Process analysis in microbial systems: biofilms as a case study

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Chapter 6

Process Analysis in Microbial Systems: Biofilms as a Case Study

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The first two-thirds of this chapter describe the principles of process analysis and in doing so serve to define and extend much of the material on modelling introduced in Chapter 2. In the last third of the chapter the author applies the methods of process analysis to bacteria growing on surfaces in biofilms, a topic of significant commercial importance—*Editorial note.*

1. Introduction

Bacteria stick firmly, and often with specificity, to almost any surface submerged in an aqueous environment. The bacterial attachment is mediated by polymeric material, primarily polysaccharide, that extends from the cell to form a tangled mass of fibres, termed a *glycocalyx* (Costerton *et al.*, 1978). The adhesion mediated by the glycocalyx may provide an attractive habitat for certain bacteria in many aquatic environments. The cells grow, reproduce and form products at the surface which increase the accumulation of the organic layer. The entire deposit is termed a *biofilm*.

Biofilm processes may be beneficial as exemplified by fixed-film wastewater treatment processes (e.g. trickling filters and rotating biological contactors). In addition, biofilms frequently play a major role in stream purification processes. In fact, microbial activity in natural waters has been found predominantly at interfaces (Marshall, 1976; Costerton *et al.*, 1978). However, biofilms can be quite troublesome in certain engineering systems. For example, biofilms in water conduits can cause energy losses resulting from increased fluid frictional resistance and increased heat transfer resistance. Numerous concerns regarding biofilms have stimulated further research and analysis of the processes (Table 1).

This chapter begins with an introduction to process analysis followed by its application to biofilm processes. The structure and properties of biofilms, especially those which contribute to stoichiometric or kinetic models, are

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Table 1. Effect and relevance of biofilms on various rate processes.

Effects	Specific process and result	Concerns
Heat transfer reduction	Biofilm formation on condenser tubes and cooling tower fill material. *Energy losses*	Power industry Chemical process industry U.S. Navy Solar energy systems
Increase in fluid frictional resistance	Biofilm formation in water and wastewater conduits as well as condenser and heat exchange tubes. Causes increased power consumption for pumped systems or reduced capacity in gravity systems. *Energy losses*	Municipal utilities Power industry Chemical process industry Solar energy systems
	Biofilm formation on ship hulls causing increased fuel consumption. *Energy losses*	U.S. Navy Shipping industry
Mass transfer and chemical transformations	Accelerated corrosion due to processes in the lower layers of the biofilm. Results in *material deterioration* in metal condenser tubes, sewage conduits and cooling tower fill	Power industry U.S. Navy Municipal utilities Chemical process industry
	Biofilm formation on remote sensors, submarine periscopes, sight glasses etc. causing *reduced effectiveness*	U.S. Navy Water quality data collection

Detachment of micro-organisms from biofilms in cooling towers. Releases *pathogenic organisms* (e.g. *Legionella* in aerosols)	Public health
Biofilm formation and detachment in drinking water distribution systems. Changes *water quality* in distribution system	Municipal utilities Public health
Biofilm formation on teeth. Causes *dental plaque and caries*	Dental health
Attachment of microbial cells to animal tissue. Causes *disease* of lungs, intestinal tract and urinary tract	Human health
Extraction and oxidation of organic and inorganic compounds from water and wastewater (e.g. rotating biological contacters, biologically-aided carbon adsorption and benthal stream activity). *Reduced pollutant load*	Wastewater treatment Water treatment Stream analysis
Biofilm formation in industrial production processes *reduces product quality*	Pulp and paper industry
Immobilized organisms or community of organisms for conducting *specific chemical transformations*	Chemical process industry
Fouling biofilm accumulation *reduces effectiveness* of ion exchange and membrane processes used for high-quality water treatment	Desalination Industrial water treatment

presented. The various rate processes which contribute to biofilm development are described and mathematical expressions are presented within the context of mass balances. Finally, the effects of biofilms on energy losses are presented, including the effect on fluid frictional resistance (momentum balances) and the effect on heat transfer resistance (energy balances).

2. Process Analysis

Process analysis refers to the application of scientific methods to the recognition and definition of problems and the development of procedures for their solution. This generally requires (i) mathematical specification of the problem for the given physical situation, (ii) development of a mathematical model and (iii) synthesis and systematic presentation of results to ensure full understanding. The *process* denotes an actual series of operations or treatment of materials as contrasted with the *model* which is a mathematical description of the process (Himmelblau and Bischoff, 1968).

2.1. Modelling (after Himmelblau and Bischoff, 1968)

A model represents a part, frequently a very simplified part, of reality. The models formulated in this book will represent only those aspects of reality which are of interest to us. Manipulation of the models improves our insight regarding the workings of the real system. Models are used in attempts to describe and understand the behaviour of real systems. However, real systems, even simple ones, are very complex and rarely understood. Since engineers and scientists have to deal with real systems, describe real systems and explain or make use of even simple real systems, models are used.

Modelling is an iterative process. First, a model is formulated in mathematical terms. The first model is usually too simple to be realistic. However, the model serves as a hypothesis and a set of experiments is designed to test the hypothesis. If the results of experiments differ significantly from predictions, the first model is modified and a new hypothesis must be formulated.

A general procedure for the analysis of complex processes has been suggested by Himmelblau and Bischoff (1968):

(1) Formulation of the problem(s) and establishment of objectives and criteria of value; delineation of performance requirements.

(2) Preliminary inspection and classification of the process to break it down into subsystems (elements).
(3) Preliminary determination of the relationships among the subsystems.
(4) Analysis of the variables and relationships to provide as simple and consistent a set as possible.
(5) Mathematical modelling (in applicable cases) of the relationships in terms of the variables and parameters; description of elements that can only be incompletely represented by mathematical models.
(6) Evaluation of how well the model represents the real process, using judgement to integrate the non-mathematical with the mathematical representations.
(7) Application of the model; interpretation and comprehension of the results.

These steps are designed to develop an approach of structuring and analysing processes wherever possible through mathematical models. This

Fig. 1. The cyclical nature of model building.

approach provides for more rigorous analysis and tends to make subjective judgements (when required) more formal and thorough. Figure 1 indicates the cyclical nature of these steps.

A model never perfectly describes reality nor is that a necessity. However, the model must describe enough of reality to answer the questions that have been posed.

2.1.1. *Types of Models*

Three types of models and their combinations can be prepared:

(1) Transport phenomena models.
(2) Population balance models.
(3) Empirical models.

Examples of transport models are the phenomenological equations of change, that is, the equations describing the conservation of mass, momentum and energy. Residence time distributions and other age distributions are examples of population balance models. Finally, examples of typical empirical models are those polynomials used to fit empirical data by the method of "least squares". Discussions in this text will focus primarily on transport phenomena models and, to a limited extent, on population balance and empirical models.

Table 2 lists the classes of models along with a brief description and reference to their application in microbial systems. More detailed discussions can be found elsewhere (Roels and Kossen, 1978).

Table 2. Description of different types of mathematical model.

Class of model	Description and application
Descriptive (black box)	Curve fitting Interpolation of data Useful within the region where the model was tested experimentally *Example*: use of the logistic equation to describe batch microbial growth
Predictive (grey box)	Extrapolation of data With care, can be used outside the region tested experimentally *Example*: Michaelis–Menten (1913) equation for describing enzyme-substrate reaction rates
Structured	Systems in a transient state Useful for modelling changes in internal structure of cells or structure of mixed microbial populations *Example*: Williams (1967) two-compartment (synthetic component and genetic component) model for biomass

Table 2. Description of different types of mathematical model.—*continued*

Class of model	Description and application
Unstructured	Does not account for internal structure Steady-state systems Lumped parameter models *Example*: Steady-state continuous flow microbial reactor (chemostat) where mass is the only property of the biotic material
Non-segregated	Considers that the biomass is homogeneously dispersed throughout the reactor fluid Mass concentration is the parameter which describes biomass Population balance models *Example*: Chemical oxidation of microbial cells by chlorine where biomass destruction or solubilization, not disinfection, is of greatest concern
Segregated	Uses a discrete parameter, the number of cells, as the parameter to describe biomass *Example*: Evaluating disinfection or sterilization processes where number of organisms alive and dead is most critical
Deterministic	Output variables have values completely determined by the structure of the system Applicable in cases where large numbers of organisms are involved ($n > 10\,000$) *Example*: activated sludge process for a chemostat in which saturation kinetics describes substrate removal and, consequently, microbial growth
Stochastic	For small numbers of organisms ($n > 100$) For cases where differences in organisms are important *Example*: different microbial species exhibit varying resistance to a sterilization process
Continuous time	Systems where events are evenly distributed in time Most microbial models *Example*: logarithmic growth rate model for a batch culture in which some micro-organisms are always undergoing division
Discrete time	Systems where events occur at a limited number of times *Example*: a synchronous culture in which all micro-organisms divide simultaneously at discrete times

2.2. Fundamentals Regarding Rate (after Churchill, 1974)

The fundamental relationships which underlie transport phenomena models are the equations for conservation of mass, momentum and energy which are a result of the laws of thermodynamics and Newton's laws of motion. The conservation equations are generally expressed in terms of intensive factors (i.e. independent of system mass) such as composition, velocity and temperature. The conservation equations also introduce quantities such as thermodynamic properties, stoichiometric coefficients, transport rates and chemical reaction rates (Table 3).

The thermodynamic properties, such as density, heat capacity and chemical equilibrium constants can be estimated with reasonable confidence from mechanistic models and can be measured with reasonable accuracy. The transport coefficients, such as viscosity, thermal conductivity and diffusivity, and the chemical reaction rate coefficients can rarely be predicted and are difficult to measure. Even the definitions of these latter quantities are somewhat arbitrary.

The equations for conservation of mass, energy, momentum and atomic species equate rate of accumulation to net rate of input by transport and net rate of input by various rate processes such as chemical reaction, diffusion, radiation, convection and viscous dissipation. Conservation equations or balance equations can be expressed in words as follows:

$$\begin{matrix}\text{net rate of}\\ \text{accumulation}\\ \text{in system}\\ \text{volume}\end{matrix} = \begin{matrix}\text{rate of}\\ \text{transport in}\\ \text{through}\\ \text{system}\\ \text{surface}\end{matrix} - \begin{matrix}\text{rate of}\\ \text{transport out}\\ \text{through}\\ \text{system}\\ \text{surface}\end{matrix} + \begin{matrix}\text{rate of}\\ \text{generation in}\\ \text{system}\\ \text{volume}\end{matrix} - \begin{matrix}\text{rate of}\\ \text{consumption}\\ \text{in system}\\ \text{volume}\end{matrix} \quad (1)$$

The change of some extensive parameter, E (an extensive quantity is proportional to the mass of the system), with time can be caused by two mechanisms:

(1) E can be generated or consumed within the system by some process, e.g. chemical reaction occurring at a rate per unit volume, r_E (r_E is negative if E is consumed).
(2) E can be transported into or out of the system through its boundary with the external environment.

2.2.1. *Process Time and the Importance of Rate*

The physical, chemical, and biological transformations which are discussed in this book are completed in a certain period of time. For example, the removal of soluble organics in a biological wastewater treatment process

Table 3. Intensive factors, physical properties and their relationships to the conservation equations.

	Conservation equations			
	Mass	Energy	Momentum	
Intensive variables	Concentration	Temperature	Velocity	
Physical properties				
Thermodynamic	Equilibrium constants	Heat capacity	Density	Easy to measure accurate, precise
Transport or reaction coefficients	Diffusivities and reaction rate	Thermal conductivity	Viscosity	Difficult to measure and define; not accurate or precise

occurs in a specified period of time; less than the hydraulic residence time within the reactor. A fouling biofilm accumulates on a heat exchanger surface over a period of time reducing heat transfer until a critical point is reached when the process unit must be shut down for cleaning operations. The time required for these specified changes to occur is inversely proportional to the rate at which the process occurs and is the most important quantity in process analysis.

2.2.2. *The Anatomy of a Balance Equation*

We will now reconsider the general balance equation presented in eqn (1) which can be reworded as follows:

$$\text{rate of accumulation} = \text{net rate of input by transport} + \text{rate of process} \tag{2}$$

The process rates are fundamental quantities in that they can be generalized and correlated simply with factors such as temperature, pressure, composition, velocity and diameter which describe the environment. Process rate includes chemical reaction rate, adsorption rate, diffusion rate, rate of heat radiation or rate of viscous dissipation in fluids.

Rate of accumulation and *net rate of input by flow* are rates of change. These rates of change are easily measured and observed quantities which may be the result of several process rates. They cannot be easily correlated or generalized with factors that describe the environment.

It is essential that *rates of change* should not be confused with *process rates*. Rates of change are generally *measured* or *observed* quantities in a system from which process rates are *inferred*.

2.2.3. *Mass Balance Equations*

Mass balance equations will be the primary tool used to analyse microbial processes. Consequently, bulk transport rate, mass transfer rate and conversion rate will be encountered frequently. These rates can be classified as follows:

transport or transfer rates,
- bulk transfer,
- mass transfer,

conversion or transformation process,
- chemical conversions.

Bulk transport or *transfer* refers to the movement of material from one location to another as a result of flows. For example, bulk transport of oil from the well to the refinery through a pipeline.

Chemical conversion or *transformation processes* refer to changes in composition as a result of chemical reactions. For example, the change in dissolved oxygen concentration in a fermenter due to respiration of bacterial cells.

Mass transfer describes the rate of material transport through a system boundary. The term mass transfer is usually reserved for interphase (e.g. solid–liquid or gas–liquid) transport processes. *Diffusion*, similar to mass transfer, generally describes intraphase transport. Consequently, the system and its boundaries must be specifically defined to analyse these processes.

One of the important criteria in defining transfer processes is that the molecular structure of the material remains unaltered. Transport processes are described by rather simple phenomenological equations containing either a concentration or concentration difference (for transport through the system boundaries) or concentration gradients (for transport within the system).

Transformation processes result in changes in molecular structure. For example, the metabolism of organisms results in the conversion of nutrients into cellular components, metabolites and heat. Transformation processes are described by *rate equations* or, more specifically, constitutive or kinetic equations of the following general form:

$$r = r(c_1, c_2, \ldots, c_n) \tag{3}$$

where c_i are concentrations of the various reacting components. Transfer and transformation processes will be combined in a general model based on the conservation equations as follows:

$$\underbrace{\text{IN} - \text{OUT}}_{\substack{\text{transfer}\\ \text{processes}}} + \underbrace{\text{CONVERSION}}_{\substack{\text{transformation}\\ \text{processes}}} = \text{ACCUMULATION} \tag{4}$$

2.3. Reactors (after Frederickson *et al.*, 1970)

Many biochemical processes involve *batch* growth of micro-organisms. After seeding a liquid medium of appropriate composition with an inoculum of living cells, nothing (except possibly some gas) is added to the culture or removed from it as growth proceeds.

However, many microbial processes of interest occur in *continuous flow* reactors which are characterized by continuous flow of reactants into the reactor while products are continuously removed. Two types of ideal

continuous flow reactors will be important in our further discussions:

continuous flow stirred tank reactor (CFSTR),
plug flow reactor (PFR).

2.3.1. *Residence Time Distribution of Fluid in Vessels*

How can we describe the flow characteristics of fluid in a vessel adequately enough to yield information useful in the design or analysis of reactors? The approach is to find out how long individual molecules stay in the vessel by using population balance models. Information on the distribution of ages of molecules or particles in the exit stream or the distribution of residence times of molecules within the vessel can be found easily and directly by a widely used experimental technique, the stimulus-response technique. This information can then be used to account for the flow behaviour of fluid in a chemical flow reactor (Levenspiel, 1972).

In developing the "language" for the treatment of flow, consider the steady-state flow, without reaction and without density change (which includes almost all liquid phase systems), of a single fluid through a vessel. Under these conditions,

$$\theta = \frac{V}{F} = \text{holding time, mean residence time or space time} \tag{5}$$

$$D = \frac{F}{V} = \text{dilution rate or space velocity} \tag{6}$$

where V = reactor volume (L^3)
F = volumetric flow rate (L^3t^{-1})

For liquid phase continuous flow systems at steady state, the mean residence time of any system component, i, can be defined as follows:

$$\theta_i = \text{mean residence time}$$

$$= \frac{\text{amount of component } i \text{ in the system}}{\text{mass flow rate of component } i \text{ out of the system}} \tag{7}$$

2.3.2. *Continuous Flow Stirred Tank Reactor (CFSTR)*

A continuous flow stirred tank reactor consists of a well-stirred tank into which there is a continuous flow of reacting material, and from which the (partially) reacted material passes continuously. The important characteristic of the CFSTR is the stirring.

In this type of reactor, culture medium and/or organisms are continuously

fed to the reactor, where growth and other processes occur. The culture within the reactor is agitated somehow; generally, a mechanical agitator is provided for this purpose. Culture is removed from the vessel at the same volumetric rate as feed is admitted, so that culture volume remains constant.

In the ideal CFSTR, agitation is assumed to be so vigorous that mixing is complete. The criteria of complete or perfect mixing may be stated in different, but equivalent ways. The usual criterion is simply that the composition of the culture in the vessel is uniform, so that the composition of the stream leaving the fermenter is the same as that of any sample from the interior of the fermenter. From a statistical viewpoint, mixing is perfect:

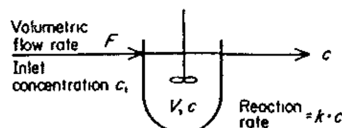
(1) The probability that a "particle" (organism, molecule) will be in a given subvolume in the culture is the same as the probability that it will be in any other subvolume of equal size regardless of location in the vessel.
(2) "Particles" move independently through the vessel. The statistical criteria are the more general, and the first criterion given can be derived from them.

No real vessel can be perfectly mixed but, in practice, the behaviour of a real vessel can be made to approach that of an ideal CFSTR very closely.

Let c be the concentration (amount per unit volume of culture) of some substance, biotic or abiotic, in a culture. Then, application of the principle of conservation of mass to the system composed of the culture (liquid plus organisms) in the CFSTR yields the following differential equation:

$$V(\mathrm{d}c/\mathrm{d}t) = F(c_i - c) + rV \tag{8}$$

RATE PROCESS ANALYSIS



MATERIAL BALANCE ON COMPONENT c

$$\frac{d(Vc)}{dt} = F(c_i - c) - k \cdot c$$

Rate of accumulation — Net rate of input by flow — Output by reaction

RATES OF CHANGE — PROCESS RATE

Fig. 2. Rate process analysis using material balances in a CFSTR.

relating the rate of change of c to the rate of flow through the vessel (F) and the rate of production of the substance per unit volume (r). In the equation, V is the volume of culture in the vessel (a constant), and c_i is the concentration of substance in the feed to the CFSTR (Fig. 2). The units of each term in the equation are mass per unit time (Mt^{-1}).

From this equation, follows the first point of importance concerning the CFSTR. Suppose that a steady state ($dc/dt = 0$) has been attained and that a substance is consumed or destroyed by reactions within the vessel ($r \neq 0$). Then we see that

$$D(c_i - c) = -r \tag{9}$$

where $D = F/V$ is the *dilution rate.* This equation shows that the composition of the feed stream undergoes a discontinuous change when the stream enters the CFSTR. Thus, if the feed of the CFSTR contains organisms, they will experience a discontinuous change, or an environmental shock, upon entering the reactor. The equation also indicates that r can be determined from D, c_i and c which are measurable quantities.

A second point of importance regarding CFSTRs is that the residence time of a particle is not fixed but is subject to statistical fluctuation. The density distribution of residence time is

$$D\,e^{-Dt} \tag{10}$$

The most probable residence time is zero. The mean residence time is D^{-1}. D^{-1} is also the standard deviation of residence time. If a cascade (series) arrangement of equal volume CFSTRs is used (Fig. 3), say m of

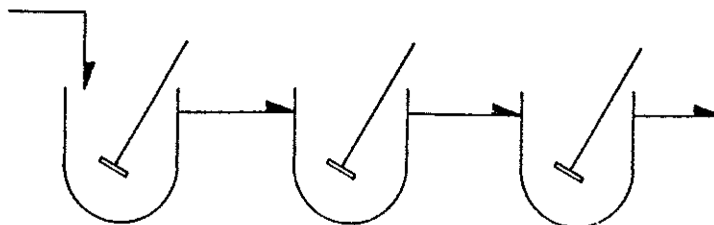


Fig. 3. A cascade (series) arrangement of equal volume CFSTRs.

them, then the residence time follows a gamma distribution with density function

$$(mD) \cdot [(mDT)^{m-1} \exp(-mDt)]/(m-1) \tag{11}$$

The mean is again D^{-1} (V is the *total* volume of all m tanks) but the standard deviation is smaller than in the one-vessel case, and is $D^{-1}m^{-1/2}$.

As the number of tanks in the cascade becomes larger and larger, while D is held constant (thus implying that the individual tanks become smaller and smaller), the standard deviation becomes smaller and smaller. In the limit of very large m, the residence time is no longer subject to statistical fluctuations, and is always D^{-1}. This arrangement of reactors is quite effective for simulating a plug flow reactor of volume V (see below).

Another point of importance concerning the CFSTR is that the probability that a particle in the vessel at some time will be "washed out" in some subsequent time interval is independent of such factors as the size of the particle or its residence time in the CFSTR. Since washout is equivalent to death so far as the population in the CFSTR is concerned, in effect, the flow through the vessel imposes a *non-selective* death rate on the population. This point becomes most important when two or more populations are growing symbiotically in a CFSTR.

2.3.3. *Plug Flow Reactor* (*PFR*)

In this type of reactor, culture medium and/or organisms are continuously fed to the reactor, where growth and other processes occur. The culture within the reactor is not stirred, since the object is to have elements of culture move progressively through the fermenter without mixing in the longitudinal direction.

In the ideal plug-flow reactor, adjacent elements of culture are assumed to move progressively through the tube without exchange of material between such elements. In addition, the composition of the culture is assumed to be uniform over any cross-section, though the composition obviously changes with distance through the reactor.

No real reactor can be an ideal PFR, but in some cases, the behaviour of real apparatus can be made to approach the ideal. A series of CFSTRs is quite effective as described above.

As before, let c be the concentration of some substance in the culture. Application of the principle of conservation of mass to a system of infinitesimal length moving with the velocity of flow through the tube (v) then yields the partial differential equation

$$(\partial c/\partial t) + v(\partial c/\partial z) = r \qquad (12)$$

where z is the axial distance from the inlet of the PFR, and r is the production rate per unit volume (Fig. 4).

In this case, the residence time of a particle is not subject to statistical fluctuations; a particle at position z has been in the vessel for a time z/v, and if the reactor has length L, the transit time will be L/v.

In many cases, mixing in the direction of flow may be important. This

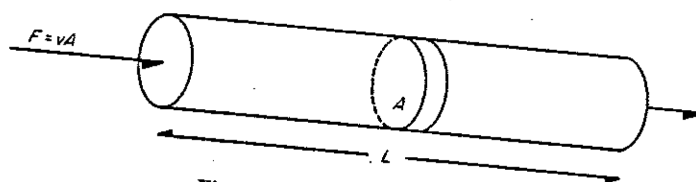


Fig. 4. A plug flow reactor.

is accounted for most easily (though only approximately) by introducing an effective axial diffusion coefficient, $\mathcal{D}$. The material balance (in this case, a differential material balance) leads to a more complicated equation:

$$(\partial c/\partial t) + v(\partial c/\partial z) = \mathcal{D}(\partial^2 c/\partial z^2) + r \quad (13)$$

where the mixing term involves the second derivative of the concentration. The diffusivity $\mathcal{D}$ is not the usual diffusion coefficient that is used in studies of transport phenomena. $\mathcal{D}$ is dependent on the flow regime in the reactor and independent of the usual (molecular) diffusion coefficient, at least if flow is turbulent.

If axial mixing does occur, then particle residence times are again subject to statistical fluctuations. No simple expression for the density of the distribution of residence times emerges in this case [though it can be found by solving eqn (13) subject to appropriate boundary and initial conditions]. However, one can say that the mean residence time will be L/v and the standard deviation of the residence time will increase as $\mathcal{D}$ increases; the distribution will be skewed, with the most probable residence time being smaller than the mean. If the dimensionless parameter $\mathcal{D}/vL$ is small compared to unity, then the distribution of residence times is approximately Gaussian, with mean L/v and standard deviation $(L/v)(2\mathcal{D}/vL)^{1/2}$.

Biologically speaking, the most important feature of the PFR is the progressive *change* in environmental conditions seen by an organism traversing the reactor. This is in marked contrast to the constant conditions seen by an organism traversing an ideal CFSTR. In fact, the situation in the ideal PFR is entirely similar to that in the batch fermenter, with residence time, z/v, replacing batch reaction time, t. Hence, the ideal PFR can presumably accomplish on a continuous, steady-state basis that which is done discontinuously in batch reactors.

2.3.4. *Segregation or Micromixing Effects*

The problem associated with the mixing of fluids is important for extremely fast reactions in homogeneous systems, as well as for all heterogeneous systems. The major concern is the *degree of segregation* of the fluid, or

whether mixing occurs on the microscopic level (mixing of the individual molecules) or the macroscopic level (mixing of clumps or aggregates of molecules). The degree of segregation does not affect batch reactors or plug flow reactors. However, it can significantly affect the performance of a CFSTR. The previously described stimulus-response techniques cannot distinguish between a CFSTR with good micromixing and another with poor micromixing.

2.4. Reaction Kinetics

The rate of reaction is characterized by a *rate equation* which is generally the result of an empirical curve-fitting procedure. The values of the rate constants must be found by experiment, even if the form of the rate equation was suggested by a theoretical analysis or mechanistic model. The determination of a rate equation usually requires a study to determine the influence of concentration, followed by the effects of pH, temperature etc. on the reaction rate coefficients.

2.4.1. *The Rate Equation*

The nth *order rate equation* has been used to great advantage in chemical reactor theory. The equation is a descriptive, two-parameter model and is useful over restricted ranges of experimental data:

$$r = kc^n \tag{14}$$

where r = reaction rate $[ML^{-3}t^{-1}]$
c = reactant or product concentration $[ML^{-3}]$
k = rate coefficient $[t^{-1}m^{1-n}L^{3(n-1)}]$
n = reaction order (dimensionless)

The equation has proven useful because of its limited number of parameters and its simplicity when used in material balance equations.

Another form of rate equation is used extensively in microbial reactors and can be described as the *saturation rate equation*:

$$r = \frac{k_1 c}{k_2 + c} \tag{15}$$

where r = reaction rate $[ML^{-3}t^{-1}]$
c = reactant or product concentration $[ML^{-3}]$
k_1 = rate coefficient $[ML^{-3}t^{-1}]$
k_2 = saturation coefficient $[ML^{-3}]$

From a practical standpoint, the numerical values of the rate coefficients are most important and will depend on the units of measurement. For example, glucose removal rate in a microbial reactor can be described by eqn (15) where c can be expressed as either glucose or organic carbon concentration. When carbon concentration is the measured variable, k_1 and k_2 will be 40% of the values obtained using glucose measurements since glucose is 40% carbon by weight. Similarly, confusion can result when molar concentration units are compared to mass concentration units. Molar units are sometimes awkward in microbial systems when dealing with microbial matter which cannot be readily characterized by a "molecular weight".

2.4.2. *Rate Equation Versus Balance Equation*

It is critically important to distinguish between a rate equation which describes a transformation and a balance equation which considers both transfer and transformation processes. In a batch reactor, where there are no transfer or transport processes, *the balance equation* for a first-order reaction is expressed as follows:

$$\underset{\text{rate of accumulation}}{V\frac{dc}{dt}} = \underset{\text{process rate}}{kcV} \qquad (16)$$

where k = rate coefficient (t^{-1}).

The expression can be confusing, especially if it is referred to as a rate equation. *The rate equation* for this process is correctly expressed as

$$r = kc \qquad (17)$$

The balance equation for the batch reactor can unambiguously be expressed by eqn (16) or as

$$V\frac{dc}{dt} = rV \qquad (18)$$

A material balance for a continuous reactor (Fig. 2) using a similar rate

expression can be expressed as follows:

$$V\frac{dc}{dt} = F(c_i - c) + rV \tag{19}$$

$$\underset{\text{rate of accumulation}}{V\frac{dc}{dt}} = \underset{\text{net rate of input by flow}}{F(c_i - c)} + \underset{\text{process rate}}{kcV} \tag{20}$$

Equation (20) reduces to the balance equation for the batch reaction (eqn (16)) process if $F = 0$.

2.4.3. *Steady State Versus Equilibrium*

In batch reaction processes, changes in composition occur with time regardless of whether spatial uniformity exists. The changes occur from moment to moment until thermodynamic equilibrium is reached (or until the reaction is completed). The continuous reaction process is different in that changes in composition occur spatially (e.g. a plug flow reactor or CFSTRs in series). Any part of the system tends toward a time–invariant state.

The movement toward a time–invariant state requires constant feed conditions, constant rate of heat removal etc. Even so, reaction systems have been observed in which concentrations of components oscillate continuously about particular values.

It is important to note that steady state *is not* an equilibrium. The term equilibrium should be reserved for the time–invariant state of closed systems. The steady state of an open system, such as a continuous reactor, depends on the flow rate, reaction rates and the size of the system. In the theory of non-equilibrium or open-system thermodynamics, the time–invariant or steady state in an open system possesses the same meaning as the equilibrium state in equilibrium thermodynamics. In brief, the steady state is the most orderly, efficient and economical state of an open system.

2.4.4. *Classification of Reactions*

A useful method of classifying reactions is according to the number and types of phases involved. A phase is that part of a system which is physically and chemically uniform throughout. A reaction is *homogeneous* if it takes place in one phase alone. A reaction is *heterogeneous* if it requires the presence of at least two phases to proceed at its characterisic rate. A phase implies uniform composition. Consequently, the choice of classification is sometimes difficult and depends on which description or model is more useful. Rigorously, all microbial reactions are heterogeneous since the

biomass constitutes a "solid" phase and the substrate being consumed is soluble, i.e. in the liquid phase. Therefore, segregated models are useful. However, at high substrate concentrations and/or low concentrations of well-dispersed biomass, distributed models are frequently satisfactory and microbial reactions can be considered homogeneous.

In heterogeneous reaction systems, reactants must be transported from one phase to another and the rate of transport may control the overall process rate observed. The transport rate is dependent on mixing intensity and system geometry. In any case, if the process consists of a number of rate processes in series (e.g. transport and then reaction), the slowest step of the series exerts the greatest influence and *controls* the overall rate.

2.4.5. *Expression of Rates*

Process rates are conveniently expressed as intensive measures (i.e. independent of system mass) so that results can be used in analysing systems of varying size. Therefore, the process rate, R, can be expressed per unit reactor volume:

$$r_v = R/V \tag{21}$$

where V = reactor volume (L^3)
R = conversion rate (Mt^{-1})

Certain other forms of expression for rate will be very useful. Conversion rate can be based on unit biomass in a dispersed, well-mixed, system:

$$r_x = R/X \tag{22}$$

where X = biomass in the system (M)
r_x = reaction rate per unit biomass ($MM_x^{-1}t^{-1}$ or possibly t^{-1})

For heterogeneous systems (e.g. biofilms), the area of reaction surface is used for expressing rate.

$$N = R/A \tag{23}$$

where A = area of reactive surface (e.g. biofilm surface area) in the system [L^2]
N = flux of reactant at the reactive surface ($ML^{-2}t^{-1}$)

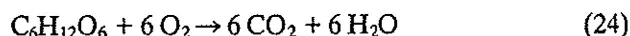
2.4.6. *Reaction Stoichiometry*

Stoichiometry is the application of the law of conservation of mass and the chemical laws of combining weights to chemical processes. In its broadest

sense, stoichiometry is a *system of accounting* applied to the mass and energy participating in a process involving chemical or physical change. It is a system of calculation which permits a surprisingly large amount of information to be obtained from a seemingly small number of facts.

Stoichiometry provides information concerning the types of changes and maximum extent of changes that can occur in a reaction process. In an abiotic system, thermodynamic calculations allow the determination of the equilibrium constant for a reaction and, hence, the actual yield or reaction products for given conditions. Such calculations require the knowledge of the exact nature of the reactants and the products which are not always determinable in a biotic environment.

In order to define the stoichiometry of a reaction, the stoichiometric coefficients in the reaction equation must be determined. For example, consider the chemical oxidation of glucose:



$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \qquad (24)$$

The stoichiometric coefficients for glucose, oxygen, carbon dioxide and water are -1, -6, 6 and 6, respectively. The units of the stoichiometric coefficient are *moles*. More generally, the relationship is expressed by a mass balance equation as follows:

$$\alpha_1 M_1 + \alpha_2 M_2 + \alpha_3 M_3 + \cdots + \alpha_k M_k = 0 \qquad (25)$$

where M_i = molecular weight of the ith component ($M\,\text{mol}^{-1}$)

α_i = stoichiometric coefficient (mol)

$\alpha_i \leqslant 0$ for reactants

$\alpha_i \geqslant 0$ for products

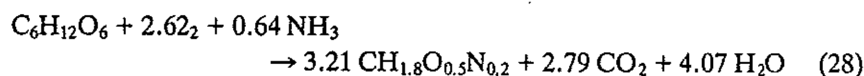
It follows that the stoichiometric relationship also provides a convenient method for comparing rates of reaction for the various reaction components. If the production rate of component i is $r^{(i)}$, then for the reaction described in eqn (24),

$$r^{(CO_2)} = r^{(H_2O)} = -0.17 r^{(\text{glucose})} = r^{(O_2)} \qquad (26)$$

For elementary reactions, the overall process rate, r, is the same regardless of the reaction component measured. Elementary reactions have rate equations which correspond to their stoichiometric equation (e.g. $A + B \rightarrow C$ and $-r_A = kC_AC_B$). Therefore, for a reaction involving k components

$$r = \frac{r^{(1)}}{\alpha_1} = \frac{r^{(2)}}{\alpha_2} = \frac{r^{(3)}}{\alpha_3} = \cdots = \frac{r^{(k)}}{\alpha_k} \qquad (27)$$

This relationship indicates that reaction rate can be determined by following the rate of appearance or disappearance of the component that is most easily detectable but which is not necessarily the one of major interest. An example of microbiological interest is the microbial oxidation of glucose. Busch (1971) has examined this process in a batch reactor and has observed the following stoichiometry at the point of glucose depletion:



$$C_6H_{12}O_6 + 2.62_2 + 0.64\,NH_3 \rightarrow 3.21\,CH_{1.8}O_{0.5}N_{0.2} + 2.79\,CO_2 + 4.07\,H_2O \quad (28)$$

or expressed in terms similar to those of eqn (25):

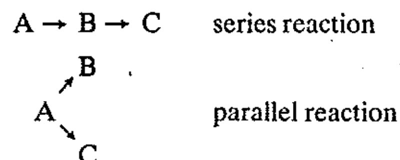
$$3.21\,A_1 + 2.79\,A_2 + 4.07\,A_3 - A_4 - 2.62\,A_5 - 0.64\,A_6 = 0 \quad (29)$$

where A_i = molecular weights of reacting components ($M\,mol^{-1}$)

$CH_{1.8}O_{0.5}N_{0.2}$ = empirical formula for biomass

The progress of this reaction can be followed by monitoring the concentration of any of the reactants or products. However, oxygen can be measured conveniently, easily and accurately and is frequently used to determine rates of aerobic, microbial reactions. From eqn (28), we see that the ratio of glucose removal rate to oxygen removal rates is approximately 3.06. However, microbial reactions are not elementary reactions and caution is advised when applying *overall process stoichiometry* such as in eqn (28).

When materials react to form products, it is usually easy to decide, after examining the stoichiometry, whether a single reaction or a number of reactions are occurring. When a single stoichiometric equation and single rate equation are chosen to represent the progress of the reaction, it is termed a *single* reaction. When more than one stoichiometric equation is used to describe the observed changes, then more than one kinetic expression is needed to follow the changing composition of the reaction components, which results in a *multiple reaction*. Multiple reactions may be classified simply as follows:



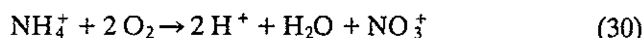
$A \rightarrow B \rightarrow C$ series reaction

$A \nearrow B$, $A \searrow C$ parallel reaction

More complicated schemes are possible.

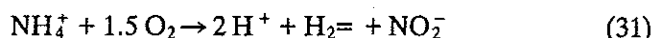
For a single, elementary chemical reaction, it is sufficient to determine

the relative amounts of reactant and product at any one time during the reaction in order to obtain the stoichiometric coefficients. However, for multiple reactions, a more detailed and cautious procedure must be used. Consider the following illustrative example. Nitrification refers to the oxidation of ammonia nitrogen to nitrate nitrogen:



$$NH_4^+ + 2\,O_2 \rightarrow 2\,H^+ + H_2O + NO_3^+ \tag{30}$$

However, the conversion is better described as a multiple reaction as follows:



$$NH_4^+ + 1.5\,O_2 \rightarrow 2\,H^+ + H_2 = + NO_2^- \tag{31}$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \tag{32}$$

Using the notation from eqn (29), the reaction stoichiometry is as follows:

$$2\,A_2 + A_3 - A_4 - 1.5\,A_5 + A_6 = 0 \tag{33}$$

$$A_1 \qquad -0.5\,A_5 - A_6 = 0 \tag{34}$$

$$A_1 + 2\,A_2 + A_3 - A_4 - 2A_5 = 0 \tag{35}$$

where A_1 = nitrate ion ($M\,mol^{-1}$)
A_2 = hydrogen ion ($M\,mol^{-1}$)
A_3 = water ($M\,mol^{-1}$)
A_4 = ammonium ion ($M\,mol^{-1}$)
A_5 = oxygen ($M\,mol^{-1}$)
A_6 = nitrite ion ($M\,mol^{-1}$)

The course of nitrification in a batch or plug flow reactor is described in Fig. 5. At any time during the reaction, the amount of NH_4^+ reacted results in production of NO_2^- and NO_3^- in a proportion that depends not only on the stoichiometry, but also on the rate of the reactions.

2.4.7. *Microbial Reactions Versus Chemical Reactions*

The methods for determining the stoichiometric and kinetic parameters for a given conversion have been developed primarily for abiotic reactions. Biochemical conversions mediated by viable organisms differ from abiotic chemical conversions in several important ways:

(1) Microbial reactions are *irreversible*. The stoichiometric end-point generally refers to the exhaustion of one of the reactants.
(2) All microbial reactions are *heterogeneous*.

(3) Relatively low concentrations of reactants and products, in addition to the heterogeneous characteristic, increase the potential for *mass transfer limitations*.
(4) The reaction, frequently represented by one stoichiometric equation, consists of many enzymatic reactions (in series and parallel) occurring in the metabolic region of the cell.
(5) The reactions are generally *autocatalytic*, i.e. biomass and related enzymes increase as the reaction proceeds.
(6) One of the reactants, biomass, has a *structure* and a *history* which can influence the stoichiometry and kinetics of the reaction.

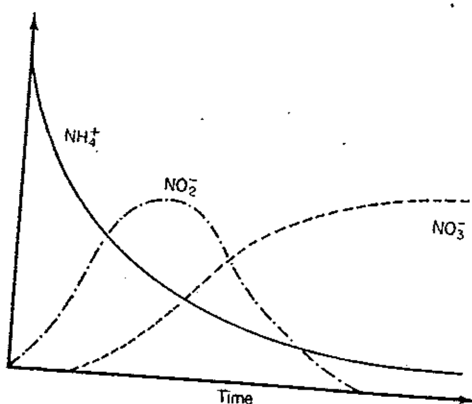


Fig. 5. Progress of the nitrification reactions in a batch reactor.

3. Modelling of Microbial Processes

The process of mathematical modelling is an iterative one in which careful study of a simple model leads to a more realistic, but more complex, model, which is itself the precursor of a better model. The test of each model requires experiments in which the model variables are measured quantities. Hence, it is important to determine which variables should be considered and which variables can be measured in the modelling of microbial processes. The variables of concern can be divided into two categories: *biotic* and *abiotic* variables.

3.1. Modelling Variables (after Frederickson *et al.*, 1970)

The biotic variables of importance depend on the complexity of the microbial process. If the process involves a monoculture, number of individuals (population density), biomass (or biomass concentration) and distribution of physiological states within the population become quantities of significant interest. If the process involves mixed cultures, then an additional class of variables which describe community structure must be considered. Such quantities include numbers and biomass of the various populations of the community, in addition to the distribution of physiological states within each population. The physiological state is quite important in modelling any microbial process and is somewhat difficult to quantify.

Abiotic variables describe the physical and chemical environment in which the microbial process is occurring. Abiotic variables describe the *state* of the abiotic phase or phases of the reaction system. The *chemical state* describes the concentrations of various chemical substances in the liquid phase such as substrate, nutrients, dissolved oxygen and pH. Another class of variables describes the *physical state* of the system which frequently exerts control on the rate of the biological and chemical processes occurring in the reactor. For example, dilution rate is an important variable controlling microbial processes in a continuous fermentor. Another example is mechanical shear which may alter the physiological state of the organisms either directly by influencing lysis rate or indirectly by influencing mass transfer rates. Exposed cellular surface area per unit reactor volume may significantly influence microbial process rates.

3.2. Physical Principles

As described earlier, mathematical models are generated in part by application of well-established principles of physical and biological sciences. Useful physical principles may be divided into three categories: conservative, thermodynamic and constitutive. These principles have been discussed previously.

3.2.1. *Conservation and Thermodynamics*

Conservation principles are essentially accounting principles. For any system, the conservation principle for a specific component may be expressed verbally as follows:

$$\begin{matrix}\text{component rate} \\ \text{of accumulation} \\ \text{in the system}\end{matrix} = \begin{matrix}\text{net rate of component} \\ \text{transfer into the system} \\ \text{across its boundaries}\end{matrix} + \begin{matrix}\text{net rate of} \\ \text{component generation} \\ \text{within the system}\end{matrix} \quad (36)$$

Components of importance to microbial process systems subject to this principle include mass, chemical and atomic species, and energy. Conservation includes the first law of thermodynamics which is the principle of conservation of energy. The second law of thermodynamics places restriction on process efficiency and the extent to which these processes can occur. Micro-organisms are so-called open systems, i.e. they are not in thermodynamic equilibrium since they exchange matter and energy with their environment. Hence, a macroscopic description of their behaviour demands the formulation of thermodynamics of irreversible processes.

3.2.2. *Constitution*

Constitutive principles deal with the *rates* of physicochemical processes. Conservation and thermodynamic principles do not deal with mechanism, but constitutive principles do; constitutive principles deal with mechanisms as influenced by the constitution of matter. Examples of constitutive principles are the laws that govern rates of transport (transport phenomena) and rates of chemical reactions (reaction kinetics).

Since conservation principles do not depend on the constitution of matter, a mathematical model expressing a conservation principle is "correct" if all flows, sources and sinks have been included. The usefulness of the model, however, depends primarily on two things:

(1) The choice of the system.
(2) Availability of constitutive principles for description of unknown quantities which appear in the model (e.g. reaction rates).

Since biological mechanisms and biological constitutions are often poorly understood, constitutive relations present the most difficulty in developing mathematical models of microbial processes.

3.3. Biological Principles

Biological principles are not as clearly defined. However, there are at least four that are of general importance in model building.

3.3.1. *Phenotype and Genotype*

The first principle is physiological and ecological and states that the activities of an organism (phenotype) and rates at which these activities are conducted are dependent not only on the organism (genotype) but also on the organism's environment. A corollary of this principle is that the constitution or state of the environment depends upon the activities of organisms contained

in it. This principle and its corollary are explicitly incorporated in most microbial process models. For example, the concept of *limiting nutrient* describes the influence of the environment on the organisms (the limiting nutrient controls metabolism) and the influence of the organism on the environment (the organism consumes the limiting nutrient thus altering the state of the environment).

3.3.2. *Past History*

The second principle is drawn from ecology and genetics. The current phenotype (constitution or state) of an organism depends not only on its genotype but also on the past history of environments experienced by the organism. Thus, cultivating an organism in a changing environment (e.g. batch culture), one may observe differing function and structure within the same genotype. Although this principle is recognized, it is seldom incorporated satisfactorily in microbial process models. If this principle is incorporated, the model is said to be *structured*; otherwise it is unstructured.

3.3.3. *Taxonomic Classification*

The third principle states that organisms can be classified based on their morphology, growth form and mode of reproduction. Thus, Eubacteriales are unicellular, exhibit intercalary growth and generally reproduce by binary fission and may be distinguished from Actinomycetales which may be filamentous, multicellular, exhibit apical growth and reproduce in several different ways. The implications of this principle are frequently not realized when modelling microbial processes. For example, the same model is frequently used for describing growth of Eubacteriales and Actinomycetales.

3.3.4. *Mutation*

The fourth principle is the mutability of organisms. Although universally recognized, the principle is rarely considered in modelling population dynamics.

There may be other biological principles of more or less interest but the four listed are generally recognized, but frequently ignored, by the model builders.

3.4. Simplifying Assumptions

The growth of even a single population of micro-organisms is a tremendously complicated process. The task of mathematically modelling such growth requires simplifying assumptions. These introduce inexactitudes

into the models since the models will not describe all of the various facets of growth that occur. However, the models will probably describe some facets of growth considered important; only continued experimental testing will lead to progress in mathematical modelling.

Many models of microbial growth make certain simplifying assumptions without stating such assumptions explicitly. It seems important that the more common hypotheses be stated more explicitly. Hence, four will be considered in this section.

3.4.1. *Neglect of the Distribution of States*

Biological populations are composed of individual organisms, and the individuals are physiologically, morphologically and genetically different. Individuals differ in size, shape, staining properties and perhaps in other characteristics, such as motility. Examination for a period of time teaches that individuals differ in "age", if we define a cell's age as the chronological time since it was formed by fission of its parent, since fissions are never fully synchronous events. More refined analyses reveal even more fundamental, if less obvious, distinctions between individuals. In summary, individuals of a population do not all exist in the same "state", but rather represent a distribution of states.

The first simplifying assumption in most microbial growth models is that the distribution of states can be ignored and the properties of the culture can be adequately described in terms of a "typical" individual whose behaviour represents an average over the distribution of states. Such assumptions at once lead to uncertainty about the validity of the model, since they imply that a whole host of parameters of the population are not important in determining the properties or activities of the population.

There are three reasons for making these assumptions; one involves a conceptual difficulty and the other two involve practical difficulties. The conceptual difficulty is: What do we mean by the "state" of an individual organism? Can we use some obvious parameter associated with a cell as a measure or index of its state? Would the age of a cell (as defined above) serve this purpose? Or can we use the size of a bacterial cell as an index of its state? Or is some more general notion of state, such as the biochemical constitution of the cell needed?

An obvious practical difficulty involved in the use of any model that recognizes a distribution of states is: How does one *measure* the states of individual organisms so that some idea of the distribution can be obtained? The idea of bacterial size as an index of state is attractive here, since electronic devices measure size distributions quickly and accurately. Many workers might prefer to use age or some other cell feature as an index of

state. The theoretical work of Frederickson *et al.* (1967) has shown that any *single* index of state is going to be inadequate in all but the most rigidly controlled growth conditions.

The other practical difficulty involved here is the fact that the equations resulting from models recognizing a distribution of states quickly became *mathematically intractable.*

For these reasons, only models where distribution of states among individual organisms is neglected will be considered.

3.4.2. *Neglect of Segregation*

In unicellular micro-organisms, life is *segregated* into structurally and functionally discrete units—cells. Hence, the *number* of individual organisms present in a population must be an important parameter for the description of the population. Such quantities as the biomass of the population must also be involved. Nevertheless, number must be a quantity of prime importance since the biological characteristics of a population composed of $2n$ organisms having total biomass M are not the same as those of a population composed of n organisms having total biomass M. ($2n$ and n as used here refer to number of organisms.)

In spite of the foregoing arguments, many models make the assumption that segregation of life into discrete units can be ignored. With such an assumption, number of organisms is not admitted as a parameter to be described by the model and, in effect, the model views the population as biomass *distributed continuously* throughout the culture. Models based on such neglect of segregation are *non-segregated* or *distributed.*

Can a non-segregated model have any success in the description of unicellular growth? There may be no *practical* need for knowing numbers of organisms present (biomass may be the quantity of practical importance), but in general, increase in number (proliferation) and increase in biomass (growth) are coupled processes so that one cannot really omit the one from a model purporting to describe the course of the other. A possible explanation for the success of some non-segregated models is that they have been applied to *balanced growth*, or nearly balanced growth situations. Under these conditions, growth and proliferation are *proportional*, so that biomass is directly related to number of organisms.

3.4.3. *Neglect of Stochastic Phenomena*

It is not possible to predict the behaviour of individual microbial cells with certainty. Thus, generation times of individual bacterial cells are not all the same, but rather show random deviations about mean values. Models

for microbial propagation generally make the simplifying assumption that the foregoing stochastic phenomena can be neglected, and that growth can be treated as a deterministic process. If this assumption cannot be made, then non-segregated models cannot be used, either; if cells divide at random times, the number of cells present must be a variable of the model.

Stochastic population models (so-called "birth-and-death processes") very quickly lead to formidable mathematical difficulties, even when one attempts to model only very simple biological phenomena. Hence, it is desirable to avoid such models whenever possible.

Fortunately, avoidance of these models is usually permissible in microbial processes because one is dealing with an enormous number of cells so that random deviations cancel out. There are situations where random deviations are important, however, and these always deal with cases where the *total number* of cells involved is small. Sterilization is one such situation and various transient growth situations, in which the population size for one reason or another becomes quite small (such as near the critical dilution rate in continuous culture) and others. Hence, stochastic phenomena will be ignored.

3.4.4. *Neglect of Biological Structure*

Two micro-organisms having the same biomass and inhabiting the same environment may nevertheless have widely different properties and activities. This is the problem of *state* again; the two organisms have different states. If the model recognizes the existence of a distribution of states, it should also recognize that distribution may change in response to changes in the environment. Or, if the model does not recognize the existence of a distribution of states, it should at least recognize the possibility that the state of the average or typical organism (which is all such models consider) can change in response to changes in the environment. This means that parameters *in addition* to population number and population biomass must in general be important for the description of population behaviour.

Many microbial process models currently used do not recognize this; in most of these models, population biomass is the sole variable employed for describing the population. Since this procedure regards organisms and population biomass as featureless, structureless entities, we shall call such models *unstructured*.

4. Biofilm Processes

Development of a systematic understanding of biofilm processes has been limited because of the interaction of several contributing rate processes.

Mechanistically, biofilm development may be described as the net result of the following:

(1) *Transport of material from the bulk fluid to the surface and attachment to the surface*. Materials can be soluble (microbial nutrients and organics) or particulate (viable micro-organisms, their detritus or inorganic particles). Also, suspended particles of sufficient mass may control films by "scouring" action.
(2) *Microbial metabolism within the film*. Microbial growth in the biofilm and extracellular polymers produced by the micro-organisms contribute to the biofilm deposit and promote adherence of inorganic suspended solids.
(3) *Fluid shear stress at the surface of the film*. Such forces can limit the overall extent of the biofilm deposit by re-entraining attached material.
(4) *Surface material and roughness*. Surface properties can influence micromixing near the surface and corrosion processes. Some metal surfaces may release toxic components into the biofilm inhibiting growth and/or attachment. Some surfaces are porous (e.g. wood) and provide environments protected from fluid shear forces.
(5) *Biofilm control procedures*. In heat exchangers and other systems where biofilms interfere with performance, chemical compounds are introduced to control or prevent them. Chlorine, the most commonly used chemical, oxidizes biofilm polymers causing disruption and partial removal. Inactivation of a portion of the microbial population also occurs. Altered biofilm "roughness" and decreased viable cell numbers will influence "regrowth" rates of the biofilm. Mechanical forces can physically remove portions of the attached film.

The physical, chemical and biological structure of the biofilm is influenced by its environment and, in turn, influences its environment (e.g. substrate removal, fluid frictional resistance and heat transfer resistance).

4.1. Properties and Composition of Biofilms

Micro-organisms, primarily bacteria, adhere to surfaces ranging from the human tooth and intestine to the metal surface of condenser tubes exposed to turbulent flow of water. The micro-organisms "stick" by means of extracellular polymer fibres, fabricated and oriented by the cell, that extend from the cell surface to form a tangled matrix termed a "glycocalyx" by Costerton *et al.* (1978). The fibres may conserve and concentrate

extracellular enzymes necessary for preparing substrate molecules for ingestion, especially high molecular weight or particulate substrate frequently found in natural waters.

The biofilm surface is highly adsorptive, partially due to its polyelectrolyte nature, and can collect significant quantities of silt, clay and other detritus in natural waters.

Physical, chemical and biological properties of biofilms are dependent on the environment to which the attachment surface is exposed. The physical and chemical microenvironment combine to select the prevalent micro-organisms which, in turn, modify the microenvironment of the surface. As colonization proceeds and a biofilm develops, gradients develop within the biofilm and average biofilm properties change. Changes in biofilm properties that occur during biofilm development must be considered when attempting to predict the effect of biofilms on mass, fluid and heat transport in turbulent flow systems. These changes have been largely ignored in past studies.

4.1.1. *Physical Properties*

Relevant *thermodynamic properties* of biofilm are its volume (thickness) and mass. In turbulent flow systems, wet biofilm thickness (*Th*) seldom

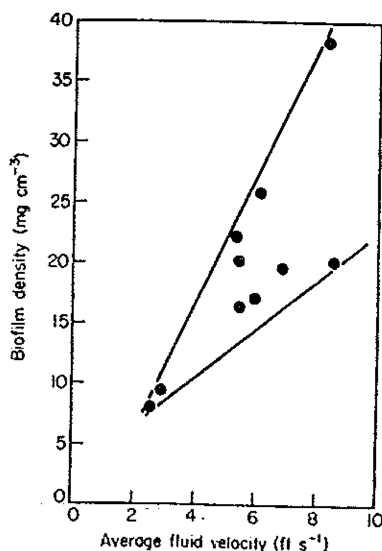


Fig. 6. Influence of fluid shear stress of biofilm density.

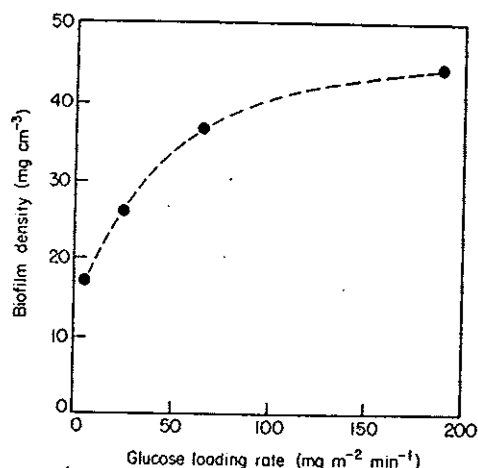


Fig. 7. Influence of glucose loading rate on biofilm density.

exceeds 1000 μm (Zelver, 1979). The biofilm dry mass density (ρ_{Th}) can be determined from the wet biofilm thickness if the biofilm mass per unit area ($\rho_{Th} Th$) is known. ρ_{Th} reflects the attached dry mass per unit wet biofilm volume and measured values in turbulent flow systems range from 10 to 50 mg cm^{-3}. ρ_{Th} increases with increasing turbulence (Characklis, 1980) and increasing substrate loading (Trulear and Characklis, 1982) as indicated in Figs 6 and 7. The increase in ρ_{Th} with increasing turbulence may be caused by one of the following phenomena:

(1) Selective attachment of only certain microbial species from the available population.
(2) Microbial metabolic response to environmental stress.
(3) Fluid pressure forces "squeeze" loosely bound water from the biofilm.

The relatively low biofilm mass densities compare well with observed water content of biofilm (Characklis *et al.*, 1981; Characklis, 1973, 1980).

The *transport properties* of biofilm are of critical importance in quantifying effects of biofilms on mass, heat and momentum transfer. Diffusion coefficients for various compounds through microbial aggregates have been reported in the literature, mostly for floc particles (Table 4). Matson and Characklis (1976) report variation in the diffusion coefficient for glucose and oxygen with growth rate and carbon-to-nitrogen ratio. In biofilms, the

Table 4. Experimental diffusion coefficient measurements from the literature (from Matson and Characklis, 1976).

Reactant	Diffusivity ($10^{-5} cm^2 s^{-1}$)	D_{floc}/D_{H_2O} $\times 100\%$	Biomass type	Growth system	Procedure	Reference
Oxygen	1.5	70	Bacterial slime	Rotating tube	Reaction products analysis	Tomlinson and Snaddon, 1966
Oxygen	0.21	8	Fungal slime	Fluidized reactor	Non-linear curve fit	Mueller *et al.*, 1966
			Zooglea ramigera			
Glucose	0.048	8	*Zooglea ramigera*	Fluidized reactor	Non-linear curve fit	Baillod and Boyle, 1970
Glucose	0.06–0.6	10–100	Mixed culture	Fluidized reactor	Two chamber	Pipes, 1974
Oxygen	2.2	90	Nitrifier culture	Fluidized reactor	Two chamber	Williamson and McCarty, 1976
Ammonia	1.3	80				
Nitrate	1.4	90				
Oxygen[a]	0.4–2.0	20–100	Mixed culture	Fluidized reactor	Two chamber	Matson, 1975
Glucose[a]	0.06–0.21	10–30				Matson, 1975

[a] Tests conducted under a variety of experimental conditions.

Table 5. Viscoelastic properties of biofilm developed at 40°C at a fluid shear stress of $3.3\,N\,m^{-2}$. Glucose was growth-limiting and was applied at $6.2\,mg\,m^{-2}\,min^{-1}$ (Characklis, 1980).

Elastic (storage) modulus	$59.5\ N\ m^{-2}$
Viscous (loss) modulus	$118\ N\ m^{-2}$

diffusion coefficient is most probably related to biofilm density. *In situ* rheological measurements indicate that the biofilm is viscoelastic with a relatively high viscous modulus as indicated in Table 5. Reported biofilm thermal conductivities are presented in Table 6. As expected from reported water content, biofilm thermal conductivity is not significantly different from water.

Table 6. Thermal conductivity of biofilm and other selected materials relevant to biofouling of heat exchangers.

Material	Thermal conductivity ($W\ m^{-1}\ K^{-1}$)	Temperature (°C)	Reference
Biofilm	0.68 ± 0.27	28.3 ± 0.3	Characklis, *et al.*, 1981
	0.71 ± 0.39	26.7 ± 0.3	
	0.57 ± 0.10	28.3 ± 0.3	
Water	0.61	26.7	Weast, 1973
	0.62	28.3	
Carbon steel	51.92	0–100	Perry and Chilton, 1973
Steel	46.86	18	Atomic Energy Commision, 1955
Stainless steel (type 316)	16.30	0–100	Perry and Chilton, 1973
Aluminium 5052	138.46	20	Perry and Chilton, 1973
	205.85	100	
Cupronickel 10% 706	44.71	0–100	Perry and Chilton, 1973
Copper	384	18	Atomic Energy Commission, 1955
Titanium (commercial pure)	16.44	0–100	Perry and Chilton, 1973
Glass	0.6–0.9	—	Weast, 1973

Table 7. Chemical properties of biofilms obtained from fouled surfaces experiencing excessive frictional losses.

	Pollard and House, 1959	Minkus, 1954	Minkus, 1954	Arnold, 1936	Characklis, 1980
Water	87	85.6	90	95	96
Volatile fraction	2.5	2.7	1.9	2.4	3.2
Fixed fraction	10.5	11.7	8.1	2.6	0.8
Si (as percentage fixed fraction)		7.0	11.8	12.5	
Fe (as percentage fixed fraction)		18.5	7.9	1.4	
Al (as percentage fixed fraction)		7.5		3.9	
Ca (as percentage fixed fraction)		1.0	5.6		
Mg (as percentage fixed fraction)		2.5		3.2	
Mn (as percentage fixed fraction)		59.5	56.3	4.9	

Table 8. Chemical composition of biofilms obtained in the field and laboratory emphasizing the primary constituents (C, N, P).

Source	Percentage dry weight						Reference
	Carbon (C)	Nitrogen (N)	Phosphorous (P)	Fixed solids	C/N	C/P	
Biofilm— power plant condenser	6.4–13.8	0.5–3.0	—	—	2–27	—	Anderson *et al.*, 1977
Biofilm— laboratory reactor	42.8	10.0	—	—	4.3	—	Kornegar and Andrews, 1967
Biofilm— laboratory reactor	19.0	9.2	1.8	20	2.1	10.5	Characklis, 1980
E. coli	50.0	14.0	3.0	—	3.6	16.7	Gunsalus and Stanier, 1960

4.1.2. *Chemical Properties*

Inorganic composition of biofilms undoubtedly varies with the chemical composition of the bulk water and probably affects the physical and biological structure of the film. Calcium, magnesium and iron affect intermolecular bonding of biofilm polymers which are primarily responsible for the structural integrity of the deposit. In fact, EDTA is effective in detaching biofilm (Characklis, 1980). In heat exchangers, corrosion products and inert suspended solids can adsorb to the biofilm matrix and influence its chemical composition. Table 7 reports the range of inorganic composition observed in selected biofilms.

The organic composition of the biofilm is strongly related to the energy and carbon sources available for metabolism. Classic papers (Herbert, 1961; Schaechter *et al.*, 1958) have demonstrated the effect of environment and microbial growth rate on the composition of the cells and their extracellular products. For example, nitrogen limitation can result in production of copious quantities of microbial extracellular polysaccharides. Trulear (1983) indicates that only 10% of the biofilm organic carbon can be attributed to the cells in a balanced growth media where glucose is the limiting growth factor. Table 8 presents data on the composition of biofilms developed in the field and in the laboratory. In terms of macromolecular composition, Bryers (1979) has measured protein-to-polysaccharide mass ratios ranging from 0 to 10 (polysaccharide concentration in terms of glucose and protein concentration based on casein) with increasing biofilm accumulation. Other chemical analyses of biofilm have been reported by Bryers and Characklis (1979).

4.1.3. *Biological Properties*

The organisms which colonize the attachment surface will strongly influence biofilm development rate and biofilm chemical and physical properties. However, organism–organism and organism–environment interactions undoubtedly shift population distributions during biofilm accumulation. Several investigators have observed succession during biofouling (Marshall, 1976; Corpe, 1978).

The first visible signs of microbial activity on a surface are usually small "colonies" of cells distributed randomly on the surface. As biofilm development continues, the colonies grow together forming a relatively uniform biofilm. The viable cell numbers are relatively low in relation to the biofilm volume (10^4–10^8 cm^{-3} biofilm) occupying only from 1 to 10% of the biofilm in dilute nutrient solutions (Characklis, 1980). Jones *et al.* (1969), present photomicrographs which corroborate these data in natural and laboratory systems.

In many cases, filamentous forms emerge as the biofilm develops further. *Hyphomicrobium*, *Sphaerotilus* and *Beggiatoa* are frequently identified. The filamentous forms may gain an ecological advantage as the biofilm develops since their cells can extend into the flow to obtain needed nutrients or oxygen which may be depleted in the deeper portions. Jenkins (1980) has demonstrated the competitive advantage of a *Sphaerotilus* sp. over a *Pseudomonas* sp. under low oxygen tension in a fermenter. Trulear (1983) has demonstrated the advantage obtained by *Sphaerotilus* over *Pseudomonas* in a reactor with a high surface area-to-volume (approx. 3 cm^{-1}).

4.2. Rate Processes Contributing to Biofilm Development

In this discussion, biofilm development will be considered to be the net result of the following physical, chemical and biological processes:

(1) Transport of organic molecules and microbial cells to the wetted surface (Fig. 8).
(2) Adsorption of organic molecules to the wetted surface resulting in a "conditioned" surface (Fig. 9).
(3) Adhesion of microbial cells to the "conditioned" surface (Fig. 10).
(4) Metabolism by the attached microbial cells resulting in more attached cells and associated material (Fig. 11).
(5) Detachment of portions of the biofilm (Fig. 16).

4.2.1. *Transport to the Wetted Surface*

When a clean surface is immersed in natural water, transport controls the initial rate of deposition (Fig. 8). In very dilute suspensions of microbial cells and nutrients, transport of microbial cells to the surface may be the rate-controlling step for long periods of time. Biofilm development in open ocean waters or distilled water storage tanks may be illustrative of these cases. Transport of molecules and particles smaller than 0.01–0.1 μm is described satisfactorily in terms of diffusion. In turbulent flow, the diffusion equation must be modified to include turbulent eddy transport. Transport of such small molecules and particles is relatively rapid compared to transport of larger particles. Consequently, adsorption of organic molecules is reported to occur "instantaneously" in many cases as schematically illustrated in Fig. 9.

Larger particles develop a sluggishness with respect to the surrounding fluid. As the particle approaches the wetted surface, eddy transport diminishes and the viscous sublayer exerts a greater influence. For soluble matter and very small particles, diffusion can adequately describe transport

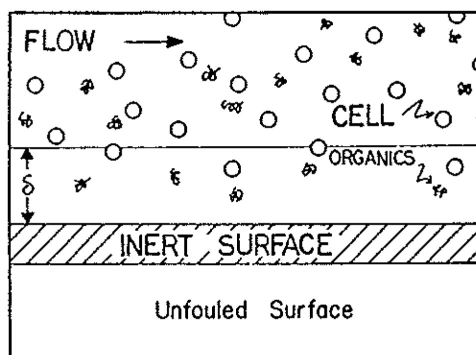


Fig. 8. A "clean" surface exposed to a turbulent flow of fluid containing dispersed micro-organisms, nutrients and organic macromolecules. δ refers to the viscous sublayer thickness.

in the viscous sublayer (Lister, 1981; Lin *et al.*, 1953; Wells and Chamberlain, 1967). For larger particles, other mechanisms must be considered to explain experimental observations.

Within a turbulent flow regime, larger particles suspended within the fluid are transported to the solid surface primarily by fluid dynamic forces. Particle flux to the surface increases with increasing particle concentration. However, particle flux is also strongly dependent on the physical properties of the particles (e.g. size, shape, density) and is influenced by many other forces near the attachment surface.

Microbial cells (0.5–10.0 μm effective diameter) can be transported from

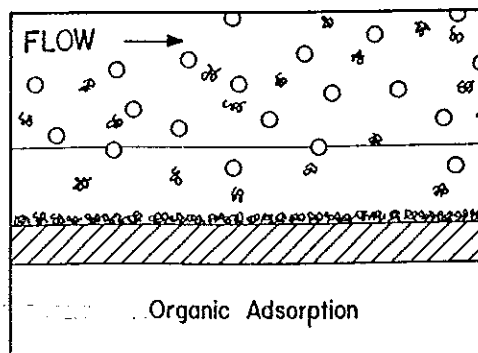


Fig. 9. Transport and adsorption of an organic monolayer on a clean surface.

the bulk fluid to the wetted surface by several processes including the following:

diffusion (Brownian),
gravity,
thermophoresis,
taxis,
fluid dynamic forces,
  inertia,
  lift,
  drag,
  drainage,
  downsweeps.

Particles in turbulent flow are transported to within short distances of the surface by eddy diffusion. Particles are propelled into the viscous (or laminar) sublayer under their own momentum. Turbulent eddies supply the initial impetus and frictional drag slows down the particle as it penetrates the viscous sublayer (Friedlander and Johnstone, 1957; Beal, 1970). For microbial cells, the inertial forces are very small because of their small diameter and density (in relation to water).

If the particle is travelling faster than the fluid in the region of the wall, the *lift force* directs the particle towards the wall (Rouhiainen and Stachiewicz, 1970). This would normally be the case if particle density is greater than fluid density and the particle is moving towards the wall. *Frictional drag forces* can be significant, especially in the viscous sublayer region. The drag force slows down the particle as it approaches the surface and is proportional to difference between particle velocity and fluid velocity.

If the mass density of the particle, ρ_p, differs substantially from the fluid density, ρ, the *gravity force* may be significant. For microbial cells in turbulent flow, the gravity force is generally negligible. *Thermophoresis* is only relevant when particles are being transported in a temperature gradient (Lister, 1981). If the surface is hot and the bulk fluid is cold, the thermophoretic force will repel the particle from the surface. *Eddy diffusion* may be instrumental in dispersing particles in the turbulent core region, thus maintaining a relatively uniform concentration in that region. However, eddy diffusion will not be significant in transporting particles to the wall. *Brownian diffusion* contributes little to the transport of microbial cells (>1.0 μm diameter) in turbulent flow. Certain microbes are capable of *motility* or *taxis* through their own internal energy. Velocities as high as 4×10^{-4} cm s^{-1} have been observed. Taxis could possibly be a significant transport process within the viscous sublayer. For particles in liquids, the *fluid drainage force* is significant (Lister, 1981). The drainage force describes

the resistance the particle encounters near the wall due to the pressure in the draining fluid film between the two approaching surfaces. This force is quite large for a microbial cell as it approaches the wall.

Recent published research on the structure of the viscous sublayer in turbulent flow indicates that "*downsweeps*" of fluid from the turbulent core penetrate all the way to the wall (Cleaver and Yates, 1975, 1976). Particles in the bulk fluid are transported all the way to the wall by these convective downsweeps. Aside from lift, this is the only fluid mechanic force directing the particle to the wall. Downsweeps are apparently quite important in terms of particle transport to the wall in turbulent flow.

For a Reynolds number = 30 000 in tube flow, the bursts resulting from the downsweeps have the following characteristics:

Burst diameter	0.11 cm
Average axial distance between bursts	0.50 cm
Mean time between bursts	0.006 s

Minimum transport rate of particles would be observed when particle diameter approximates 0.1×10^{-4} cm under constant fluid flow conditions. At this diameter, *Brownian diffusion* starts exerting a significant effect. Calculated particle flux in the pipe for Reynolds number = 30 000 and a bulk fluid particle concentration 10^4 particles cm^{-3} is approximately 0.1 particles $cm^{-2} s^{-1}$.

Surface roughness significantly influences transport rate and microbial cell attachment for several reasons including the following:

(1) Increases convective mass transport near the surface.
(2) Provides more "shelter" from shear forces for small particles.
(3) Increases surface area for attachment.

If surface roughness elements are larger than the viscous sublayer, the roughness can be measured quantitatively by hydraulic methods. If surface roughness elements are smaller than the viscous sublayer (i.e. microroughness), measurements of roughness are difficult to quantify and interpret. Brown (1974) reports that particle deposition from gases is very sensitive to roughness too small to be detected by fluid frictional resistance.

When a "clean" surface first contacts water with biological activity, organic substances and microbial cells must be transported to the surface before biofilm development can begin. Consequently, the rate of transport determines the length of the "induction" period, i.e. the initial period during which no macroscopic effects of the biofilm are evident. In very dilute solutions (e.g. open ocean), the rate of transport may control the overall rate of biofilm development for long periods. Rate of transport is

proportional to the concentration difference between the bulk fluid and the surface. In dilute solutions, this difference is small. The flow regime (zero, laminar or turbulent) also significantly influences transport rates and should be defined carefully in any experimental system used for biofilm studies. Surface characteristics are also critical to the repeatability and applicability of the results because a rough surface will increase transport and attachment rates. Which rate controls—rate of transport or rate of adhesion?

4.2.2. *Adsorption of Organic Molecules to the Wetted Surfaces*

Figure 8 illustrates an initially "clean" surface exposed to turbulent flow of a fluid containing dispersed micro-organisms, nutrients and organic macromolecules. Micro-organisms select their habitats on the basis of many factors, including the nature of the wetted surface (material of construction and surface roughness). Adsorption of an organic monolayer occurs within minutes of exposure as shown in Fig. 9 and changes the properties of the wetted surface. Investigations have shown that materials with diverse surface properties (e.g. wettability, surface tension, electrophoretic mobility) are rapidly conditioned by adsorbing organics when exposed to natural waters with low organic concentrations. These organic molecules are usually polysaccharides or glycoproteins. Loeb and Neihof (1975) and DePalma *et al.* (1979) have measured adsorption rates of organic molecules in seawater, and Bryers (1979) has observed adsorption rates in a laboratory system. Rates and extent of adsorption in these investigations are presented in Table 9. Maximum accumulation from molecular fouling is less than 0.1 μm. The rate of molecular fouling can be considered instantaneous since it is much greater than the rate of microbial fouling. Based on "thickness" measurements, molecular fouling can have no significant effect on fluid flow or heat transfer. Nevertheless, the surface properties resulting from adsorption of an organic film may affect the sequence of microbial events which follow.

Costerton *et al.* (1978) have discussed the pronounced specificity of some bacteria that attack only a particular animal host tissue and suggest that specificity may be explained by the specificity of the host-tissue glycocalyx. It remains to be seen whether an abiotic surface, wetted by the adsorption of organic molecules indigenous to that environment, will be initially colonized by a *specific* microbial cell.

Brash and Samak (1979) present experimental evidence that significant turnover occurs in molecular (proteinaceous) films developed on polyethylene. Protein molecules in the bulk fluid are continuously exchanging with adsorbed proteins. This suggests that dispersed microbial cells in the

Table 9. Maximum rate and extent of molecular fouling.

Maximum rate ($nm\ min^{-1}$)	Maximum accumulation (nm)	Maximum accumulation ($\mu g\ COD\ cm^{-2}$)	Surface	Reference
0.15–0.45	30–80		Pt[a]	Loeb and Neihof, 1975
0.004	7.1		Ge[b]	DePalma *et al.*, 1979
0.004	77.3		Ti[b]	
0.01[5]	13.5[c]	1.5	glass[d]	Bryers, 1979
0.22[5]	22.5[c]	2.5	glass[e]	

[a] Immersed in quiescent Chesapeake Bay water (3–4°C) containing 2.3 mg carbon l^{-1}, salinity between 9 and 16‰ and pH between 7.9 and 8.2.
[b] Gulf of Mexico water (22°C) flowing past the surface at a fluid shear stress of 7.1 $N\ m^{-2}$. Salinity was 34‰. Carbon concentration not reported.
[c] Estimated from measurements of chemical oxygen demand (COD) adsorbed per unit area. Assumed DOC of protein is 0.855 mg COD mg^{-1} protein and protein density is 1.3 g protein cm^{-3}.
[d] Medium consisted of sterile 1:1 w/w trypticase soy broth–glucose mixture (34°C; pH 8). The glass surfaces were immersed in tubes placed in a mechanical shaker. Carbon concentration was approximately 80 mg carbon l^{-1}.
[e] Medium was effluent (30°C; pH 8) from a chemostat (10–20 mg l^{-1} COD, 3 mg l^{-1} polysaccharide) with no primary substrate remaining. Micro-organisms were present (approximately 10^6 cells ml^{-1}) but no cells attached during the period of interest. Fluid shear stress was 3.8 $N\ m^{-2}$.

bulk fluid and their associated extracellular material may be continually exchanging with biofilm material at the wall.

4.2.3. *Adhesion of microbial cells to the wetted surface*

Previous research (Marshall *et al.*, 1971; Zobell, 1943) suggests the existence of a two-stage adhesion process: (1) reversible adhesion followed by, (2) an irreversible adhesion. Reversible adhesion refers to an initially weak adhesion of a cell which no longer exhibits Brownian motion but is readily removed by mild rinsing. The adhesive forces which hold the cell at the wall during reversible adhesion probably include electrostatic, London–van der Waals, interfacial tension and covalent bonding. Conversely, irreversible adhesion is a permanent bonding to the surface, usually aided by the production of extracellular polymers. Cells attached in this way can only be removed by rather severe mechanical treatment. Marshall (1976) and Corpe (1970) have implicated polysaccharides and glycoproteins in irreversible adhesion (Fig. 10).

Most of the research on cell adhesion has been conducted at very low fluid shear stress or in quiescent conditions (Fletcher, 1977). Under these

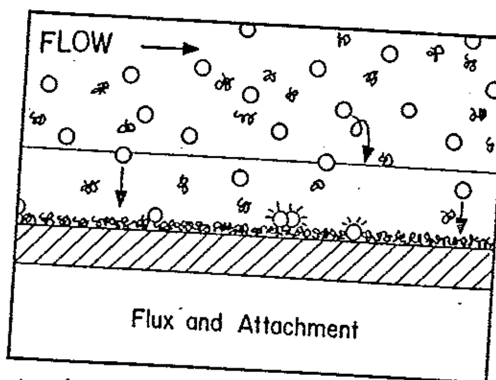


Fig. 10. Transport and attachment of microbial cells to the conditioned surface.

conditions sedimentation or diffusion may control the rate of adhesion. There is yet to be a demonstration of reversible adhesion in turbulent flow.

In turbulent flow, the *net* rate of adhesion is the quantity most easily measured. The net rate of adhesion is the difference between the rate of adhesion and rate of detachment. Detachment results from several forces including fluid dynamic forces, shear forces, lift (upsweeps) and taxis. Upsweeps are analogous to the downsweeps discussed previously. Upsweeps result in turbulent bursts which move away from the surface into the bulk flow. Upsweeps generate a lift force normal to the surface which can influence detachment. Drag or viscous shear forces act in the direction of flow on attached cells and are approximately 1000 times greater than the lift forces acting on attached cells. Note that although viscous shear may dislodge a particle, unless a lift force is present, the particle will

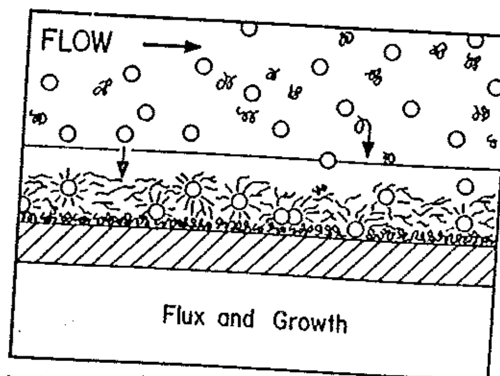


Fig. 11. Continued transport and attachment of microbial cells as well as growth and other metabolic processes within the biofilm.

Table 10. A matrix representation for the fundamental microbial rate processes.

Process rate / Fundamental process		Stoichiometry							
		Reactants			Products				
		Substrate	Nutrient	Electron acceptor	Biomass		Product		Metabolite
Process	rate	s	z	e	x_T	x_d	p_e	p_i	a
Growth	μ	−	−	−	+		+	(+)	+
Maintenance									
Exogenous	m	−	−	−			+		+
Endogenous	k_e		+	−	−	(+)	+	−	+
Product formation	k_p	−	−	−			+	+	+
Death									
Loss of viability	k_d				−	+			
Lysis	k_L	(+)	(+)		−	(+)	+		
Observed rate		q_s	q_z	q_e	μ_n		q_p		q_a

where q = specific production or removal rate (t^{-1})
μ = specific growth rate or specific biomass production rate (t^{-1})
x_T = total biomass concentration (ML^{-3})
x_d = inert solids concentration (ML^{-3})
p_e = extracellular microbial product concentration (ML^{-3})
p_i = intracellular microbial product concentration (ML^{-3})
s = substrate concentration (ML^{-3})
z = nutrient concentration (ML^{-3})
e = electron acceptor concentration (ML^{-3})
μ_n = net solids production rate (t^{-1})

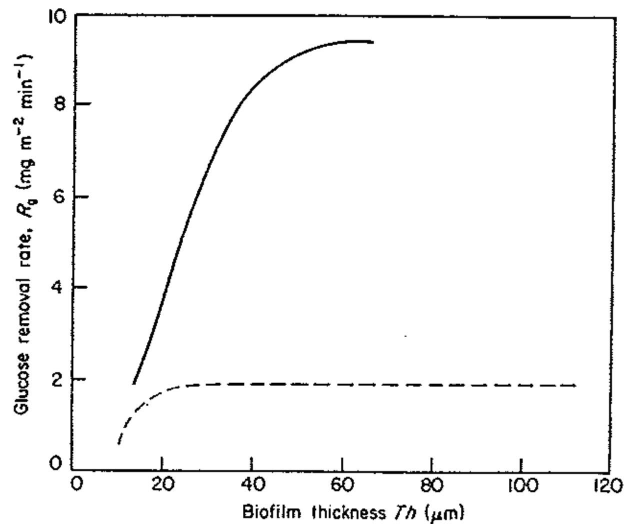


Fig. 12. The influence of biofilm thickness and glucose loading rate on glucose removal rate by a biofilm. Solid curve $R_L = 9.9 \text{ mg m}^{-2} \text{ min}^{-1}$. Dashed curve $R_L = 2.0 \text{ mg m}^{-2} \text{ min}^{-1}$.

presumably roll along the surface until another surface adhesion site is found.

The nature of the surface is an important factor affecting adhesion. Wettability or critical surface tension, is the property used most frequently to describe surface characteristics in microbial attachment studies (Dexter, 1976; Fletcher and Loeb, 1979). In seawater, cell attachment increased with increasing critical surface tension of the surface (including glass, copper, polyethylene, teflon) with the exception of the copper surface on

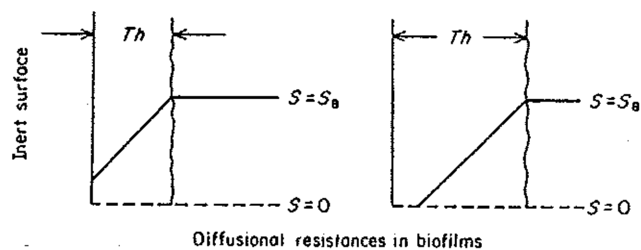


Fig. 13. As biofilm thickness increases beyond the depth of substrate (or oxygen) penetration, substrate (or oxygen) removal rate becomes independent of biofilm thickness.

which fewer cells attached. The copper may inhibit cell attachment by inhibiting a metabolic process necessary for attachment. Even so, there are many examples of biofilm formation on cupronickel condenser surfaces.

The presence of multivalent cations (expecially Ca^{2+}, Mg^{2+} and Fe^{3+}) also influence the attachment process, possibly by altering surface characteristics or by bridging cellular anionic polyelectrolytes to anionic polyelectrolytes adsorbed on the wetted surface.

4.2.4. *Metabolism by the Attached Microbial Cells*

Restricting our discussion to chemosynthetic organisms, the attached microbial cells assimilate reduced organic or inorganic compounds, nutrients and oxygen or some other electron acceptor. The process yields energy with which the cells reproduce, maintain their internal structure and form extracellular products (Fig. 11). Therefore, *growth*, *maintenance* and *product formation* are *fundamental processes* carried out by microbial cells in the presence of sufficient nutrients (Table 10). If nutrients are depleted, or toxic substances are present, *death* and/or *lysis* may occur.

The rates of the fundamental microbial processes are difficult to measure. The *observed rates* (last row, Table 10) are usually rate of substrate (the growth-limiting nutrient) removal, electron acceptor (usually oxygen) removal, biomass production or product formation.

The stoichiometry of each fundamental process can be measured in certain laboratory systems (e.g. chemostats). The rows in Table 10 qualitatively represent the stoichiometry of each fundamental process (− refers to reactant and + refers to product).

Analysis of rate and stoichiometry of processes within a biofilm are frequently complicated by significant mass transfer resistances in the liquid or diffusional resistances within the biofilm.

Trulear and Characklis (1982) have observed substrate removal rate in an experimental biofilm reactor. The substrate removal rate increases in proportion to biofilm thickness up to a critical thickness beyond which removal rate remains constant (Fig. 12). The critical, or "active", thickness is observed to increase with substrate concentration. This behaviour is confirmed by other investigators (LaMotta, 1976; Kornegay and Andrews, 1968) and is attributed to nutrient diffusional limitations within the biofilm. Once the biofilm thickness exceeds the depth of substrate (or oxygen) penetration into the biofilm (Fig. 13), the removal rate is unaffected by further biofilm accumulation.

Observed substrate removal rate cannot be used to distinguish between growth, maintenance, product formation and death. It seems clear from other data (Bryers, 1979) that product formation (primarily polysaccharide)

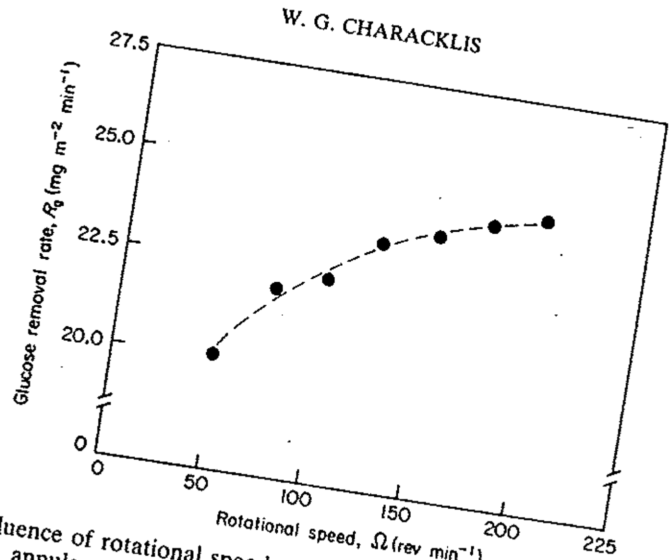


Fig. 14. Influence of rotational speed on glucose removal rate by a biofilm in an annular reactor. $Th = 112\ \mu m$. $R_L = 27.0\ mg\ m^{-2}\ min^{-1}$.

is significant in the early stages of biofilm formation. Maintenance requirements or biomass decay become important as the biofilm gets thicker and substrate does not entirely penetrate the biofilm. These other process rates have not been measured and are critical for determining stoichiometric coefficients and predicting biofilm development rates.

The substrate removal rate is also dependent on fluid velocity (Fig. 14). At low fluid velocities, a relatively thick mass transfer boundary layer (δ_m) can cause a liquid phase diffusional resistance which decreases substrate concentration at the liquid–biofilm interface and thereby decreases substrate removal rate (Fig. 15).

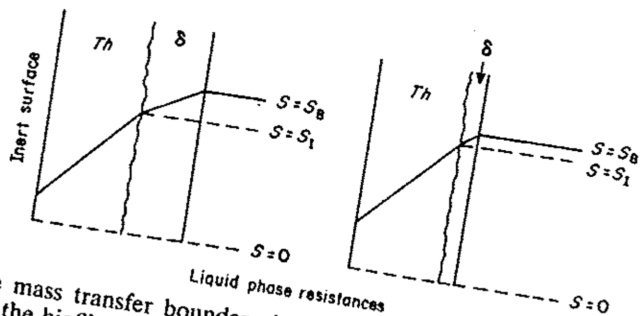


Fig. 15. The mass transfer boundary layer (δ_m) decreases with increasing fluid velocity past the biofilm interface resulting in a higher effective substrate concentration at the biofilm–fluid interface (S_I).

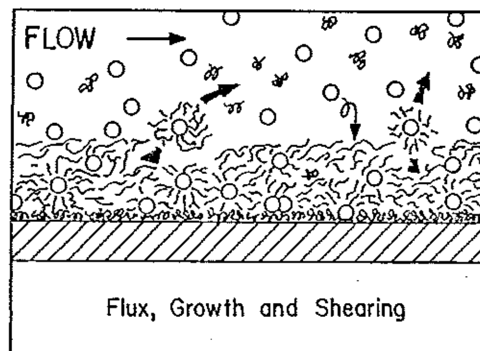


Fig. 16. Transport, attachment and growth increase the accumulated mass of the biofilm while detachment processes decrease the attached mass.

4.2.5. *Detachment of Biofilm*

As the biofilm grows thicker, the fluid shear stress at the biofilm interface generally increases. Also as biofilms grow thicker, the potential for substrate, oxygen or nutrient limitation in the deeper portions is great. These limitations may weaken the biofilm matrix and cause detachment (Fig. 16). Trulear and Characklis (1982) report that the biofilm detachment rate increases with increasing biofilm mass (Fig. 17) and that detachment rate increases with fluid shear stress (Fig. 18).

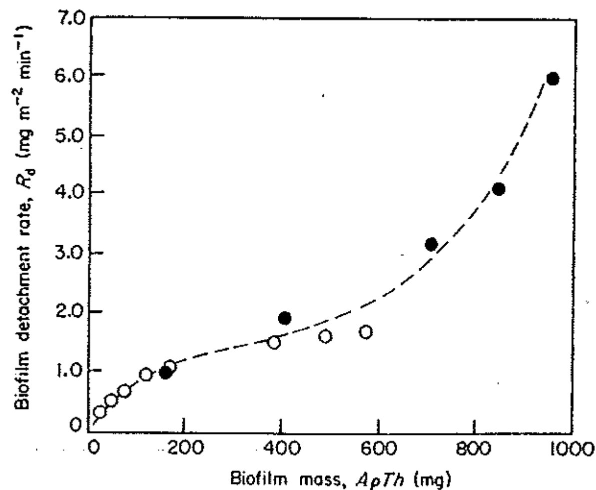


Fig. 17. Influence of biofilm mass on biofilm detachment rate at a constant fluid shear stress. ●, $R_L = 37.2$ mg m^{-2} min^{-1}; ○, $R_L = 4.2$ mg m^{-2} min^{-1}.

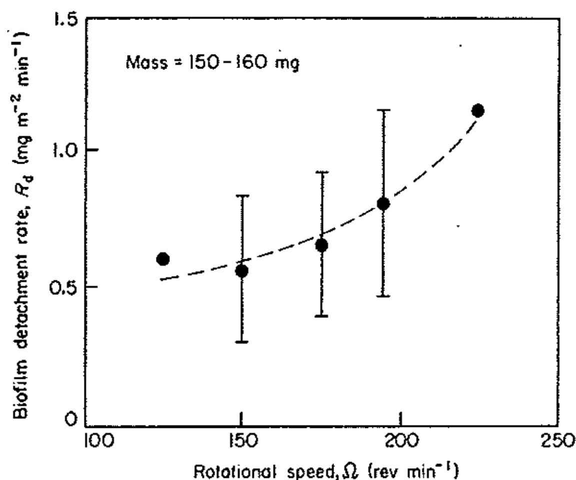


Fig. 18. Influence of fluid shear stress on biofilm detachment rate at a constant attached biomass.

Techniques for determining strength of adhesion and strength of deposit are necessary to further understanding of the detachment process.

4.2.6. *Overall Rate of Biofilm Development*

A general mathematical model for microbial processes in a continuous stirred tank reactor (CSTR), based on material balances, is presented in Table 11. The model considers microbial activity in the bulk fluid as well as the reactor surfaces.

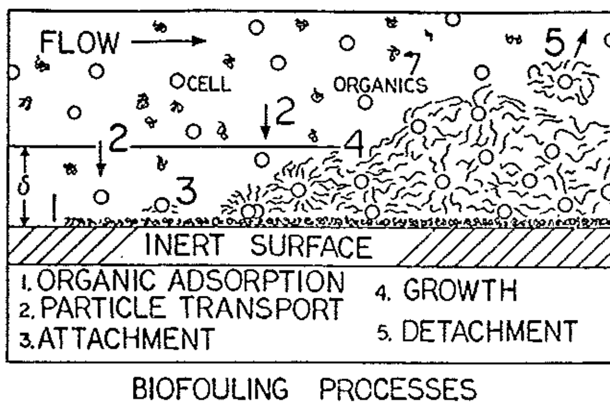


Fig. 19. A summary diagram of processes contributing to biofilm accumulation.

Table 11. Mathematical representation of microbial processes, including biofilm formation, occurring in a CSTR (see Fig. 19).

Substrate material balance

$$V\frac{\mathrm{d}s}{\mathrm{d}t} = F(s_1 - s) - NA - \frac{(\mu - m)xV}{Y_x} \tag{37}$$

Suspended biomass material balance

$$V\frac{\mathrm{d}x}{\mathrm{d}t} = F(x_i - x) + \mu xV - R_A A + R_D A \tag{38}$$

Accumulation of biofilm

$$\frac{\mathrm{d}B}{\mathrm{d}t} = NAY_B - R_D A + R_A A - R_E B \tag{39}$$

Accumulation of total reactor biomass

$$\frac{\mathrm{d}M_t}{\mathrm{d}t} = V\frac{\mathrm{d}x}{\mathrm{d}t} + \frac{\mathrm{d}B}{\mathrm{d}t} = F(x_i - x) + \mu xV + NAY_B - R_E B \tag{40}$$

where A = wetted surface area (L^2)
B = biofilm mass (M_B)
F = volumetric flow rate (L^3t^{-1})
m = maintenance coefficient (t^{-1})
M_t = total reactor biomass (M)
N = substrate flux into the biofilm ($M_S L^{-2}t^{-1}$)
R_A = rate of suspended biomass adsorption onto the biofilm ($M_x L^{-2}t^{-1}$)
R_D = rate of biofilm detachment ($M_B L^{-2}t^{-1}$)
R_E = rate of biofilm decay (e.g., lysis, endogenous respiration) (t^{-1})
$s(s_i)$ = (input) substrate concentration ($M_s L^{-3}$)
t = time (t)
$x(x_i)$ = (input) suspended biomass concentration ($M_x L^{-3}$)
Y_B = yield coefficient for biofilm ($M_B M_s^{-1}$)
Y_x = yield coefficient for suspended biomass ($M_x M_s^{-1}$)
V = reactor volume (L^3)
μ = specific growth rate of suspended biomass (t^{-1})

Biofilm development is the net result of several processes occurring in series and parallel (Fig. 19). The development of a biofilm is adequately described by a sigmoidal-shaped curve (Fig. 20). The slope of this curve at a particular time is the *net* biofilm *development rate* and is also plotted in Fig. 20. The rate increases to a maximum value corresponding to the sigmoidal inflection and then decreases to zero. Net biofilm development rate is expressed as follows (Table 11):

$$\frac{\mathrm{d}B}{\mathrm{d}t} = NAY_B - R_D A + R_A A - R_E B \tag{39}$$

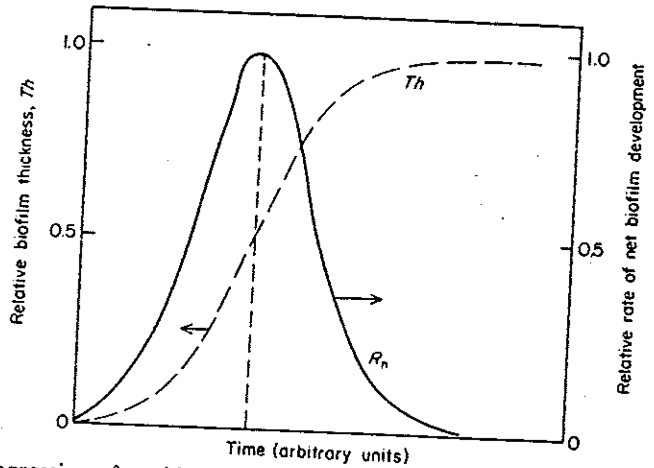


Fig. 20. Progression of net biofilm development is described by a sigmoidal-shaped curve. Net biofilm development rate is the slope of the sigmoidal-shaped curve at any time.

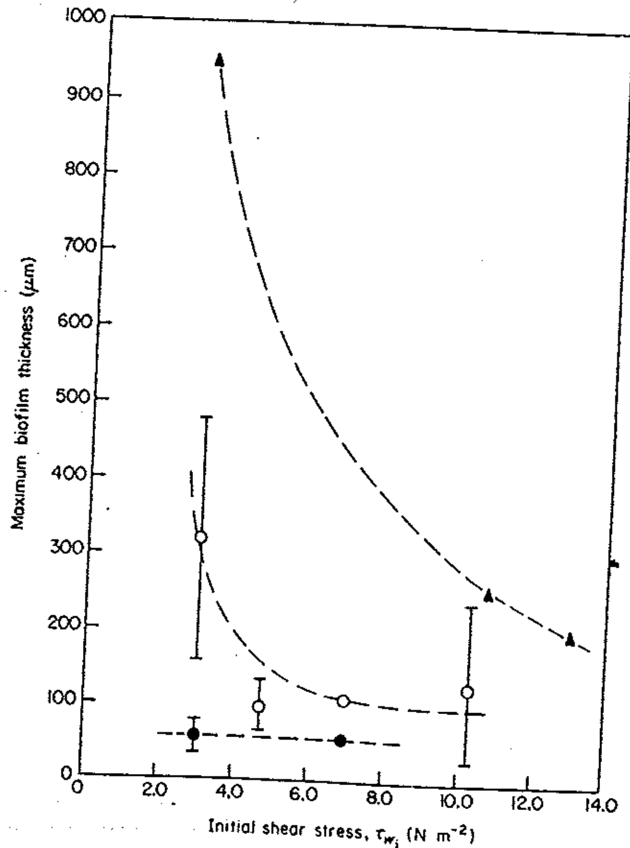


Fig. 21. Influence of fluid shear stress and substrate loading on plateau (or maximum) biofilm thickness. R_L = 18.30 mg m^{-2} min^{-1}. R_L = 2.6–7.0 mg m^{-2} min^{-1}. R_L = 1–15 mg m^{-2} min^{-1}.

where NAY_B is the attached biomass production rate, R_DA is the biofilm detachment rate, R_AA is the adsorption rate of cells and R_EB is the endogenous respiration rate. At steady state, thickness remains constant.

The effect of fluid velocity on the plateau (or steady state) biofilm thickness is illustrated in Fig. 21 for various substrate loadings. At high substrate loadings, increasing fluid velocity increases biofilm detachment rate which minimizes the plateau biofilm thickness. However, at low substrate loadings, fluid velocity seems to have no measurable effect on the plateau thickness. Trulear and Characklis (1982) have demonstrated that plateau biofilm mass exhibits a maximum when fluid velocity is increased. At low fluid velocities, mass transfer limits the rate of biofilm production. Therefore, increasing fluid velocity increases substrate flux into the biofilm and net biofilm development rate increases. As fluid velocity continues to increase, biofilm detachment rate becomes the dominant process and net biofilm development begins to decrease.

4.3 Effects of Biofilms on Fluid Frictional Resistance

Increase in fluid frictional resistance due to biofilm accumulation when flow rate is maintained constant causes an increase in pressure drop and power requirements for pumping as shown in Fig. 22 (Picologlou *et al.*,

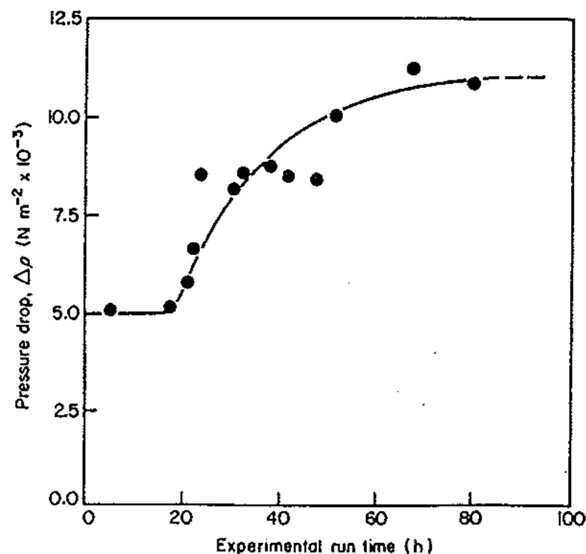


Fig. 22. Change in pressure drop with time due to biofilm formation. Experiment was conducted at constant fluid velocity. Initial $\tau_w = 6.8\ \mathrm{N\,m^{-2}}$. $\bar{v} = 150\ \mathrm{cm\,s^{-1}}$.

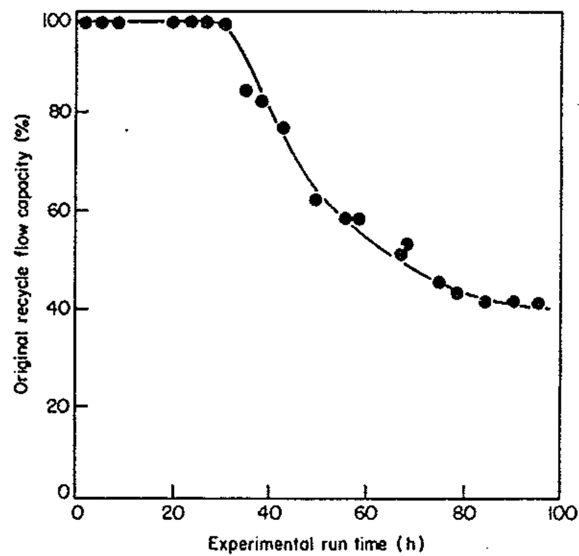


Fig. 23. Change in volumetric flow rate with time due to biofilm formation. Experiment was conducted at constant pressure drop. $\tau_w = 7.9\ \mathrm{N\,m^{-2}}$. Initial $\bar{v}$ = 185 cm s^{-1}.

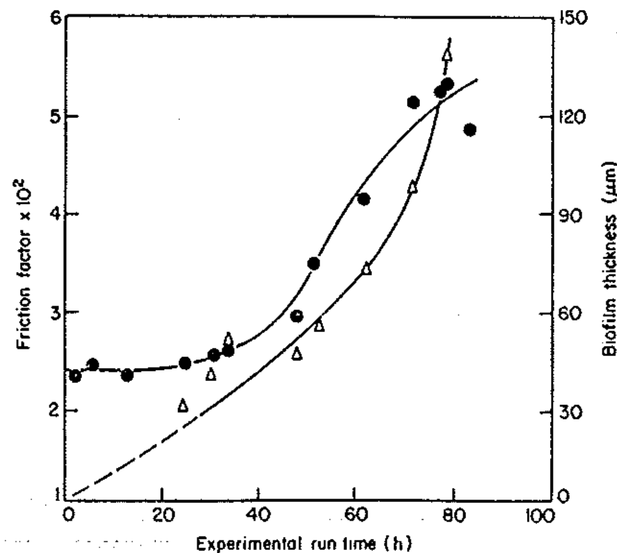


Fig. 24. Change in friction factor and biofilm thickness with time for an experiment conducted at constant pressure drop. $\tau_w = 7.9\ \mathrm{N\,m^{-2}}$. Initial $\bar{v}$ = 180 cm s^{-1}. ●, friction factor; △, biofilm thickness.

1980). Conversely, if pressure drop is held constant, flow capacity is reduced. Figure 23 indicates that flow capacity was reduced to 42% of the original capacity in a 100-h laboratory experiment (Picologlou *et al.*, 1980).

Frictional resistance can be represented by a dimensionless friction factor given by the following equation:

$$f = 2.0 \frac{d}{L} \frac{\Delta_p}{\rho \bar{v}^2} \tag{41}$$

where f = friction factor (dimensionless)
d = tube diameter [L]
ρ = fluid density [ML^{-3}]
$\bar{v}$ = average fluid velocity [Lt^{-1}]
Δp = pressure drop along length L [$ML^{-1}t^{-2}$]
L = length between pressure ports [L]

The change in friction factor and biofilm thickness with time is shown in Fig. 24 for a laboratory tubular reactor. Dehart (1979) has observed similar behaviour in a tubular reactor in the field (Fig. 25).

The friction factor is related to the Reynolds number and the equivalent sand roughness k_s through the empirical Colebrook–White relation. This equation correlates friction factor to the Reynolds number for various "commercially rough" pipes throughout the hydraulically smooth, transition and fully rough regimes. The Colebrook–White equation, solved for the equivalent sand roughness k_s yields

$$k_s = \frac{d}{2} 10^{(0.87 - 0.50 f^{-1/2})} - \frac{18.70}{\mathrm{Re}\, f^{1/2}} \tag{42}$$

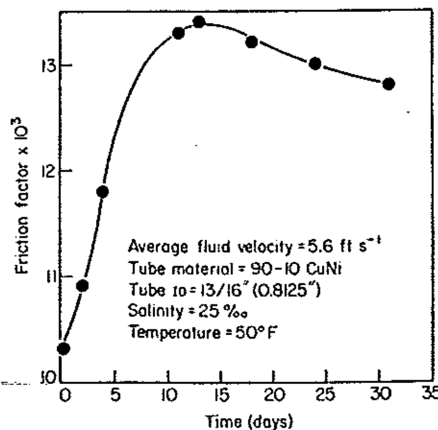


Fig. 25. Change in pressure drop due to biofilm formation at a field location (Dehart, 1979).

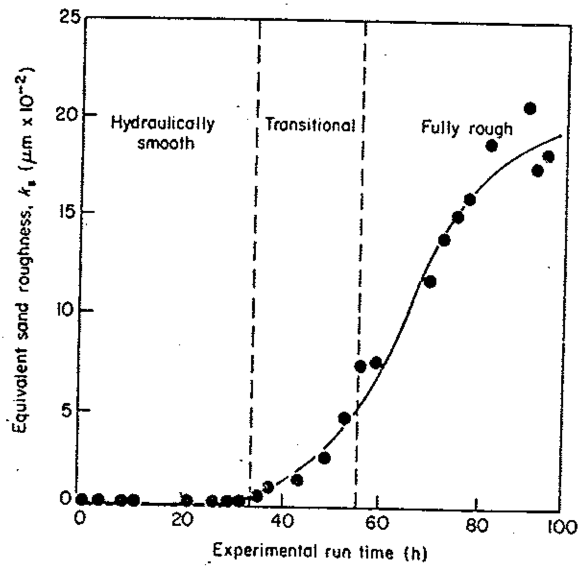


Fig. 26. Change in equivalent sand roughness with time due to biofilm formation. Experiment was conducted at constant pressure drop. $\tau_w = 7.9 \text{ N m}^{-2}$. Initial $\bar{v}$ = 163–185 cm s^{-1}.

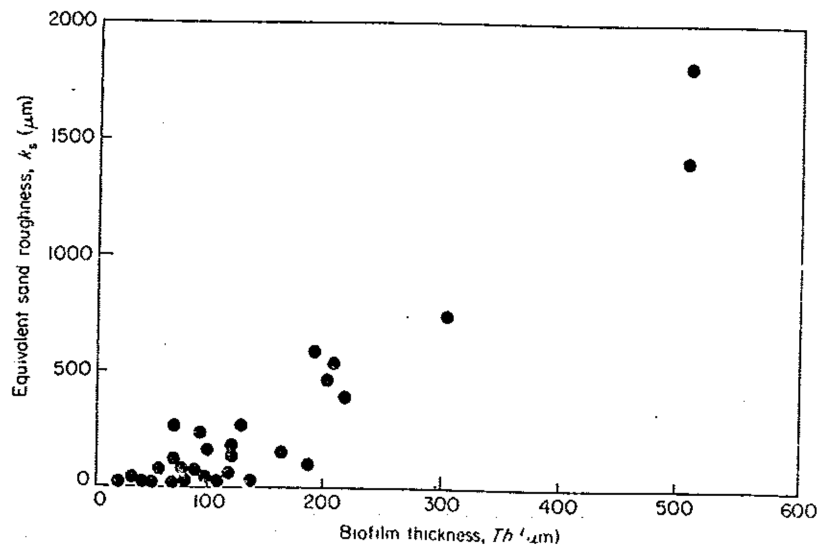


Fig. 27. Change in calculated equivalent sand roughness with biofilm thickness for several experiments conducted at constant pressure drop. $\tau_w = 6.5\text{–}7.9 \text{ N m}^{-2}$. Initial $\bar{v}$ = 163–185 cm s^{-1}.

where d = tube diameter [L]

Re = $\bar{v}d/\nu$ = Reynolds number (dimensionless)

ν = kinematic viscosity [L^2t^{-1}]

This expression can be used to compute an equivalent sand roughness for the biofilm from a measurement of the flow rate and pressure drop. Figure 26 indicates the progression of k_s with time and Fig. 27 presents the change in k_s with biofilm thickness for the range of shear stress investigated by Picologlou *et al.* (1980).

Determination of the flow regime (smooth, transitional or fully rough) depends on the magnitude of k_s relative to the size of the viscous sublayer (δ_l):

$$\delta_l = \frac{10d}{\text{Re}}\left(\frac{f}{2}\right)^{-0.5} \tag{43}$$

More specifically, when $k_s < \delta_l$, the pipe is considered hydraulically smooth; when $14\delta_l > k_s > \delta_l$ the flow is in the transitional regime; when $k_s > 14\delta_l$ the flow is in the fully rough regime (Schlichting, 1968).

Frictional resistance of biofilms grown under constant pressure drop (i.e. constant shear stress) have been compared to the frictional resistance of pipes with a rigid roughness as given by the Colebrook–White equation. The following was observed:

(1) Frictional resistance due to biofilms shows a similar dependency on Reynolds number as frictional resistance due to commercially rough pipe surface.
(2) Frictional resistance is dependent on biofilm thickness.
(3) Frictional resistance does not increase above the hydraulically smooth pipe value until a critical biofilm thickness is attained.

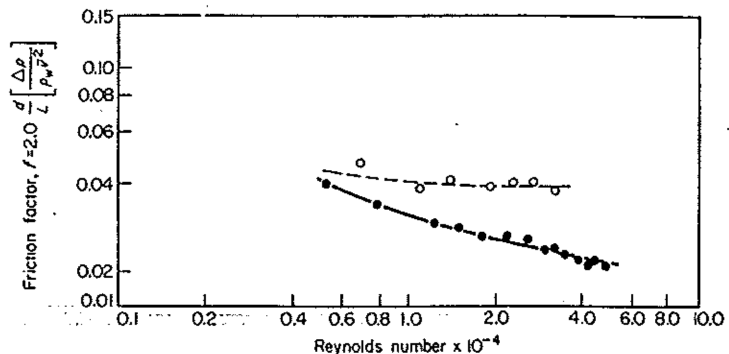


Fig. 28. Change in friction factor as a function of Reynolds number and roughness due to biofilm formation. ○, k_s/d = 0.008; Th = 320 µm. ● clean tube.

The Blasius–Stanton or Moody diagram (Moody, 1944) can be used to compare frictional resistance due to biofilm with frictional resistance of rigid rough surfaces. The Blasius–Stanton diagram is a plot of friction factor versus Reynolds number for a series of pipes with different equivalent sand roughness; the friction factor in a pipe with a rigid rough surface depends on both the relative roughness and the Reynolds number.

The relationship between friction factor and Reynolds number for a fouled circular tube is presented in Fig. 28. The friction factors and Reynolds numbers presented have not been corrected for the pipe constriction resulting from the biofilm. This figure shows the dependency of friction factor on Reynolds number is the same as for a tube with a rigid rough surface within the range of Reynolds number investigated (5000–48 000). This data was obtained by reducing, in steps, the shear stress from its initial value in a given experiment and calculating friction factor and Reynolds number at each step. The shear stress was reduced from the initial condition to minimize detachment of biofilm during the experiment.

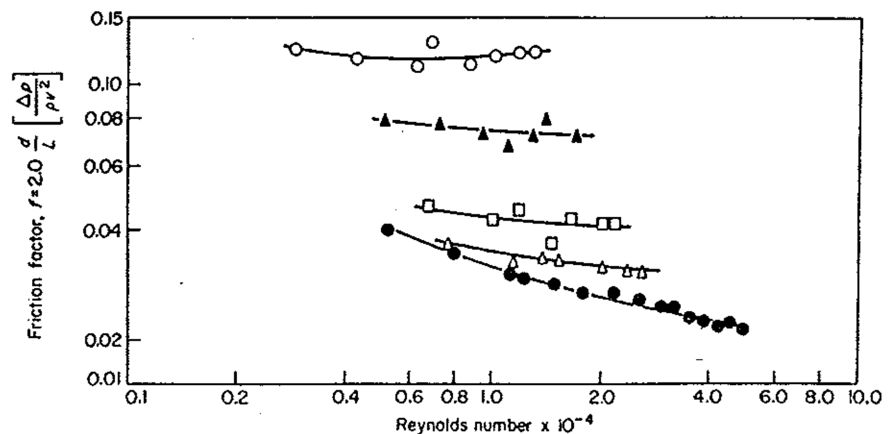


Fig. 29. Change in friction factor as function of Reynolds number and roughness at different stages of biofilm development. ○, $k_s/d = 0.157$; $Th = 500\ \mu m$. ▲, $k_s/d = 0.062$; $Th = 300\ \mu m$. □, $k_s/d = 0.014$; $Th = 165\ \mu m$. △, $k_s/d = 0.002$; $Th = 40\ \mu m$. ●, clean tube.

Figure 29 indicates the relationship between friction factor and Reynolds number within a single experiment at different stages of biofilm development; friction factor increases with biofilm thickness. The relationship between biofilm thickness and friction factor at a wall shear stress from 6.5–7.9 $N\,m^{-2}$ is shown in Fig. 30. Friction factor is dependent on biofilm

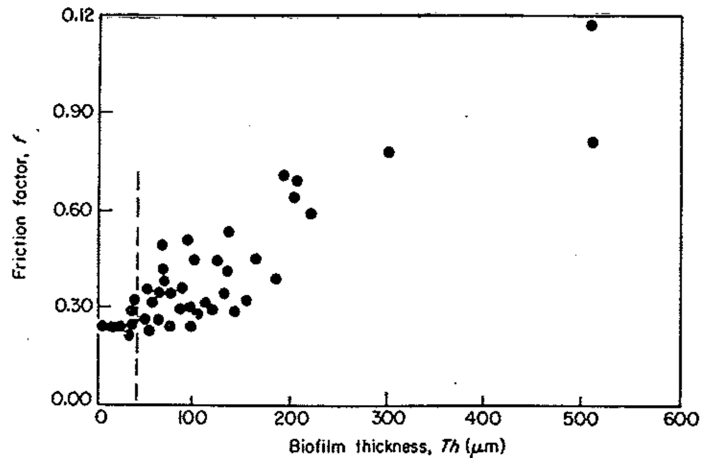


Fig. 30. Change in friction with biofilm thickness at constant pressure drop indicating viscous sublayer thickness. $\tau_w = 6.5$–$7.9\ \mathrm{N\,m^{-2}}$. Initial $\bar{v} = 163$–$185\ \mathrm{cm\,s^{-1}}$.

thickness after a critical thickness (Th_c) approximately equal to the thickness of the viscous sublayer (δ_l) is attained.

Conceptually, Th_c corresponds to the stage of biofilm development at which surface irregularities protrude through the viscous sublayer. Until this stage, the biofilm lies completely within the viscous sublayer ($k_s < \delta_l$) and friction factor does not increase (the tube is hydraulically smooth). For a wall shear stress of 6.5–7.9 $\mathrm{N\,m^{-2}}$, the viscous sublayer is approximately equal to 40 μm; this compares well with the observed $Th_c = 30$–35 μm for the same wall shear stress range.

Although the frictional resistance effects of biofilm can be adequately described by formulae and concepts suitable for rigid rough surfaces, the conclusion should not be drawn that indeed the biofilm presents a rigid rough surface to the flow. Such a notion is an oversimplification and cannot account for all experimental observations (Sherwood *et al.*, 1975).

Finally, frictional resistance measurements provide a relatively simple method for determining liquid mass transfer resistance in some biofilm systems since frictional resistance and liquid mass transfer resistance are related (Sherwood *et al.*, 1975).

4.4. Effects of Biofilms on Heat-Transfer Resistance

Biofilm development and resulting fluid frictional resistance have been discussed and both influence heat transfer. Changes in heat-transfer resistance arise from the combined effects of increased biofilm thickness (con-

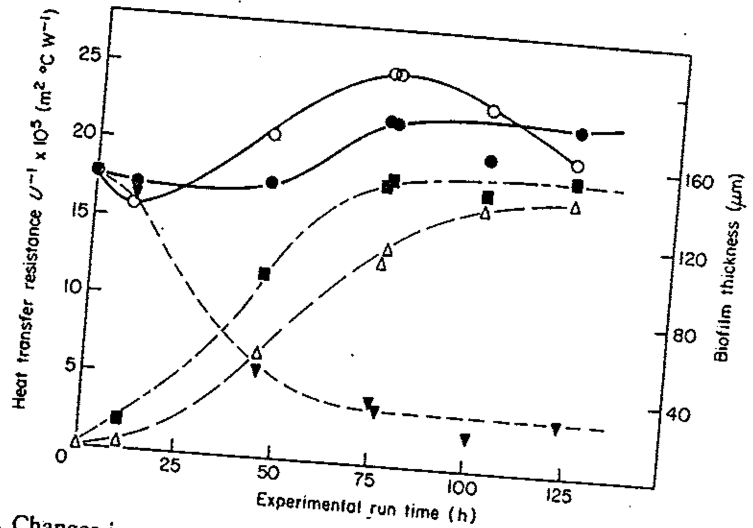


Fig. 31. Changes in convective and conductive heat transfer resistance as a result of biofilm development. ○, measured overall resistance. ●, total resistance. ■, conductive resistance. △, biofilm thickness. ▲, convective resistance.

ductive heat transfer) and increased frictional resistance (convective heat transfer).

Conductive heat transfer can be related to biofilm thickness and its effective thermal conductivity. Experimental biofilm thermal conductivity determinations indicate no significant difference from that of water at the

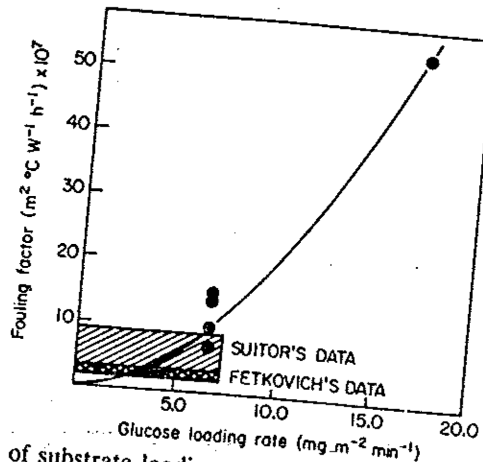


Fig. 32. Influence of substrate loading rate on heat transfer fouling reactor. The ranges measured by others are indicated for comparison (see Table 12).

same temperature (see Table 6). This is not surprising since biofilm is approximately 98–99% water.

Convective heat transfer results from fluid mixing or motion, and can be related to momentum transfer or frictional resistance. Colburn (1933) correlated convective heat transfer in tubes to friction factor and properties of the fluid. The Colburn relationship is only useful when the biofilm is thicker than the viscous sublayer.

Overall heat-transfer resistance due to biofouling film development can then be calculated if the following are known:

(1) Biofilm thickness and biofilm thermal conductivity.
(2) Frictional resistance.
(3) Wall temperature and bulk temperature.

Figure 31 describes a typical experiment (Characklis *et al.*, 1981) in a tubular reactor and illustrates the relative effects of conductive and convective heat-transfer resistance on overall heat-transfer resistance.

Heat-transfer resistance was consistently observed to decrease upon initial exposure to the fouling fluid. Presumably, a very thin biofilm causes a decrease in convective heat-transfer resistance which is larger than the resulting increase in conductive heat-transfer resistance.

The rate of change in heat-transfer resistance is strongly dependent on substrate loading. R_f is a fouling factor traditionally used by engineers to quantify the influence of biofilms (and other deposits) on heat-transfer rate. Rate of change in R_f measured in natural seawater systems are compared to laboratory freshwater data in Fig. 32. Table 12 describes the experimental conditions in which the data were obtained. Neither carbon nor nutrient concentrations were determined in the latter two studies. However, soluble organic carbon concentrations are estimated at between 0.5 and 10 mg l^{-1}.

Kirkpatrick *et al.* (1980) have modelled the heat and mass transfer occurring in a heat exchange tube as a biofilm develops. In a typical heat exchanger, results indicate a significant decrease in heat transfer. For

Table 12. Description of experimental systems for R_f measurements reported in Fig. 32.

	Characklis *et al.*, 1981	Ritter *et al.*, 1977	Fetkovich *et al.*, 1978
Surface	Al 6061-T6	Titanium	Cupronickel
Surface temperature (°C)	39–45	26–38	21
Fluid velocity (cm s^{-1})	81	60–120	90–180

systems of interest, the biofilm is relatively uniform over the length of the heat exchange tube. In tubes with combined heat and mass transfer, the biofilm thickness varies appreciably with fluid temperature. The assumed relationships between temperature and biofilm development rates in their model have been partially verified by Stathopoulos (1981).

5. Summary

The basis and methods for a process analysis of biofilm development have been presented. The purposes were as follows:

(1) To familiarize the reader with process analysis based on conservation and constitutive principles.
(2) To present the assumptions frequently made in such an analysis.
(3) To present a framework for analysis of the rate of biofilm development, extent of biofilm development and influence of biofilms on energy losses.

Biofilms are emerging as a most critical factor affecting natural aquatic systems, water distribution systems, wastewater treatment systems, heat exchangers, shipping and human disease. More attention is being directed to this behaviour.

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