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Biopolymer particulate turnover in biological waste treatment systems: a review

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Abstract. Microorganisms – the major component in most biological waste treatment processes and a number of industrial fermentations – are not able to directly assimilate biopolymeric particulate material. Such organic particulates must first be solubilized into soluble polymers or monomers before they can diffuse through the capsular slime layer surrounding most bacteria, then transported across the cell membrane, to be used as either a carbon, energy or other essential nutrient source. Throughout these events, new cells are synthesized, which are themselves biopolymer particulates.

The turnover of biopolymer particulates in biological treatment systems has not been examined with respect to its impact on system performance and culture physiology. The aim of this paper is to review the observations of particulate turnover in various biological treatment systems and to identify those fundamental mechanisms which govern microbial conversion of biopolymer particulates.

1 Introduction

The breakdown and utilization of biopolymeric particulate matter of microbial origin as carbon, energy, and essential nutrients sources by microbial communities is a common occurrence in both natural and engineered systems. Here, we define biopolymeric particulates as intact bacterial cell mass, particulate debris resulting from cell lysis, and extracellular polymeric floc (i.e., aggregates of lipopolysaccharides and glycoproteins). This definition excludes other common forms of colloidal matter such as cellulosic fibers, proteinaeous debris, insoluble hydrocarbons (e.g., fats, oils, and lipids) and inorganic precipitates and silicates, frequently found in waste streams and surface waters. Despite the prevalence of particulate biomass in both natural and engineered biological processes, practically all studies concerning microbial physiology, industrial fermentation, and biological wastewater treatment consider only soluble organic compounds as carbon-energy substrates. Even that portion of biological wastewater treatment research dealing specifically with particulate degradation (i.e., sludge digestion, using either aerobic and anaerobic technology) has ignored the fundamental mechanisms involved in biopolymeric particulate degradation, choosing to model the complex mechanisms as a single reaction first order rate process. Such a situation is understandable, since historically microbiological research has generally been concerned with growth phenomena and increases in biomass. Situations creating low growth, starvation, and death, and either subsequent or spontaneous lysis of cells have been studied, but with antiquated methods that are not capable of either completely characterizing such processes or differentiating between the various particulate entities.

Consequently, there is little fundamental physiological or practical engineering information concerning cellular starvation and death, cell lysis, hydrolysis of cellular biomass, particulate lysis products, and extracellular polymers, and the subsequent metabolism of such lysis products as electron donor, carbon sources, and nutrient supplies.

This review will examine the processes involved in biopolymeric particulate turnover in engineered systems, attempting to define the various mechanisms governing microbial conversion of such particulates, and will provide a methodology for the evaluation of how operating conditions might affect the relative contributions of each mechanism in the net particulate turnover in full-scale systems.

2 Biopolymer particulate turnover in engineered systems

Bacteria – the major microbial component in most biological waste treatment processes and certain industrial fermentations – are not able to directly assimilate biopolymeric particulate material. Such organic particulates must first be broken down into soluble poly- or monomeric molecules, before bacteria can transport the material through the polymeric floc matrix entrapping cells,

across the cell wall/membrane, and use the material as a substrate. Thus particulate hydrolysis or solubilization is a prerequisite step in the degradation and turnover of complex biopolymeric substrates. In the course of this process (Fig. 1) new cells, which are themselves biopolymeric particulates, are synthesized. The turnover of such biomass particulates in virtually all engineered systems has not been examined with respect to its impact on system performance and culture physiology.

2.1 Biomass recycle systems

Microorganisms suspended in an appropriate medium will grow at a rate dictated by their physiological status and the prevailing environmental conditions (e.g., limiting substrate concentration, nutrient and electron acceptor supply, pH-value). If a well-mixed system is operated continuously, i.e., as with a chemostat, the growth rate of the culture is dictated by the reactor mean hydraulic residence time. Cell concentration for any one residence time is a function of the influent concentration of growth limiting substrate. Should either the reactor mean residence time decrease below the maximum generation time of the microbial culture or should the microbial generation time suddenly increase, cells will be washed-out of the reactor. At a particular influent substrate concentration, the "wash-out" point limits the maximum substrate removal rate and the productivity possible in a bioreactor.

In waste treatment processes, the objective is to remove the maximum amount of pollutant at a minimum reactor residence time and minimum biomass production. In the biotechnological sector, the objective is to maximize productivity. Such objectives are in conflict with the chemostat wash-out criterion. Consequently, engineered biological processes are not carried out in chemostats, but rather, in suspended culture systems where biomass is separated from the effluent and returned to the bioreactor. Cell recycle elevates the homogeneous biomass concentration in the system and thus the potential overall substrate removal rate and system productivity. External biomass recycle forms the basis for the activated sludge process in its many variations. Although employed in waste treatment for more than 60 years, cell recycle has only recently been identified as a means to intensify process productivity in the industrial microbiology sector [1].

The theoretical analysis of a chemostat with recycle can be first attributed to Herbert [2] and was subsequently extended by Powell and Lowe [3]. Predicted performance from these early theories did not provide a good description of the activated sludge process since several real system complexities were ignored. Modeling efforts concerning the activated sludge process have expanded since Herbert's work and several "unified" models, based upon either mean cell residence time or substrate loading rate, are currently recognized by environmental engineers

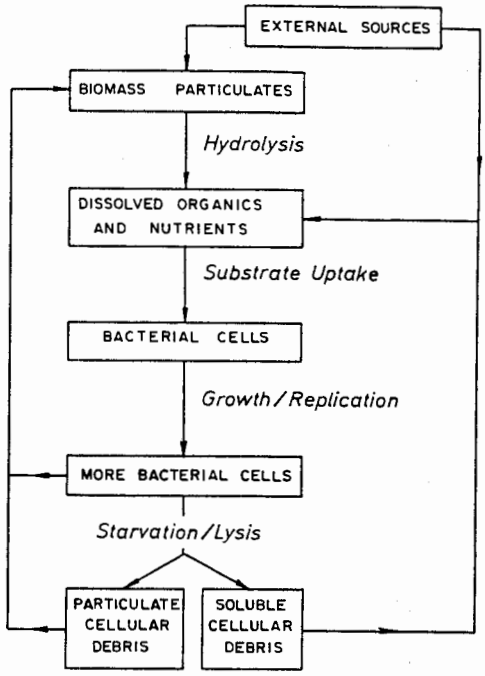

Fig. 1. Hypothetical sequence of events involving biomass turnover in a microbial culture

[4–7]. These models have their positive and negative features, but most, if not all, ignore cellular physiology and predict, for soluble influent substrates only, a steady state cellular biomass that is 100% viable and active. Provided that cellular biomass is artificially maintained by either a cell recycle or a cell retention system, at concentrations higher than could be accomplished in a non-recycle bioreactor due to growth solely, it is reasonable to expect a certain portion of the recycle biomass to be either starving, essentially inactive, or present as polymeric particulates. Indeed, a review of research literature substantiates that particulate cellular biomass is continually being degraded within microbial growth systems.

Postgate and Hunter [8] showed for a pure culture of Klebsiella aerogenes growing in a chemostat, that, depending on the residence time, as few as 38% of the bacteria were viable on the basis of slide culture counts; at higher growth rates a larger percentage was viable. Banks et al. [9] found that the viability of sludges from 10 different municipal activated sludge systems ranged from an estimated 2–60% of the volatile mass, based on plate counts.

Employing relatively more sophisticated measurements, such as ATP, dissolved O_2-uptake, cell count, and dehydrogenase activity, Weddle and Jenkins [10] analyzed pilot- and full-scale activated sludge systems for cell viability and activity. Conventional and low-rate activated sludge processes were found to consist largely of nonviable cells and inert non-biodegradable biomass with a viable aerobic heterotrophic microorganism content be-

tween 10–20% (based on ATP measurements), which increased with increasing net growth rate. Dehydrogenase activity per viable cell (basis: ATP content) was essentially the same at all net growth rates, but the total number of viable cells increased with increasing growth rate.

Grady and Roper [11] derived a model for the activated sludge process which predicted cell viability as a function of mean cell residence time by incorporating the processes of cell death, maintenance, and decay into material balances for viable and non-viable cells. Death, decay, and maintenance were assumed, without verification, to be first order autocatalytic functions of the appropriate cell concentration. Although mentioned in their derivation, Grady and Roper chose not to incorporate processes of cell and particulate debris solubilization and subsequent growth by intact cells on the soluble lytic products "... because of the difficulties inherent in parameter evaluation". At that time, analytical methods were not available to distinguish the cellular particulate debris, being used as substrate, from newly synthesized biomass.

Jones [12] first suggested the presence of non-viable but active substrate metabolizing bacteria, which could account for the discrepancy between observed metabolic activity and lower values for activity calculated based on the number of viable bacteria, although this discrepancy could more likely be attributed to inherent inaccuracies of plate count methods. Walker and Davies [13] pursued this line of research by measuring an increasing respiratory activity and unit viability with increasing net growth rate. However, they found, unlike the results of Weddle and Jenkins, that apparent respiration rates per viable cell were not constant but rather increased at low growth rates, being approx. 6 times greater at a net growth rate of $0.1\,d^{-1}$ than expected from observed viability. This discrepancy could be due to the different dissolved O_2-uptake rate techniques employed in the two studies. Weddle and Jenkins most likely measured the cells' endogenous respiration rate since their method did not provide an exogenous substrate during the test. The O_2-uptake rate method of Walker and Davies did employ a supplementary substrate, which suggests that they actually measured an energy-spilling, growth-decoupled substrate oxidation, common at low growth rates [14]. Walker and Davies interpret their results by assigning part of the activity in the activated sludge to non-viable but metabolically active cells.

Nelson and Lawrence [15] also report viability and substrate utilization rate per viable cell, on an ATP basis, but suggest the activated sludge biomass be divided into three fractions: active and viable cells, inert microbial debris and non-viable but biodegradable microbial solids. Nelson and Lawrence ignored the concept of non-viable but active cells proposed by Walker and Davies. Consideration of viability in Nelson's and Lawrence's work does not significantly affect estimates of the yield and maximum growth rate constants for activated sludge processes, but markedly affects estimates of endogenous decay and saturation coefficient values.

Grady and Lim [16] extend the earlier model of Grady and Roper [11] to predict cell viability in activated sludge processes receiving an influent consisting of soluble organic substrates, microbial cells, and non-viable biodegradable particulates. However, only growth, natural cell death, and decay processes were considered with particulate solubilization ignored. Additional assumptions made in their modified version were that upon entering the reactor influent cells were indistinguishable from cells grown in the vessel, and that biodegradable particulates were also indistinguishable from growing cells, thus serving to merely increase the total biomass in the system and lower the relative viability. The growth rate of viable cells was considered dependent upon soluble organic substrate only. No experimental verification of the revised model was provided. The assumptions mentioned above are a classical example of process modeling being constrained by a lack of analytical techniques, i.e., the ability to distinguish intact cells from particulate cell debris.

A novel alternative to the common cell recycle activated sludge treatment reactors is the sequencing batch reactor (SBR) system. An activated sludge SBR system may consist of a single vessel or multiple vessels, the exact number depending upon the specific pollution problem. Basically, each tank is operated through a succession of up to five modes or periods: fill, react, settle, draw, and idle (Fig. 2). The length of time for each period and for the total sequence (reactor cycle time) may be constant or variable depending upon the selected control strategy. The principle advantage of an SBR system is its ability to simulate reaction conditions ranging from ideally plug flow behavior to completely mixed tank behavior or any intermediate situation. As one can see, the microbial population in an SBR system is subjected to a wide range of feeding patterns providing either growth or starvation conditions. Chiesa et al. [17] examine the effects of periodic feast-to-famine conditions, mixed culture population dynamics, and physiology. Various factors including cell morphology, sludge activity, viability, and starvation resistance were investigated in relation to population selection in low net growth rate systems. Results indicate that properly balanced feast/famine conditions selected for organisms with both high substrate assimilation rates and high resistance to starvation. By creating repeated conditions of steep substrate gradients and long endogenous periods, sequential batch reactors "force substrate" to those microorganisms with both high substrate uptake rates and the ability to use intracellular reserve materials.

2.2 Biomass digestion systems

Conventional wastewater treatment processes produce large quantities of biological solids, i.e., sludge, that cannot be immediately disposed of without further treatment.

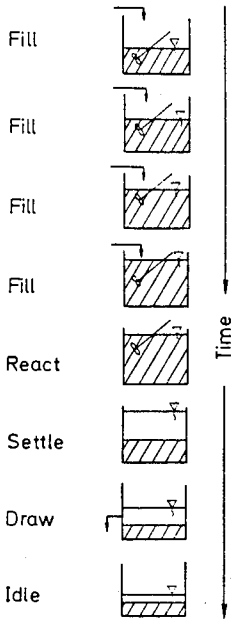

Fig. 2. Operation of a Sequenced Batch Reactor System

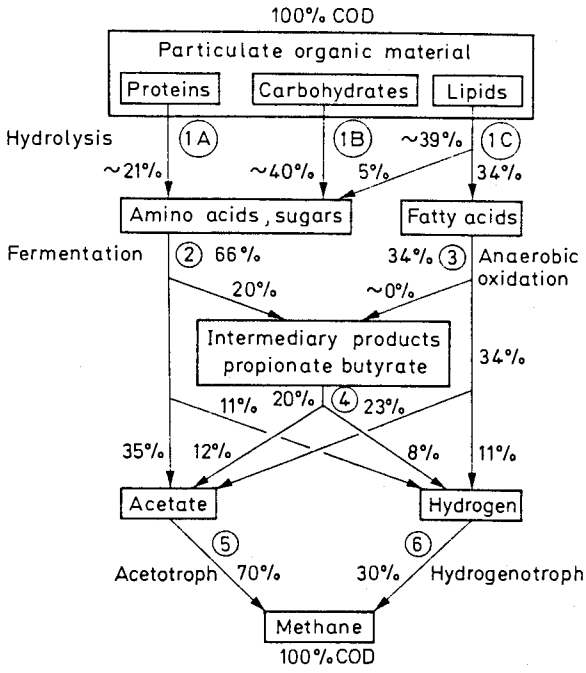

Fig. 3. Proposed reaction scheme for the anaerobic digestion of domestic sludge. Adapted from Kaspar and Wuhrmann [23] by Gujer and Zehnder [73]. Percentages indicate substrate flow (stoichiometrically) in the form of COD or CH_4 equivalents. Only the net flow of substrates (degradation minus biomass formed) through cell external pools is indicated. Numbers in circles identify different processes

Such sludges are thixotropic fluids containing from 0.25 to 12% solids depending on the characteristics of the raw wastewater and the treatment process. Sludge, composed mainly of bacterial cell mass, extracellular flocculant polymeric material, cellulosic materials, inorganic salts and silicates, still contains large amounts of potentially offensive pollutants and thus must be stabilized prior to disposal. Here stabilization means the reduction of the pathogenic organism content, the offensive odor-creating material, the organic carbon content, and the water content of the sludge, thereby facilitating ultimate sludge disposal.

Traditionally, sludge stabilization or digestion is accomplished biologically using anaerobic processes, but aerobic treatment is currently gaining in favor. Specific mechanisms involved in the degradation of sludge biomass in either digestion process are addressed in more detail in the separate sections below. However, in either process, a digester is merely a reactor, fed biomass particulates, that is operated at a sufficient residence time to stabilize the sludge; a microbial process in which significant biomass particulate breakdown is a prerequisite.

2.2.1 Anaerobic digestion

Bryers [18] has presented a structured model of the anaerobic digestion of biomass particulates based upon the substrate flow scheme proposed by Kaspar [19]. This model is shown in a modified form in Fig. 3. Six distinct processes may be identified in the anaerobic digestion process:

1. Hydrolysis or solubilization of complex biopolymeric particulates
   - 1 a. Hydrolysis of proteins
   - 1 b. Hydrolysis of carbohydrates
   - 1 c. Hydrolysis of lipids
2. Fermentation of amino acids and sugars
3. Anaerobic oxidation of long chain fatty acids and alcohols
4. Anaerobic oxidation of intermediate products such as volatile acids (with the exception of acetic acid)
5. Conversion of acetic acid to methane
6. Conversion of hydrogen to methane.

While recent microbiological research has focused on processes 4, 5 and 6 [20–22], some information is available on most of the six processes. Although a prerequisite for the remaining five processes, the first step – hydrolysis of biopolymeric particulates – has received only cursory attention. Consequently, the meager amount of data existing concerning anaerobic particulate digestion is mostly circumstantial.

Fluxes in Fig. 3 are expressed as chemical oxygen demand (COD) as determined by the dichromate method. Digester gas contains predominantly methane and carbon dioxide. If carbon, rather than either sulfur or nitrogen prevails as a sink of either electrons or hydrogen, the

production of methane is due to the reduction of COD. However, predicting CO_2 gas formation is complicated by the fact that CO_2 either remains dissolved in the digester liquor or is converted into bicarbonate depending upon the prevailing ammonium concentration. Thus, the composition of digester gas depends mainly on the mean oxidation state (= 1.5 COD/TOC – 4) of the carbon in the influent biomass, the CO_2 saturation, and the nitrogen content in the organic material degraded as shown in Fig. 4. Bryers [18] makes theoretical considerations for ammonia release from organic particulates and its effects on digester stability. These and other results indicate that the performance of a mature digester can be linked directly to the stoichiometry of the various processes involved in biomass breakdown.

Bacteria are not able to assimilate particulate organic material until it is first broken down into either soluble polymers or monomers. Consequently, solubilization is the first step required in the microbial utilization of complex biopolymeric particulates. In a digester treating predominantly biomass solids from an activated sludge process, substrate input into the anaerobic digestion scheme is the hydrolysis products, and only indirectly, the particulates themselves.

During digestion, new cells, which are themselves particulates, are produced. Based on substrate flux data and reported cellular yield values, the largest production of biomass may be expected during fermentation, which is indicated by process 2 in Fig. 3. The biomass formed will itself be subjected to relatively high degradation rates. Therefore, newly produced bacterial biomass must be included in the pool of particulate material in the reactor. Gas (CH_4, CO_2) is produced directly from dissolved compounds. The gas production rate can be predicted based on the net decay rate of particulate material plus the accumulation rate of soluble compounds, if any. Literature data indicate that the net decay rate is lower than the actual particulate solubilization rate, since the net rate is reduced by the amount of organics used in biomass production. Consequently, without a means to distinguish between biomass used as nutrient and biomass subsequently synthesized, most studies describe only the net rate of biopolymer removal and thus data cannot be used to estimate directly solubilization rates.

In a mature digester operated to stabilize domestic sludge, for a minimum mean residence time of 12 days at a temperature of 33 °C, soluble organic compounds do not accumulate to any significant extent. Compared to the total organic carbon (TOC) in the reactor, less than 10% is dissolved organic carbon (DOC). Kaspar and Wuhrmann [23] reported very short mean residence times for both acetic acid (approx. 1 hour) and propionic acid (0.7 hour) in a digester operated at a mean residence time of 40 days at 33 °C. Soluble COD, from volatile acids only, was found by O'Rourke [24] to be less than 7% of the total biodegradable COD in reactors with biomass residence times

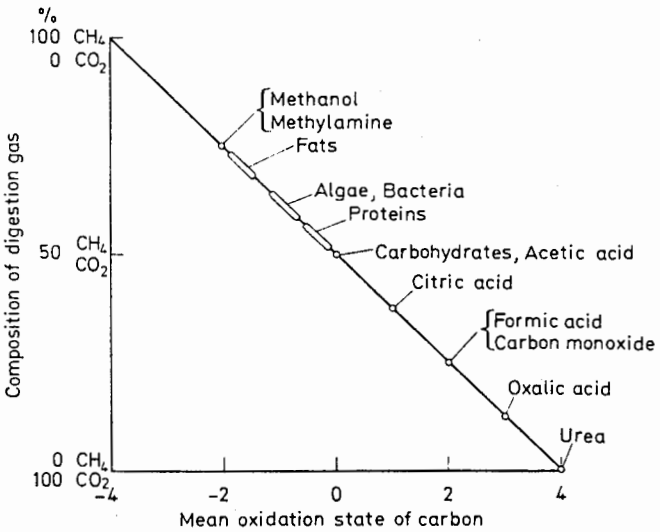

Fig. 4. Composition of the digestion gas depending on the mean oxidation state of the carbon in the substrate, assuming total mineralisation of the substrate [73]

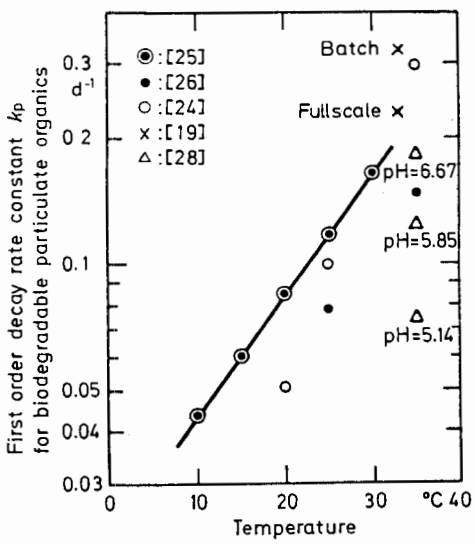

Fig. 5. First order decay rate constant k_p as a function of temperature, for the net decay of biodegradable particulate organic material in the anaerobic digestion of domestic sludge. Calculated from data of the different authors indicated by [73]

≧ 7.5 days at 35 °C. Thus it is reasonable to assume that methane production in a mature digester is limited by the net rate of particulate turnover.

Unfortunately, most work in assessing stoichiometry and kinetics in anaerobic systems has a black box approach. Based on batch studies, Imhoff and Fair [25] reported gas production rate to be first order with respect to remaining particulate organic material available for degradation:

$$r_{gas} = r_{degradation} = k_d P \qquad (1)$$

where $r_{degradation}$ is the net rate of particulate degradation in kg m^{-3}s^{-1}; k_d is the first order rate constant in s^{-1} and P is biodegradable organic material in kg m^{-3}.

Most kinetic treatments of particulate degradation are dependent upon an estimate of the fraction of volatile solids that is resistant to biodegradation. This fraction is determined with long-term batch tests.

Figure 5 illustrates the temperature dependence and absolute magnitude of k_d reported by Imhoff and Fair [25] and others. Pfeffer [26] reports that volatile solids in an digester operated at different biomass residence times can be approximated with a first order decay rate with a rate constant of 0.15 d^{-1} at 35 °C and 0.077 d^{-1} at 25 °C, assuming 20% inerts (see Fig. 6). Kaspar [23] and Gerritsen [27] provided carbon balances for a digester operated at a 40 day mean residence time and at 33 °C; feed solids were 22.4 kg/m^3 of organic carbon and the effluent contained 8.7 kg/m^3 of organic carbon. Gas production rate in this system was 0.34 kg m^{-3} d^{-1} of organic carbon which, assuming gas is 60.4% CH_4, results in a methane production rate of 0.7 mMol dm^{-3} h^{-1}. Kaspar's data [23] indicate a first order decrease in CH_4 production rate in batch degradation tests on samples taken directly from both laboratory- and full-scale digesters (Fig. 7). Both k_d and r_{gas} were considerably higher in the laboratory-scale reactor than in the full-scale system. Since both systems were operated under identical conditions as to pH-value and temperature, it is likely that the differences above are due

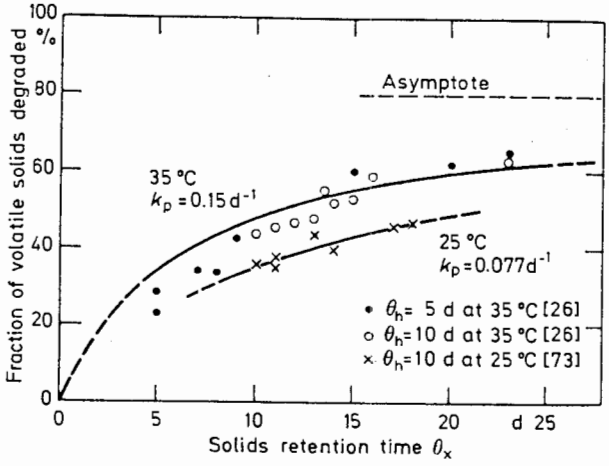

Fig. 6. Degradation of particulate volatile solids in raw sludge as a function of solids retention time. Continuous lines are predicted by a first order decay model with an assumption of 20% non-biodegradable solids. θ_h is the hydraulic retention time

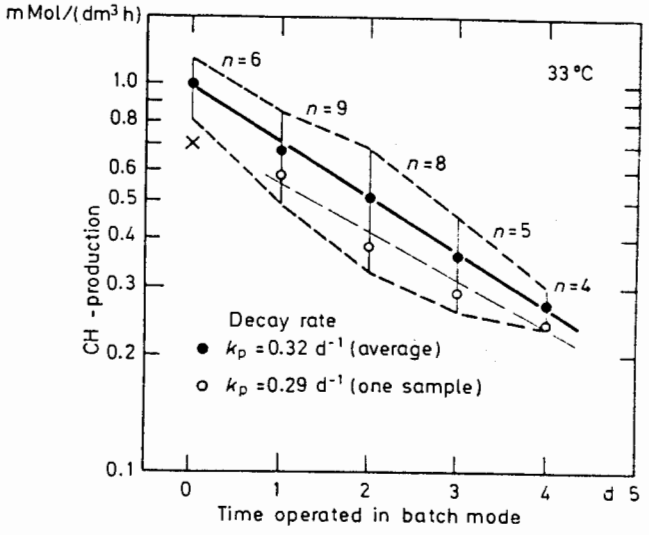

Fig. 7. Methane production of digested sludge, taken from the effluent of a full scale digester and kept in a laboratory fermenter in batch mode. Dashed lines indicate the range of results. n indicates the number of experiments on different days. x denotes methane production in the full scale digester. Data from Kaspar [23] adapted by [73]

Table 1. Variation in the hydrolysis rate as a function of pH as predicted from data by Eastman and Ferguson [28] at 35 °C with raw domestic sludge calculated by [73]

Solids retention time θ_x	pH-value	Feed concentration (COD) p_0	Influent p_0	Effluent soluble (COD)	Gas production (COD)	Net particulate COD degradation	Residual (COD) p_1	First order decay rate constant k_p
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
[d]	–	[kg/m^3]*	[kg/m^3]*	[kg/m^3]*	[kg/m^3]*	[kg/m^3]*	[kg/m^3]*	[d^{-1}]
1.5	5.14	52.0	35.4	3.45	0.22	3.67	31.73	0.077
1.5	5.85	52.0	35.4	5.29	0.30	5.59	29.81	0.125
1.5	6.67	52.0	35.4	6.92	0.68	7.60	27.80	0.182

(4) = (3) · 0.68 assuming 68% of the feed COD is biodegradable
(7) = (5) + (6)
(8) = (4) − (7)
(9) k_p as predicted from a steady state mass balance: $\frac{p_1}{p_0} = \frac{1}{1 + k_p \theta_x}$
* All in kg COD per m^3 sludge treated

Table 2. Apparent first order degradation rate constant k_d in d^{-1} calculated by [73] from data of O'Rourke [24] for steady state, continuous flow laboratory scale digesters

Component	Temperature [°C]	Solid retention time				
		5 [d]	10 [d]	15 [d]	30 [d]	60 [d]
Lipids	35	0.01	0.17	0.11	0.06	0.04
	25	0	0.01	0.09	0.07	0.03
	20	0	0	0.02	0.05	0.03
	15	–	0	0	0	0
Cellulose	35	1.95	1.21	0.62	0.38	0.21
	25	0.29	0.27	0.27	0.34	0.16
	20	0.09	0.14	0.13	0.14	0.10
	15	–	0.05	0.03	0.10	0.08
Protein	35	0.10	0.05	0.03	0.02	0.01
	25	0.09	0.04	0.03	0.02	0.01
	20	0.08	0.04	0.03	0.02	0.01
	15	–	0.03	0.02	0.01	0.01

Table 3. Hydrolysis of biopolymers under anaerobic condition [73]

Biopolymer	Hydrolysis products	Organism O and/or oxoenzymer E involved in hydrolysis	Apparent hydrolysis rate k_d [d^{-1}]	Temperature [°C]	Reference *
Lipids	Fatty acids Glycerol Alcohols	E: lipase O: clostridia	0.04 – 0.6 (fatty acid esters)	35 – 40	[29]
			0.6 – 1.7 (greases)	35 – 40	[29]
			0.12 – 0.19 (greases)	33 – 40	[30]
			0.08 (lipids)	34	[31]
Proteins	Polypeptides Oligopeptides Amino acids	E: protease E: peptidase O: Proteus vulgaris different clostridia	0.02	34	[31]
			0.03	35	[32]
Cellulose	Polysaccharides Oligosaccharides Glucose	E: cellulase O: fungi different clostridia Acetovibrio cellulitic-us – many others	0.04 (crude cellulose)	35	[32]
			0.13	34	[31]
Hemicellulose (xylans, pentosans)	Polysaccharides Oligosaccharides Hexoses Pentoses	E: hemicellulase xylanase O: fungi different clostridia	0.54	35	[32]

* The apparent rate (k_d) has been calculated based on data given in these articles assuming all material is biodegradable

to the greater turbulence in the laboratory-scale vessel, a factor frequently ignored in anaerobic digestion studies.

Eastman and Ferguson [28] investigated organic particulate solubilization in domestic sludge during only the acid phase of anaerobic digestion, and determine a first order decay rate with respect to the remaining biodegradable particulates. Their work serves to illustrate the effect of pH on solubilization rate (Table 1, Fig. 5). No study has determined the particular group of bacteria, acid formers, propionic acid degraders, acetic acid degrading methanogens, or hydrogen-converting methanogens responsible for solubilization. From Eastman and Ferguson [28], it can be deduced that acid forming bacteria alone will mediate particulate breakdown but this is only a circumstantial argument, since chemical dissolution at reduced pH-values may be partially responsible.

O'Rourke [24] differentiated between the degradation of lipids, cellulose, and proteins in continuous lab-scale digesters fed domestic sludge and found significantly different degradation rates for each group (Table 2). Moreover, a first order decay rate does not accurately describe his data.

It is apparent that only cursory information on the stoichiometry and kinetics of biopolymer particulate turnover in anaerobic digestion exists. The indirect information that does exist indicates that particulate breakdown possibly limits the rate of the overall process (Table 3). Further intensive research into the fundamental mechanisms of biopolymer turnover is warranted since such information can be used 1) to define circumstances under which process instabilities can occur, 2) to predict more reliably gas production, and 3) to estimate the maximum degree of biomass stabilization.

2.2.2 Aerobic digestion

Aerobic digestion, like anaerobic digestion, seeks to stabilize influent biomass solids but without the production of methane. Proposed advantages/disadvantages of aerobic versus anaerobic digestion have been enumerated elsewhere [33]. Aerobic digestion does not suffer the operational instabilities inherent to the anaerobic digestion sequence and its associated carbonate equilibrium chemistry. On the other hand, reactions involved in the stabilization of biomass solids, with the exception of the solubilization process, are better defined, both kinetically and stoichiometrically, for anaerobic systems.

Which biological process will dominate during the overall aerobic stabilization of biomass particulates is dependent upon the nature and condition of the feed biomass, i.e., on whether the influent particulates are either primary or secondary sludge, or a mixture of both. Primary sludges provide a markedly larger supply of reduced organic carbon and essential nutrients and thus can support more aerobic heterotrophic growth and respiration than secondary activated sludge biomass. At extended digester residence times readily available organics are consumed and, without an exogenous energy source, the bacteria will consume endogenous reserve material. Such starvation leads to eventual cell death and lysis, releasing both soluble and particulate cell debris into the surroundings. Most studies consider around 80% to be the maximum solids reduction biologically feasible, assuming the remaining 20% of the cell mass to be inert or nonbiodegradable. However, all such estimates are based on dry weight measurements of biomass, i.e., volatile or total suspended solids, which also includes the cellular biomass that has been synthesized via growth on particulate debris. Consequently, most existing particulate degradation kinetic estimates ignore growth, thus underestimating both lysis and hydrolysis process rates.

Historically, this neglect of growth on particulate organics is mainly due to the lack of analytical methods to distinguish between the original particulate substrate and the subsequently produced biomass. Typically, aerobic digester designs are based upon a first order kinetic expression describing biomass decay, i.e., on net suspended biomass reduction basis. There is very little, if any, direct work concerning the relative contribution of cell starvation, death, lysis, growth on soluble lytic products, and the solubilization of particulate biomass to the overall endogenous decay rate.

Adam et al. [34] propose the most popular form of design equation for aerobic digestion, where changes in biodegradable volatile suspended solids, (VSS_b), are described using a first order decay process, so that

$$d(VSS_b)/dt = -k \cdot VSS_b . \quad (2)$$

Biodegradable VSS are defined by Adams et al. as the difference between the initial total VSS and the final VSS remaining after an extended period of digestion of about 30 days. Such a kinetic description cannot be universally applied since it requires an a priori estimate of the VSS_b for the particular system, ignores solubilization of nonvolatile organic solids, and neglects biomass solids production. In addition, the parameter VSS_b is not a measure of the viability or reactivity of a microbial culture and thus does not reflect changes in culture physiology with changing conditions. Consequently, VSS_b proves to be an insensitive and, due to the duration of the analysis, an impractical control parameter.

Randall [35] and Benefield and Randall [36] attempt to incorporate activity into a first order decay expression by assuming only an active fraction of the total suspended biomass participates in endogenous decay. Unfortunately, the authors provided no experimental means for estimating the instantaneous active fraction, and thus resorted to assuming it to be a constant portion (approx. 77%) of the total biomass. The use of total biomass alone only as a measure of decay rate agrees with the authors' mathematical concepts, but contradicts their experimental observations that significant fixed particulate organics are being removed, which is possibly due to the solubilization of dead cellular material.

Grady and Lim [16] extend their bio-oxidation concepts to aerobic digestion by developing material balances for two types of cells, viable and non-viable. Non-viable cells are the result of the death of viable cells. Both viable and non-viable cells are assumed to lyse, but at characteristically different rates. Having proposed such concepts, Grady and Lim state that the estimation of these various types of cells was not experimentally feasible, and thus they simplified their initial approach by considering only the decrease in biodegradable biomass. Their approach tacitly allows for the direct conversion of biodegradable biomass to soluble substrates, which are then instantly scavenged by active cells. Grady and Lim did ignore possible bacterial growth on the soluble products that may occur due to lysis.

2.3 Biofilm systems

One common alternative to suspended culture cell recycle systems (i.e., in waste treatment the activated sludge process) is the heterogeneous biofilm reactor.

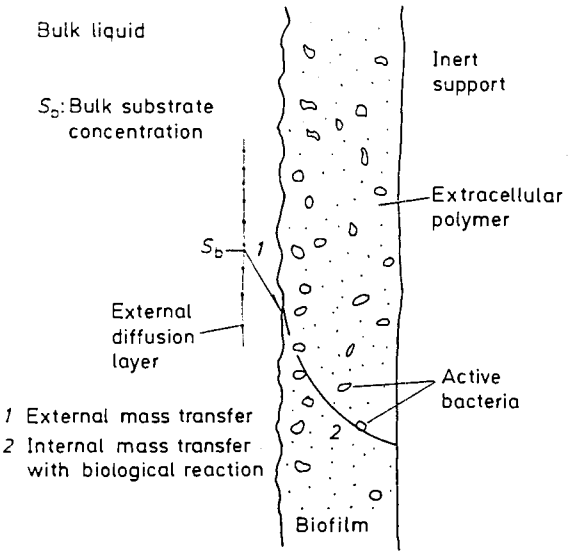

Fig. 8. Substrate concentration profiles through an idealized biofilm

Biofilms are a collection of microorganisms and their extracellular by-products associated with an interface or inert surface. Biofilms can form either naturally due to a microorganism's proclivity to adhere, or by way of a man-made entrapment method (e.g., immobilized whole-cell biocatalysts or biomass support particles). Biofilm reactors are manifested in the form of packed bed (trickling filter) reactors, fluidized bed or slurry reactors, and rotating biological contactors. In natural aquatic environments, the majority of microbial activity is now considered to be associated with an interface [37]. All biofilms pose the situation of a heterogeneous system where substrate(s) must be transported to and into the biofilm before any reaction can occur. The classical one-dimensional biofilm substrate removal scenario is illustrated in Fig. 8.

Design of fixed film reactors should be based upon an overall per volume removal rate of limiting substrate as depicted in Fig. 8. A multitude of steady-state biofilm kinetic models have defined overall substrate removal rates as functions of reactor geometry, biological reactivity parameters, and internal and external mass transfer resistances. All such biofilm substrate removal kinetics are based solely on soluble substrates (i.e., dissolved organic carbon, NH_4^+–N, NO_3^-–N, O_2). Both Bryers [38] and Grady [39] provide extensive reviews on the modeling of biofilm formation processes and biofilm substrate removal kinetics, respectively.

However, for most wastewaters the organic carbon and nitrogen loading to a once-through biofilm reactor can be 50–80% particulate. This percentage can be increased, if solids recycle to the fixed film is practiced. Turnover of organic material in natural biofilm systems also involves a large fraction of particulates. Regrettably, no fundamental study has been attempted to quantify the mechanisms of either particulate interactions with a biofilm or turnover of particulates in a fixed film reactor. Thus, neither practical design procedures nor recycle operating protocols for particulate turnover in a biofilm reactor exist. Some questions that need to be addressed are:

1. How are biomass particulates removed in a biofilm system, by deposition and entrapment only, or are particles actually degraded?
2. If hydrolysis of biomass particulates occurs at the biofilm surface, which microorganisms are responsible for it, those that deposit from the liquid phase with the particulates or those entrapped within the biofilm?
3. What are the rates and stoichiometry for particulate turnover in a biofilm system and what is the rate limiting step in biomass particulate degradation in such systems?
4. To what extent does particulate biomass influence the biological character or genetic make-up of a biofilm? (Experience indicates that certain biofilm communities can be displaced by invading bacteria, e.g., lactic acid bacteria supplanting bound Streptococcus spp. in the "butter milk gargle" treatment of Strep-throat, or the yogurt treatment of infant cholic.) Can slower growing plasmid-recombinant bacteria be maintained within a biofilm reactor allowing competition with faster growing non-plasmid containing bacteria?
5. If hydrolytic processes exist at biofilm surfaces, by what mechanisms do cells distinguish between external biopolymeric particles and the exopolymer that comprises the biofilm gel-matrix?
6. Does recycled biomass actually maintain desired biological activity within a biofilm (e.g. recycling nitrifying biomass during the winter to maintain biofilm nitrification), or is the majority of activity in the recycled liquid?

3 Processes involved in biopolymer particulate turnover

3.1 Cell decay

3.1.1 Historical concepts of maintenance, starvation, and viability

Cellular processes (mechanical, chemical or osmotic) require energy, and, unless a supply is available, essential processes will stop and the cell will die. Microbial cells orchestrate highly sophisticated chemical reactions and many of the intermediates of these reactions have higher free energies than their original substrates. Energy must be supplied to counteract a natural tendency toward disorder. That energy required to maintain the basic necessities of cellular activity, i.e., the status quo, is termed maintenance energy.

When an exogenous energy supply exists, a portion is diverted intracellularly to meet maintenance requirements and the remainder used for cell synthesis. In this widely held scenario culture mass increases since cellular growth for the culture is a net positive process. If energy supplied equals that required for maintenance, the culture appears

not to increase in mass, i.e., net growth is zero (this approach ignores lysis!). When the energy supplied to a culture is less than the maintenance requirement, the difference will be met by utilization of internal energy supplies, i.e., endogenous metabolism. In this case, the culture decreases in mass. Deprived of exogeneous energy sources, cells would derive all maintenance requirements from internal sources until the cells die.

Mallete [40] discussed in detail the concept of microbial maintenance energy and the potential for low concentrations of substrates fed to microbial cultures not to be able to support growth. Data were presented indicating that intermittent additions of small amounts of substrate to otherwise starved bacteria substantially increased culture viability over long periods of time, and implying the existence of an energy of maintenance. Marr et al. [41], using E. coli, demonstrated that a certain portion of the energy generated from the metabolism of a carbon source was diverted from the synthesis of new cells. They found this maintenance energy to be independent of growth rate over a wide range of growth rates. Temperature was found to have a pronounced effect on the energy required for maintenance with low temperatures resulting in low maintenance energy requirements.

Pirt [42] presented mathematical methodology for determining the maintenance energy of growing microbial cultures. The following equation was used to graphically determine the maintenance coefficient:

$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_G}, \tag{3}$$

where Y is the observed growth yield,
m is the maintenance coefficient,
μ is the specific growth rate, and
Y_G is the true growth yield.

Straight line relationships for plots of $1/Y$ versus $1/\mu$ were found for a number of microbial species when m and Y_G were assumed to be constant. A major question concerning this early concept of maintenance is whether m is the same constant during both periods of energy excess and energy deprivation. Pirt [43] modifies the model above by incorporating a growth-rate dependent maintenance energy into the specific substrate utilization equation. Thus:

$$q = \mu / Y_g + m_1 + m^* (1 - k\,\mu), \tag{4}$$

where q is the specific substrate uptake rate (mass substrate per mass cells h^{-1}), Y_g is the true growth yield, m_1 the constant maintenance energy coefficient, m^* the growth rate dependent maintenance energy coefficient (mass substrate per mass cells h^{-1}) and K is a constant. The group $m^*(1-k\mu)$ is the growth rate dependent maintenance energy and m^* its value when $\mu = 0$. Tempest and Neijssel [14] query whether m_1 should be constant and suggest that at low growth rates, cultures are affected more by apparatus effects which can result in uncoupling catabolism from metabolism.

Viability is historically defined as the ratio of viable cells to total cells in a system where the total cell count is the sum of the viable and non-viable cells [8]. Viability has been traditionally based on the arcane method of plate counts tacitly equating viability to reactivity, and the ability to replicate. Also such a definition demands estimates of only viable and total cells obviating the difficult problem of defining quantitatively a dead cell. Investigation of starvation, maintenance energy, and viability prove to be extremely difficult due to several experimental problems, e.g. to obtain steady state information at a net dilution rate of 0.004 h^{-1}, Tempest et al. [44] had to operate a chemostat for a minimum of 52 days in order to obtain reliable steady state data. Postgate and Hunter [45] maintained cultures of Aerobacter aerogenes in chemostats at dilution rates which varied between 0.232 h^{-1} and 0.0038 h^{-1}. Culture viability ranged from 96.6% at the highest growth rate to 37.8% at the lowest. The mean doubling time of the bacteria at a dilution rate of 0.0038 h^{-1} was found to be 82 hours, a value substantially higher than would be expected from the imposed dilution rate.

Relatively few microbial ecosystems provide a continuous renewal of all required nutrients and thus many cells are subjected to periods of nutrient starvation. Depending upon the system, this nutrient deprivation could be either for minutes or for months. As illustrated in section 2.1, even within industrial fermentation systems operated at apparently uniform nutrient supply ecological niches of insufficient nutrient flux may exist creating local areas of starvation (e.g. within cell separation and recycle stages). A cell's response to such starvation is unique to the cell and its cultivation history.

Strange et al. [46] investigated the ability of Aerobacter aerogenes, from the stationary phase of a batch culture, to maintain viability in a buffered sodium chloride solution. Long term viability was correlated with the medium used for growth. Bacteria cultured in complex media, that demonstrated prolonged viability during starvation, contained significantly higher amounts of carbohydrate and protein and less RNA than did starvation-susceptible bacteria. Glycogen, RNA and protein were degraded during starvation with 50% or more of one or more constituents being degraded without significant losses in viability. Also addressed was the concept of growth on secondary substrates, whereby long term viability is prolonged through growth of active cells on the lysis and hydrolysis products of dead cells, although no meaningful data were generated on the topic.

Tempest et al. [44] studied the growth of a culture of glycerol-limited Aerobacter aerogenes at dilution rates of between 0.004 h^{-1} and 0.24 h^{-1}. Observed yield, the percentage of RNA and DNA decreased with decreasing dilution rate. The protein and carbohydrate fractions remained constant or increased slightly with decreasing dilution rate. Viability was shown to be strongly depen-

dent on dilution rate with the doubling time of viable cells asymptotically approaching a maximum value of about 80 h^{-1}, a value that was extremely temperature dependent. Dilution rate was also shown to influence culture morphology with high dilution rates resulting in single cells, whereas low dilution rates resulted in "snake-like" multicellular filaments. A large number of investigators have examined the role of the classical storage products, poly-β-hydroxybutyrate (PHB) and glycogen, in prolonging the viability of microorganisms during starvation. Macrae and Wilkinson [47] demonstrated that PHB delayed the death and autolysis of Bacillus megaterium. Similar results have been reported by Sierra and Gibbons [48] for Micrococcus halodenitrificans, Sobek et al. [49] for Azotobacter agilis and Stokes and Parson [50] for Sphaerotilus discophorus. Glycogen has been shown to prolong survival in cultures of Aerobacter aerogenes [46], Escherichia coli [51] and Streptococcus mitis [52]. However, the presence of relatively large amounts of the two classical storage compounds does not insure that bacteria containing lower amounts will necessarily die faster. Under certain growth conditions, cells may contain large quantities of other intracellular macromolecules, e.g. RNA and protein, which may be used more efficiently as long term reserve materials [52]. An excellent review on the role and regulation of energy reserve polymers in microorganisms is provided by Dawes and Senior [54].

Kjelleberg and Hermansson [55] and Kjelleberg et al. [56] followed changes in bacterial surface hydrophobicity, charge, size and shape, and degree of irreversible binding to glass surfaces of several marine bacteria under nutrient-enriched and nutrient-deficient conditions. Reduction in cell size and increase in both cell number and endogenous metabolism preceded a marked enhancement of bacterial attachment rates upon starvation.

For further specific examples of starvation on bacterial cells or growth at low nutrient environments the reader is directed to a number of somewhat dated review articles: Lamanna et al. [57], Tempest and Neijssel [58], Strange [59], Postgate and Hunter [45], Dawes [60], Morita [61], and Postgate [62].

One should be aware that while concepts of bacterial life and death are based on the life cycle of an individual cell, most observations of these concepts are based upon culture-averaged measurements of net growth, increases or decreases in cell mass, cell number, or culture RNA or PHB content percentage. In reality, unless the exogenous energy supply is maintained in excess, all microbial cultures are growing at less than their maximum rate and always a certain percent of cells are lysing; this portion may or may not be statistically significant in certain cases, but in otherwise natural energy-limited or stressed environments a portion of starved cells will exist.

Postgate [62] discussed the various methods used to assess the viability of microbial cultures which included, as of 1967, staining and dye-uptake, optical density, detection of intracellular materials leaked into solution, enzyme activity and various plating and counting methods. Slide culture techniques were regarded as the fastest, most flexible determination for relative viability, but one should note that they are based on the cell's ability to replicate. In a short discussion of factors affecting viability during starvation, auto-degradation of RNA beyond a certain base level was concluded to be the most lethal factor in the ultimate demise of starved cells. More recent studies of starvation and death, propose for the first time a potential quantitative definition of cell death based upon the chemiosmotic transport state of a cell. The immediate result of biological oxidations via the electron transport system is the expulsion of protons (H^+) from the cell. This process creates a hydrogen ion gradient, which can be detected as a pH difference between the aqueous phases on either side of the cell membrane, and an electric potential difference. The proton motive force (PMF) or total chemiosmotic force across a membrane can be calculated from

$$\mathrm{PMF} = \mathrm{F}\,\Delta\psi - 2.3\,\mathrm{R}T\,\Delta\mathrm{pH} \tag{5}$$

where ΔpH is the pH gradient across the membrane, $\Delta\psi$ is the electric potential across the membrane, F is Faraday's constant, R is the gas constant and T is the temperature. Several researchers [63–65] suggest both zero $\Delta\psi$ and ΔpH may be a good criteria for death since a zero PMF parallels concomitant decreases in poly-β-hydroxyalkonates and ATP concentration and culture viability. Analytical methods for assessing cell activity, viability, and death are historically numerous and often create operational definitions of death. Various existing and potential analytical procedures will be discussed in a separate work of the authors [74].

3.1.2 Lysis

As prolonged starvation forces a cell to deplete its endogenous energy supplies, vital functions cease (growth, replication, osmosis) until the cell wall abruptly disintegrates and its internal components are released into the suspending fluid. Lysis of individual cells is almost never studied; rather lytic events are observed en masse as a decrease in optical density, cell number, or biomass. In general, starvation itself does not initiate lysis which is fortuitous since microbes frequently face nutrient depletion. What stage of endogenous metabolism initiates lysis and by what mechanism the intracellular mureinases are regulated during starvation to create cell wall break-down is not yet clear.

Almost, if not all, bacteria synthesize a murein-based cell wall and thus require a perfectly balanced system of murein-hydrolyzing and synthesizing enzymes. It is not surprising that such a sensitive system would be disrupted by various stresses, leading to autolysis, the spontaneous lysis of a cell without the influence of any exogenous

agent. A variety of exogenous agents of microbial origins are known to cause lysis of living bacteria, dead cells, or cell wall fragments [66]. Among these are murein-destroying enzymes released during normal growth and autolysis, certain antibiotics which interfere with cell wall synthesis, and metabolic products (e.g. gramicidin and tyrocidin).

3.2 Particulate hydrolysis and secondary organic scavenging

Once a cell lyses, the remnants consist of particulate, debris (cell wall fragments, exopolymers, flocculant lipopolysaccharidic floc material), soluble organics (monomers and low molecule weight polymers, e.g. proteins, amino acids, monosaccharides), and colloidal organics (higher molecular weight polymers, e.g. RNA, DNA, glycogen, larger proteins). Certain soluble organics can be taken directly into another actively growing cell without difficulty and can either be incorporated into the biosynthesis machinery or oxidized for energy. Secondary organic scavening is the term applied to the metabolism and growth of one cell on either the leakage or lysis products of another.

Hydrolysis is the breaking of a chemical bond by the addition of a proton (H^+), either chemically or enzymatically. Cell wall components are comprised of repeating units of peptidoglycan, which are unique biopolymers in that they are crosslinked in two dimensions, in one direction by β-1,4-glycosidic bonds and in the other dimension by a peptide bond between the amino acid components of the tetrapeptide side chain.

Slime and capsular layers of Gram-negative bacteria are comprised of a lipopolysaccharide complex of three major parts: (1) the core polysaccharide composed of a variety of sugars, attached at one end to (2) a complex lipid material containing glucosamine residues with the other end attached to (3) strands of "O – specific" polysaccharide. Hydrolysis of lipopolysaccharide capsular material would require breakage of β-1,4-glycoside bonds, peptide bonds, and phosodiester linkages.

Protein hydrolysis would encompass breakage of a multitude of peptide bonds resulting in free amino acid production, while nucleotide hydrolysis would form nucleoside monophosphates once the phosodiester bonds are cleaved.

The rate of hydrolysis for each of these components could be limited in several ways: (1) by the rate of specific hydrolytic enzyme synthesis, (2) in the rate of contacting the enzyme to the biopolymer, and (3) in the specific hydrolysis reaction itself. The overall rate of hydrolysis of biomass may also be influenced by chemical or physical factors, i.e., high temperature, acid pre-treatment, particulate disruption.

There are only a few studies concerning the kinetics of microbial growth on cellular components. Gaudy [67] demonstrated that microbial cells can serve as usable substrate for other cells. Gaudy et al. [68] report that cytoplasmic contents can serve as a usable carbon source. Mitchell and Nevo [69] report on a microbial culture adapted to a medium employing cell wall components as the carbon-energy source, although growth rate and the degree of conversion were not determined.

In practical aerobic systems, a substantial slime layer (extracellular polysaccharides) can form a flocculant matrix about a groups of cells. Obayashi and Gaudy [70] report that various exopolymers were utilized as limiting carbon substrates by mixed cultures growing batch-wise in a medium supplimented with sufficient nutrients. Rates of growth on polymer ranged from 0.14 to 0.32 h^{-1} with yield values ranging from 0.32 to 0.55 g COD cells/g COD polymer, both quantities depending upon the origin of the polymer.

Such studies would indicate that no component of a cell would inhibit metabolism and that all components can be metabolized at reasonable rates. Then why does the literature perennially report the accumulation of biological particulates in aerobic processes? Why are such processes related to decay based upon such extremely low rates? One source of these discrepancies may lie in the methods employed in the relatively few experiments discussed above. First, cellular components were reconstituted from disrupted cells and cell fragments, purified, and resolubilized to provide uniform growth conditions. Such preprocessing may have eliminated the critical rate-limiting step in particulate turnover, i.e., hydrolysis. Secondly, all essential nutrients were provided in excess, such that the particulate cell components acted only as a limiting carbon source. This may not be the actual situation in practical systems since either soluble nitrogen sources may be stoichiometrically limiting or nitrogen may be in the organic form, itself awaiting hydrolysis and conversion to ammonia-nitrogen. According to Wilkinson [71], organisms in nitrogen-deficient media, are not able to metabolize extracellular polysaccharide of their own making. This may be due either to nitrogen limiting the production of required polymer-hydrolyzing enzymes or to the antibacterial nature of the polysaccharides.

The overall stoichiometry of – or the amount of electrons available for – cellular growth on a cellular component could be estimated using the half-reaction method of McCarty [72] once a cellular yield is assumed. However, such approaches assume energy consumption and utilization reactions are coupled, i.e., they assume balanced-growth, which is not the situation in endogenously metabolizing cells. No theoretical basis for estimating the stoichiometry during uncoupled growth currently exists.

3.3 Summary

The above discussions establish that cells can experience growth, death, and lysis. In addition, growth itself can be divided into biosynthesis and maintenance activities, the

relative significance of maintenance being dependent upon exogenous substrate levels. All these processes combined influence the steady-state viable cell concentration in a microbial culture, influence estimates of cellular yield parameters, and determine the required rate of electron acceptor transfer.

Mathematically, the description of the above processes has been handled rather simply. Since all these processes lead to a reduction in viable cell mass, they are typically considered as one lumped event termed microbial decay. Microbial decay is modelled as a first order rate in viable cell concentration and thus acts to reduce the maximum possible cellular growth rate, as is stated in the following equation:

$$dx/dt = \mu x - b x , \tag{6}$$

where b = overall first order decay constant in s^{-1}.

Optimization of microbial processes will require a more sophisticated approach to modeling the mechanisms governing net decay. Careful examination of all available literature reveals that very little experimental work has been specifically carried out on the kinetics and mechanisms of particulate degradation. This was mainly due to a past lack of analytical tools to distinguish between the various particulate entities in such complex systems. Such tools are now available and, consequently, researchers are expanding the concept of decay into its fundamental components. Mason et al. [75] propose a structured model, based on Fig. 9, for an aerobic heterotrophic bacterial culture, which incorporates not only cell growth and replication but also cellular inactivation, cell death, cell lysis, metabolism of soluble lysis products, and hydrolysis of particulate lysis products. They report good agreement between model predictions and experimental results of respiring versus non-respiring cell concentrations in a continuous culture of Klebsiella pneumoniae.

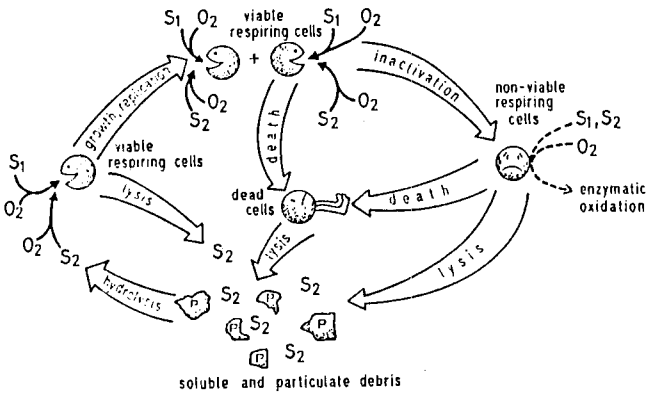

Fig. 9. Proposed scenario for biomass turnover in pure culture system

Closing remarks

Research reviewed here is concerned with biopolymeric particulate turnover within microbial systems. This paper has attempted to quantify the significance of microbial growth, starvation, lysis, particulate hydrolysis, secondary organics scavenging within both pure and mixed cultures. Continued research in these topics is needed and would impact directly and indirectly on the technology base of the following areas:

Biological treatment processes: Design of aerobic wastewater treatment processes to remove soluble organic carbon is straight-forward and there is very little fundamental research that will impact upon the design of such systems.

However, most of these designs are static in that they consider constant loading and composition of an influent pollutant. As man's needs to treat an increasingly complex, time-dependent wastewater for carbon, nitrogen and phosphorous removal, optimization and control of aerobic systems cannot be relegated to existing design concepts. More fundamental models will be mandatory. The degradation of biodegradable particulate matter is increasingly more important in activated sludge and aerobic digester systems because such particulates are primarily responsible for the attainment of realistic space-time and real-time dependent electron acceptor profiles. Careful examination here of all available literature reveals very little experimental work has been carried out specifically on the kinetics and mechanisms of biopolymer particulate turnover.

Fundamental microbial physiology: In both natural and engineered systems, mixed populations co-exist to mediate a number of processes involved in the cycling of C, N, P, and O_2. How transient, non-uniform growth conditions will create selection pressures on these mixed populations is not well understood. Profitable use of transient conditions to select for desired biological conversions will depend upon our scientific knowledge of an organism's ability to survive feast/famine situations.

Understanding mechanisms of starvation and death in well controlled experimental conditions will add to our understanding of microorganism survival in natural systems.

Biotechnological processes: The industrial biotechnological sector has only recently realized the potential of productivity enhancement afforded by cell recycle systems. Examples of recycling both wild-type cells, plasmid-recombinant bacteria, and mammalian cells are ever-increasing. As the concentration of such cells is artifically elevated by recycle, it is not unrealistic to expect starvation and death of a certain fraction of the culture. The release and turnover of both genetic material and particulate nutrients could severely influence certain genetically-engineered processes.

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