



Processes of aerobic/anaerobic biofilm development  
by Steven Owen Schaftel

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE  
in Civil Engineering  
Montana State University  
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**Abstract:**

The constitutive response of an aerobic/anaerobic composite annular biofilm reactor was modeled with fundamental kinetic parameters obtained independently from a completely aerobic, reactor utilizing glucose, a completely anaerobic reactor utilizing glucose, and a completely aerobic reactor utilizing products formed in the anaerobic reactor. The model satisfactorily predicted the biofilm areal carbon density dependence of the specific glucose removal rate, the specific suspended biomass production rate, and the specific oxygen removal rate. Specific product formation rate and specific biofilm accumulation rate were not predicted satisfactorily as a function of biofilm areal carbon density.

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Date August 25, 1982

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by

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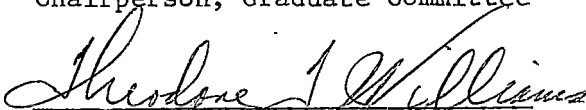
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in

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Approved:

  
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## Table of Contents

	<u>pg.</u>
List of Tables	vi
List of Figures	viii
Introduction	1
Background	1
Preliminary Studies	2
Theory	9
Organization of Material Balances	12
Primary Substrate Removal	18
Oxygen Removal	23
Soluble Product Formation	25
Soluble Products Utilization	25
Biofilm Detachment	26
Aerobic/Anaerobic Biofilm Analysis	27
Methods	31
Apparatus	31
AFR and Control Systems	31
Support Systems	34
Media	38
Inoculum	41
Experimental Protocol	41
Cleaning	41
Sterilization	41
Start-up	42
Analytical Techniques	42
Sampling	42
Analytical Methods	43
Results	46
Experimental Conditions	46
Time-Smoothing of Data	46
Glucose Removal	50
Biofilm Development	50
Product Formation	63
Oxygen Removal	63
Product Reactor	69
Product Removal	69
Biofilm Development	69

## Table of Contents, cont.

Oxygen Removal	73
Discussion	79
Literature Review	79
Determination of Kinetic and Stoichiometric Coefficients	80
Application of Aerobic/Anaerobic Model	82
Net Product Formation	90
Product Utilization	92
Re-evaluation of the Model	93
Oxygen Penetration	95
Sloughing	98
Carbon Removal	99
Applications	100
Suggestions for Further Model Development	101
Conclusions	103
Bibliography	104
Appendices	109
A. Notation	110
B. Description of Analytical Methods	114
C. Experimental Data and Rates From Material Balances	118
D. Calculation of Kinetic Constants	125
E. Data From Lactate Studies	133
F. Reaeration Studies	135
G. Oxygen Transient Studies	136
H. Computer-Generated Data for the Model	137
I. Computer Program for the Model	141
J. Chromatogram from VFA Analysis	142
K. Time-Smoothing Constants	143

## List of Tables

		<u>pg.</u>
Table 2.1	Reaction rates and stoichiometries.	13
Table 2.2	Individual material balances.	16
Table 3.1	Dimensions of the annular reactor.	33
Table 3.2	Media used for the batch induction period in the aerobic, anaerobic, and composite AR's.	39
Table 3.3	Media used during continuous flow conditions.	40
Table 3.4	Analytical methods.	44
Table 3.5	Preservation methods.	45
Table 4.1	AR influent conditions.	47
Table 4.2	Steady state experimental results.	48
Table 4.3	Comparison of the rate of accumulation of glucose in the aerobic AR to the transport of glucose in the aerobic AR.	52
Table 5.1	The calculated kinetic and stoichiometric coefficients used in predicting the response of the composite reactor.	81
Table 5.2	A comparison of substrate removal by reaction in the bulk liquid and by transport in the aerobic reactor.	83
Table 5.3	Progression of calculated data versus exper- imental data for the composite reactor.	84
Table A.1	Progression of measurements from the aerobic AR study.	118
Table A.2	Progression of measurements from the anaerobic AR study.	119

Table A.3	Progression of measurements from the anaerobic products AR study.	120
Table A.4	Progression of measurements from the composite AR study.	121
Table A.5	Calculated rates from material balances for the aerobic reactor.	122
Table A.6	Calculated rates from material balances for the anaerobic reactor.	123
Table A.7	Calculated rates from material balances for the anaerobic products reactor.	124
Table A.8	Measured data from long term lactate experiment number 2.	132
Table A.9	Measured data from long term lactate experiment number 3.	133
Table A.10	Progression of measurements from the study to determine $k_{\ell}a$ of the composite AR.	134
Table A.11	Progression of measurements from the oxygen transient studies done in the aerobic and anaerobic products AR's at steady state.	136
Table A.12	Time-smoothing constants, $k'$ .	143

## List of Figures

		<u>pg.</u>
Figure 1.1	Progression of lactate consumed by a fixed-film. Lactate was the sole carbon and energy source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.	3
Figure 1.2	Progression of biofilm thickness by a fixed-film. Lactate was the sole carbon and energy source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.	4
Figure 1.3	Progression of effluent soluble product formed by the fixed-film. Lactate was the sole carbon and energy source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.	6
Figure 1.4	Progression of the ratio of oxygen consumed to lactate consumed by the fixed-film. Lactate was the sole carbon and energy source. The horizontal dashed line indicates ratio for completely aerobic lactate consumption. Vertical dashed line indicates estimated time for initiation of anaerobic activity.	7
Figure 2.1	Processes of development in an aerobic/anaerobic biofilm.	10
Figure 2.2	CSTFFR with wall growth.	11
Figure 2.3	Simplified biofilm geometry.	17
Figure 2.4	Biofilm concentration profiles calculated from equations 2.10 & 2.11. (Harramos, 1978).	22

Figure 3.1	Annular reactor.	32
Figure 3.2	AR and support systems.	35
Figure 3.3	External aerator assembly. The bubbles which entered the AR were vented through the top of the AR. Not drawn to scale.	36
Figure 3.4	Inline sand filter between the anaerobic and anaerobic products AR's. Not drawn to scale.	37
Figure 4.1	Progression of glucose removed in the aerobic, anaerobic, and composite AR's. (Time smoothed)	51
Figure 4.2	Progression of observed glucose removal rate in the aerobic, anaerobic, and composite AR's. (Time smoothed)	53
Figure 4.3	Progression of biofilm thickness in the aerobic, anaerobic, and composite AR's.	54
Figure 4.4	Progression of biofilm areal carbon density in the aerobic, anaerobic, and composite AR's. (Time smoothed)	55
Figure 4.5	Progression of biofilm areal mass density in the aerobic, anaerobic, and composite AR's.	57
Figure 4.6	Progression of suspended biomass carbon in the aerobic, anaerobic, and composite AR's. (Time smoothed)	58
Figure 4.7	Progression of suspended solids in the aerobic, anaerobic, and composite AR's.	59
Figure 4.8	Progression of observed biofilm detachment rate in the aerobic, anaerobic, and composite AR's. (Time smoothed)	60
Figure 4.9	Relationship of suspended biomass carbon to suspended solids in the aerobic, anaerobic, and composite AR's.	61

Figure 4.10	Relationship of biofilm areal carbon density to biofilm areal mass density in the aerobic, anaerobic and composite AR's.	62
Figure 4.11	Progression of product formed in the aerobic, anaerobic, and composite AR's.	64
Figure 4.12	Progression of the observed product formation rate in the aerobic, anaerobic, and composite AR's.	65
Figure 4.13	Progression of reactor oxygen concentration for the anaerobic and composite AR's.	67
Figure 4.14	Progression of oxygen concentration in the aerobic AR after aerator was turned off at $t=0$ . (Time smoothed)	68
Figure 4.15	Progression of oxygen removal rate in the composite reactor. (Time smoothed)	70
Figure 4.16	Progression of product removed in the anaerobic products AR.	71
Figure 4.17	Progression of the observed product removal rate in the anaerobic products AR.	72
Figure 4.18	Progression of biofilm thickness, areal mass density and areal carbon density in the anaerobic products AR.	74
Figure 4.19	Progression of suspended biomass carbon formed in the anaerobic products AR.	75
Figure 4.20	Progression of suspended biomass carbon formed in the anaerobic products AR.	76
Figure 4.21	Progression of observed biofilm detachment rate in the anaerobic products AR.	77
Figure 4.22	Progression of oxygen concentration in the anaerobic products AR after aerator was turned off at $t=0$ . (Time smoothed)	78

Figure 5.1	Calculated and experimental relationship of the specific substrate removal rate with biofilm areal density in the composite AR.	86
Figure 5.2	Calculated and experimental relationship of the specific suspended biomass carbon production rate with biofilm areal density in the composite AR.	87
Figure 5.3	Calculated and experimental relationship between specific oxygen removal rate and biofilm areal density in the composite AR.	88
Figure 5.4	Calculated and experimental relationship between specific biomass carbon accumulation rate and the log of the biofilm areal density in the composite AR.	89
Figure 5.5	Calculated and experimental relationship of the specific product formation rate with biofilm areal density in the composite AR.	91
Figure 5.6	Relationship of specific substrate removal rate to biofilm areal density when assuming product formation is occurring in the aerobic layer of biofilm in the composite AR.	96
Figure 5.7	The relationship of the specific product formation rate to biofilm areal density when aerobic product formation is considered in the composite AR.	97
Figure A.1	The relationship of the substrate removal rate to zero order and half order substrate removal kinetics.	126
Figure A.2	Determination of $k_{an}$ .	127
Figure A.3	Calculation of biomass yield from glucose in the aerobic AR.	129
Figure A.4	Calculation of the product formation rate in the aerobic AR.	131

Figure A.5 Determination of  $k_0 a$  for the AR's.

## ABSTRACT

The constitutive response of an aerobic/anaerobic composite annular biofilm reactor was modeled with fundamental kinetic parameters obtained independently from a completely aerobic reactor utilizing glucose, a completely anaerobic reactor utilizing glucose, and a completely aerobic reactor utilizing products formed in the anaerobic reactor. The model satisfactorily predicted the biofilm areal carbon density dependence of the specific glucose removal rate, the specific suspended biomass production rate, and the specific oxygen removal rate. Specific product formation rate and specific biofilm accumulation rate were not predicted satisfactorily as a function of biofilm areal carbon density.

## INTRODUCTION

### Background

A biofilm is an attached microbial mat which is composed of both cells and an adhesive polysaccharide material termed glycocalyx. In natural environments such as rivers and streams, the relative number of attached cells per square centimeter of biofilm is as much as 3 to 4 orders of magnitude greater than the number of suspended cells per cubic centimeter of liquid. In a polluted stream, the attached bacterial areal density is as much as 4 orders of magnitude greater than in an unpolluted stream (Costerton, et al, 1978).

Therefore, due to the relatively enormous cell density, biofilm process rates are much greater than suspended cell process rates. The wastewater treatment industry has taken advantage of these high process rates through the use of fixed film reactors such as trickling filters (Eckenfelder, 1961) and rotating biological contactors (Bunch, 1976). Trickling filters and rotating biological contactors (RBC's) receive oxygen from air. In some cases, the effluent is recycled to reduce the likelihood of oxygen limitations within the biofilm. Recycling, then increases the amount of waste removed.

In previous studies using fixed film reactors (Eckenfelder, 1961; Antonie et al, 1971; Wu et al, 1980; Yeun et al, 1981), measurement of influent and effluent organic carbon was done on the basis of BOD, COD,

or TOC. However, these analytical methods provided only a gross measure of the biological processes occurring. Only by accounting for the individual components of the influent (and effluent) can a quantitative evaluation of fixed film processes be made.

### Preliminary Studies

A more quantitative evaluation of fixed film processes was completed as a preliminary study to aid in experimental design. (The tabular data is presented in Appendix E). Lactate was used as the sole carbon and energy source. After 100 hours, lactate consumption rate increased to a plateau value approximately 75% of the feed rate (Figure 1.1. In all figures error bars indicate one standard deviation.). However, lactate consumption rate increased again at approximately 120 h until all lactate in the reactor was consumed. Though several explanations are possible, the second phase of growth was probably due to lactate consumption within a developing anaerobic region of the biofilm.

Further evidence of a developing anaerobic region was observed in the progression of biofilm thickness (Figure 1.2). The rate of biofilm accumulation appeared to slow at approximately 120 h. This observation could be accounted for by anaerobic biofilm development because anaerobic processes are typically less efficient than aerobic processes and would result in slower biofilm growth.

Anaerobic biofilm development was also the probable reason for

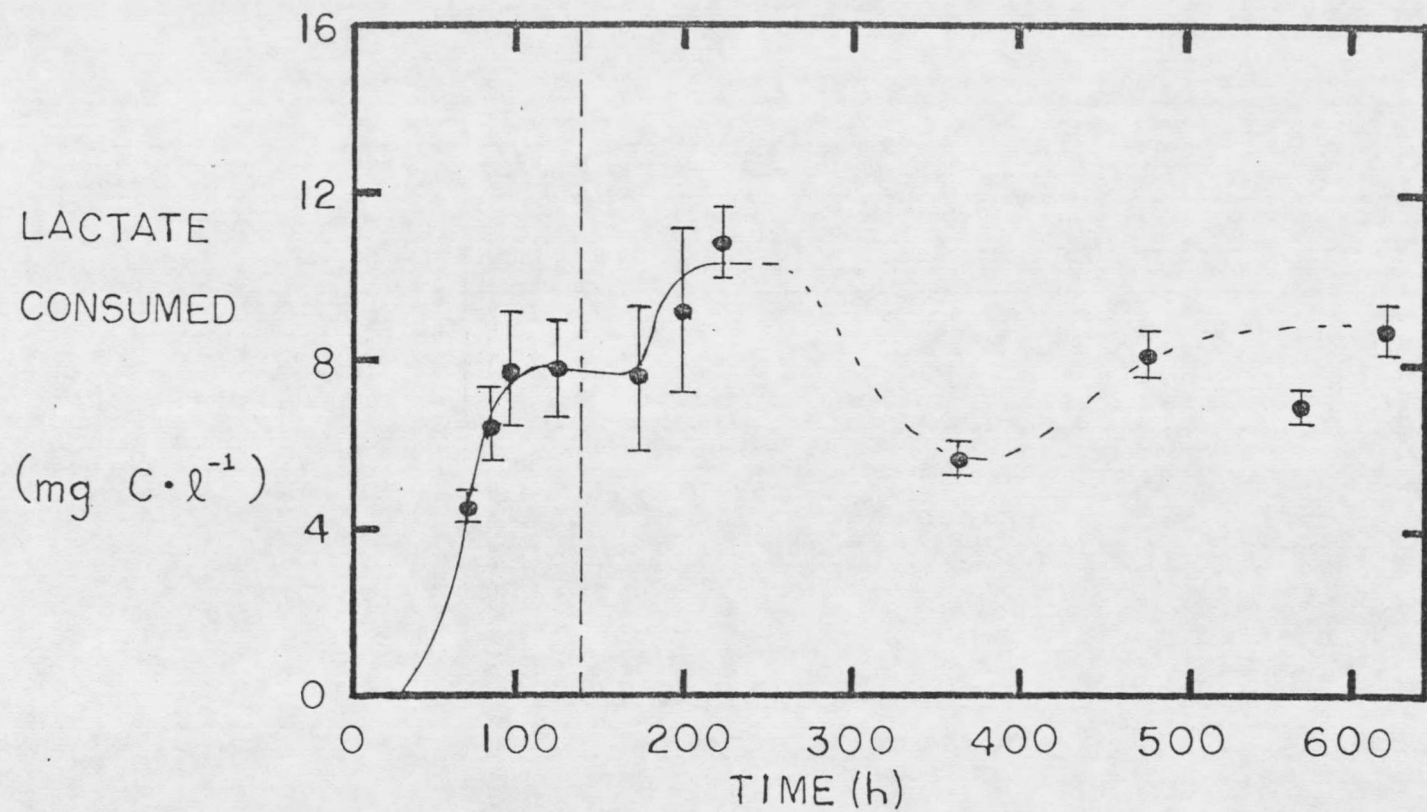


Figure 1.1 Progression of lactate consumed by a fixed film. Lactate was the sole carbon and energy source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.

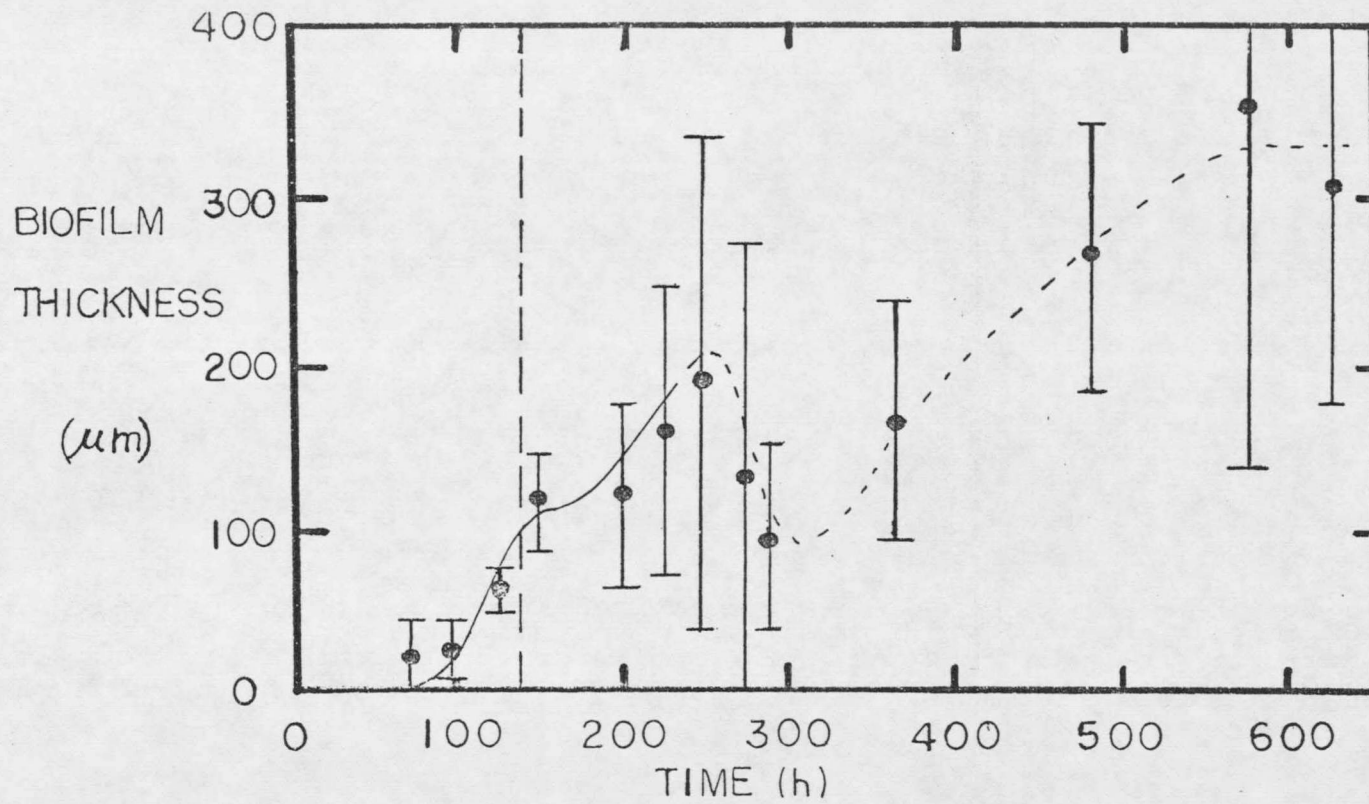
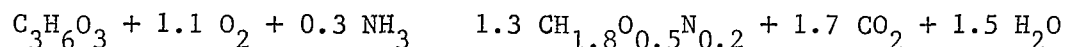


Figure 1.2

Progression of biofilm thickness by a fixed film. Lactate was the only carbon source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.

detection of soluble organic products in the reactor effluent (Figure 1.3).

The best evidence for the initiation of anaerobic biofilm development at about 120 h was the ratio of oxygen consumed to lactate consumed (Figure 1.4). At about 120 h this ratio dropped from 1.0 to 0.5. A ratio of 1.0 indicates complete aerobic utilization of lactate based on the following theoretical stoichiometry:



where 1.3 moles biomass per mole lactate represents a reasonable aerobic yield (Grady and Lim, 1980). The observed decrease in the oxygen consumption ratio indicates that lactate was being removed anaerobically and aerobically.

From the experimental evidence presented above, there is strong indication for the following:

- 1) the biofilm initially developed aerobically during which time lactate was completely oxidized to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ;
- 2) anaerobic activity was significant after 120 h as indicated by production of soluble organic products as well as a reduction in the overall stoichiometric oxygen consumption ratio;
- 3) biofilm accumulation was slower in the anaerobic layers than in the aerobic layers, as evidenced by the steeper slope during aerobic biofilm development (Figure 1.2).

Though oxygen limitations with resulting anaerobic activity have been observed before (Trulear, 1980; Harris and Hansford, 1976; Tomlin-

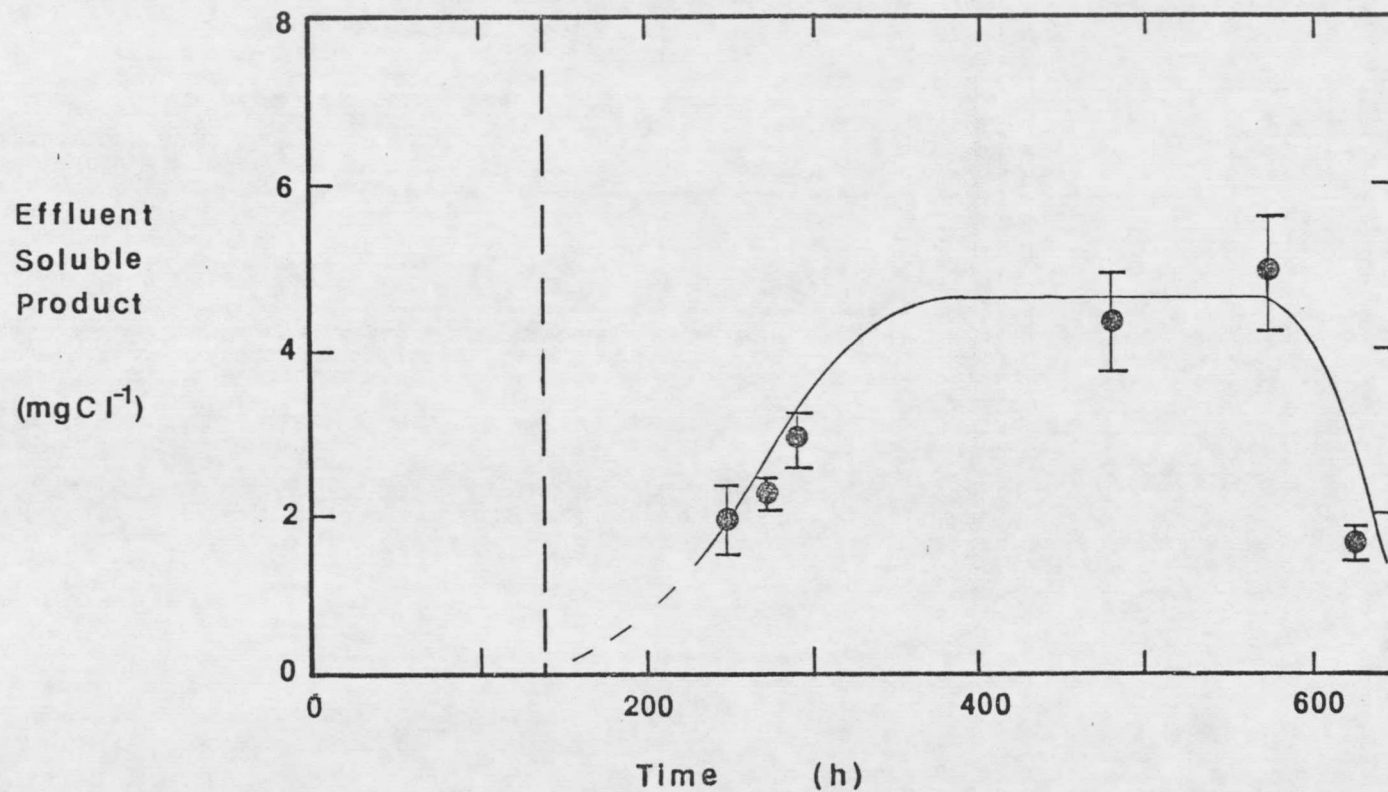


Figure 1.3 Progression of effluent soluble product formed by the fixed film. Lactate was the sole carbon and energy source. Vertical dashed line indicates estimated time for initiation of anaerobic biofilm layer.

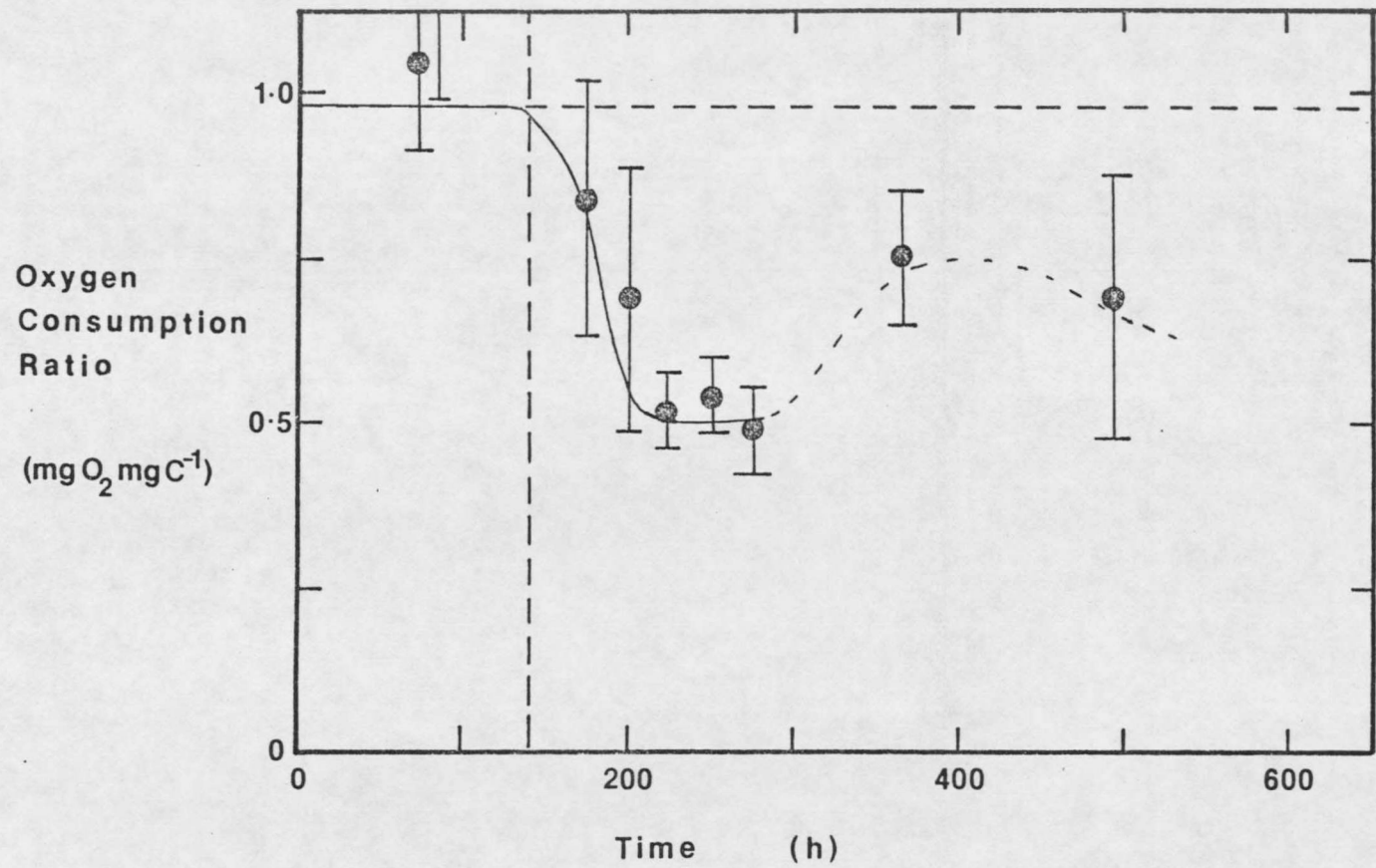


Figure 1.4

Progression of the ratio of oxygen consumed to lactate consumed by a fixed film. Lactate was the sole carbon and energy source. The horizontal dashed line indicates ratio for completely aerobic lactate consumption. Vertical dashed line indicates estimated time for initiation of anaerobic activity.

son and Snaddon, 1966), a composite system (aerobic/anaerobic) has not been analyzed so as to differentiate between aerobic and anaerobic biofilm development rates. The lactate experimental results indicate that such a discriminating analysis is necessary for a fundamental understanding of fixed film reactor processes.

An understanding of aerobic/anaerobic interactions and rates would benefit both wastewater treatment, where oxygen limitation may reduce efficiency of aerobic fixed film processes, and other industries such as the pharmaceutical and food industries which engineer specific microbial transformations of specific organic substrates.

This research was undertaken to identify fundamental kinetic parameters for aerobic and anaerobic biofilm processes and then to apply these kinetic parameters to an aerobic/anaerobic composite biofilm system in order to predict the constitutive nature of biofilm development in the composite system.

## THEORY

A biofilm exposed to oxygen and a stoichiometric excess of substrate will develop to the point at which oxygen becomes limiting in the lower layers of the biofilm, provided development is not first limited by shear stress at the biofilm surface. Despite the oxygen limitation, the biofilm continues to accumulate because the aerobic and anaerobic regions continue to metabolize substrate. As a result of anaerobic activity, soluble organic products are released and may be consumed within the aerobic region as they diffuse outward. Therefore, three processes presumably occur over two regions (Figure 2.1):

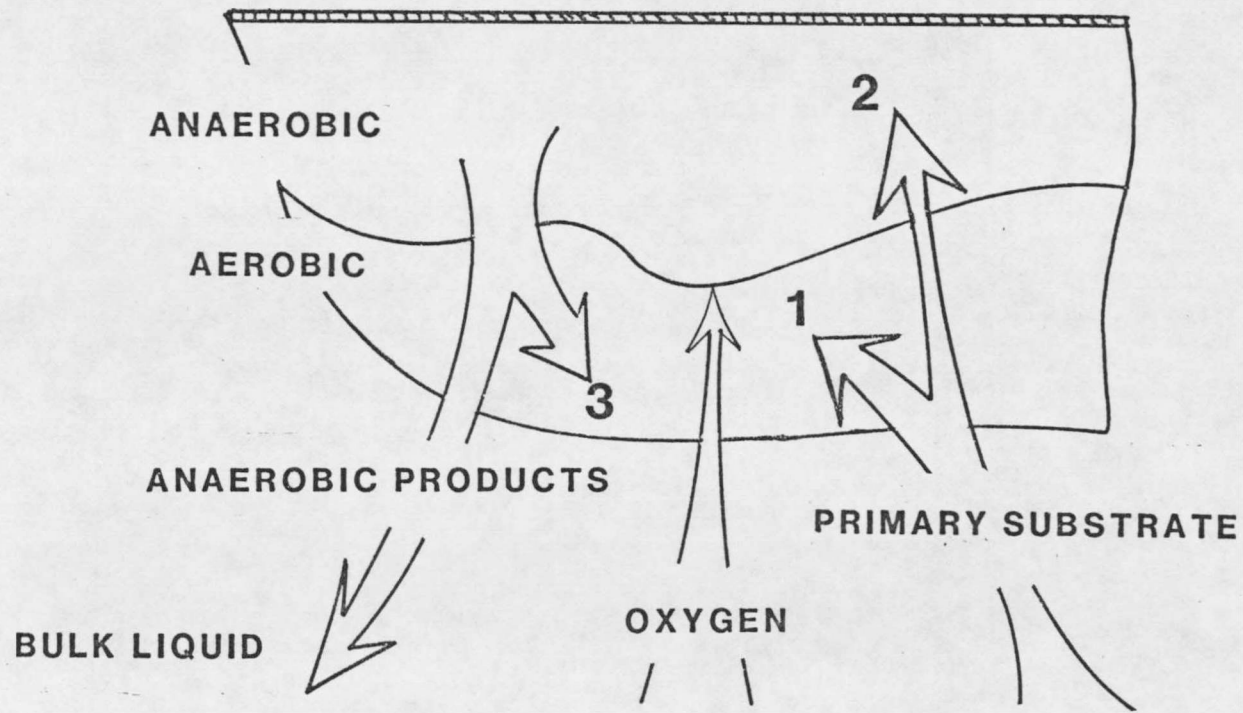
- (1) aerobic substrate removal,
- (2) anaerobic substrate removal and product formation, and
- (3) aerobic removal of the products formed in the anaerobic film environment.

### Hypothesis:

The development of an aerobic/anaerobic biofilm can be modeled with the fundamental kinetic parameters determined from completely aerobic and completely anaerobic biofilm reactors.

To test this hypothesis, it is convenient to mathematically model the system as a continuous stirred tank fixed film reactor (CSTFFR) (Figure 2.2). Biofilm stoichiometries and reaction rates can be deter-

THE TOTAL BIOFILM ACCUMULATES VIA  
THREE IDENTIFIABLE PROCESSES



10

Figure 2.1 Processes of development in an aerobic/anaerobic biofilm.

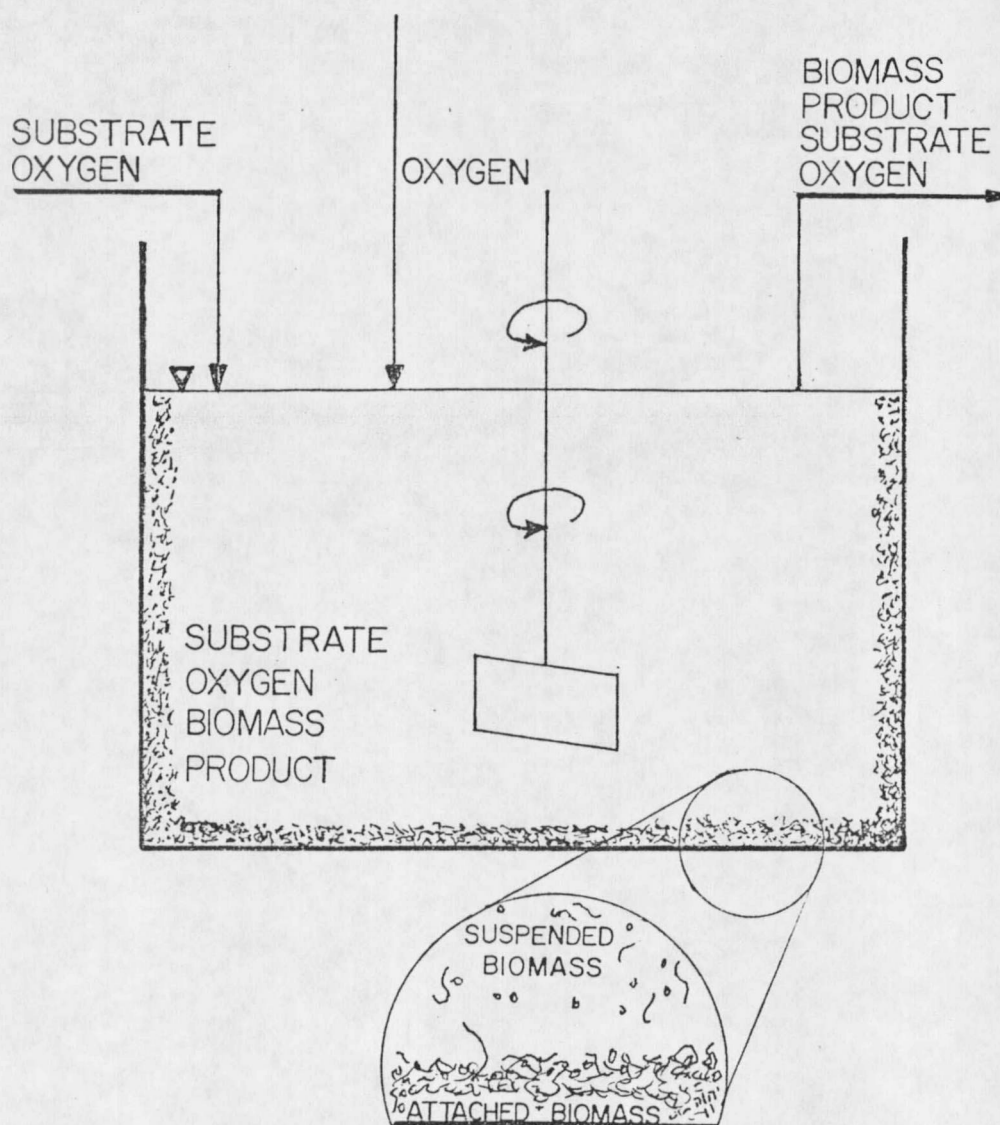


Figure 2.2 A CSTFFR with wall growth

mined by applying the principle of mass conservation. Reactions in the bulk liquid can be shown to be negligible. These stoichiometries and reaction rates are presented in Table 2.1. Notation is described in the Appendix A.

### Organization of Material Balances

A material balance for any component, C, across a CSTFFR results in the following:

$$\begin{array}{rcl} \text{Net Rate of} & \text{Net Rate of} & \text{Net Rate of} \\ \text{Accumulation} & = & \text{Transport} & + & \text{Transformation} \end{array}$$

or

$$V \frac{dC}{dt} = F(C_i - C) + A \sum_{i=1}^n R_i \cdot \quad 2.1$$

Dividing through by V yields:

$$\frac{dC}{dt} = D(C_i - C) + \frac{A}{V} \sum_{i=1}^n R_i \cdot \quad 2.2$$

To obtain Equation 2.2 for each parameter in Table 2.1, it is convenient to use matrix notation (Aris, 1969). In matrix form, the element  $\frac{dC}{dt}$  is a component of the vector  $\frac{dC}{dt}$ ;  $(C_i - C)$  is a component of the vector  $T$ ;  $\sum_{i=1}^n R_i$ , is a component of the vector  $X \cdot R$

TABLE 2.1

## Reaction Rates and Stoichiometries

Description	Stoichiometry	Process Rate
Biomass formed	$Y_{x/p} p + Y_{x/s}^e + Y_{x/s}^{an} s \rightarrow x_b$	$R_{px} + R_x^e + R_x^{an}$
Product formed	$Y_{p/s} s \rightarrow p$	$R_p$
Biofilm detached	$x_b \rightarrow x_\ell$	$R_d$
Oxygen consumed	$Y_{e/p} p + Y_{e/s} s \rightarrow x_b$	$R_e + R_{ep}$

Table 2.1 continued

$R_d$	biofilm detachment rate	$ML^{-2}t^{-1}$
$R_e$	oxygen removal rate because of glucose consumption	$ML^{-2}t^{-1}$
$R_{ep}$	oxygen removal rate because of anaerobic product consumption	$ML^{-2}t^{-1}$
$R_{px}$	biofilm growth rate from product consumption	$ML^{-2}t^{-1}$
$R_x^{an}$	anaerobic biofilm growth rate	$ML^{-2}t^{-1}$
$R_x^e$	aerobic biofilm growth rate	$ML^{-2}t^{-1}$
$s$	AR glucose concentration	$ML^{-3}$
$x_b$	biofilm carbon mass per unit of biofilm	$ML^{-3}$
$x_b^*$	biofilm areal carbon density	$ML^{-2}$
$x_l$	reactor concentration of suspended biomass carbon	$ML^{-3}$
$Y_{e/p}$	the mass ratio of oxygen consumed to product consumed in the aerobic biofilm layer	$MM^{-1}$
$Y_{e/s}$	the mass ratio of oxygen consumed to substrate consumed in the aerobic biofilm layer	$MM^{-1}$
$Y_{p/s}$	product yield from glucose	$MM^{-1}$
$Y_{x/p}$	biomass yield from product	$MM^{-1}$
$Y_{x/s}$	biomass yield from glucose	$MM^{-1}$
$Y_{x/s}^{an}$	anaerobic biomass yield from glucose	$MM^{-1}$
$Y_{x/s}^e$	aerobic biomass yield from glucose	$MM^{-1}$

where,

$T \equiv$  transport vector

$$= \{s_i - s, p_i - p, 0, x_{\lambda_i} - x_{\lambda}, (e_i - e) + \frac{k_1 a}{D} (e^* - e)\},$$

$X \equiv$  stoichiometry matrix

$$= \begin{bmatrix} -Y_{x/s}^e - 1 & -Y_{x/s}^{an} - 1 & 0 & -Y_{p/s}^{-1} & 0 & 0 & 0 \\ 0 & 0 & -Y_{x/p}^{-1} & 1 & 0 & 0 & 0 \\ 1 & 1 & 1 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & -1 \end{bmatrix},$$

$R \equiv$  reaction rate vector

$$= \{R_x^e, R_x^{an}, R_{px}, R_d, R_e, R_{ep}\}.$$

The general mass balance equation is:

$$\frac{dC}{dt} = D T + X \cdot R \frac{A}{V}. \quad 2.3$$

Equation 2.3 is expanded for the composite reactor in Table 2.2 to show all the material balances.

TABLE 2.2

Equation 2.3 Expanded to Show all the Material  
Balances That Presumably Apply to the Composite AR

$$\begin{bmatrix} \frac{ds}{dt} \\ \frac{dp}{dt} \\ \frac{dx_b^*}{dt} \\ \frac{dx_\ell}{dt} \\ \frac{de}{dt} \end{bmatrix} = D \cdot \begin{bmatrix} s_i - s \\ p_i - p \\ 0 \\ x_i - x \\ (e_i - e) + \frac{k_\ell a}{D} (e^* - e) \end{bmatrix} + \begin{bmatrix} -\frac{R_x^e}{Y_{x/s}} - \frac{R_x^{an}}{Y_{x/s}^{an}} - \frac{R_p}{Y_{p/s}} \\ R_p - \frac{R_{px}}{Y_{x/p}} \\ R_x^e + R_x^{an} + R - R_d \\ R_d \\ -R_e - R_{ep} \end{bmatrix} \cdot \frac{A}{V}$$































































































































































































































































