



Thickness and density measurements in biofilm with a fiber optic sensor
by Gabriele Sabine Walser

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Environmental Engineering
Montana State University
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Abstract:

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Biofilm thickness was related to shear stress.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Environmental Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 1990

N378
W1678

APPROVAL

of a thesis submitted by

Gabriele Sabine Walser

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

July 16, 1990
Date

John J. Swenson
Chairperson, Graduate Committee

Approved for the Major Department

16 July '90
Date

Shedue E. Long
Head, Major Department

Approved for the College of Graduate Studies

July 25, 1990
Date

Henry J. Parsons
Graduate Dean

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ACKNOWLEDGEMENTS

I wish to express my appreciation to the following:

Zbigniew Lewandowski for his guidance in the experimental work of this thesis which made my work productive and enjoyable.

Warren Jones for his advice, support and encouragement and Bill Characklis for his advice and comments throughout my work.

The whole "IPA gang", Vivek, Robert, Brent, Ewout, Bill, Feisal, Whon Chee, Satoshi and Bryan, for being such a fun group to work with.

Paul and Ann, for always being ready to help and answer questions.

Wendy, Diane, Lynda, Jamie, Susan, Mary, Frank, Al, and Dick for their support.

TABLE OF CONTENTS

	Page
APPROVAL	ii
STATEMENT OF PERMISSION TO USE	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vii
LIST OF FIGURES	viii
ABSTRACT	xiii
INTRODUCTION	1
Goal of Research	2
Objectives of Research	2
BACKGROUND	3
Models for the Influence of Shear Stress on Biofilm Accumulation	3
The Influence of Shear Stress on Biofilm Thickness and Density	5
Shear Stress on a Rotating Disk	7
Fiberoptic Sensors in Biofilm Research	9
MATERIALS AND METHODS	18
The Rotating Disk Reactor	18
The Fiber Optic Sensing Device	20
Procedures	22
RESULTS	25
Location of the Biofilm-Water Interface	25
Biofilm Thickness	29
Biofilm Density	32
Biofilm Thickness and Extinction Coefficient versus Shear Stress	35

TABLE OF CONTENTS-Continued

	Page
DISCUSSION	39
Fiber Optic Sensor Construction and Application	39
The Delineation of the Biofilm and the Water Phase	40
The Determination of the Biofilm Thickness	41
The Determination of the Biofilm Density	43
The Relation between Biofilm Thickness, Density and Shear Stress	45
CONCLUSIONS	47
REFERENCES CITED	48
NOMENCLATURE	51
APPENDICES	
A. The Calibration of the Micromanipulator	55
B. The Variation of Absorbance with Distance to the Substratum for Density Calibration	57
C. The Variation of Absorbance with Distance to the Substratum for 4-Day-Old Biofilm	62
D. The Variation of Absorbance with Distance to the Substratum for 7-Day-Old Biofilm	81
E. The Variation of Absorbance with Distance to the Substratum for Biofilm Grown under Non-Laminar Flow Conditions	94
F. Light Intensity Measurement in Water	108

LIST OF TABLES

Table		Page
1.	Literature data about the influence of shear stress on biofilm thickness	6
2.	Comparison of volumetric measured density with optically measured density	33
3.	Step length versus micromanipulator settings	56
4.	Step frequency for different micromanipulator settings	56

LIST OF FIGURES

Figure	Page
1. Biofilm schematic	3
2. Velocity profile for Couette-flow	9
3. A sensing device in general form and in the form used for the fiber optic sensor	11
4. Schematic of an intensity-modulated sensing device	11
5. Path of a ray through a fiber optic cable	14
6. Bending effects on critical angle	15
7. Frequency modulation with a sinusoidal modulating wave	16
8. Photograph of the rotating disk reactor	19
9. Schematic diagram of measurement device setup	21
10. Enlarged picture of the fiber optic sensor tip	22
11. The variation of light intensity with distance from the substratum	26
12. The variation of light intensity with distance from the substratum	27
13. The variation of light intensity with distance from the substratum	28
14. The variation in light intensity with distance from the substratum	30
15. The variation in light intensity with distance from the substratum	31
16. Absorbance of homogenized biofilm versus biomass concentration ("biofilm density") measured with a spectrophotometer at 660 nm	32
17. Absorbance in a biofilm versus distance	34
18. Biofilm thickness versus shear stress/velocity for a 4-day-old biofilm	36

LIST OF FIGURES-Continued

Figure	Page
19. Biofilm thickness versus shear stress/velocity for a 7-day-old biofilm	37
20. Biofilm thickness vs. flow velocity for a biofilm grown under non-laminar flow conditions	37
21. Extinction coefficient versus shear stress for a 7-day-old biofilm grown under laminar flow conditions	38
22. The variation of light intensity with distance from the substratum in a fluffy or diffuse biofilm	42
23. Biofilm with distinct base and surface film	45
24. Absorbance measurements in biofilm sample A	58
25. Absorbance measurements in biofilm sample B	60
26. Absorbance measurements in 6 cm distance from the center of the disk	63
27. Absorbance measurements in 7 cm distance from the center of the disk	64
28. Absorbance measurements in 8 cm distance from the center of the disk	65
29. Absorbance measurements in 9 cm distance from the center of the disk	66
30. Absorbance measurements in 11 cm distance from the center of the disk	67
31. Absorbance measurements in 12 cm distance from the center of the disk	68
32. Absorbance measurements in 13 cm distance from the center of the disk	69
33. Absorbance measurements in 14 cm distance from the center of the disk	70

LIST OF FIGURES-Continued

Figure	Page
34. Absorbance measurements in 16 cm distance from the center of the disk	71
35. Absorbance measurements in 17 cm distance from the center of the disk	72
36. Absorbance measurements in 18 cm distance from the center of the disk	73
37. Absorbance measurements in 21 cm distance from the center of the disk	74
38. Absorbance measurements in 23 cm distance from the center of the disk	75
39. Absorbance measurements in 24 cm distance from the center of the disk	76
40. Absorbance measurements in 26 cm distance from the center of the disk	77
41. Absorbance measurements in 27 cm distance from the center of the disk	78
42. Absorbance measurements in 28 cm distance from the center of the disk	79
43. Absorbance measurements in 29 cm distance from the center of the disk	80
44. Absorbance measurements in 11 cm distance from the center of the disk	82
45. Absorbance measurements in 16 cm distance from the center of the disk	83
46. Absorbance measurements in 17 cm distance from the center of the disk	84
47. Absorbance measurements in 18 cm distance from the center of the disk	85

LIST OF FIGURES-Continued

Figure	Page
48. Absorbance measurements in 19 cm distance from the center of the disk	86
49. Absorbance measurements in 21 cm distance from the center of the disk	87
50. Absorbance measurements in 22 cm distance from the center of the disk	88
51. Absorbance measurements in 23 cm distance from the center of the disk	89
52. Absorbance measurements in 24 cm distance from the center of the disk	90
53. Absorbance measurements in 26 cm distance from the center of the disk	91
54. Absorbance measurements in 27 cm distance from the center of the disk	92
55. Absorbance measurements in 28 cm distance from the center of the disk	93
56. Absorbance measurements in 7 cm distance from the center of the disk	95
57. Absorbance measurements in 11 cm distance from the center of the disk	96
58. Absorbance measurements in 12 cm distance from the center of the disk	97
59. Absorbance measurements in 13 cm distance from the center of the disk	98
60. Absorbance measurements in 14 cm distance from the center of the disk	99
61. Absorbance measurements in 16 cm distance from the center of the disk	100

LIST OF FIGURES-Continued

Figure	Page
62. Absorbance measurements in 17 cm distance from the center of the disk	101
63. Absorbance measurements in 18 cm distance from the center of the disk	102
64. Absorbance measurements in 19 cm distance from the center of the disk	103
65. Absorbance measurements in 21 cm distance from the center of the disk	104
66. Absorbance measurements in 22 cm distance from the center of the disk	105
67. Absorbance measurements in 24 cm distance from the center of the disk	106
68. Absorbance measurements in 26 cm distance from the center of the disk	107
69. Light intensity measurement in water	109

ABSTRACT

The purpose of this study was to measure biofilm thickness and density using a fiber optic sensor. Biofilm thickness was related to shear stress.

Biofilm was grown in a horizontal rotating disk reactor under laminar flow conditions. Measurements of biofilm thickness and density were performed with an intensity modulated fiber optic sensor.

The biofilm-water interface and the substratum-biofilm interface were detected with the sensor and the biofilm thickness was determined. The sensor was calibrated to measure biofilm density. The results for biofilm grown in the rotating disk reactor indicate that biofilm thickness depends on shear stress. The maximum biofilm thickness was found for a shear stress of approximately 0.08 Nm^{-2} . Biofilm thickness decreases for higher and lower shear stresses.

INTRODUCTION

Inert surfaces immersed in water become colonized with microorganisms forming biofilms. Biofilms are undesirable in drinking water systems, where they pose a threat to the hygienic safety, as well as they are undesirable in industrial water systems, where biofilms deteriorate the quality of the product. Biofilm also obstruct the heat transfer in cooling towers. Conversely, biofilms are desirable in many biotechnological applications where microbial cells are preferred fixed to a substratum in order to resist washout. With growing interest in the role of biofilms, a growing interest in the modeling of biofilm systems has been reported (Grady, 1982). New models have to be verified by testing their variables in experiments. Biofilm thickness and density are variables used in most models, therefore experimental methods must be found to determine thickness and density. If thickness and density can be measured, they can be related to other parameters in the model, as was done in this thesis where a correlation between biofilm thickness and shear stress was found. A measurement system based on fiber optics was chosen because fiber optic sensors have several advantages over other sensors. They are durable and immune from electromagnetic interference and the small size of the fiber optic sensor makes it especially attractive for measurements in biofilm.

Goal of Research

The goal of the research was to measure thickness and optical density of biofilms using a fiber optic sensor.

Objectives of Research

The specific objectives of the research related to sensor application to measure biofilm thickness and density were as follows:

1. Determine the position of the biofilm-water interface using a fiber optics sensor.
2. Measure the biofilm thickness using a fiber optic sensor.
3. Determine the extinction coefficient of the biofilm.
4. Measure the density of the biofilm.
5. Correlate biofilm thickness, extinction coefficient and density to shear stress in a rotating disc reactor.

BACKGROUND

Models for the Influence of Shear Stress
on Biofilm Accumulation

Biofilm accumulates on surfaces immersed in water. It is assumed that biofilm consists of one or more homogeneous layers of biomass (Figure 1).

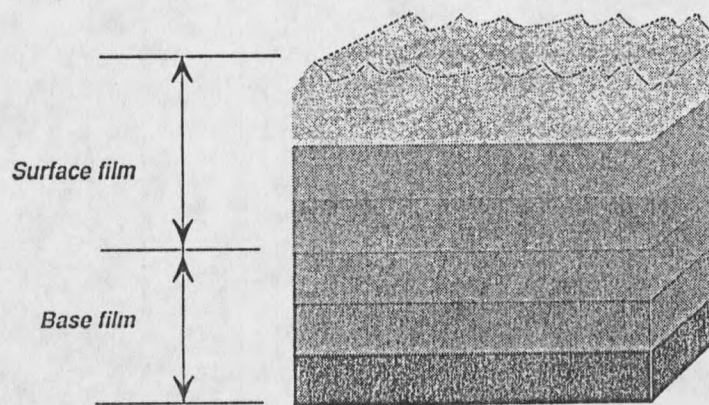


Figure 1. Biofilm schematic. The density of the biomass changes throughout the biofilm.

A numerical model for the prediction of biofilm accumulation and activity has been developed (Characklis et al., 1988). The model predicts biofilm accumulation as the net result of several physical, chemical and microbiological processes. A biofilm material balance encompasses growth, attachment and detachment, as shown in equation (1):

$$\text{accumulation} = \text{growth} + \text{attachment} - \text{detachment} \quad (1)$$

Detachment consists of erosion and sloughing. Sloughing occurs when large areas of biofilm detach from the wall. Sloughing usually removes all the biofilm down to

the substratum. Detached pieces of biofilm are removed by the flow of the bulk liquid. Erosion is the constant removal of single cells or a small group of cells from the biofilm, and is largely attributed to shear stress at the biofilm-fluid interface. Erosion rate has been expressed through the following formula (Characklis et al., 1988):

$$R_e = k_e \sigma \rho_f AL \quad (2)$$

where R_e is the erosion rate, k_e the erosion coefficient, σ the shear stress, ρ_f the film density, A the surface area of the biofilm and L the biofilm thickness. The erosion coefficient can be determined if a correlation between the biofilm thickness and the shear stress is established.

Rittmann (1982) developed an expression for detachment which encompasses erosion and sloughing. Since detachment of biofilm is a surface phenomenon, the rate of detachment, R_d , is defined as a surface rate:

$$R_d = b_d X_b L \quad (3)$$

where b_d is the surface detachment coefficient, X_b the biofilm density and L the biofilm thickness.

The detachment coefficient, b_d , can be expressed as a function of shear stress. Different expressions for the relation between shear stress and detachment coefficient could be found, depending on the biofilm thickness and biofilm roughness. Other factors might also influence the relation.

For a biofilm thinner than $30\mu\text{m}$ the following expression could be found (Rittmann, 1989):

$$b_d = 3.62 * 10^{-6} \sigma^{0.58} \quad (4)$$

For thicker biofilms the following equation held true:

$$b_d = 3.62 * 10^{-6} \{ \sigma / [1 + 0.0443(L - 30)] \}^{0.58} \quad (5)$$

The Influence of Shear Stress
on Biofilm Thickness and Density

Erosion controls the extent of biofilm accumulation and thus determines the biofilm thickness. As shown in the models above, erosion is controlled by shear stress. Most previous experiments, however, investigated the influence of flow velocity on biofilm accumulation (Table 1). While flow velocity and shear stress are related through the system geometry, it is the shear stress that directly affects the development of the biofilm.

Kornegay and Andrews (1967) measured biofilm thickness versus shear stress. They found a strong decline in the biofilm thickness when the shear stress was increased from 1 to 3 Nm^{-2} . In a study of biofilm accumulation in turbulent flow, Characklis (1980) found that biofilm thickness is dependent on shear stress and glucose loading rate. The dependence of biofilm thickness on shear stress is smaller for lower substrate loading rates. Only a small decrease in biofilm thickness was found for an increase in shear stress from 2 to 3 Nm^{-2} , when the substrate loading rate was low.

Zelver et al. (1982) did not find a significant difference in biofilm accumulation between fluid velocities of 0.30 ms^{-1} and 0.50 ms^{-1} . Harty and Bott (1981) measured the effect of increasing velocity on maximum biofilm thickness on a simulated heat exchanger surface. Their results showed that an increase in velocity of 450% from 0.1 ms^{-1} to 0.55 ms^{-1} caused a decrease in the maximum biofilm thickness of 90%. A similar reduction was found for the accumulation rate. Bland et al. (1978) evaluated the accumulation of slime in drainage pipes. Up to 0.7 kg dry matter m^{-2} was deposited in pipes close to a turbulent inlet at a velocity of 0.5 ms^{-1} , whereas at a velocity of 2.4 ms^{-1} , deposition was seldom greater than 0.05 kg m^{-2} . Conversely, Pedersen (1982b) observed a significant

increase in rate of biofilm accumulation in seawater when the water velocity was increased from 0.005 ms^{-1} to 0.15 ms^{-1} . At these low velocities, biofilm accumulation is probably mass-transfer-limited for the substrate, and an increase in velocity should increase the biofouling rate.

Table 1. Literature data about the influence of shear stress on biofilm thickness.

Reference	Experimental condition	System variable	Parameter measured	Results
Kornegay and Andrews, 1967	RotoTorque turbulent flow	shear stress $1 - 3 \text{ Nm}^{-2}$	biofilm thickness	shear stress $\uparrow$ thickness $\downarrow$
Characklis, 1980	RotoTorque turbulent flow	shear stress $2-3 \text{ Nm}^{-2}$ glucose loading	biofilm thickness	shear stress $\uparrow$ thickness $\downarrow$
Zelver et al., 1982	tube reactor turbulent flow	fluid velocity $0.3 - 0.5 \text{ ms}^{-1}$	accumulation rate	velocity $\uparrow$ rate = const.
Harty and Bott, 1981	plug flow reactor turbulent flow	fluid velocity $0.1 - 0.55 \text{ ms}^{-1}$	maximum thickness	velocity $\uparrow$ thickness $\downarrow$
Bland et al., 1978	drainage pipes turbulent flow	fluid velocity $0.5 - 2.4 \text{ ms}^{-1}$	acc. solids dry mass	velocity $\uparrow$ dry mass $\downarrow$
Pedersen, 1982b	square cell, lamellar glass laminar flow	fluid velocity $0.005 - 0.15 \text{ ms}^{-1}$	accumulation rate	velocity $\uparrow$ rate $\uparrow$

While not studied as extensively as the relationship between thickness and shear stress, biofilm density is also influenced by shear stress. Characklis (1980) investigated the influence of shear stress on biofilm density. An increase of biofilm density with an increase in shear stress was observed. Kornegay and Andrews (1967) conducted

experiments in a RotoTorque reactor under different shear stresses. Increasing shear stress had no significant influence on biofilm density under high substrate loading rates.

Shear Stress on a Rotating Disk

One of the objectives of this thesis was to correlate biofilm thickness to shear stress; thus, an experimental system was needed in which the shear stress could be calculated precisely. A reactor in which the disk revolves in a very tight housing under laminar flow conditions meets this condition.

For laminar flow the Reynolds number, Re , has to be smaller than 10^5 . The Reynolds number can be calculated in the following manner:

$$Re = R^2 \omega / \nu \quad (6)$$

where R is the radius of the disk, θ the radial velocity and ν the kinematic viscosity.

The width of the gap between the rotating disk and the housing, s , has to be smaller than the boundary layer, b . The boundary layer thickness can be calculated from velocity and continuity equations for a disk in an infinite water bath.

We state that all velocities are independent of the angular coordinate, θ , for reasons of symmetry. The following velocity equations and continuity equations then hold true (von Kàrmàn, 1921):

$$v_r \frac{\delta v_r}{\delta r} + v_z \frac{\delta v_x}{\delta x} - \frac{v_r^2}{r} = -\frac{1}{\rho} \frac{\delta p}{\delta r} + \nu \left(\frac{\delta^2 v_r}{\delta r^2} + \frac{\delta}{\delta r} \left(\frac{v_r}{r} \right) + \frac{\delta^2 v_r}{\delta x^2} \right) \quad (7)$$

$$v_r \frac{\delta v_t}{\delta r} + v_x \frac{\delta v_x}{\delta x} - \frac{2v_t v_r}{r} = \nu \left(\frac{\delta^2 v_t}{\delta r^2} + \frac{3\delta}{r} \frac{v_t}{\delta r} + \frac{\delta^2 v_t}{\delta x^2} \right) \quad (8)$$

$$v_r \frac{\delta v_x}{\delta r} + v_x \frac{\delta v_x}{\delta x} = -\frac{1}{\rho} \frac{\delta p}{\delta x} + \nu \left(\frac{\delta^2 v_x}{\delta r^2} + \frac{1}{r} \frac{\delta v_x}{\delta r} + \frac{\delta^2 v_x}{\delta x^2} \right) \quad (9)$$

$$\frac{\delta V_r}{\delta r} + \frac{V_r}{r} + \frac{\delta V_z}{\delta x} = 0 \quad (10)$$

The system can be solved with the following functions:

$$v_r = r f(x) \quad v_t = r g(x) \quad v_x = h(x) \quad p = p(x) \quad (11)$$

Thus, equations (7) through (10) become equations (12) through (15), respectively.

$$f^2 - g^2 + h \, df/dx = \nu \, d^2f/dx^2 \quad (12)$$

$$2fg + h \, dg/dx = \nu \, d^2g/dx^2 \quad (13)$$

$$dh/dx + 2f = 0 \quad (14)$$

$$h \, dh/dx = -1/\rho \, dp/dx - \nu \, d^2h/dx^2 \quad (15)$$

Boundary conditions for the numerical integration are:

$$f(0) = 0 \quad f(\infty) = 0 \quad g(0) = 0 \quad g(\infty) = 0 \quad h(0) = 0$$

After integration the thickness of the boundary layer can be found to be:

$$b = 2.58 \sqrt{(\nu/\omega)} \quad (16)$$

If the gap between the disk and the housing is smaller than the boundary layer, the variation of the tangential velocity across the gap becomes linear in the manner of Couette-flow. Couette-flow is defined as flow between two parallel walls, one of which is at rest, the other moving along its own plane with a constant velocity.

The equation for the shear stress (17) can be solved exactly.

$$\sigma = \mu \, dv/dh \quad (17)$$

with the following boundary equations:

$$\text{for } s = 0 : v = 0$$

$$\text{for } h = s : v = r \, \omega$$

$$\text{so: } \sigma = \mu \, r \, \omega \, s^{-1} \quad (18)$$

The shear stress is a linear function of the radius. A linear velocity profile develops (Figure 2).

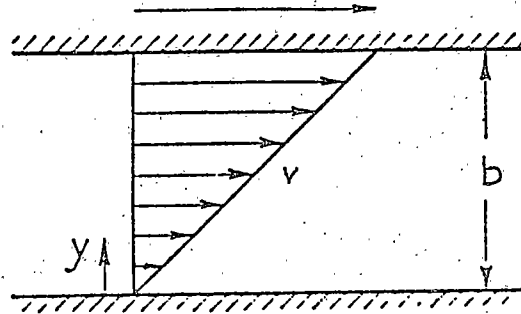


Figure 2. Velocity profile for Couette-flow (Schlichting, 1960).

Fiber Optic Sensors in Biofilm Research

A literature search was performed to find useful information in previous applications of fiber optic sensing systems for the intended research. Fiber optic sensor technology is a young field; thus only one very recent published book (Krohn, 1988) could be found which encompasses mainly sensor technology. Personick (1985) writes in some detail about sensing systems. A broad range of specialized applications for fiber optic and laser sensors can be found in the proceedings of the meeting of "The International Society for Optical Engineering" (De Paula and Udd, eds., 1987). Especially interesting was one paper by Zhong and Li (1987), which describes the use of an optical fiber sensor for dust concentration measurements. Here absorption of light following the Lambert-Beer Law is used effectively to measure particle concentration. This is similar to our intention to measure the thickness of biofilm.

Two examples for absorption measurements in biofilms were found in the literature. Pedersen (1982a) used the absorbance of stained biofilm to measure biofilm thickness. However, he used a spectrophotometer to measure the absorbance of stained biofilm rather than sensors. The absorbance of biofilm was successfully used to determine its thickness. Jørgensen (1989) measured light penetration and absorption in bacterial mats. He employed a fiber optic microprobe to detect radiance gradients in the bacterial mat. By using the fiber optic microprobe it was possible to measure the spectral quantum flux within small clusters of cells. Quantum flux is the most important light parameter for microbial photosynthesis.

Sensing systems detect physical or chemical conditions and pass this information in suitable form to an operator or to another system or device. A sensing device is composed of four essential components: one or more sensors, processors, an output device and communication links.

A sensor is an input device that transduces a physical or chemical parameter into an electrical or optical signal. A processor converts sensor information into a form suitable for output. An output device presents the processed information to a user, to an actuator or to another system. A communication link provides a path for the transmission of an electrical or optical signal from the sensor to the processor or from the processor to an output device. Typically the path is a wire or optical fiber. The fiber optic sensing device used in this research is assembled in an analogous manner (Figure 3).

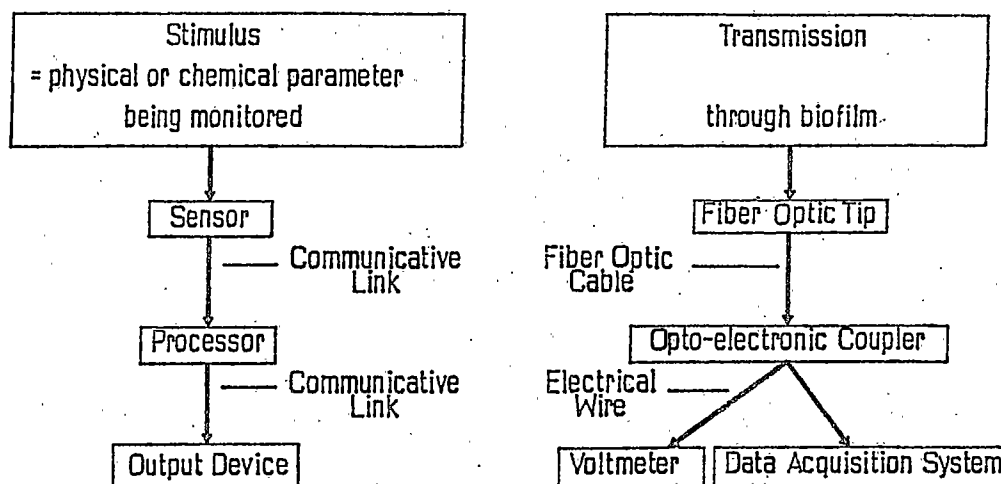


Figure 3. A sensing device in general form and in the form used for the fiber optic sensor.

The sensing device measured light-intensity changes caused by the biofilm. It therefore can be called an intensity-modulated sensor. Intensity-modulated sensors are defined in Krohn (1988) as sensors that detect variations in light intensity. Light intensity variations can be associated with the perturbing environment, i.e., transmission and reflection in biofilm (Figure 4). Intensity-modulated sensors are generally analog devices.

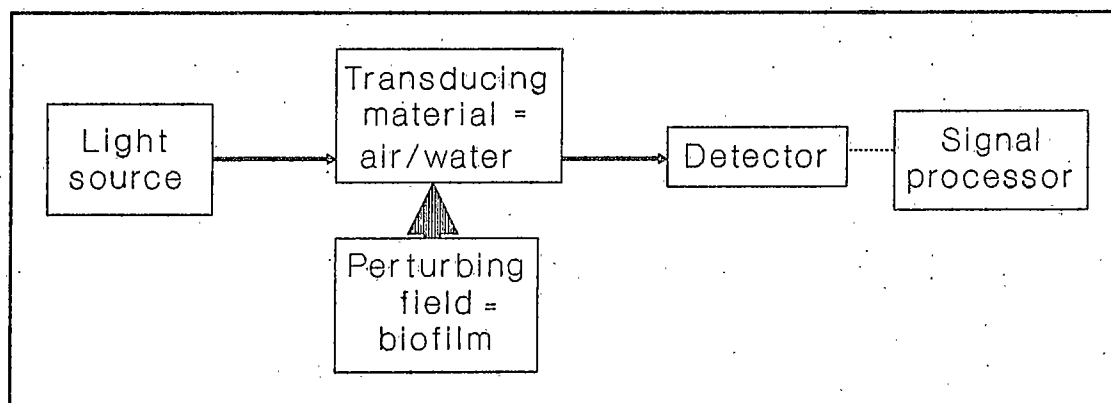


Figure 4: Schematic of an intensity-modulated sensing device.

The light intensity modulation can be described qualitatively by the laws of absorption. Lambert's Law states that the rate of change of light intensity in passing through a homogeneous medium is proportional to the light intensity at any point within that medium, or:

$$dI/dx = -e I, \quad (19)$$

where I = light intensity

e = extinction coefficient

x = distance

Upon integration, this equation yields:

$$\ln(I/I_0) = -e * d \quad (20)$$

where I_0 is the light intensity at the point where the distance, d , equals 0. Thus, if the logarithm of light intensity is plotted as a function of distance through a homogeneous medium, a line of constant slope " e " would result. Where the extinction coefficient changes abruptly from one value to another, an abrupt change in the slope of the line would be expected. It is this phenomenon which is to be used to detect the biofilm-water interface. Biofilm has a high extinction coefficient owing to the presence of a variety of light absorbing organic compounds, while the bulk water has a very low extinction coefficient. As the sensor is moved through the bulk water toward the light source, a line of very low slope would be presumed. As the sensor moves through the biofilm, a line of much steeper slope results, corresponding to the extinction coefficient of the biofilm. At the interface between these two media, a distinct break in the slope of the line would be expected.

In a solution, the absorption depends upon the concentration and thickness of the layer traversed. Unit layer and unit concentration absorb equal light as a layer twice

as thick but with half the concentration. Calling the absorption coefficient of unit concentration ϵ , the thickness d and the concentration c , we have:

$$I = I_0 * e^{-\epsilon cd} \quad (\text{Beer's Law}), \text{ or} \quad (21)$$

$$\ln(I_0/I) = \epsilon cd \quad (22)$$

The $\ln(I_0/I)$ is called absorbance. Beer's Law states that absorbance is linearly proportional to concentration. Thus, a relationship between the biofilm density and the slope of the absorbance curve is expected.

The changes in light intensity, caused by absorption, were registered with a fiber optic sensor. Extrinsic and intrinsic capabilities of the sensor were used to determine biofilm thickness. Extrinsic fiber optic sensors use the optical fiber as a transmission line to convey modulation of light intensity. In intrinsic sensors the optical fiber changes physically in response to the physical or chemical parameter being monitored. A physical change of the fiber is bending of the fiber, which occurs as a response to touching of the substratum surface. The optical principles of refraction and reflection describe the detection of light with a fiber optic sensor.

Refraction occurs when light passes from one homogenous isotropic medium to another. In our case the two media considered are air and the glass of the optic cable. The light ray will bend at the interface between the two media. The mathematic expression that describes the refraction phenomena is known as Snell's Law, which can be derived from Maxwell's Equations.

$$n_0 \sin \alpha_0 = n_1 \sin \alpha_1 \quad (\text{Snell's Law}) \quad (23)$$

where n_0 = the index of refraction of the medium in which the light is initially travelling

n_1 = the index of refraction of the second medium

α_0 = the angle between incident ray and the normal to the interface

$\alpha_1 =$ the angle between refracted ray and the normal to the interface

In the case of light passing from a high index medium to a low index medium refraction is occurring, but a certain portion of the incident ray is reflected.

The transmission of the light ray through the fiber optic cable is explained by total internal reflection. If the incident ray hits the boundary at ever increasing angles, a value of $\alpha_0 = \alpha_c$ will be reached at which no refraction will occur. The angle, α_c , is called the critical angle. The refracted ray propagates along the interface, not penetrating the lower index medium. So $\alpha_1 = 90^\circ$ and therefore, $\sin \alpha_c = n_1/n_0$. For incident angles greater than the critical angle the ray is entirely reflected at the interface and no refraction takes place. This phenomena is known as total internal reflection.

In Figure 5 the refraction of the ray can be seen as it enters a flat-ended fiber cable. Total internal reflection can be observed as the ray propagates along the cylinder of the cable.

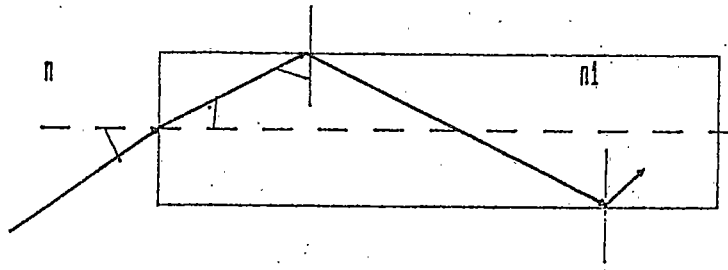


Figure 5. Path of a ray through a fiber optic cable.

The fiber bends when it touches the surface of the substratum. This can be detected, because the ray propagation is disturbed in a curved fiber. For a straight fiber, the angle between the light ray and the normal to the plane of reflection is defined by the angle ϕ . However, when the fiber is bent, the plane of reflection and the reflective angle

rotate by the angle δ (Figure 6). Therefore, for a curved fiber, the angle between the reflected and the tangent at the reflection point is $\phi - \delta$. In a straight fiber, for $\phi > \phi_c$, the rays will be totally internally reflected. In a bent fiber the effective critical angle is reduced by δ . Therefore, rays incident between ϕ_c and $\phi_c - \delta$ will be lost. The effective critical angle is reduced in a bent fiber, and the amount of light that can be detected at the end of the fiber is reduced.

A second mechanism adds to decrease the amount of transmitted light further. In this study the light source was in direct line with the sensor. When the sensor is moved into a hard surface, it not only starts bending, but the sensor tip will also move sideways. Thus, the sensor is not pointed in the direction of the light source anymore and the possible light uptake is decreased. Both effects are responsible for a decrease in registered light intensity.

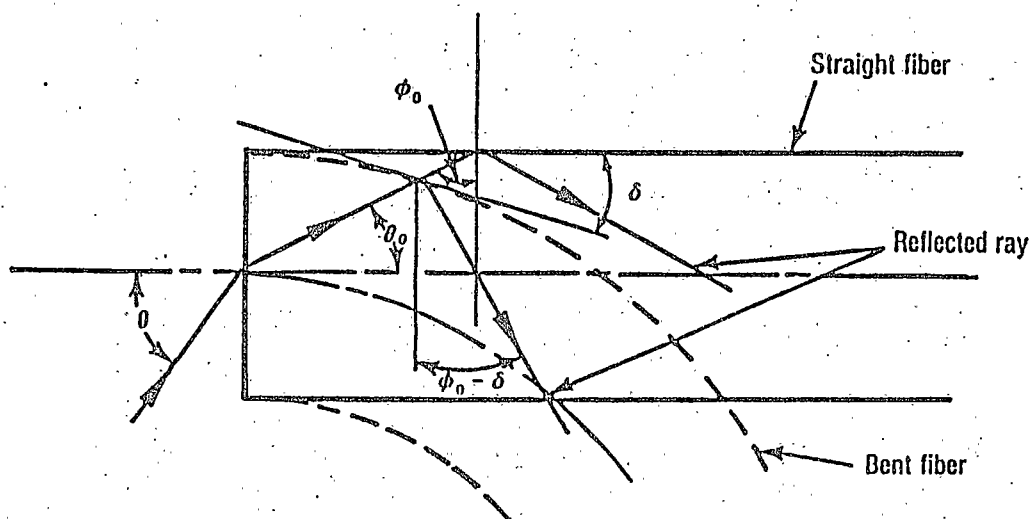


Figure 6. Bending effects on critical angle (Krohn, 1988).

Ambient light was prevented from interfering with the measurements through frequency modulation. The light source was frequency modulated with a frequency of

1000 Hz. Modulation is a systematic alteration of the wave which carries the message. An illustration of frequency modulation can be seen in Figure 7. In analog modulation the modulated parameter varies in direct proportion to the modulating signal. Modulation is a reversible process, so the message can be retrieved at the receiver by the complementary operation of demodulation, where unmodulated light signals are discarded. Thus, ambient light is filtered out.

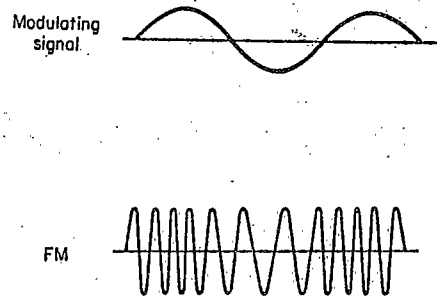


Figure 7. Frequency modulation with a sinusoidal modulating wave. The detector and the light source are modulated at the same frequency, eliminating the effects of ambient light on the measurement.

The optical signal produced with the fiber optic sensor had to be converted to an electrical signal to allow easy recording of the measured data. The device which converts the optical signal into a voltage or current is called the optical detector. A detector with its interfacing electronic circuit is called a receiver. In this experiment, the light intensity is converted into a voltage signal.

The approach to implementing an optical detector is to allow the incident power to illuminate a semiconductor device, resulting in the generation of hole-electron pairs by

absorbed photons. These pairs can in turn flow in the presence of an electric field to produce an observable current. The current can be measured, or else the corresponding potential.

MATERIALS AND METHODS

The experimental setup and the procedures used for the research are described in the following sections.

The Rotating Disk Reactor

A mixed population biofilm was grown in a rotating disk reactor (Figure 8). The reactor vessel was a square container made out of transparent polycarbonate with a side length of 70 cm and a reactor height of 20 cm. A disk with a radius of 30 cm rotated in the reactor. Radial grooves were cut into the lower side of the disk, where transparent polycarbonate slides could be inserted. The disk rotated at a distance, s , of 2 mm from the bottom of the vessel and was submerged 5 cm. An electrical motor was used to turn the disk at a rotational speed, ω , of 1.05 s^{-1} . An electric thermostat held the water temperature constant at 20°C , resulting in an absolute viscosity of $0.01 \text{ g cm}^{-1} \text{ sec}^{-1}$ and a kinematic viscosity of $1.01 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$. The Reynolds number for this condition is 9×10^4 , which indicates laminar flow and a boundary layer thickness of 2.5 mm. Thus, a linear velocity distribution existed in the reactor; the conditions for laminar Couette-flow were met, and the shear stress could be calculated.

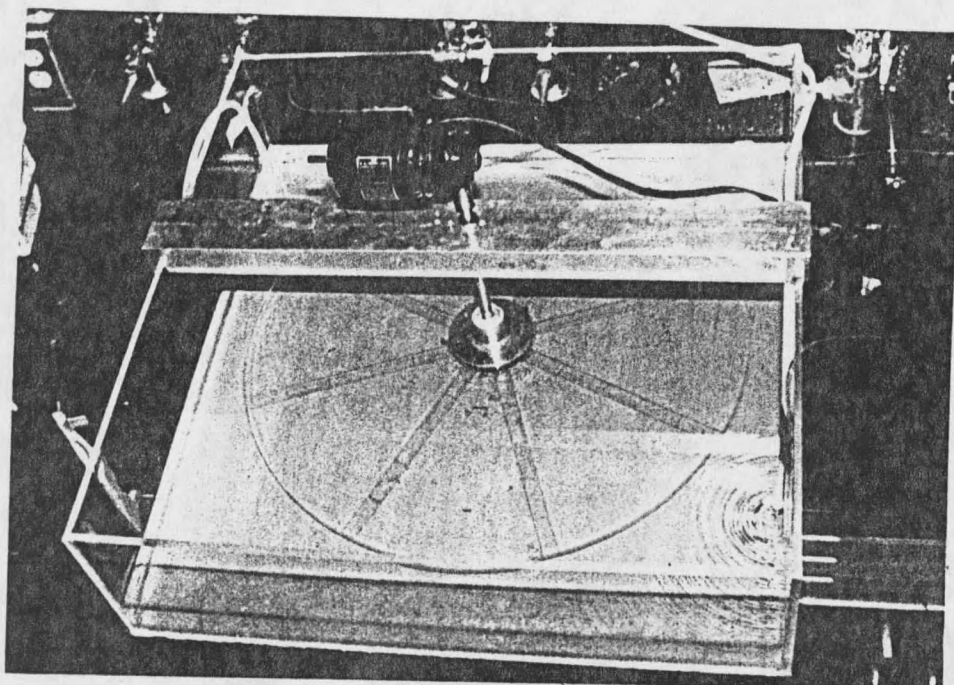


Figure 8. Photograph of the rotating disk reactor.

A flow rate 1 l min^{-1} of tap water through the reactor resulted in a residence time of 20 minutes. A mixed population of bacteria was grown as biofilm on the disk and the short residence time ensured minimal growth in the bulk water. The feed solution contained 20 mg l^{-1} glucose, 7 mg l^{-1} ammonium chloride and trace elements given in 0.25 mg l^{-1} yeast extract dissolved in tap water. The reactor was run for three weeks without the slides, until a robust biofilm culture was established in the reactor. After the initial growth period the slides were inserted. The reactor was stopped and slides removed four days and seven days after insertion so thickness measurements could be performed.

The Fiber Optic Sensing Device

The fiber optic sensing device was developed to detect changes in light intensity. A red light diode with a wave length of 660 nm and an infrared light diode with a wave length of 920 nm were the two primary light sources, modulated at 1000 Hz. The sensor and the following communicative link were one integrated fiber optic cable, glass fiber 125/85, United Detector Technology. One inch of the fiber was mechanically exposed and, depending on the application, tapered or cut to obtain a flat, defined surface. The shape of the tip defined the maximum intensity of light which is taken up. The light was transmitted to an optic-electronic coupler, where the light signal was transformed into an electrical signal. The voltage output was proportional to the incoming light intensity. The detector was a Standard High Responsivity Photodetector, PIN-HR020, United Detector Technology. A custom made device compared the incoming signal to the modulation of the light source. Only those parts of the incoming light in the same modulation interval as the light source were transmitted to the output device. This ensured independence of the measurement from ambient light. Two output devices were used. The first one was a volt meter to observe a digital output during the measurement. The second was a commercial available data acquisition system which recorded and stored the data automatically on disk. The final setup of the measurement device is shown in Figure 9.

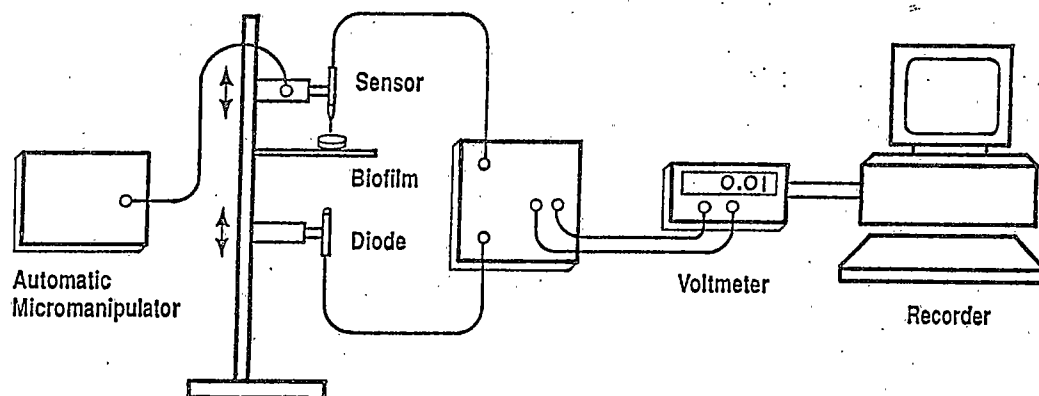


Figure 9. Schematic diagram of measurement device setup.

The sensor was mounted to an automatic micromanipulator. The micromanipulator allowed motorized movements in three dimensions. Continuous movements, step by step movements or single steps, started by hand were possible, and step length, speed, and step interval can be set by hand.

In these experiments, step by step movement in the vertical direction was used. The micromanipulator moved the sensor downward, so it would slowly penetrate the biofilm from above. The micromanipulator was calibrated for different settings (Appendix A). Through use of the automatic micromanipulator and the data acquisition system, a full measurement could be performed without direct operator control.

The exposed tip of a fiber optic cable was used as a sensor. The tip could be tapered to obtain a needle formed tip with a tip diameter of $10\ \mu\text{m}$. Before tapering the sensor, the protective isolation was removed with a razor blade. Hydrofluoric acid (HF) was used to taper the sensor. A syringe tip cover was put upright in a rubber stopper,

and partially filled with HF. The sensor was placed in a holder, and inserted about 5 mm into the acid. Tapering occurs automatically with time when the acid evaporates. After 30 minutes all the acid should be evaporated, and the sensor can be carefully removed. The sensor tip then must be checked under the microscope. Usually an evenly shaped tip can be seen (Figure 10).

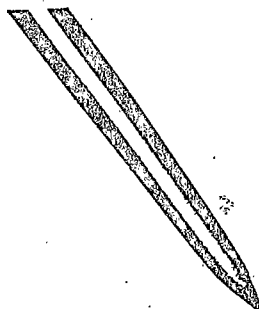


Figure 10. Enlarged picture of the fiber optic sensor tip.

Procedures

The locations of the biofilm-water interface and the biofilm-substratum interface were found through light intensity measurements while moving the sensor through the film. A slide with biofilm was taken out of the reactor and put in the measurement device, mounted above the light source. The sensor was lowered through the bulk water above the biofilm into the biofilm until the sensor touched the slide. The light transmission was recorded for every step of the sensor. The difference in the extinction coefficients of biofilm and the overlying bulk water was used to find the biofilm-water interface, through an abrupt change in the slope of the light intensity versus distance curve. When the sensor touches the surface of the slide, it starts bending, and the light transmission

through the sensor decreases abruptly. The data acquisition system records the time between the beginning and the end of the measurement. The distance is related to the time through the speed, step length and frequency of movement of the step motor. Finally, a curve of intensity versus distance could be prepared.

From the same measurement, the extinction coefficient can be calculated by using Lambert's Law and graphing $\ln(I_0/I)$ versus the distance which the sensor travelled. The extinction coefficient can be found as the slope of the curve.

The biofilm density can be calculated from the extinction coefficient, if the absorption coefficient of biofilm is known. Combining equations (20) and (22) yields:

$$e = \epsilon * c, \quad (24)$$

It can be seen that the extinction coefficient, e , divided by the absorption coefficient gives the biofilm density. The absorption coefficient was determined from representative samples of biofilm from the experimental system. A sample of biofilm was homogenized and different dilutions of the mixture were prepared. The absorbance of the diluted samples was measured with a Varian DMS 90 UV-VISIBLE Spectrophotometer at a wavelength of 660 nm and a band width of 4 nm. The cell constant, d , of the spectrometer is 1 cm. After measuring the absorbance of each sample, the dry weight per ml homogenized biofilm was determined. This is defined as biofilm density or, using the notation of Beer's Law, a concentration of mass per unit volume. Thus, density or concentration, c , times cell constant, d , could be graphed versus absorbance. A regression could be done, where the slope of the regression line gives the absorption coefficient, ϵ .

A volumetric density measurement was used to obtain independent density values for a comparison. The biofilm thickness was measured with the fiber optic sensor, and

the area was determined with a ruler. Next the dry weight was taken. The procedure to measure the dry weight followed the standard method no. 2540, "Total, Fixed and Volatile Solids in Solid and Semisolid Samples" (Clesceri et al., 1989). The average biofilm density was determined as:

$$\rho_F = \text{dry weight} / (\text{film thickness} * \text{area}) \quad (25)$$

This value was compared to a mean density, obtained from six fiber optic measurements on the same biofilm.

RESULTS

In the following sections, results of the research are presented consistent with the objectives stated earlier. The biofilm-water interface was located with the sensor, as was the biofilm-substratum interface, enabling determination of biofilm thickness. Biofilm absorption coefficients were obtained, and these were used to approximate biofilm density. Finally, relationships between density, thickness and shear stress are presented.

Location of the Biofilm-Water Interface

All biofilms investigated showed absorption of infrared and visible light. Light absorption by water was negligible compared to absorption by the cell mass. Thus, a delineation between biofilm and water was possible using optical methods (Figures 11 through 13). The direction of the sensor movement is shown on the graphs. The distance the sensor travels is displayed on the x-axis as distance relative to the substratum. On the y-axis the relative light intensity is graphed in millivolts, a measurement unit which is directly proportional to the light intensity and was given by the detector. The light intensity increases as the sensor enters the biofilm and moves through the film. The biofilm-water interface is marked on the graphs where the light intensity starts to increase. The first objective to find the biofilm interface could thus be fulfilled.

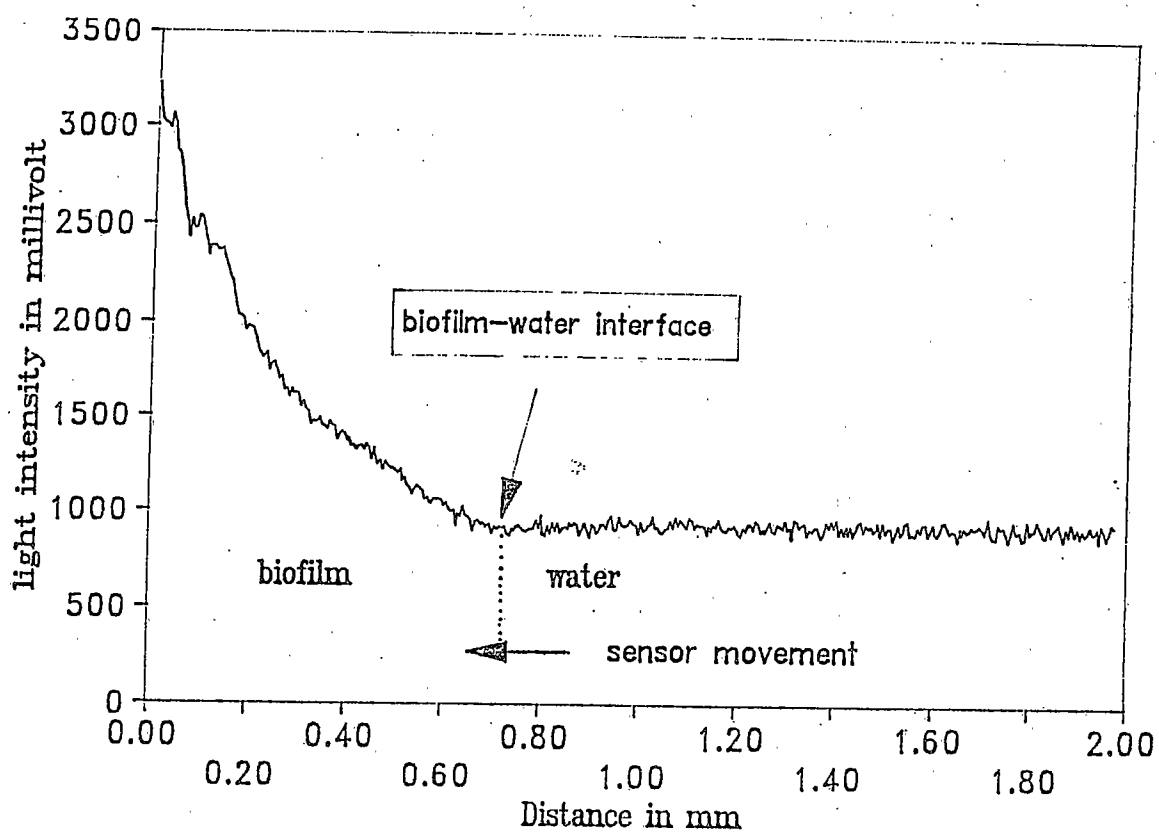


Figure 11. The variation of light intensity with distance from the substratum. The rapid change in slope represents the biofilm-water interface. The data are representative for a dense biofilm with thickness of $700\text{ }\mu\text{m}$.

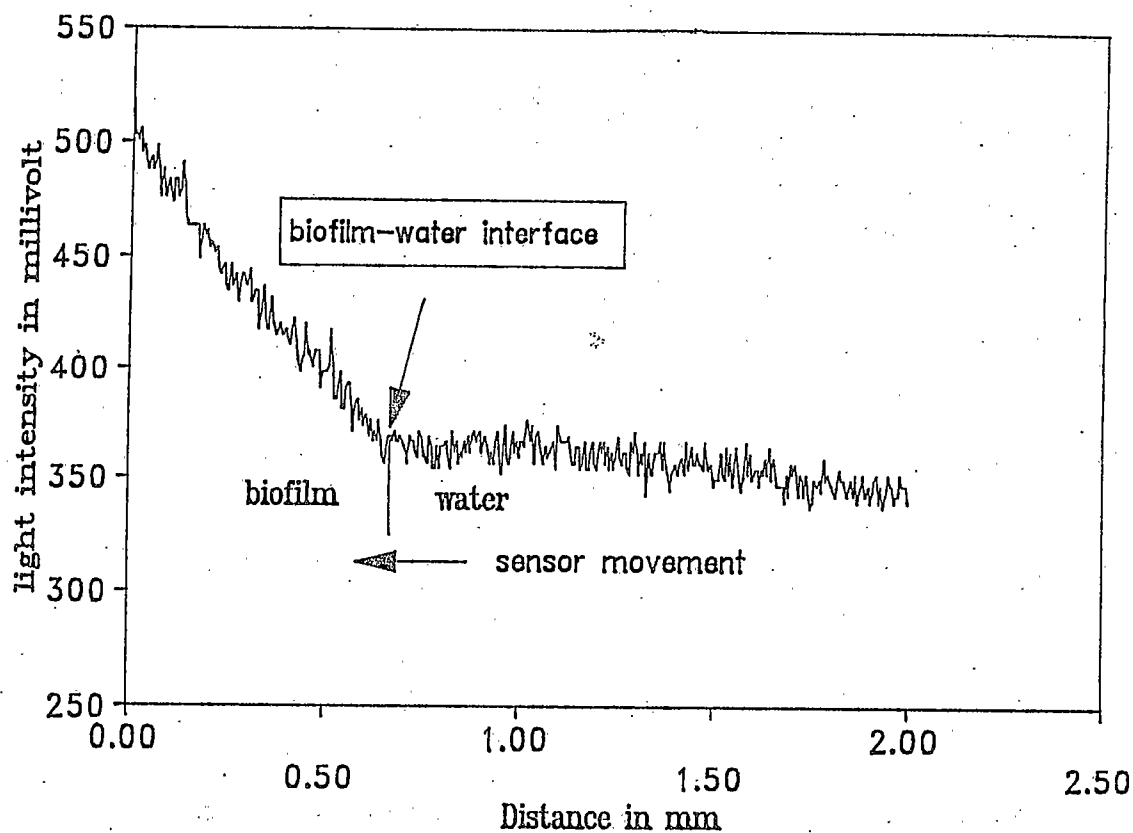


Figure 12. The variation of light intensity with distance from the substratum. The rapid change in slope represents the biofilm-water interface. The data are representative for a medium dense biofilm with thickness of 700 μm .

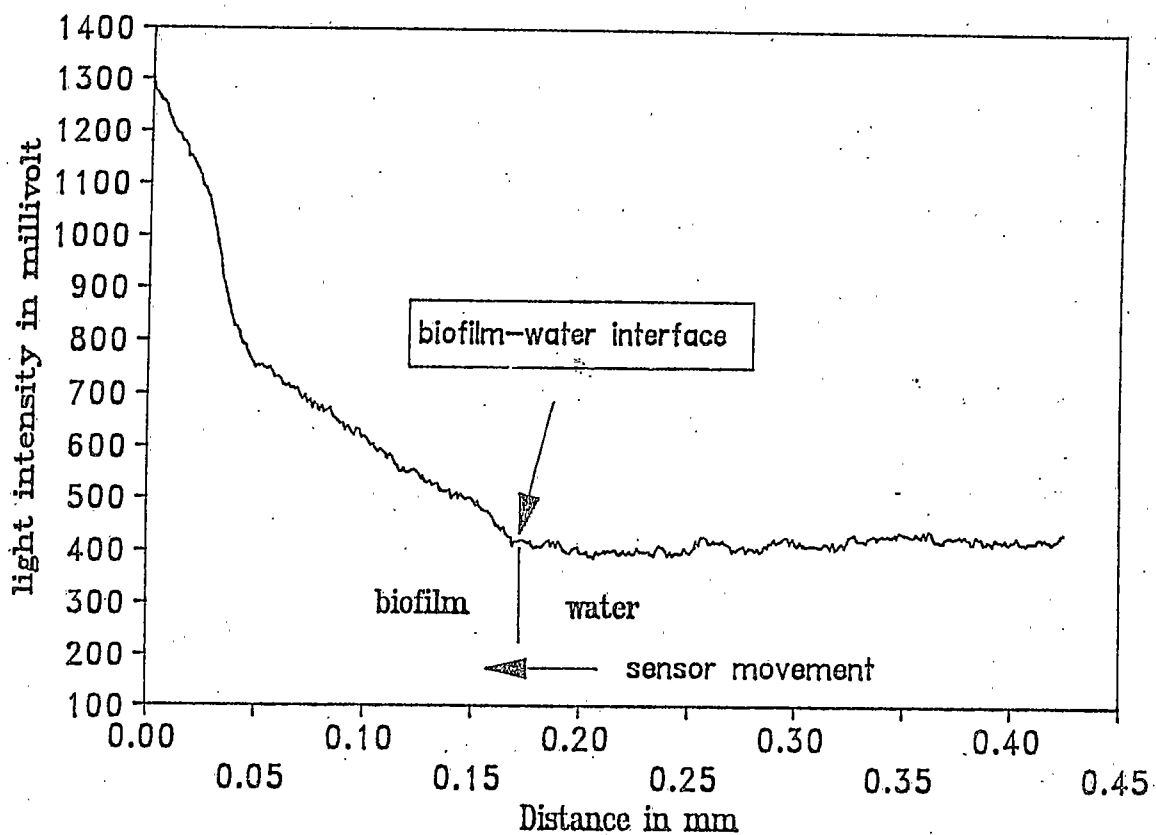


Figure 13. The variation of light intensity with distance from the substratum. The rapid change in slope represents the biofilm-water interface. The data are representative for a biofilm with a fluffy surface film and an overall thickness of $170\text{ }\mu\text{m}$.

Biofilm Thickness

Biofilm thickness was determined by locating the biofilm-water and the biofilm-substratum interfaces. The biofilm-water interface was found as shown above, and the biofilm-substratum interface was indicated when the sensor touched the substratum. This was manifested as a decrease in light intensity, due to the bending of the fiber. The distance between the biofilm-water interface and the biofilm-substratum interface was the biofilm thickness (Figure 14).

Biofilms which had a distinctly different extinction coefficient for base and surface film were also investigated. The three different slopes in Figure 15 represent three different extinction coefficients for base film, with the highest extinction coefficient, surface film, with a lower extinction coefficient, and water with an extinction coefficient close to zero. Base and surface film thickness can be read from the graph.

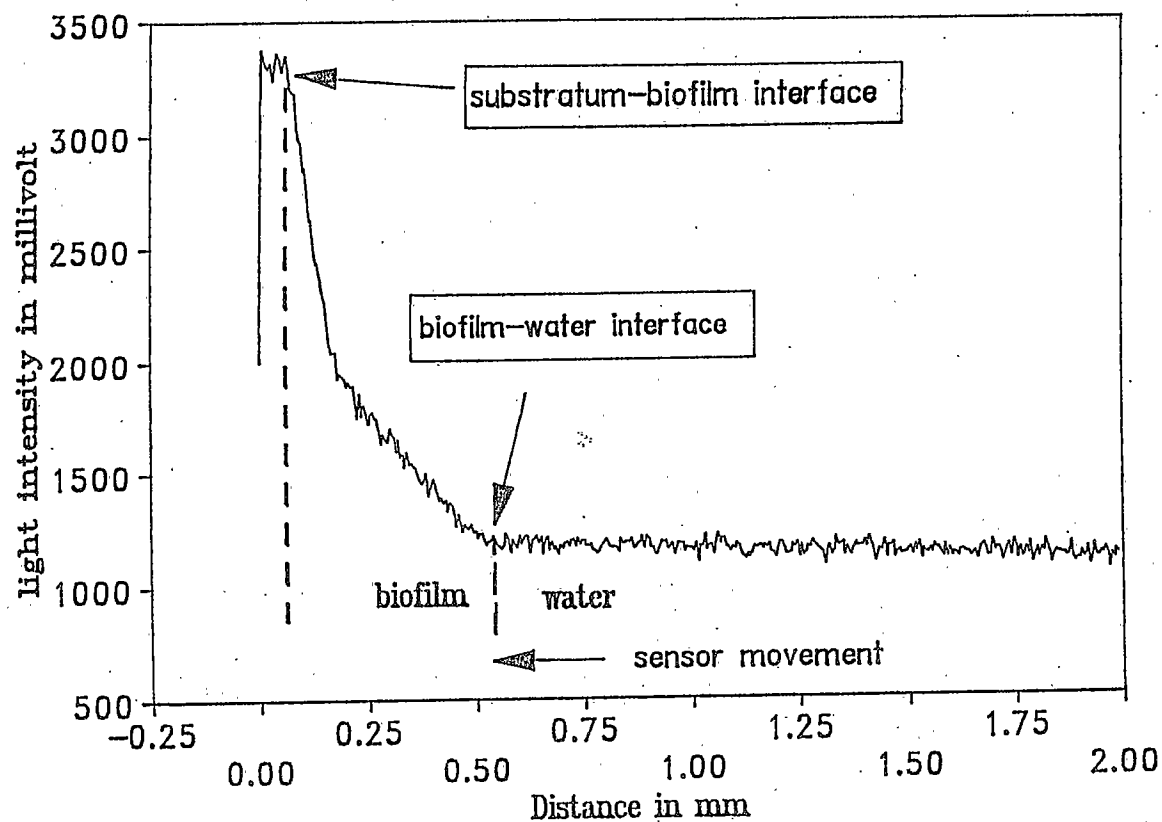


Figure 14. The variation in light intensity with distance from the substratum. The increase in light intensity marks the biofilm-water interface, while the rigid decrease marks the biofilm-substratum interface.

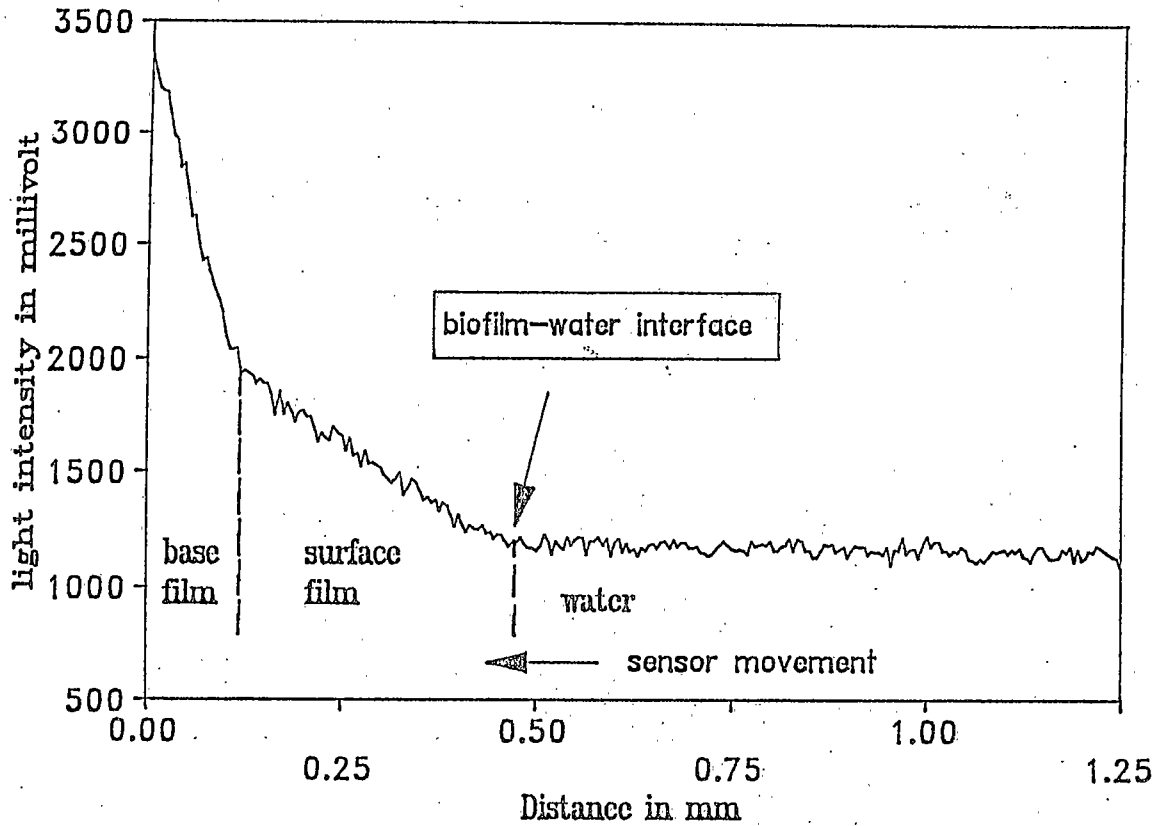


Figure 15. The variation in light intensity with distance from the substratum. The first increase in light intensity marks the biofilm-water interface, while the second marks the transition from surface film to base film. The location where the sensor touches the substratum is used as a zero reference for distance.

Biofilm Density

The absorbance of homogenized biofilm samples was measured with the spectrophotometer, and plotted as a function of the film dry mass density (Figure 16).

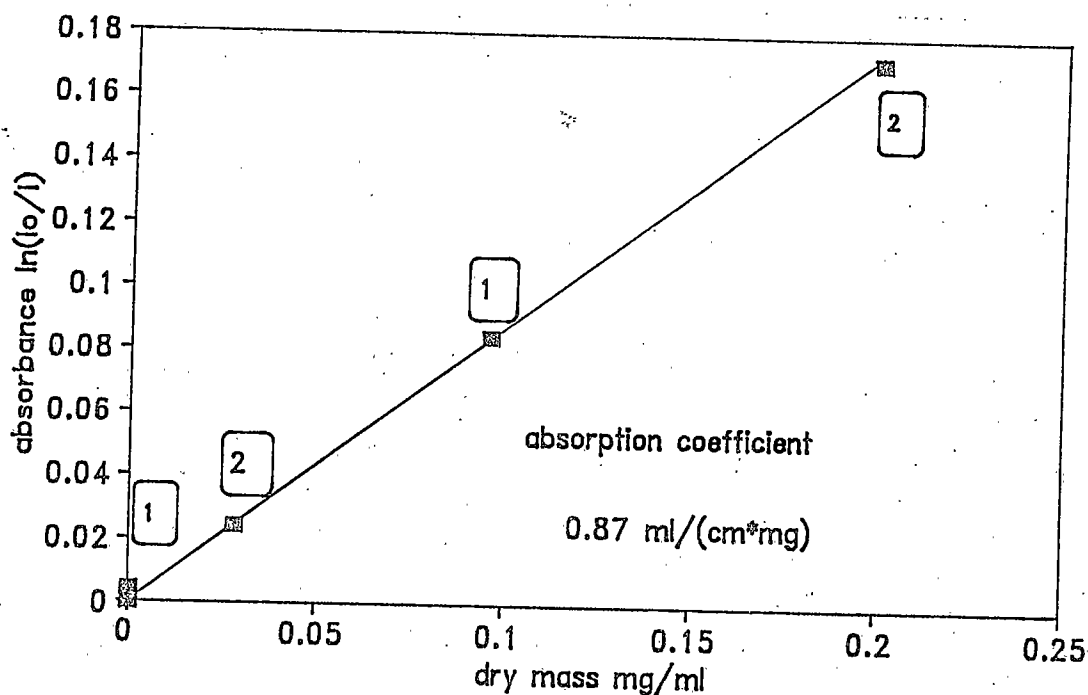


Figure 16. Absorbance of homogenized biofilm versus biomass concentration ("biofilm density") measured with a spectrophotometer at 660 nm.

Absorbance appears to be directly proportional to biomass concentration (density). Assuming that biomass absorbs light according to Beer's Law, the absorption coefficient was determined to be $0.87 \text{ ml}/(\text{cm} \cdot \text{mg})$ or $8.7 \text{ m}^2 \text{ kg}^{-1}$. Data were obtained both from high

shear stress regions (marked with "2") and low shear stress regions (marked with "1"). Though using biofilm from two different locations, the results indicate no difference in absorption coefficient as a function of shear stress. Thus, shear stress does not influence the absorption coefficient.

The thickness and the density of the biofilm were measured with the fiber optic sensor on randomly selected sites in a 2.4 cm * 1.5 cm large area of biofilm. The measurements can be seen in the appendix. Figure 17 shows one of the measurements. The absorbance, $\ln(I_0/I_1)$ is graphed as the y-axis. Following Beer's Law, the slope of the graph is now related to the concentration or biofilm density. The biofilm thickness can be read from the graph; the mean slope was obtained by fitting a regression line through the data points which indicated the biofilm. Two independent series of measurements were completed (Table 2).

Table 2: Comparison of volumetric measured density with optically measured density.

	sample A	sample B
# of measured profiles	6	4
average slope (cm^{-1})	8.8	11.1
absorption coefficient ($\text{ml cm}^{-1} \text{mg}^{-1}$)	0.87	0.87
optically estimated density (mg ml^{-1})	10.1	12
average thickness (mm)	0.29	0.33
volume (cm^3)	0.10	0.12
dry mass (mg)	0.78	0.82
volumetric estimated density (mg ml^{-1})	7.8	7.0

The optical measured densities were 2.3 mg ml^{-1} and 5 mg ml^{-1} higher than the volumetric determined densities, a deviation of 23% and 40%, respectively.

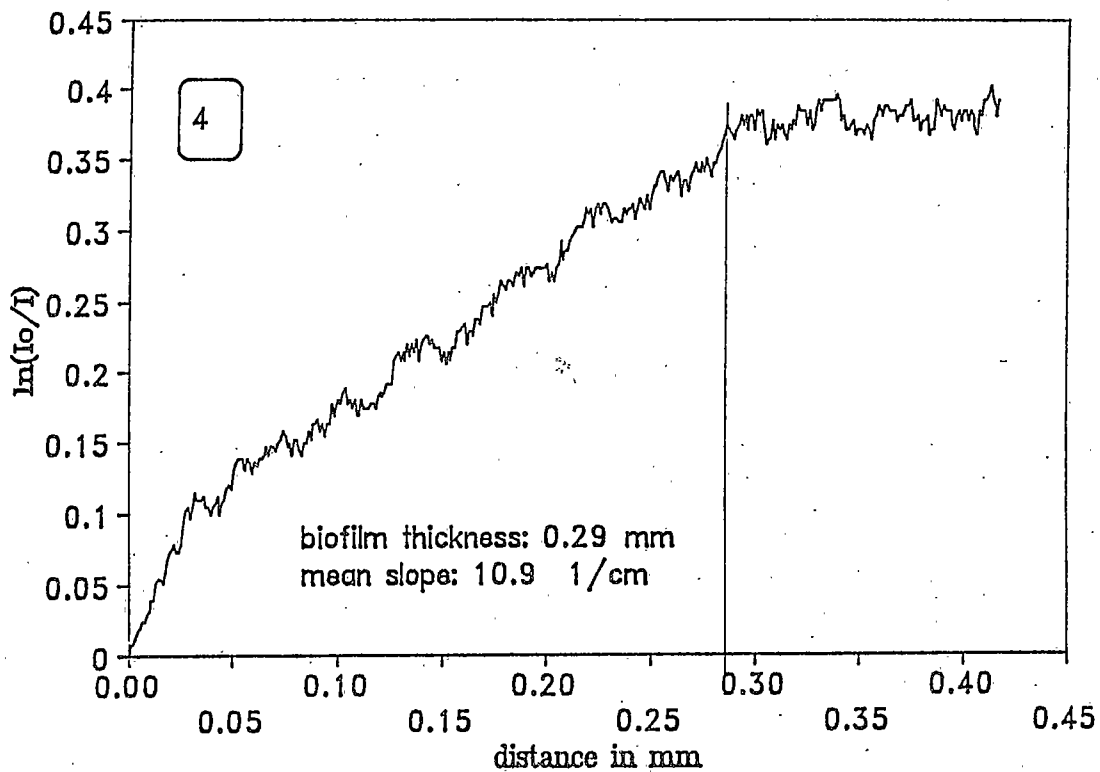


Figure 17. Absorbance in a biofilm versus distance. The measurement is representative for the measurements conducted to determine the biofilm density. The biofilm thickness was determined and the slope of the regression line was calculated using the points which indicate the biofilm.

Biofilm Thickness and Extinction
Coefficient versus Shear Stress

Transmission measurements were conducted to obtain the biofilm thickness as a function of shear stress. For each measured radial location on the disk (corresponding to one defined shear stress), three measurements were performed and averaged. The measurements were conducted on a 4-day-old biofilm (Figure 18). The error bars give the standard deviation of the measurements. The flow velocity is plotted in the graph as a second x-axis. This permits a comparison with the findings from literature, which all were given as biofilm thickness versus flow velocity. It can be seen that the biofilm thickness varies around a constant value for a shear stress smaller than 0.07 N m^{-2} . The biofilm thickness reaches a maximum at a shear stress of approximately 0.08 N m^{-2} and decreases again for higher shear stress. The same pattern can be seen in a measurement of a 7-day-old biofilm (Figure 19), where biofilm thickness increases until it reaches a maximum at a shear stress of approximately 0.08 N m^{-2} , and subsequently decreases as shear stress exceeds 0.08 N m^{-2} . The maximum biofilm thickness is also higher for 7-day-old biofilm.

Another measurement series was performed for a higher flow velocity. Shear stress could not be calculated, since the conditions for laminar flow were no longer met. However, it was of interest to investigate biofilm thickness for higher flow velocities. The results confirm a decrease in biofilm thickness for higher velocities (Figure 20).

The extinction coefficient was also calculated for the measurements on 7-day-old biofilm grown under laminar flow conditions (Figure 21). Employing the relation that the

extinction coefficient equals the absorption coefficient times the biofilm density, a mean biofilm density was calculated using the absorption coefficient from earlier spectrophotometer measurements. The mean density of the biofilm was determined to be 34 mg ml^{-1} .

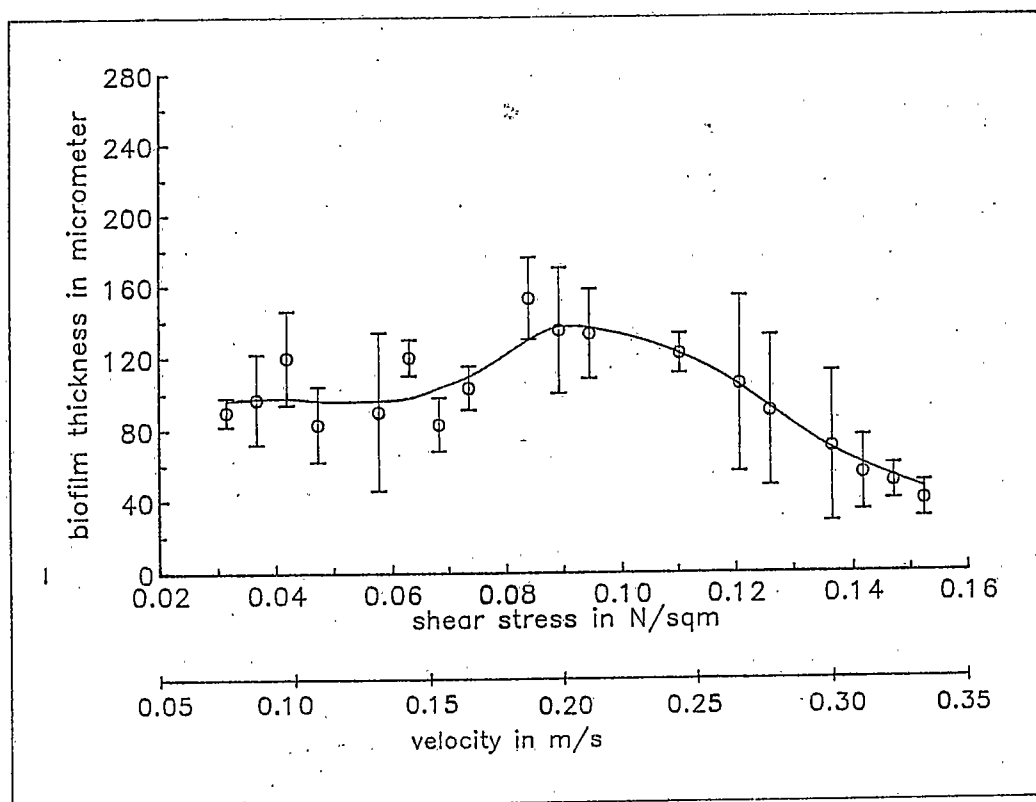


Figure 18. Biofilm thickness versus shear stress/velocity for a 4-day-old biofilm.

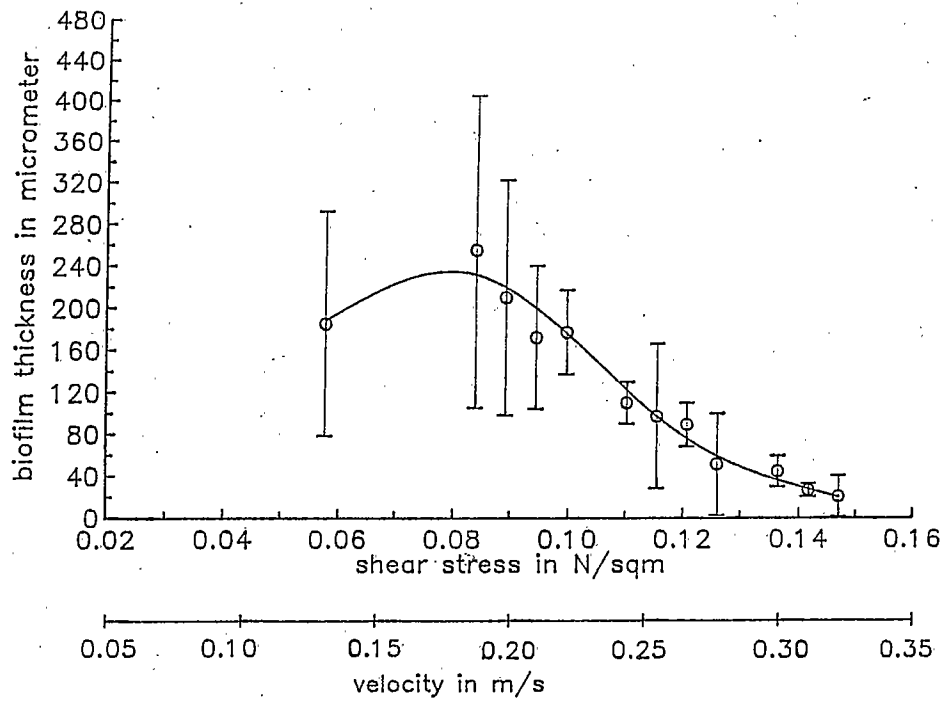


Figure 19. Biofilm thickness versus shear stress/velocity for a 7-day-old biofilm.

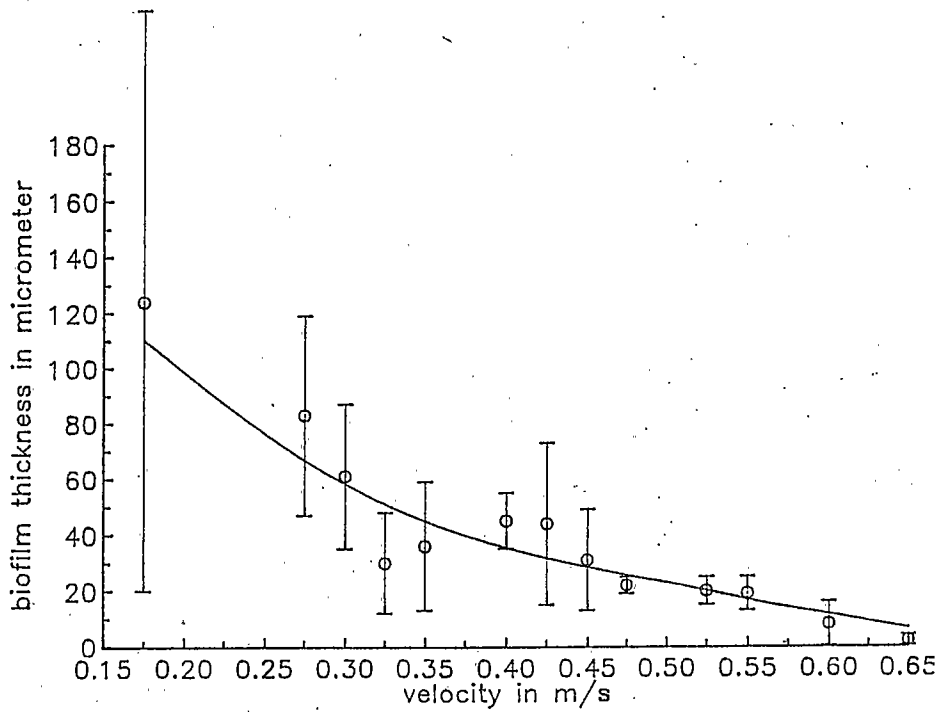


Figure 20. Biofilm thickness versus flow velocity for a biofilm grown under non-laminar flow conditions.

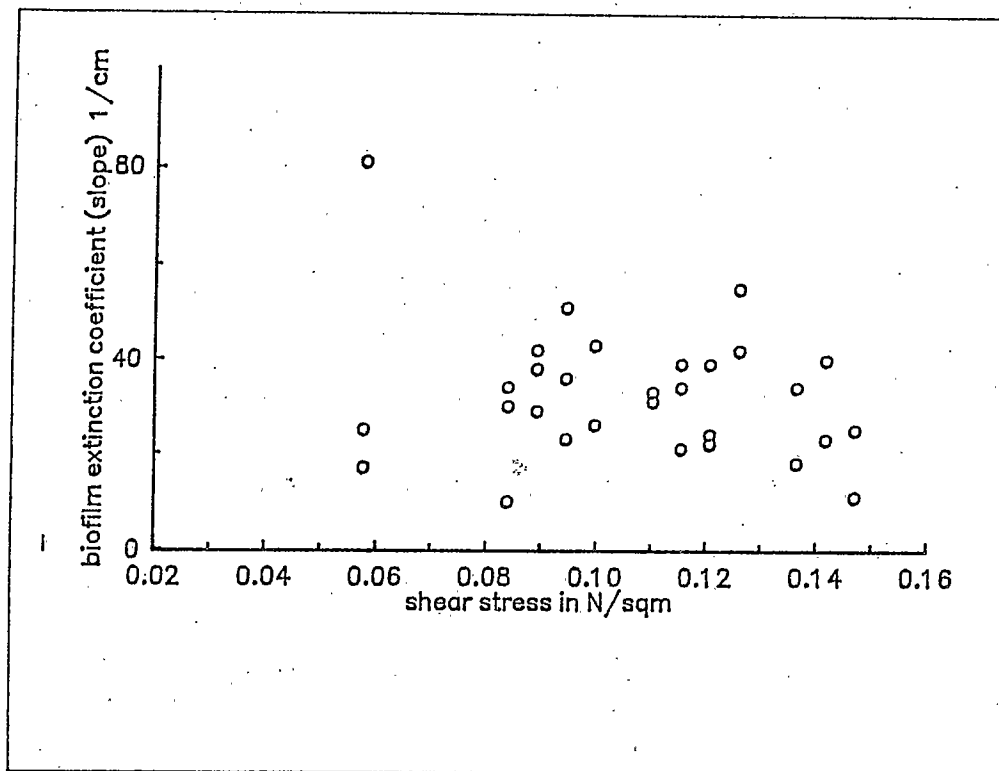


Figure 21. Extinction coefficient versus shear stress for a 7-day-old biofilm grown under laminar flow conditions.

DISCUSSION

A fiber optic sensor was used to measure biofilm thickness and density. The following sections discuss the usefulness of the fiber optic sensor as a tool for biofilm research. The results obtained with the measurements are evaluated.

Fiber Optic Sensor Construction and Application

The sensor described in these experiments used a light emitting diode as light source. The light intensity of a diode decreases proportional to the distance squared. The measurements were conducted in almost constant distance from the light source, since the movement of the sensor was in the range of a few millimeters. It was shown that the change in light intensity over a distance of 2 mm in water was smaller than the noise level (see Appendix F). For measurements over larger distances, however, a different light source could be used, such as a laser diode which works as a beam emitter and the light intensity stays nearly constant with distance.

Another problem with an LED as light source was to line the sensor up so it did not move out of the area of maximum intensity of the light source during measurements. When the sensor and diode were misaligned, a decrease in light intensity could be detected, even though the sensor moved closer to the diode. This problem could be avoided for a laser diode with a lens system to emit a wide beam of constant light

intensity. The problem was avoided in the present study by repeated test-measurements and readjusting of the sensor.

The fiber optic sensor was shown to be a useful tool for finding the biofilm-water interface, and for measurements of biofilm thickness and density, when applied to biofilms grown on transparent slides. To increase the applications for the fiber optic sensor, a device where light source and sensor are coupled might be developed in the future. This might open possibilities for in-situ measurements.

The Delineation of the Biofilm and the Water Phase

The difference in the extinction coefficients of biofilm and the overlying bulk water was successfully used to distinguish the biofilm/water interface, through an abrupt change in the slope of the light intensity versus distance curve.

To describe transport phenomena of nutrients and oxygen in the biofilm and in the bulk liquid, the biofilm water interface has to be located simultaneously with the nutrient concentration. This is possible by combining any sensor with the fiber optic sensor. Concentration gradients can then be evaluated in direct association with the position of the interface.

The Determination of the Biofilm Thickness

The fiberoptic sensor can be used to measure biofilm thickness for biofilm grown on transparent slides. In-situ measurements are possible, if the system to be measured is configured in such a way that the biofilm grows on a transparent substratum and the diode can be placed underneath the substratum, while the sensor can be moved into the biofilm.

The determination of the biofilm thickness can be problematic, however, for very fluffy biofilms where the biofilm-water interface is very difficult to detect. In this type of biofilm the surface film incorporates more water than a denser film. Hence, a slow transition occurs from zero absorption (zero slope) to positive absorption (measurable slope). The light intensity in the biofilm increases only very slowly, the biofilm-water interface is very difficult to find (Figure 22), and the biofilm thickness cannot be measured.

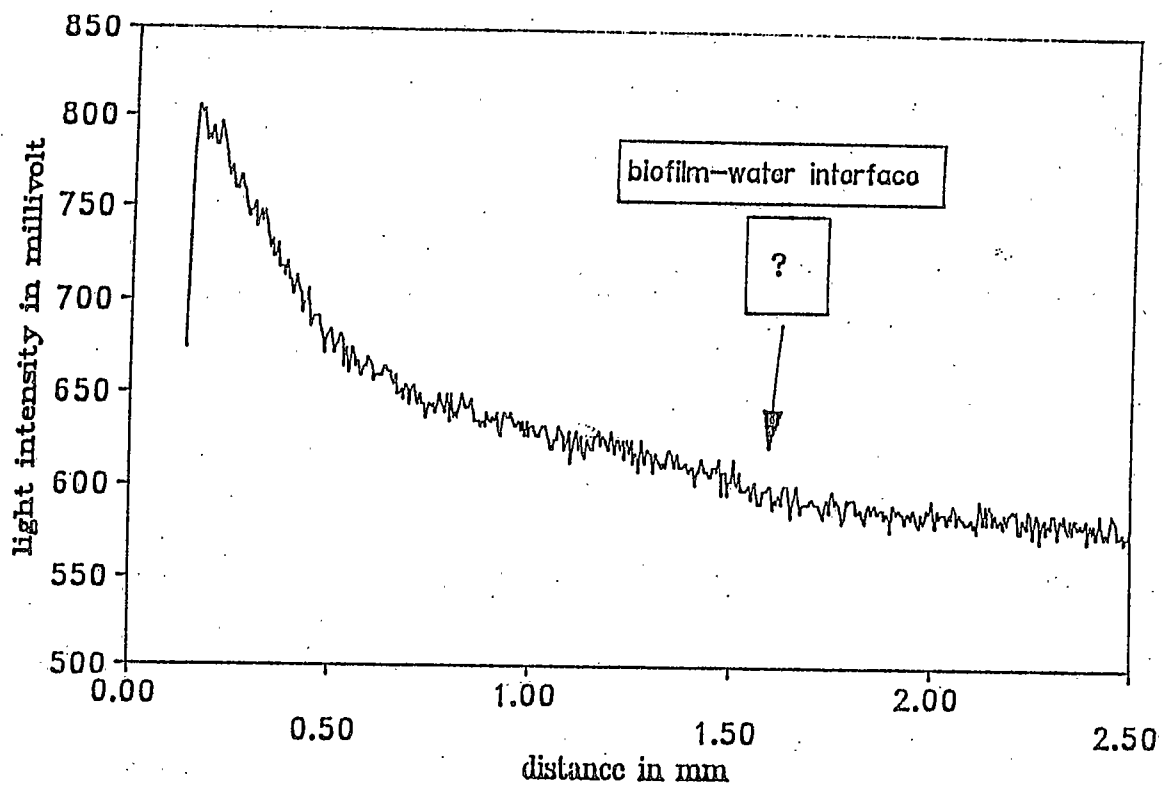


Figure 22. The variation of light intensity with distance from the substratum in a fluffy or diffuse biofilm. The transition from water to surface film is very hard to determine.

The Determination of the Biofilm Density

The fiber optic sensor is a useful tool for monitoring biofilm density. For many applications where biofilm density has to be monitored, only the changes in biofilm density are of interest. Here the movable fiber optic sensor can be used to monitor absorbance versus distance. The slope of a graph from this data gives the extinction coefficient, which in turn can be used as a measure of biofilm density. Changes in the extinction coefficient are directly proportional to changes in the biofilm density.

For cases where it is important to know the absolute density of a biofilm, the absorption coefficient of the specific biofilm must be measured. This can be easily done with a spectrophotometer, as described above. The absolute biofilm density can then be calculated from the measurement.

The following assumptions had to be made for the calibration of the fiber optic sensor with the spectrophotometer. First, biomass is assumed to absorb light according to Beer's Law. Second, the absorption coefficient must be the same for a homogenized biofilm sample as it is for an intact biofilm. The third assumption is that the absorption coefficient in a biofilm does not change with depth and is thus constant in the whole biofilm. It cannot be assumed that the absorption coefficient is constant for biofilms formed by different species or under different growth conditions. A separate calibration has to take place for every biofilm.

As shown in Figure 16 of this study, a single absorption coefficient was found to apply to biofilm exposed to the full range of shear stress from this study. However, it is

possible that under turbulent conditions the ratio of cell mass to mass of extracellular polymer changes significantly, which might also change the absorption coefficient. The possibility that the absorption coefficient changes with biofilm depth cannot be excluded. Slices of biofilm from different depths would be necessary to investigate this possibility.

Density measured with the volumetric method was found to differ from the density measured with the fiber optic sensor. For repeated measurements, the density found with the volumetric method was about 25% lower than the density calculated from the fiber optic measurement. This difference may result from a difference in the absorption coefficient measured with the spectrophotometer to the actual absorption coefficient seen by the fiber optic sensor. The absorption coefficient is a function of wavelength. The wavelength for which the absorption coefficient was measured in the spectrophotometer was 660 nm with a band width of 4 nm. The wavelength of maximum intensity of the diode was 660 nm, but the band width of the emitted light was about 40 nm. It is possible that the absorption coefficient for wavelengths other than 660 nm is higher, and therefore the extinction coefficient measured with the photo spectrometer at 660 nm is somewhat lower than the overall extinction coefficient between 640 nm and 680 nm. This means that the density found with the fiber optic measurement would be slightly too high. A laser diode with an exactly characterized emitting wavelength could be used to avoid this problem.

The main advantage of this method, however, is that the density of any arbitrarily chosen sublayer of the biofilm can be estimated. Density for base film and surface film can be compared quantitatively. For most investigated biofilms, from this study, the density of the base film was higher than the density of the surface film (Figure 23).

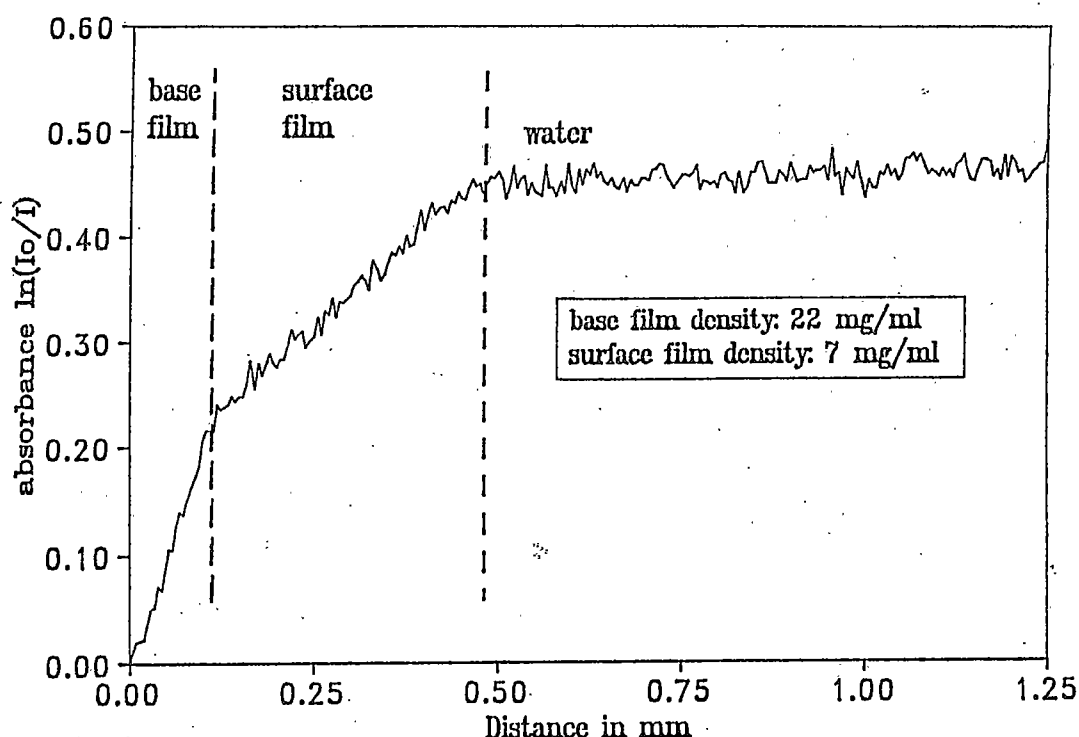


Figure 23. Biofilm with distinct base and surface film.

The Relation between Biofilm Thickness, Density and Shear Stress

In this study, a maximum biofilm thickness was found to occur at a critical shear stress of 0.08 N m^{-2} for the mixed population biofilm grown. Biofilm thickness decreases for higher and lower shear stresses. The knowledge of this value for other systems can be used to optimize biofilm reactors to obtain the maximum biofilm thickness, or in industrial pipelines to avoid maximum biofilm thickness.

Biofilm thickness was a maximum at a velocity of 0.2 ms^{-1} . This corresponds to the findings of Pedersen (1982b), who observed an increase in the rate of biofilm accumulation when the water velocity was increased from 0.005 ms^{-1} to 0.15 ms^{-1} . The

decrease in biofilm thickness for very small velocities can be attributed to mass-transfer limitations for substrate. This might slow the biofilm growth and lead to increased sloughing. Increased sloughing can also explain the encountered large variation in biofilm thickness for small velocities.

The decrease in the biofilm thickness for higher shear stress can be attributed to erosion. The higher the shear stress, the higher the erosion and the smaller is the biofilm thickness. The finding that the biofilm thickness decreases when flow velocity increases was supported by other authors in various experiments (Characklis and Marshall, 1989), who conducted studies with velocities greater than 0.3 ms^{-1} . Kornegay and Andrews (1967) observed a strong decline in biofilm thickness when the shear stress was increased from 1 to 3 Nm^{-2} . Characklis (1980) also found a decrease in biofilm thickness when the shear stress was increased from 2 to 3 Nm^{-2} . These results confirm the findings of this study.

A limitation of this experiment was that only a small range of low shear stress was investigated. A higher shear stress could not be obtained with the geometry of the reactor under the conditions of laminar flow. Turbulent flow conditions were not investigated. In the future the experimental setup can be used to perform similar measurements on industrial relevant biofilms and obtain additional valuable data.

A statistical test was performed on the mean values for the density of the biofilm versus shear stress. A t-test for the slope of the line showed that for a 5% level of significance the slope is not different from 0. The probability that the slope is different from 0 is only 47%. The coefficient of correlation is 0.2. Thus a correlation between shear stress and biofilm extinction coefficient or density could not be concluded. Density of biofilms is probably independent of shear stress for laminar flow.

CONCLUSIONS

The fiber optic sensor system is a well suited tool for biofilm research. The biofilm absorbs visible and infrared light better than water. The difference in the extinction coefficient can be detected and permits detection of the biofilm-water interface.

The substratum surface can be detected by touching the substratum with the sensor, which leads to bending of the sensor; hence, most of the light is not transmitted, but escapes. A very sudden decrease in measured light intensity therefore indicates the substratum surface.

Through finding the biofilm-water interface and the substratum surface, the biofilm thickness can be determined.

The sensor can be calibrated for biofilm density measurements by measuring the absorption of a defined mass of biofilm suspended in a small volume of water with a commercial spectrophotometer. Thus, mass concentration of the biofilm can be measured and a gradient in cell mass concentration or "density" of the biofilm can be found. Changes in the biofilm density can be detected.

Biofilm thickness depends on shear stress in laminar flow. Maximum biofilm thickness was observed for a shear stress of 0.08 Nm^{-2} .

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NOMENCLATURE

NOMENCLATURE

- A surface area of disk $[L^2]$
 b boundary layer thickness $[L]$
 b_d surface detachment coefficient $[1/T]$
 c concentration $[M/L^3]$
 d thickness of layer passed by light $[L]$
 e extinction coefficient $[1/l]$
 I light intensity $[I]$
 I_0 incident light intensity $[I]$
 k_D detachment coefficient $[1/T]$
 K_s saturation coefficient for Monod kinetics $[M/L^3]$
 L biofilm thickness $[L]$
 n refractive index
 r radial coordinate in a cylindrical system
 R radius of disk $[L]$
 R_d detachment rate $[M/L^2]$
 R_e erosion rate $[M/(L^3T)]$
 Re Reynolds number
 p pressure $[N/L^2]$
 s gap between rotating disk and housing $[L]$
 S substrate concentration $[M/L^3]$
 x axial coordinate in a cylindrical system
 X biomass concentration in suspension $[M/L^3]$
 X_b biofilm mass concentration $[M/L^3]$
 v velocity $[L/T]$
 $v_{r,x,t}$ radial, axial, tangential velocity component
 α angle between light ray and surface normal
 δ angle of rotation
 ϵ absorption coefficient $[L^2/M]$
 ϕ angle between light ray and reflection plane normal
 μ absolute viscosity $[M/(LT)]$
 μ_m max. specific growth rate $[1/T]$
 ρ density of fluid $[M/L^3]$
 ρ_F density of biofilm $[M/L^3]$
 σ shear stress $[N/L^2]$
 ν kinematic viscosity $= \mu/\rho$ $[L^2/T]$
 ω radial velocity $[1/T]$
 θ angular coordinate in a cylindrical system

UNITS:

I light intensity units
L length units
M mass units
N force units $[ML/T^2]$
T time units

APPENDICES

APPENDIX A

THE CALIBRATION OF THE MICROMANIPULATOR

The distance travelled by the sensor per step was recorded for different manipulator settings (Table 3).

Table 3: Step length versus micromanipulator settings.

	SPEED	STEP LENGTH	ACTUAL STEP LENGTH (μm)
high	6	10	94
adj	7	5	24.7
adj	5	4	13.5
adj	4	4	9
adj	3	4	5
adj	4	2	1.1

Additionally the frequency of the steps was measured and calibrated for different micromanipulator settings (Table 4).

Table 4: Step frequency for different micromanipulator settings.

INTERVAL	FREQUENCY (s^{-1})
1	3.16
2	1.43
3	0.40
4	0.24
4.5	0.20

APPENDIX B

THE VARIATION OF ABSORBANCE WITH DISTANCE FROM THE SUBSTRATUM FOR DENSITY CALIBRATION

The measurements were conducted on 2 biofilm samples from the rotating disk reactor. The measurement was repeated six times in the first sample, four times in the second sample. The mean extinction coefficient was calculated for each sample.

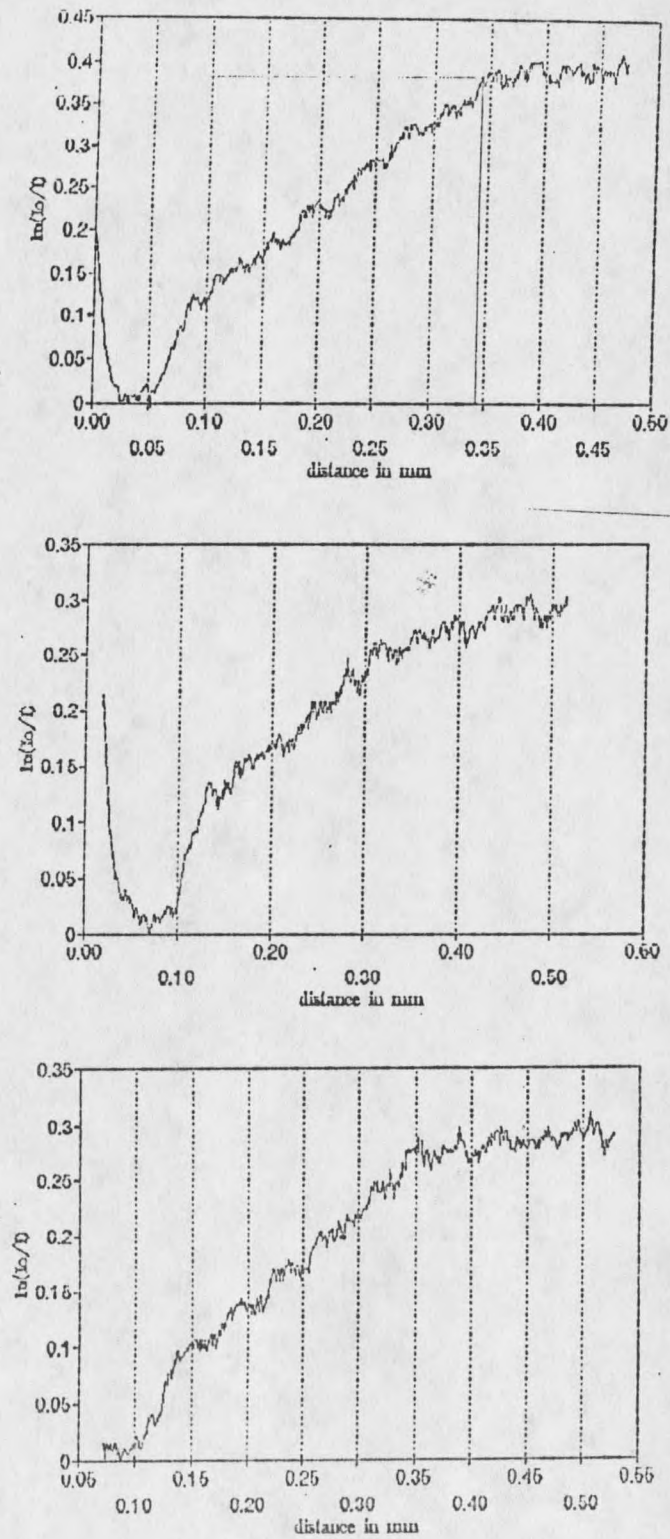


Figure 24. Absorbance measurements in biofilm sample A.

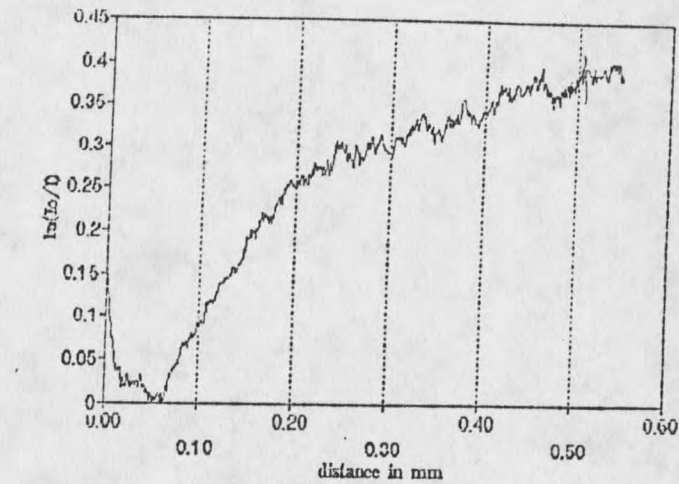
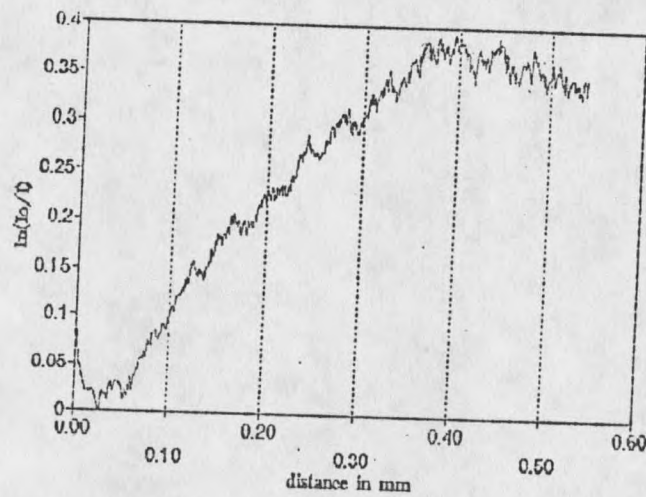
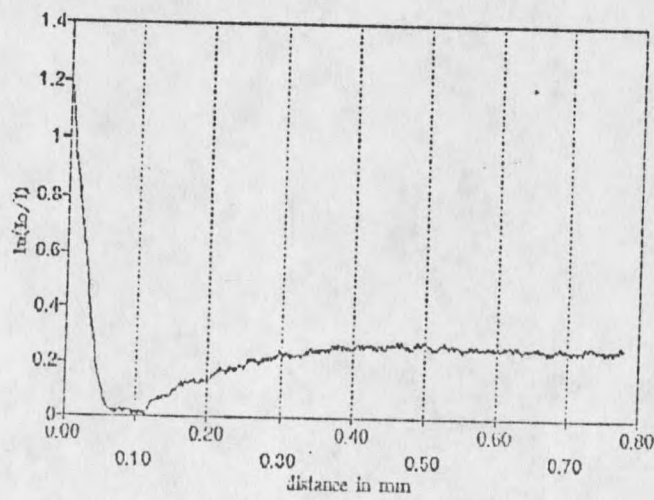


Figure 24-Continued. Absorbance measurements in biofilm sample A.

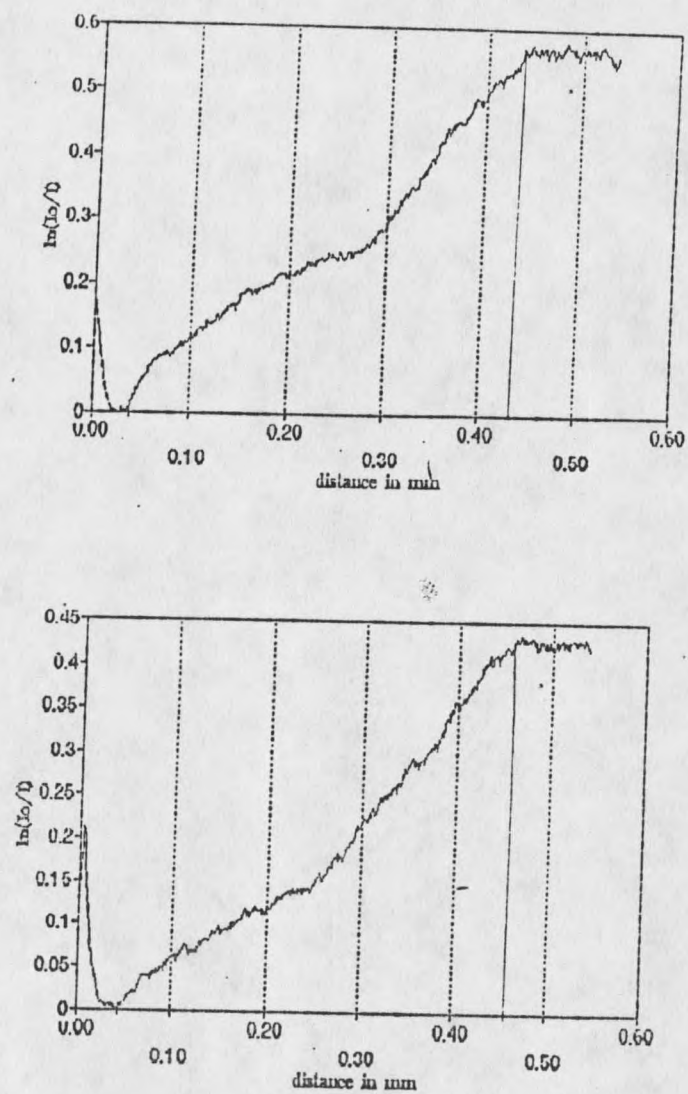


Figure 25. Absorbance measurements in biofilm sample B.

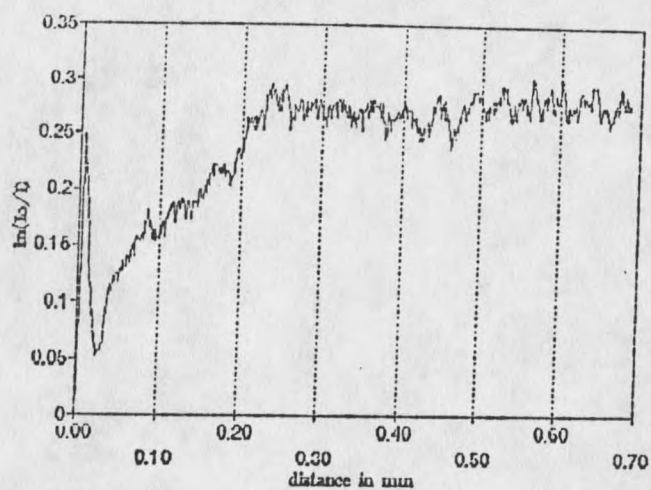
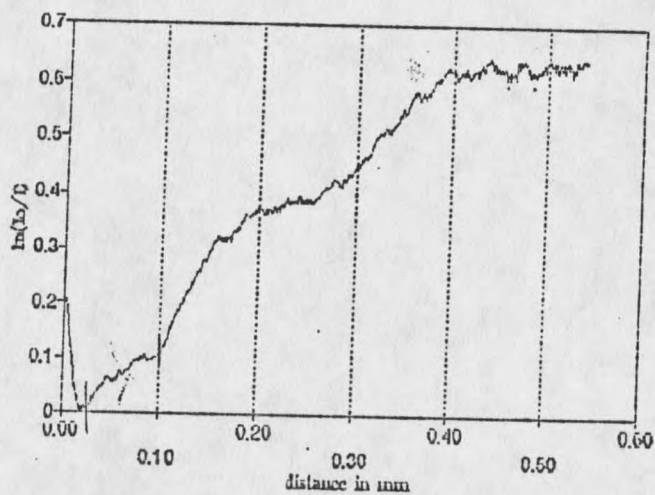


Figure 25-Continued. Absorbance measurements in biofilm sample B.

APPENDIX C

THE VARIATION OF ABSORBANCE WITH DISTANCE FROM THE SUBSTRATUM FOR A 4-DAY-OLD BIOFILM

The measurements were conducted on biofilm samples of 4-day-old biofilm from the rotating disk reactor. The number in the upper left hand corner of the graph signifies the location from where the sample was taken. It gives the distance from the center of the disk in cm.

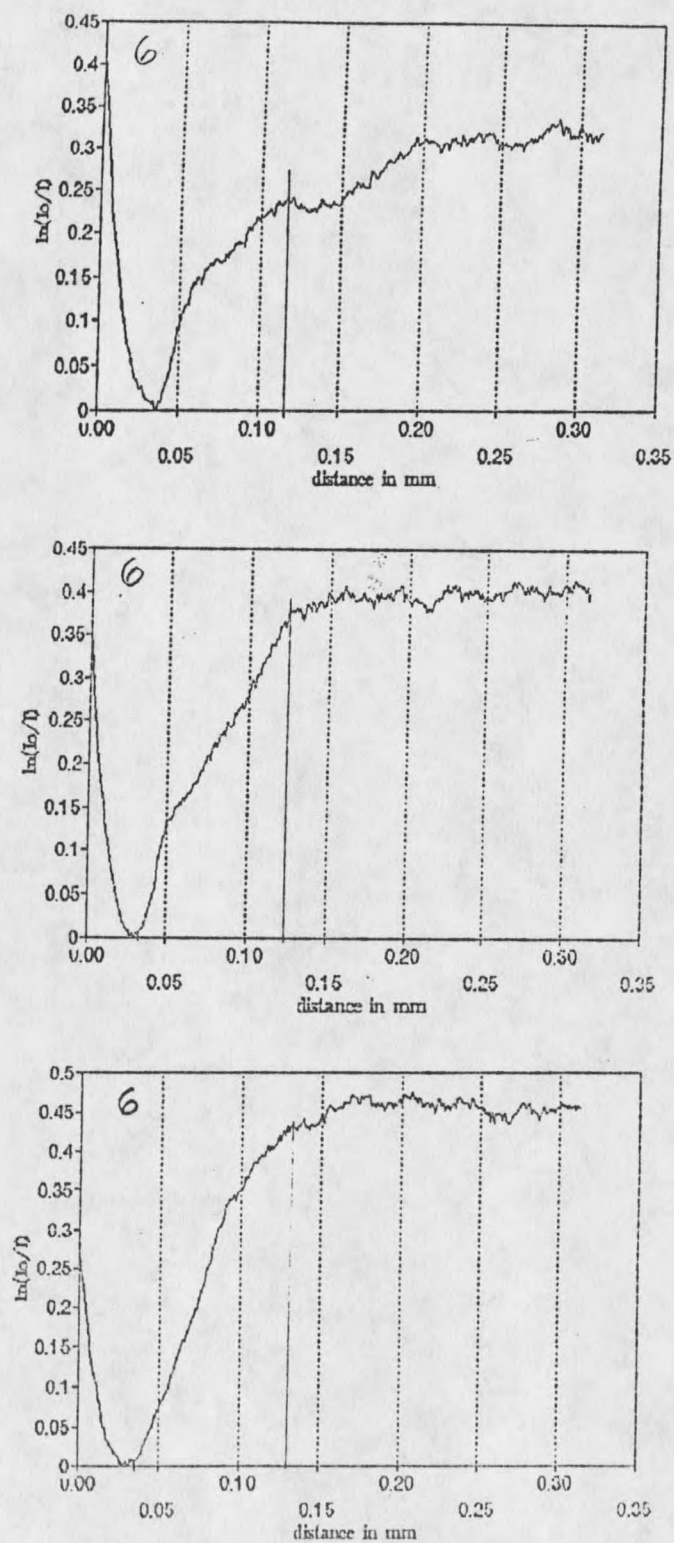


Figure 26. Absorbance measurements in 6 cm distance from the center of the disk.

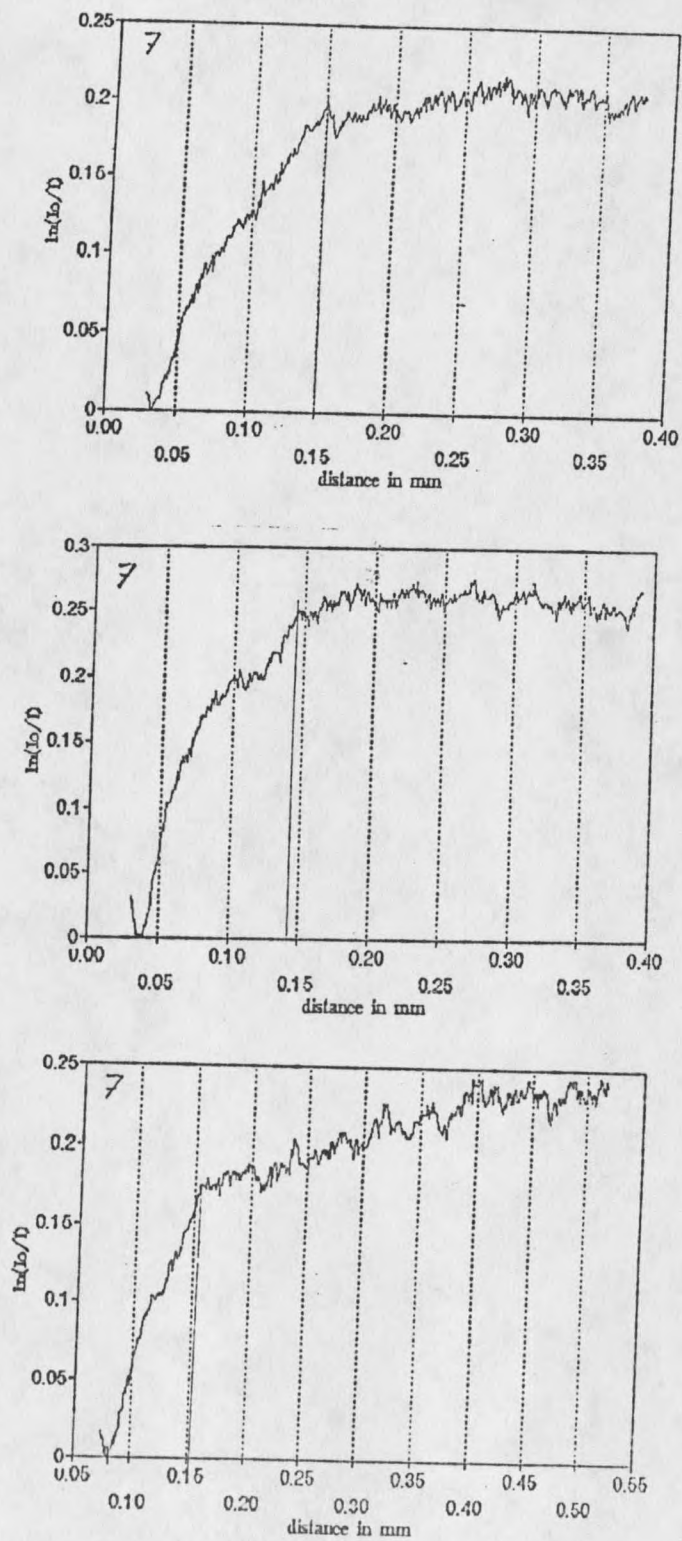


Figure 27. Absorbance measurements in 7 cm distance from the center of the disk.

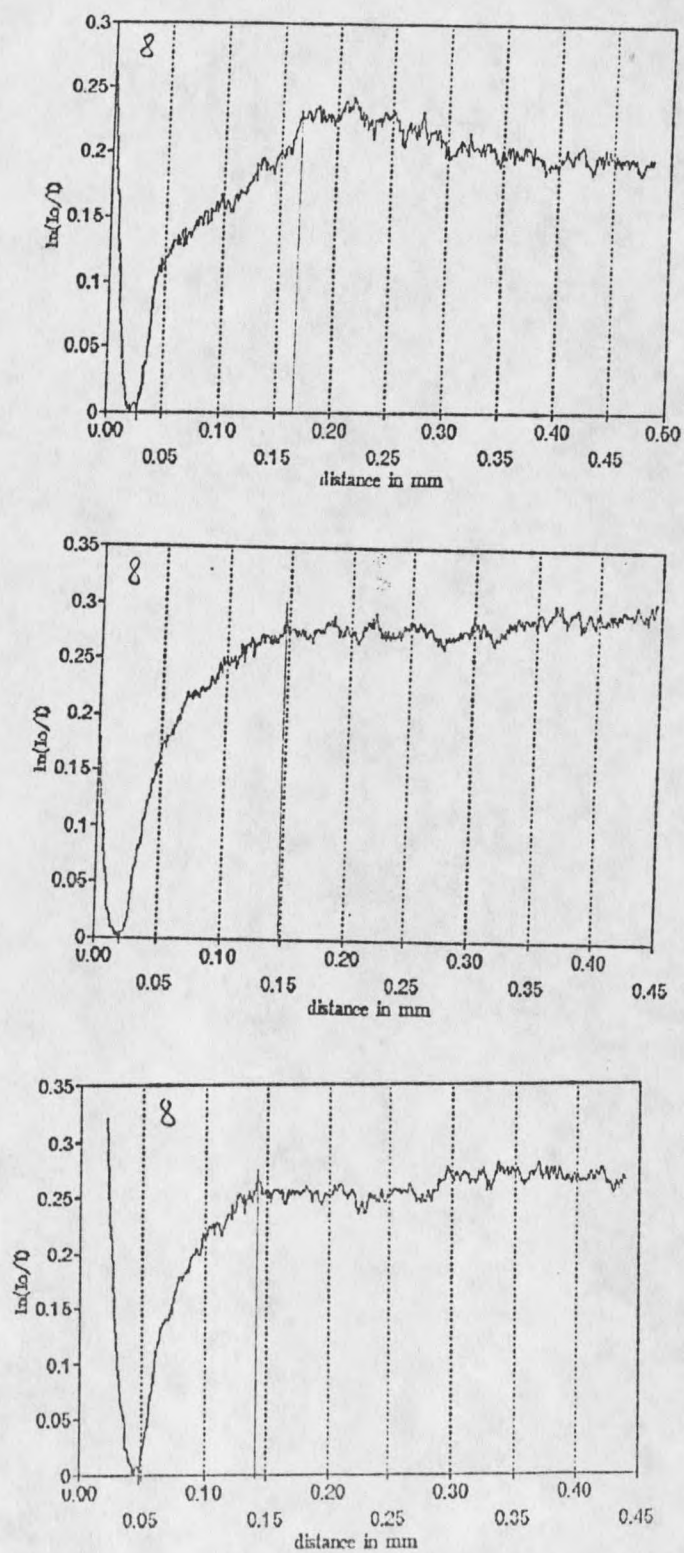


Figure 28. Absorbance measurements in 8 cm distance from the center of the disk.

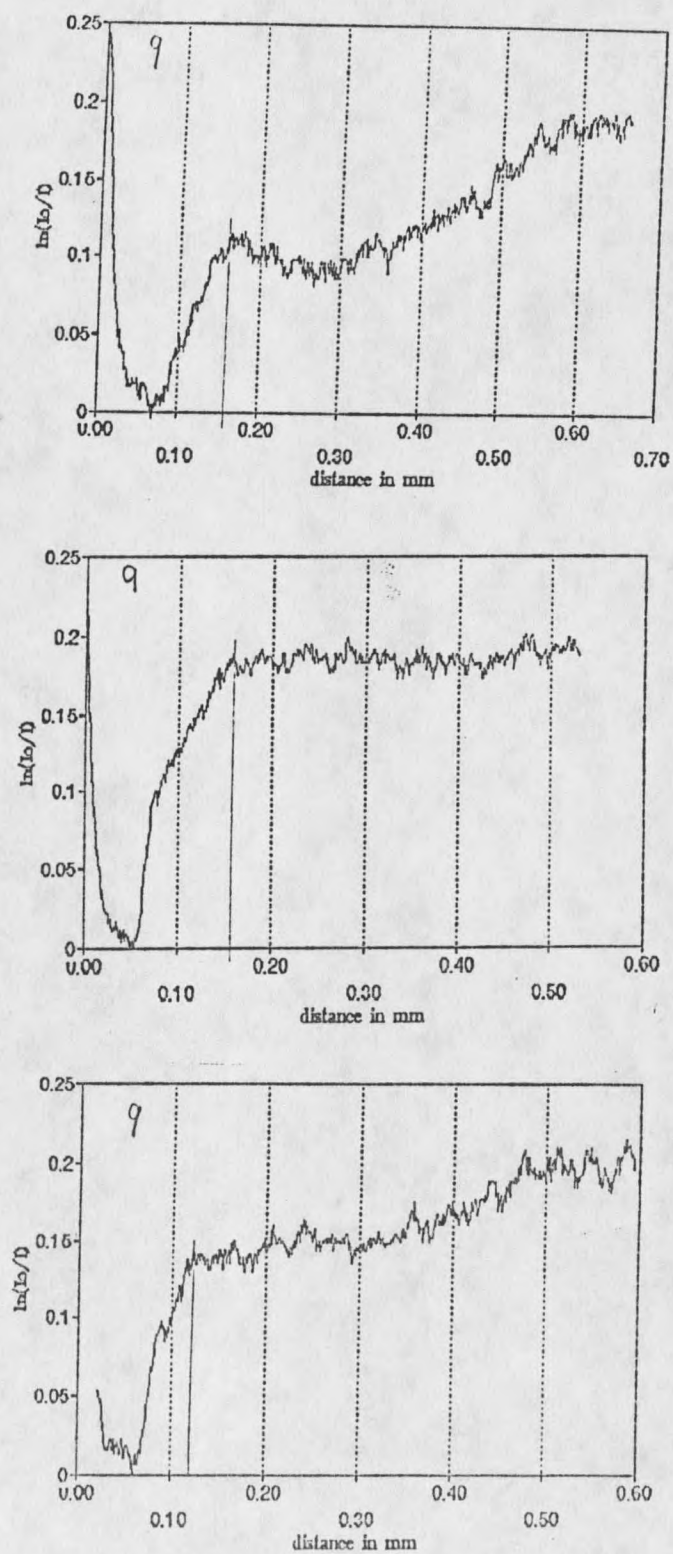


Figure 29. Absorbance measurements in 9 cm distance from the center of the disk.

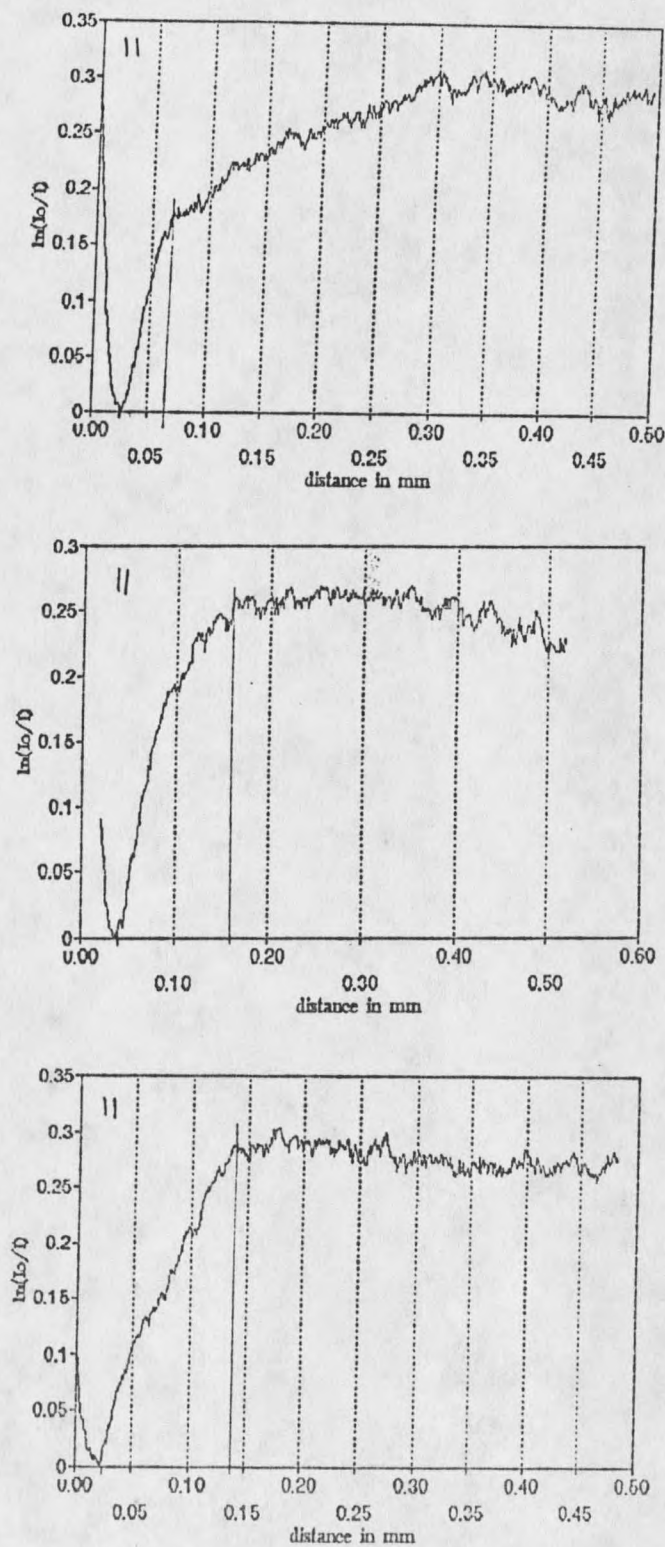


Figure 30. Absorbance measurements in 11 cm distance from the center of the disk.

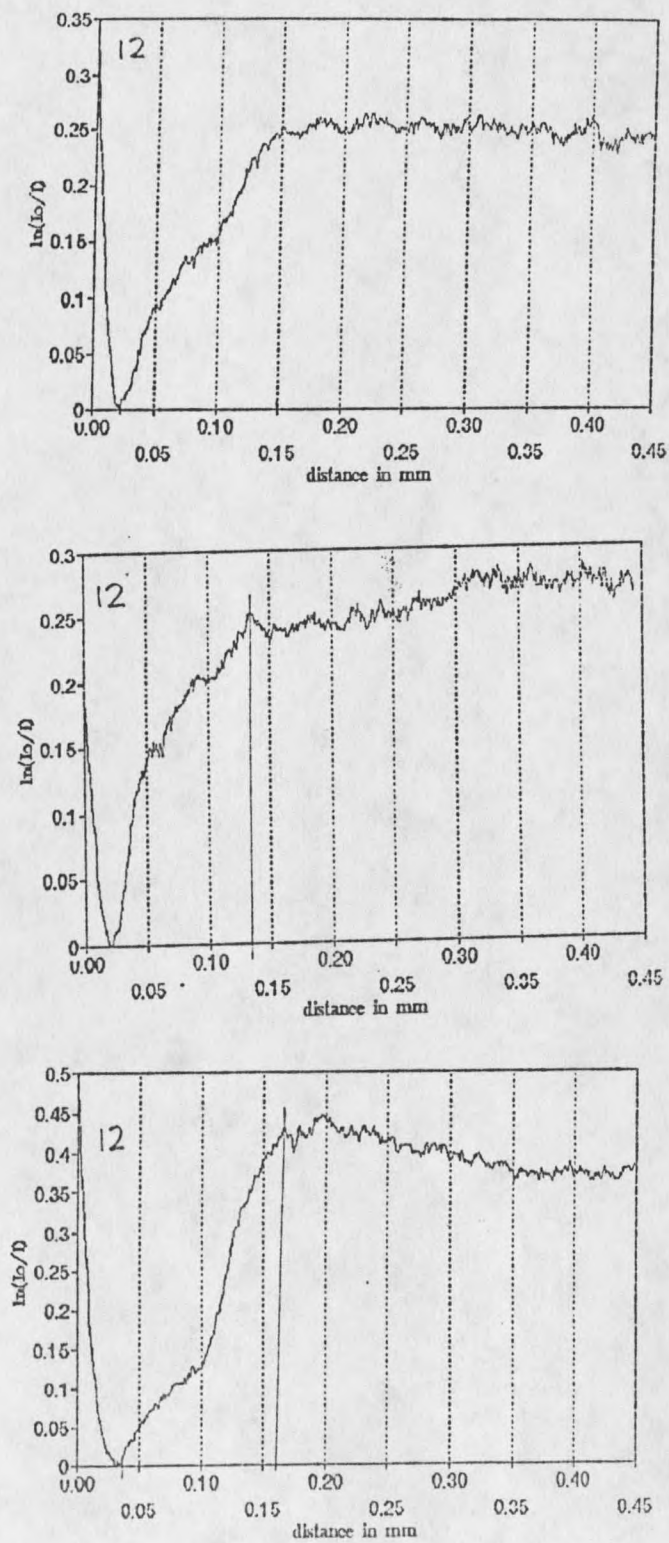


Figure 31. Absorbance measurements in 12 cm distance from the center of the disk.

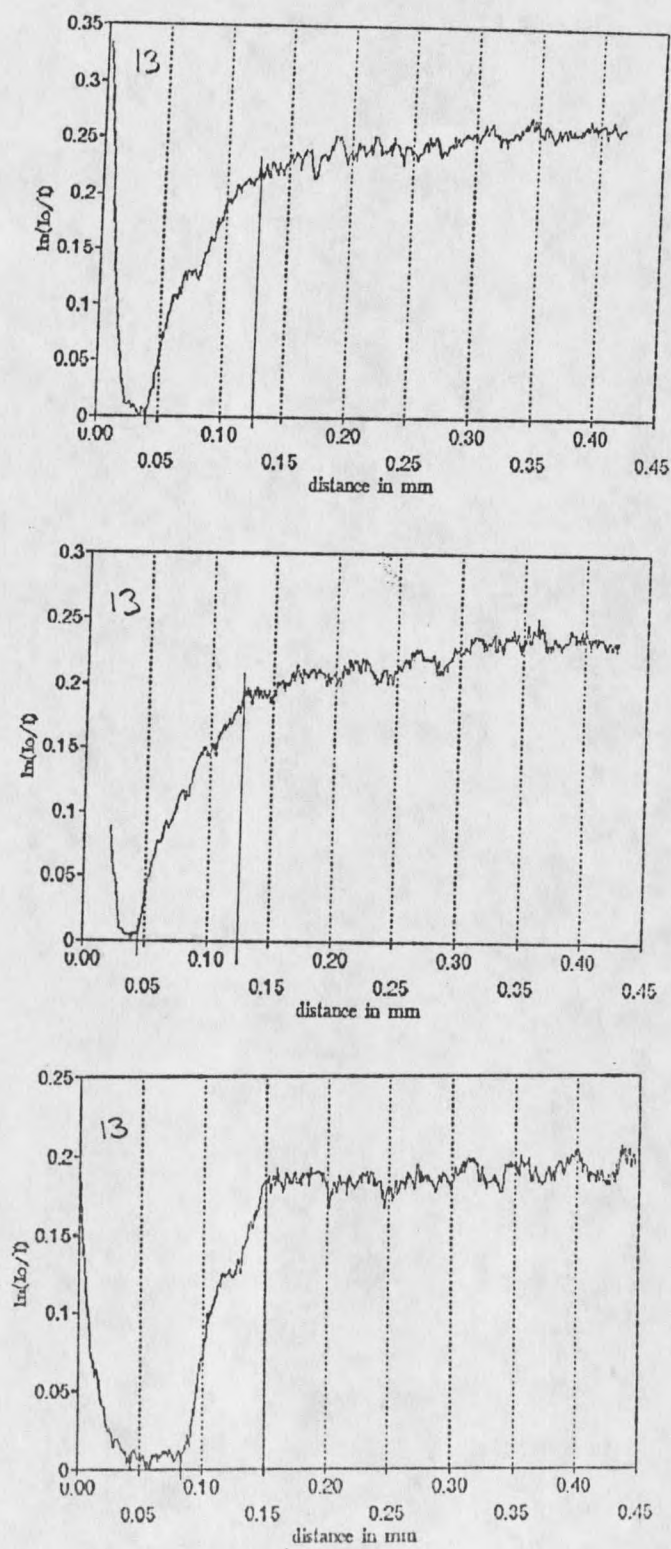


Figure 32. Absorbance measurements in 13 cm distance from the center of the disk.

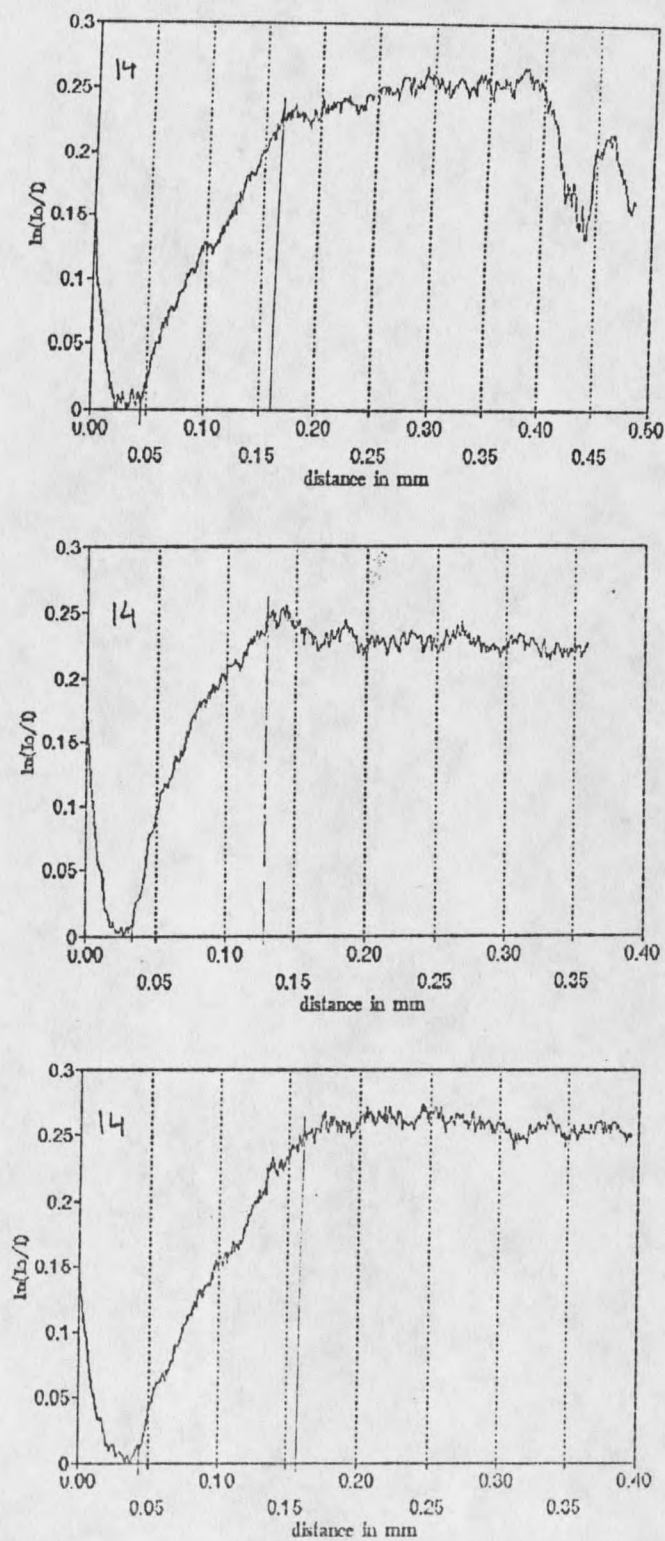


Figure 33. Absorbance measurements in 14 cm distance from the center of the disk.

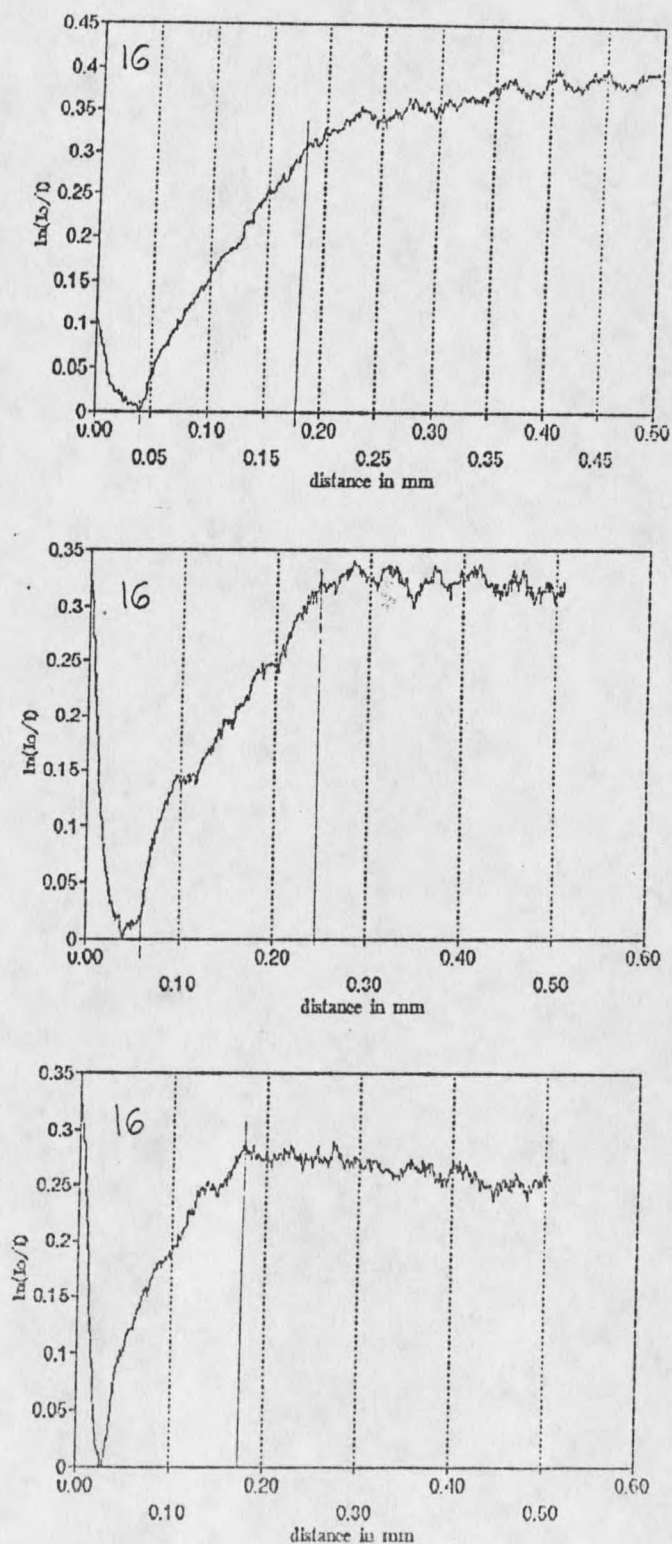


Figure 34. Absorbance measurements in 16 cm distance from the center of the disk.

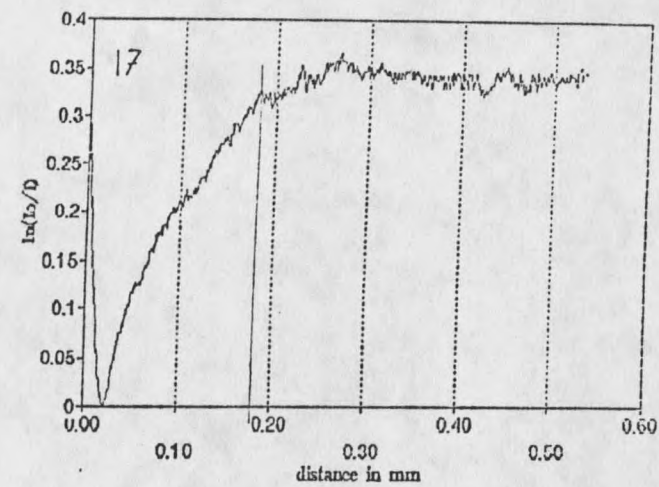
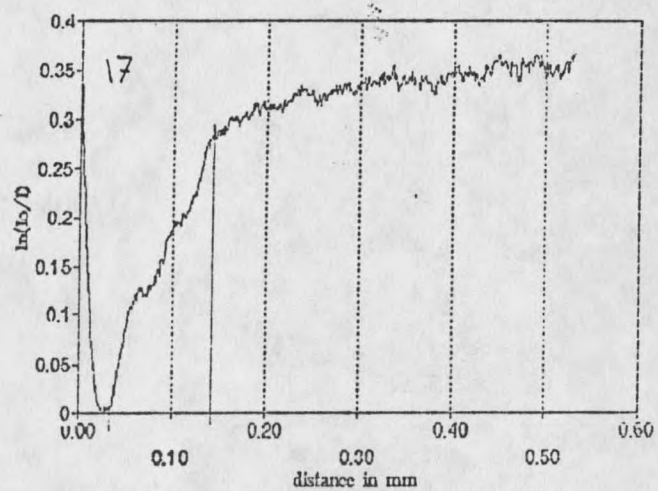


Figure 35. Absorbance measurements in 17 cm distance from the center of the disk.

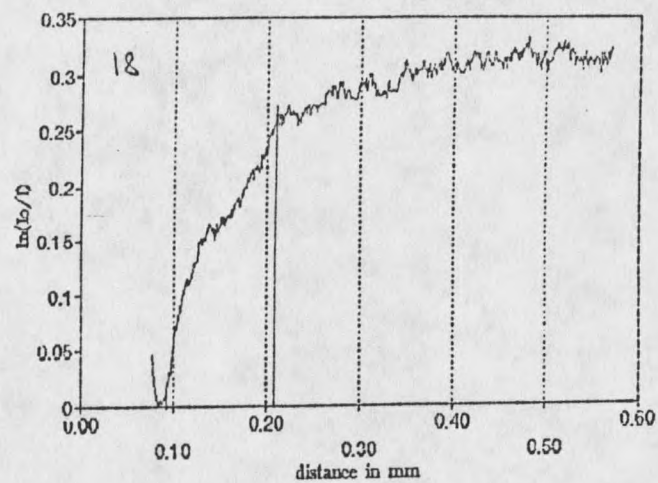
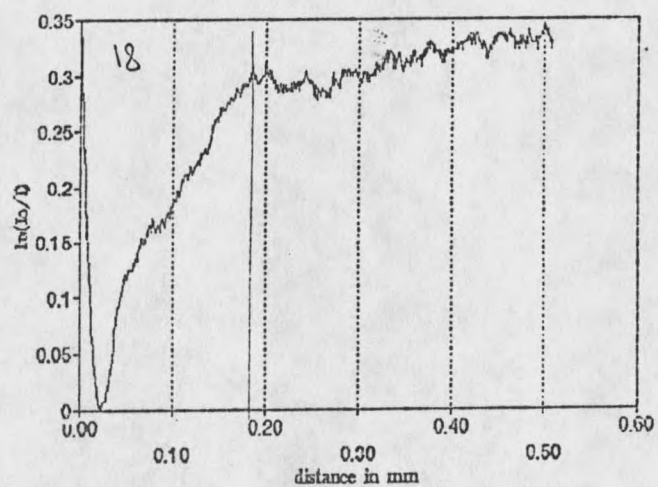
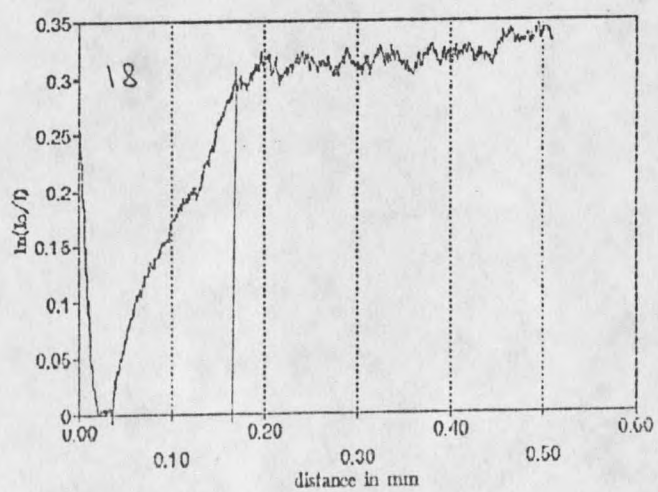


Figure 36. Absorbance measurements in 18 cm distance from the center of the disk.

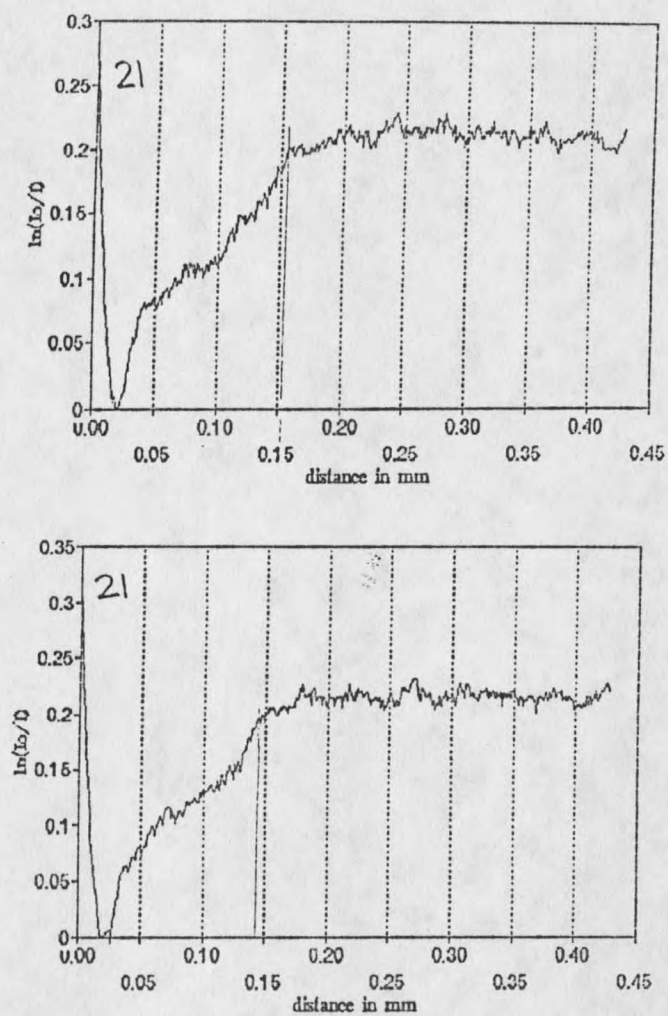


Figure 37. Absorbance measurements in 21 cm distance from the center of the disk.

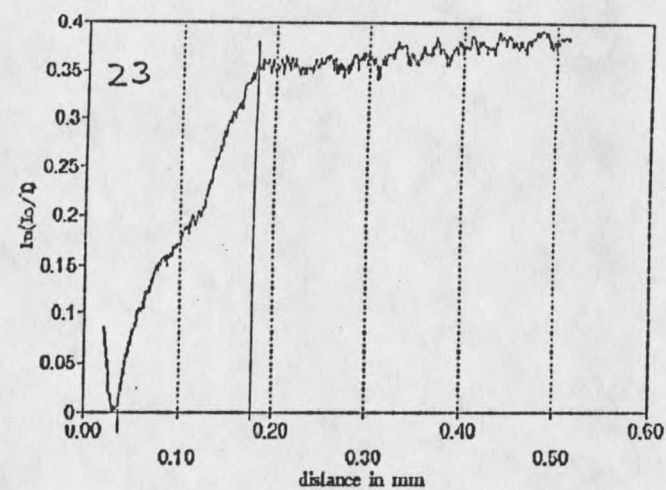
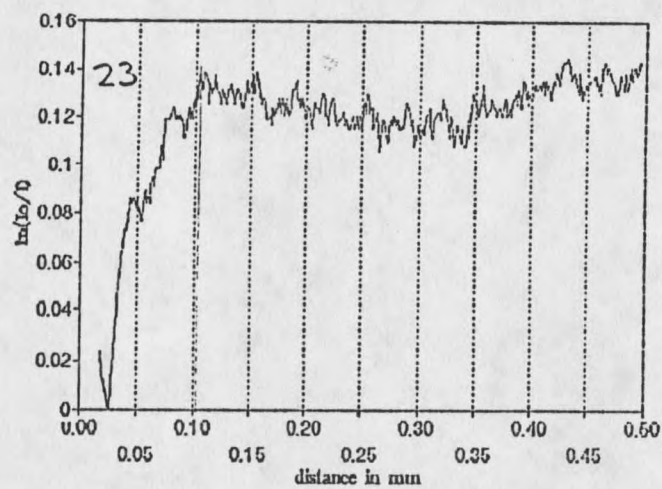


Figure 38. Absorbance measurements in 23 cm distance from the center of the disk.

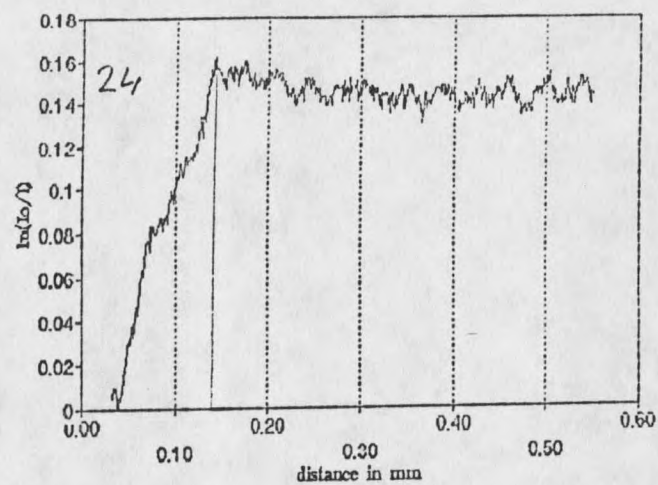
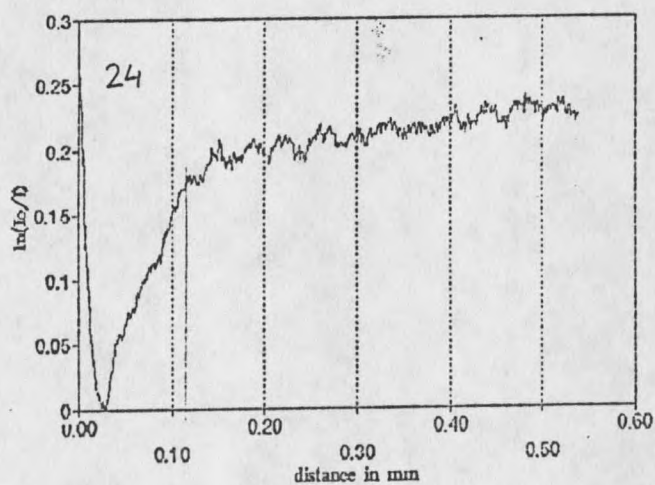
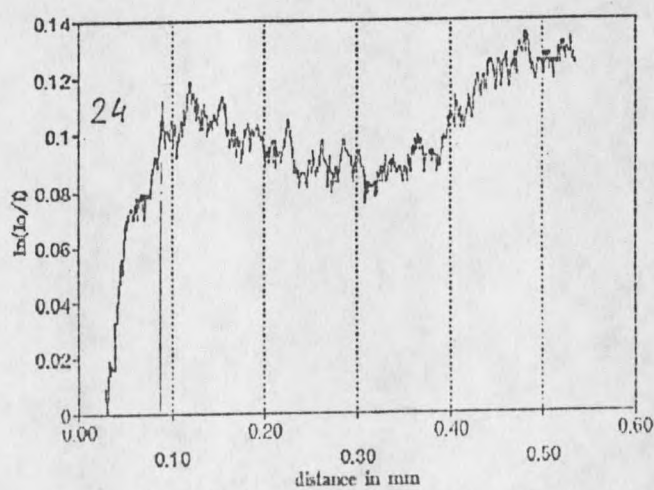


Figure 39. Absorbance measurements in 24 cm distance from the center of the disk.

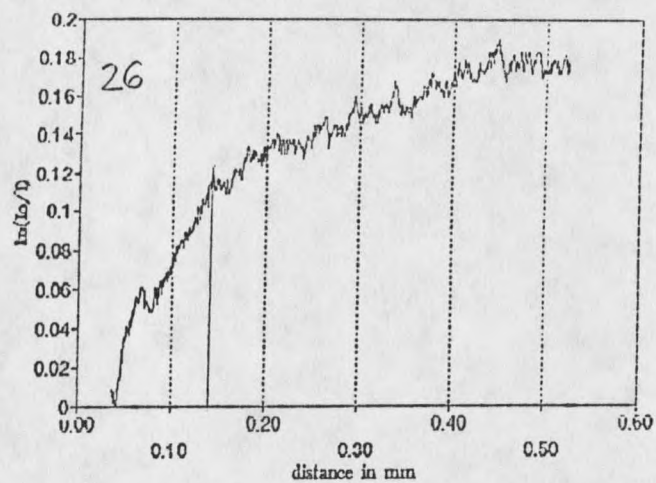
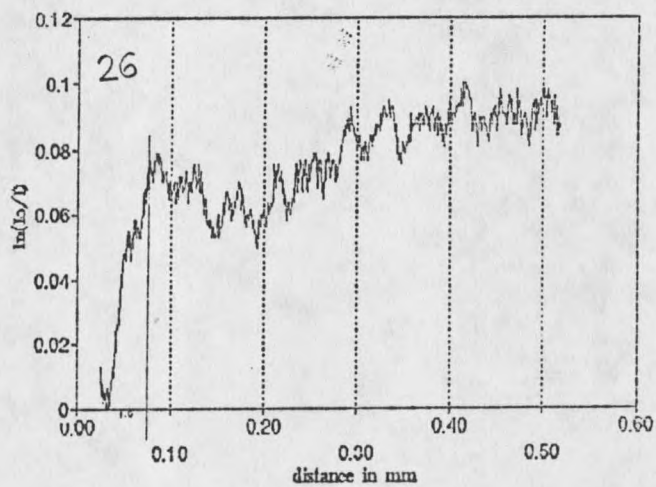


Figure 40. Absorbance measurements in 26 cm distance from the center of the disk.

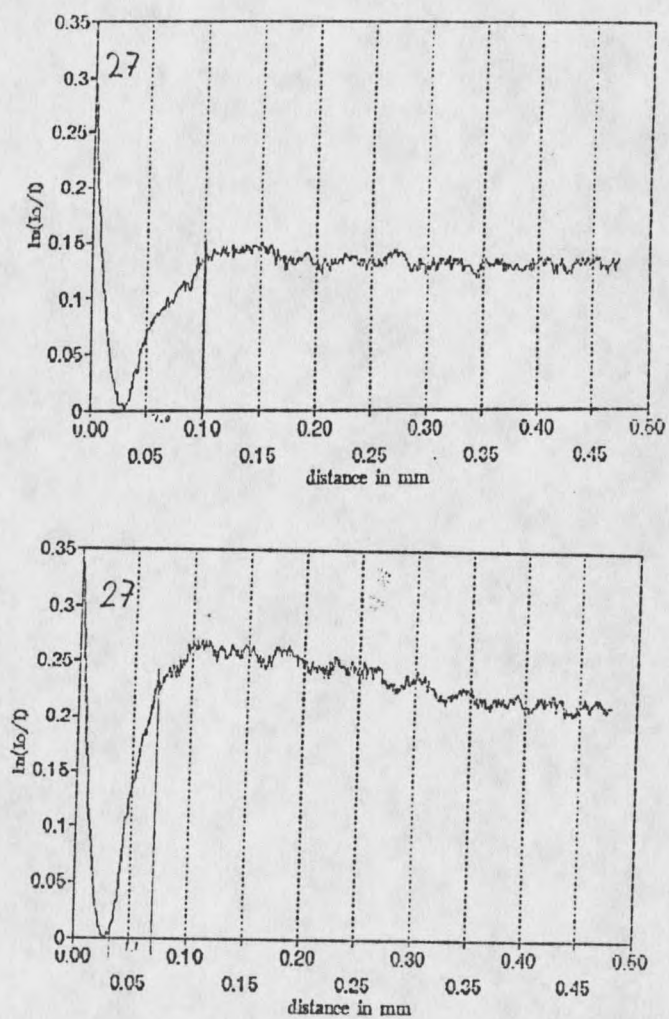


Figure 41. Absorbance measurements in 27 cm distance from the center of the disk.

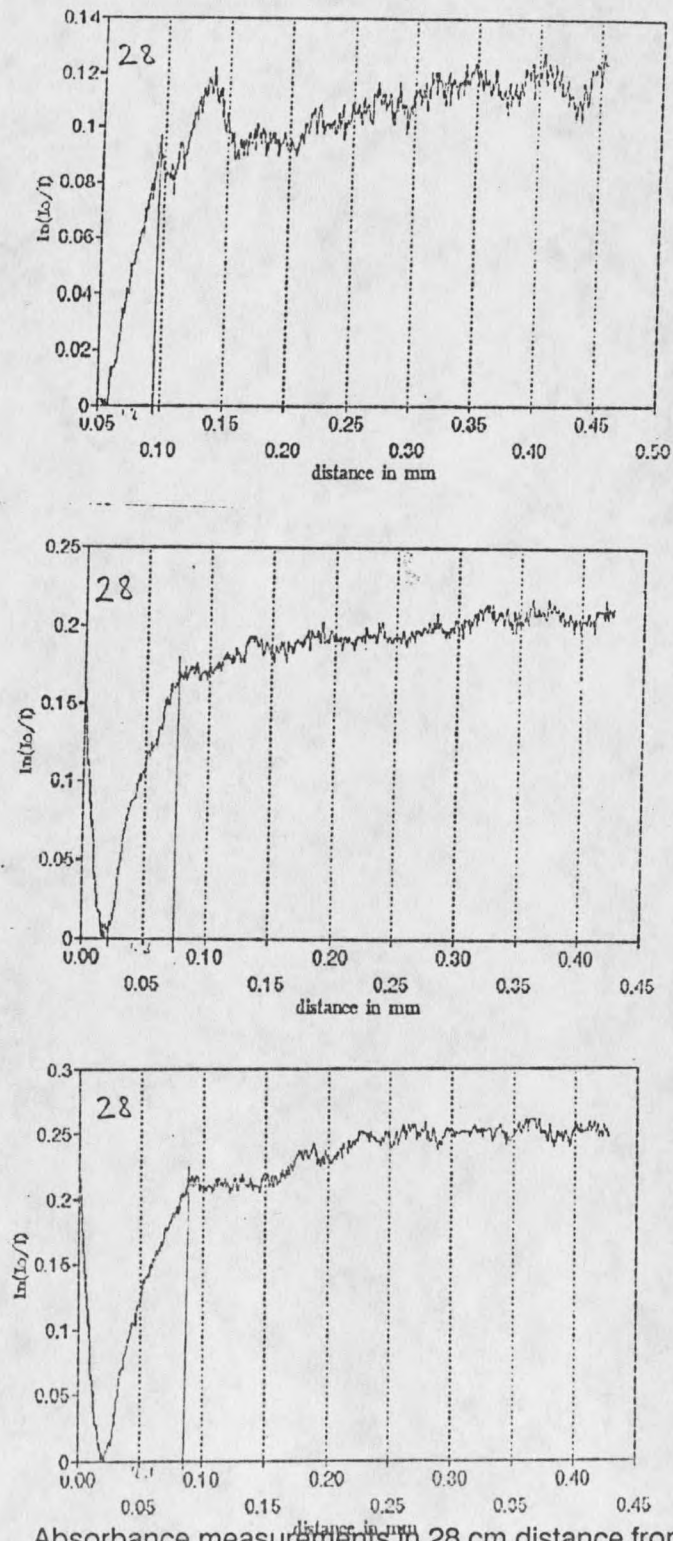


Figure 42.

Absorbance measurements in 28 cm distance from the center of the disk.

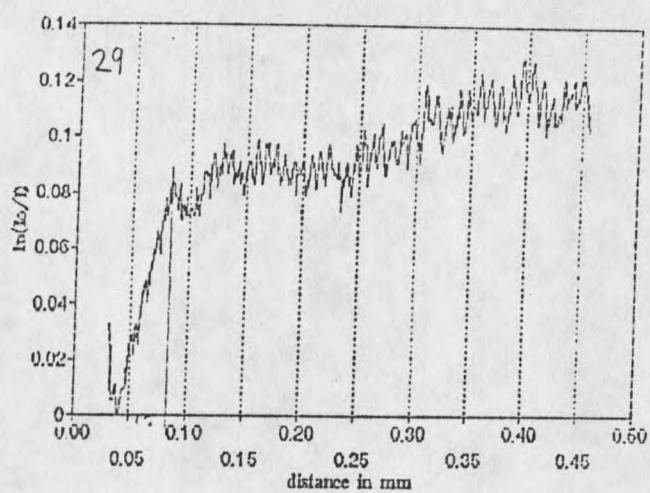


Figure 43. Absorbance measurements in 29 cm distance from the center of the disk.

APPENDIX D

THE VARIATION OF ABSORBANCE WITH DISTANCE FROM THE SUBSTRATUM
FOR A 7-DAY-OLD BIOFILM

The measurements were conducted on biofilm samples of 7-day-old biofilm from the rotating disk reactor. The number in the upper left hand corner of the graph signifies the location from where the sample was taken. It gives the distance from the center of the disk in cm.

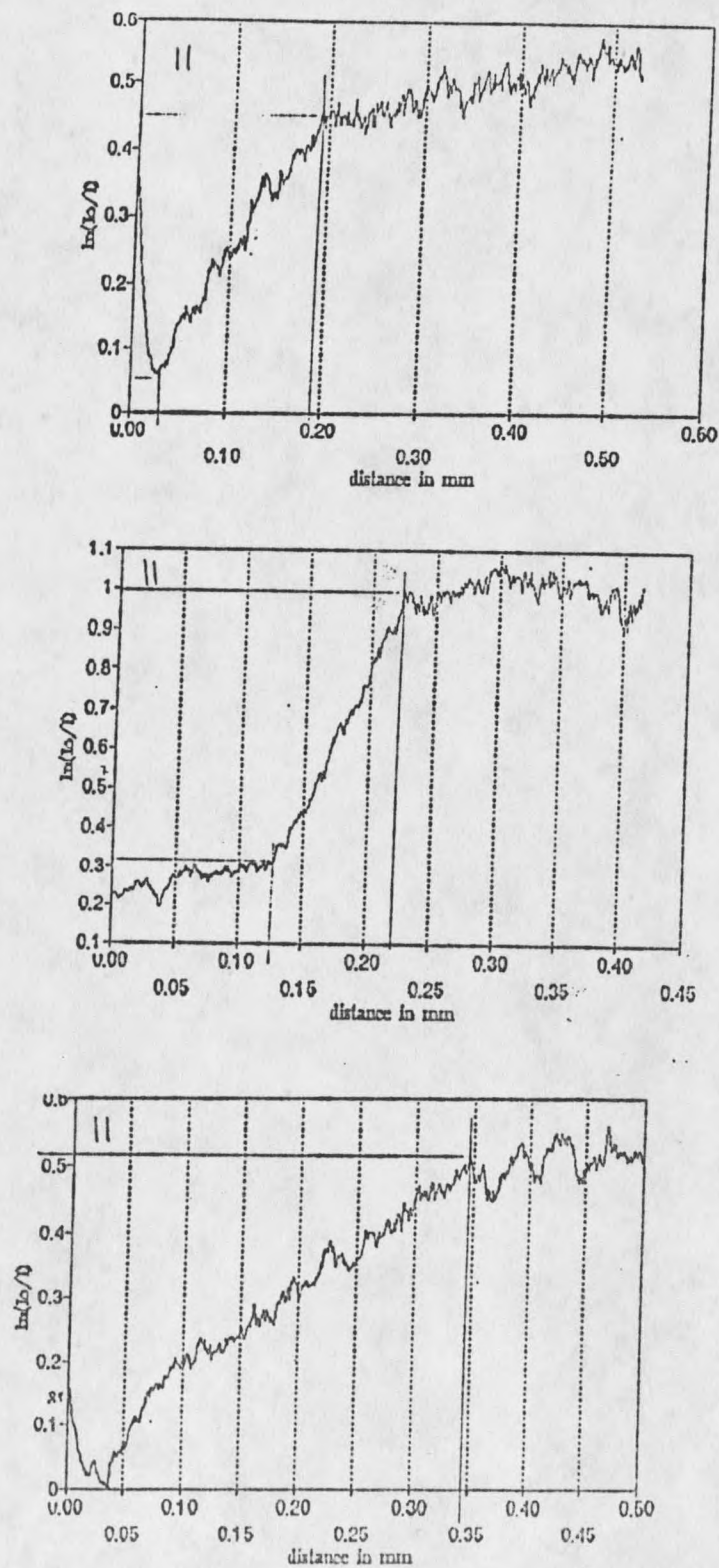


Figure 44. Absorbance measurements in 11 cm distance from the center of the disk.

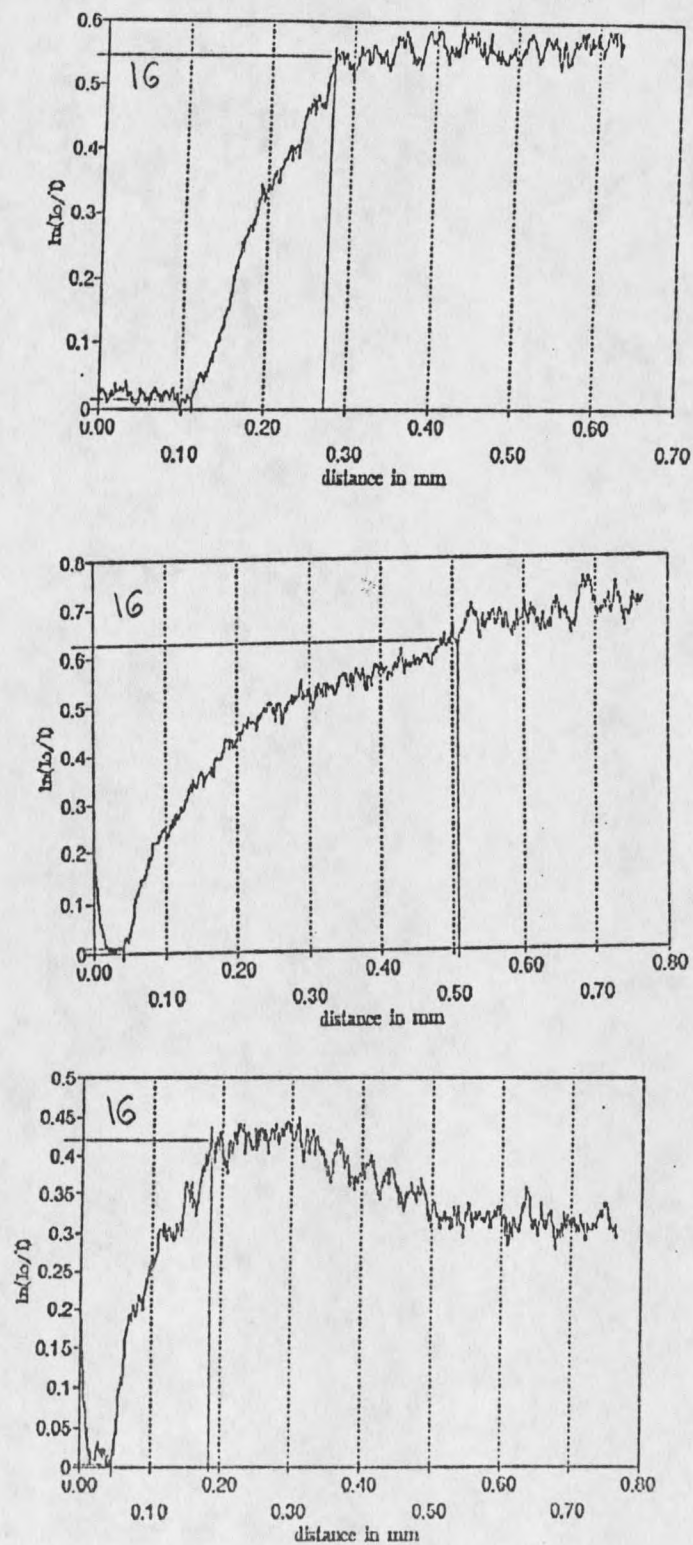


Figure 45. Absorbance measurements in 16 cm distance from the center of the disk.

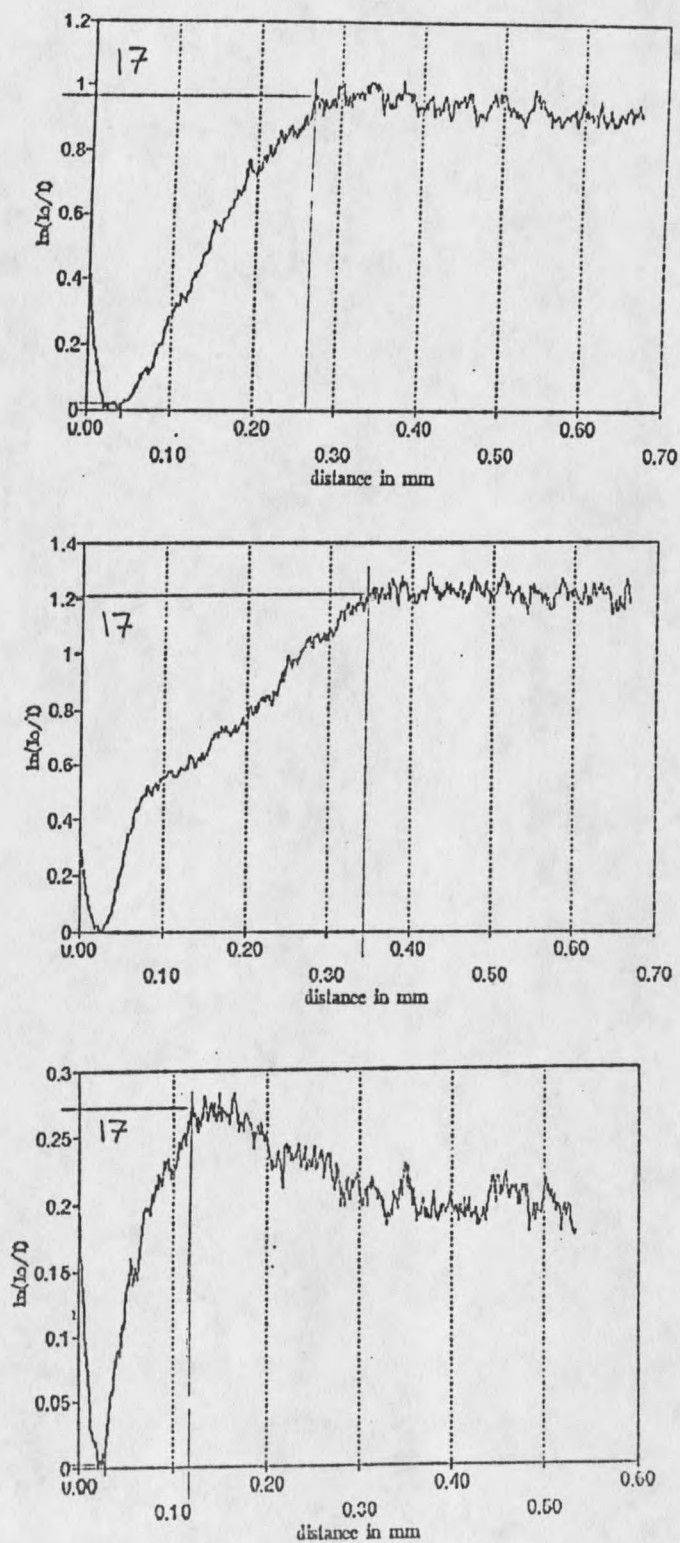


Figure 46. Absorbance measurements in 17 cm distance from the center of the disk.

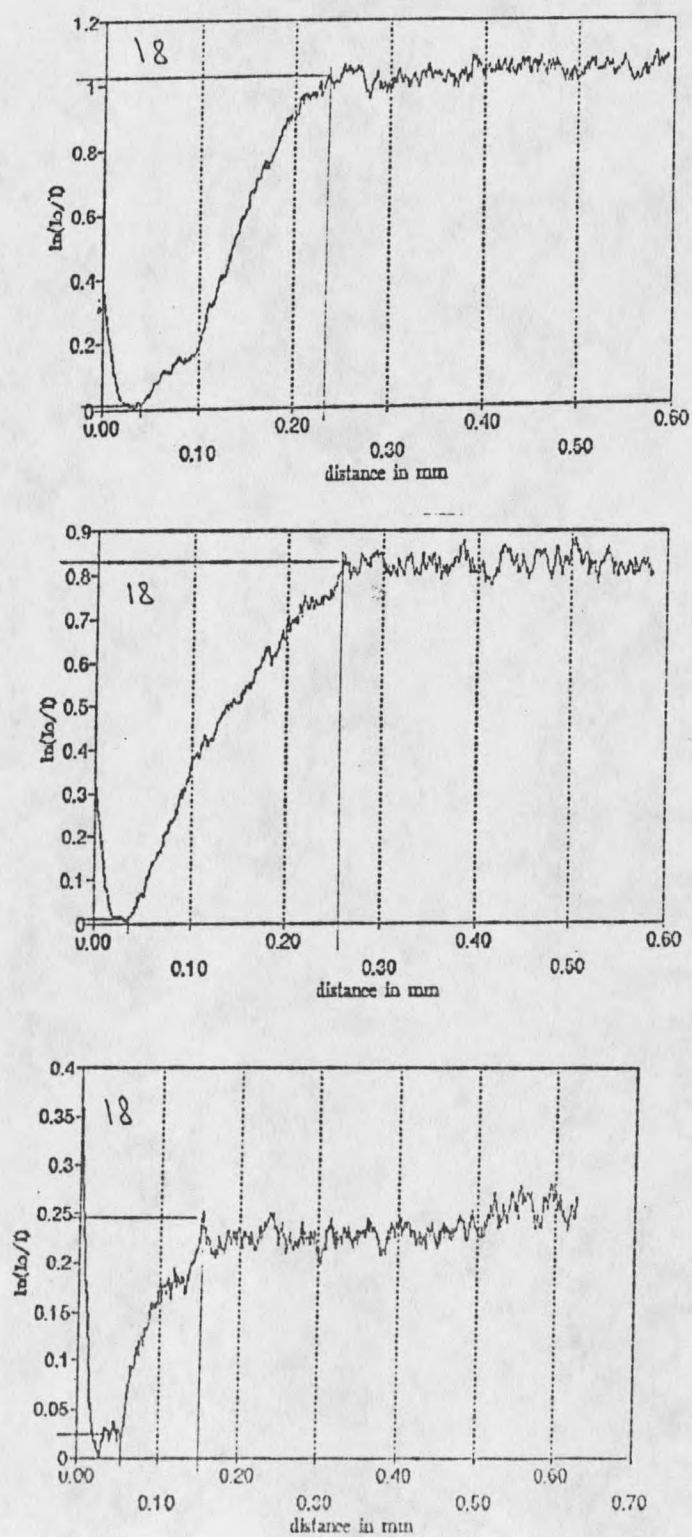


Figure 47. Absorbance measurements in 18 cm distance from the center of the disk.

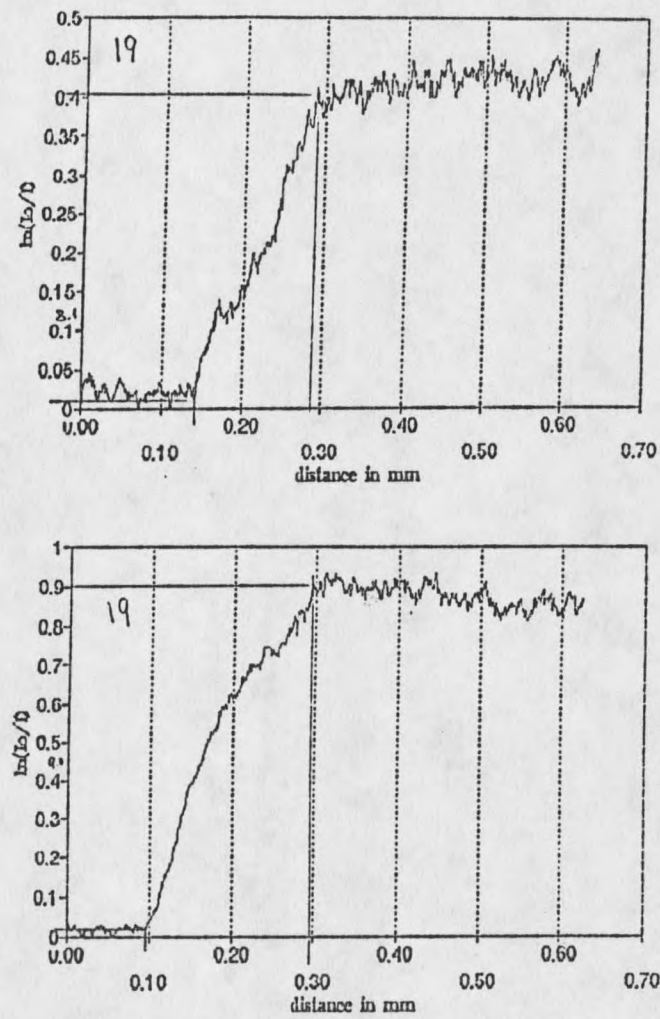


Figure 48. Absorbance measurements in 19 cm distance from the center of the disk.

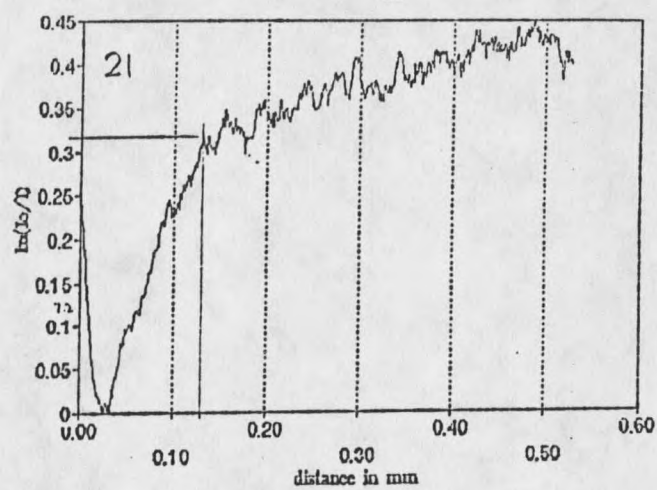
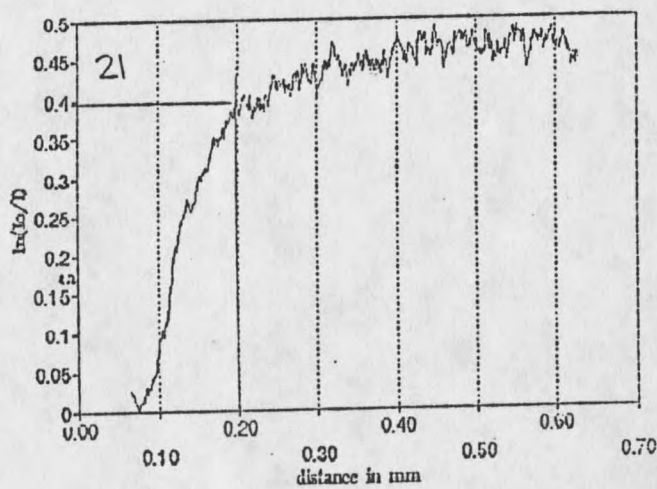


Figure 49. Absorbance measurements in 21 cm distance from the center of the disk.

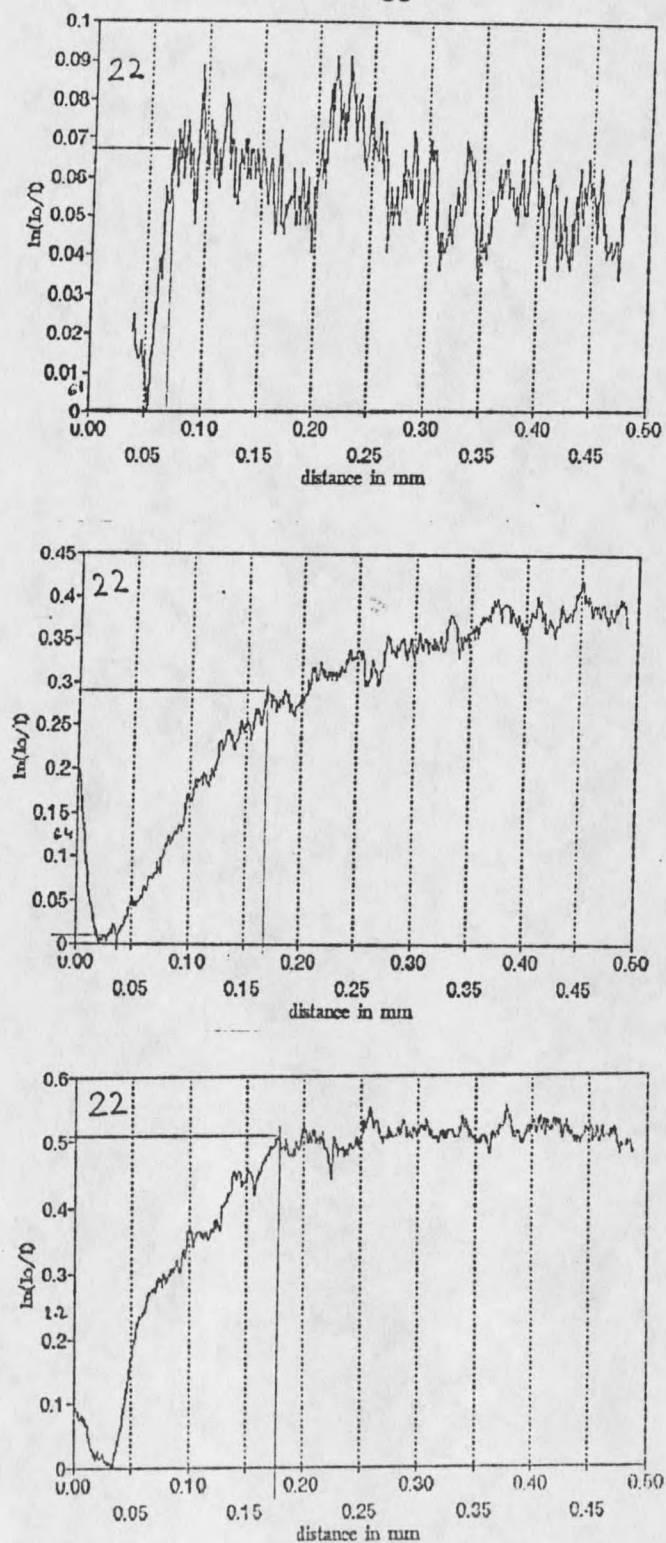


Figure 50. Absorbance measurements in 22 cm distance from the center of the disk.

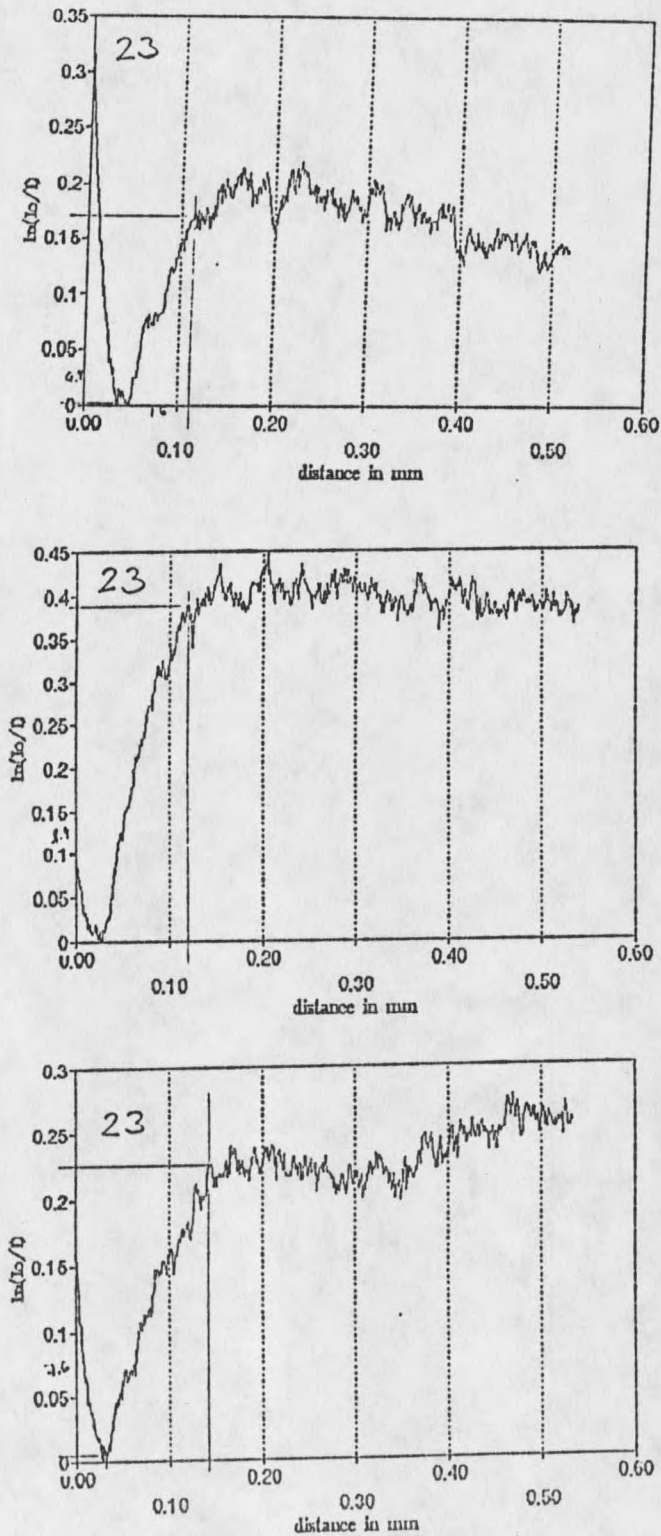


Figure 51. Absorbance measurements in 23 cm distance from the center of the disk.

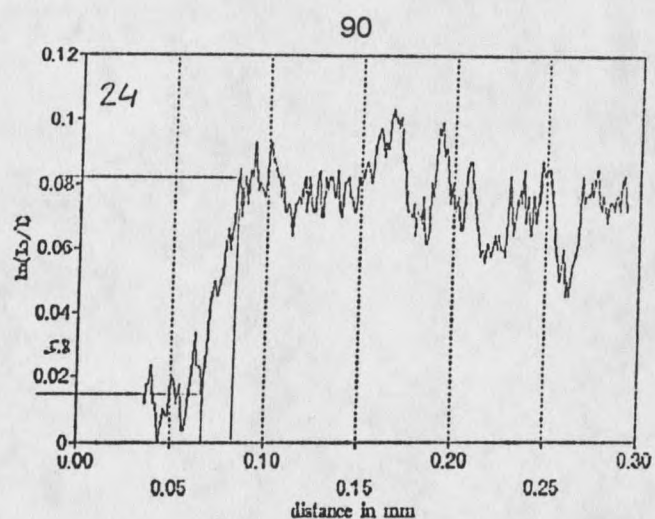
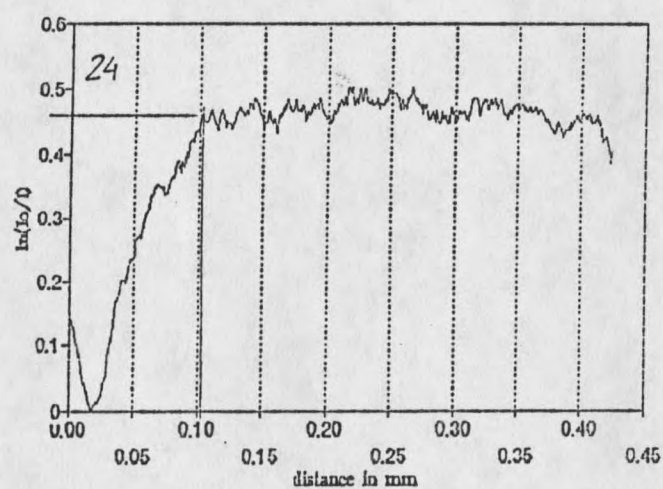


Figure 52. Absorbance measurements in 24 cm distance from the center of the disk.

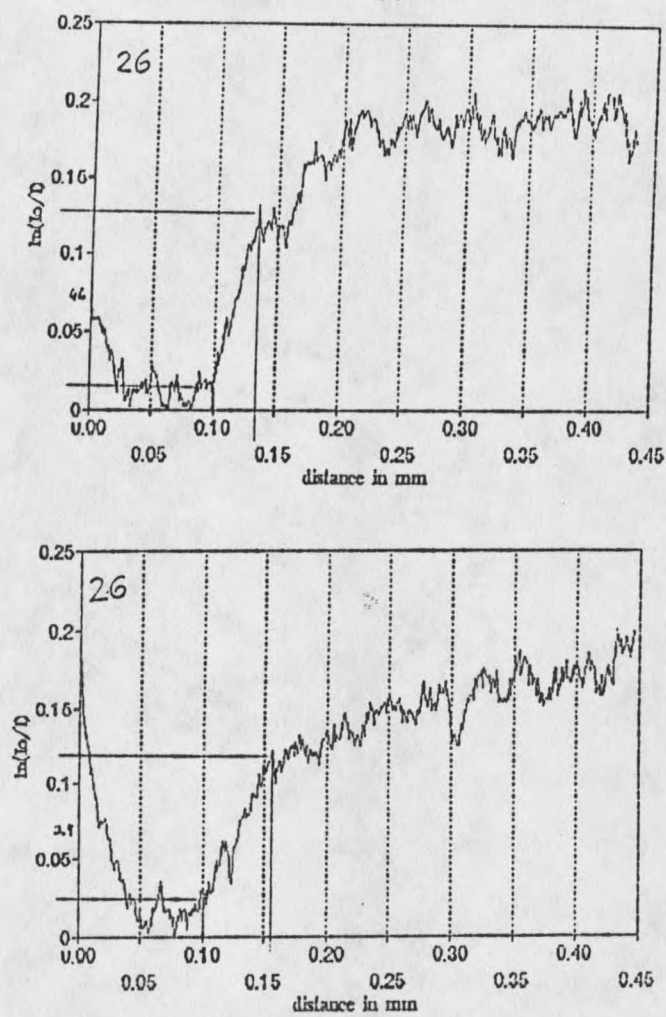


Figure 53. Absorbance measurements in 26 cm distance from the center of the disk.

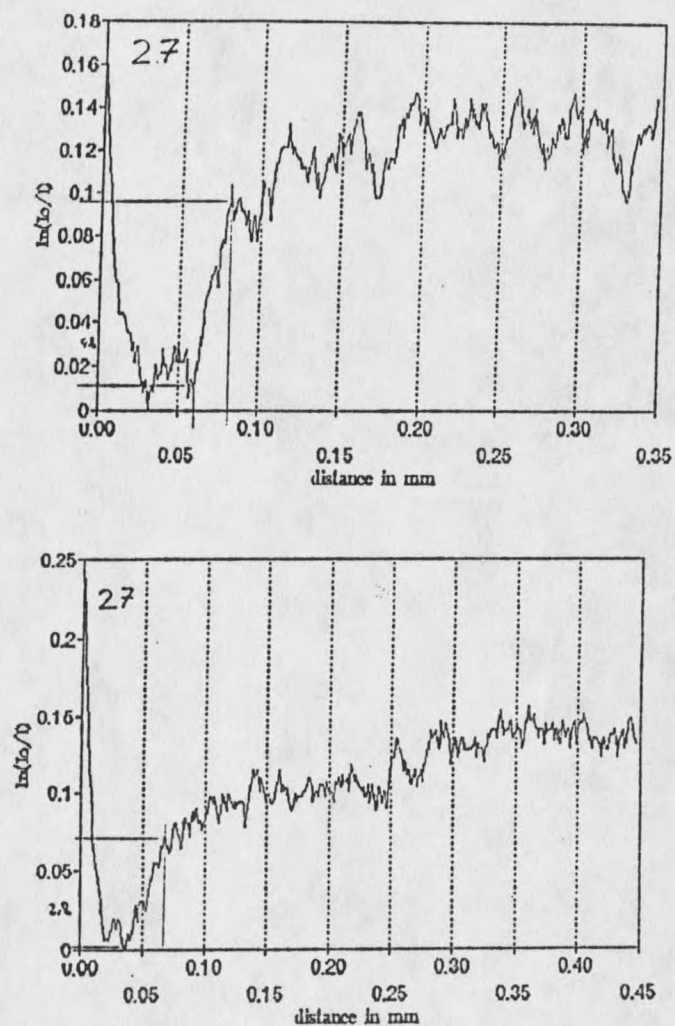


Figure 54. Absorbance measurements in 27 cm distance from the center of the disk.

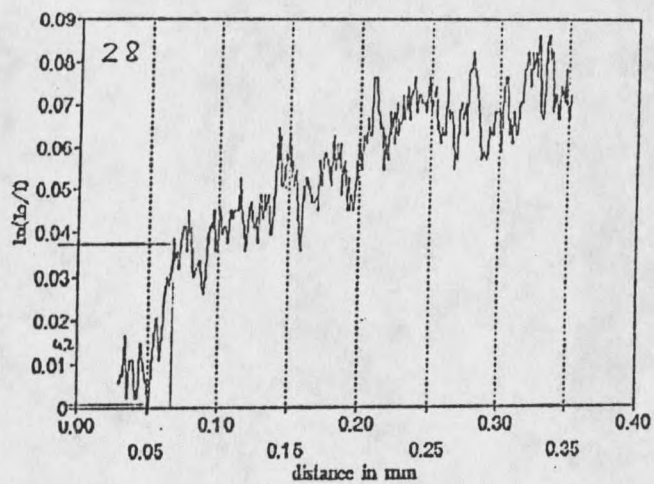
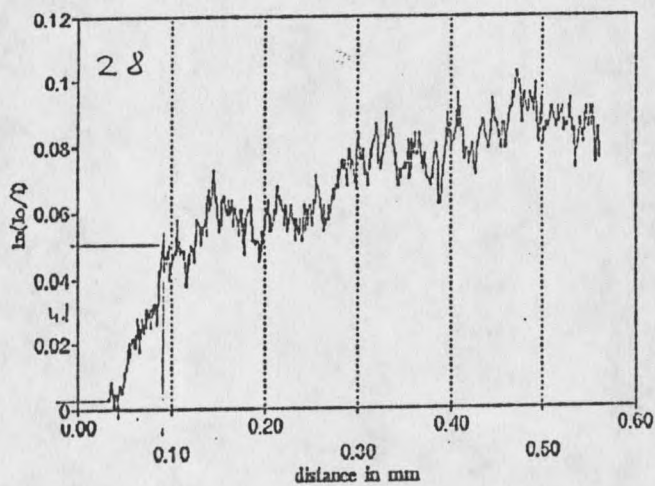


Figure 55. Absorbance measurements in 28 cm distance from the center of the disk.

APPENDIX E

THE VARIATION OF ABSORBANCE WITH DISTANCE FROM THE SUBSTRATUM
FOR A BIOFILM GROWN UNDER NON-LAMINAR FLOW CONDITIONS

The measurements were conducted on biofilm samples of biofilm grown under non-laminar flow conditions in the rotating disk reactor. The number in the upper left hand corner of the graph signifies the location from where the sample was taken. It gives the distance from the center of the disk in cm.

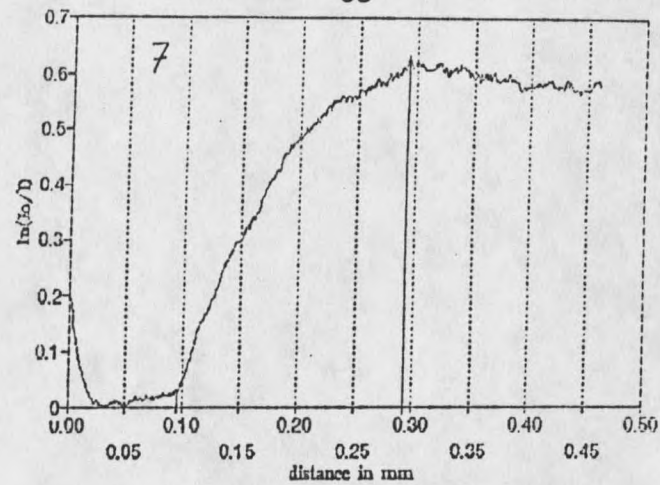
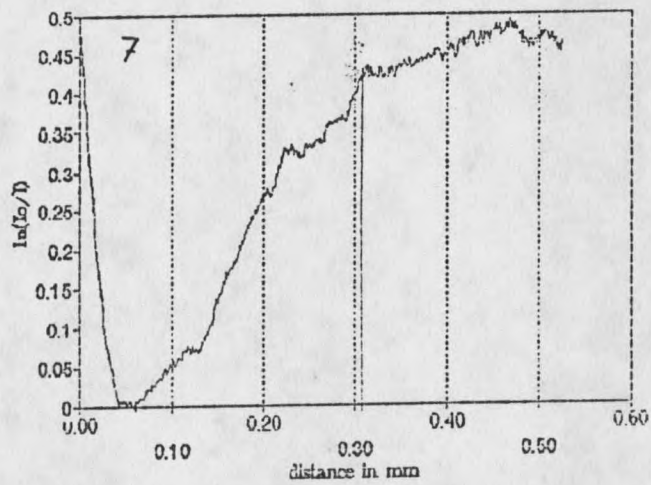
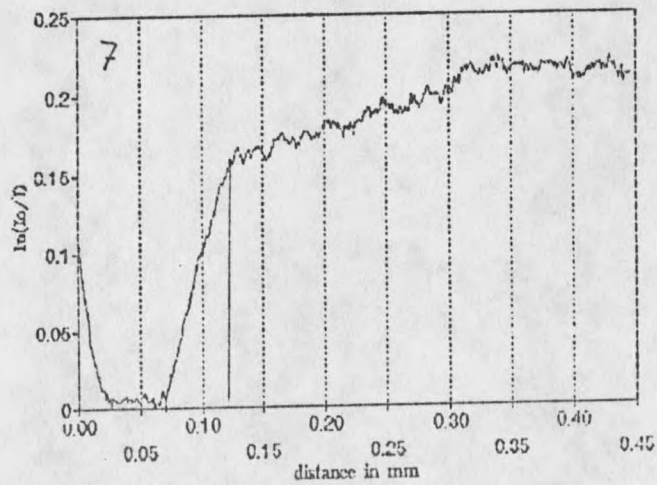


Figure 56. Absorbance measurements in 7 cm distance from the center of the disk.

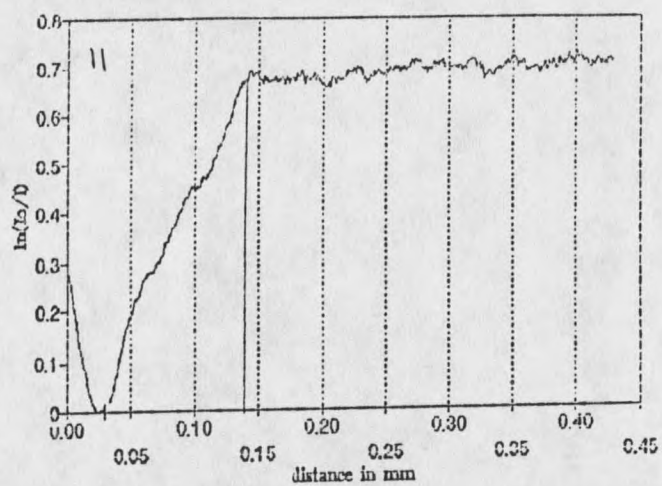
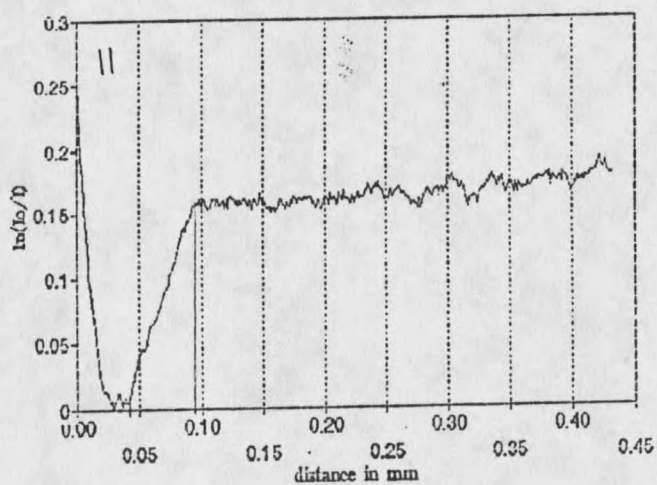


Figure 57. Absorbance measurements in 11 cm distance from the center of the disk.

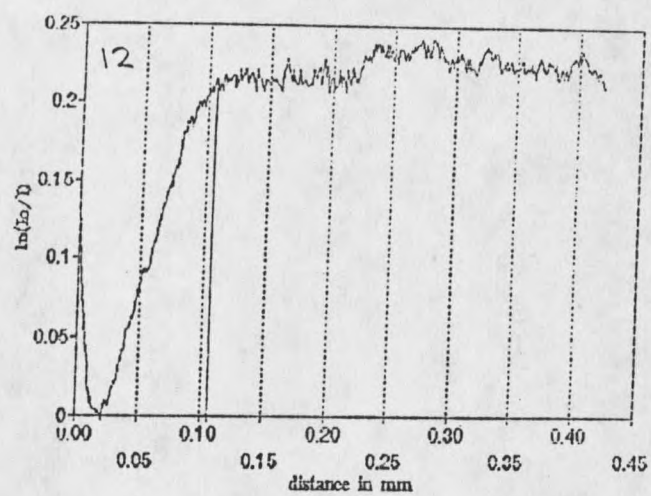
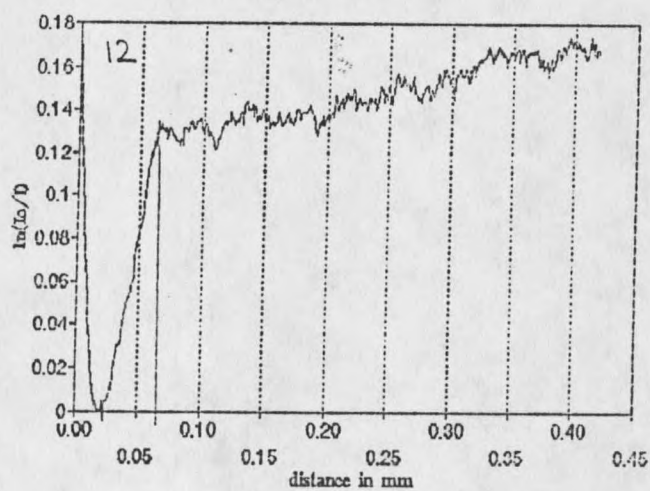


Figure 58. Absorbance measurements in 12 cm distance from the center of the disk.

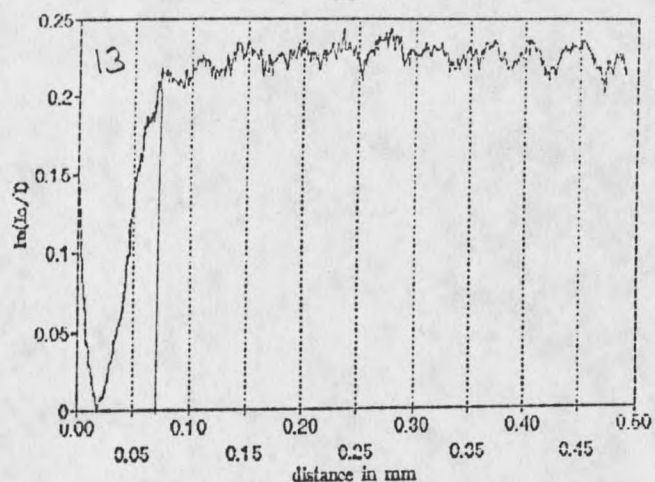
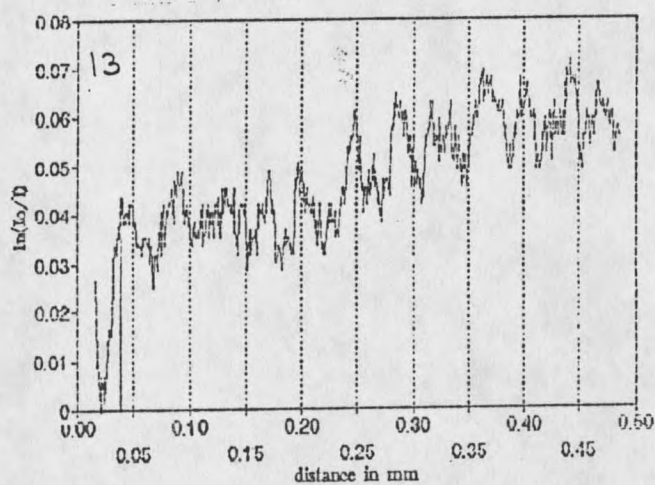
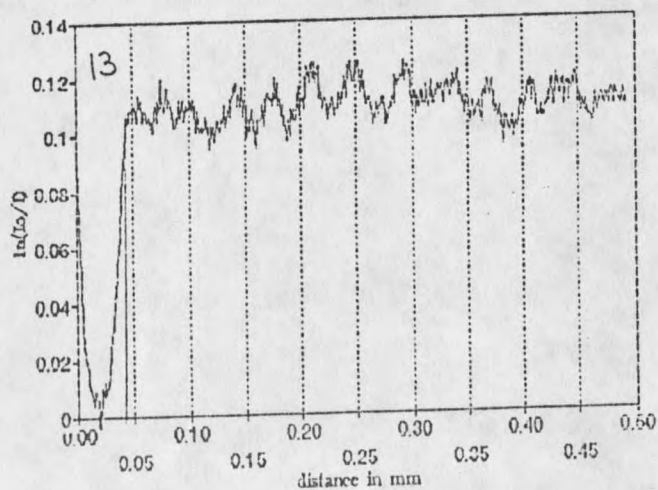


Figure 59. Absorbance measurements in 13 cm distance from the center of the disk.

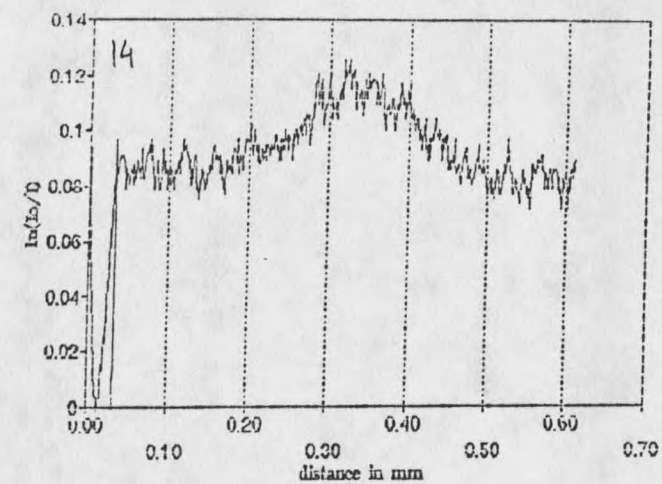
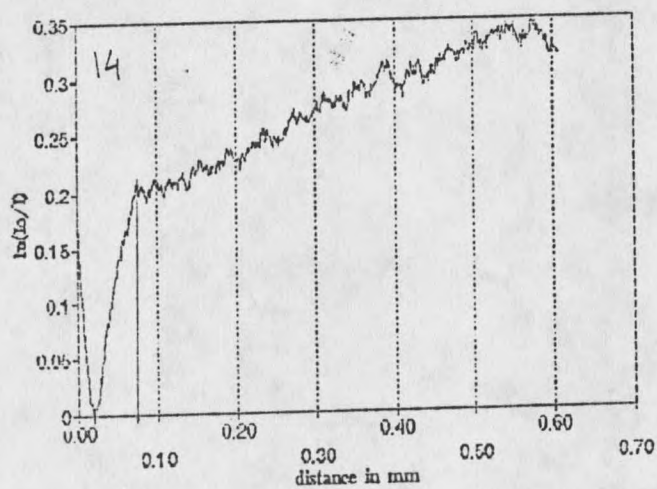


Figure 60. Absorbance measurements in 14 cm distance from the center of the disk.

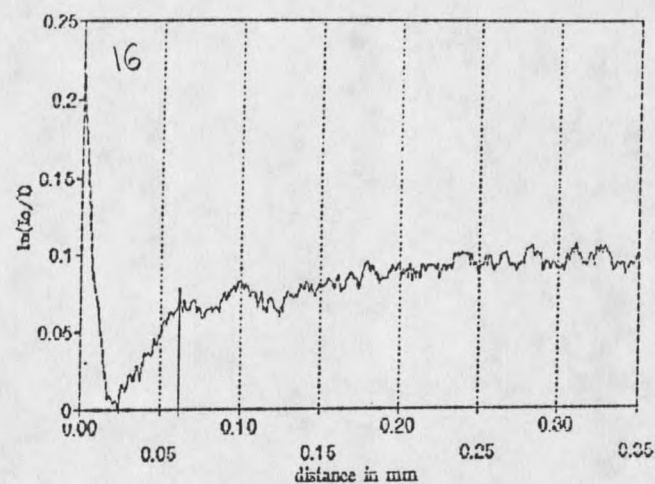
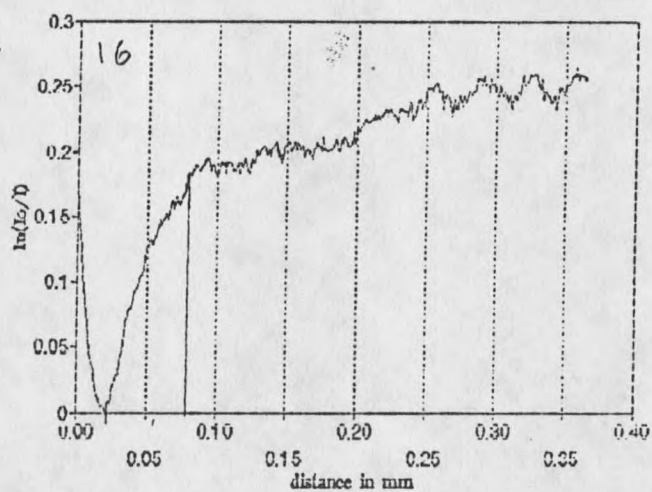
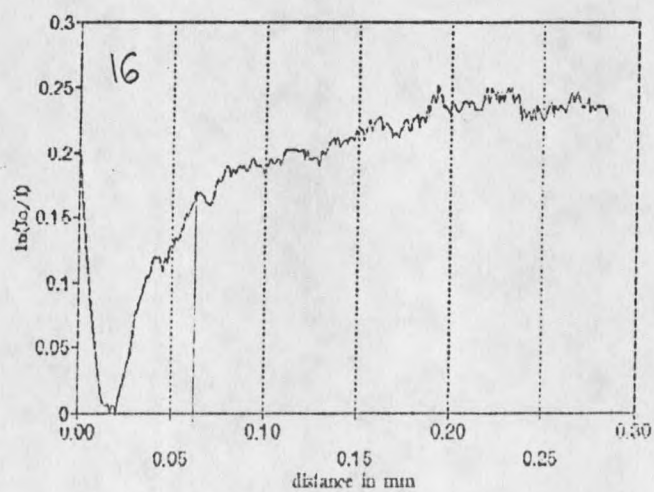


Figure 61. Absorbance measurements in 16 cm distance from the center of the disk.

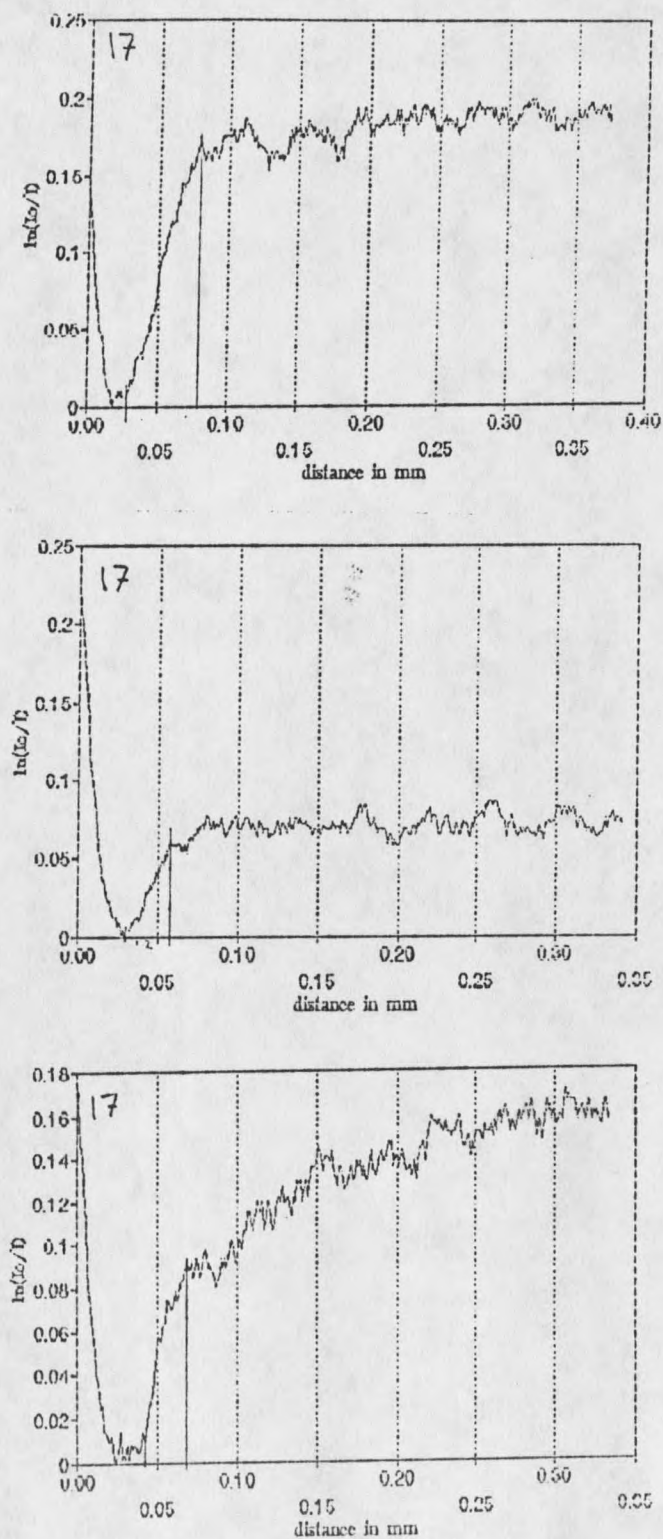


Figure 62. Absorbance measurements in 17 cm distance from the center of the disk.

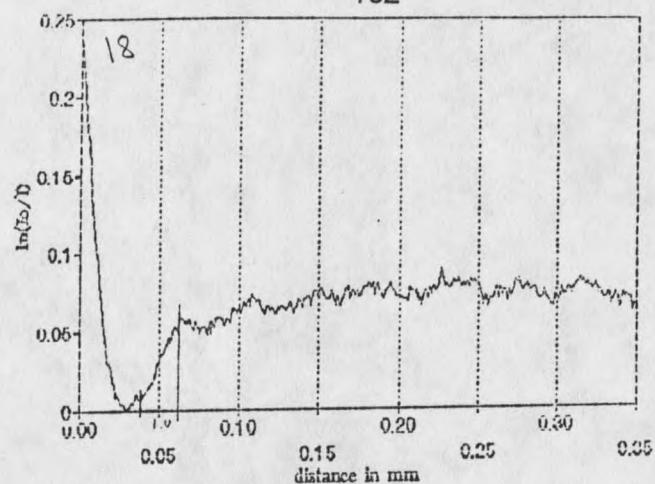
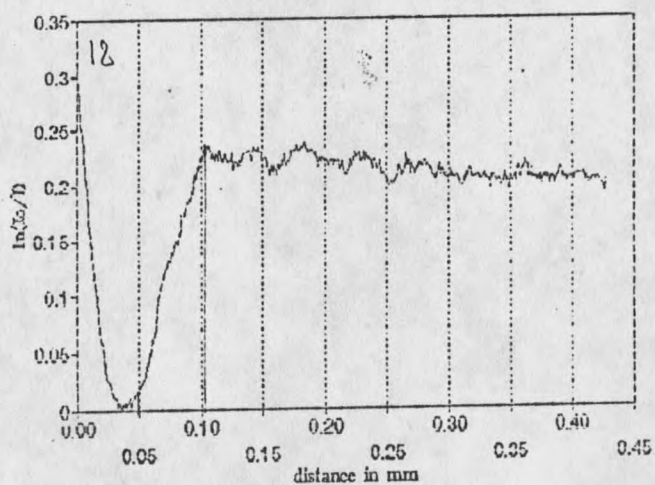
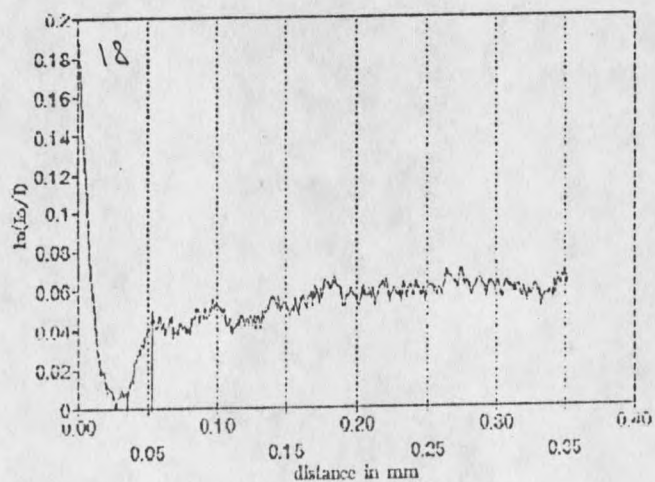


Figure 63. Absorbance measurements in 18 cm distance from the center of the disk.

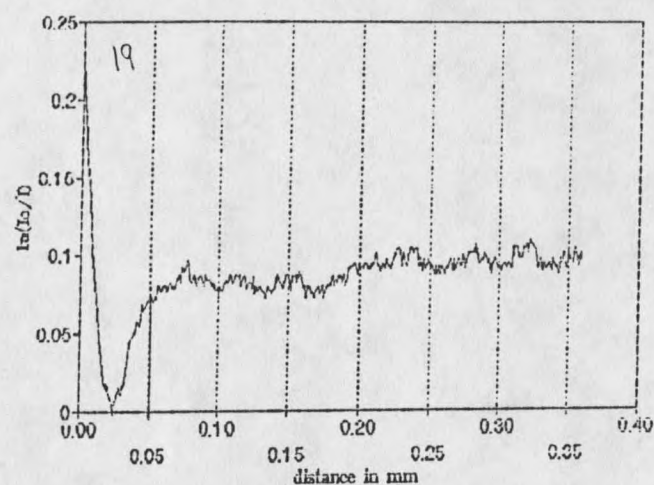
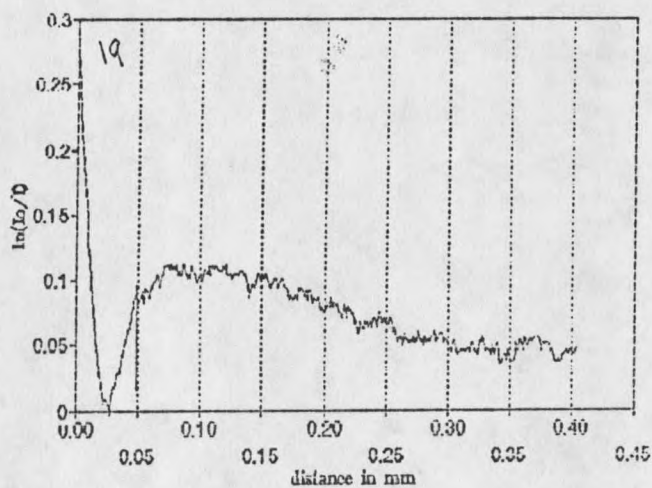
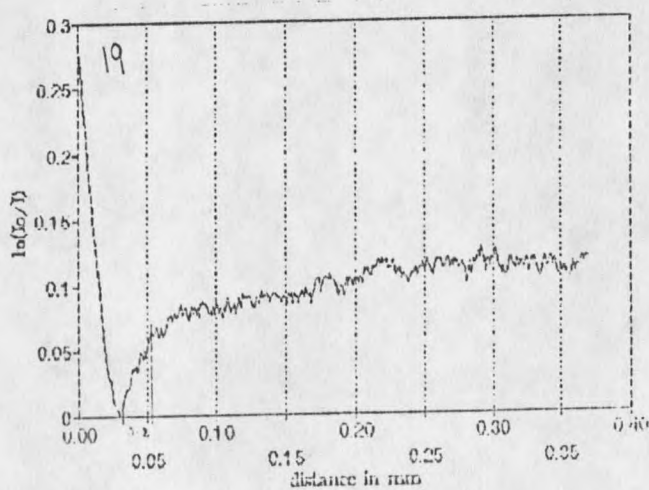


Figure 64. Absorbance measurements in 19 cm distance from the center of the disk.

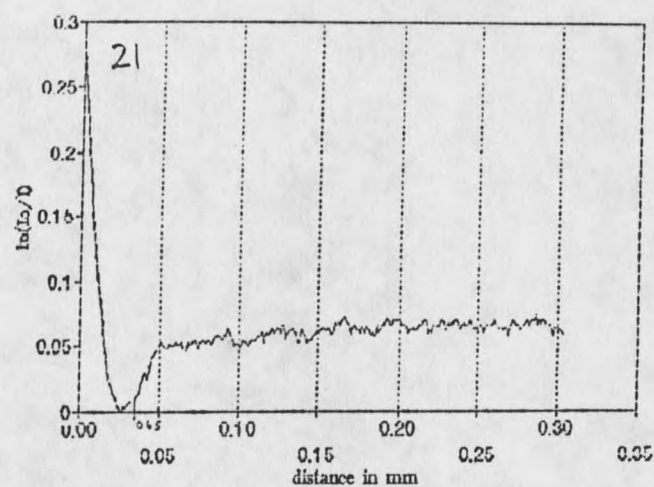
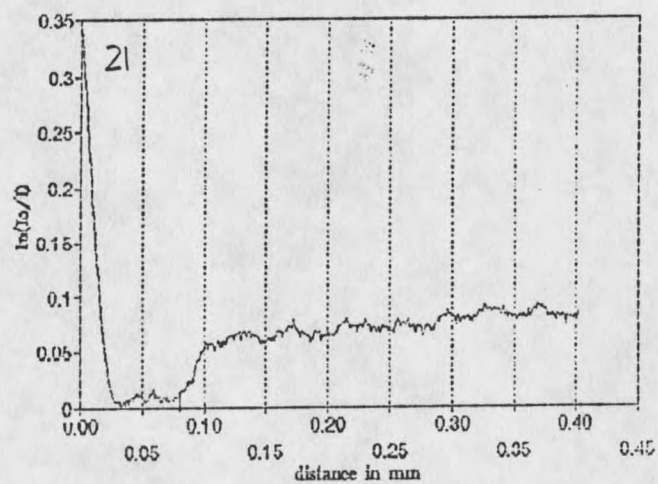


Figure 65. Absorbance measurements in 21 cm distance from the center of the disk.

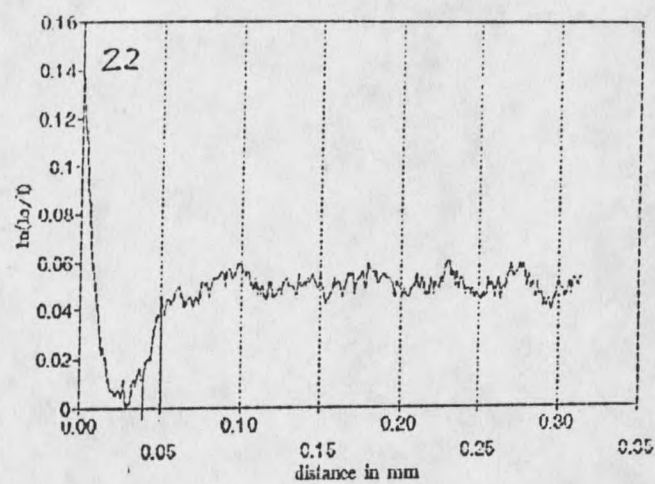
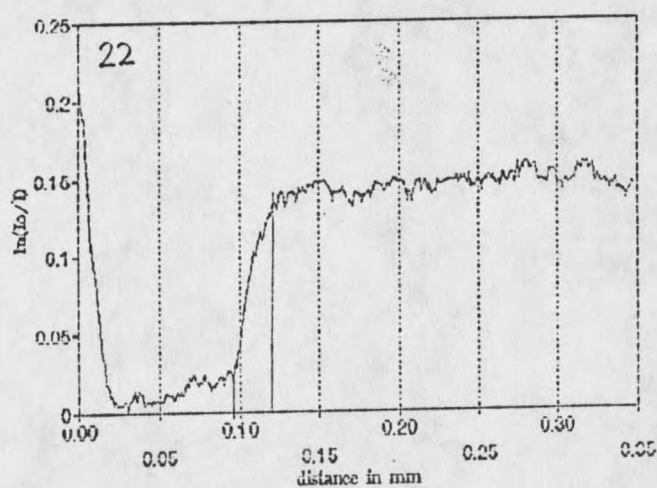
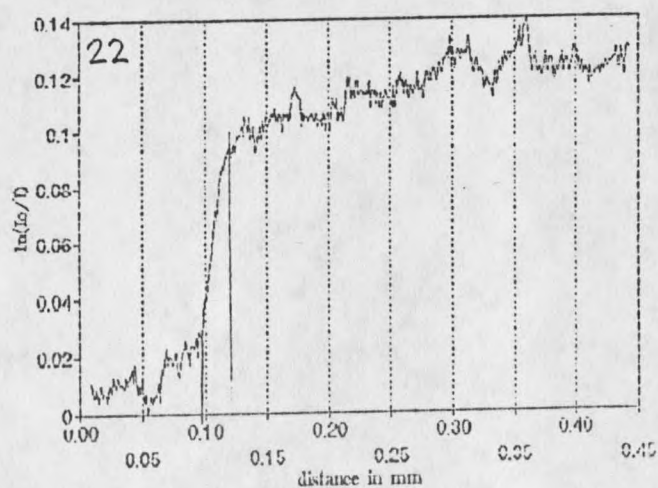


Figure 66. Absorbance measurements in 22 cm distance from the center of the disk.

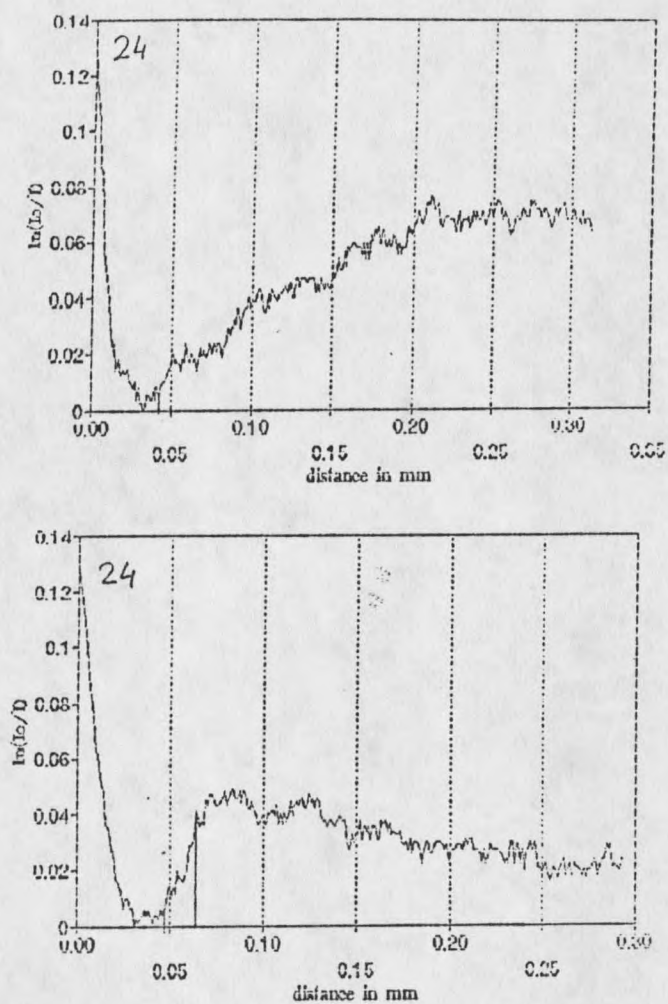


Figure 67. Absorbance measurements in 24 cm distance from the center of the disk.

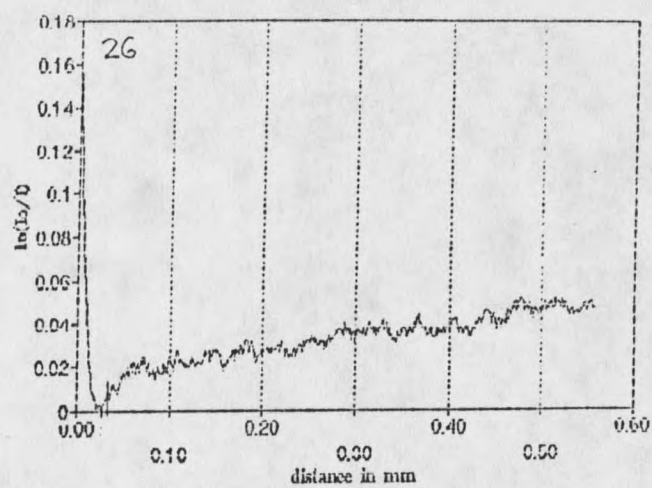
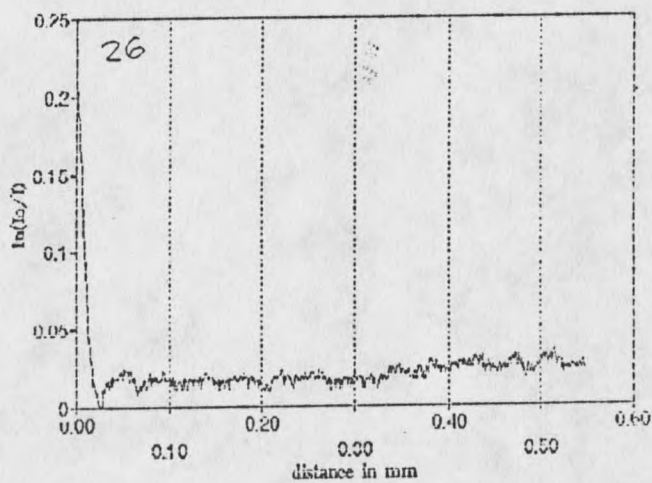


Figure 68. Absorbance measurements in 26 cm distance from the center of the disk.

APPENDIX F

LIGHT INTENSITY MEASUREMENT INTENSITY IN WATER

This measurement indicates the noise level of the sensor. The sensor was moved repeatedly up and down in the water above the biofilm. It did not touch the biofilm. The sharp falls in light intensity indicate a change in the direction of the movement. These falls were created by covering the light source while changing the direction of the sensor movement.

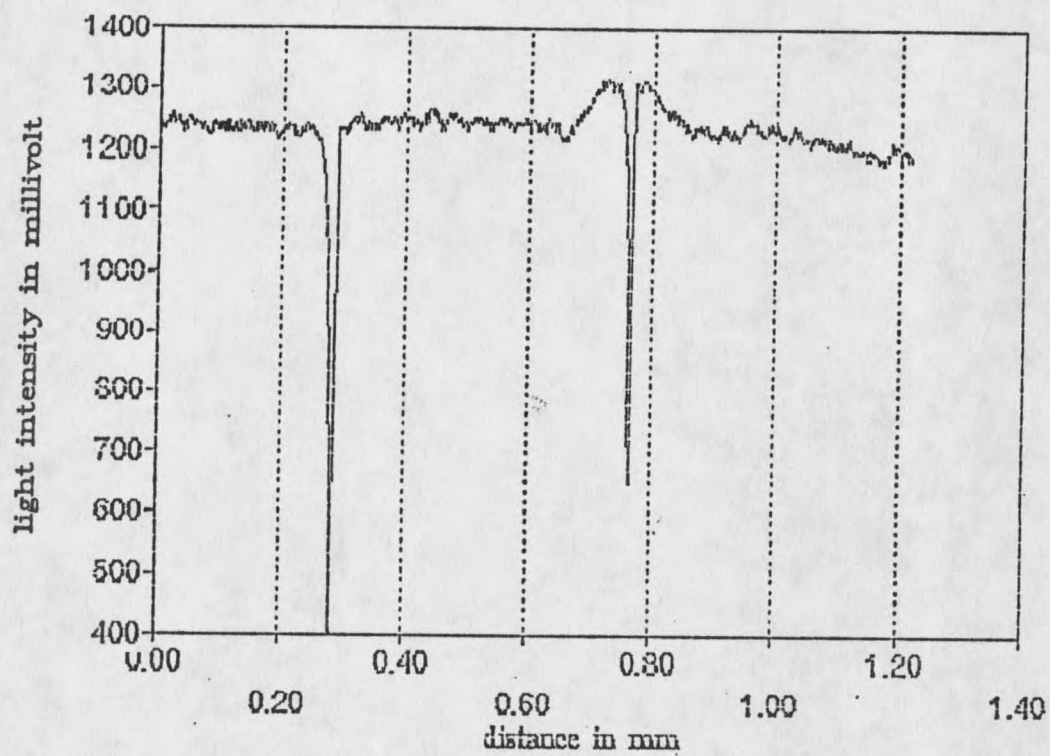


Figure 69. Light intensity measurement in water.

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