic waste water; (—), theoretical values with the model 1.

A similar death rate was obtained by Dold & Marais (1986) introducing the "death regeneration" process in AS. Considering that the material arising from death (except for inert fraction) becomes available as slowly biodegradable particulate substrate, they found a death rate (K_D) of 0.62 d^{-1} which is consistent with what is obtained in the current study. Using equation (12) with ATP measurements for a culture of Brevibacterium linens under starvation (Boyaval et al., 1985), a death rate (K_D) of 1.2 d^{-1} is obtained. Based on the same method for a slide cultures of A. aerogenes cells under starvation (Postgate & Hunter, 1962), the obtained death rate (K_D) is 0.8 d^{-1}.

Estimation of γ

Pavlostathis & Gossett (1986) used a lysis coefficient (γ) of 0.3 gBOD/gBOD. They did estimate this value by autoclaving AS, in order to break the cells and measure the released soluble COD. Note that the experimentalist needs to be cautious as the released COD may not be linked with the total amount of cells but with viable and non lysed dead cells. By taking a fresh sample of AS of small solid retention time (less than a day) one may consider that most of the VSS is composed of viable and non lysed dead cells.

Ultimate degradable fraction of an AS (fd)

A degradable fraction (fd) of 0.77 was found by Kountz and Forney (1959) – they found that the inert non biodegradable fraction was composed of polysaccharide material. This result was confirmed by other studies - a degradable fraction in the same order was found by Quirk and Eckenfelder (1986).

Evaluation of the model hydrolysis rate (K_H)

Solving equations (3) and (4) (with K_D = 0.6 d^{-1}, and b = 0.13 d^{-1} for a 10 day AS), a lysis/hydrolysis rate of dead cells under aerobic conditions (K_H) of 0.17-0.19 d-1 is obtained. This result is in the range of values found by prior investigators: Based on a OUR method, Eliosov & Argaman (1995) found a first order rate coefficient (K_H) of 0.16 to 0.20 d^{-1} for hydrolysis of organic particles in aerobic AS. Balmat (1957) found a first order rate hydrolysis (K_H) of 0.22 d^{-1}, for raw sewage and colloidal particles (0.08-1 μ in size) in AS. Pavlosthatis and Gossett (1985) obtained a hydrolysis rate of dead AS cells under anaerobic conditions of 0.16 d^{-1}, based on batch studies with autoclaved sludge inoculated with anaerobic cultures.

Estimation of growth yield coefficient on lysis/ hydrolysis substrate (Y^H)

The growth yield coefficient on lysis/hydrolysis substrate (Y^H) was estimated by performing a calibration with equations (3), (4), (5), (7), and (8a), on Xvss versus solid retention time experimental data published by Metcalf & Eddy (1991). This calibration led to a growth yield coefficient on lysis/hydrolysis substrate (Y^H) of 0.28 gVSS/gBOD, and a growth yield on influent substrate from sewage (Y) of 0.62 gVSS/gBOD.

Note that the growth yield (Y^H) obtained with this method is close to the cryptic growth yield found by previous investigators: Hamer (1985) used a cryptic growth yield of 0.23 gVSS/gCOD for aerobic thermophilic treatment of waste sludge; and in his thesis, Mason (1986) used a cryptic growth yield of 0.18 gVSS/gCOD for Klebsiella pneumonae cells.

Estimation of the lysis rate (Kl) and intrinsic hydrolysis rate (Kh)

An OUR method was developed for the calibration of the lysis and intrinsic hydrolysis rate, and to double check the prior estimates for the model hydrolysis rate and the growth yield on lysis/hydrolysis substrate. The rationale behind the present method is based on the consideration that "endogenous respiration" represents oxygen requirement to oxidize the soluble substrate released from lysis/hydrolysis under starvation. In order to implement this approach, discrete model equations for the batch have been derived earlier.

For this calibration, the experimental data from Gossett & Belser (1982) were used. Initial conditions were obtained by solving equations (3), (4), (6) and (7) for the feeding AS (Xa_o = 3000 mg/L; Xv_o = 830 mg/L; Xd_o = 2170 mg/L). For model 1, equations (15) and (16) were used to compute viable cells (Xv) and dead cells (Xd) at each time step, and the corresponding theoretical OUR with equation (14).

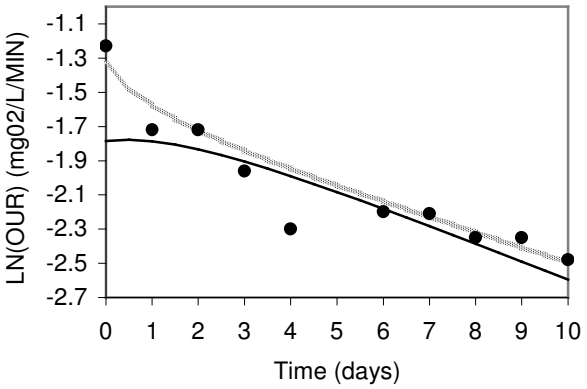

Figure 4. Calibration using an OUR method: (●), data from Gossett & Belser (1982) with an AS fed with synthetic sewage; (—), ln(OUR) with model 1; (▒▒▒), ln(OUR) with model 2 including lysis.

From figure 4 one can see that the computed OUR values using model 1 do not fit very well with the measurements as the lysis process was not taken into account – model 2 gives a much better fitting. Actually using the unification relationship (27) for model 1 by computing (K_H) at each time step, the result would reasonably fit the curve. The OUR calibration with both models 1 and 2 confirm the estimate for the growth yield on lysis/hydrolysis substrate (Y^H) of 0.28 gVSS/gCOD. In addition, for model 2, a good fitting was obtained with a hydrolysis rate (Kh) of 0.13 d^{-1}, and a lysis rate (Kl) of 2.0 d^{-1}. The intrinsic death rate (Kd) of 0.6 d^{-1} (from the calibration figure 3), and the γ of 0.30 d^{-1} estimated by Pavlostathis & Gossett (1986) were used for model 2.

For an aerobic batch under starvation containing an AS, one shall consider that the only source of substrate available in the medium is the soluble organic material released during lysis and hydrolysis of dead bacteria, which is the substrate oxidized by viable bacteria as measured with the Oxygen Uptake Rate. The above result (figure 4) constitutes a strong case to support the viability approach of this study.

Kappa, lambda and b versus solid retention time

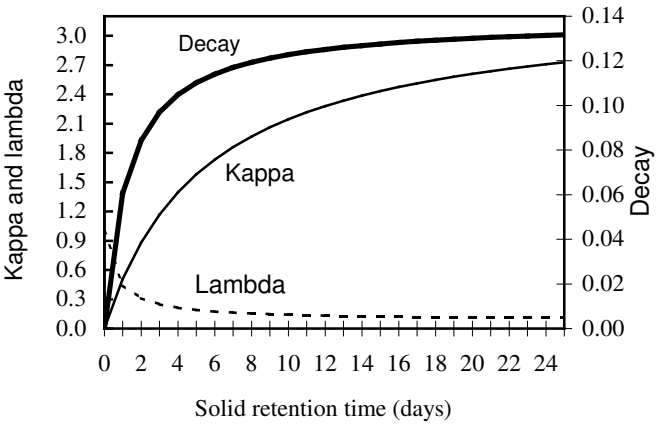

Figure 5. Kappa, lambda and b versus solid retention time (CSTR); equation (3), (4) and (17).

In figure 5, the decay coefficient (b) is fairly constant for long solid retention time (> 2-3 days). For smaller solid retention time (θc) the decay (b) tends to zero.

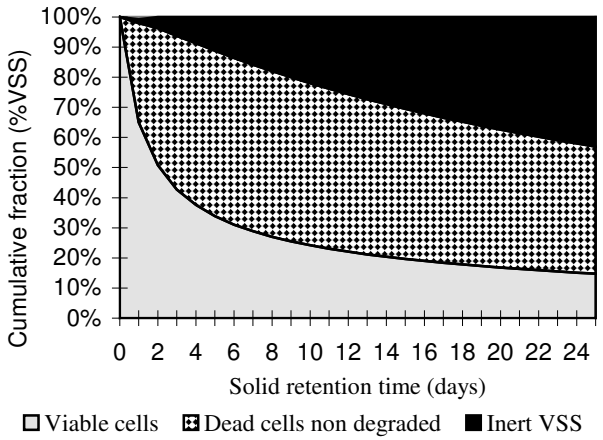

Figure 6. VSS cumulative composition of an activated sludge (CSTR)

Figure 6 shows the three phases in an AS, respectively viable cells, dead cells non degraded, and inert VSS. The composition of an AS is expressed as a percentage of total VSS function of the solid retention time.

4. Discussion

In this paper, was proposed a new approach for AS modeling: Instead of the conventional approach considering two phases (active biomass and inert VSS), was introduced a three phase model (viable cells, dead cells and inert VSS). Not anymore based on a single decay coefficient and the growth on influent substrate, the present model encompasses the following processes: the death rate of viable cells; hydrolysis of dead cells; growth on influent substrate; cryptic growth on substrate released by lysis and hydrolysis.

In addition, was suggested a method to test the hypothesis whether bacteria have an aging process (internal clock). If the hypothesis is positive the following death rate domains may be defined for a CSTR: for solid retention time smaller than $\tau/2$, the death rate should converge towards $1/\tau$; and, for solid retention time higher than τ, the death rate should be close to $2/\tau$. This analysis could be performed using indirect measurements of decay with equation (7) to estimate the observed death rate for different solid retention times. In absence of tangible experimental data it is difficult to conclude at this stage. On the other hand one shall reject this hypothesis as it is in contradiction with the common view that bacterial death is caused by viral infections and predation (bacteriophages). The author believes that further experiments would be required to investigate this hypothesis.

From the mass balances on viable cells, dead cells and inert VSS, simple mathematical expressions were obtained to describe the system for a CSTR (Table 1); furthermore, the decay coefficient – constant in the conventional model – is function of the death rate, the lysis/hydrolysis rate of dead cells, and the solid retention time.

Afterward, the model of the study was confronted with experimental data taken from previous investigations; and the following was undertaken:

1. First, the viability expression of model 1 (equation 12) was fitted with viability data based on ATP measurements (figure 3). Based on this approach the death rate (K_D) was estimated to be close to 0.6 d^{-1}.
2. Then, an OUR method for batch tests under starvation was proposed (figure 4). In order to solve this system, discrete mathematical equations had to be derived (equations 15 and 16 for model 1; equations 19, 20, and 21 for model 2). Calibrations were performed with OUR measurements, and the "endogenous respiration" observed under starvation appears to correspond to the oxygen consumption for oxidation of the soluble substrate released during lysis and hydrolysis of dead cells. This calibration was in agreement with the estimated growth yield on lysis/hydrolysis substrate (Y^H) of 0.28 gVSS/gCOD. A rough estimation for the model 1 hydrolysis rate of dead bacteria (K_H) was in the range of 0.16 to 0.19 d^{-1}. Finally, the γ of 0.3 (Pavlostathis & Gossett, 1986) was used, and the intrinsic death rate (Kd) of 0.6 d^{-1} (from the calibration figure 3; and equation 27). For model 2, the intrinsic hydrolysis rate (Kh) was estimated at 0.13 d^{-1}, and the lysis rate (Kl) at 2.0 d^{-1}.

From figure 4 can be inferred that (K_H) is sensitive to the time scale, whereas the first order kinetics (Kh) and (Kl) are not; therefore, a unification of model 1 with model 2 was necessary (equations 26 and 27).

5. Conclusion

In general it can be drawn that the models developed in this study have good abilities to explain viability processes in aerobic AS, and the results validate the approach based on the death rate of viable bacteria and lysis/hydrolysis of dead cells. Furthermore, the model parameters estimated in the present study are consistent with parameters obtained by previous investigators. Finally, the results from the present study provide experimental evidence of regulatory processes controlling death and lysis of bacteria.

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