

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**GROWTH AND SHEAR LOSS CHARACTERISTICS
OF
AN AEROBIC BIOFILM**

**TIAM TENG SEE
1990**

**GROWTH AND SHEAR LOSS CHARACTERISTICS
OF
AN AEROBIC BIOFILM**

A Thesis

submitted in partial fulfilment
of the requirements for the degree of
MASTER OF TECHNOLOGY in Biotechnology
at Massey University

by

Tiam Teng See

Department of Biotechnology
Massey University
Palmerston North
NEW ZEALAND

March, 1990

ABSTRACT

The application of biofilms in fermentation and waste treatment processes has been increasingly considered in recent years due to several inherent advantages over suspended growth systems. For example, they enable higher biomass hold-up providing larger quantity of cell per unit reactor volume which allows high loading rates. The biofilm systems, with fixed or immobilised cells, avoid washout conditions. The often difficult problems of sludge thickening, separation, recycle, and wasting associated with suspended growth systems are eliminated for biofilm systems. However, the major drawback lies in the control of film thickness in order to maintain high reactor productivities.

The attached film thickness depends on both the biological parameters such as growth rate, and physical parameters such as hydrodynamic shear. The understanding of the growth and shear loss characteristics is a prerequisite for effective film thickness control.

The main objective of this work therefore is to investigate the growth and shear loss characteristics of an aerobic biofilm utilizing phenol in a concentric cylindrical bioreactor. The growth and detachment of the

biofilm was studied at different shear stresses, and their relationships were established. Detachment by shear was studied under two different conditions. One was examined simultaneously with growth under a constant shear stress where the biofilm detachment and growth occurred at the same time in the bioreactor. The other was examined via a separate shear test performed on the biofilm initially grown at a shear stress lower than that applied during the test. A method for measuring the torque exerted on the biofilm surface was first developed to enable computation of the related shear stress necessary for the study.

The effect of film thickness on torque at film surface for a constant rotational speed was not significant. Shear stress can be conveniently determined from a quadratic relationship between torque and rotational speed for the range of film thickness studied.

The substrate consumption is directly proportional to film thickness up to about 0.050 to 0.100 mm only, and beyond that it becomes independent of film thickness.

The mass transfer resistance in the liquid phase appears to reach a minimum at shear stress greater than 3.44 N/m^2 coinciding with the maximum steady-state substrate removal rate.

The shear loss resistance of the biofilm increases with increasing shear stress during growth. The ultimate shear loss rate and shear stress relationship follows approximately:

$$R_s = (40.82 - 2.75\sigma + 0.15\sigma^2 - 31.83e^{-0.61\sigma}) \times 10^{-2}$$

The net growth rate varies with shear stress according to a parabolic function which predicts a shear stress of 19 N/m^2 is required to achieve zero net growth.

The biofilm-support adhesion must remain stronger than the film layer adhesion, otherwise, detachment will occur at the film-support interface rendering it impossible to control the film thickness.

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Dr S. M. Rao Bhamidimarri for his advice, guidance and inspiration during the course of this study, and to the Department of Biotechnology for providing the facilities to enable this research.

I also like to extend my appreciation and gratitude to the staff of Biotechnology Department and fellow students who had given me their help and support generously whenever required.

My special thanks to Mr John Algers for his technical assistance and expeditious job in organizing the experimental equipment, without whom, the timely completion of this project will not be possible. Thanks also for his pleasant friendship and sense of humour that made my stay at Massey most comfortable and enjoyable.

The invaluable assistance of Mr Sridhar Susarla and Mr Wayne Mallet in the use of computers are gratefully acknowledged.

Last of all, my heartfelt thanks to my wife, Hong Kiow, for her love, patience, and cheerful endurance during the

many evenings and weekends when I have to work late on this project; and also to my parents for their never-ending support throughout the course of this graduate study.

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	xii
CHAPTER 1 : INTRODUCTION	1
CHAPTER 2 : LITERATURE REVIEW	5
2.1 BIOFILM GROWTH AND DEVELOPMENT ON SURFACE	5
2.1.1 Environmental Factors Affecting Growth	7
2.1.2 Microbial Attachment to Surface	11
2.2 TRANSFER OF MATERIAL INTO BIOFILM	14
2.2.1 Diffusion in the Liquid Phase	14
2.2.2 Diffusion Within the Biofilm	17
2.3 BIOFILM CHARACTERISTICS	17
2.3.1 Thickness	18
2.3.2 Density	22
2.4 BIOFILM DETACHMENT	27
2.5 CONCLUDING REMARKS	30
CHAPTER 3 : MATERIALS AND METHODS	31
3.1 MICROBIAL CULTURE AND LIQUID MEDIUM	31
3.2 MEASUREMENT OF CELL CONCENTRATION	32
3.3 MEASUREMENT OF PHENOL CONCENTRATION	35

3.4	THE BIOREACTOR	35
3.5	EXPERIMENTAL SYSTEM AND PROCEDURES	39
3.5.1	Measurement of Biofilm Growth	44
3.5.2	Shear Test	45
3.5.2.1	Measurement of Torque and Determination of Shear Stress	45
3.5.2.2	Measurement of Biofilm Loss due to Shear	49
3.5.3	Biofilm Dry Density Measurement	50
CHAPTER 4 :	RESULTS AND DISCUSSIONS	51
4.1	TORQUE ON SUPPORT SURFACE WITH AND WITHOUT BIOFILM	51
4.2	BIOFILM GROWTH AND ACCUMULATION	56
4.2.1	Substrate Removal or Consumption	60
4.2.2	Net Biofilm Accumulation	64
4.3	BIOFILM LOSS DURING GROWTH	68
4.4	EFFECT OF SHEAR CONDITIONS DURING GROWTH	76
CHAPTER 5 :	CONCLUSIONS	82
CHAPTER 6 :	RECOMMENDATIONS FOR FURTHER STUDY	84
	NOMENCLATURE	86
	REFERENCES	88
APPENDIX 1 :	THEORY OF TORQUE AND SHEAR STRESS IN A CONCENTRIC CYLINDRICAL REACTOR SYSTEM	95
APPENDIX 2 :	SIZING OF SHAFT DIAMETER FOR TORQUE MEASURING DEVICE	101
APPENDIX 3 :	BIOREACTOR DIMENSIONS	102

APPENDIX 4 :	DERIVATION OF FUNCTIONS FOR COMPUTATION OF VOLUME AND THICKNESS OF BIOFILM	103
APPENDIX 5 :	DATA FOR ESTABLISHING STANDARD CURVES TO DETERMINE THE BIOREACTOR CELL MASS AND PHENOL CONCENTRATIONS	105
APPENDIX 6 :	TORQUE AND ROTATIONAL SPEED (RPM) RELATIONSHIP DATA	110
APPENDIX 7 :	DATA OF NET BIOFILM ACCUMULATION ON BIOREACTOR SURFACE	115
APPENDIX 8 :	DATA OF SUBSTRATE CONCENTRATION IN BIOREACTOR DURING GROWTH	137
APPENDIX 9 :	DATA OF BIOFILM TOTAL GROWTH AND LOSS RATES DURING GROWTH PHASE	147
APPENDIX 10:	SHEAR TEST DATA	149
APPENDIX 11:	DATA FOR BIOFILM DRY DENSITY DETERMINATION	163

LIST OF FIGURES

Figure No.	Title	Page
2.1	Diagram illustrating biofilm main features	8
2.2	Effect of pH on enzyme reaction velocity (Greenfield, 1987)	8
2.3	Oxygen concentration profile inside and outside a biofilm in bulk liquid (Chen and Bungay, 1981)	16
2.4	Idealized biofilm and stagnant liquid layer illustrating concentration profile of the organic substrate as electron donor (D) and oxygen as electron acceptor (A)	16
2.5	Rate of COD (representing substrates) removal as a function of film thickness (Hoehn and Ray, 1973)	20
2.6	Effect of biofilm thickness on the density of the biofilm growing on a rotating drum (Hoehn and Ray, 1973)	24
2.7	Effect of fluid shear stress and organic loading (R_L) on the maximum biofilm thickness attained in an annular reactor (Data from Zolver, 1979 as presented by Characklis, 1981)	24
2.8	Influence of fluid shear stress on biofilm density (Data from Zolver, 1979 as presented by Characklis et al, 1982)	26
2.9	Effect of dissolved oxygen (D.O.) on dry density of biofilm (Huang et al, 1985)	26
2.10	Effect of rotational speed in an annular reactor on the detachment rate of a biofilm with a mass of 150-160 mg (Trulear and Characklis, 1982)	28
2.11	Effect of biofilm mass (proportional to thickness) in an annular reactor rotating at constant speed on the detachment rate of biofilm. R_L refers to the organic loading rate. (Trulear and Characklis, 1982)	28

3.1	Relation between dry cell mass concentration and absorbance	33
3.2	Photograph of vibrating equipment	34
3.3	Relation between phenol concentration and absorbance	36
3.4	Diagram of a concentric cylindrical bioreactor	37
3.5	Schematic diagram of the experimental set-up	40
3.6	Photograph of the overall arrangement of the experimental equipment	41
3.7	Schematic diagram of the torque measuring device	46
3.8	Photograph of the torque measuring device for shear test	47
3.9	Photograph of 'Turn-Table' of the Torque Measuring Device with Bioreactor	48
4.1	Variation in torque with rotational speed without biofilm	52
4.2	Variation in torque with rotational speed with biofilm	54
4.3	Effect of biofilm thickness on torque	55
4.4	Torque variation with rotational speed for different surface types	57
4.5	Photograph of activated carbon coated surface reactor with biofilm	58
4.6	Photograph of activated carbon coated surface reactor without biofilm	59
4.7	Variation in substrate removal with film thickness for various rotational speeds	61
4.8	Effect of shear stress on steady-state substrate removal	62

4.9	Variation in net biofilm accumulation with time	65
4.10	Variation in biofilm thickness with time	66
4.11	Effect of shear stress on net biofilm growth rate	67
4.12	Variation in biofilm total growth rate with shear stress	69
4.13	Variation in biofilm total loss rate with shear stress	71
4.14	Effect of shear stress on biofilm growth and loss rates	72
4.15	Photograph of net biofilm accumulation for 'thick' film (after 4 days of growth, at 350 rpm)	75
4.16	Variation in biofilm loss with shearing time for different rotational speeds	77
4.17	Variation in initial loss rate of biofilm with film thickness for different rotational speeds	79
4.18	Variation in initial loss rate per unit film thickness with shear stress	80
A1.1	Diagram of concentric cylindrical reactor with inner cylinder rotating	95
A1.2	Co-ordinate system for bottom surface of a bioreactor inner cylinder in rotation	99
A3	Diagram of bioreactor dimensions without biofilm	102
A4	Diagram of bioreactor dimensions with biofilm	103

LIST OF TABLES

Table No.	Title	Page
2.1	Temperature ranges of microorganisms	9
3.1	Composition of aqueous phenol solution	31
3.2	Dimensions of reactor	38
3.3	Operating parameters	42
3.4	Operating speeds of inner cylinder of reactor	43
A3	Details of bioreactor dimensions	102
A5.1.1	RAW DATA: Absorbance at 620 nm wavelength for various dry cell mass concentrations	105
A5.1.2	Calculated dry cell mass for various absorbance at 620 nm wavelength	106
A5.2	Absorbance at 279 nm wavelength (UV) for various phenol concentrations	108
A6.1(a)	Measured torque at various rotational speeds (rpm) with no biofilm on carbon coated surface bioreactor	110
A6.1(b)	Calculated 95% confidence interval of the mean torque values in Table A6.1(a)	111
A6.2(a)	Measured torque at various rotational speeds (rpm) with biofilm on carbon coated surface bioreactor	112
A6.2(b)	Calculated 95% confidence interval of the mean torque values in Table A6.2(a)	
A7.1	RAW DATA: Rise in liquid height, Δh mm, representing cumulative increase in cell quantity (volume) attached on bioreactor surface at different days of growth	115
A7.2	Calculated net biofilm accumulation (growth) in terms of thickness, z and volumetric quantity at various days of growth	118

A7.3	Computed biofilm net attached growth rate at various speeds (rpm) and shear stresses	135
A8.1	RAW DATA: Absorbance at 279 nm representing substrate (phenol) concentration in the bioreactor at different days of growth for various constant rotational speeds (rpm)	137
A8.2	Calculated substrate (phenol) consumption or removal in bioreactor during growth at various film thicknesses for different constant rotational speeds (rpm)	141
A8.3	Steady-state substrate (phenol) consumption or removal in bioreactor at different rotational speeds and shear stresses	145
A9	Calculated total growth and loss rates at different shear stresses	147
A10.1	RAW DATA: Absorbance representing cell concentration in reactor due to shear loss at various rotational speeds (growth at 35 rpm)	149
A10.2	Calculated biofilm shear loss at rotational speeds higher than the speed (35 rpm) adapted during growth	152
A10.3	Calculated shear loss rate at various rotational speeds and film thicknesses	161
A11.1	RAW DATA: Dry mass and wet volume for biofilm density measurement	163
A11.2	Calculated biofilm dry density, wet volume and thickness from bioreactor operating at various speeds	164

CHAPTER 1**INTRODUCTION**

In an aquatic environment, there is a tendency for the microorganisms to adsorb and colonize on submerged surfaces. The immobilized cells grow to form layer-like aggregates known as biofilm. Biofilms present in the natural environment consist of a complex community of primarily bacteria with other organisms such as fungi, algae and protozoa, and utilize the nutrients present in the bulk liquid medium for growth and reproduction. Such activity has its importance in the recycling of organic and inorganic substrates in the natural environment. These processes have been exploited to considerable industrial advantage in various fermentation and wastewater treatment systems e.g. trickling filters, rotating biological contactors and fluidized bed reactors.

Although biofilm or fixed-film biological processes found early application to wastewater treatment, their use declined with the development of suspended growth systems. The earliest application of biofilm system was that of trickling filter and later the rotating biological contactor was developed to achieve higher degree of treatment, eliminating some of the disadvantages of

trickling filters. The fluidized bed being the most recently introduced process, is essentially a hybrid of suspended growth and attached growth system. However, in recent years with the increasingly stringent discharge standard and high strength effluents from expanding manufacturing and processing industries, the application of biofilm or fixed-film has been increasingly considered due to certain inherent advantages over suspended growth systems. For example, they enable higher biomass hold-up, thus providing larger amount of cell per unit reactor volume, and thereby permitting high loading rates and treatment of inhibitory wastes and avoiding washout conditions. The often difficult problems of sludge thickening, separation, recycle, and wasting associated with suspended growth systems are eliminated for biofilm or fixed-film systems.

The major operational problem in fixed film systems is the control of film thickness. Biofilm thickness is thought to affect the performance of the system for a given loading rate and there is an optimum thickness beyond which substrate utilization rate will not increase any further. The mechanisms which act to remove the biomass such as decay, sloughing and attrition are still generally beyond operational control and in many cases they are not sufficient for removal of all excess

biomass. In the foregoing aerobic systems, the rapid consumption and stabilization of the organic substrate inevitably result in rapid cell growth. Thus problem of plugging or clogging of filter beds such as in trickling filters arises due to excessive growth and irregular dislodging/sloughing of thick films, whilst in other fixed-film systems periods of unsteady state system may arise resulting in fluctuating performance.

The accumulation or net attachment of biofilm on surfaces depends on both the biological parameters such as growth rate, and physical parameters such as hydrodynamic shear. The understanding of the growth and shear loss characteristics is a prerequisite for effective control of the film thickness. The existing knowledge of these is limited and no universal model has yet been developed for such control.

The objectives of this research are therefore to:

1. develop a reactor system in which biofilms of required thickness can be obtained under defined shear conditions,
2. develop a method for measuring shear stress in the reactor,
3. study the aerobic biofilm growth characteristic under different shear conditions,

4. study the aerobic biofilm loss characteristic due to hydrodynamic shear,
5. establish relationships between growth rate and shear stress, detachment or shear loss rate and shear stress.