BIOFILMS IN POROUS MEDIA

by

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ABSTRACT

IPA has established a long-term fundamental research program intended to provide a better understanding of microbial, chemical and hydraulic processes in porous media. Plans include an extension of results and application of techniques from previous research on fundamental biofilm processes to porous media flow systems. The intent is to also develop instrumentation and methods for monitoring porous media fouling in field applications. Finally, we intend to develop useful, practical models for describing biofilm accumulation in porous media.

This paper describes the proposed programmatic approach including the conceptual description of biofilm accumulation and the perceived manner in which biofilms interact with the porous media to influence hydraulic resistance. Application of several methods, unique to the study of microbial activity in porous media, are also discussed. Several issues related to modelling are also considered.

INTRODUCTION

The first serious study of biofilms was perhaps that of Zobell (1943), who recognized the possibility of macromolecular conditioning films modifying surfaces prior to microbial colonization and suggested a two step process for colonization, an initial reversible step followed by an irreversible binding of the cell to the surface. Progress in biofilm research was relatively slow until the early 1970's when researchers in various fields became conscious of almost universal association of microorganisms with surfaces or with each other (Marshall, 1976). Research and understanding of biofilm processes has progressed rapidly in the last decade and was summarized succinctly in the proceedings of a recent workshop (Marshall, 1984).

One of the ultimate aims in studying biofilms is to evolve the means for manipulating these processes to our own advantage. Thus, we wish to control fouling of a ship hull or heat exchange surface, control dental plaque or periodontal disease, control cholera and other intestinal

diseases, enhance biofilm formation in fixed film fermenters, or to modify microbial associations in natural environments to establish more effective consortia.

Biofilms are contrasted to suspended growth microbial systems primarily because of the critical role of transport processes which generally are rate-controlling for the process. The transport processes of concern may occur in the liquid phase (influenced by mixing) as well as in the biofilm (diffusion limitations). Thus, process analysis techniques have been applied to biofilm systems to evaluate the relative importance of the various processes contributing to biofilm accumulation (Characklis, 1981; Bakke et al, 1984).

BIOFILM: A Definition

Microbial cells attach firmly to almost any inanimate surface submerged in an aquatic environment. The immobilized cells grow, reproduce, and produce extracellular polymers which frequently extend from the cell forming a tangled matrix of fibers which provide structure to the assemblage termed a **BIOFILM.** Biofilms sometimes provide a relatively uniform coverage of the wetted surface and in other locations are sometimes quite "patchy". Biofilms can consist of less than a monolayer of cells or can be as thick as 300-400 mm thick as observed in algal mats. A biofilm can consist of many microenvironments for microbial growth as a result of concentration gradients which become established within the film. In fact, the clear distinction between biofilms and other microbial systems is the heterogeneity in its microenvironment which makes transport processes and gradients so important. For example in thick biofilms, the biofilm can contain both aerobic and anaerobic environments due to oxygen diffusion limitations within the biofilm. In the case of algal mats, the aerobic and anaerobic regions change diurnally because of the contribution of photosynthetic processes. Thus, the term biofilm reflects a surface accumulation which is not necessarily uniform in time or space.

In the simplest case, biofilms are composed of microbial cells and their products (e.g., extracellular polymers). Such a biofilm generally is a very adsorptive and porous ($\geq$ 95% water) structure. As a result, biofilms observed in many waters consist of a large fraction of adsorbed and entrapped materials such as solutes and inorganic particles (e.g., clay, silt). Consequently, the term biofilm may reflect a surface deposit which is composed of a significant fraction of inorganic or abiotic substances held together by a matrix of biotic origin.

BIOFILM SYSTEMS

The International Association for Water Pollution Research and Control Task Group on Modelling of Biofilms has developed a systematic description of the biofilm system. Biofilms are composed of microorganisms immobilized at a substratum (i.e., the support surface) generally in association with an organic polymer matrix (Figure 1). In some cases, the biofilm contains degradable and/or inert particles and sometimes may include

macroorganisms. The biofilm consists of two phases: the base film and the surface film.

The biofilm system can be classified in terms of "phases". Each phase is characterized by thermodynamic and transport properties as well as by the transport and transformation processes which dominate within the phase (Figure 2).

System Phases

As many as five "phases" can be defined in a biofilm system (Figure 3): 1) the substratum, 2) the base film, 3) the surface film, 4) the liquid, and 5) the gas. The substratum plays a major role in biofilm processes during the early stages of biofilm accumulation. The base film ~~is the phase most often considered in biofilm observations and~~ consists of a rather structured accumulation with relatively well-defined boundaries. The surface film provides a transition between the liquid phase and the base film. The surface film essentially provides for gradients in biofilm properties with distance away from the substratum. For example, biofilm density probably decreases with distance from the substratum as fluid flow processes continually entrain parts of the film increasing its porosity near the liquid interface. In some cases, the surface film may extend to the substratum, especially if a preponderance of filamentous microorganisms are present. In other cases, the surface film may not exist at all as observed in certain monoculture biofilm systems (Figure 1). Liquid phase processes affect biofilm system behavior primarily as a result of the mixing or flow patterns resulting from its geometry. The gas phase, absent in some biofilm environments, provides for aeration and/or removal of gaseous microbial reaction products (e.g., CO_2, CH_4).

Interaction Between System Phases

The interaction between the various phases in a biofilm system occurs via transport processes (Figure 4). Liquid phase transport processes deliver substrate (particulate or soluble) and microorganisms to the surface film while carrying away detached cells and other products of metabolism. Transport between the surface film and base film occurs primarily by molecular diffusion for soluble components and by volumetric displacement for particulate components. There may be other means of transport for particulate components within the biofilm such as cell motility and macroorganism grazing.

POROUS MEDIA CONCERNS

Biofilms extract contaminants from water in various porous media environments thus "purifying" the water. Degradation occurs in groundwater systems as well as granular media (e.g., sand, activated carbon, ion exchange) filters used for treating drinking water and wastewater. Microorganisms, purposefully injected into oil-bearing formations as a means for enhancing oil recovery, form and excrete products which aid in the release of immobilized oil within the porous media.

However, biofilms also significantly influence the hydraulic resistance in porous media. So although biofilm microorganisms degrade various contaminants in a groundwater formation, their accumulation in the pore space, if significant, increases hydraulic resistance in the formation. The highest hydraulic resistance in the formation is frequently observed near the well where the microorganisms can also dramatically increase corrosion of the metal well components. Increased hydraulic resistance, "clogging", also influences oil field production when microorganisms (and their products) accumulate in the oil-bearing formation. Granular media filter performance can also deteriorate as a result of biofilm accumulation in the interstitial space resulting in shorter run times. Biofilms are also believed to influence groundwater-surface water interaction in natural streams as a result of their accumulation on the stream bottom.

GOALS

IPA has established a long-term fundamental research program intended to provide a better understanding of microbial, chemical and hydraulic processes in porous media. Major goals include the following:

1. Extend results and apply techniques from previous research on fundamental biofilm processes (i.e. transport, attachment, detachment, growth, accumulation) to porous media flow systems.

2. Develop instrumentation and methods for monitoring porous media fouling.

3. Develop useful, practical models for describing biofilm accumulation in porous media.

Achieving these goals requires the combined efforts of investigators specializing in several relevant disciplines including engineering, microbiology, and chemistry. Accordingly, the project has been organized so as to coordinate the interdisciplinary expertise and facilities necessary to address the following objectives:

Objective 1: Determine the major variables affecting transport processes and microbial activity in saturated porous media systems. Previous research suggests that cell concentration, fluid flow regime, nutrient supply, and porous media characteristics be addressed initially.

Objective 2: Evaluate the influence of biofilm accumulation on the hydraulic resistance in porous media. We will initially focus ~~specifically~~ on the effects of biofilm accumulation on specific media characteristics including porosity, tortuosity, frictional resistance, and hydraulic conductivity.

Objective 3: Evaluate the influence of transport processes and microbial metabolism at the substratum on biotransformation in porous media.

Objective 4: Use results from previous objectives to define engineering parameters useful in the design, operation, and/or renovation of porous media systems.

The remainder of this paper will present the research approach presently being pursued as well as some results of recent investigations addressing objectives listed above.

BIOFILM PROCESSES

The focus of our efforts in biofilm processes is the identification of relevant parameters and quantification of their influence on the rate and extent of microbial cell adsorption, desorption, and growth on surfaces in porous media. The long range goal of the program is the formulation of a model to identify the rate-limiting processes for microbial cell accumulation under different environmental and physiological conditions (e.g., fluid dynamics and nutrient concentration in the fluid) as an extension of more macroscopic characterizations. Similar analyses of other biofilm systems have already been conducted in this laboratory (Characklis, 1984; Bakke et al, 1984; Nelson et al, 1985). In all cases, open ecosystems (i.e., continuous flow systems) with a measurable fluid shear stress at the substratum are being considered because of their relevance to aquatic ecosystems and technological applications.

The overall progression of biofilm accumulation can be described by a logistic function comprised of three arbitrarily determined periods (Figure 5): 1) the induction period which encompasses the initial colonization of the substratum, 2) logarithmic accumulation period during which growth processes dominate, and 3) steady state where attrition rate equals formation rate.

INDUCTION PERIOD

Transport and Transformation Processes

Net accumulation of bacterial cells at a surface is the result of transport of cells to the substratum, adsorption, desorption, attachment, detachment, and growth (transformation) processes (Figure 6). Adsorption/desorption refer to processes at the substratum whereas attachment/detachment refer to processes occurring at the biofilm-liquid interface. Immobilized colonies can separate on the substratum but do not influence the number of biofilm cells.

Because of past limitations in experimental methods, cellular accumulation rate at a substratum has been observed and then equated to cellular adsorption rate (R_A) approximated by a saturation law. Thus,

$$\begin{matrix}\text{rate}\\ \text{of}\\ \text{accumulation}\end{matrix} = \frac{dX_b}{dt} = R_A = kX\left(1 - \frac{X_b}{k_s}\right) \qquad [1]$$

where X = cell number in the fluid (# L^{-3})

X_b = cell number on the substratum (# L^{-2})

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

k_s = saturation coefficient (# L^{-2})

Since net accumulation of bacterial cells at a substratum is the result of several processes including sorption and growth-related processes, the rate equation for cell accumulation should include at least the following four rate expressions (Characklis and Escher, 1986):

$$\frac{dX_b}{dt} = \begin{matrix}\text{rate}\\ \text{of}\\ \text{accumulation}\end{matrix} = \begin{matrix}\text{rate}\\ \text{of}\\ \text{adsorption}\end{matrix} - \begin{matrix}\text{rate}\\ \text{of}\\ \text{desorption}\end{matrix} + \begin{matrix}\text{rate}\\ \text{of}\\ \text{growth}\end{matrix} - \begin{matrix}\text{net rate}\\ \text{of}\\ \text{detachment}\end{matrix} \qquad [2]$$

Image Analysis

Capabilities. We have developed a non-destructive assay procedure for the initial events in the cell-substratum interaction using in situ video microscopy of the accumulation process occurring within in a rectangular glass capillary (Figure 7). Colonization processes are analyzed with an image analyzer which determines time of adsorption, desorption, shape, size, spatial coordinates, and number of cells in each colony-forming unit (CFU). A CFU is a convenient variable for adsorption studies and includes single cells as well as aggregates of more than one cell immobilized at the substratum. The image analyzer records CFU in addition to cell numbers immobilized at the substratum. Additional sorting routines calculate rates of adsorption, desorption, separation of colonies, growth, and erosion of cells within each CFU (Escher and Characklis, 1986). Additional calculations include the frequency distributions for cell residence time at the substratum, growth rates of each CFU, and rate and direction of cell movement on the substratum.

Our assay procedure independently measures adsorption, desorption, detachment, cfu separation, and multiplication so the rate expressions in Eq. [2] can be assessed. Since the assay method also distinguishes between

cfu and cells, two coupled differential equations are necessary to describe the net accumulation of cfu and cells at the surface.

Some relevant applications. The assay method offers some intriguing opportunities for assessing the effectiveness of various methods for inhibiting or enhancing substratum colonization. Not only can we assess the effect of a control technology on overall colonization but we can determine which independent process is most affected by the treatment. The potential for using this assay method in development of biofilm control technologies is astonishing.

Transformation (Growth) Processes are Dominant

Recent results from image analysis experiments indicate that rates of adsorption, desorption, and detachment are independent of time (Figure 8). Growth rate at the substratum is dependent of cell numbers at the substratum but, even at low substrate concentration (less than 0.5 mg glucose/liter), growth rate was the dominant contributing process to biofilm accumulation within two hours of exposure. Thus, colonization of a substratum seems more closely related to growth than to sorption interactions in these experiments.

Interestingly, very few (<1%) cell "hits" at the substratum result in a "stick" and the "sticking efficiency" decreases with increasing fluid shear stress. Are the infrequent "sticks" a result of random capture of the substratum or are only some of the organisms in the population capable of sticking? Since these results are from experiments using pure cultures (Pseudomonas aeruginosa), the possibility of genetic heterogeneity within the population may have to be considered.

The assay procedure, in conjunction with the kinetic analysis, permits the rapid, quantitative, and visual analysis of the microbial colonization of a surface as well as the determination of the rate-controlling steps in any environmental condition.

Modeling Issues

With the new technology at our disposal, we now have the ability to observe and quantify parameters of individual processes. This presents an opportunity for an entirely new kind of modeling of biofilm processes.

Previous models could only treat the net balance between the attachment, detachment, and growth processes (Eq. [1]), whereas we are now in a position to quantify these processes and others independently (Eq. [2]). Further, we are not constrained to dealing with these processes as macroscopic deterministic mean rates pertaining to the entire population (Eq/UBIN). Instead, we may utilize models which explicitly represent each individual cell in the system, and which treat the attachment, detachment, and growth events in terms of probabilities...the demography of chance extinction (Goodman, 1986a and b).

Thus, we can construct stochastic models which describe not only the mean rates (equivalent to the gross deterministic rates of the macroscopic models), but also the statistics of these rates such as the variances of the rates, or the probability distribution of events in small samples (e.g., properties of small surface areas). These statistics are central to an adequate understanding of the vitally important "patchiness" phenomena observed in porous media environments.

The new class of models, now accessible, suggests prospects for considering options for fouling/corrosion prevention and control at a new, more detailed, level of mechanisms. Consider, for example, such possibilities as:

> A 2-stage adsorption process, in which the first stage is "loose" (reversible) adsorption, followed by "firm" (irreversible) adsorption. The first phase may be more vulnerable to prevention and control measures, so there would be advantage to learning the determinants of the transition from stage 1 to stage 2.
>
> A "critical mass" effect, in which aggregations of cells of a certain threshold density have distinctly more severe corrosion properties, and/or are more difficult to remove by usual cleaning measures. Then we would want to know how aggregates form, and how their formation might be slowed, so there would be advantage to learning the factors which control the spatial distribution of attachment, detachment, and growth events.

LOGARITHMIC AND STEADY STATE PERIODS

There have been reports in the literature that the biofilm environment alters organism behavior. For example, there have been some reports that residence in the biofilm is a survival mechanism for microorganisms. Others suggest that specific cellular activity increases when a cell resides in a biofilm. Others report that specific cellular activity decreases in a biofilm. Some of these phenomena can probably be explained by gradients (e.g., substrate or oxygen concentration) within the biofilm or mass transfer rate limitations in the liquid phase. The effects of such physical phenomena can be determined. However, does residence in the biofilm per se alter cellular activity?

Transport Processes and "Apparent" Physiology

The major transport processes in the logarithmic and steady state periods are mass transfer from the liquid to the biofilm and diffusion within the biofilm. These topics have been addressed in the past by others (e.g., Trulear and Characklis, 1982).

Diffusion Gradients. The specific activity of a microorganism is related to the substrate concentration (and other environmental factors) sensed by the organism in its immediate environment. For example, the saturation

rate law predicts the following influence of substrate concentration on specific growth rate:

$$u = \frac{u_m S}{K_u + S} \qquad [3]$$

In a biofilm system, the liquid phase concentration, the parameter most easily measured, is not necessarily the concentration in the cell's immediate environment. Thus, the specific activity of a biofilm cell may not be directly related to the liquid phase substrate concentration. If a diffusion resistance exists, specific growth rate will decrease with increasing depth of the biofilm.

We have fabricated microelectrodes for the purpose of probing the microenvironment of the biofilm. Dissolved oxygen and pH microelectrodes with tip diameters of approximately 2-10 um are presently being used to establish gradients in experimental flow systems. Oxygen and pH (acids and bases) play such pivotal roles in microbial metabolism that these probes will suffice for many studies. However, we are pursuing the manufacture of other specific microelectrodes including those for eH and sulfide measurement.

In a mixed culture biofilm with diffusion resistance, environmental conditions change with biofilm depth. As a consequence, different microbial species may dominate at different depths. Thus, filamentous forms may emerge as a biofilm accumulates because a filamentous form of growth emerges under oxygen limitations. We are presently conducting research with a two species microbial biofilm to compare with mixed species mathematical models developed by Wanner and Gujer (1985).

Mass Transfer. A microbial cell in aqueous suspension has little inertia and, as a result, may possess a significantly thick "stagnant" water layer around it. The same cell adsorbed on a relatively large particle possesses an inertia characteristic of the particle. In most natural aquatic systems, the inertia of the particle will be significantly greater than the inertia of the microbial cell and will result in higher mass transfer rates from the liquid to the cell. A cell adsorbed to a stationary surface with fluid flowing past the surface also experiences a higher mass transfer rate than if suspended in planktonic form in the water. Hence, adsorption does confer an apparent higher activity to the adsorbed cell if, by virtue of the higher mass transfer rates, a higher rate of substrate uptake occurs.

Biofilm Physiology

Growth and product formation. Robinson et al (1984) and Bakke et al (1984) report on experiments with Ps. aeruginosa in a chemostat and in a biofilm reactor (rotating annular reactor). A two compartment model was used to analyze the experimental results. The two compartments were 1) cellular mass and 2) extracellular polymeric substances (EPS). EPS was distinguished from cell mass in the chemostat even if it was still trapped

in the slime intimately associated with the cells. Kinetic and stoichiometric coefficients were determined in the chemostat operating with glucose as the sole carbon source and limiting nutrient. Using the material balance for substrate, mean specific substrate removal rate was observed to be a linear function of mean specific growth rate (u_b in the biofilm was calculated from the cell balance in the biofilm) in the chemostat and in the biofilm. The results clearly indicate no significant difference between activities in planktonic and biofilm forms with regard to energy metabolism. The slope reflects the fractional substrate conversion for cell and EPS production and is not different for planktonic versus biofilm cells growing at the same average rate. Turakhia (1986) has observed similar results with Ps. aeruginosa and has also defined more concisely the stoichiometry of microbial metabolism in the biofilm.

Starvation. The effects of "starvation" may be significant in some biofilms especially in the early stages when individual cells can be discriminated (Morita et al, 1982). Starvation may also be significant in the lower depths of a thick biofilm where substrate is absent.

Biofilm Genetics

Genetic transfer is reportedly enhanced when cells are immobilized on a surface. Presumably, the short distances between immobilized cells for long periods enhances the transfer mechanisms. There are reports of increased rate of genetic material transfer when cells are adsorbed to a surface (Suzuki et al, 1984). Recently, several biofilm microorganisms which exhibit extraordinary physiological diversity have been isolated from mixed culture environments (Kuenin, personal communication; Bock, personal communication). These organisms contain one or more plasmids. Did the biofilm environment contribute to the characteristics of these organisms or the method by which the organisms mere modified?

HYDRAULIC RESISTANCE DUE TO BIOFILMS IN POROUS MEDIA

A porous medium can be considered as a packed column reactor. There are two main conceptual approaches for studying fluid flow (e.g., pressure drop) through a packed bed. One method considers the packed column as a collection of submerged objects and pressure drop is calculated by summing up the resistances of the submerged objects. The other method regards the packed column as a bundle of tangled tubes of irregular cross section. The theory is then developed by applying the results of flow in single straight tubes to the collection of "tortuous" tubes. Since we have an experimental system which permits in situ, non-destructive observation of biofilm processes in capillaries, we will pursue the latter method.

Biofilms and Hydraulic Resistance in Tubes

Biofilms increase hydraulic resistance in tubes by two mechanisms: 1) reduce the cross section area for flow and 2) increase the roughness of the tube. Picologlou et al (1980) observed that the major cause of hydraulic resistance in turbulent flow was increased ~~in~~ roughness.

the influence of biofilm on

Biofilms and Hydraulic Resistance in Porous Media

In porous media, laminar flow is the rule and reduced cross section is expected to be the major cause of resistance. We have demonstrated this effect in a laboratory experiment with a simulated porous media. The results are diagrammatically presented in Figure 9. The figure clearly indicates the "patchiness" in the biofilm distribution with major accumulations occurring on the leading side of the medium particles. The accumulation having the greatest effect on hydraulic resistance was located in the narrow channel between media particles through which the flow passed. The permeability dropped dramatically after approximately 10 days exposure (Figure 10). The drop in permeability was matched by decrease in equivalent diameter (Figure 11). Permeability was a linear function of equivalent diameter and, thus, increased roughness of the particles was probably insignificant.

Investigations documenting permeability reduction due to biofilm accumulation in porous media have been reported by practitioners in petroleum recovery, disposal of treated wastewater, and recharge/recovery for water supply. ~~Specifically~~, Studies which have monitored permeability decrease in laboratory test columns subjected to biofilm growth have been reported by Shaw et al (1985), Raiders et al (1986), Raleigh and Flock (1965), Hart et al (1960) and Cerini et al (1946). The reports cover a variety of porous media including core samples from field sites as well as synthetic media (e.g., glass spheres). Pure cultures as well as mixed cultures ~~were~~ served as inoculum. Substantial reduction in permeability (65-99%) occurred in all cases. Raiders et al (1986) report decreased permeability due to biofilm accumulation in effective pore space. Extracellular polymeric substances were presumed to play an important role in the plugging phenomena in some of these experiments.

Nonuniform or "patchy" accumulation of biofilm along the flow path is frequently observed in column investigations. Most of the biofilm occurs within a few centimeters of the column entrance. Similar observations have been reported from field investigations by Peterson and Oberdorfer (1985) with injection of treated wastewater and van Beek (1984) with water well clogging. Raiders et al (1986), using a parallel core system, reported that permeability reduction occurred faster in cores with higher intrinsic permeability.

The first eleven (11) days of the experiment was characterized by permeability reduction caused by biofilm accumulation, primarily resulting from adsorption and growth of cells at the substratum (Figure 10). However after eleven (11) days, the rate of permeability reduction is significantly increased. The substantial permeability increase was caused by "filtration" of suspended biomass sloughed from upstream porous media locations.

Modelling of Hydraulic Resistance in Porous Media

Biofilm accumulation in porous media is the net result of microbial cell adsorption, desorption, growth on surfaces, and detachment from the porous

media. Several, if not all, of these processes are strongly influenced by hydraulic characteristics in the porous media. For example, pore velocity influences cell and substrate flux into the media. Tortuosity influences the "filtration" rate of the media. Since biofilm accumulation will influence porosity, pore velocity or pressure head will change with additional accumulation of biofilm. Thus, there is a **COUPLED RELATIONSHIP** between biofilm accumulation and hydraulics within the porous medium.

Similar analyses of biofilm systems in other geometries have already been conducted in this laboratory (Kirkpatrick et al, 1980; Characklis, 1984; Bakke et al, 1984; Nelson et al, 1985; Escher, in preparation). These models all involve coupling biofilm accumulation equations with hydrodynamic equations for a given geometry. Our first task is characterizing and describing the porous media geometry (e.g., effective pore volume and tortuosity) so that we can relate the hydraulics to the biofilm equations.

The long range goal of the program is the formulation of a model to identify the rate-limiting processes for microbial cell accumulation under different environmental and physiological conditions (e.g., fluid dynamics and nutrient concentration in the fluid) as an extension of more macroscopic characterizations. In all cases, open ecosystems (i.e., continuous flow systems) with a measurable fluid shear stress at the substratum are being considered because of their relevance to aquatic ecosystems and technological applications.

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FIGURE TITLES

1. Transmission electron micrograph of a Pseudomonas aeruginosa biofilm.

2. The biofilm system modelling matrix including "phases" (e.g., base film), modelling components (e.g., base film properties). The third dimension, termed mixing/geometry, describes the fluid flow regime in the biofilm "reactor".

3. The biofilm system including the following five "phases": 1) substratum, 2) base film, 3) surface film, 4) bulk liquid, and 5) gas. The base film and surface film comprise the biofilm.

4. The interaction between the various phases in a biofilm system occurs via transport processes.

5. The generalized progression of biofilm accumulation.

6. The image analysis system including the support equipment for providing a continuous flow of microorganisms to the rectangular capillary.

7. A schematic representation of the independent processes distinguished by the image analysis system.

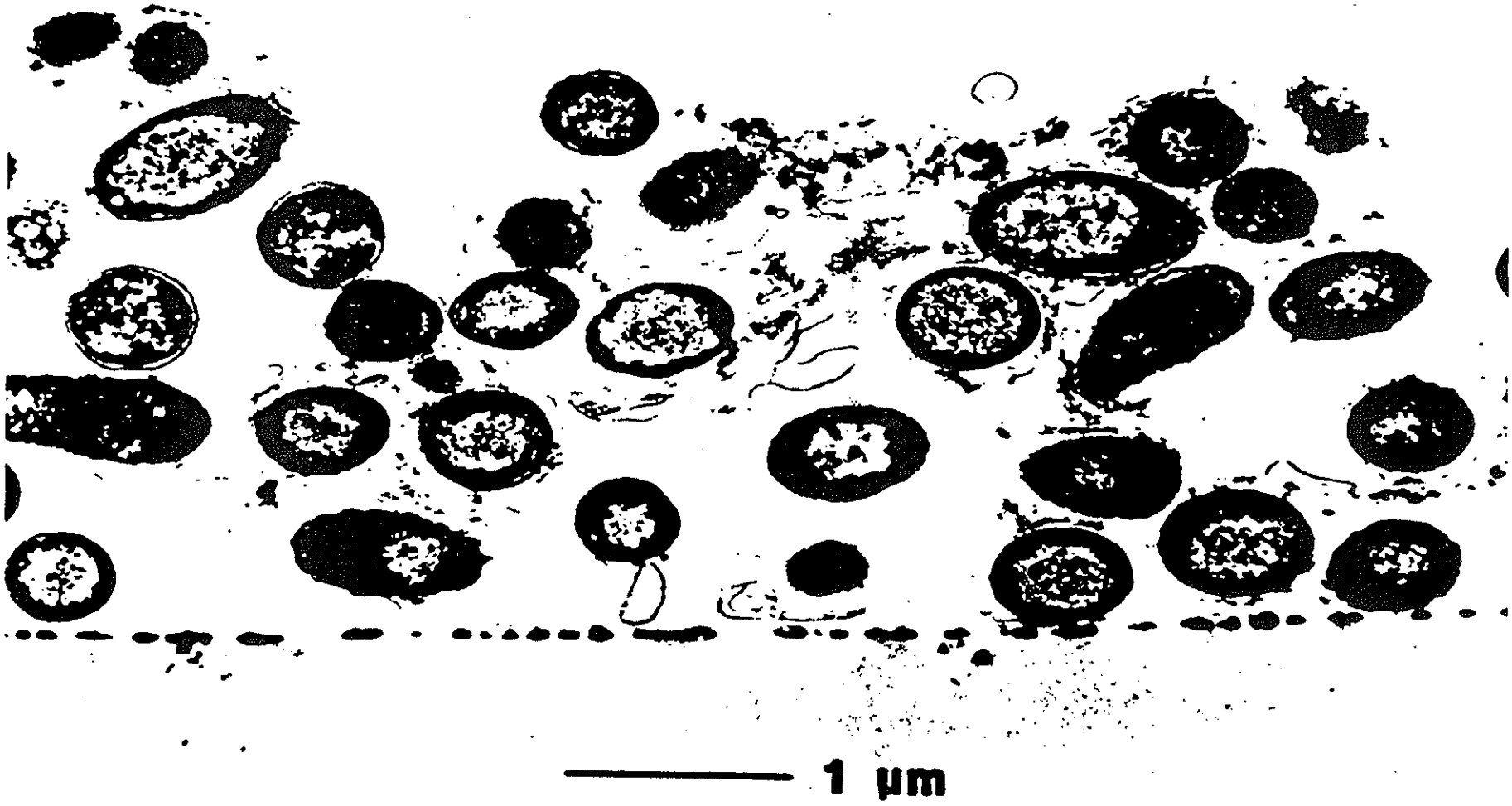
8. Typical results from an image analysis experiment indicating the cumulative cell numbers at the substratum resulting from the various processes of adsorption, desorption and growth.

9. Experimental system for simulating the hydraulic resistance resulting from biofilm accumulation in porous media.

10. A diagrammatic representation of the spatial distribution of biofilm accumulation in the simulated porous medium.

11. Decrease in permeability in the simulated porous medium due to biofilm accumulation.

12. The relationship of permeability to equivalent diameter indicating that permeablility changes were due primarily to reduced pore volume.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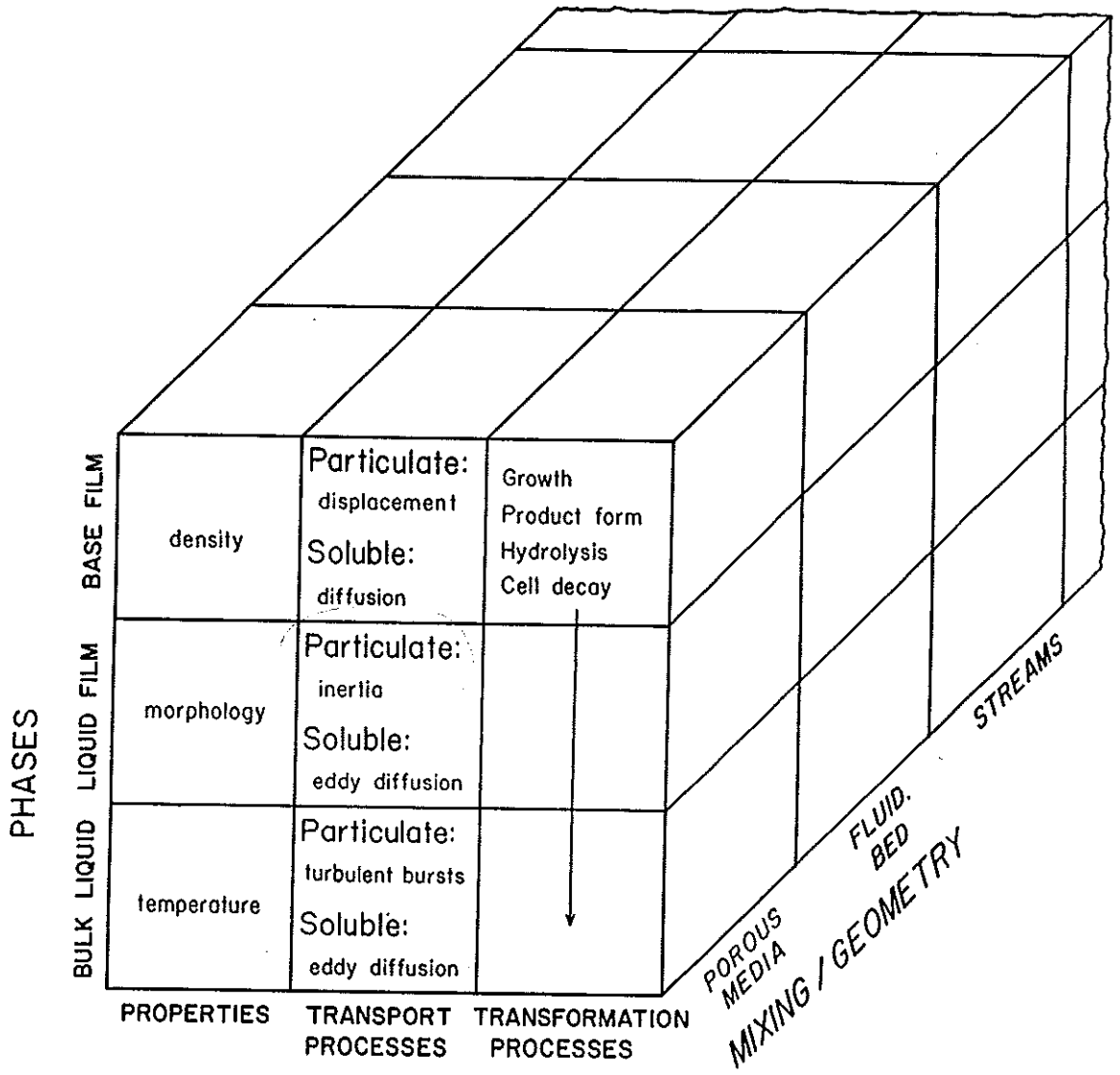
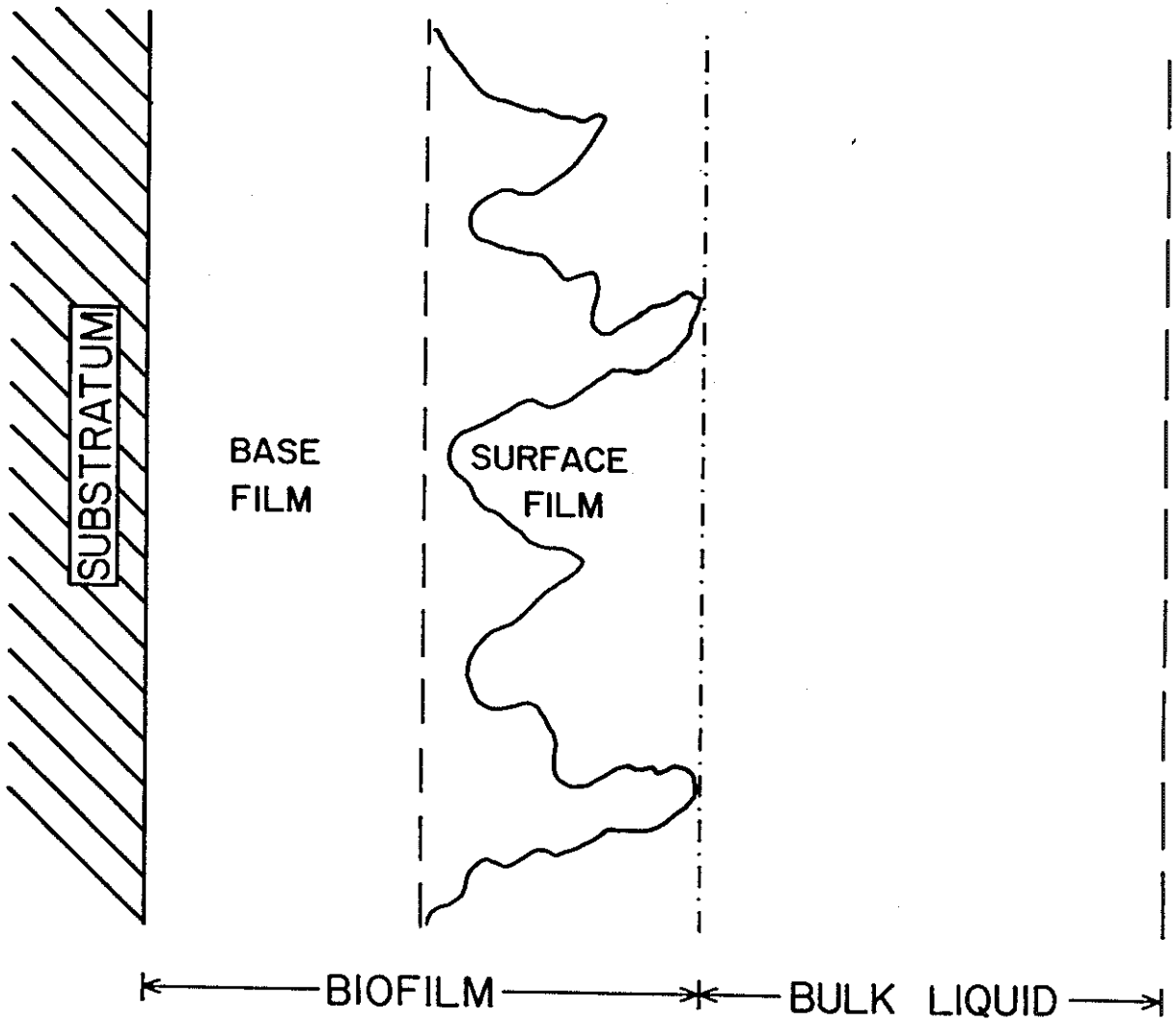


MODELING COMPONENTS


SUBSTRATUM
BASE
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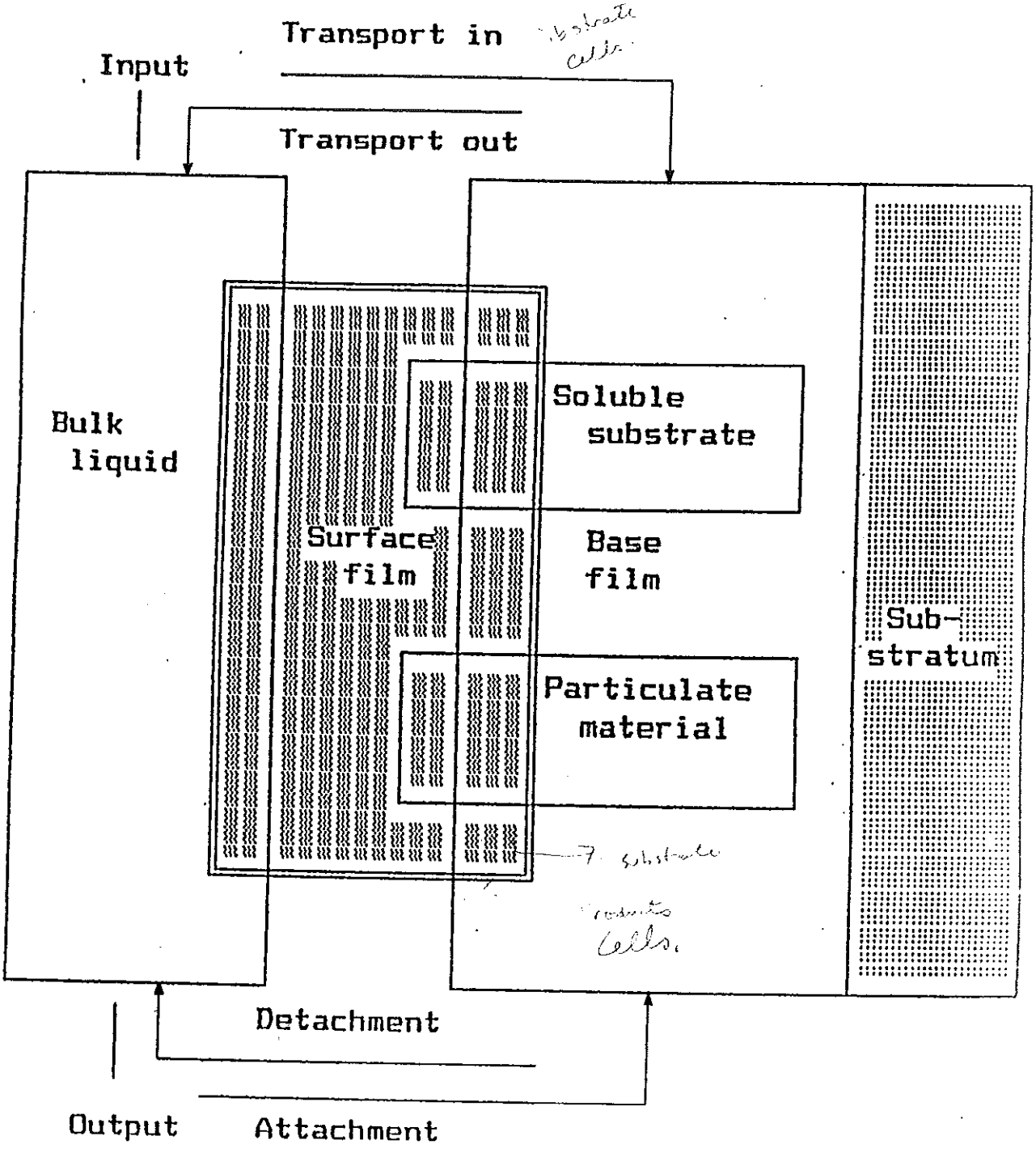
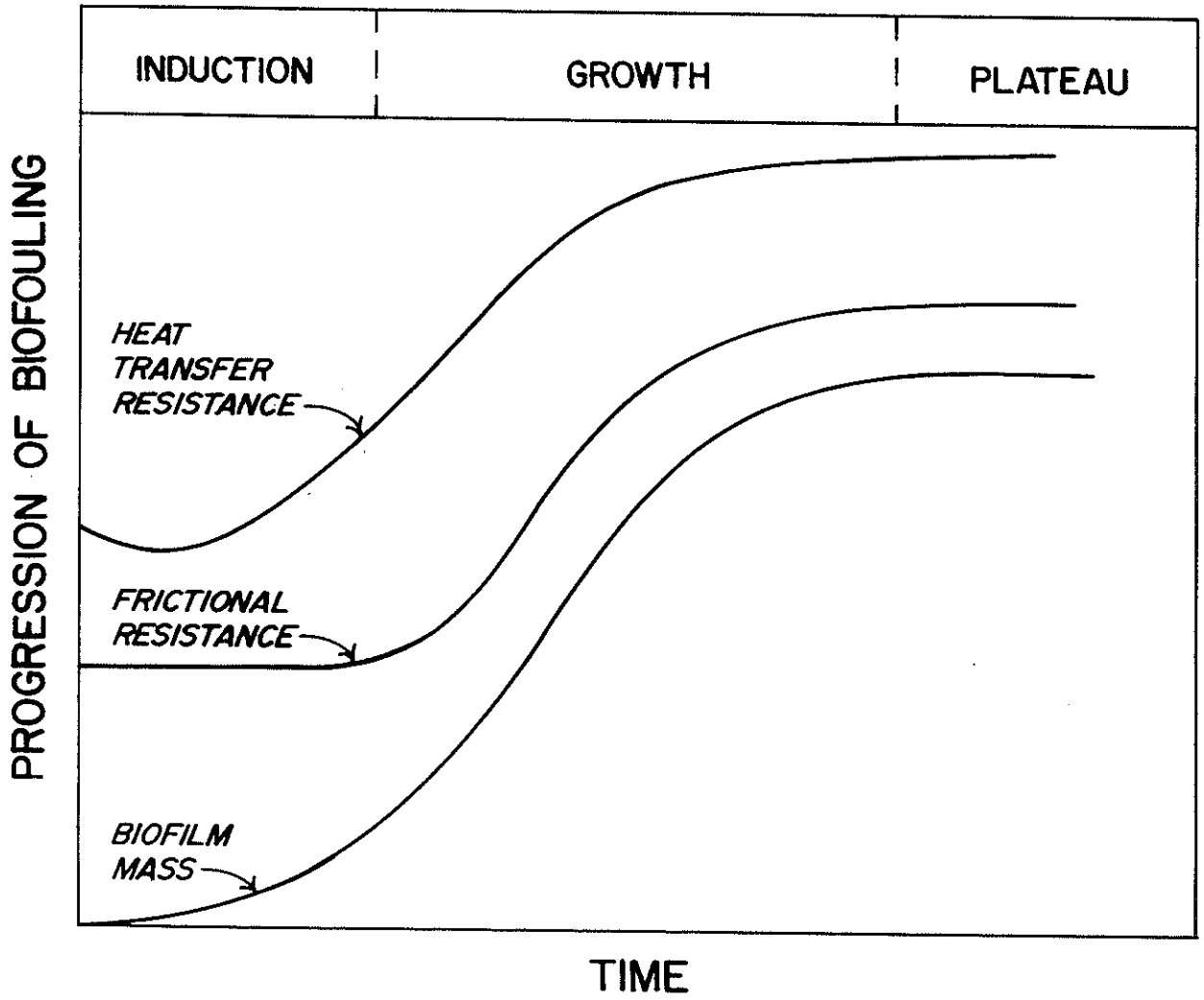
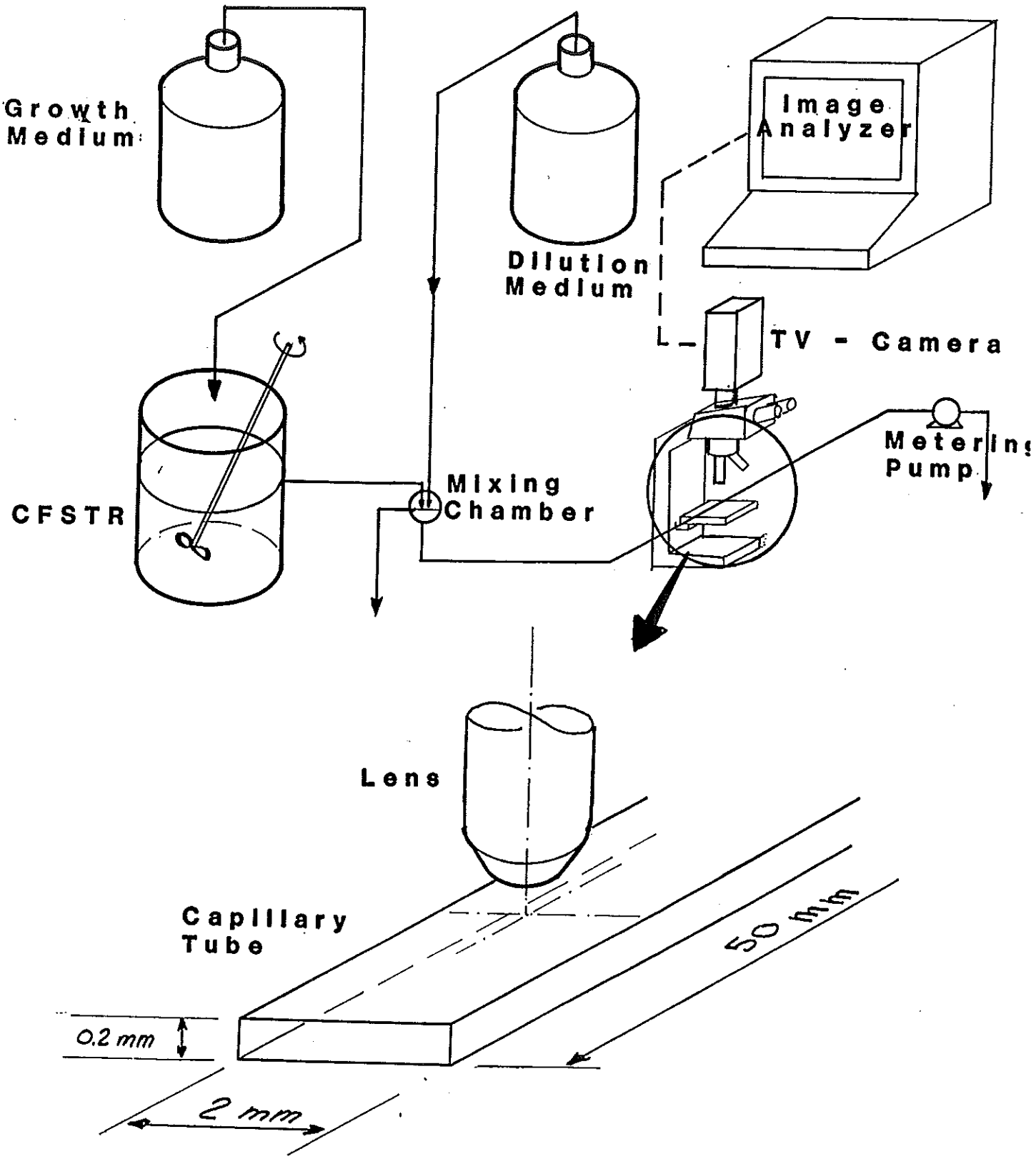
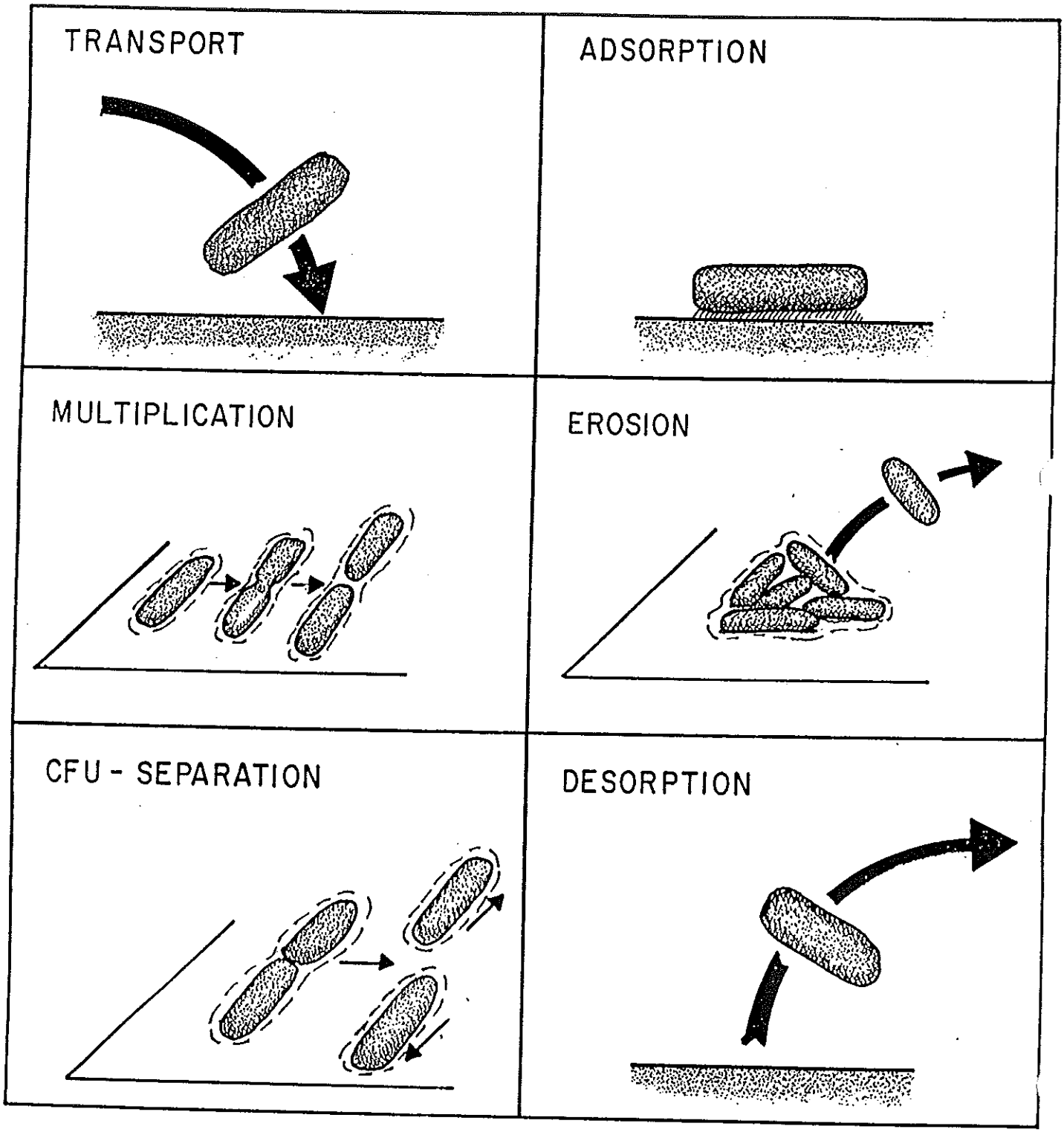
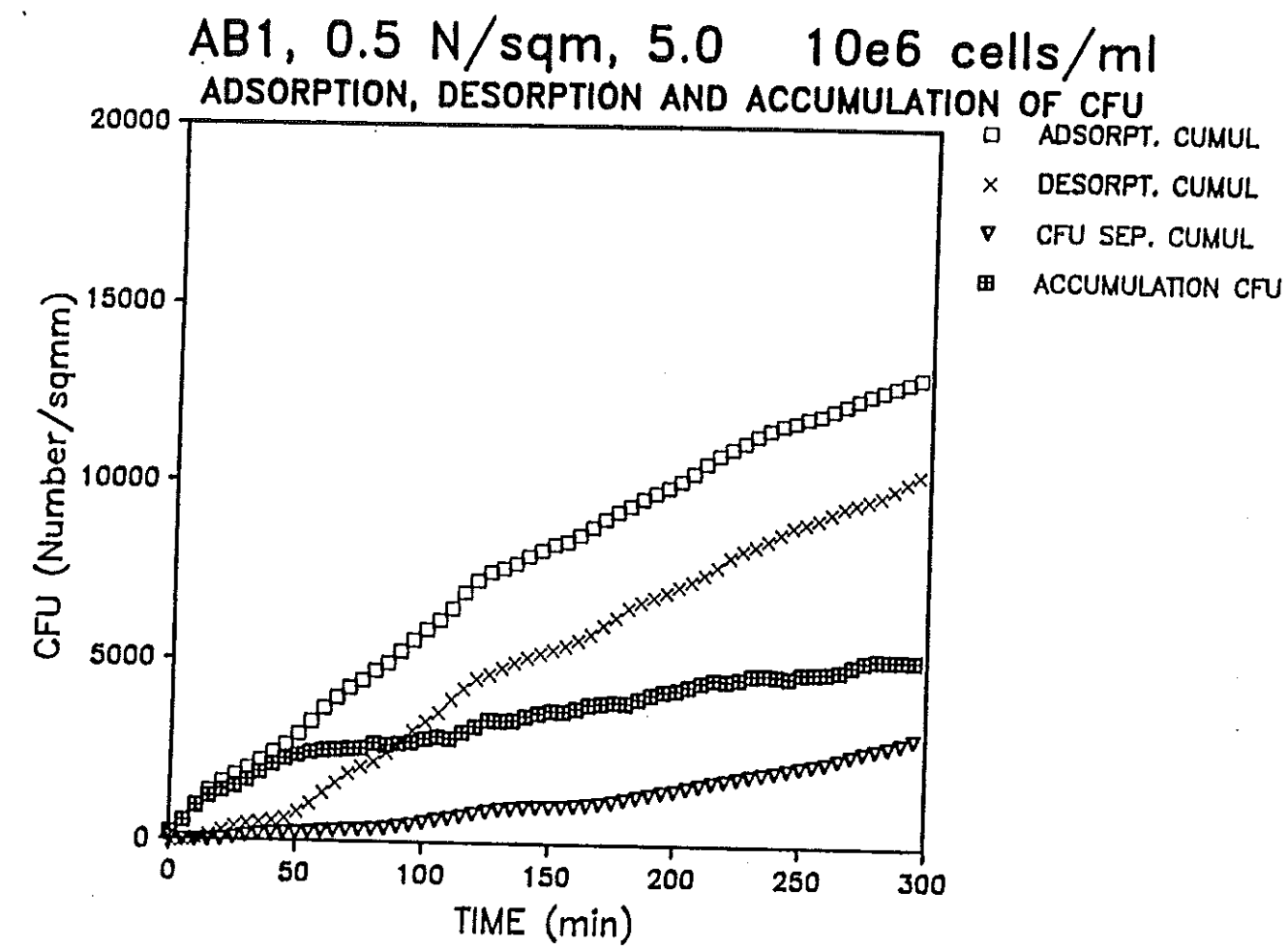
FILM
BIOFILM
BULK LIQUID

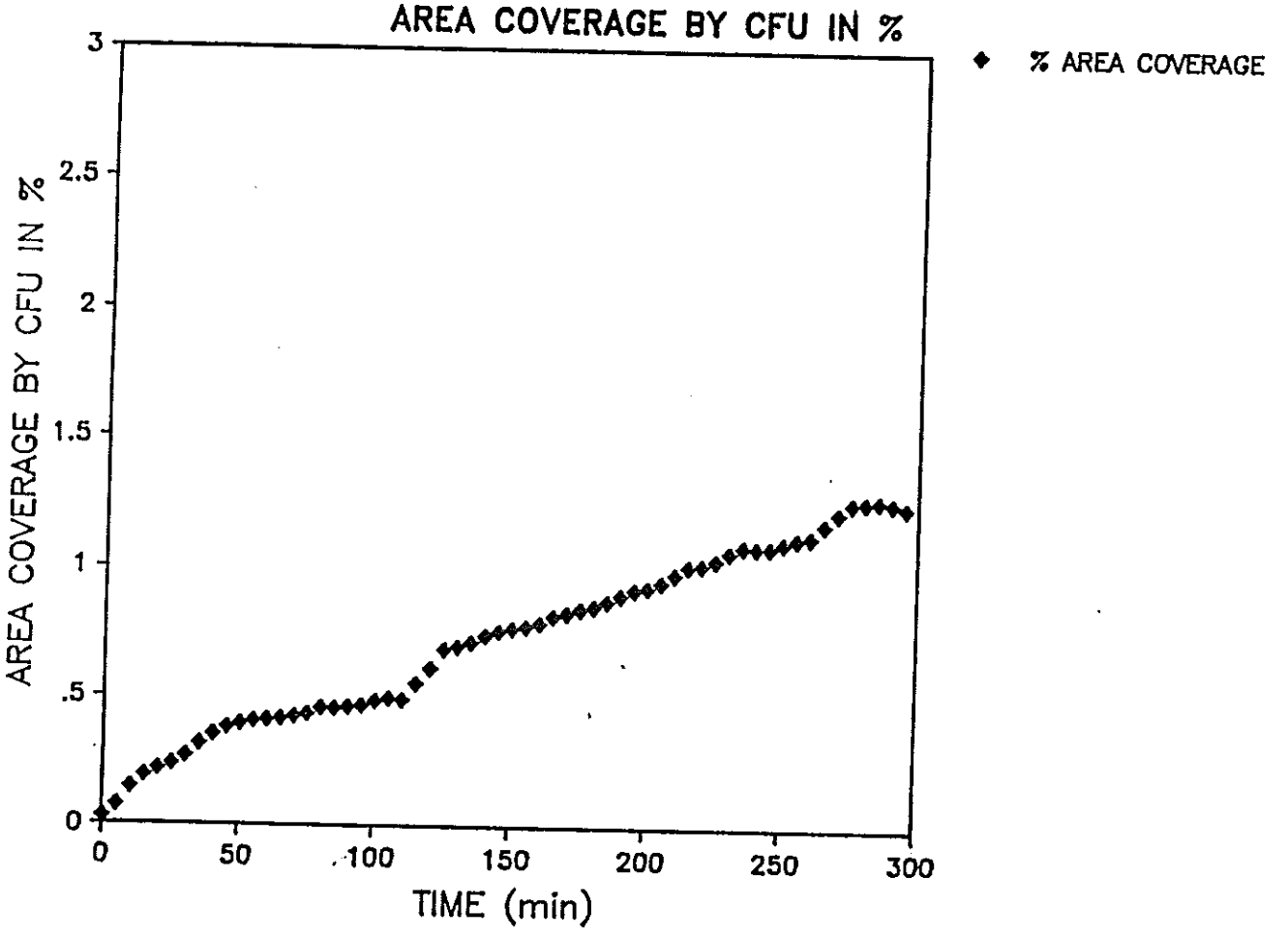

Transport in
Input
Transport out
Bulk liquid
Soluble substrate
Surface film
Base film
Particulate material
Sub-stratum
Detachment
Output
Attachment

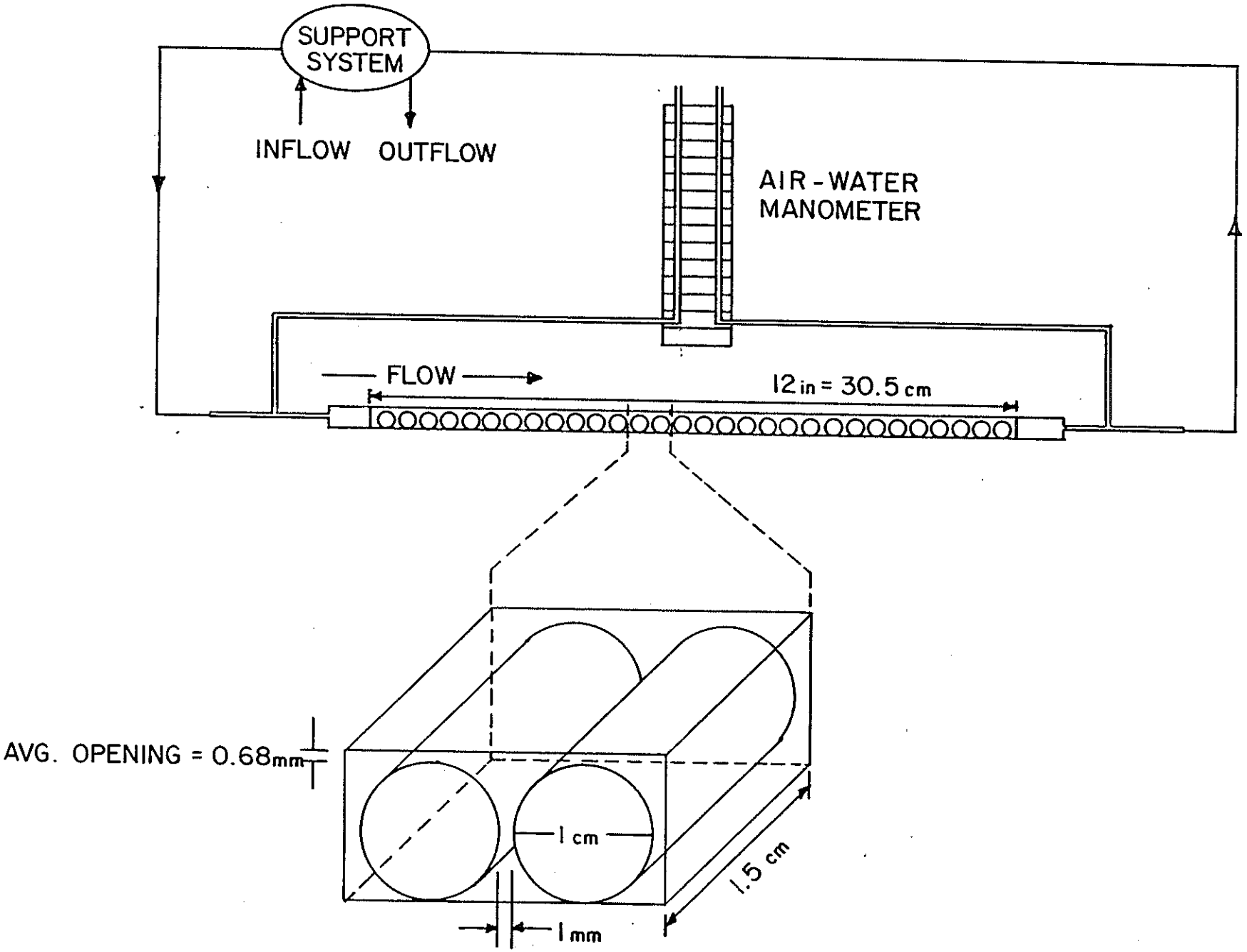

INDUCTION
GROWTH
PLATEAU
PROGRESSION OF BIOFOULING
HEAT TRANSFER RESISTANCE
FRICTIONAL RESISTANCE
BIOFILM MASS
TIME

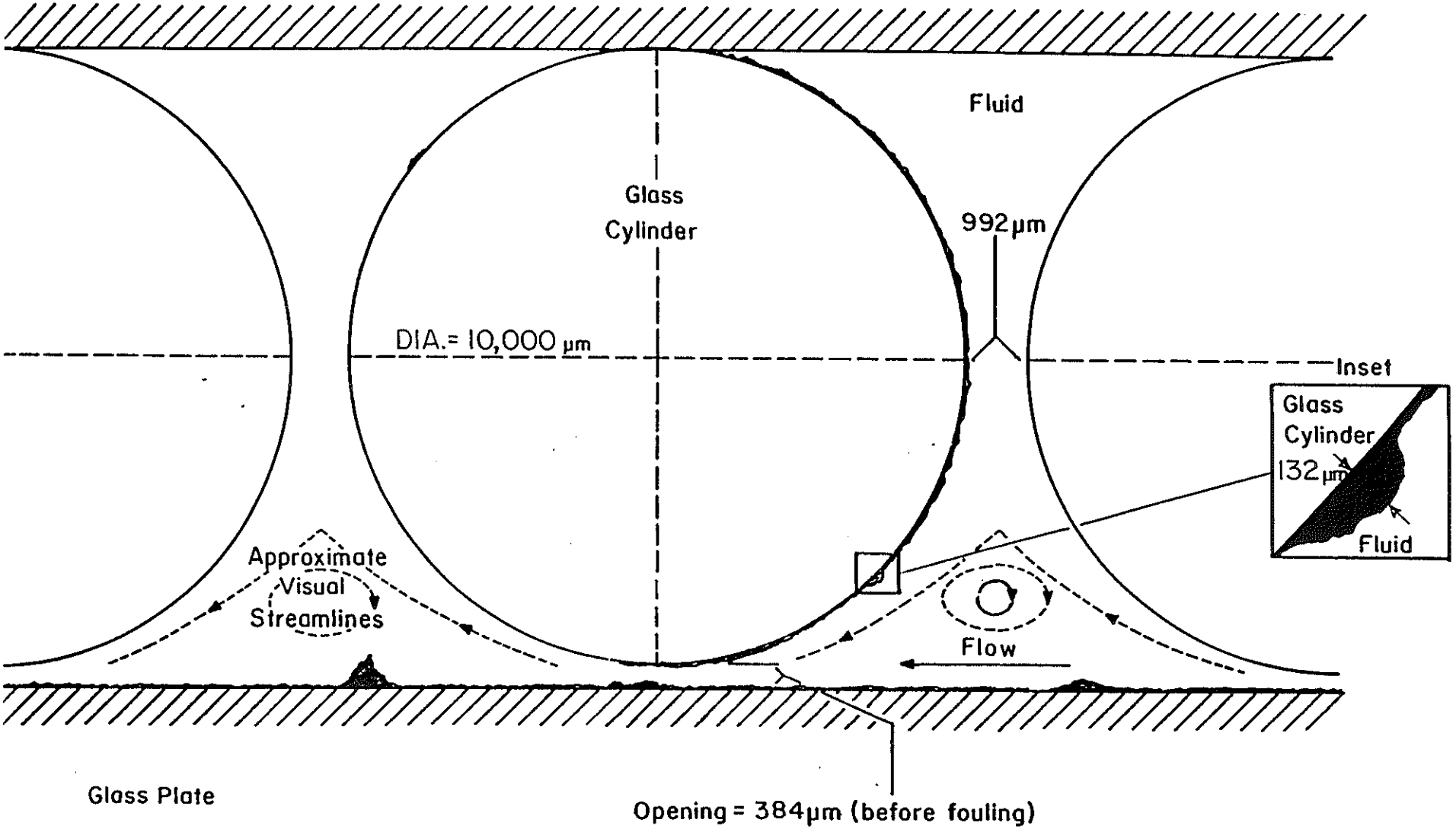

Growth Medium
Dilution Medium
Image Analyzer
TV - Camera
CFSTR
Mixing Chamber
Metering Pump
Lens
Capillary Tube
0.2 mm
2 mm
50 mm


TRANSPORT
ADSORPTION
MULTIPLICATION
EROSION
CFU - SEPARATION
DESORPTION


AB1, 0.5 N/sqm, 5.0 10e6 cells/ml
ADSORPTION, DESORPTION AND ACCUMULATION OF CFU
ADSORPT. CUMUL
DESORPT. CUMUL
CFU SEP. CUMUL
ACCUMULATION CFU
CFU (Number/sqmm)
20000
15000
10000
5000
0
0
50
100
150
200
250
300
TIME (min)

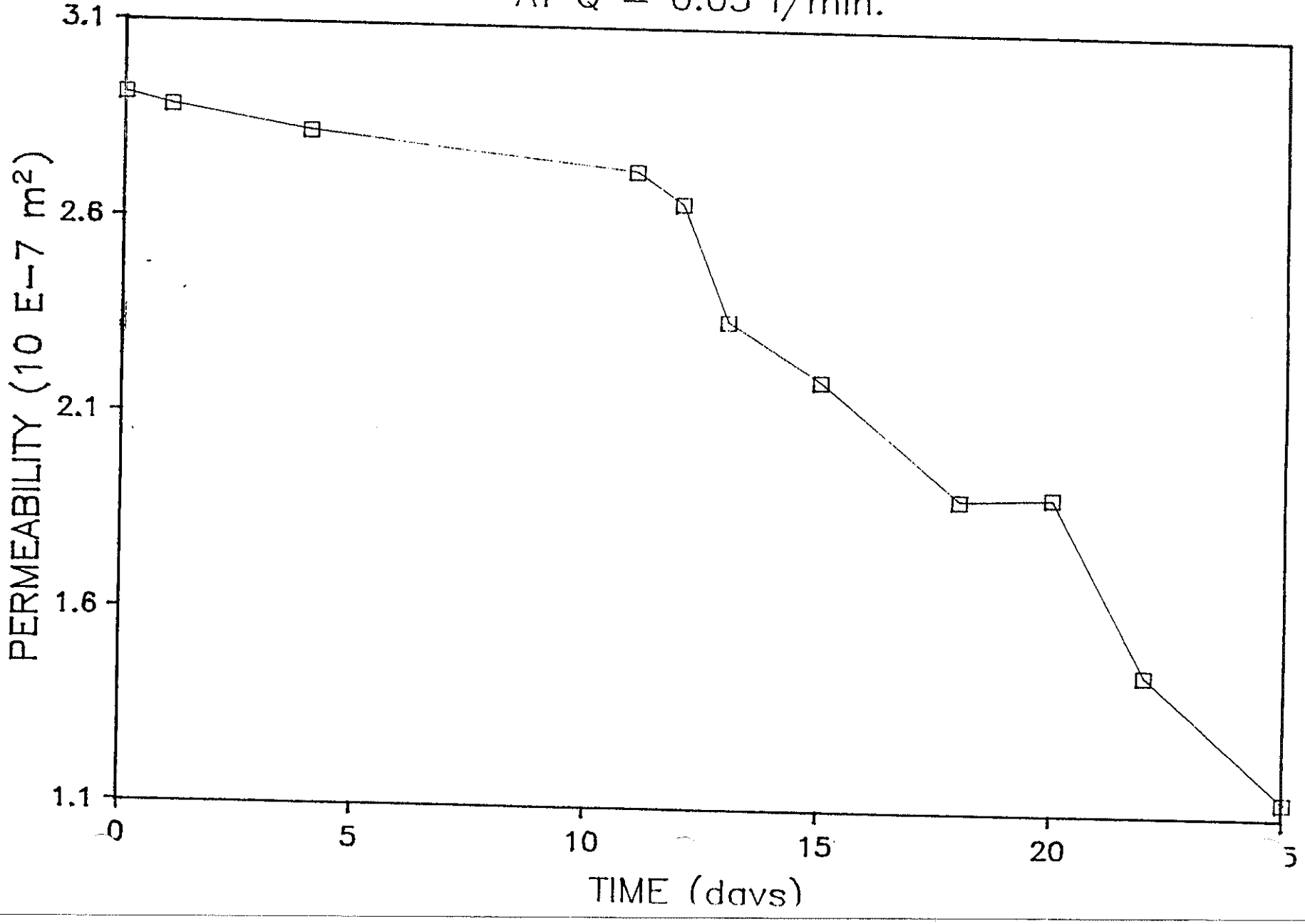

AREA COVERAGE BY CFU IN %
% AREA COVERAGE
AREA COVERAGE BY CFU IN %
3
2.5
2
1.5
1
.5
0
0
50
100
150
200
250
300
TIME (min)


SUPPORT SYSTEM
INFLOW
OUTFLOW
AIR - WATER MANOMETER
FLOW
12 in = 30.5 cm
AVG. OPENING = 0.68mm
1 cm
1.5 cm
1 mm


Fluid
Glass
Cylinder
992 µm
DIA.= 10,000 µm
Inset
Glass
Cylinder
132 µm
Fluid
Approximate
Visual
Streamlines
Flow
Glass Plate
Opening = 384µm (before fouling)

PERMEABILITY vs. TIME

AT Q = 0.05 l/min.



EQUIVALENT DIAMETER vs. PERMEABILITY

AT Q = 0.05 l/min.