Growth kinetics of Pseudomonas aeruginosa
by Suet Nee Chen

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

Abstract:
The growth kinetics of Pseudomonas aeruginosa in continuous culture was investigated. A chemostat was used to grow Pseudomonas aeruginosa, and steady state glucose, dissolved oxygen concentrations and utilization rates, and microorganism concentrations were measured. The continuous system experiments were carried out at 25°C, pH = 7.2, with a stirrer agitation rate of 350 rpm. The growth of Pseudomonas aeruginosa was found experimentally to be limited by glucose and dissolved oxygen concentrations in the chemostat. The data taken from the steady state chemostat measurements were used to calculate growth kinetic parameters through nonlinear analysis using a multiple-substrate growth model of Pseudomonas aeruginosa. A dual-substrate growth kinetics model, using Tessier kinetics for both oxygen and glucose, was found to show good agreement with the experimental data. Respective growth kinetic parameters were evaluated to be μmax = 0.29 h-1, KgT = 26.9 mg/L, Kot =1.18 mg/L, Yx/g = 0.628 mg microorganism/mg glucose, and Yx/0 = 0.635 mg microorganism/mg oxygen. Maintenance factors for both glucose and oxygen were found to be mg = 0.0078 g glucose consumed/g microorganism hour and m0 = 0.014 g oxygen consumed/g microorganism hour. The coefficient for oxygen and maintenance factors for glucose and oxygen were evaluated from the constructed model for Pseudomonas aeruginosa.

The oxygen coefficients for Pseudomonas aeruginosa biofilms were calculated from the dissolved oxygen concentration profiles, measured by dissolved oxygen microelectrodes in the artificial Pseudomonas aeruginosa colony biofilms. Both the Tessier and Monod kinetic models were used to calculate the coefficients for oxygen. The Tessier coefficient for oxygen was 0.012 ± 0.003 mg/L and the Monod coefficient for oxygen was 0.18 ± 0.02 mg/L.

The oxygen coefficients calculated using both the Tessier and Monod kinetic models for Pseudomonas aeruginosa colony biofilms and for planktonic cultures were compared. The results from either Tessier or Monod kinetic model showed that the oxygen coefficient for a colony biofilm was lower than the oxygen coefficient for a planktonic culture. In conclusion, the growth kinetic parameters derived from planktonic cultures should not be applied in biofilms.

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Bozeman, Montana

November 2001

APPROVAL

Of a thesis submitted by

Suet Nee Chen

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.

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Dr. John T Sears John T. Sears (Signature) 9/26/01 Date

Approval for the College of Graduate Study

Dr. Bruce McLeod Bruce R. McLeod (Signature) 11-28-01 Date

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ACKNOWLEDGMENTS

First of all, I would like to express my gratitude to my research advisor Dr. Lewandowski for his guidance and support that enabled me to achieve my goal. I would like to particularly thank Dr. Beyenal for all his guidance, support, counsel, "patience" and assistance in experimental design. I would also like to thank Dr. Sears and Dr Stewart for their counsel.

I would especially like to acknowledge my parents, Kok Kong Chen and Mei Chin Kuan for their boundless support and love across the sea from Malaysia. "Dad and mum, thank you for your sacrifices and love in sending me to school in the United States."

Last but not least, Nurdan Beyenal, Gary Jackson, Kevin Braughton, Frank Roe and everyone at the Center for Biofilm Engineering, that has made my years at the Center a WONDERFUL time.

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NOMENCLATURE

A	Preexponential factor or frequency factor
b	Dimensionless half saturation constant
B_g	Constant in Contois model for glucose
B_i	Constant in Contois model for substrate *i*
B_o	Constant in Contois model for oxygen
c	Constant in Equation 2.20 (h^{-1})
CO_2	Carbon Dioxide
$C_5H_7NO_2$	Biomass
$C_6H_{12}O_6$	Glucose
C_{Nf}	Influent ammonium concentration (mg/L)
C_{Ne}	Effluent ammonium concentration (mg/L)
D	Dilution rate (h^{-1})
De	Diffusion coefficient of oxygen in water (cm^2/sec)
E	Activation energy (J/mol)
H_2O	Water
K_{gT}	Tessier coefficient for glucose (g/L)
K_{oT}	Tessier coefficient for oxygen (g/L)
K_{SM}	Monod coefficient (g/L)

K_{ST}	Tessier coefficient (g/L)
K_{SMz}	Mozer coefficient (g/L)
K_{SA}	Andrews coefficient (g/L)
K_{SE}	Edwards coefficient (g/L)
K_{SL}	Luong coefficient (g/L)
K_{STW}	Tseng and Wayman coefficient (g/L)
K_{Si}	Coefficient for substrate i (g/L)
K_{iA}	Andrews substrate inhibition constant (g/L)
K_{iE}	Edwards substrate inhibition constant (g/L)
L_f	Biofilm thickness (μm)
m_g	Maintenance factor for glucose (h^{-1})
m_i	Maintenance factor for limiting substrate i (h^{-1})
m_o	Maintenance factor for oxygen (h^{-1})
mo	Microorganism
n	Exponential constant for Equation 2.19
na	not available
NH_4^+	Ammonium
O_2	Oxygen
OUR	Oxygen uptake rate (mg oxygen/h)

Q	Flow rate (L/h)
R	Gas constant, 8.314 J/mol·K
Rr	Reaction rate, (g/h)
S	Substrate concentration (g/L)
S_{ei}	Substrate concentration in effluent stream (g/L)
$S_{experiment}$	Experimental substrate concentration (g/L)
S_{fg}	Concentration of glucose in fresh feed (g/L)
S_{fi}	Substrate concentration in influent stream (g/L)
S_{fn}	Concentration of ammonium sulfate in fresh feed (g/L)
S_g	Concentration of glucose in chemostat (g/L)
S_i	Concentration of substrate i (g/L)
S_m	Substrate concentration above which growth is completely inhibited (g/L)
S_o	Concentration of dissolved oxygen (g/L)
S_{os}	Concentration of dissolved oxygen at the surface of the colony biofilm measured using dissolved oxygen microelectrode (g/L).
SOUR	Specific oxygen uptake rate (g oxygen/g microorganism/h)
$S_{predicted}$	Predicted substrate concentration (g/L)
S*	Substrate concentration under which microorganism can not grow in equation 2.20 (g/L)

S^*	Dimensionless substrate concentration
t	Time (sec)
T	Temperature (C^o)
V	Reactor volume (L)
X	Microorganism concentration in chemostat (g/L)
X_f	Biofilm density (g microorganism/L)
$Y_{x/g}$	Yield coefficient for glucose (g microorganism/g glucose)
$Y_{x/o}$	Yield coefficient for oxygen (g microorganism/g oxygen)
$Y_{x/si}$	Yield coefficient for limiting substrate *i* (g microorganism/g limiting substrate)
z	Space coordinate in biofilm (μm)
z*	Dimensionless space coordinate in biofilm

Greek letters

μ	Specific growth rate (h^{-1})
$\mu_{experimental}$	Experimental specific growth rate (h^{-1})
μ_i	Specific growth rate for limiting substrate *i* (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
μ_{model}	Theoretical specific growth rate (h^{-1})

λ_g	Mozer's constant for glucose (g/L)
λ_i	Mozer's constant for substrate i (g/L)
λ_o	Mozer's constant for oxygen (g/L)
Φ	Dimensionless Thiele Modulus

ABSTRACT

The growth kinetics of *Pseudomonas aeruginosa* in continuous culture was investigated. A chemostat was used to grow *Pseudomonas aeruginosa*, and steady state glucose, dissolved oxygen concentrations and utilization rates, and microorganism concentrations were measured. The continuous system experiments were carried out at 25°C, pH = 7.2, with a stirrer agitation rate of 350 rpm. The growth of *Pseudomonas aeruginosa* was found experimentally to be limited by glucose and dissolved oxygen concentrations in the chemostat. The data taken from the steady state chemostat measurements were used to calculate growth kinetic parameters through nonlinear analysis using a multiple-substrate growth model of *Pseudomonas aeruginosa*. A dual-substrate growth kinetics model, using Tessier kinetics for both oxygen and glucose, was found to show good agreement with the experimental data. Respective growth kinetic parameters were evaluated to be μ_{max} = 0.29 h^{-1}, K_{gT} = 26.9 mg/L, K_{oT} = 1.18 mg/L, $Y_{x/g}$ = 0.628 mg microorganism/mg glucose, and $Y_{x/o}$ = 0.635 mg microorganism/mg oxygen. Maintenance factors for both glucose and oxygen were found to be m_g = 0.0078 g glucose consumed/g microorganism·hour and m_o = 0.014 g oxygen consumed/g microorganism·hour. The coefficient for oxygen and maintenance factors for glucose and oxygen were evaluated from the constructed model for *Pseudomonas aeruginosa*.

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The oxygen coefficients calculated using both the Tessier and Monod kinetic models for *Pseudomonas aeruginosa* colony biofilms and for planktonic cultures were compared. The results from either Tessier or Monod kinetic model showed that the oxygen coefficient for a colony biofilm was lower than the oxygen coefficient for a planktonic culture. In conclusion, the growth kinetic parameters derived from planktonic cultures should not be applied in biofilms.

CHAPTER 1

INTRODUCTION

Literature Review

Pseudomonas aeruginosa is often used in biofilm studies and in modeling biofilm accumulation (Robinson et al., 1984; Bakke et al., 1984; Wanner et al., 1997; Wirthanem et al., 1999), probably because microbial geneticists have been studying this organism intensively, and its physiology and genetics are well known. Growth kinetic parameters for microbial growth of *Pseudomonas aeruginosa* have been determined in biofilms by Bakke et al., (1984), and in planktonic cultures by Robinson et al., (1984). However, in both papers, the growth parameters of *Pseudomonas aeruginosa* have been determined at relatively low glucose concentrations, less than 7.5 mg/L in the chemostat (Bakke et al., 1984) and less than 1.4 mg/L in the biofilm reactor (Robinson et. al., 1984). The only reasonable conclusion as to why both authors used such low glucose concentrations was to assure that glucose – NOT oxygen – was the limiting substrate.

A number of studies have shown that biofilm accumulation is related to the growth rate of the planktonic microorganisms before their attachment to the surface (Anwar et. al., 1991). Bakke et. al., (1984) has shown that *Pseudomonas aeruginosa* does not behave differently in biofilms than in suspension at steady state. In contrast, it has also been suggested by Fletcher et. al., (1983), and Brown et. al., (1990), that the growth kinetic parameters in biofilms are different from the growth kinetic

parameters derived from planktonic cells. Nonetheless, there are no consistent results predicting how the microbial growth would be different between planktonic and biofilm cells.

It is well known that substrate concentrations decrease in the deeper parts of biofilms, due to mass transfer limitations and substrate consumption by the microorganisms. Therefore, in biofilm, the growth of *Pseudomonas aeruginosa* may be simultaneously limited by more than a single substrate, (Livingston et. al., 1989, Bailey and Ollis, 1986; Keen and Prosse, 1987). In this case, multiple-substrate growth kinetics of microorganisms must be employed to describe and to represent the growth of the microorganism.

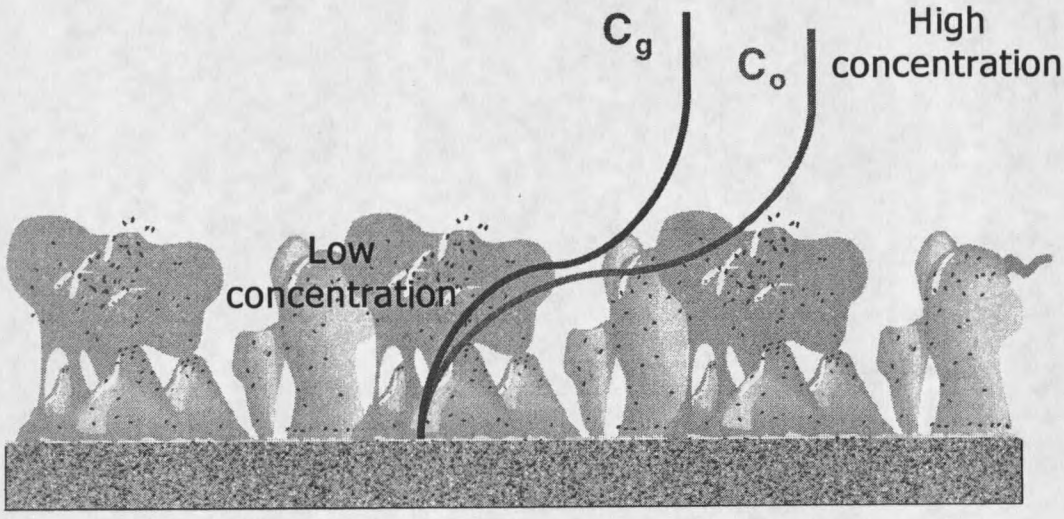

Figure 1.1. Concentration distribution in biofilms.

However, there are inherent difficulties associated with developing relevant multiple-substrate growth models. These difficulties stem from the necessity of providing relevant experimental data and solving non-linear equations. Appropriate techniques to build such models are available (Venkatesh et al., 1997; Beyenal and Tanyolac, 1997). A model can be provided and solved with a reasonable amount of experimental data. The growth dependence of microorganisms on both substrates can be predicted from independent data sets where only a single substrate is limiting.

Several techniques have been proposed in the literature for determining the growth kinetic parameters of biofilms. A published computer simulation program for biofilms, AQUASIM, has been used by several authors to compare experimental data to computer-simulated data (Wanner et. al., 1995; Arcangeli et. al., 1999; Horbel et. al., 1999). However, models derived from AQUASIM are applicable only when the given influent substrate concentration is changing slowly and dissolved oxygen concentration is treated as a state variable. Another technique involved in the measurement of growth kinetic parameters using biofilm cultures is to treat the biofilm as a pseudo suspended culture. This is done by disrupting the biofilm culture (Jih et. al., 1994; Cao et. al., 1995). However, it was shown by de Beer et. al., (1993), that the constituents and the structure of biofilm, such as extracellular polymeric substances (EPS), cell structure, channels and voids, all greatly affected the substrate distribution in the biofilm. In the 1970's and 1980's, dissolved oxygen microelectrodes were introduced into the field of microbial ecology, and became the popular tools for *in situ* analysis of oxygen distribution and microbial respiration in

biofilms (Bungay et. al., 1969; Revsbech et. al., 1986). Dissolved oxygen microelectrodes are able to measure very low concentrations. Their high sensitivity allows them to detect small oxygen concentration changes in the biofilms and has provided investigators with quality in situ experimental data with minimal mass loss and disruption to the structure of the biofilms (Riefler et. al., 1997). In addition, the application of dissolved oxygen microelectrodes in measuring the dissolved oxygen concentration in biofilms has resolved inherent experimental errors from chemical specific analyses and increased the accuracy of growth kinetic parameters estimation. Therefore, microelectrodes are the recent most accurate instruments for evaluating the growth kinetics of biofilms.

From an engineering perspective, a mathematical growth model would have significant importance in predicting the rate and extent of biofilm growth in bioreactors and the feasibility of instruments used in chemical industry. In order to predict the rate and extent of biofilm growth correctly, one must address the question: Could the growth kinetic parameters calculated from planktonic cultures be used in predicting the growth rate and extent of biofilm cells? Thus, the research objectives, hypotheses and methods of this study were designed to answer the above question.

Research Objectives

The objectives of this study were:

1) To produce experimental growth data of *Pseudomonas aeruginosa* in planktonic form.
2) To derive a multiple-substrate growth model for the planktonic cultures of *Pseudomonas aeruginosa.*
3) To measure dissolved oxygen concentration profiles in *Pseudomonas aeruginosa* biofilms using dissolved oxygen microelectrodes.
4) To calculate the coefficient of oxygen in *Pseudomonas aeruginosa* biofilms from the measured dissolved oxygen concentration profiles.
5) To compare the coefficient of oxygen for *Pseudomonas aeruginosa* in planktonic cultures and in biofilms.
6) To answer the question: Can we apply the growth kinetic parameters derived from planktonic cultures in biofilms?

Hypotheses

My hypotheses are:

1.) Glucose (also a limiting substrate to the growth of *Pseudomonas aeruginosa*) should be included in kinetic models to better describe the growth of planktonic cultures of *Pseudomonas aeruginosa.*

2.) The coefficient of oxygen in *Pseudomonas aeruginosa* colony biofilm is different from the half rate coefficient of oxygen in planktonic cultures.

Research Methods

A continuous system chemostat was used to measure glucose, dissolved oxygen concentrations and consumption rates, ammonium sulfate, and microorganism concentration at steady state. These results were used to derive multiple-substrate growth model for *Pseudomonas aeruginosa*. Chemostats have long been utilized in the laboratory to grow bacteria. The pH and the temperature in the chemostat were changed to calculate the optimum growth condition for *Pseudomonas aeruginosa*. Under steady state conditions, the dilution rate is equal to the growth rate of the bacteria. The experimental data collected at steady state, at optimum pH and temperature, and at different dilution rates were then used to develop a multiple substrate growth model for *Pseudomonas aeruginosa* using non-linear solution techniques.

For *Pseudomonas aeruginosa* biofilm, the coefficient for oxygen was extracted from dissolved oxygen concentration profiles measured with a dissolved oxygen microelectrode. Artificial *Pseudomonas aeruginosa* colony biofilms were grown on the surface of the black polycarbonate membrane filters placed on enriched agar. After the colony biofilms reached maturity, the dissolved oxygen concentration was measured using a dissolved oxygen microelectrode. These dissolved oxygen

concentration profiles were then used to extract the coefficients of oxygen in *Pseudomonas aeruginosa* colony biofilms.

CHAPTER 2

BACKGROUND

Physiology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative, aerobic, rod-shaped, environmentally adaptable bacterium, belonging to the bacterial family *Pseudomonadaceae,* and comprise the informal group of bacteria known as **Pseudomonads**. *Pseudomonas aeruginosa* is an opportunistic pathogen of humans that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in the victims of severe burns, and in cancer and AIDS patients who are immunosuppressed.

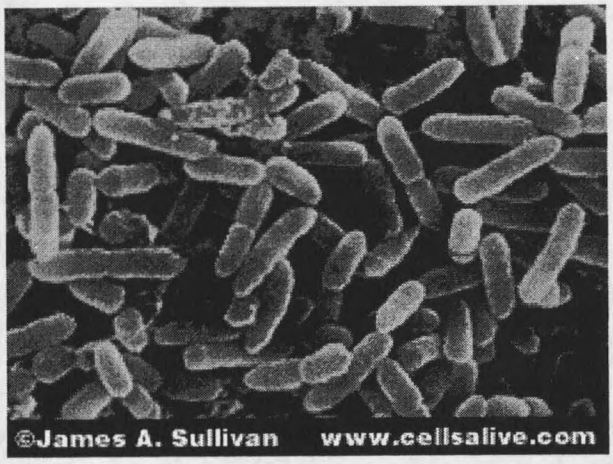

Figure 2.1. *Pseudomonas aeruginosa.*