THESIS

This is to certify that the

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The Development of a Biochemical Engineering Teaching Laboratory

presented by

Andrew Burkett Kinney

has been accepted towards fulfillment of the requirements for

M.S. degree in Chem. Engr.

Robert Mark Worden

Major professor

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THE DEVELOPMENT OF A BIOCHEMICAL ENGINEERING TEACHING LABORATORY

By

Andrew Burkett Kinney

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Chemical Engineering

1992

ABSTRACT

THE DEVELOPMENT OF A BIOCHEMICAL ENGINEERING TEACHING LABORATORY

By

Andrew Burkett Kinney

Biotechnology is an expanding field with great economic potential for engineers able to apply chemical engineering fundamentals to problems in microbiology and biochemistry. There is a shortage of engineers trained in the life sciences for monitoring, designing and scaling-up biotechnology processes. To meet the educational needs of the growing biotechnology industry, a undergraduate biotechnology laboratory was developed. The experiments for the laboratory were designed to minimize operating costs, waste disposal problems, and demonstrate applications of chemical engineering principles in biotechnology. This thesis incorporates six experiments: batch ethanol fermentation, enzyme kinetics, fermentation mass transfer, fermentation power transfer, immobilized biocatalysts and membrane filtration. Each experiment is a chapter of the thesis with its own abstract, introduction, background, theoretical analysis, results, conclusions, recommendations, and an appendix containing specific experimental protocols.

"October knew, of course that the action of turning a page, of ending a chapter or of shutting a book, did not end a tale.

Having admitted that, he would also avow that happy endings were never difficult to find: 'It is simply a matter,' he explained to April,'of finding a sunny place in the garden, where the light is golden and the grass is soft; somewhere to rest, to stop reading and be content.'"

-Neil Gaiman, *The Season of Mists*

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INTRODUCTION

Biotechnology is a rapidly growing field with great economic potential for engineers who can integrate chemical engineering fundamentals into the context of microbiology and biochemistry. Engineers must respond to opportunities in biotechnology by applying analytical approaches like unit operations and transport phenomena (Wei, 1988). Many studies have indicted a shortage of "well-trained bioprocess engineers" for monitoring, designing and scaling-up biotechnology processes. The development of biotechnology requires individuals who are trained to operate in an interdisciplinary environment of biology, biochemistry and engineering (*Commercial*, 1984). To produce engineers to accomplish this task, the curriculum taught in universities must be revised to teach new concepts (Wei, 1988). Most existing universities' bioprocess engineering programs focus primarily on graduate level education (*Commercial*, 1984). Recently universities have anticipated the needs of a mature biotechnology industry and have designed curricula at the B.S. level which focus on recombinant DNA techniques and bioengineering (Carter, 1987).

Commercializing biotechnology requires innovative chemical engineers with strong life- science and process-engineering backgrounds to achieve large-scale implementation of biotechnology. The new university curricula must be developed to coordinate academic programs in engineering with the life sciences to facilitate interdisciplinary collaborations. Chemical engineers are needed to work in interdisciplinary groups to transform the results of health and biotechnology research into practical products and applications. Some of the areas identified for research and

modeling are: biological interactions, biological surfaces and interfaces, bioprocessing, separations, and engineering analysis of complex biological systems. In addition, facilities for education and research in biotechnology must be financed and constructed (*Frontiers*, 1988).

To meet the educational needs of the growing biotechnology industry, Michigan State University expanded its undergraduate biotechnology course offerings. A substantial investment was made to develop an undergraduate biochemical engineering laboratory, which is now one of the best equipped laboratories of its type in the country. The general course content of the laboratory was developed based on the needs of the biotechnology industry and the biotechnology curricula that existed in other departments. The experimental topics of the laboratory were formulated as nine summaries that briefly described the topics and goals of each experiment. Based on those abstracts, the laboratory experiments were developed over a two-year period by a graduate student as a Master's thesis project. The project entailed selecting, purchasing, installing, operating, and designing equipment for the laboratory as well as utilizing equipment previously purchased for the laboratory. Each experiment described in the abstract was developed through literature and experimental research, and designed to minimize operating costs and waste disposal problems, while demonstrating applications of chemical engineering principles in biotechnology. Ultimately, the list was expanded to ten laboratory topics: aseptic techniques, batch ethanol fermentation, continuous and annular chromatography, continuous sterilization, enzyme kinetics, fermentation mass and power transfer, immobilized biocatalysts, membrane filtration, and plasmid stability.

The biotechnology laboratory also emphasizes modernization of the curricula, in that four of the experiments utilize computerized data acquisition and control, and other experiments use microprocessor controlled fermentors and motors. The laboratory also focuses on scale-up techniques for mass and power transfer using a 100 gallon pilot-scale fermentor for large-scale experiments.

This thesis consists of six chapters, each of which contains one of the following experiments: batch ethanol fermentation, enzyme kinetics, fermentation mass transfer, fermentation power transfer, immobilized biocatalysts and membrane filtration. Time limitations prevented the complete design and optimization of all of the experiments so only the six that were fully completed are included in the thesis. The experiments are presented in a format similar to the laboratory reports required for the class but include specific experimental protocols in the appendix of each chapter.

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Batch Ethanol Fermentation

Summary

Glucose was converted to ethanol by the microorganism *Zymomonas mobilis* in a microprocessor controlled fermentor. The fermentor was steam-sterilized, inoculated, and maintained at a pH 6.5, 30°C, and agitated at 150 rpm. The concentrations of glucose, cell mass, and ethanol were monitored for a several-hour period of the fermentation. The specific growth rate, the specific ethanol-production rate, and the cell and ethanol yield coefficients were calculated. The results calculated from the experimental data varied significantly from the literature values in some cases. The experimental results indicated that the method of determining glucose concentration was in error.

Introduction

Fermentations use microorganisms to catalyze chemical reactions that produce substances such as pharmaceuticals, organic acids, and alcohols. Fermentations can be used to produce a large variety of products, but their commercial applications are limited by their economic competitiveness with alternative processes. Advances in recombinant DNA technology allow fermentations to produce complex biomolecules such as human insulin that cannot be synthesized by other means.

The most widely known fermentation process is the production of ethanol. Batch yeast fermentations have been used for hundreds of years to produce alcoholic beverages such as beer and wine. Ethanol can be produced from a wide variety of plant-derived

starting materials as is evidenced by the large selection of alcoholic beverages for human consumption. Ethanol can also be produced from agricultural wastes such as the chaff from wheat or the remnants of sugar cane after the sugar has been removed.

Ethanol has many advantages for use as an alternative to non-renewable fossil fuels. It is a liquid and easily transported, has a heating value 2/3 that of gasoline, and can be blended with gasoline for use as an additive to increase the octane rating of the fuel. Ethanol was used as a primary fuel before and during World War II, but was replaced by cheaper petroleum and petrochemical products. Acetone and butanol production by fermentation were also displaced by petroleum distillates (Bailey and Ollis, 1986). The unstable global economic forces that control petrochemical prices make ethanol fuel production by fermentation a high economic risk since a drop in oil prices could bankrupt an ethanol-fuel producer. However, the need for renewable energy resources may spur an increase in the production of ethanol from agricultural products by fermentation for use as a fuel.

Innovative processing strategies are required to make ethanol production economically competitive with petrochemical products. New developments such as continuous fermentations with a cell recycle and vacuum operation allow a twelve-fold increase in productivity over conventional processes (Bailey and Ollis, 1986). Tower fermentors are used to retain flocculent yeast, minimizing process costs by eliminating the need for auxiliary separation devices and a cell recycle to maintain high cell concentrations (Bailey and Ollis, 1986). Yeasts are sometimes replaced by bacteria that reproduce faster and give higher yields of ethanol (Worden, 1982).

Background

Fermentations that are used to produce a specific product must be carried out in a pure culture to assure that only the desired product is produced. Contamination of a fermentation by unwanted microorganisms can result in inconsistencies in the product or failure to produce the products. Contamination is minimized in the initial phases of the fermentation by sterilizing the reactants and any components of the fermentor that will come in contact with the fermentation. The procedures that prevent contamination of the fermentation by foreign organisms after sterilization are known as aseptic techniques.

Typically the fermentor is sterilized to kill any organisms present and then it is inoculated by aseptically adding a small quantity of the microorganisms selected for the fermentation. After inoculation, a batch fermentation goes through four phases: the lag phase, the exponential growth phase, the stationary phase, and the death phase. The lag phase is the time period of adjustment by the cells to their new environment before they begin growth after inoculation. The cell concentration increases exponentially in exponential or logarithmic growth phase until the substrates are used up or high product concentrations inhibit the reaction. Cell growth ceases in the stationary phase, and cells begin to die in the death phase.

A fermentation is monitored by measuring the substrate, product, and cell concentrations during the fermentation. Common methods for determining glucose and ethanol concentrations use spectrophotometric measurements of enzymatic reaction products. Unknown samples are combined with enzymatic reagents, and the optical density of the mixture is measured and compared to a calibration curve constructed using

known standards. Cell concentrations are usually measured by either the optical density of the fermentation broth or the cell dry weight. The optical density (O.D.) of a sample of fermentation broth is often referred to as the turbidity or the absorbance. Absorbance measurements are quicker and more precise than dry weight determinations but must be converted to cell concentration units using a calibration curve. Cell dry weights provide a direct measurement of cell mass, which is needed for carbon and electron balances. The dry cell weight is determined by removing a known volume of fermentation broth, centrifuging and rinsing the cells to remove nonvolatile salts and substrates, and then drying and weighing the cells. The cell dry weight is the weight of the dried cells divided by the volume of the original sample of fermentation broth. Dry-weight measurements are time consuming and imprecise at low concentrations. However they become more accurate at the high cell concentrations present in the late exponential phase where they can be used to create a calibration curve for O.D. versus cell concentration.

Theoretical Analysis

A rod shaped, anaerobic, gram negative, bacterium known as *Zymomonas mobilis* (ATCC 10988) is often used to produce ethanol instead of yeasts because it grows and produces ethanol faster than yeasts (Worden, 1982). *Z. mobilis* grows well at high temperatures and is often used to brew alcoholic beverages in tropical climates. The general reaction catalyzed by the cells is shown below:

$$\textit{ammonia} + \textit{glucose} \xrightarrow{\textit{cells}} \textit{ethanol} + \textit{cells} + \textit{carbon dioxide} \qquad (1)$$

Zymomonas mobilis grows well between pH 5.5 and 8 in solutions containing up to 20% glucose and 1% yeast extract, a powdered nutrient obtained from yeast. The carbon dioxide produced by the fermentation acidifies the medium, inhibiting the fermentation, unless the pH is maintained by adding base or buffering the fermentation broth (Worden, 1982).

Batch fermentations are autocatalytic in nature because as the reaction proceeds the concentration of the reaction catalyst, cells, increases exponentially with time. The rate equation for cell growth is:

$$\frac{dx}{dt} = \mu x \tag{2}$$

where x is the cell concentration and t is time. The specific growth rate, μ, is a function of temperature, pH, and the substrate and product concentrations. The specific growth rate is constant early in the fermentation until the substrate is consumed and inhibitory products accumulate to a significant level. When μ is constant, Equation (2) can be integrated to yield:

$$\ln(x) = \mu t + c \tag{3}$$

where c is an integration constant. Equation (3) indicates that a plot of ln(x) versus t should be linear with a slope equal to the specific growth rate. Excellent linear fits for μ are not uncommon for the growth of *Z. mobilis* over a several-hour period (Worden, 1982).

The mass of product per unit mass of substrate is given by the yield coefficient,

$Y_{p/s}$. The slope of a plot of the product concentration versus the substrate concentration gives the yield coefficient $Y_{p/s}$. Alternatively, $Y_{p/s}$ may be determined using substrate and product concentrations from any two times during the fermentation. When using this method, the changes in the substrate and product concentrations should be as large as possible to minimize the error due to imprecisions in the concentration data.

An electron balance can be used to evaluate the relative chemical-energy contents of the fermentation products and substrates to determine the reaction efficiency. The electron balance entails comparing the total number of available electron equivalents contained in the substrate consumed to that in the products formed. The balance can be performed using initial and final concentration data. The electron balance is a combination of elemental mass balances because it is based upon reaction stoichiometry. Electron balances use the reductance degrees of the substrates and products relative to their combustion products. The reductance degree, γ, of a compound is defined as the number of equivalents of electrons available for transfer to molecular oxygen during combustion on a C-mole basis. One C-mole is the mass of substance containing 1 mole (12 g) of carbon. Reductance degrees are calculated using values of 4, 1, -2, and -3 for the number of available electrons from carbon, hydrogen, oxygen, and nitrogen atoms, respectively. For example, the reductance degree of the glucose ($C_6H_{12}O_6$) is calculated below.

$$\gamma = \left[\frac{6\ C}{C_6H_{12}O_6}\frac{4e^-}{C} + \frac{12\ H}{C_6H_{12}O_6}\frac{1e^-}{H} + \frac{6\ O}{C_6H_{12}O_6}\frac{-2e^-}{O}\right]\frac{C_6H_{12}O_6}{6\ Cmol} = \frac{4e^-}{Cmol}$$

where e^- = electron equivalent

The chemical formula of *Z. mobilis* cells can be assumed to be $CH_{1.8}O_{0.5}N_{0.2}$, indicating that 1 C-mole of *Z. mobilis* cells is 24.6 grams. Table 1 shows a sample calculation for a typical set of experimental data. The cell and ethanol yields are expressed in g product produced per g glucose consumed. Ammonia and carbon dioxide are not considered in this balance, because their reductance degrees are zero. Yeast extract is ignored in the balance, because *Z. mobilis* does not metabolize carbon sources within the yeast extract to a significant degree. Since 1 gram of glucose contains 0.133 electron equivalents, the electron balance for this example would be within 2% of converting all of the glucose to ethanol and cells.

Table 1. Available Electrons in Fermentation Products

	Product Yield	Yield	γ	Electron Equivalents
Ethanol	0.480	0.0209	6	0.125
Cells	0.032	0.0013	4.2	0.005
		Total Electron Equivalents		0.130

The electron balance can also be interpreted as a type of energy balance because heats of combustion are directly proportional to reductance degrees by a factor of 27.5 kcal/e^- for a wide range of organic materials, such as alcohols, sugars, alkanes and biomass. The energy-recovery efficiency of the fermentation can be evaluated using the enthalpy of the reactants and products. In the example above, 94% of the available electrons were recovered in the ethanol, and only 4% in the biomass.

Experimental Equipment and Procedure

The fermentation broth for the experiment consisted of 2 wt % glucose and 1 wt % yeast extract in a 0.01 M succinate buffer at pH 7. The glucose was autoclaved separately from the yeast extract and the succinate buffer to prevent darkening of the solution. The yeast extract and succinate were autoclaved in a 1000 ml flask equipped with an aseptic transfer apparatus shown in Figure 1. The fermentation vessel was autoclaved as shown in Figure 2, containing 500 ml of 4% glucose dissolved in the succinate buffer. Four 250 ml flasks, two of which contained 50 ml and two containing 150 ml of 10% glucose and 2% yeast extract, were also autoclaved with cotton stoppers. Two Pasteur pipettes with cotton plugs near the top were autoclaved wrapped in paper towels to preserve sterility for aseptic transfer.

A culture of *Z. mobilis* (ATCC 10988) was revived from freezed dried storage by aseptically transferring the cell pellet and resuspending the cells in 5 ml of sterile 5% glucose and 1% yeast extract solution. The 5 ml sample was incubated at 35°C for three days until a significant increase in bubbling and turbidity was observed. Portions of the 5 ml revived culture were transferred to two 250 Erlenmeyer flasks containing 25 ml of sterile medium containing 5% glucose and 1% yeast extract. One of the flasks was used as a storage culture and the other was used as a working culture. The working culture was used to inoculate a flask containing 100 ml of fermentation broth which was used as the inoculum culture for the fermentor.

The fermentor used was a microprocessor controlled, New Brunswick

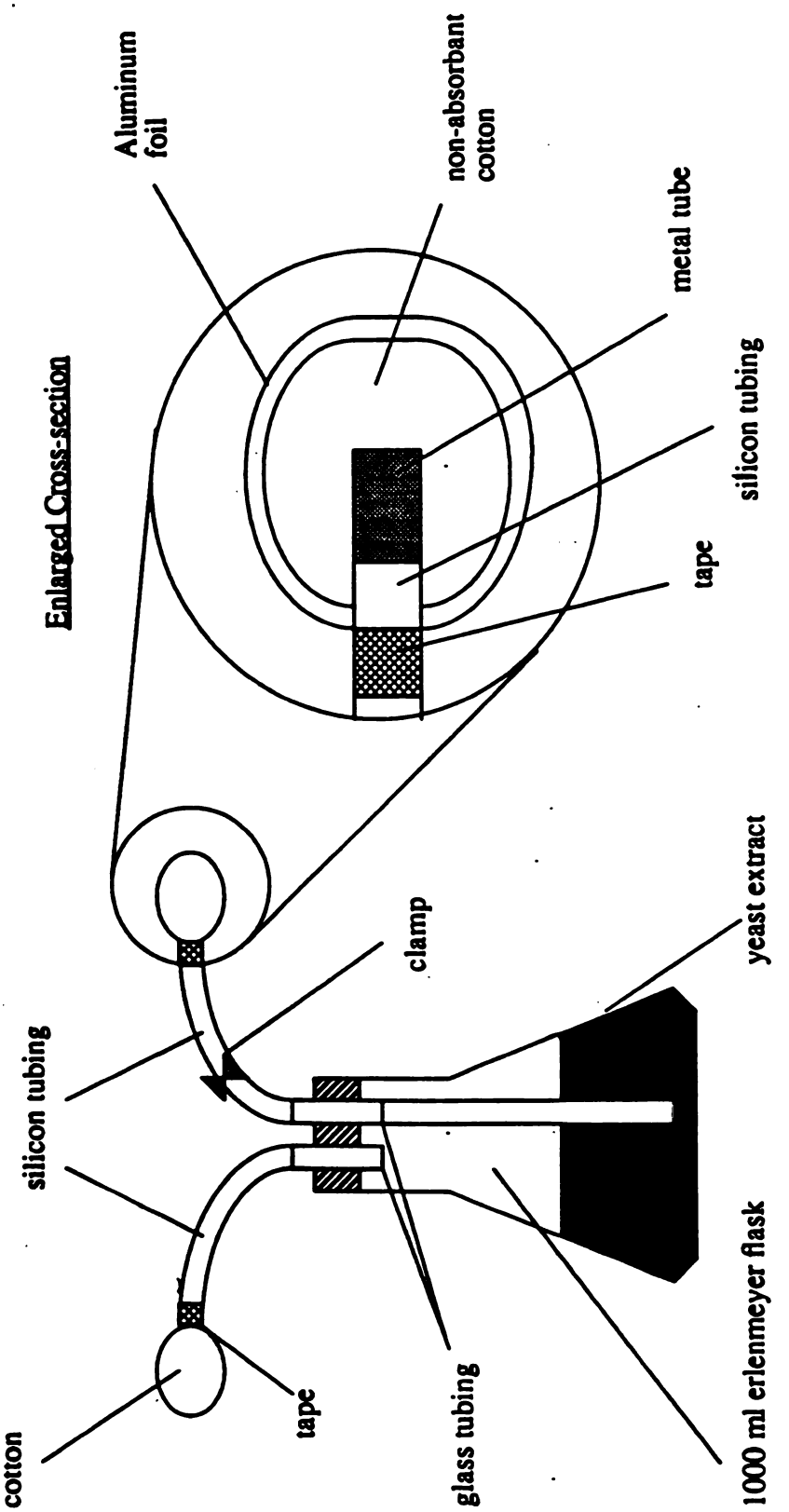

Figure 1. Setup for Autoclaving and Aseptic Transfer of Yeast Extract Fraction

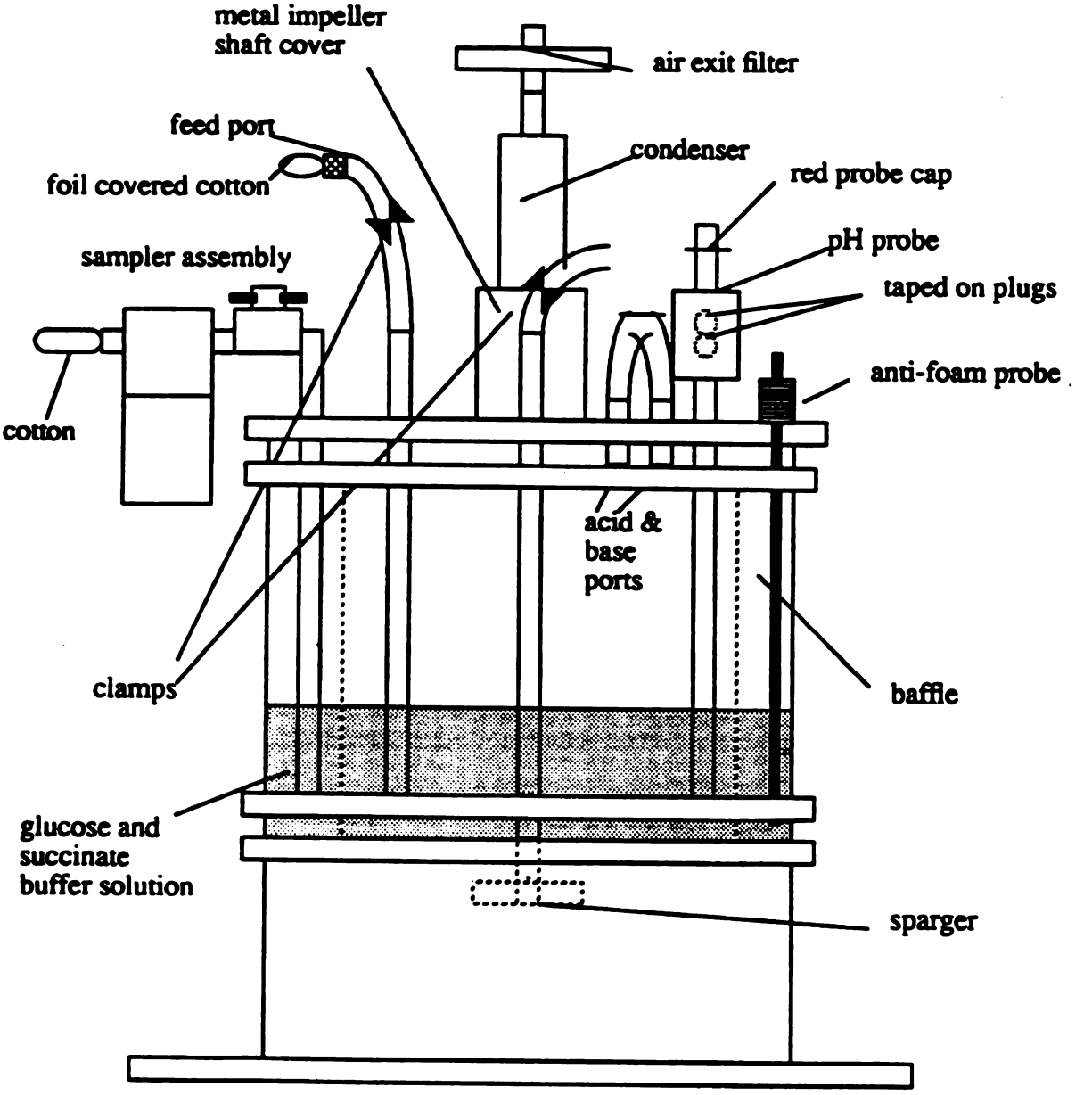

Figure 2. Simplified View of Fermentor Setup for Autoclaving

BioFloIIC$_{m}$, which is capable of maintaining a set pH, temperature, and agitation rate. The pH was measured by a steam-sterilizable pH probe. The temperature was maintained at 35°C and was controlled by a heat exchanger unit contained in the bottom of the fermentation vessel. The two equally-spaced Rushton, 6-bladed turbines were used agitate the fermentor at 150 rpm. The cell, ethanol, and glucose concentrations were measured and recorded at various times during the fermentation. The cell concentration was assayed using both optical density and dry weight measurements. The ethanol concentrations were determined using a YSI autoanalyzer which was calibrated against known standards. The glucose was assayed using the YSI autoanalyzer, which was also calibrated against known standards.

Results and Discussion

The results of the experiment varied from the literature values significantly in most cases. Figure 3 shows the cell growth curves for both dry weight and optical density. The specific growth rate was determined by linear regression from the slope of a plot of ln(O.D.) versus time. The specific growth rate determined was 0.33 ± 0.01 hr^{-1} at 35°C. The literature value for the specific growth rate of *Z. mobilis* is 0.526 hr^{-1} at 36°C (Worden, 1982). The optical density data taken throughout the experiment were correlated with the dry weight measurements as shown in Figure 4 to estimate cell mass concentrations for the electron balance. The line was forced through zero for the linear regression, since the Lambert-Beer law indicates that absorbance should be directly proportional to concentration (Boyer, 1986).

Figure 5 illustrates the concentration profiles of glucose, cells and ethanol during the course of the fermentation as well as a glucose concentration profile calculated using the electron balance and the experimental ethanol and cell concentrations. Figure 6 demonstrates the scatter of the glucose data and the difference between the experimental glucose concentration and the glucose concentration calculated from the electron balance. The total number of electron equivalents calculated from the experimental data was not constant over time, conflicting with the definition of a closed batch system. Figure 7 shows the relative contribution of each component of the fermentation to the total number of electron equivalents present during the course of the fermentation. Figure 7 clearly demonstrates that the experimental glucose concentration contributes most of the fluctuation in the total number of electron equivalents until the last two hours of the fermentation. The most likely explanation is that the glucose membrane for the YSI autoanalyzer was not allowed sufficient time to equilibrate before the glucose assays were performed. The fluctuation in the ethanol concentration was attributed to the slow degeneration of the membrane due to operation above the specified temperature for the membrane. The equipment lacked the temperature control component designed to lower the operating temperature of the unit to increase the operating life of the ethanol membrane.

The ethanol yield coefficient, $Y_{EtOH/glucose}$ ($Y_{p/s}$), was estimated from the experimental data to be 0.735 $\pm$ 0.134 g ethanol produced per g glucose consumed and the fit to the data is shown in Figure 8. Similarly, Figure 9 shows the experimental cell yield to be 0.181 $\pm$ 0.019 g cells produced per g glucose consumed. The literature data

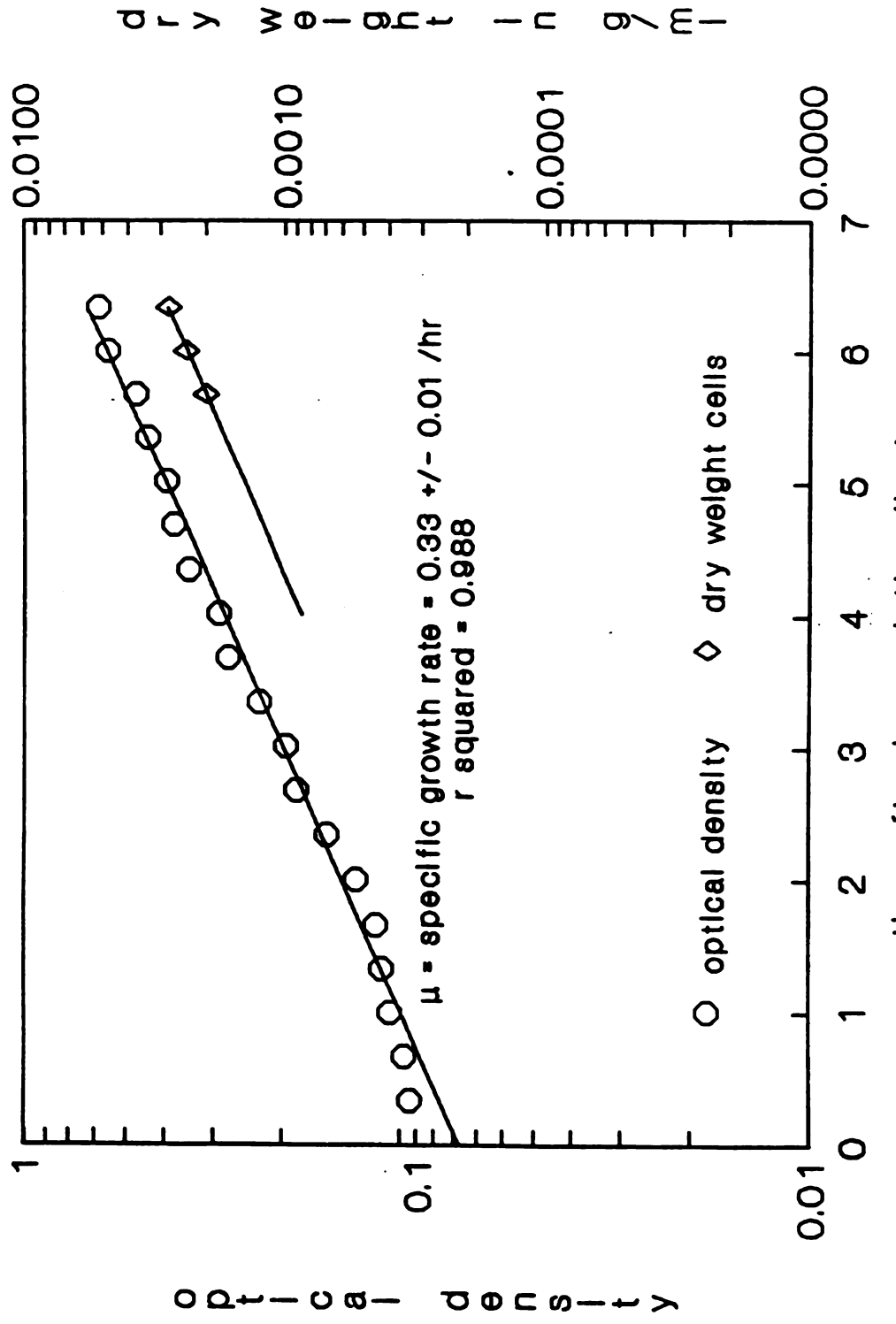

Figure 3. Cell Growth Curves using Dry Weight and Optical Density

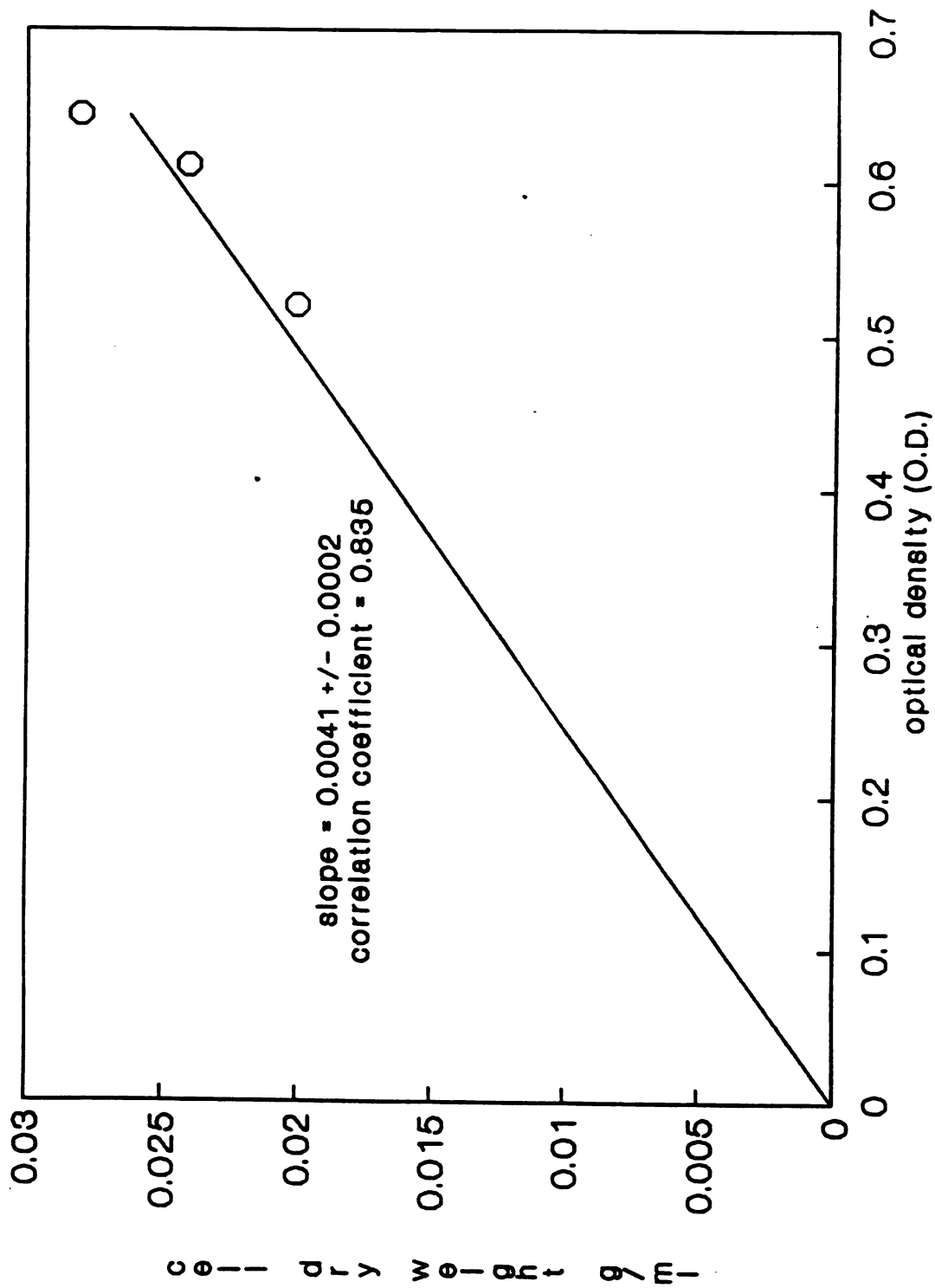

Figure 4. Cell Dry Weight versus O.D.

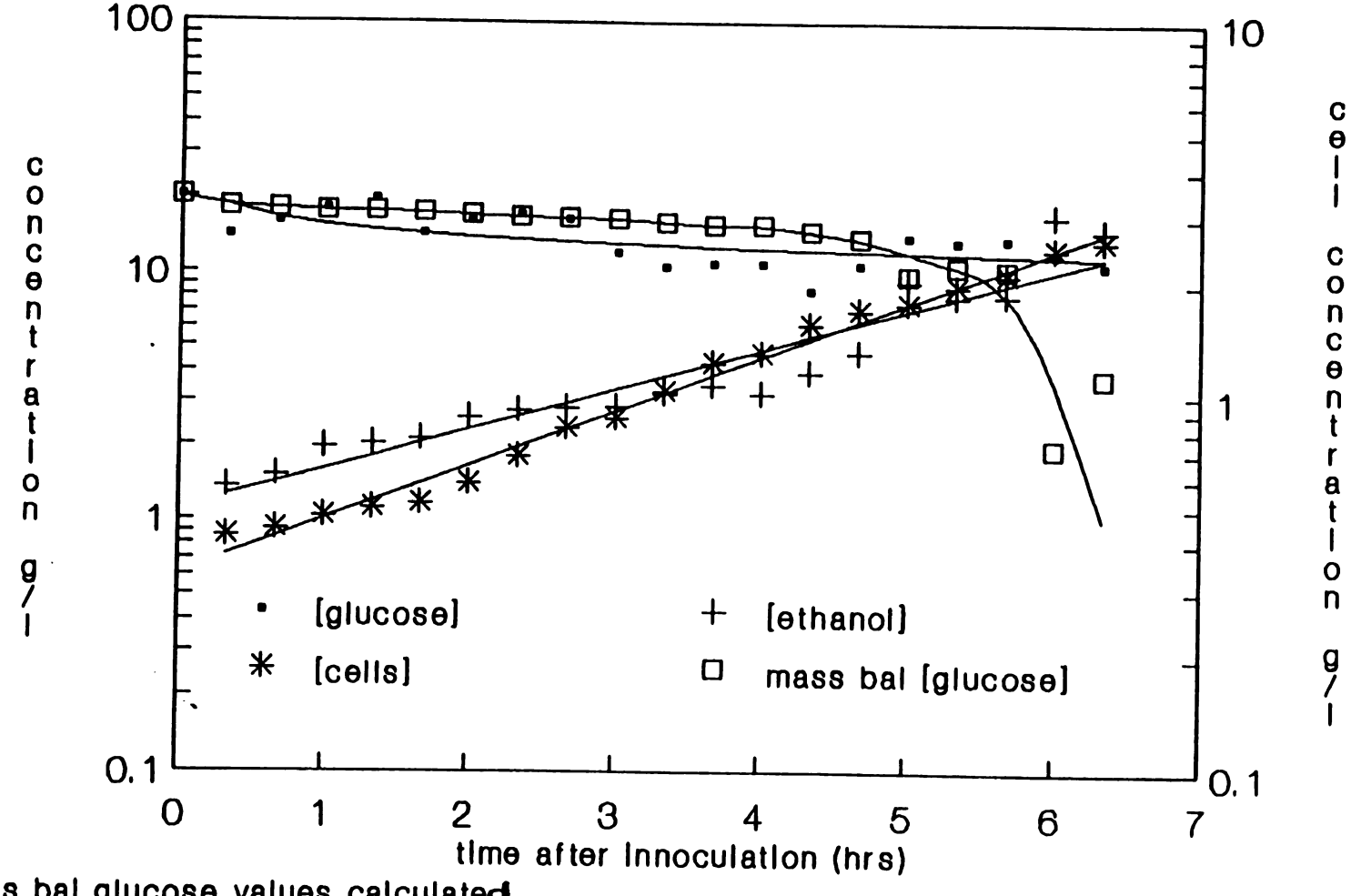

Figure 5. Fermentation Concentration Profiles versus Time

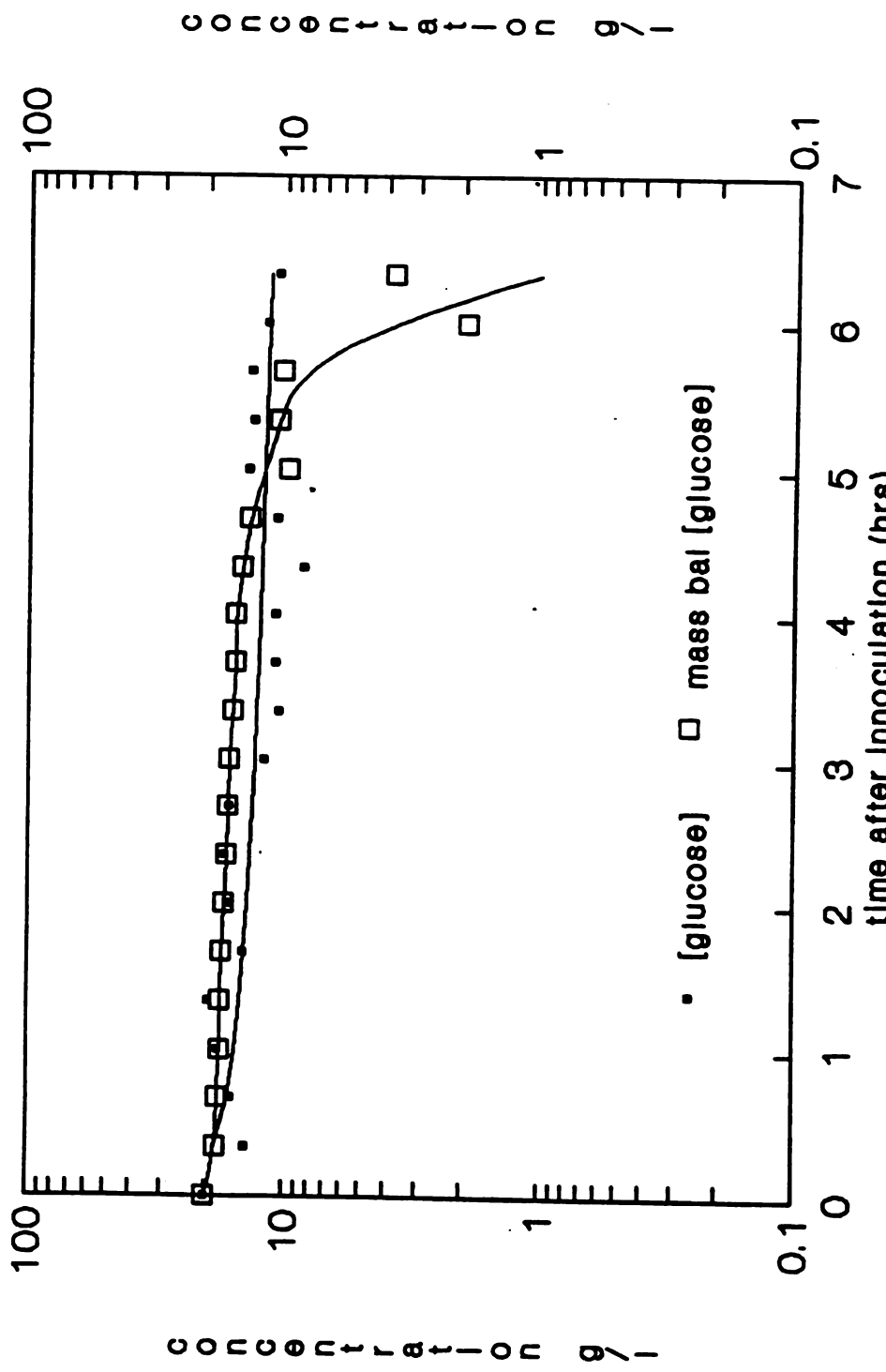

Figure 6. Comparison of Experimental and Calculated Glucose Concentrations

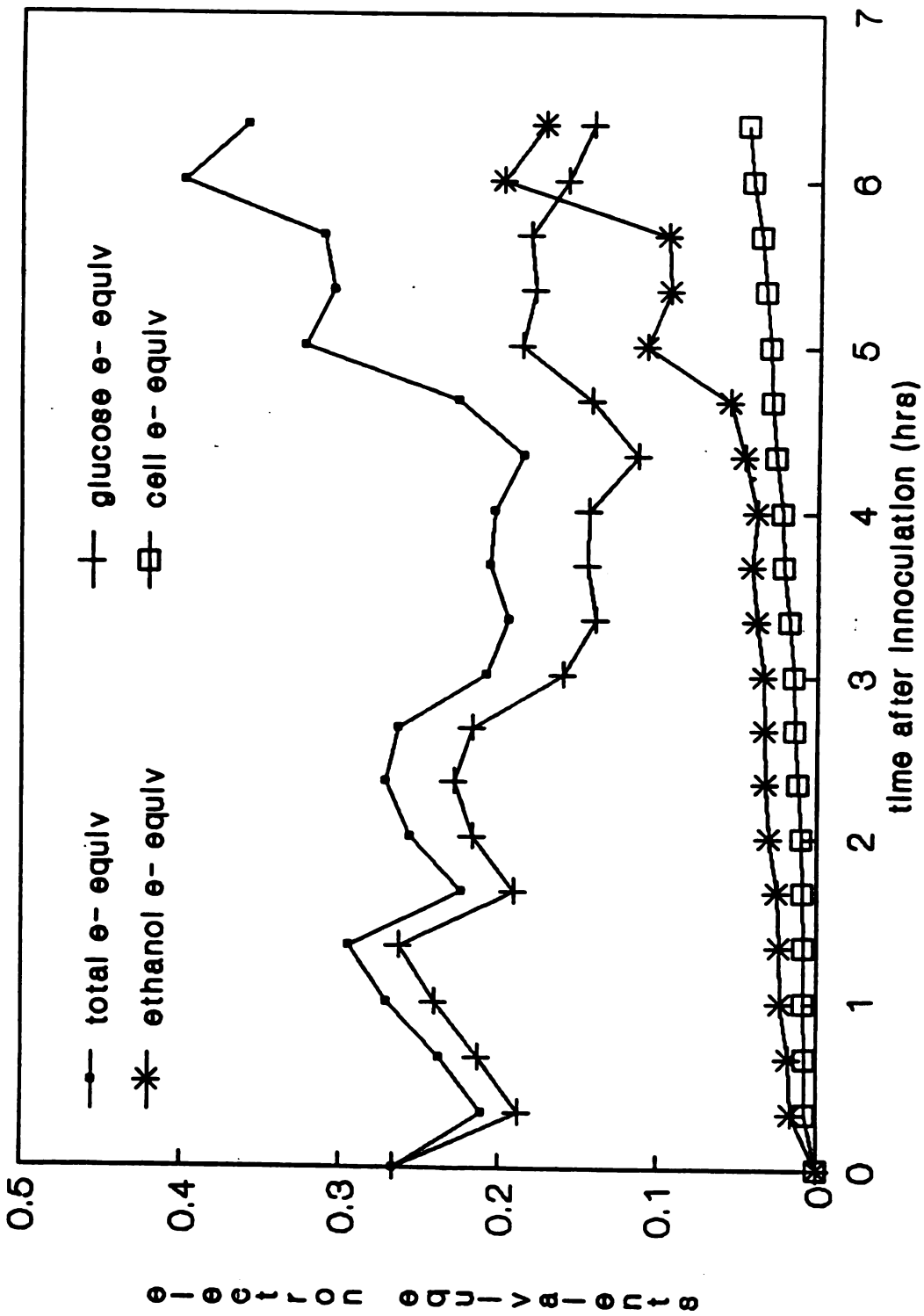

Figure 7. Electron Equivalents Present versus Time

for the ethanol and cell yields for *Z. mobilis* at 34°C are 0.48 and 0.031 respectively in units of g product per g glucose consumed (Lee, 1981). Estimates of the yield coefficients were obtained using the glucose concentrations calculated from the electron balance and the experimental ethanol and cell concentration data. Figure 10 shows less data scatter and results in ethanol and cell yields of 0.49 and 0.099 respectively. The specific ethanol production rate was calculated to be 1.49 g ethanol/g cells hr and is shown in Figure 11. The literature value for the specific ethanol production rate is 8.0 g ethanol per g cells hr at 36°C (Worden, 1982).

The yields calculated for ethanol and cells using the glucose concentration determined from the electron balance are much closer to the literature values than the experimental data. This indicates that most of the experimental error was in the glucose assay. This conclusion is also supported by Figure 7 which demonstrates the large contribution of the glucose concentration to the failure of the electron balance. The difference between the literature and experimental values of the specific growth rate and the cell yield calculated from the electron balance indicates some potential experimental error in the measurement of the biomass concentration. Fluctuations in the ethanol concentration also produced error but were not significant until late in the fermentation (see Figure 7).

Conclusions and Recommendations

The experiment has the potential to produce results with a good correlation to the literature values as is indicated by the closeness of the estimated yields to the literature

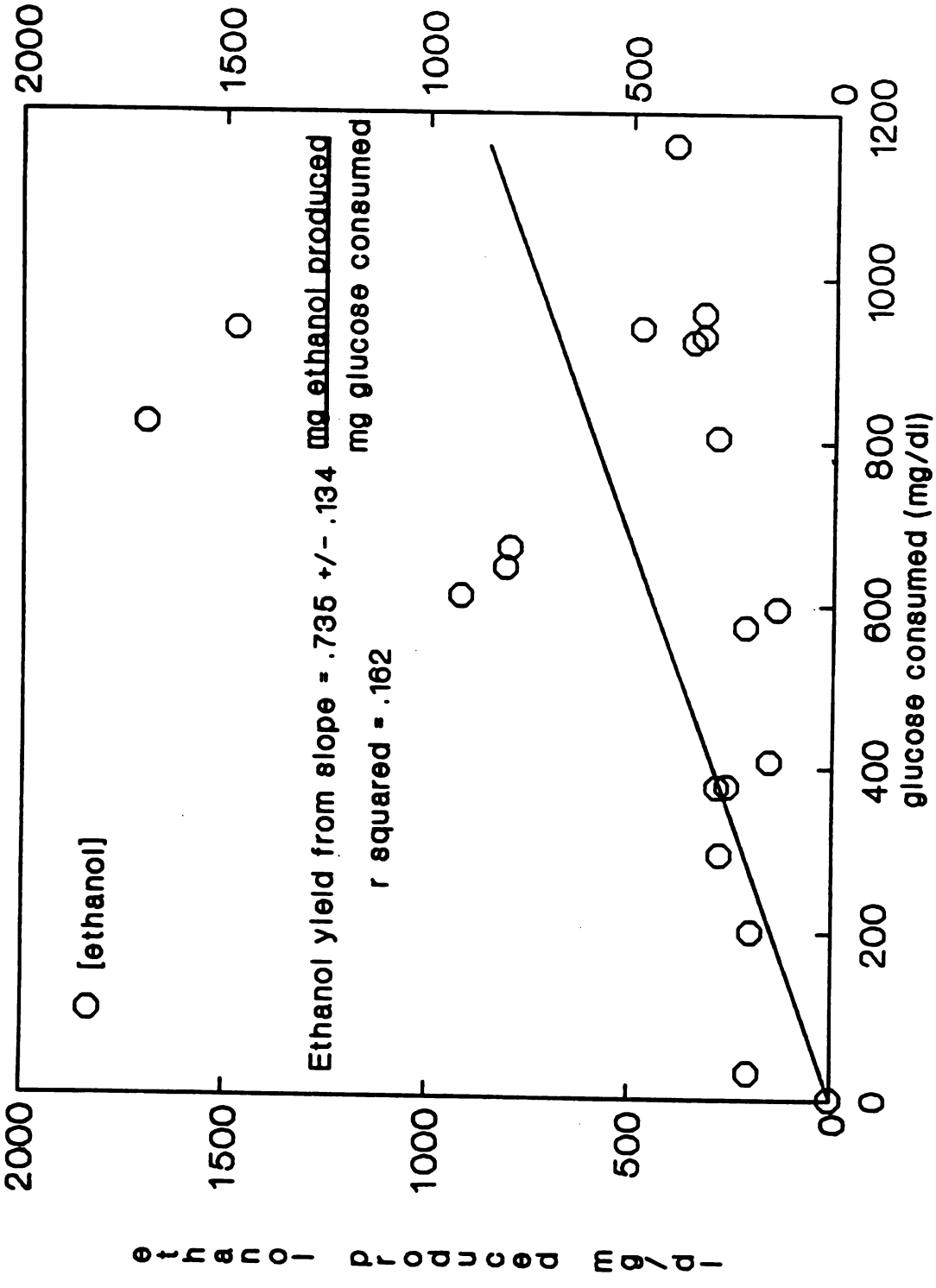

Figure 8. Determination of the Experimental Ethanol Yield

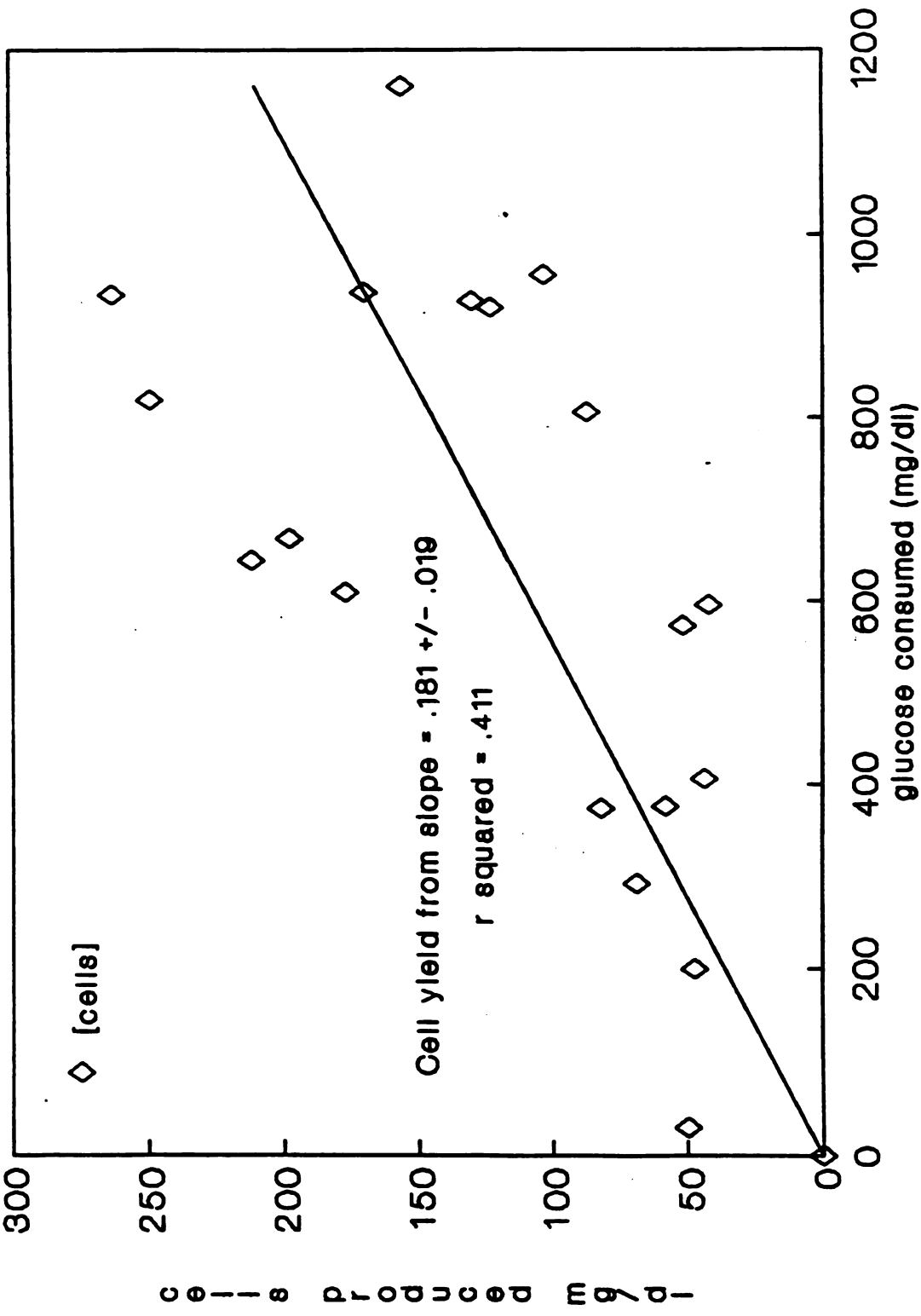

Figure 9. Determination of the Experimental Cell Yield

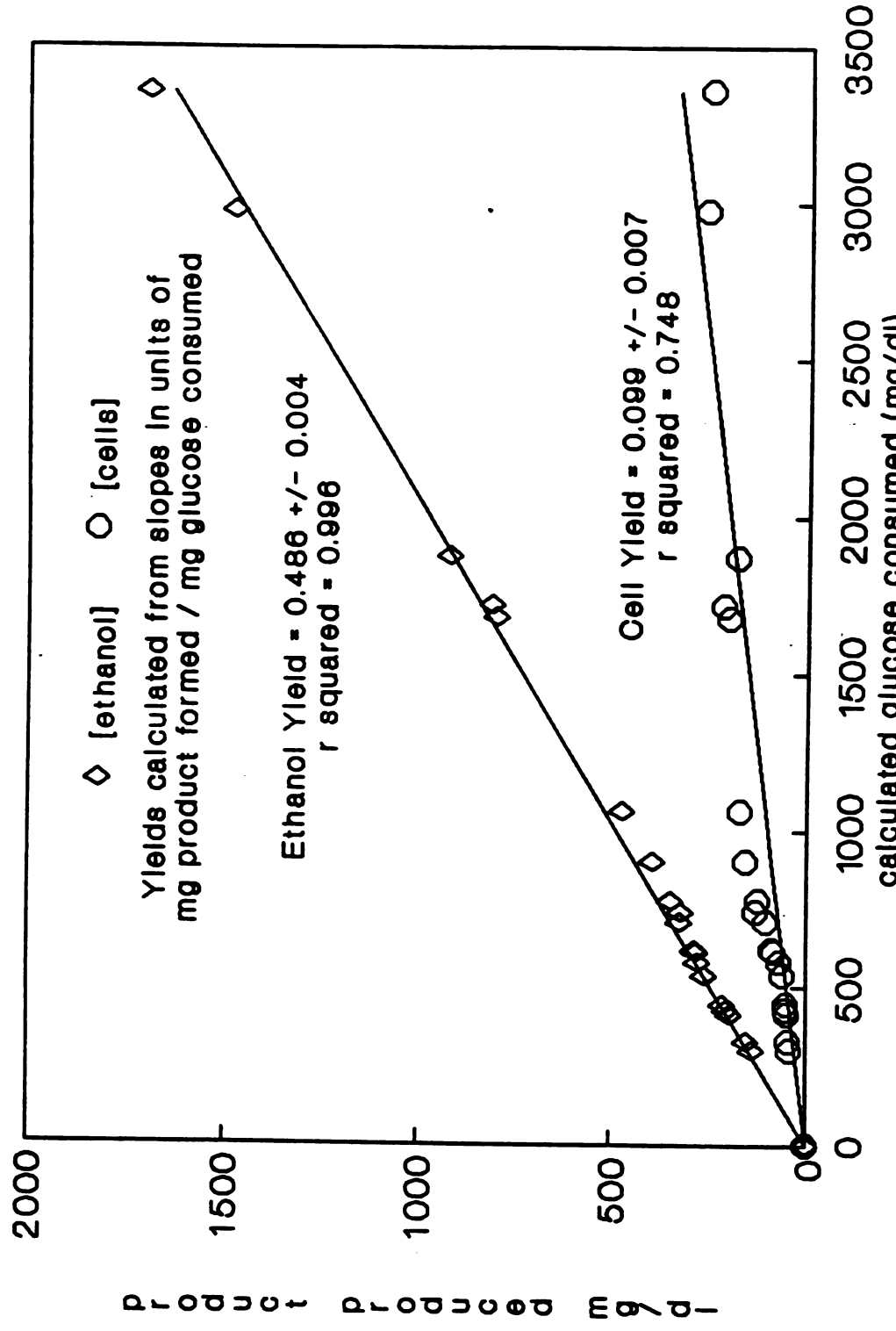

Figure 10. Determination of Yields based on the Calculated Glucose Concentration

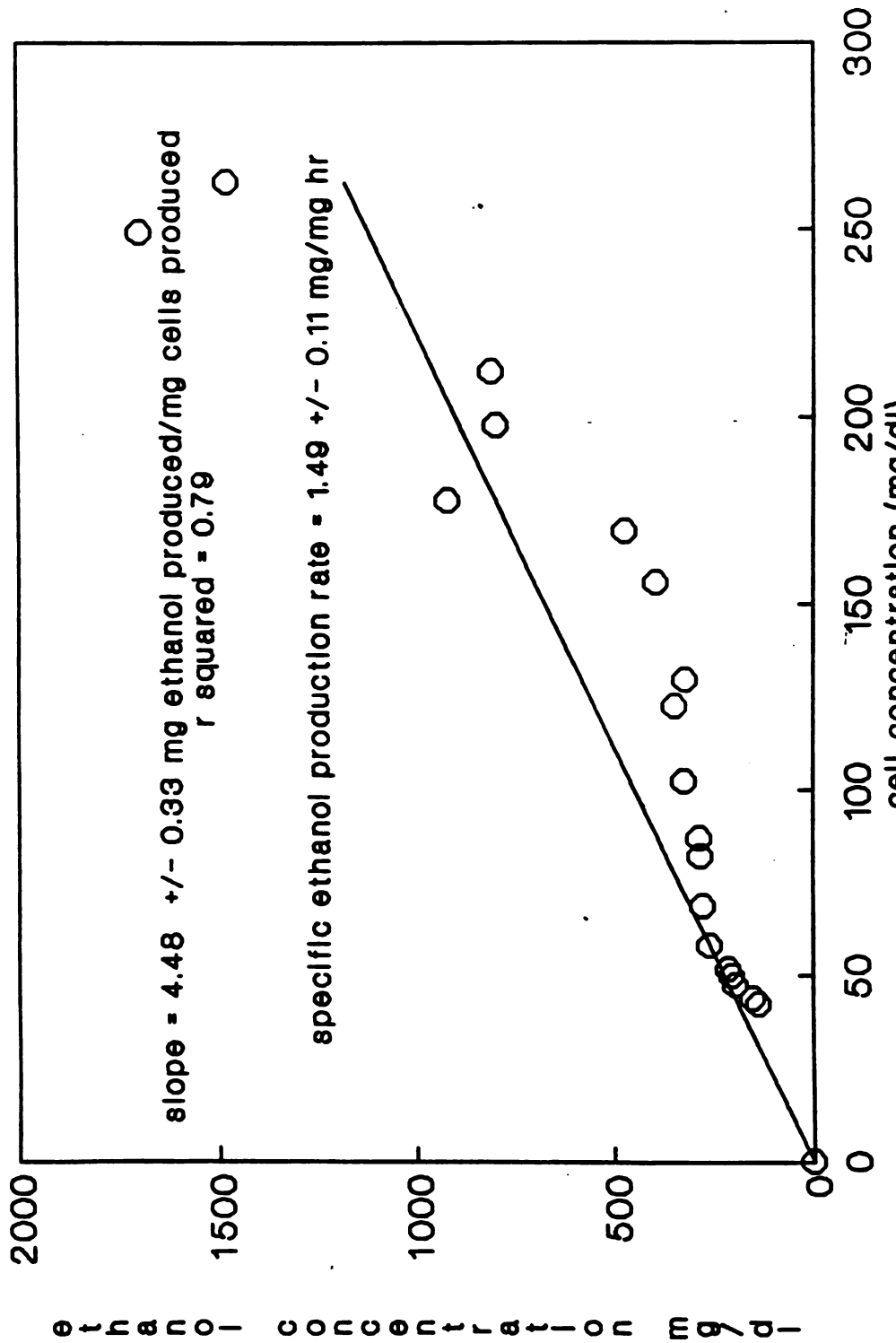

Figure 11. Determination of Specific Ethanol Uptake Rate for *Z. mobilis*

values. The glucose and ethanol assays can be effective using the YSI autoanalyzer but the manufacturer's protocols must be followed carefully. Both YSI assays are more economical, quicker, and easier than their reagent based counterparts but it is still advisable to retain samples for verifying the YSI method using reagent kits.

Use of the YSI autoanalyzer is recommended for convenience and economy and may prove to be a useful analytical tool. The instructions as related to membrane life and reagent expiration dates should be followed closely. The ethanol membrane temperature adapter should also be installed whenever ethanol assays are performed as well.

Symbols

γ = reductance degree
μ = specific growth rate
c = integration constant
e^- = electron equivalent
t = time
x = cell concentration
$Y_{EtOH/glucose}$ = ethanol yield coefficient
$Y_{p/s}$ = general product yield coefficient

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Appendix

Experimental Procedure

I. Day one

A. Preparation of Growth Media

1. The yeast extract concentration in solution should be about 1 % (10 g/l) and 10 g should be added to 500 ml of water since half the volume of the media will be added as the yeast extract solution and the other half will be added as the glucose solution. Prepare the yeast extract in a 1000 mL erlenmeyer flask stoppered with the apparatus shown in figure 1.

2. Make 500 ml 0.02 M succinate buffer by adding succinic acid (succinate) to 300 - 400 ml of reverse osmosis water. Make a 1 N NaOH solution and add the base solution to the succinate solution while checking the pH with a pH meter until it is 7.0.

3. The glucose concentration will be 2% (20 g/l) to keep the fermentation short. The glucose solution should also be prepared at 40 g/l (i.e. add 20 g to 500 ml of succinate buffer). The glucose concentration for the inoculum culture should be higher (e.g., 50 g/l) to provide a higher initial cell concentration. The glucose and yeast extract solutions are prepared separately because when they autoclaved together the medium darkens considerably.

4. Remove the top of the fermentor by gently sliding out the pH probe and then loosening the three head plate knobs and carefully lift off the top. Note that the pH probe enters the vessel in the area where there are no baffle supports to prevent any obstruction from damaging the glass probe. Clean up the fermentor and add the glucose and buffer solution.

5. Replace the top carefully seating the top against the seal. Make sure that the pH probe port is in the over the part of the vessel with no obstructions. Lightly coat the pH probe with glycerol and gently slide the probe into place.

6. Similarly, autoclave the inoculum medium in two 50 mL portions in separate 250 mL Erlenmeyer flasks stoppered with wads of cotton. Do not combine the glucose and yeast extract fractions until it is time to use them. The solutions will remain sterile indefinitely until one of the vessels is opened and then they may

be contaminated by airborne microbes.

B. Calibration of pH Probe

1. Connect electrode to probe cable.

2. Turn agitation switch to OFF.

3. Make sure that water is flowing and the pressure is less than 30 psi. Turn on POWER switch.

4. Check the level of the electrolyte. It should be about 1 cm below the level of the filling orifice. Remove the rubber plugs and set them aside where they will not get lost.

5. Set the selector switch to pH and set the MODE switch to zero.

6. Immerse the pH probe into an external pH 7.0 buffer solution. Set the display to read the pH value of the buffer with the INC/DEC switch.

7. Immerse the pH probe in a basic standard pH solution. Set the MODE switch to SPAN. Set the display to read the value of the second buffer solution with the INC/DEC switch.

C. Preparation for Autoclaving

1. Remove the motor drive from the top of the vessel and place it on the motor mount on top of the cabinet while placing the stainless steel autoclaving cap where the motor rested over the bearings.

2. Calibrate the pH probe as per calibration instructions above. The pH probe should be autoclaved in place (without the cable), with the refill plug taped closed, and the red cap in place.

3. Disconnect all probes and remove all cables from the fermentor. Make sure the anti-foam probe cable is disconnected at the top of the probe and that it is pushed all the way down into the fermentor.

4. Disconnect the air line on the vessel side of the filter and clamp off the tubing.

5. Disconnect the jacket and exhaust condenser water lines.

6. Remove the sampler bulb and insert cotton into the port for the bulb. Close the valve on the sampler bulb.
7. Any openings through which microbes could enter should also be covered with cotton and then wrapped tightly in aluminum foil. Masking tape can be used to hold the aluminum foil. Make sure the feed tube of the fermentor is clamped and stoppered with cotton and wrapped in aluminum foil because it will be used later in the aseptic transfer of the yeast extract fraction into the fermentor.

8. Tubes whose ends are submerged in the liquid medium should be sealed, silicon tubing can be clamped to prevent loss of liquid during autoclaving. Use a small piece of silicon tubing to connect the acid and base ports as shown in Figure 2.

9. Note that silicon tubing can withstand the heat of autoclaving, while Tygon tubing will melt.

10. Check the autoclaving setup against those in figures 1 and 2.

11. If in doubt, show the reactor to the instructor prior to autoclaving.

D. Sterilization

1. The following items need to be autoclaved sealed with glass wool:

   a. Fermentor vessel containing 500 mL of glucose solution and succinate buffer as in figure 2.

   b. 1 L Erlenmeyer flask containing 500 mL of yeast-extract solution with aseptic transfer setup attached as shown in Figure 1.

   c. 250 mL Erlenmeyer flask containing 50 mL of glucose solution

   d. 250 mL Erlenmeyer flask containing 50 mL of yeast-extract solution

   e. Several glass sample bottles with screw-top lids (lids should be screwed on loosely to allow pressure equilibration).

2. Open the upper access door to the sterilizer make sure that the chamber pressure is at or near 0 psi (gauge). Make sure the manual control knob is turned to "off". Place aluminum foil

beneath the rack on the bottom of the autoclave this will aid in cleanup if anything should spill or boil over. Talk with other groups and coordinate so that all the items that need to be autoclaved can be sterilized in one batch.

3. Slide down the door beneath the silver "warning" plate on the left front of the autoclave. Set the POWER and CONTROL switches to ON. The control panel will then light up to demonstrate that all the lights are functioning. The printer records the date and that the power is turned on.

4. Check that the correct time and date is listed by pressing the "time" or "date" button. To change either the time or date press the forward or reverse button until the correct time or date is displayed.

5. Check to make sure there is paper and that the pen is recording well.

6. Set the STERILIZE timer thumbwheels to 15 minutes. The set time will be displayed on the primary control panel. Once the cycle has been started the time cannot be changed using the thumbwheels and the process must be restarted.

7. Check to make sure that the thumbwheel above the printer is set to 121 for 121°C. If not, turn the thumbwheels to 121. If the temperature is outside the allowable range a buzzer will be sounded until an allowable temperature is selected. Once the cycle has started the temperature cannot be changed using the thumbwheels and the cycle must be stopped and restarted to change the set temperature.

8. Check to make sure that the chamber pressure is sufficiently low by opening the upper access door and reading the gauge labeled chamber. Open the chamber door and load the sterilizer. The printer will record that the door was opened. If the door will not unlock, turn the door wheel slightly clockwise and press the center black button on the wheel. Since the door will not open if the pressure is too high it is important to check the chamber pressure before attempting to open the door. Turn the handwheel counter clockwise and open the door.

9. Check to make sure that all containers within the sterilizer are able to equalize pressure through cotton and are not completely sealed.

Close the door and press on the left hand side of the door to insure a good seal. Then turn the door wheel clockwise to lock the door. The DOOR UNLOCKED light on the panel should shut off at this time. Once the chamber is pressurized, an integral pressure lock will prevent the door from being opened. Turn on the steam and water valves. Turn the HI-LO knob to LO.

10. Touch the liquids cycle selector on the lighted panel. It should increase in brightness. The CONDITION and WARNING HOT LIQUIDS lights should come on. If you press the wrong cycle selector simply press the white reset button and re-depress the liquids button.

11. After the CONDITION phase is completed, the STERILIZE light lights for the duration of the sterilization phase. The STERILIZE TIME readout begins to countdown once the temperature and pressure has been reached. The printed record will show when the sterilize time began.

12. When the sterilization time runs out the Exhaust light comes on and the chamber slowly begins to cool and depressurize. The printed record will show the time, temperature and pressure the exhaust phase begins.

13. Upon completion of the exhaust phase the buzzer will sound and the COMPLETE light will come on. Crack open the door about 1/2" and leave it cracked for about 10 minutes. Do not fully open the door at this time.

14. When the door is opened the buzzer will stop and the WARNING HOT LIQUIDS light will begin to flash. At the end of ten minutes the buzzer will sound again and the WARNING HOT LIQUIDS will stop flashing but remain lit. press the reset button to silence the buzzer.

15. Open the chamber door and carefully remove the load from the sterilizer. Press the reset button to shut off the warning hot liquids light and tear off the paper form the printer. Shut off the power and control switches for the sterilizer and shut off the steam and water.

II. Day two: this should be performed the afternoon before your second laboratory period

A. Preparation of Inoculum Culture

1. Aseptically combine your two flasks containing the yeast extract and glucose solutions and then redivide them into two 50 ml portions.

2. Aseptically transfer 5 mL of refrigerated *Z. mobilis* "working" culture to one of the flask using a 10 mL, disposable, sterile, plastic pipette. Prepare two inoculum cultures, one receiving 5 mL of storage culture and the other receiving 0.5 mL. The second culture will reach maturity several hours later than the first. This precaution will help ensure that one of the flasks will have a culture at the proper stage of development when it is time to inoculate the reactor. The area where the culture is transferred should have as little air flow as possible to minimize contamination. The pipette should not be removed from the wrapping until absolutely necessary and the tip of the pipette should not be touched by anything but the media being transferred. To help maintain sterility, whenever the cotton is removed or replaced on a flask, the opening of the flask should be "flamed" with a Bunsen burner. The instructor can demonstrate this technique.

3. Incubate the inoculum culture at 30°C in the incubator shaker until the reactor is inoculated. Do not shake the flask.

4. The inoculum culture is sufficiently mature when it is highly turbid and bubbles form when the flask is swirled. Culture maturity should occur from 10 to 24 hours after addition to growth medium at the proper temperature, depending on the condition of the refrigerated culture.

III. Day three

A. Operating the Fermentor

1. Aseptically combine the glucose and yeast extract fractions into the fermentor and turn on the agitation to mix. This can be accomplished either by using filtered air to force the yeast extract into the fermentor vessel or by using a large syringe to produce a siphon. The connection of the tubes should be performed by wrapping the tube in an alcohol soaked paper towel for 10 minutes and then cutting it in the center of the paper towel and sliding it onto the connecting tube. Remove the rack and wipe out the autoclave with a moist sponge. Check the drain plug for obstructions and empty it.

2. Adjust the values for fermentor pH, temperature, and agitation rate to 7.0, 30°C, and 150 rpm, respectively. This is performed by turning the selector knob to the desired variable (pH, temperature, etc.) and turning the mode knob to set point. The control selection on the mode knob shows the present value of the function on the selector knob on the LED display.

3. Take a sample of the growth medium with a sterile pipette and check the pH on a separate pH meter. If this value does not agree with that given by the fermentor, adjust the desired setpoint slightly to account for the difference. This fermentation is not strongly affected by pH between 6 and 8, so errors of less than half a pH unit are tolerable.

4. Transfer approximately 50 mL of the medium to the sterile, screw-top sampling bottles to be used later as a spectrophotometer blank and to dilute fermentation broth samples. Refrigerate until needed to avoid possible microbial growth. Warm to room temperature before using.

5. Transfer the inoculum culture (50 mL) into the fermentor to initiate the fermentation.

B. Sampling the Fermentor

1. Using a squeeze bulb, force some air through the sampling tube before taking each sample, to ensure that the sample is fresh. Record the pH.

2. Three mL samples should be taken for the turbidity assay approximately every 20 minutes once growth has begun. The samples should be immediately transferred to a cuvette and read in the colorimeter at 710 nm versus a sterile growth medium blank. Keep a running plot of ln(OD) versus elapsed time to follow the progress of the fermentation.

3. Two milliliter samples are adequate for the glucose and ethanol assays, and the same sample can be used for both.

4. Filter-sterilize the sample immediately using a disposable, 0.2 or 0.45 mm syringe filter. Fill the syringe with 1-2 ml of fermentation broth collected from the sampling port. Attach the syringe by screwing the filter onto the end of the syringe. Point the nozzle of the filter into the sample vial or test tube and slowly

press the plunger to filter the liquid into the vial.

5. Collect the clarified filtrate in a small test tube, cap and label the tube, and refrigerate for later analysis. Because ethanol is volatile, it is important to control evaporative losses by minimizing exposure of the samples to air. One sample should be taken soon after inoculation to give the initial conditions. An appropriate sampling strategy should be planned to give accurate measurements of glucose consumption, as discussed previously. As an approximate rule of thumb, 1% glucose is consumed for every 0.6 OD increase in the cell concentration during the exponential growth phase.

C. Operation of the colorimeter

1. Turn on the colorimeter using the switch on the left hand side of the colorimeter.

2. Select the absorbance mode by pressing the MODE button in the lower left until the display reads ABS in the right hand side of the LED display. Turn the dial on the top to the desired wavelength (710 nm) and insert your sample where in the front cuvette holder. Allow 15 minutes for the optics to warm up.

3. Fill a cuvette with reverse osmosis (RO) water and place in the rear cuvette holder and close the sample area lid. When handling cuvettes, touch only the frosted sides of the cuvette and wipe down the clear sides of the cuvette with a kimwipe before taking absorbance readings. Turn the dial on the top of the colorimeter towards the word blank until it stops. Then press the CAL button.

4. Fill another cuvette with RO water, place it in the front sample holder and read the absorbance. If the absorbance is zero set aside the cuvette for use and label it on the frosted side in some way. This process can be repeated so that a whole series of cuvettes can be used without washing them until you have used the last absorbance matching cuvette. An alternative method is to record the absorbance if it is not zero and label the cuvette so that you may subtract off that "zero" absorbance form any readings taken with that cuvette.

5. Remove the RO water from the cuvette and place your blank in the rear cuvette holder. Turn the dial to blank and press CAL.

6. Place your sample in a cuvette and put the cuvette in the front sample holder and close the lid.

7. Turn the dial to sample and record your absorbance reading. The lid should be closed whenever cuvettes are not be added or removed form the sample chamber.

D. Turbidity assay

1. Place a 3 mL sample of fermentation broth in the sample cuvette immediately after it is withdrawn from the bioreactor.

2. Insert into the spectrophotometer and read the OD (710 nm) versus a sterile growth medium blank.

3. If the OD is greater than 1.0, dilute quantitatively with sterile growth medium to bring the OD value below 1. When analyzing the data, multiply the OD value by the dilution factor to put all values on an equal basis.

4. Occasionally check the blank for signs of microbial growth (cloudiness), and replace if necessary. Increases in the OD of the blank may also be interpreted as growth but may merely be recorded and accounted for while the absorbance reading is small.

E. YSI Autoanalyzer Setup (this may be necessary every two weeks)

1. Set the RUN/STANDBY switch to STANDBY. Plug the autoanalyzer into the wall socket. Only after you have plugged in the autoanalyzer to the wall socket should the probe cable be connected to the socket labeled probe.

2. Empty the supply bottle and rinse with RO water. Prepare the buffer by dissolving the buffer concentrate in the water according to the directions on the package. Pour the buffer into the supply bottle and screw on its cap.

3. Purge the sample chamber by pressing the CLEAR button and holding it down until the buffer drains into the waste container. If the pump does not begin pumping, the pump will have to be primed. To prime the pump fill the bottle completely with buffer and squeeze the bottle or use a buffer filled syringe to force fluid into the pump.

4. Hold the clear button down for about another 30 seconds. Switch

from STANDBY mode to RUN, and press the clear button. Allow the instrument to go through its clear cycle. When the ZERO/INJECT light comes on, press the clear button. Check for leaks and correct any that are found. Press the clear button twice more and switch to standby. Remove the waste container, empty it, rinse with water, and reinstall.

5. With the instrument in standby, unscrew the probe retainer from the upper port and gently remove the Sensor Probe from the temperature block, retrieving the O-ring from the block if it does not come out with the probe. Remove the previous membrane, being careful not to scratch the probe. Note that the membrane is attached to the rubber o-ring and that a the new membrane will also be attached to an o-ring.

6. Rinse the probe surface with distilled water and wipe clean with a kimwipe removing any dirt or lint on the surface.

7. Remove the membrane foil pouch and snap open a cavity of the plastic membrane holder. Place a drop of YSI 2392 NaCl solution on the probe face. Using the plastic membrane holder, press the O-ring membrane assembly onto the probe face with the membrane side toward the probe surface. Return the plastic membrane holder to the foil pouch and store as directed on the pouch label.

8. Inspect the probe and membrane assembly for hairs, dirts, bubbles, wrinkling of the membrane and other signs of trouble. The O-ring must fit snugly behind the guard at the tip. Replace the probe back into the upper port on the temperature block being careful in seating the probe.

F. Ethanol Assay using the YSI Autoanalyzer

1. Flush the sample chamber with buffer until no more air bubbles can be seen by holding down the clear button continuously and switching between STANDBY and RUN every five seconds or so.

2. Allow the instrument to warm up until the small red light in the sample chamber lights up.

3. Whenever the instrument is not being operated switch the unit to standby. This keeps the pump running and prevents the membrane from drying out. The digital display should read three eights (888) in the standby mode but a minus sign or a one may lead the eights. Any other meter reading indicates a loose connection or a defective

meter and all connections should be checked.

4. While in standby make sure that the sample chamber contains no air bubbles and press the clear button. Switch to RUN and press CLEAR and release.

5. The syringepet should be filled by depressing the plunger button at the top and inserting the needle into the solution to be measured. Slowly release the plunger carefully avoiding drawing up air bubbles. Rinse the syringepet in RO water by filling and emptying it three times, then rinse the syringe three times with 300 mg/ml (.3 % w/v) ethanol solution. If the 5 μl syringepet is not available for use on the ethanol assay a hamilton syringe will work well if air bubbles are avoided. If a hamilton syringe is used make sure that you activate the read function by gently pressing on the injection port until the display reads wait.

6. Fill the syringepet with 300 mg/dl (0.3 % w/v) standard and when the ZERO/ INJECT light comes on turn the knob so that the display reads zero and then inject the sample. The syringepet should be inserted into the injection port through the rubber septum and the plunger should be pressed firmly and evenly. Pull the syringepet out the port keeping the plunger depressed to avoid drawing out the sample.

7. When the READ light appears press the calibrate button and turn the CAL knob until the display reads 300.

8. Press the clear button. Five seconds after the ZERO/INJECT light comes on the reading should be between -1 and +3. If it is not, press clear again and then adjust the zero after the second clear cycle. Repeat the calibrations until they are stable within +/- 2.

9. Check the calibration by injecting 5 μl of water. The reading should be between -2 and +5.

10. Check the linearity of the instrument by injecting 100 mg/dl (0.1 % w/v) and 50 mg/dl (0.05 % w/v).

11. Once the calibration procedures have been carried out, all that is required is the injection of the sample into the instrument.

G. Operation of the spectrophotometer

1. Turn on the spectrophotometer, and allow it to warm up for 15 min.

2. Set the desired wavelength by entering wavelength (in nm) on the key pad and pressing the Go To key 660 nm.

3. Fill both cuvettes with the blank solution (e.g., sterile growth medium in the case of the turbidity cell mass assay). Insert the cuvettes into the spectrophotometer and close the cover. Handle the cuvettes only by the frosted glass sides. The clear glass windows must remain clean and dry; if they are not wipe them with a kimwipe.

4. Zero the instrument by pressing the Zero key.

5. One of the cuvettes is the "sample" cuvette (the closest one), and the other is the "blank". Empty the sample cuvette, taking care not to get any liquid on the outside of the clear glass windows. Rinse it with distilled water, and gently touch the mouth of the inverted cuvette to a paper towel to siphon off any remaining water. You may check to find cuvettes with identical absorbances as above.

6. Transfer the sample into the sample cuvette. Insert it into the spectrophotometer and close the cover. Record the OD.

7. To analyze another sample, repeat steps 5 and 6.

H. Glucose Assays Using the Sigma Diagnostic Reagent Kit

1. Prepare the glucose trinder reagent according to the instructions.

2. Set the spectrophotometer to 505 nm and the absorbance to zero with respect to a water blank.

3. Set up a series of test tubes labeled for reagent, blank, standard, controls and experiment samples.

4. Pipet 3.0 ml of Glucose trinder reagent in to each tube and warm to assay temperature.

5. At timed intervals, add 10μL (0.01 ml) of reverse osmosis water, standard, controls, and experimental samples to the appropriately labeled test tubes.

6. Incubate each tube for exactly 18 minutes at ambient temperature. If the assay temperature is 30°C or 37°C, incubate for 15 and 10 minutes respectively.

7. Read and record the absorbance of all tubes at 505 nm using the same timed intervals from step 5.

8. Subtract the absorbance of the blank from the absorbance of the experimental sample, standard, and controls to obtain the change in absorbance due to glucose.

9. The calculation of the glucose concentration can use the following equation:

$$\frac{A_{sample} - A_{blank}}{A_{standard} - A_{blank}} x\, Concentration\ of\ Standard \qquad (5)$$

10. If the results indicate concentration greater than 750 mg/dl, dilute 1 part sample with 1 part RO water and re-assay, multiplying the results by two.

11. An alternate method involves running a series of known glucose concentrations and recording their absorbances. The absorbances for the known solutions are then linearized and used to determine the concentration of the unknown samples. Both methods can be utilized. Use a sterile pipette when removing amounts of the glucose standard solution.

12. Compare the results of the two methods.

I. Alternate Glucose Assay using the YSI Autoanalyzer

1. Replace the ethanol membrane with a glucose membrane using the procedures outlined in the YSI Autoanalyzer Setup Procedure above.

2. Use a sample volume of 25 μL and the 25 μl syringepet.

3. Calibrate the analyzer with the glucose standard solution supplied and check the linearity by diluting a small volume of the standard as outlined in the ethanol procedure above. Sigma glucose standards my also be used if they are available.

J. Dry-weight assay

1. Withdraw an accurately measured sample (e.g., 25.0 mL) of

fermentation broth and place on ice in a 30 mL centrifuge tube.

2. Centrifuge at 8000 rpm for 10 min in the refrigerated centrifuge.

3. Remove the supernatant from above the cell pellet with a pipette. Save a sample for glucose and ethanol assays.

4. Re-suspend the cell pellet in approximately 20 mL of deionized water and centrifuge again, as before.

5. Repeat the deionized water wash (steps 3 and 4).

6. Weigh an aluminum weighing pan on the analytical balance.

7. Quantitatively rinse the cells into the weighing pan using a small amount of deionized water.

8. Dry the sample overnight in a 105°C drying oven. The oven should be set to about 3 with the vent slightly open.

9. Once dry, cool the pan in a desiccator to prevent water absorption from the air.

10. Reweigh the pan on the analytical balance and calculate the mass of dry cells by difference.

Enzyme Kinetics

Summary

The kinetics of the enzyme fumarase which catalyzes the conversion of fumarate to L-malate, were studied spectrophotometrically at 300 nm using polymethacrylate UV grade cuvettes and initial rate kinetic assays. The kinetic parameters, K_m and V_{max}, were determined for fumarate, alone and in the presence of 22.4 mM citrate and 67 mM phosphate, using Lineweaver-Burk and Hanes-Woolf plots. Neither linear analysis technique produced superior fits to the experimental data in all of the cases studied. Citrate was shown to be a competitive inhibitor, which agreed with the literature and phosphate demonstrated mixed-inhibitor behavior contrary to the literature.

Introduction

A catalyst is a substance that increases the rate of a chemical reaction but is not consumed in the process. Catalysts increase the rate of reaction by lowering the activation energy without shifting the equilibrium of the reaction. Enzymes are catalysts that are produced by living systems to minimize the energy requirements for the reaction of a substrate. A substrate is a reactant for the reaction catalyzed by the enzyme. Enzymes often have the ability to distinguish between very similar substrates and produce highly specific products. Because of these properties, enzymes are useful in the production of pharmaceuticals and other high-purity products such as a specific stereoisomer of a compound. To design and optimize a reactor that utilizes an enzymes

as its catalyst, the enzyme's kinetics must be known. An enzyme's kinetics can be determined by measure the reaction rate as a function of the substrate concentration. These data are modeled using a the appropriate rate form, which for most enzymes is the Michaelis-Menten equation.

Background

Michaelis and Menten were pioneers in the field of enzyme kinetic studies who classified enzyme behavior into two regimes: low and high substrate concentration. Enzymes demonstrated first order behavior with respect to substrate concentration at low substrate concentrations and zero order behavior at high substrate concentrations. Michaelis and Menten developed a two-parameter model that emulated enzyme behavior in both regimes. The two parameters, designated K_m and V_{max}, have physical significance related to the enzyme activity. The Michaelis constant, K_m, which is also known as the half-saturation coefficient, corresponds to the substrate concentration that produces one half of the maximum reaction rate (Bailey and Ollis, 1986). The affinity of the substrate for the enzyme is also represented by the value of its K_m. The smaller the value of K_m, the higher the affinity of the enzyme for the substrate and vice versa. The maximum reaction rate, V_{max}, represents the maximum possible reaction rate that would occur at an infinitely high substrate concentration for a specific amount of enzyme. Graphical and numerical techniques based on the saturation-kinetics model proposed by Michaelis and Menten can be used to evaluate K_m and V_{max} from experimental reaction-rate data.

Reaction-velocity assays must be carried out to obtain reaction rate data to fit to

the kinetic model. An assay is an experiment to determine the concentration of a chemical species. Reaction-velocity or enzyme-activity assays measure the rate of change of the concentration of a species. An assay may be direct or indirect. A direct spectrophotometric assay measures the concentration of a light-absorbing product or reactant of the enzymatic reaction of interest. An indirect assay is required when the reaction of interest does not contain a light absorbing compound called a chromophore. The reaction of interest is chemically coupled to a second reaction that involves a chromophore. The rate of the first reaction can be followed indirectly by observing the rate of appearance or disappearance of the chromophore. The reaction of interest must be the rate-limiting step for the series of coupled reactions carried out for the indirect assay technique to be valid. The electron carrier NAD^+/NADH is commonly used to couple reactions, since both the reduced and oxidized forms of NAD are chromophores at different wavelengths. Both forms of NAD are usually compatible with enzymes since they are often used as electron acceptors/donors within living organisms.

The enzyme fumarase catalyzes a reaction whose kinetics can be determined by a direct assay. Fumarase catalyzes the hydration of fumarate to L-malate in the Citric Acid Cycle. Fumarate's double bond absorbs light in the ultraviolet spectrum at 300 nm. A direct spectrophotometric assay can be used to monitor the concentration of fumarate because on the absorbance of the solution at 300 nm decreases as its double bond is hydrated to form L-malate. The fumarase reaction is very stereospecific; only a trans configuration of fumarate will react with the enzyme to yield L-malate as the sole product as shown in Figure 1 below (Dixon, 1979).

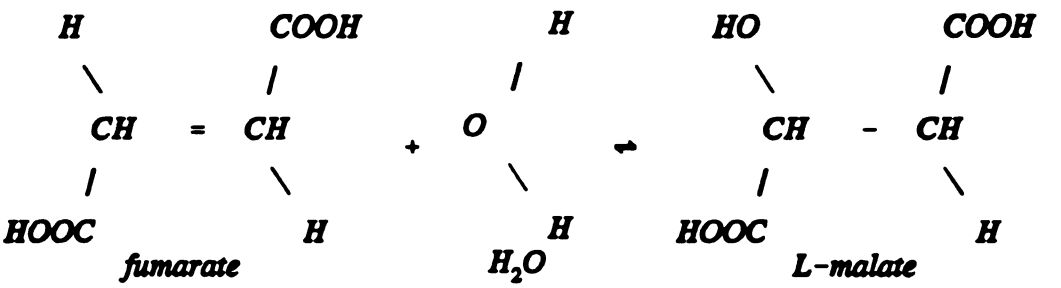

Figure 1. Reaction Catalyzed by the Enzyme Fumarase

Fumarase is classified as a lyase because it removes water from fumarate by simple extraction rather than by hydrolysis or oxidation.

Acids such as glycine, malate, citrate, adipate, trans-aconitate, glutarate, succinate, and malonate can act as inhibitors of fumarase. An inhibitor is a substance that interacts with the enzyme and reduces the rate of reaction.

Theoretical Analysis

The Lambert-Beer law relates the absorbance of fumarate to the concentration by the following equation:

$$A = \log(\frac{I}{I_o}) = abc \qquad (1)$$

where A = absorbance
I_o = intensity of the incident light
I = intensity of the transmitted light
a = molar extinction coefficient
b = length of the light path
c = concentration in moles

The molar extinction coefficient, a measure of how strongly the chromophore absorbs light, is a constant that depends on the wavelength of the light. The equation shows that the absorbance is directly proportional to the concentration of the chromophore in the

solution. Application of the spectrophotometric method requires the use of a blank. A blank is identical to the unknown solution except for the compound whose concentration is to be measured. The blank's absorbance is due to all the other species in solution and is therefore subtracted from the absorbance of the unknown solution. Thus, only the absorbance of the compound of interest is measured (Robyt and White, 1986).

Initial velocities for the enzymatic reactions are determined using a dynamic version of the Lambert-Beer law given by the following equation:

$$v = \frac{(\Delta c)}{\Delta t} = \frac{(\Delta A)}{(\Delta t)(a)(b)} = \frac{(\Delta A)(chart\ speed)}{(distance)(a)(b)} \qquad (2)$$

where
- v = reaction velocity
- t = time elapsed
- a = molar extinction coefficient
- b = length of the light path (cuvette width)
- c = concentration in moles
- A = absorbance

The molar extinction coefficient is either determined experimentally using known standards or obtained from the literature. The length of the light path is 1 cm for most cuvettes. The enzyme is mixed with the substrate solution in the cuvette, and the cuvette is placed in the spectrophotometer. As the reaction proceeds, the concentration of the chromophore changes, causing the absorbance of the solution to increase or decrease. The absorbance can be recorded as a function of time on chart paper or by computerized data acquisition. The reaction rate can then be determined from the slope of the absorbance-versus-time curve using the Lambert-Beer law. The initial slope of the absorbance curve is used to determine the reaction rate characteristic of the initial

substrate concentration. When the slope is determined by a graphical procedure from chart-recorder data, the chart speed is chosen such that the slope of the absorbance-versus-time curve is between 30 and 60° from the x axis on the chart paper. Alternatively, for computerized data acquisition, numerical differentiation can be used to determine the reaction rate. Once reaction velocities are determined, they can be fit to the Michaelis-Menten model.

The Michaelis-Menten equation for an enzyme catalyzed reaction of S → P is as follows:

$$v = \frac{V_{max}S}{K_m + S} = -\frac{dS}{dt} = \frac{dP}{dt} \qquad (3)$$

where
- v = velocity of the reaction
- V_{max} = maximum reaction velocity
- K_m = half saturation or Michaelis constant
- S = substrate concentration
- P = product concentration
- t = time

The Michaelis-Menten equation and its various forms were derived from elementary-step mechanisms and rearranged into simplified forms that combined the rate constants of the elementary steps. The Michaelis-Menten equation can be rearranged into several linear forms whose slopes and intercepts are composed of the kinetic constants of the enzyme. The constants can then be evaluated by using graphical or analytical line fitting techniques. Two common linearized forms of the Michaelis-Menten equation are the Lineweaver-Burk and the Hanes-Woolf plots (Segel, 1986; Robyt and White, 1986).

The Lineweaver-Burk equation is obtained by inverting both sides of the Michaelis-Menten equation and algebraically rearranging it into the form of a line. The

Lineweaver-Burk equation is given below:

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \quad (4)$$

The slope and intercept of a plot of 1/v versus 1/[S] are used to determine the K_m and V_{max}. The Hanes-Woolf equation is obtained by diving both sides of the Michaelis-Menten equation by [S] before inverting and rearranging it into linear form. The Hanes-Woolf equation is:

$$\frac{[S]}{v} = \frac{1}{V_{max}}[S] + \frac{K_m}{V_{max}} \quad (5)$$

The parameters for the Hanes-Woolf equation, determined from a plot of [S]/v versus [S], are slightly different from those determined from the Lineweaver-Burk plot.

The effects of enzyme inhibition can be modeled if more parameters are added to the Michaelis-Menten equations. The Michaelis-Menten equation for modeling inhibition replaces V_{max} and K_m with the apparent maximum velocity, $V_{max,app}$, and the apparent Michaelis constant, $K_{m,app}$. These apparent kinetic constants are functions of the inhibition constant, K_I; the inhibitor concentration, [I]; and the mechanism of inhibition. If the presence of the inhibitor makes $K_{m,app}$ larger than K_m for the substrate alone, the enzyme is competitively inhibited by that compound. The larger $K_{m,app}$ is, the lower the affinity of the enzyme for the substrate in the presence of the inhibitor. If the inhibitor makes $V_{max,app}$ less than V_{max}, then the enzyme is noncompetitively inhibited. A mixed inhibitor makes both $K_{m,app}$ and $V_{max,app}$ smaller than K_m and V_{max}, respectively but not necessarily by the same amount. An uncompetitive inhibitor decreases $K_{m,app}$ and $V_{max,app}$

by the same amount and is a special case of mixed inhibition. The forms of $K_{m,app}/V_{max,app}$ and $1/V_{max,app}$ are given in Table 1 in terms of K_m; V_{max}; the inhibitor concentration, [I]; and α, a factor of the decrease in $V_{max,app}$ relative to $K_{m,app}$.

Table 1. Values of $K_{m,app}/V_{max,app}$ and $1/V_{max,app}$ for Different Types of Enzyme Inhibition

Type of Inhibition	$K_{m,app}/V_{max,app}$	$1/V_{max,app}$
Competitive	$(1 + [I]/K_I)K_m/V_{max}$	$1/V_{max}$
Uncompetitive	K_m/V_{max}	$(1 + [I]/K_I)/V_{max}$
Mixed	$(1 + [I]/K_I)K_m/V_{max}$	$(1 + [I]/(\alpha K_I))/V_{max}$
Noncompetitive	$(1 + [I]/K_I)K_m/V_{max}$	$(1 + [I]/K_I)/V_{max}$

The mechanism of inhibition can often be determined from the Lineweaver-Burk and Hanes-Woolf plots. Bailey and Ollis (page 128) illustrate how the various types of inhibition appear on the Lineweaver-Burk and Hanes-Woolf plots.

Experimental Equipment and Procedure

A Perkin Elmer Lambda 3A spectrophotometer and R100A chart recorder were used to determine the molar extinction coefficient of fumarate and measure initial reaction velocities. The absorbance of the fumarate was determined at 300 nm using polymethacrylate UV-grade cuvettes. The molar extinction coefficient was determined using the same fumarate concentrations used in the kinetics study. Reaction velocities were determined from the slope of the absorbance-versus-time data that were recorded by the chart recorder. The slope of the initial linear region of the absorbance-versus-time

curve was estimated graphically using a ruler and a clear straight edge. The reaction velocity was calculated by multiplying the slope by the molar extinction coefficient and the length of the light path. The chart-recorder speed was adjusted to keep the slope of the line between 30° and 60° from the x axis to minimize the error of the graphical slope determination. The absorbance of the blank was used to zero the chart recorder. The absorbance scale of the chart paper was set by marking the absorbance of the fumarate solution without enzyme present at the corresponding position on the chart paper. A small volume of enzyme was transferred from a refrigerated stock solution to a small test tube to avoid the risk of contamination or denaturation of the stock. The enzyme was kept on ice to avoid denaturation after removal from the stock solution. A Labsystems Finnpipette (Helsinki, Finland) 5-40μL repeat pipettor delivered reproducible 10 μL aliquots of the enzyme from the tube to the cuvettes. After addition of the enzyme, the cuvette was quickly capped with clean plastic film and rapidly inverted 3 to 4 times. This mixing protocol minimized bubble formation which could denature the enzyme and interfere with the absorbance measurements. Once mixed, the cuvette was immediately placed in the spectrophotometer, and the absorbance was recorded on the chart paper as a function of time.

The standard assay solution contained 2 ml of fumarate solution adjusted to pH 7.0 with 0.1 N NaOH and 1 ml reverse osmosis (RO) water. The fumarate concentrations used for the velocity assays and the determination of the molar extinction coefficient were 0.498, 0.622, 1.246, 2.492, 2.865, 4.983, 7.475, 9.967, 12.458 mM. The concentrations stated for the assays are given in terms of the actual concentration in

the cuvette, not the concentration of the solution added to the assay. The concentrations of the stock solutions for each of the assay ingredients were higher than the stated cuvette concentrations to adjust for the dilution upon combination of the solutions in the cuvette. The activity of the fumarase was also measured in the presence of 22.4 mM citrate and 66.4 mM phosphate, both of which were adjusted to pH 7 with 0.1N NaOH. The inhibition experiments substituted 0.5 mL of the citrate or phosphate solutions for 0.5 mL of RO water present in the standard solution. The fumarase used for this experiment was Sigma Chemical (St. Louis, MO) extracted from porcine heart tissue. The 10 μL aliquots of enzyme correspond to 2.4×10^{-4} activity units of fumarase. One activity unit is defined as the amount of enzyme that will convert one μmole of L-malate to one μmole of fumarate per minute at 25°C and a pH of 7.5. This activity is for the reverse of the reaction studied but should make substitution of fumarase from another source simple.

Experimental Results and Discussion

The molar extinction coefficient for fumarate was determined to be 0.0363 absorbance units/cm mM by linear regression of the data shown in Figure 2. The kinetic data were analyzed using both the Lineweaver-Burk and Hanes-Woolf plotting techniques (Boyer, 1986). The kinetic constants were determined using linear-regression analysis for both types of plots. The Lineweaver-Burk plot of the experimental data is shown in Figure 3. The types of inhibition present are not clearly discernable from analysis of Figure 3. Figure 4 shows the Hanes-Woolf plot of the experimental data. The lines with

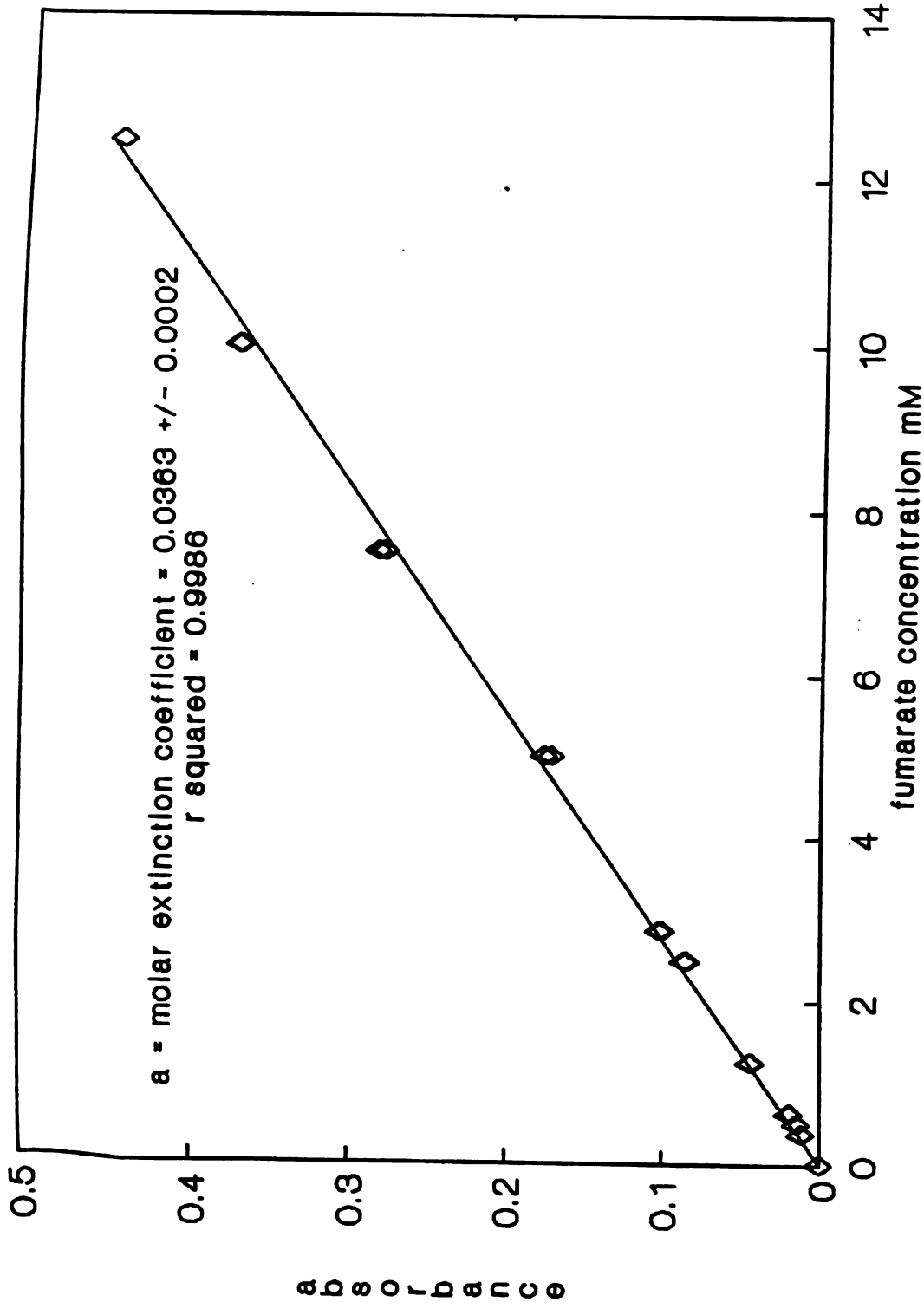

Figure 2. Calibration Curve for Fumarate Absorbance at 330 nm

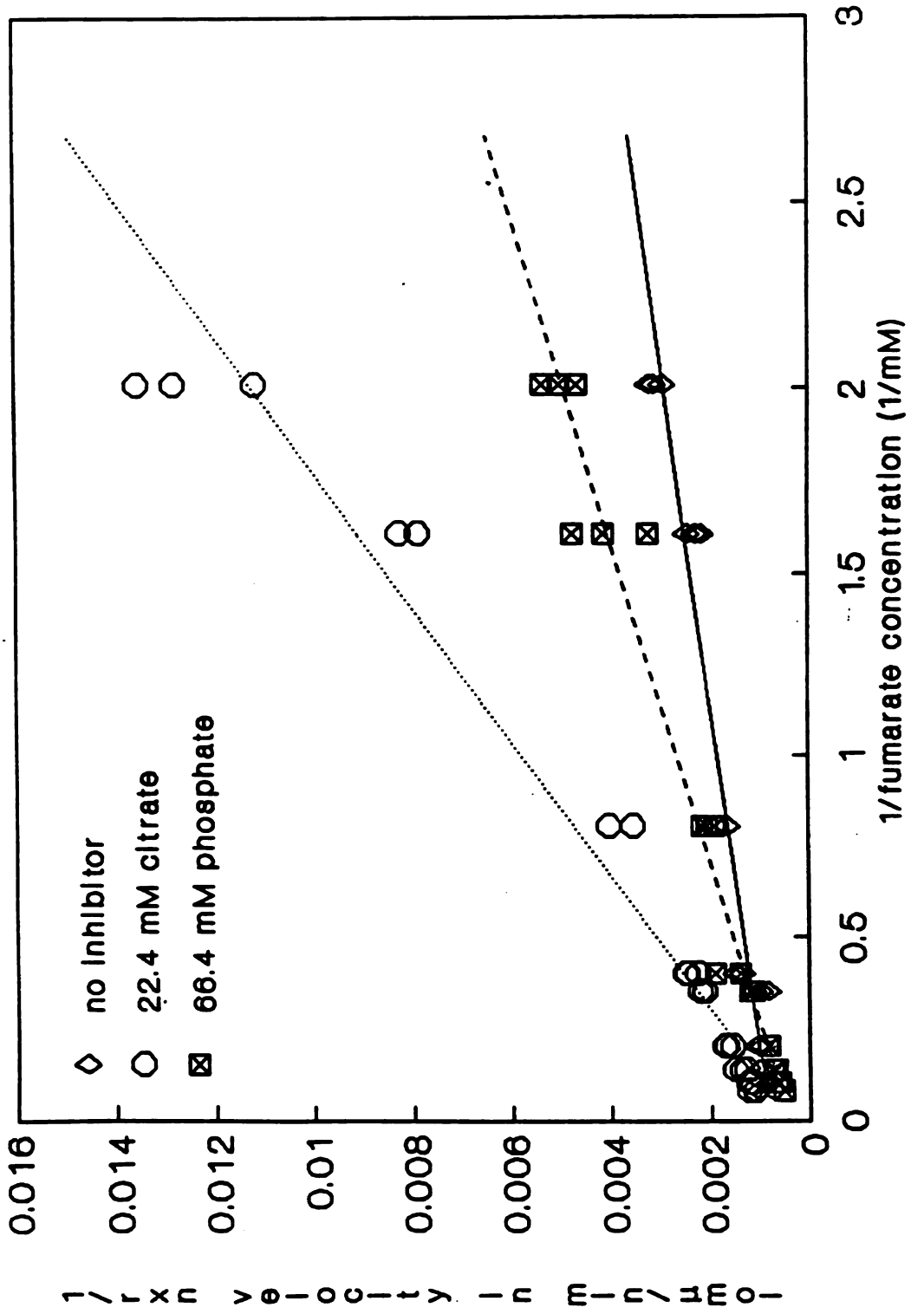

Figure 3. Lineweaver-Burk Plot for Determination of Kinetic Constants

and without citrate are nearly parallel, suggesting competitive inhibition by citrate, which agrees with Massey's findings (Massey, 1952). The lines for the phosphate and the fumarate intersected above the x-axis on the Lineweaver-Burk plot, which suggests behavior resembling mixed inhibition. Figure 5 shows the reaction velocities calculated by the two models and experimental data plotted versus the fumarate concentration. The K_m, V_{max}, and the r^2 values calculated by linear regression for both plots are given in Table 2 below:

Table 2. Kinetic Constants for Fumarase Determined by Different Plotting Techniques

Plot Technique	K_m	V_{max}	r^2 value
Lineweaver-Burk	1.336	1271	0.9498
Hanes-Woolf	1.219	1236	0.9481

The r^2 values suggest that the Lineweaver-Burk plot constants may fit the data slightly better than the Hanes-Woolf constants. The plot of v versus [S] shown in Figure 6 demonstrates that the constants derived from the Hanes-Woolf plot model the competitive-inhibition data for 22.4 mM citrate better than those from the Lineweaver-Burk analysis. Bock and Alberty obtained a K_m value of 1.37 ± 0.14 mM for fumarate using a Lineweaver-Burk plot (Bock and Alberty, 1971). This value is within the experimental error of the present study. The values of $K_{m,app}$, $V_{max,app}$, K_I and the r^2 values for citrate are given below in Table 3 for both the linear plots.

The value for K_I determined by Massey for citrate at pH 6.35 and 23°C was 3.5 mM.

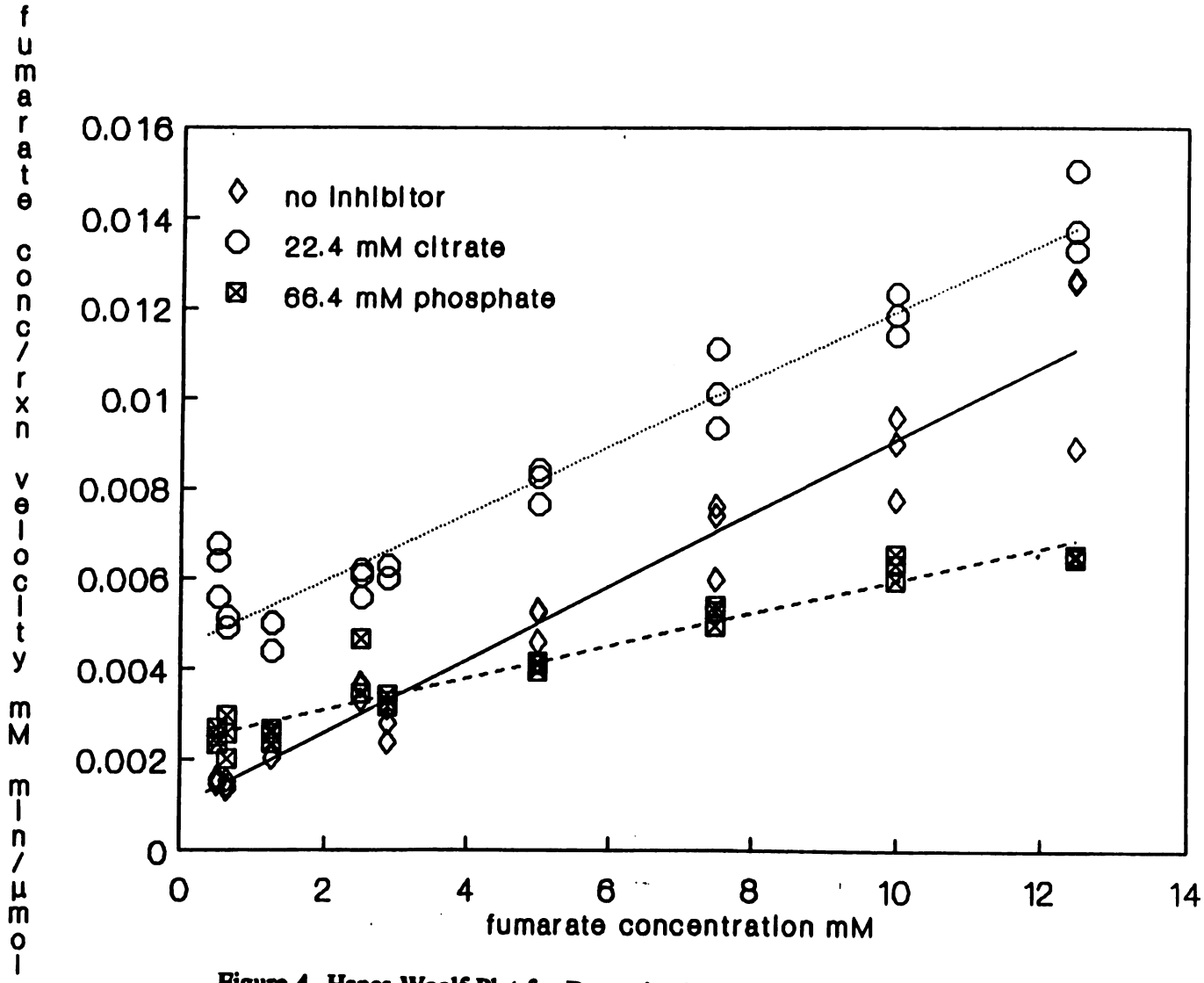

Figure 4. Hanes-Woolf Plot for Determination of Kinetic Constants

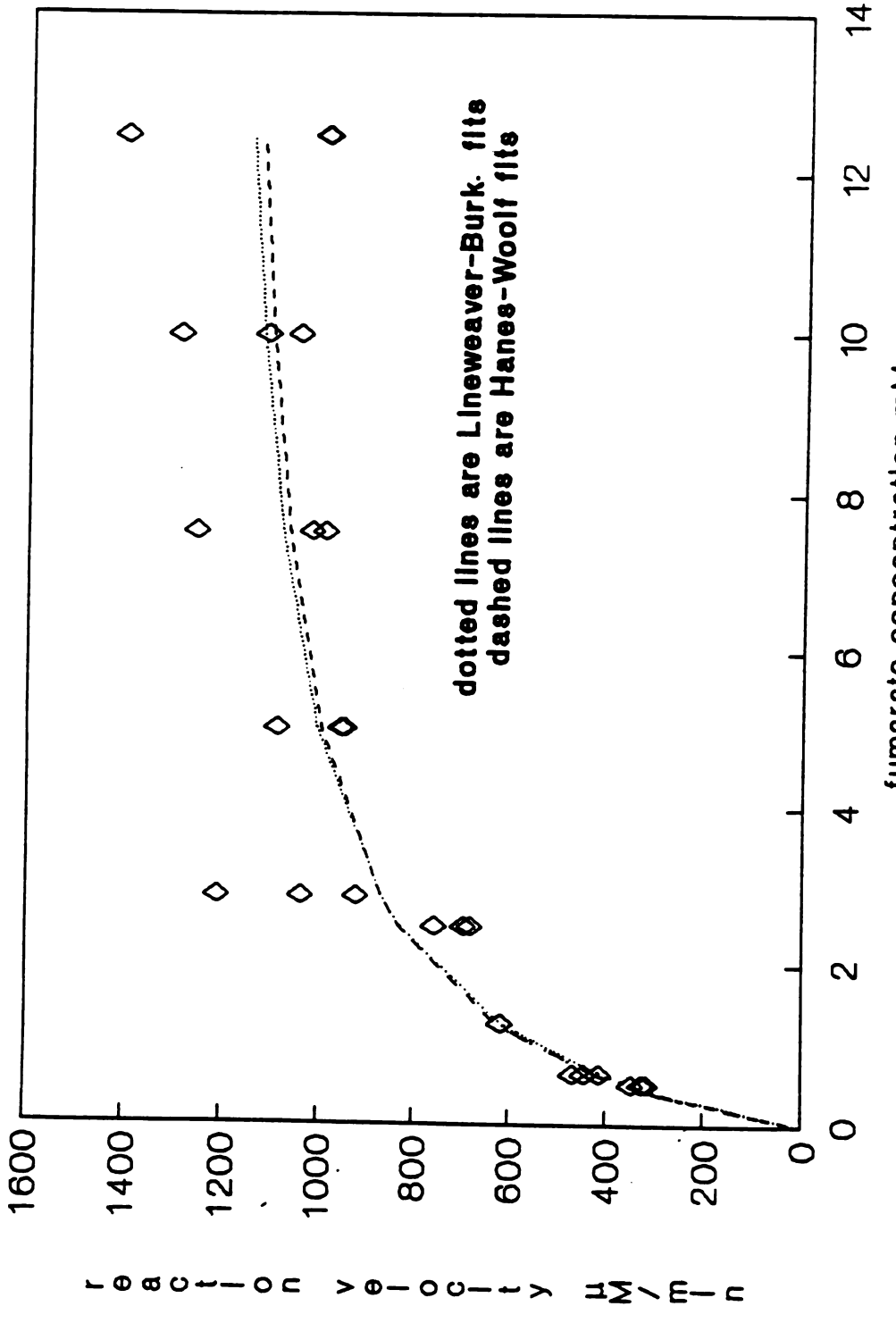

Figure 5. Reaction Velocity versus Substrate Concentration for Fumarate

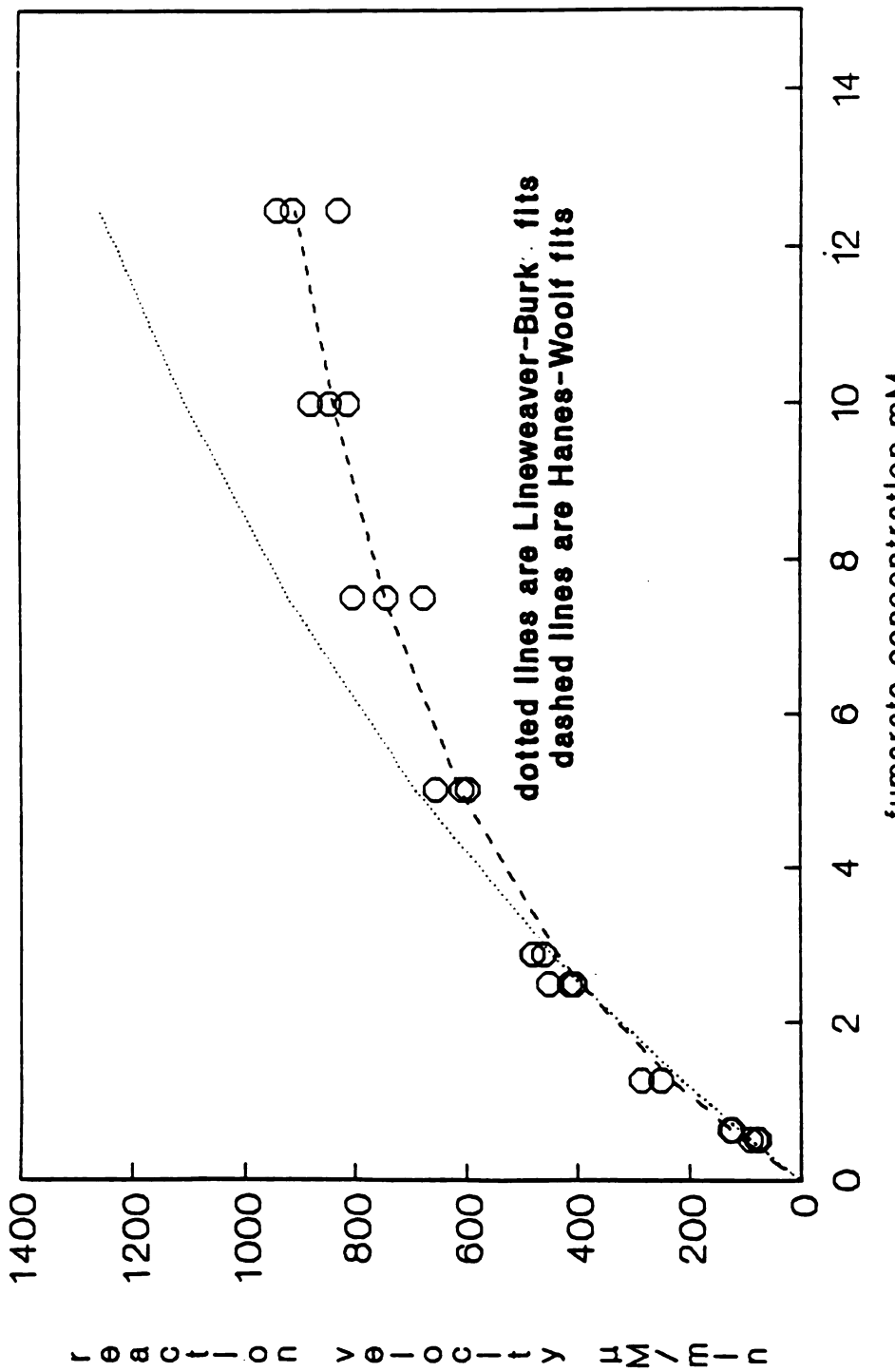

Figure 6. Reaction Velocity versus Substrate Concentration for Fumarate with 22.4 mM Citrate Present

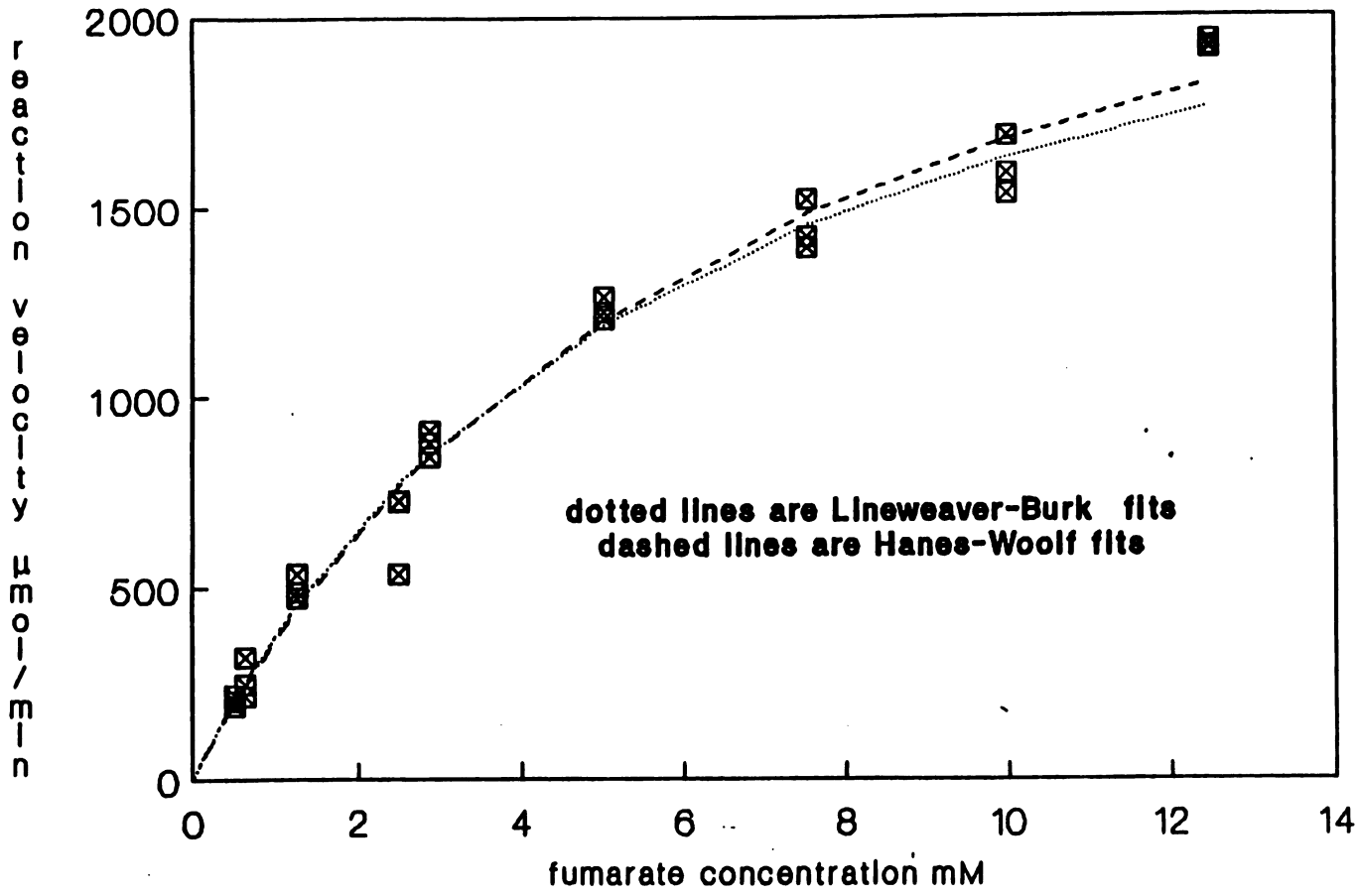

Figure 7. Reaction Velocity versus Substrate Concentration for Fumarate with 66.4 mM Phosphate Present

Table 3. Inhibition Constants for the effect of Citrate on Fumarase Activity

Plot Technique	$K_{m,app}$	$V_{max,app}$	K_I	r^2 value
Lineweaver-Burk	15.03	2768	2.187	0.9586
Hanes-Woolf	6.030	1345	5.678	0.9460

Experimental error and pH differences may account for the between the literature and the present study. One of the experimentally determined values for K_I is slightly larger, and the other is slightly smaller than the literature value, indicating that the determination was reasonably accurate. Figure 7 shows the experimental and model reaction velocities for phosphate in which neither plotting technique produces a clearly superior fit to the data. Table 4 shows the kinetic constants derived from the experiment with phosphate. The phosphate causes behavior resembling mixed inhibition by increasing the $K_{m,app}$ but it also significantly increases the $V_{max,app}$ of the system. Massey's data for the effect of phosphate on fumarase show a different trend: K_m was unaffected by phosphate but $V_{max,app}$ was increased (Massey, 1952). Massey also indicated that high phosphate concentrations decreased the enzyme activity due to high solution ionic strength.

Table 4. Inhibition Constants for the effect of Phosphate on Fumarase Activity

Plot Technique	$K_{m,app}$	$V_{max,app}$	K_I	α	r^2 value
Lineweaver-Burk	5.877	2591	17.38	3.488	0.968
Hanes-Woolf	6.637	2796	14.95	3.523	0.931

Figures 2 through 7 illustrate the scatter in the reaction-velocity data, which is

attributed primarily to the error involved in the determination of the slope of the absorbance-versus-time data. Several previous experiments also demonstrated the need for uniform delivery of the correct volume of enzyme to reduce data scatter.

Experimental error affects the two types of plots in different ways. The reaction velocity assay exhibits the greatest error at low substrate concentrations. The data at low substrate concentrations are the most heavily weighted by the Lineweaver-Burk analysis since the technique uses the reciprocal of the substrate concentration. Figure 3 demonstrates that the points that correspond to higher fumarate concentrations are located near zero and that the lower concentrations are located much farther from zero. Thus, the slope determination, and hence the K_m value is most strongly affected by the data with greatest amount of experimental error. In contrast, Figure 4 shows that all of the substrate concentrations are given equal weight by the Hanes Woolf Plot due to their even spacing. The error of the Hanes-Woolf analysis is greatest when the reaction velocity is small because the uncertainty in the measurement of the velocity becomes significantly large. This occurs at low substrate concentrations and has a stronger effect when inhibition is studied.

Conclusions and Recommendations

The enzyme fumarase has been shown to follow Michaelis-Menten kinetics. Citrate has been shown to be a competitive inhibitor of fumarase, and phosphate appears to be a mixed inhibitor. The Hanes-Woolf technique presented a clearly superior fit to the Lineweaver-Burk plot in only one case. The Lineweaver-Burk plots produced

slightly better linear fits to the data, but both plots yield kinetic constants that fit the data well. The delivery of the correct amount of enzyme and the solution pH is crucial for good experimental results.

It is recommended that the computer-controlled and monitored spectrophotometer be used to carry out this experiment for the teaching laboratory. The computer-controlled spectrophotometer possesses software which is designed specifically for kinetic assays and uses linear-regression techniques to obtain reaction velocities. It is also recommended that a repeat pipettor be used for the crucial step of addition of enzyme to the cuvette because of its speed and reproducibility.

Many further optional studies for this experiment are available. Several documented inhibitors exist for inhibition studies. The reaction could also be studied in the reverse direction by starting with L-malate and monitoring the reaction with and without the presence of the inhibitors.

Symbols

α, α' = constants that are functions of the type of inhibition
A = absorbance
a = molar extinction coefficient
b = length of the light path (cuvette width)
c = concentration in moles
I = intensity of the transmitted light
I_o = intensity of the incident light
K_I = inhibition constant
K_m = half saturation or Michaelis constant
$K_{m,app}$ = apparent Michaelis constant
P = product concentration
S = substrate concentration
t = time
v = reaction velocity
V_{max} = maximum reaction velocity
$V_{max,app}$ = apparent maximum reaction velocity

[I] = inhibitor concentration

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Appendix

Equipment and reagents needed:

fumarate	fumarase
L-malate	citric acid
spectrophotometer and recorder	polymethacrylate cuvettes
pH calibration solutions	pH meter
Na or K phosphate	volumetric flasks
gloves	parafilm

pipettes (5μL, 10μL, 20 μL, 2 mL, and 5 mL)

Experimental Procedure

I. Preparation of Stock Fumarate Solutions

1. Weigh out sufficient fumaric acid for 500 ml of 18.75 mM solution of fumaric acid.

2. Adjust the pH of the fumaric acid using a dilute NaOH solution to 7.0.

3. Dilute the stock fumarate solution into more solutions following table 5 below checking and adjusting the pH if necessary.

Table 5. Dilution Schedule for the Fumarate Solutions

Stock Conc (mM)	Cuvette Conc (mM)	% stock in solution	volume made (ml)	stock added (ml)	water added (ml)	# of runs available
18.75	12.45847	100	500	500	0	79
15	9.966777	80	100	80	20	50
11.25	7.475083	60	100	60	40	50
7.5	4.983389	40	100	40	60	50
4.3125	2.865449	23	100	23	77	50
3.75	2.491694	20	100	20	80	50
1.875	1.245847	10	100	10	90	50
0.9375	0.622924	5	100	5	95	50
0.75	0.498339	4	100	4	96	50

II. Determination of the Standard Curve

1. Remove any cuvettes from the sample and reference beams.

2. Turn on the spectrophotometer's UV bulb using the UV ON switch on the front lower right of the spectrophotometer.

3. Turn on the power switch located on the back of the unit and allow 30 minutes for the spectrophotometer to warm up.

4. Turn on the computer and type "PECSS".

5. Once the computer screen states "ready for next command", press Shift-F10 and use the arrow keys to move the cursor to "PARAM". After ORD type A for absorbance, after speed type 120, after Autosave type Y, and after Autoprint type Y unless the printer is not connected in which case type N and hit enter.

6. Hit Shift-F10 again and this time move to autozero. Always hold cuvettes by the frosted sides and wipe the clear sides with a kimwipe before inserting them into the spectrophotometer to obtain a reading.

7. Insert the cuvette containing the blank solution (RO water for the calibration curve) in the reference (rear) position and place a cuvette containing the blank solution in the front position.

8. Under autozero type A after "mode" for absorbance and 300 after "wave" for the wavelength which is 300 nm. When the cuvettes are in spectrophotometer and the lid is closed type Y to begin the autozero procedure.

9. Press Shift-F10 again and move to the CALIB menu. After "method" type fumarate and after "nwave" type 1. After "curve" type 1 this chooses a linear calibration curve that passes through the origin.

10. After "wavelength w1_" appears type 300 for 300 nm.

11. When the screen displays "Change or save the instrument parameters (Y/N)?" type N.

12. Place the unknown in the front sample holder and record the spectrophotometer reading and the concentration of the stock solution as well as the concentration of the fumarate in the cuvette. The unknowns should contain 2 ml of each of the stock solutions from table 1 and 1 ml of water. Mix the contents of the cuvette by placing a clean piece of parafilm over the end of the cuvette and gently inverting it several times. Practice mixing quickly producing as few bubbles in the solution as possible.

13. When prompted "Ready (Y/N)? enter a sample id of Cal01 and the concentration of the sample in the cuvette after it is displayed. The next sample should automatically call itself CAL02 so all you will need to do is type Y under "Ready (Y/N)?" and change the concentration under "conc". When all the standards have

been recorded type N after "Ready (Y/N)?".

14. The computer will then calculate a calibration curve and display it on the screen.The slope is the molar extinction coefficient and is in the 2 nd column after "calib prms:" at the top of the screen. Record the slope and the residual error ("resid err:").

15. Use the data to plot the concentration on the y-axis and plot the absorbance data on the x-axis. Find the slope of the data using either a graphical technique or a least squares fitting (preferred). The slope determined is the molar extinction of the fumarate at 300 nm as defined by the Beer-Lambert Law.

III. Determination of K_m and V_{max} for Fumarate

1. Make sure that the spectrophotometer is on and PECSS is started and autosave is on under PARAM as outlined in section III. Autozero the spectrophotometer with the two cuvettes you will be using by pressing Shift-F10 and moving the arrow to AZERO. Enter A , 300 and Y to begin.

2. Obtain an ice bucket and fill with ice. Obtain gloves for handling of the enzyme. The enzyme poses no real health danger, but skin has many proteases and other substances on it that could denature the enzyme.

3. With a clean, sterile pipette remove a small aliquot of enzyme solution from the bottle in the refrigerator. Place the aliquot on ice immediately and make sure the main vial of enzyme is sealed tightly and replaced in the refrigerator.

4. Using all the solutions in listed in table 1 prepare solutions containing 2 ml of the given fumarate solution and 1 ml of water in the sample cuvette. Cut out squares of parafilm to serve as caps for the cuvettes during mixing of the cuvettes.

5. Press Shift-F10 and move the arrows to TDRIVE. Under "reg" type x, after "wave" type 300, under "NoPts" type 1000, and after "int" type 0.5 to have a time interval of 0.5 seconds between the recording of each slope and press enter.

6. Use the arrows to move past "ymin" and "ymax". Under sample ID enter something that corresponds to the sample since each sample must have a different sample id. A sample id of "15r1ak" could be used to indicate the 1 st run of the 15 mM fumarate by someone whose initials are A.K. Remain on this screen and move to the "Ready (Y/N)" put Y on the screen but do not press enter until the cuvette is placed in the spectrophotometer.

7. Rinse the pipet used for the enzyme thoroughly with water. Measure out 10 μL of fumarase and quickly add it to the cuvette. Cap the cuvette with the parafilm

square and mix by gently but quickly inverting the cuvette, taking care not produce bubbles.

8. Immediately place the cuvette in the sample holder close the lid and press Y and Enter under "Ready (Y/N)" in TDRIVE. After a few minutes after the line displayed on the screen is clearly no longer linear press Esc to stop the run.

9. Type N to leave the TDRIVE menu so you may determine the reaction velocity using the VIEW and SLOPE commands.

10. Press F9 to use the VIEW command. Enter x under "reg" and press enter unless yo wish to change the defaults. Once you have looked at the graph attempt to narrow down the linear region of the absorbance versus time curve. Press F9 again and enter the times during which the slope appears linear under "start" and "end". Continue focusing in until you are satisfied with the linearity of the slope. Ignore the initial linear period that may last about 1 second or so. Look for the initial linear period after that.

11. When you are satisfied that you have isolated the linear region record the start and end times and press Shift-F3 to use the slope command. After "reg" type x and enter the start and end times you just determined under start and end and enter 1 for the factor to receive the slope in A/sec or 60 to receive the absorbance in A/min. and press enter.

12. The slope is given by the "SL" , the residual error is given by "RE", and a linearity factor is given by "LF", and F is the factor by which the slope was multiplied(1 for A/sec, 60 for A/min).

13. Return to TDRIVE enter a new file name and repeat the process when ready to quit type stop and hit enter which will exit the program.

IV. Determination of K_I for Citric Acid for Fumarate

1. Prepare a stock solution of 42 mM Citrate buffer to pH 7.

2. Autozero the spectrophotometer using a new blank consisting of 2.5 ml water and 0.5 ml citrate.

3. Run activity assays for all the solutions in table 1 with 2 ml of fumarate solution, 0.5 ml of citrate stock solution, and 0.5 ml RO water. Use the procedures outlined above in section III.

V. Determination of the Effect of Phosphate on Fumarase Activity

1. Prepare a stock solution of 400 mM phosphate buffer to pH 7.

2. Autozero the spectrophotometer using a new blank consisting of 2.5 ml water and 0.5 ml phosphate.

3. Run activity assays using 2 ml fumarate solution, 0.5 ml phosphate stock solution, and 0.5 ml water use the procedures outlined in section III.

Fermentation Mass Transfer

Summary

Experiments were performed to determine the volumetric mass-transfer coefficient, k_La, for oxygen in water in a 1 L fermentor. The concentration of oxygen was monitored as a function of time using a polarographic dissolved-oxygen probe after a step change was made in the gas-phase oxygen concentration. The dissolved-oxygen data were analyzed using unsteady-state mass balances to determine the k_La. The experiment will eventually be performed in a 100 gallon stainless-steel fermentor that is currently under renovation.

Introduction

Fermentation can be loosely defined as the production of chemicals from a substrate using microorganisms as catalysts for the reaction. Fermentation is used to produce a variety of commercial products including alcohol and pharmaceuticals. Many fermentations are carried out using aerobic microorganisms, which consume dissolved oxygen from the fermentation broth. Oxygen is transferred to the liquid phase by sparging air or pure oxygen through the fermentation broth. The ability to accurately measure and predict the oxygen mass-transfer rate is critical in bioreactor design since oxygen transfer is often rate-limiting in commercial fermentations (Roberts et al., 1992).

Background

Fermentation rates are often limited by the amount of dissolved oxygen available to the cells growing in the fermentor. Oxygen mass transfer to the liquid phase is inherently slow due to its low solubility (Bailey and Ollis, 1986). The solubility of oxygen in water in equilibrium with air at ambient conditions is about 10 parts per million (ppm), and a typical aerobic yeast population requires over 750 times that amount (Atkinson, 1983).

The rate of oxygen transfer to the liquid increases with the rate of agitation. Impeller shear induces bubble breakup, thus decreasing the bubble rise velocity and increasing the residence time for the gas bubbles in the liquid. The increased residence time allows more mass transfer to occur before the bubbles exit the liquid. The reduction in bubble size also enlarges the interfacial area of the bubbles available for mass transfer, which further increases the rate of mass transfer. Other important variables that affect the rate of mass transfer include the gas flow rate, the geometry of the mixing vessel, the physical properties of the fluid, the type of impeller used, and the power input to the system from mixing and gas sparging. The geometry of the mixing tank, power input and the impeller style are specified during the design of the system. The tank geometry may be characterized by several geometric ratios known as "shape factors" (McCabe, Smith, and Harriot, 1985). Mixing vessels are often designed to match typical "shape factors" given in the literature for which correlations exist.

The type of impeller used for agitation strongly affects both the mass-transfer coefficient and power requirements of a system. Impellers are usually classified as either axial or radial flow. Axial-flow impellers displace the fluid downward along the axis of

rotation, and radial-flow impellers push the fluid outward in the radial direction. Radial-flow impellers generate higher shear rates and better gas dispersion, but they are more costly to operate since they consume more power than axial-flow impellers (Oldshue, 1990).

Traditionally, commercial fermentors have used radial-flow impellers because of their excellent mass-transfer capabilities. The high operating cost of radial-flow impellers has initiated the development of advanced axial-flow impellers that promote mass transfer and yet require less power than radial-flow impellers. These new impellers were developed using complex hydrodynamic modeling and laser-doppler anemometry (Oldshue, 1990).

Theoretical Analysis

The aqueous solubility of oxygen is controlled by the partial pressure of oxygen, the temperature and the presence of other solutes. Sparingly soluble gases such as oxygen are often modeled by Henry's law:

$$C_s = \frac{p_G}{H} \tag{1}$$

where C_s = liquid phase concentration
p_G = the partial pressure of the gas
H = Henry's law constant

The solubility of oxygen is strongly affected by the presence of other solutes in a fermentation broth that affect bubble coalescence and breakup. The accuracy of mass

transfer studies is significantly increased by using a fluid with properties as similar to the fermentation broth as possible.

An unsteady-state mass balance on oxygen in the liquid phase of a stirred tank is given below:

$$\frac{d(C_{O_2}V)}{dt} = k_L a(C_{O_2}^{*} - C_{O_2})V - (-r_{O_2})V \qquad (2)$$

where k_L = mass-transfer coefficient (length/time)
a = specific interfacial area (area/liquid volume)
C_{O2}^{*} = liquid-phase oxygen concentration in equilibrium with the gas composition (mass/volume)
C_{O2} = liquid-phase oxygen concentration (mass/volume)
t = time
V = liquid volume in the reactor
$-r_{O2}$ = rate of oxygen consumption (mass/liquid volume time)

The volumetric mass-transfer rate is given by the product of a mass-transfer coefficient and a concentration driving force. The rate of consumption of oxygen, $-r_{O2}$, is represented by a kinetic expression in terms of oxygen concentration. If the rate of oxygen consumption is approximately constant, a quasi-steady state assumption can be made for the system. The change in the partial pressure of oxygen with time can be assumed to be approximately zero, resulting in the following solution for k_La (Roberts et al., 1992):

$$k_L a \approx \frac{-r_{O_2}}{C_{O_2}^{*} - C_{O_2}} \qquad (3)$$

This method of determining k_La is sometimes called the steady-state method.

The static gassing out method is used to determine the k_La in this experiment.

The value of r_{O2} is equal to zero since no oxygen-consuming reaction takes place, and the mass balance becomes:

$$\frac{dC_{O_2}}{dt} = k_L a(C_{O_2}^* - C_{O_2}) \tag{4}$$

The method consists of allowing a saturation concentration, C_{O2}^*, to be achieved in the liquid phase and then making a step change in the gas-phase concentration and hence the oxygen saturation concentration, C_{O2}^*. The liquid-phase oxygen concentration is then monitored as a function of time using a dissolved-oxygen electrode. The dissolved-oxygen data may be analyzed using the mass balance without a reaction term in either its differential form or one of its integrated forms. The integrated form of the equation appropriate while sparging the fermentor with air is given by (Atkinson, 1983):

$$\ln(1 - \frac{C_{O_2}}{C_{O_2}^*}) = -(k_L a)t \tag{5}$$

The integrated form of the equation for sparging with nitrogen is:

$$\ln(\frac{C_{O_2}}{C_{O_2,air}^*}) = -k_L a t \tag{6}$$

where $C_{O2,air}^*$ = oxygen solubility in equilibrium with air

The mass-transfer coefficient, $k_L a$, is a function of the tank and impeller geometry, the agitation power input to the system, the rate and method of sparging, and many factors pertaining to mass transfer such as the bubble size. The mass-transfer coefficient is often correlated in terms of sparging rate and power input in the following

form:

$$k_L a = \alpha \left(\frac{P}{V}\right)^{\beta} Q_{air}^{\gamma} \qquad (7)$$

where α, β, γ = constants dependant on hydrodynamic properties and the vessel geometry
P/V = agitator power per reactor volume
Q_{air} = volumetric flowrate of air

The volumetric flowrate of air can results in either of two air flow regimes: free-bubble-rise, which occurs in large scale equipment; and high-turbulence, which occurs in small scale laboratory fermentors. More data exist for the high turbulence regime than for the free-bubble-rise due to the large-scale equipment required and proprietary nature of fermentor design (Atkinson, 1983). The agitator power is a function of agitator rpm for a fermentor containing a Newtonian fluid. Hence, the $k_L a$ for a Newtonian fermentation broth and a constant air flow rate can be correlated in terms of the impeller rate by the following equation (Roberts et al., 1992):

$$k_L a = \alpha'(N)^{\beta'} \qquad (8)$$

where α', β' = constants
N = impeller rate , revolutions per minute

Experimental Equipment and Procedure

The equipment used for this experiment included a 1 L New Brunswick MultiGen (New Brunswick, NJ) fermentor, an Ingold model 170 percent O_2 amplifier with a polarographic electrode (Wilmington, MA), a nitrogen cylinder, and a Dow Camile data acquisition and control system (Midland, MI). Figure 1 illustrates the experimental setup

of the equipment. The impellers used in the fermentor were two equally spaced, 6 cm diameter, 6-bladed, Rushton, radial-flow impellers. The tank was 4.5 inches in diameter and contained two 0.5 inch wide baffles.

The O_2 probe and amplifier were calibrated after the fermentor reached 30°C. Air was sparged through the fermentor at 2 L/min, and the agitation was set at 1000 rpm. The O_2 amplifier had 4-20 mA outputs which were connected to the data-acquisition system. The mA output of the amplifier was monitored using the data-acquisition system until steady state was reached. Then the amplifier was calibrated to 20.9 percent oxygen in air by turning the screw on the front of the unit. The amplifier was calibrated for zero percent O_2 by sparging nitrogen into the fermentor until a steady value of zero was reached. The data-acquisition system was then calibrated with respect to the amplifier to convert the mA signals to percent O_2. The fermentor was sparged with air and nitrogen at 1000 rpm repeatedly to verify that the calibration of the amplifier and data-acquisition system was stable.

The k_La was determined at 400, 600, 800, and 1000 rpm for air and nitrogen flowrates of 2 L/min. Experiments began by sparging with air until oxygen saturation was achieved. Then the air flow was replaced by nitrogen flow at the same rate. The decrease in the dissolved-oxygen (DO) concentration was monitored and recorded using the data-acquisition system. After the DO concentration reached zero, the experiment was run in reverse by sparging with air. The k_La for each run was determined using the appropriate form of the static-gassing-out equation. The slopes of the DO-concentration-versus-time data used in the equations were determined using linear regression.

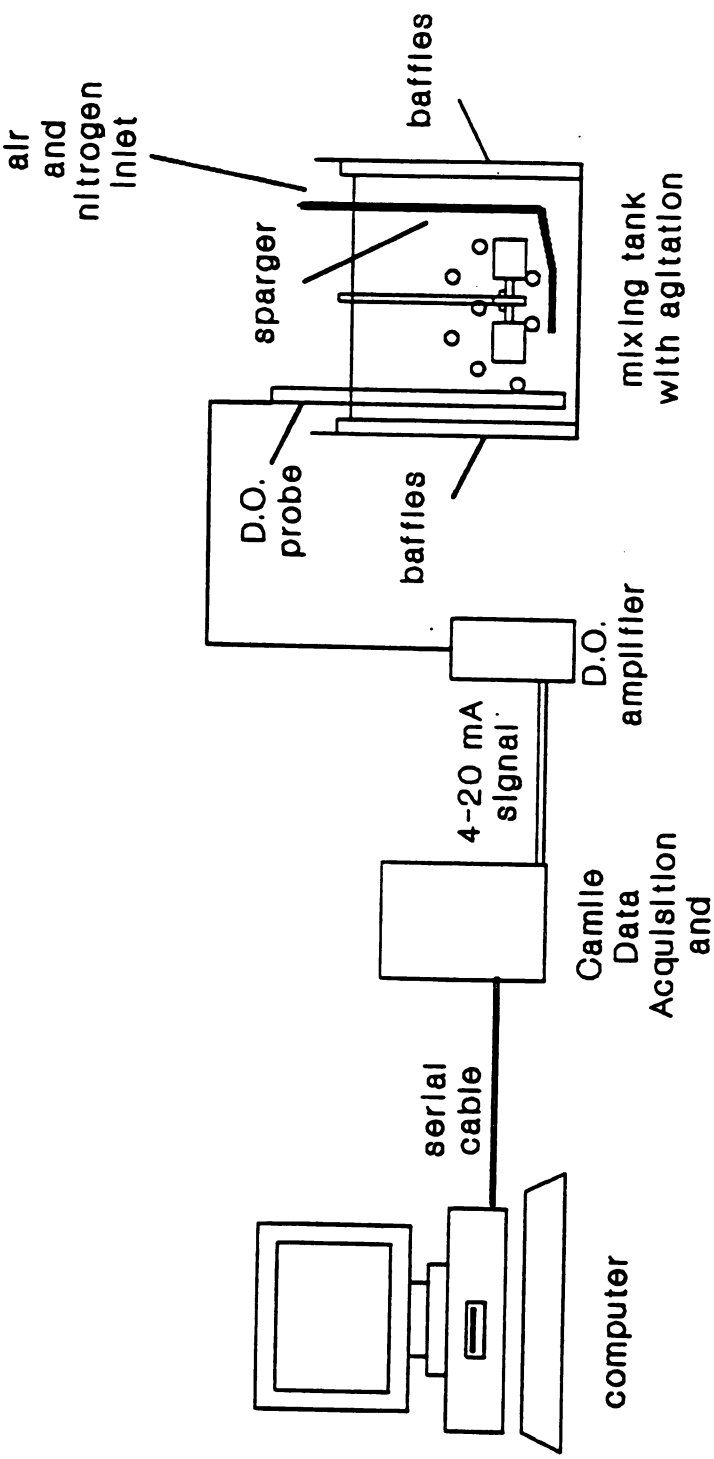

Figure 1. Experimental Setup for Mass Transfer Experiment

Experimental Results and Discussion

Figure 2 shows four sets of DO data at different impeller speeds for the determination of k_La when sparging an air saturated fermentor with nitrogen. The slope, and therefore k_La, increased substantially with the impeller rate. Figure 3 illustrates the fit of the experimental data to Equation (9) above. The constants α' and β' were determined to be 1.6×10^{-4} min^{-1} and 1.4 ± 0.1 for agitation rate in units of min^{-1} (rpm). The correlation coefficient of the least squares fit used to determine α' and β' was 0.923.

Experimental data collection about every 3 seconds was found to provide sufficient accuracy while avoiding excessively large data files that can cause retrieval and storage problems. The linear portion of the DO curves was usually about 40 seconds long, so recording data every three seconds allowed about 12 data points for the linear regression. The scatter in the data in Figure 3 is due to slight differences in the slopes determined for each run. The variation of the slopes between runs is due primarily to the differences in the selections of the linear regions chosen for each run.

Conclusions and Recommendations

The static gassing out method is a relatively quick method for estimating the k_La for a system. The k_La also is a function of impeller rate for Newtonian fluids at a constant air flowrate. This experiment demonstrates the basic principles of oxygen mass transfer in a stirred tank and the correlation between agitation rate and k_La. The experiment requires few reagents and little maintenance, making it an excellent experiment to operate

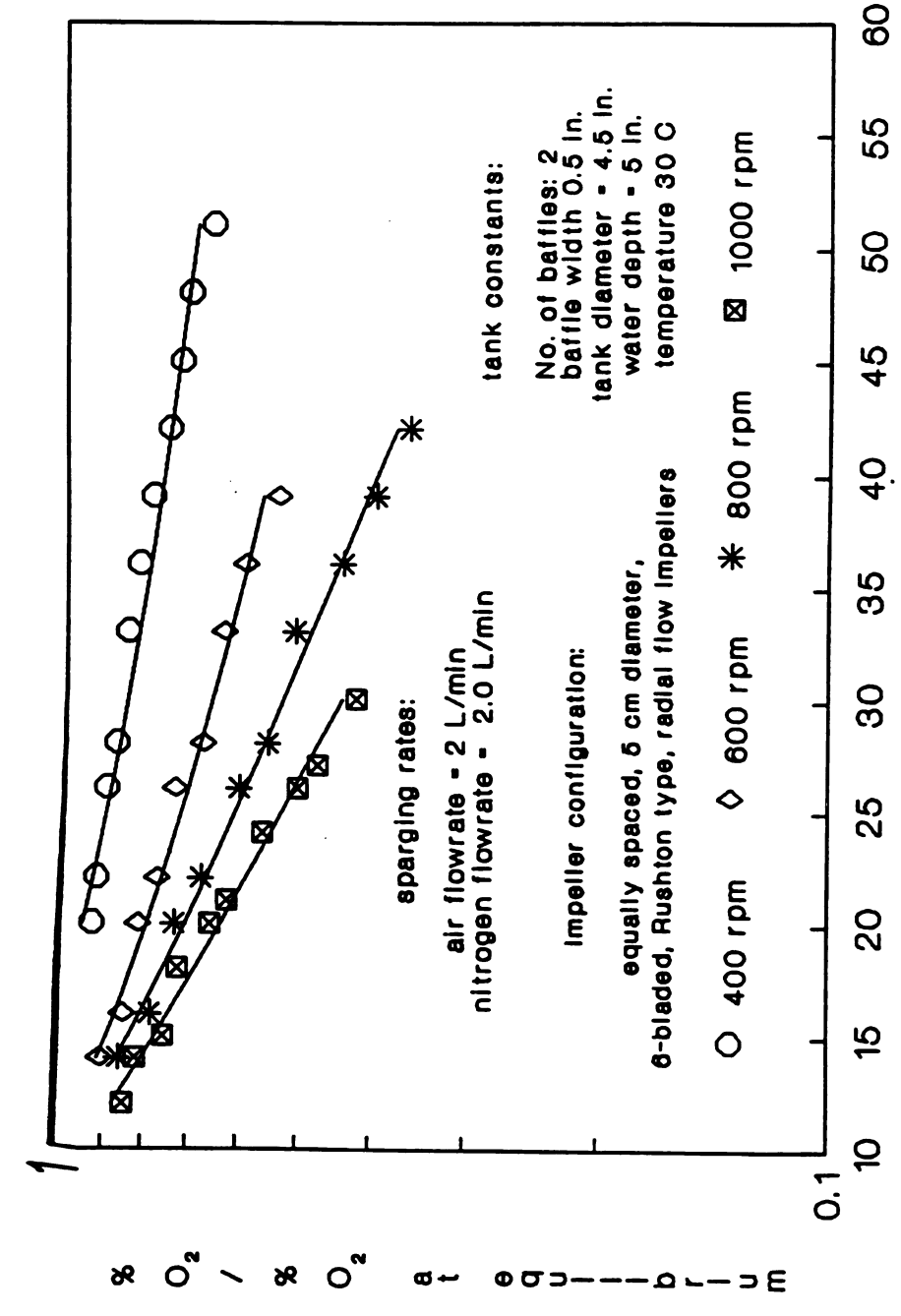

Figure 2. Dynamic Gassing Out Method of Determining k_La

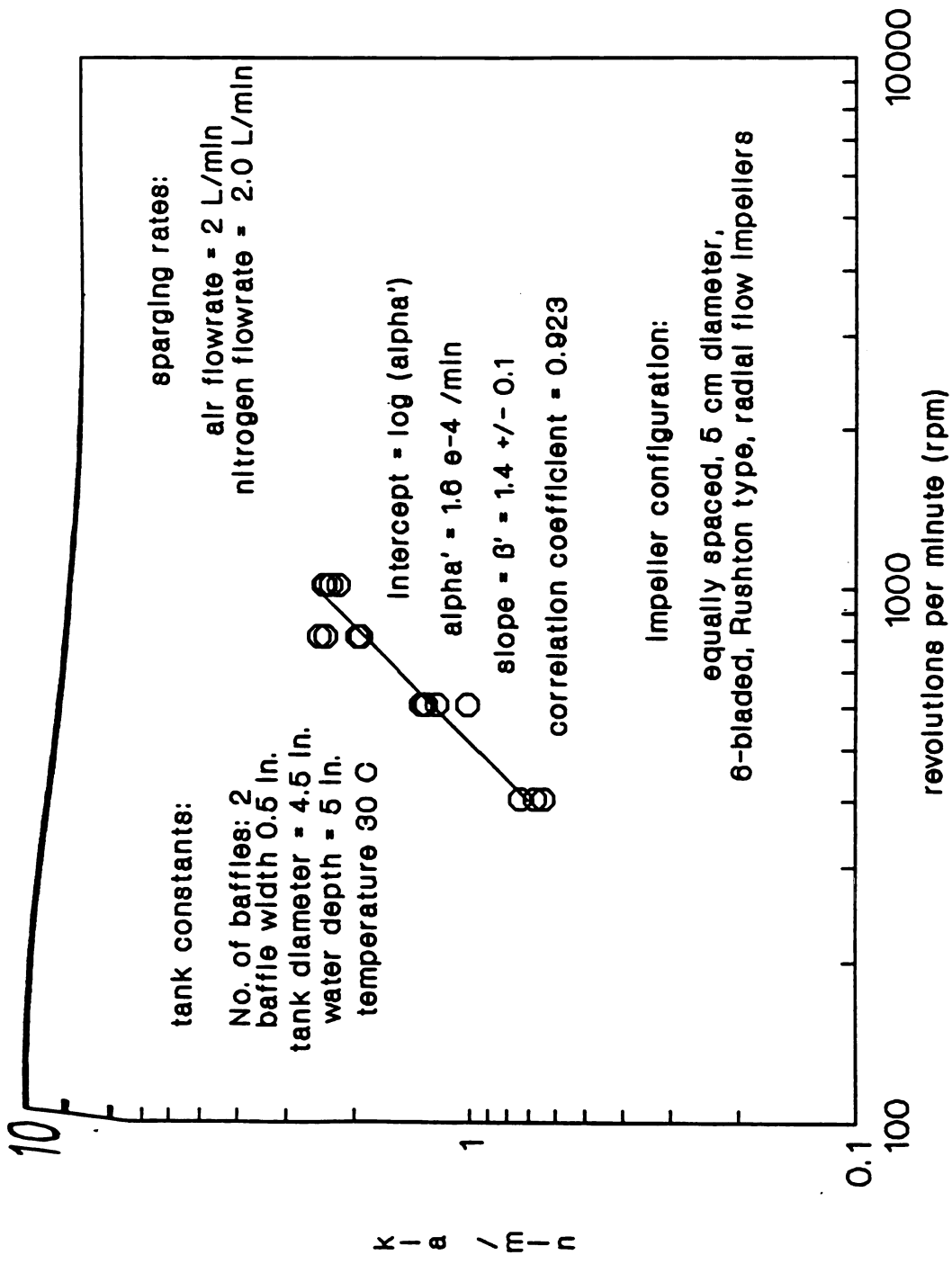

Figure 3. ka versus RPM

on a long-term basis. This experiment is relatively straightforward and produces high-quality data that can be analyzed using simple mass-balance equations. It is recommended that the 100 gallon fermentor currently being renovated for the laboratory be used for this experiment to observe the characteristics of mass transfer of a much larger scale fermentor. Determinations of k_La in fermentors of two different sizes would allow scale-up calculations to be performed and broaden the scope of this experiment.

Symbols

α, β, α', β', γ = constants dependant on hydrodynamic properties and the vessel a = interfacial area of the bubbles
a = specific interfacial area (area/liquid volume)
C_{O2} = liquid phase oxygen concentration (mass/volume)
C_{O2}^* = liquid phase oxygen concentration in equilibrium with the gas composition (mass/volume)
C_s = liquid phase concentration
H = Henry's law constant
k_L = liquid phase mass-transfer coefficient (length/time)
N = impeller rate , revolutions per minute
p_G = the partial pressure of the gas
P/V = agitator power per reactor volume, unit power per unit volume
Q_{air} = volumetric flowrate of air
$-r_{O2}$ = rate of oxygen consumption (mass/liquid volume time)
t = time
V = liquid volume in the reactor

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Appendix

Experimental Equipment
Materials needed:
dissolved-oxygen probe
Multigen fermentor
Camile data-acquisition system
nitrogen cylinder and regulator

Experimental Procedure

I. Operation of the Multigen fermentor

1. Turn on the power with the switch on the far right.

2. Turn on the switches for the air, agitation, and heat.

3. Adjust the dial for the temperature to the desired temperature and turn the dial for the agitation until the desired rpm reading appears on the gauge on the upper left.

II. Operation of the Camile System and Calibration of the Dissolved-oxygen probe

1. Consult with the other groups using the Camile system so that it may be used by all the groups. The immobilized biocatalysts, and the power and mass transfer experiments use the Camile system. The capabilities of the system for multiple users is limited so cooperation between lab groups is important.

2. If the camile system is not already started, start up the Camile system by turning on the computer and typing "cd\camile" to get to the Camile directory. Then type "menu" and the Camile logo will appear. Press any key and the main menu will be displayed. Use the arrow keys to highlight "run" and hit ENTER. Then use the arrow keys to highlight the database called "HEATTRAN" and hit ENTER. The computer will then ask you if you wish to initialize the Camile control constants. Type "y" and hit enter.

3. When the computer prompts for the default log directory "C:\camile\logs" should be present and highlighted. If not, backspace and type it in then hit enter. The log file screen should now be on the monitor. Use the arrows to move to log file 6 and type the name you have chosen for your log file. Make sure that "BIN" and "APP" appear in the fields to the right of the log file name.

4. Wait for the other groups to enter their filenames before pressing the END key. When all the groups have entered their filenames press END. If you have repeated any log file name currently on file, the computer will prompt you to

change it. The computer will then ask you to enter a title. This and the comments it prompts for next are optional, but your names and a few brief words describing your experiment might save considerable confusion later. Leave a blank line to finish and hit ENTER.

5. The computer will then inform you that the printer does not respond and will ask you if you wish to retry. Type N if there is no printer.

III. Calibration of the Dissolved-oxygen probe and the Camile system

1. Press the F4 button and then PAGE UP or PAGE DOWN until you are on the screen that has the tag name "oxygen". Look in the lower right hand corner next to the bar graph. Check to see that the slope is equal to 1 and that the intercept and filter constant are both equal to zero. If they are not you may reset these values by typing "SL 1","IN 0","FL 0" to change the slope, intercept and filter constant, respectively.

2. Turn on the air and open the valve on the rotameter all the way. Also set the agitation to 100 rpm. Record the flowrate of the air as given by the rotameter. You may use the on-line trending (F3) to make sure that the dissolved-oxygen probe is fully equilibrated before adjusting the meter.

3. Once the response from the meter becomes constant, turn the screw on the front of the dissolved oxygen meter to adjust the reading to 20.9 which is the percentage of oxygen in air. It is important the meter is calibrated for each temperature data is collected at since oxygen solubility is a function of temperature.

4. On the F4 screen of the Camile system labeled "oxygen", calibrate the camile system for the 0 - 20 mA output of the dissolved oxygen meter into units of % oxygen. Once the DO meter has equilibrated at 20.9 type "CAL HI 20.9", which records the amperage that corresponds to 20.9% oxygen.

5. Shut off the air and carefully open the regulator by slowly turning the bar to the right. Use the regulator to adjust the pressure and flow with the rotameter valve open. Record the flowrate of the nitrogen.

6. Allow the nitrogen to sparge through the fermentor until the dissolved oxygen content read by the meter stops changing. At that point type "CAL LO" and the value of the % oxygen read by the meter.

7. Type "CAL" again and the computer will calculate the a slope and an intercept which are necessary to convert the mA signal from the meter into % oxygen.

IV. Determination of k_La at a specific rpm and flowrate

1. Set the fermentor at the desired temperature for the experiment. Calibrate the oxygen probe for that temperature, remember to set the slope and intercept to 1 and 0 respectively before beginning the calibration.

2. If one of the other groups has not already started the question and answer menu press F6 and type "S2 ON". This activates a question and answer sequence that will prompt you for the parameters needed to log the data. You may leave a question and answer screen at any time by pressing another one of the function keys. The F1 key is help and a list of the commands for the function keys is given by ALT-F1. You can then return to the question and answer screen by hitting ALT-F8 at any time.

3. Set and record the rpm for the experiment and adjust the air flowrate for the experiment.

4. Allow the oxygen concentration to reach steady state.

5. Once the oxygen concentration has reached steady state, make preparations to begin logging data for your experiment by using the menus to get to the screen that asks if you wish to begin logging for the oxygen mass transfer experiment.

6. Move through the screen into the highlighted areas to and type in the values you desire. The logging period should be 3 seconds and the maximum length of the experiment should be around 2 minutes. Change the "Do you wish to begin logging?" to YES by typing "y". Nothing will occur from the changes made on any of the question and answer screens until the END key is pressed. Make sure to press the END key when you are ready.

7. Begin logging just before the air is shut off and the nitrogen is started unless the number of users on the Camile allows you access to begin logging as the nitrogen gas is started. You may record the rpm and gas flowrates in the log file by pressing the F8 key and typing them in. When typing in comments include your initials so that they may be distinguished from the comments recorded by the other groups using the camile system.

8. Monitor the progress of you experiment by using the on-line trend screens (F3). You may explore the functions of the Camile at this time as most functions that will harm your experiment are denied access without a password. Do not change your slope and intercept. An interesting feature of the on line trending screens of the Camile system is the history trend. The history trend appears as graph 9 in the on-line trending screens and can be reached by paging up or down after hitting F3. The history trend displays the data already logged into your file. To

view a history trend type "Read" while above graph 9 and then the cursor will move to the middle of the screen next to the word tagname. Type in "oxygen", the tagname for the dissolved oxygen experiment, and then hit enter. The cursor will move to the next row and which will say "log file". Enter 6 and press END. At this point the computer should begin reading your data from the log file and displaying it on the screen. If you or another lab group wishes to use the history trend later you must first type "Clear" and then re-enter the pertinent information.

9. After you have been logging data for the time you specified on the question and answer screens, the Camile will automatically stop logging data. If you wish to stop logging before the time you specified runs out press ALT-F8 to get the question and answer main menu which will offer to stop logging for your experiment. Enter the number corresponding to stopping logging for that experiment on the next menu that is displayed and your group's security code. The security code is to prevent other groups from accidentally stopping logging data for your experiment.

10. Repeat the experiment two or three times for each set of conditions and choose a variety of rpms for each flowrate and use several air flowrates or temperatures if time allows.

11. To get your log file off the hard disk for analysis you will have to exit run and use the DATA subprogram to convert your binary log file into a comma separated value ASCII file. At this time ask the teaching assistant or the instructor for the password. Once it has been entered by pressing the F9 key and typing "LEVEL ENGINEER" and the password, you may type "BYE" and exit run.

III. Data Analysis

1. Use the arrows to move the highlighting to DATA and press ENTER. The screen should say "output file type:ASCII" and "Report all time points:yes" then hit END. A whole list of options will then be provided for you to change. Highlight a particular selection and then press the space bar to view the various options and hit ENTER when the option you desire is shown. Make sure you include comments, tag values, elapsed time, date, and the time of day. The column delimiter must be a comma for easy retrieval of the log file into a spreadsheet program. When the appropriate options have been selected, press END. Highlight the two tag names (oxygen and timedo) and press the space bar. An arrow should be present on the left of the names and then press END.

2. The computer will then show the prompt "EDIT filename:" and your log file title with a ".prn" extension will be displayed. Hit END and you will exit DATA and then press escape to exit the Camile menu.

3. Import the log file into a spreadsheet as a comma separated value format (CSV). (i.e. \\Import,CSV,\camile\logs\oxygen.prn). You may do this from SuperCalc4 from cd\bin\sc4 or SuperCalc5 (cd\bin\sc5).

4. You will then need to find the time data and convert it from seconds to minutes. Once the time data has been converted to minutes you must create a file that contains only the time data in the left hand column and the concentration data in the right hand column.

5. If the computer indicates that the memory is full when attempting to import the data into the spreadsheet several things could have gone wrong. The log file may become too long to import if the filename of the logging file was repeated, by adding data to an already existing file; too short a logging period produced too much data; or too many runs were performed. If this happens don't panic simply import the file into a editor such as WordPerfect and save the file in several smaller pieces.

Fermentation Power Transfer

Summary

Power-transfer rates were studied for 3 different impellers in a clear plexiglass mixing tank that was constructed to match geometric ratios specified in literature correlations. A Lightnin Mixer LabMaster$_{\text{TM}}$ 2510 (Lightnin Mixing Co., Rochester, N.Y.) was used to measure power consumption outfitted with three impellers: a 6-bladed, Rushton-style radial-flow impeller; a traditional marine-type axial-flow impeller; and an Lightnin A310 high-performance axial-flow impeller. The power consumption was correlated to the rotational speed for each impeller. The power number was also correlated to the Reynolds number for all three impellers.

Introduction

Fluid mixing is an important industrial operation whose economics are affected by the power consumed by the process. The power and mass-transfer characteristics of a particular impeller style, rate, and tank geometry can be predicted using correlations. The impeller style used can have significant effect on the economics of a process since the power consumed by two different types of impellers operating at the same impeller rate may differ by an order of magnitude. In biochemical applications, the choice of impeller can be crucial to the economics of the process because of the dual requirements of minimal operating costs and adequate oxygen mass transfer or mixing. The design

of a fermentation process must reflect the power consumption, gas dispersion, and the shear characteristics of the impeller (Dickey, 1992).

Typically, a mixer consists of a motor, a drive mechanism, and a shaft with one or more impellers mounted on it. Mixing power is transferred from the motor to the fluid via the shaft and impeller. The amount of power transferred to the fluid is a function of the impeller speed and type, the geometry of the mixing vessel, and the physical properties of the fluid being mixed (Rushton, 1950). There are many types of impellers such as propellers, shrouded turbines, rakes, disk turbines, gates and paddles. Each type of impeller requires a specific amount of power to overcome its hydrodynamic resistance during rotation; thus different impeller types exhibit different power-consumption characteristics (Rushton, 1950). There are two broad categories for characterizing impellers: axial and radial flow (Oldshue, 1990).

Axial-flow impellers displace the fluid downward from the impeller, parallel to the axis of rotation. Typical axial-flow impellers include marine-type propellers and any impeller with pitched blades. Axial-flow impellers are more economical to operate than radial-flow impellers because they produce more fluid motion per unit of power consumed (Oldshue, 1990).

Radial-flow impellers push the fluid outward from the impeller in the radial direction (Oldshue, 1990). Radial-flow impellers consume more power than axial-flow impellers but typically have superior mass-transfer characteristics. Radial-flow impellers produce higher shear rates, resulting in increased bubble breakup which improves gas

dispersion and mass transfer. Typical radial-flow impellers include square paddles and the 6-bladed Rushton turbine.

Theoretical Analysis

The correlations from Rushton, Costich, and Everett's 1950 dimensional analysis of fluid mixing are still widely used for estimating impeller power consumption (McCabe, Smith, and Harriot, 1985). The dimensionless groups that describe mixing power consumption are the Power number, the Reynolds number, and the Froude number which are given below:

$$P_n = \textit{Power number} = \frac{Pg_c}{\rho_l N_i^3 D_i^5} = \frac{\textit{drag forces}}{\textit{inertial forces}} \tag{1}$$

$$Re = \textit{Reynolds number} = \frac{D_i^2 N \rho}{\mu} = \frac{\textit{inertial forces}}{\textit{viscous forces}} \tag{2}$$

$$Fr = \textit{Froude number} = \frac{N_i^2 D_i}{g} = \frac{\textit{inertial forces}}{\textit{gravity forces}} \tag{3}$$

where
- P = power output of mixer
- g_c = Newton's Law unit conversion constant
- ρ_l = liquid density
- N_i = impeller rate
- D_i = impeller diameter
- g = acceleration of gravity
- μ = liquid viscosity

The Power number is correlated as a function of the Froude number and the Reynolds

number and other dimensionless groups that represent the geometry of the tank and impeller. The form of the correlation is given below:

$$P_n = K\, Re^m Fr^n \left(\frac{T}{D_i}\right)^t \left(\frac{H}{D_i}\right)^h \left(\frac{C}{D_i}\right)^c \left(\frac{S}{D_i}\right)^s \left(\frac{L}{D_i}\right)^l \left(\frac{W}{D_i}\right)^w \left(\frac{J}{D_i}\right)^j \quad (4)$$

where
- T = tank diameter
- D_i = impeller diameter
- H = liquid depth
- C = height of impeller off bottom
- S = pitch of impeller
- L = length of impeller blades
- W = width of impeller blades
- J = width of baffles
- B = number of blades on impeller
- B_r = number of blades on the reference impeller
- R = number of baffles
- R_r = number of baffles on reference tank

The lower-case letters are the unknown exponents for the terms shown (Rushton, pt I, 1950). The characteristic dimensions for the tank and the impeller are shown in Figure 1. The last seven terms of the above equation are geometric parameters that have been rearranged into dimensionless "shape factors". These "shape factors" designated S_1 through S_6, are defined below, along with some common values used in correlations (McCabe, Smith, and Harriot, 1985):

$$S_1 = \frac{D_i}{T} = \frac{1}{3} \quad S_2 = \frac{C}{D_i} = 1 \quad S_3 = \frac{L}{D_i} = \frac{1}{4} \quad S_4 = \frac{W}{D_i} = \frac{1}{5} \quad S_5 = \frac{J}{T} = \frac{1}{12} \quad S_6 = \frac{H}{T} = 1 \quad (5)$$

If all the geometric parameters are fixed for a set of experiments, the constant K absorbs all the geometric constants yielding an equation of the form:

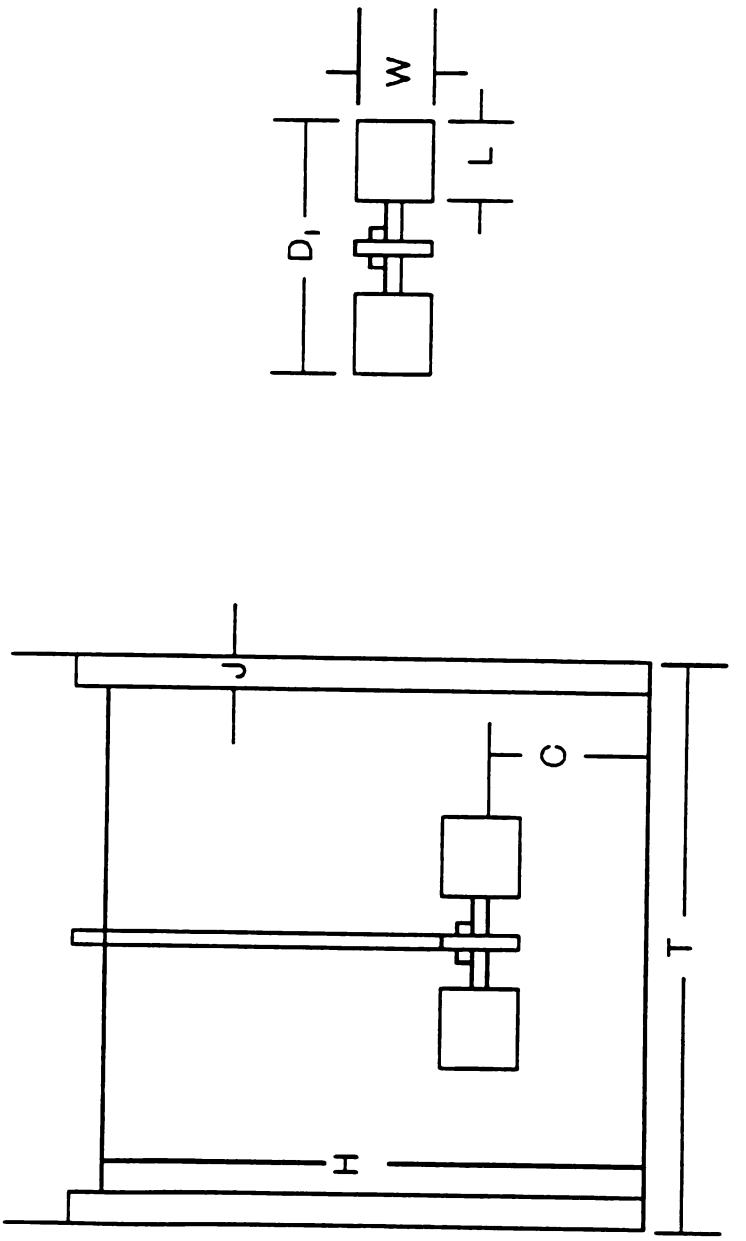

Figure 1. Tank and Impeller Geometry Constants

$$P_n = K\, Re^m Fr^n \qquad (6)$$

The group $\log_{10}(P_n/Fr)$ is often plotted versus the $\log_{10}(Re)$, and the slope gives the exponent of the Re term. The effect of the Froude number is negligible when the tank is baffled (i.e. $n \approx 0$), and the value of the $(Fr)^n$ is equal to 1.0 (Rushton et al., 1950).

The mixing conditions may be characterized into flow regimes defined by the Reynolds number in a manner analogous to the friction factor. Viscous conditions exist at Reynolds numbers below 10, with a transition range between Re values between 10 and 20,000. At Re greater than 20,000, the flow becomes turbulent, and the power consumed by a radial-flow impeller in a baffled tank containing a Newtonian fluid is proportional to the cube of the impeller rate. Log-log plots of power versus rpm in this regime should yield lines with slopes equal to 3 for Rushton-style impellers.

Experimental Equipment and Procedure

The equipment used for the experiment consisted of a plexiglass tank fitted with removable baffles, a Lightnin Mixer LabMaster™ 2510 (Lightnin Mixing Co., Rochester, NY) outfitted with Lightnin Mixer impellers of several different types, and a Camile 2000 data-acquisition system (Dow Chemical Co., Midland, MI). The setup of the equipment is shown in Figure 2. The impellers used were A100 marine-type propeller with a power number of 0.87, a R100 six-bladed paddle turbine with a power number of 6, and an A310 high-performance axial-flow impeller with a power number of 0.3.

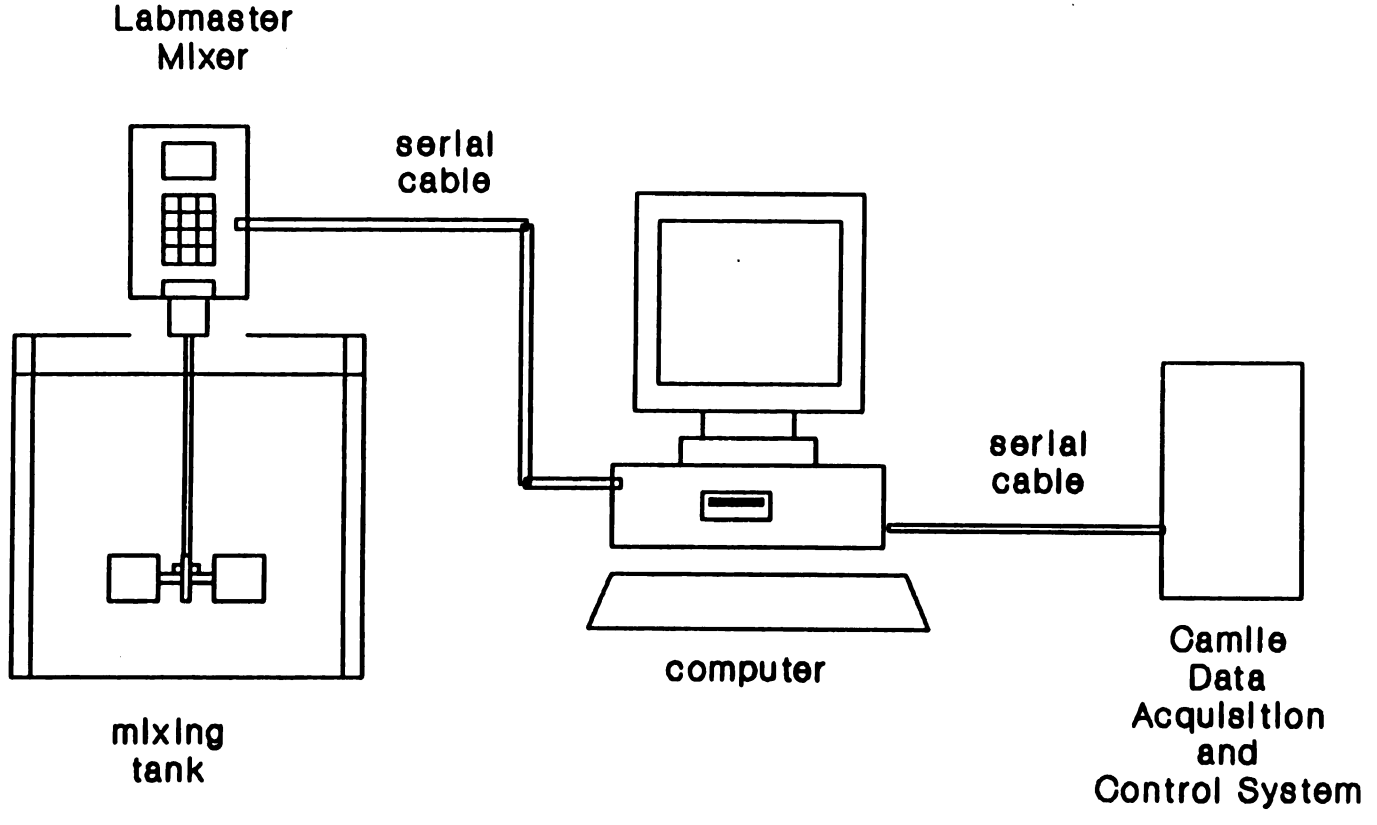

Figure 2. Experimental Setup for Mixing Power Equipment

The LabMaster$_{m}$ mixer motor measures the impeller speed and the power consumed and sends the data to the Camile data-acquisition system via a serial cable. The mixer auto-calibration procedure was followed before acquiring power-consumption data. The power consumption of the impeller, shaft, and bearings was measured in air to determine the losses due to friction within the mechanical couplings of the system for each impeller. The power consumption for the same series of speeds was then measured in water for each impeller.

Results and Discussion

The power consumption of the shaft and impeller in air was essentially zero for all speeds and impellers tested, with the limitation that the set screw used to hold the impeller on the shaft be as small as possible. Experiments performed with an excessively large set screw produced vibratory deflections of the shaft and impeller at high impeller speeds causing power fluctuations. Figure 3 shows the results of the power-measurement experiments. The literature indicates that the power-meter data should increase with the cube of the impeller rate for a Newtonian fluid in the turbulent regime (Oldshue, 1990). However, linear regression of the power data produce slopes greater than 3 as shown in Figure 3. Figure 4 contains a plot of the Power number versus the Reynolds number. The R100, A310, and A100 impellers should asymptotically approach Power numbers of 6, 0.3, and 0.87 respectively. The power numbers calculated from the experimental data are about 1.5 times smaller than the asymptotic Power numbers. This may be due

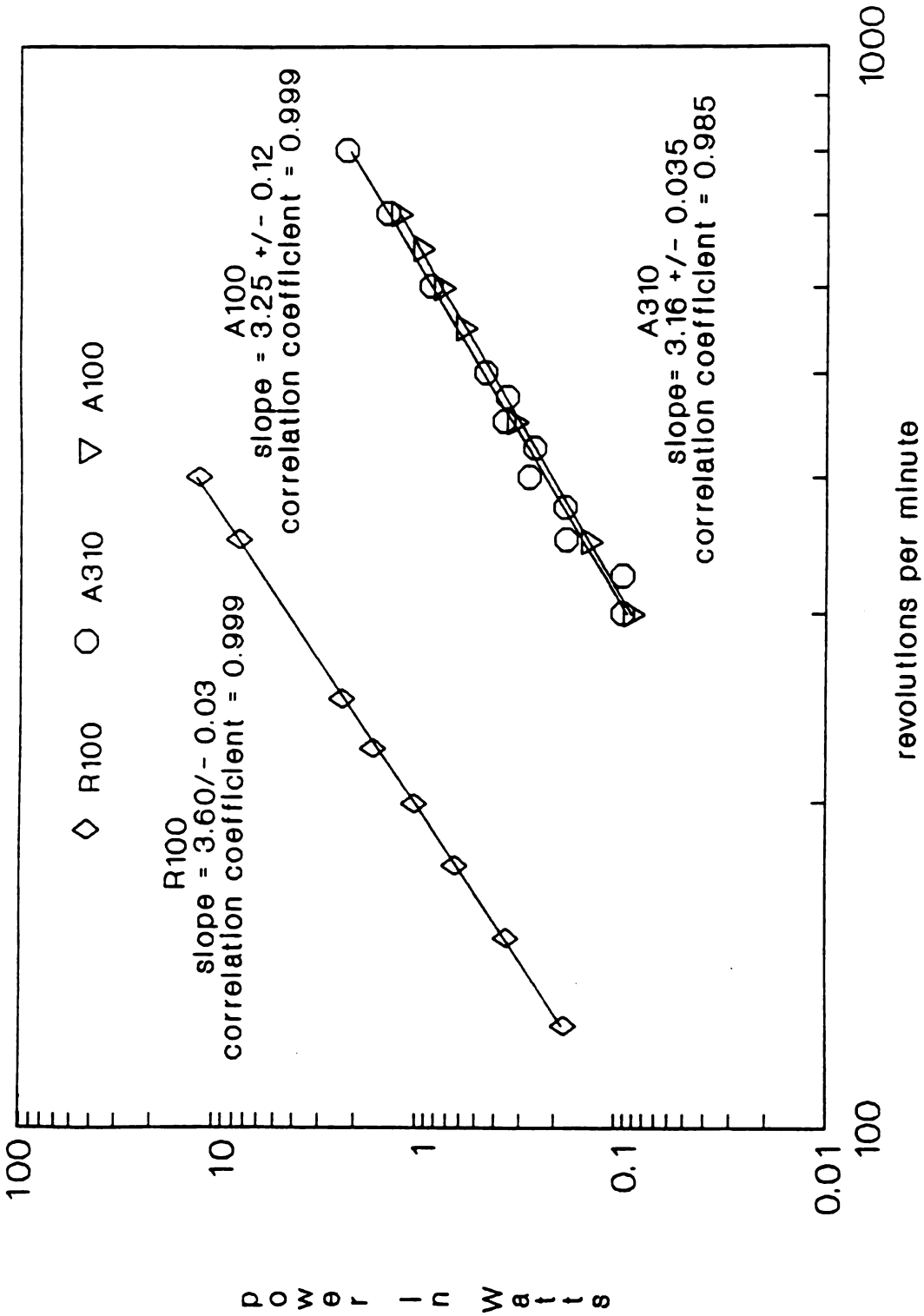

Figure 3. Power versus RPM

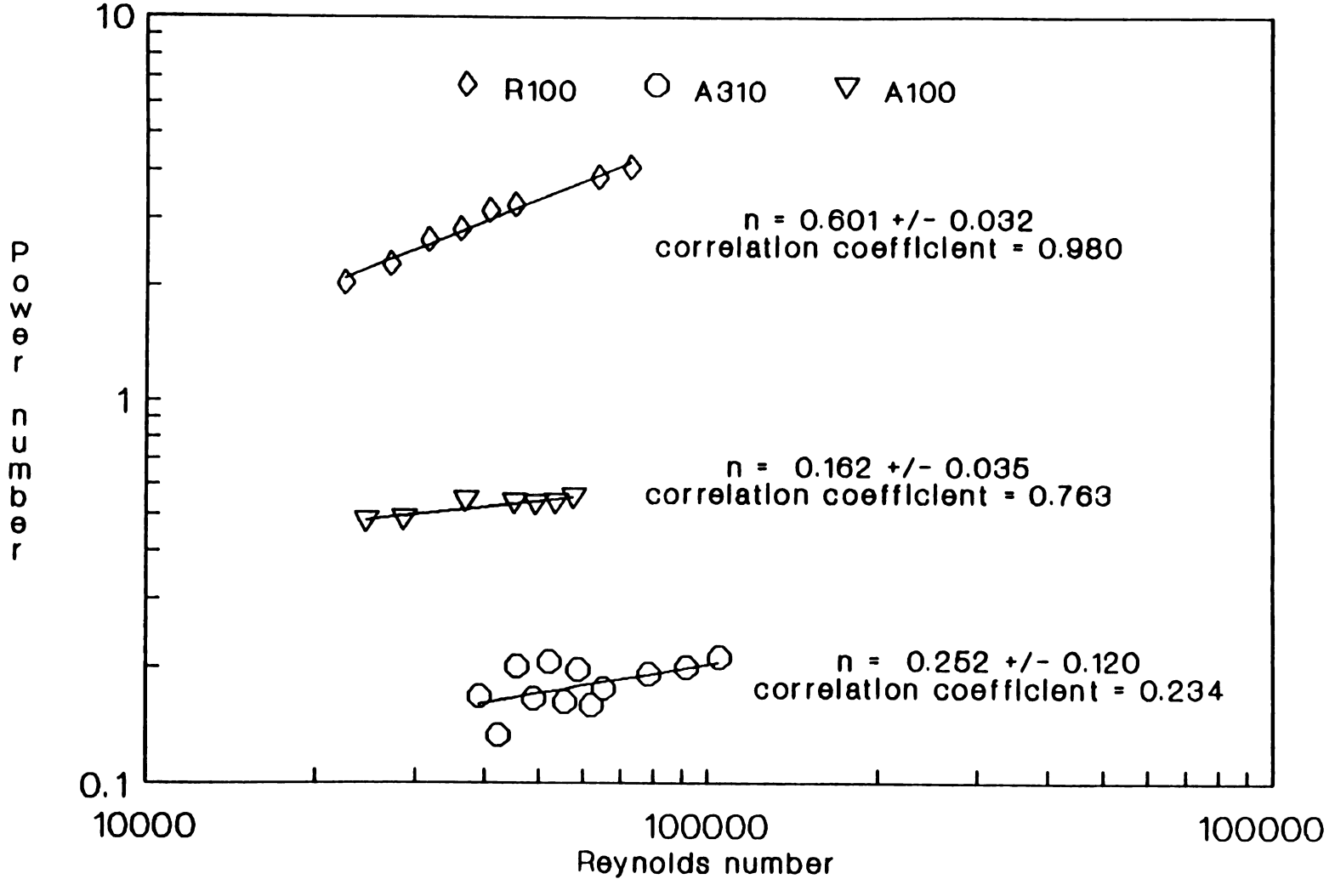

Figure 4. Power Number versus Reynolds Number

in part due to the inability of the current system to reach Re greater than 200,000 where all impellers are said to reach their asymptotic Power number regardless of design (Oldshue, 1990). Power measurements can be taken only between Re of 11,000 and about 100,000 using water as the mixing fluid because of the limitations of the power meter and the mixer. In addition, the power cannot be measured in water by the mixer below a certain range of impeller speeds, which varies with the type of impeller. At low speeds, the power meter is not sensitive enough to detect a difference in power consumption between water and air. Thus, the viscous regime of Reynolds numbers is unavailable for study unless much more viscous fluids are used. Alternatively, Reynolds numbers above 100,000 are difficult to achieve since the power consumed reaches the maximum capacity of the motor and the power meter for the radial-flow impeller. The axial-flow impellers approach the critical shaft speed above Re of 100,000 and shaft vibration and deflection begins to become significant.

The mixer's method of power measurement utilizes a microprocessor that may be reprogrammed using input from the keypad and a series of commands (see Appendix I). Specifically, it may be possible to change the torque constant used by the microprocessor to calculate power-consumption values. This adjustment may allow the mixer to produce data that more accurately fit the existing correlations for the Power number versus the Reynolds number. A few preliminary runs after changing the torque constant indicate that this method may produce the desired results, but much more analysis is required.

Conclusions and Recommendations

The mixer produces data that are highly precise but do not correspond to the literature values. The power meter of the mixer should be calibrated to determine its accuracy with respect to the literature. A trial and error method could be utilized to adjust the torque constant of the mixer to obtain the desired literature values for all the impellers.

Both more- and less-viscous Newtonian fluids than water should be used to obtain lower and higher Reynolds numbers. The use of higher-viscosity solutions such as sucrose would decrease the relative contribution of the mechanical losses in the power consumption of the system by increasing the overall power consumption. The use of lower viscosity fluids may allow operation at higher Reynolds numbers to ensure operation in the turbulent regime.

Symbols

μ = liquid viscosity
ρ_l = liquid density
B = number of blades on impeller
B_r = number of blades on the reference impeller
C = height of impeller off bottom
D_i = impeller diameter
D_i = impeller diameter
Fr = Froude number = $N_i^2 D_i / g$
g = acceleration of gravity
g_c = Newton's Law unit conversion constant
H = liquid depth
J = width of baffles
L = length of impeller blades
N_i = impeller rate

P = power output of mixer
P_a = Power number = $Pg_c/(\rho_l N_i^3 D_i^5)$
R = number of baffles
Re = Reynolds number = $D_i N\rho/\mu$
R_r = number of baffles on reference tank
S = pitch of impeller
T = tank diameter
W = width of impeller blades

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Appendix

Equipment and reagents needed:
Lightnin Labmaster TS2510
three impellers
tanks with removable baffles
Camile Data Acquisition and Control system
Glycerol
sugar (optional)

Experimental Procedure
I. Determination and Adjustment of Tank Geometric Constants

1. Measure all the appropriate pieces of equipment to be used in the experiment to obtain the values of the shape factors.

2. Look at the diagrams in the instruction manual to identify the each of the types of impellers. The A310 impeller has a diameter of 3.4" with a Power number of 0.3. The A100 impeller has a diameter of 2.7" and a Power number of 0.87. The R100 is a 4" impeller..

3. Adjust the height of the impeller relative to the tank bottom according to the values of the shape factors raise the water level to the appropriate height.

II. Operation of the Labmaster Mixer

1. The power meter of the mixer must be autozeroed before collecting data for the day. The shaft must be removed from the mixer to perform the autozero procedure.

2. Loosen the two knobs on the arm that supports the mixer enough slide the mixer up and down the track it is mounted on. Slide the metal bar out of the way above and replace it once the mixer is above the level of the hole. Rest the mixer on the bar above the tank.

3. Press the white shaft lock button located at the bottom center of the front of the mixer. If the button does not go all the way in rotate the chuck slowly with your fingers until the button travels all the way in. Never attempt to stop the mixer with the shaft lock button always use the "RUN/STOP" button to stop the mixer. The "RUN/STOP" button will have no effect in remote mode so the power must be turned off in an emergency.

4. Loosen the chuck by turning it counterclockwise until the shaft glides freely out of the mixer.

5. Press the "ON" button and the "RESET" button at the same time and then release the "RESET" button and then release the "ON/OFF" button. After the LCD readout on the mixer says "diagnostic", press "6" and then press "ENTER". The mixer will then perform a series of pre-set speeds and calibrate its power meter. Be sure to keep hands and clothing away from the rotating parts of the mixer.

6. Replace the shaft into the mixer using the reverse of the above procedure. Make sure that the chuck is hand tight but do not overtighten the chuck.

7. Press the "ON/OFF" button twice to get to normal operating mode.

8. Use the "MENU" key to move the flashing asterisks from speed to the temperature/power window. Use the item key pressed several times in succession to show the temperature in °C and °F and the power in Watts. Use the "MENU" key to move the asterisks to the pumping capacity and use the item key to select the type of impeller used and enter the impeller diameter to one decimal place. Enter the values from part A step 2 for both the A310 and the A100 and 4" for the R100 impeller.

9. Press the RUN/STOP button to start and stop the motor.

III. Measurement of Power Consumption

1. Locate the impeller at the proper depth in the tank by sliding the shaft up and down in the chuck while the mixer rests on the lowest bar in the track. It may be necessary to raise the mixer to adjust the shaft. The knobs that hold the mixer in the track must be tightened before operation.

2. Fill the tank with reverse osmosis (RO) water.

3. Measure the power with RO water in tank for all the speeds that were used for the empty tank and record the rpm's, temperature, and power allowing the system to equilibrate at each speed. Measure the power consumption and rpm of each impeller in the empty tank at a range of speeds between 50 and 400 for the R100 and speeds between 50 and 800 rpm's for the other two impellers. Allow the system to equilibrate at each speed. Install the R100 impeller with the hub facing up. Install the A100 and the A310 with the convex side of the blades facing up.

IV. Operation of the Camile system with the Labmaster Mixer

1. Consult with the other groups using the Camile so that it may be used by everyone. The three experiments that use the camile system are: immobilized biocatalysts, and the power and mass-transfer experiments. The capabilities of the system for multiple users is limited so cooperation between lab groups is important.

2. If the Camile system is not already started, start up the Camile system by turning on the computer and typing "cd\camile" to get to the Camile directory. Then type "menu" and the Camile logo will appear. Press any key to display the main menu. Use the arrow keys to highlight "run" and hit ENTER. Then use the arrow keys to highlight the database called "HEATTRAN" and hit ENTER. The computer will then ask you if you wish to initialize the Camile control constants. Type "y" and hit enter.

3. When the computer prompts for the default log directory "C:\camile\logs" should be present and highlighted. If not, backspace and type it in then hit enter. The log file screen should now be on the monitor. Use the arrows to move to log file 6 and type the name you have chosen for your log file. Make sure that "BIN" and "APP" appear in the fields to the right of the log file name.

4. Wait for the other groups to enter their filenames before pressing the END key. When all the groups have entered their filenames press END. If you have repeated any log file name currently on file, the computer will prompt you to change it. The computer will then ask you to enter a title. This and the comments it prompts for next are optional, but your names and a few brief words describing your experiment might save considerable confusion later. Leave a blank line to finish and hit ENTER.

5. The computer will then inform you that the printer does not respond and will ask you if you wish to retry. Type N if the printer is not connected.

6. If one of the other groups has not already started the question and answer menu press F6 and type "S2 ON". This activates a question and answer sequence that will prompt you for the parameters needed to log the data. You may leave a question and answer screen at any time by pressing another one of the function keys. The F1 key is help and a list of the commands for the function keys is given by ALT-F1. You can then return to the question and answer screen by hitting ALT-F8 at any time.

7. Select the number for the power transfer experiment on the main menu, enter it in the highlighted space and press the END key. Move through the screens into the highlighted areas to and type in the values you desire. The logging period should be 30 seconds.

8. The speed of the mixer can only be changed in manual mode. To change the speed of the mixer press ALT-F8 to return to the question and answer screens. Move through the menus like when beginning logging and then enter "n" after the question that asks if you want the mixer in remote mode and press "END". When you have set the desired rpm on the mixer go back to that screen and type "y" for both the remote mode and whether you wish to begin logging.

9. Before restarting the motor look at the shaft below the chuck and check for scoring of the shaft, if there is consult with the teaching assistant. Make sure to press the END key when you are ready.

10. Check the trend screen periodically to see if the values have become constant. Go to the F6 screen and see if the number next to sequence "4. labmaster" is constantly changing. If the number after "labmaster" is constant, the communications sequence must be restarted. To restart the communication sequence, press F6 and type "S4 OFF" and then type "S4 ON".

11. You may record the type of impeller and whether or not the reading is a calibration in water or air in the log file for your data by pressing the F8 key and typing in any relevant notes. When typing in comments include your initials to distinguish them from the comments recorded by the other groups using the camile system.

12. Monitor the progress of you experiment by using the on-line trend screens (F3). You may explore the functions of the Camile at this time as most functions that will harm your experiment are denied access without a password. Do not change your slope and intercept. An interesting feature of the on line trending screens of the Camile system is the history trend. The history trend appears as graph 9 in the on-line trending screens and can be reached by paging up or down after hitting F3. The history trend displays the data already logged into your file. To view a history trend type "Read" while above graph 9 and then the cursor will move to the middle of the screen next to the word tagname. Type in "power" or "rpm", the tagnames for the your experiment, and press enter. The cursor will move to the next row and which will say "log file". Enter 1 and press END. At this point the computer should begin reading your data from the log file and

displaying it on the screen. If you or another lab group wishes to use the history trend later you must first type "Clear" and then re-enter the pertinent information.

13. When ready to stop logging data press ALT-F8 to get the question and answer main menu which will offer to stop logging for your experiment. Enter the number corresponding to stopping logging for that experiment on the next menu that is displayed and your group's security code. The security code is to prevent other groups from accidentally stopping logging data for your experiment.

14. Repeat the experiment with a variety of rpms for each impeller and with and without baffles if time allows.

15. To get your log file off the hard disk for analysis you will have to exit run and use the DATA subprogram to convert your binary log file into a comma separated value ASCII file. At this time ask the teaching assistant or the instructor for the password. Once it has been entered by pressing the F9 key and typing "LEVEL ENGINEER" and the password, you may type "BYE" and exit run.

V. Data Analysis

1. Use the arrows to move the highlighting to DATA and press ENTER. The screen should say "output file type:ASCII" and "Report all time points:yes" then hit END. A whole list of options will then be provided for you to change. Highlight a particular selection and then press the space bar to view the various options and hit ENTER when the option you desire is shown. Make sure you include comments, tag values, elapsed time, date, and the time of day. The column delimiter must be a comma for easy retrieval of the log file into a spreadsheet program. When the appropriate options have been selected, press END. Highlight the tag names (power and rpm) and press the space bar. An arrow should be present on the left of the names and then press END.

2. The computer will then show the prompt "EDIT filename:" and your log file title with a ".prn" extension will be displayed. Hit END and you will exit DATA and then press escape to exit the Camile menu.

3. Copy the file that is produced ".prn" to a 3.5" diskette.

4. Import the ".prn" file into a spreadsheet as a comma separated value format (CSV). (i.e. \\Import,CSV,mixer.prn).

VI. Adjustment of the Labmaster$_{DL}$ Mixer Torque Constant

1. Put the mixer in diagnostic mode using the directions for calibration above.

2. Type "90" on the keypad and press the enter key.

3. Type "1723" on the keypad and press the enter key.

4. Type "75" on the keypad and press the enter key. The display should now read "ARG".

5. Press the enter key to see the current value of the torque constant. The factory setting is "519".

6. Press the "on/off" key.

7. To change the torque constant repeat steps 1 through 4 and enter in the new torque constant using the keypad and press enter.

8. Press the "on/off" key.

9. Re-enter the diagnostic mode and perform a calibration using the instructions above. A calibration must be performed each time the torque constant is changed.

Immobilized Cell Biocatalysts

Summary

The diffusive properties of $CaCl_2$ in alginate gel spheres were studied in an unsteady state experiment by following the decrease in concentration of $CaCl_2$ in a well stirred solution of finite volume. The alginate gel spheres were produced by dropwise addition of sodium alginate into a $CaCl_2$ solution. A known volume of gel spheres was added to a 10 g/l $CaCl_2$ solution, and the rate of disappearance of salt was measured using a conductivity meter. A computer program determined the effective diffusion coefficient by fitting a mathematical model to the experimental data and minimizing the error between the model and the data.

Introduction

Enzymes and cells are often immobilized by attachment to a support to allow them to be reused continuously. Immobilization techniques can be classified into two classes for biocatalysts: chemical and physical. Chemical immobilization uses a covalent bond between the immobilization material and the biocatalyst, which is typically an enzyme. Physical immobilization often traps the biocatalyst using other types of interactions such as entrapment within a porous matrix, containment by a membrane, or surface adsorption.

Cells are most commonly immobilized by physical structures such as polymer gels. The polymer gel is usually a bead which is formed by mixing the biocatalyst and

the monomer of the gel and then dripping the solution into a crosslinking solution which polymerizes the monomer, entrapping the biocatalyst. The most common forms of polymer immobilization are polyacrylamide, κ-carrageenan, and sodium alginate. Polyacrylamide is crosslinked using chemical agents that are toxic to cells, but κ-carrageen and sodium alginate are both polysaccharides that can be crosslinked by calcium solutions under mild conditions (Bailey and Ollis, 1986).

Alginate is a linear polysaccharide which acts as the skeletal component of the seaweed and algae, from which it is normally obtained (Wang, 1990). The addition of a polyvalent cation such as Ca^{+2} to a solution of alginic acid causes the formation of an ionic-network polymer which is crosslinked by the Ca^{+2} ions between carboxyl ions. Thus dropwise addition of the sodium salt of alginic acid to a solution of calcium chloride will result in the formation of crosslinked alginate beads upon the exchange of calcium for sodium in solution. The pore size, molecular permeability, and degree of crosslinking of the beads depend primarily on the molecular weight of the alginic acid used (Wang, 1990). The mild polymerization conditions for the alginate and its stable structure between 0 and 50°C make it an ideal medium for immobilizing cells. However, citrate and phosphate scavenge the Ca^{+2} ions, thus breaking the crosslinks. High concentrations of Na^{+} ions also reverse the crosslinking reaction by replacing the Ca^{+2} with Na^{+}.

Immobilization of biocatalysts presents advantages over non-immobilized biocatalysts in separations and reaction engineering. A non-sterile feed may be used for fluidized and packed-bed reactors because any contaminants not entrapped in the beads

will wash out of the reactor (Petersen, 1991). Downstream separation of the biocatalysts is not necessary since they are retained in the reactor. The retention of the biocatalysts in the reactor also increases their concentration within the reactor, increasing the rate of reaction and decreasing the need for renewal of the biocatalysts (Bailey and Ollis, 1986; Petersen, 1991).

Immobilization can present disadvantages for the engineer as well. Mass-transfer limitations of the substrates and products can limit the reaction rate of the biocatalysts. Systems that form inhibitory products may have a lower reactor performances than a non-immobilized systems. Covalently attaching an enzyme to a support may decrease its intrinsic kinetic properties (Bailey and Ollis, 1986).

The advantages and disadvantages of immobilization of biocatalysts vary with the types of biocatalysts and immobilization technique used. The transport properties for all the important species in the reaction must be considered before the system is immobilized. The determination of effective diffusion coefficients for reactants and products within the immobilization media is often required to analyze the transport phenomena involved.

Theoretical Analysis

Simple diffusion without convection is most commonly described by Fick's law. Fick's law relates the flux of molecules by diffusion to the concentration gradient by a proportionality constant known as the effective diffusion coefficient. The form of Fick's law for radial diffusion in spherical coordinates is (Cussler, 1984):

$$J_i = -D_e \frac{\partial C_i}{\partial r} \tag{1}$$

where J_i = the diffusive flux of component i
D_e = the effective diffusion coefficient
C_i = the concentration of component i
r = radial coordinate

Equations describing the unsteady behavior of a diffusional system are derived by using Fick's law in the mass balance.

The basic unsteady-state mass balance equation describing the diffusion of a solute into a sphere is the following:

$$\frac{\partial C_s}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 D_e \frac{\partial C_s}{\partial r}) \tag{2}$$

where C_s = solute concentration in the beads
t = time
r = radial coordinate
D_e = effective diffusion coefficient

The equation assumes that convective mass transfer within the sphere is negligible. The following initial and boundary conditions are used for the diffusion of a solute into spheres from a well stirred solution of limited volume (Crank, 1975):

$$at\ t=0;\quad 0<r<R;\quad C_s=0 \tag{3}$$

$$at\ t=0;\quad r>R;\quad C_L=C_{Lo} \tag{4}$$

$$at\ t>0;\quad r=0:\quad \frac{\partial C_S}{\partial r}=0 \tag{5}$$

$$t>0;\quad r=R;\quad V_L\frac{\partial C_L}{\partial t}=K_p A_s D_e \frac{\partial C_s}{\partial r}\Big|_{r=R} \tag{6}$$

where
- R = radius of the beads
- C_L = concentration in the liquid phase
- C_{Lo} = initial concentration in the liquid phase
- V_L = volume of the liquid phase
- K_p = partition coefficient between liquid and beads
- A_s = surface area of the beads

This model assumes negligible mass transfer resistance in the liquid film surrounding the spheres. Crank gives the following series solution for the liquid phase concentration of solute in terms of the diffusion coefficient, the ratio of the liquid volume to the bead volume (α), the radius of the beads, and several q_n values:

$$C_L=\frac{\alpha C_{Lo}}{1+\alpha}\{1+\sum_{n=1}^{\infty}\frac{6(1+\alpha)\exp(\frac{-D_e q_n^2 t}{R^2})}{9+9\alpha+q_n^2\alpha^2}\} \tag{7}$$

Values for the q_n's are given by the non-zero solutions of the following equation:

$$\tan(q_n)=\frac{3q_n}{3+\alpha q_n} \tag{8}$$

Determination of the effective diffusion coefficient for $CaCl_2$ within an alginate

gel sphere requires that the other mass transfer resistances must be made negligible. The limiting transport step in the system must be the diffusion of the $CaCl_2$ into the polymer itself. The film theory of mass transport states that a significant amount of resistance to mass transfer may exist within the film that surrounds any object immersed in a fluid. Film theory also states that the film thickness decreases with the velocity of the fluid motion across a surface. Therefore to reduce the film resistance, the velocity of the alginate spheres relative to the solution must be high; this is accomplished by rapidly swirling the beads in solution.

Film theory defines the film thickness (δ) with the following equation (Nguyen, 1986):

$$\delta = \frac{D_m}{k} \tag{9}$$

where D_m is the diffusivity of $CaCl_2$ into free water and k is the external mass transfer coefficient. The external mass transfer coefficient is often defined by the following relation in terms of the total flux of component i, N_i:

$$N_i = k(C_L - K_p C_S) \tag{10}$$

The equilibrium concentrations of the liquid and solid phases are related by the partition coefficient K_p. Correlations for the value of k are given in terms of the Schmidt number, the Reynolds number, and the Sherwood number:

$$Sh = f(Re,Sc) \tag{11}$$

where Sh = Sherwood number = kd/D_m
Sc = Schmidt number = $\mu/\rho D_m$
Re = Reynolds number = $\rho ud/\mu$
d = bead diameter
μ = fluid viscosity
ρ = fluid density

The variable u is the liquid velocity relative the to beads, and it is dependent on the shaker speed, orbit size of the shaker, the density of the beads, and the density and viscosity of the fluid in which they are immersed.

Experimental Equipment and Procedure

The equipment used to produce the alginate beads included a magnetic stirrer, plastic tubing, a funnel, a plastic nozzle, and a clamp for the tubing. The equipment was set up as shown in Figure 1. The clamp on the tubing was adjusted until the alginate flowed out the nozzle in a dropwise fashion into the $CaCl_2$ solution where it was crosslinked into beads. The beads were incubated in the $CaCl_2$ solution at room temperature for about an hour after the alginate was completely added to the solution.

The beads were then repeatedly rinsed with reverse osmosis (RO) water and placed in the shaker at 400 rpm and 30°C with fresh RO water. The water was replaced every 5-10 minutes until its conductivity matched that of pure RO water for 15 minutes. Once the beads contained no detectable free $CaCl_2$, 25 beads were measured to determine the average diameter.

The equipment used in the determination of the effective diffusion coefficient, D_e,

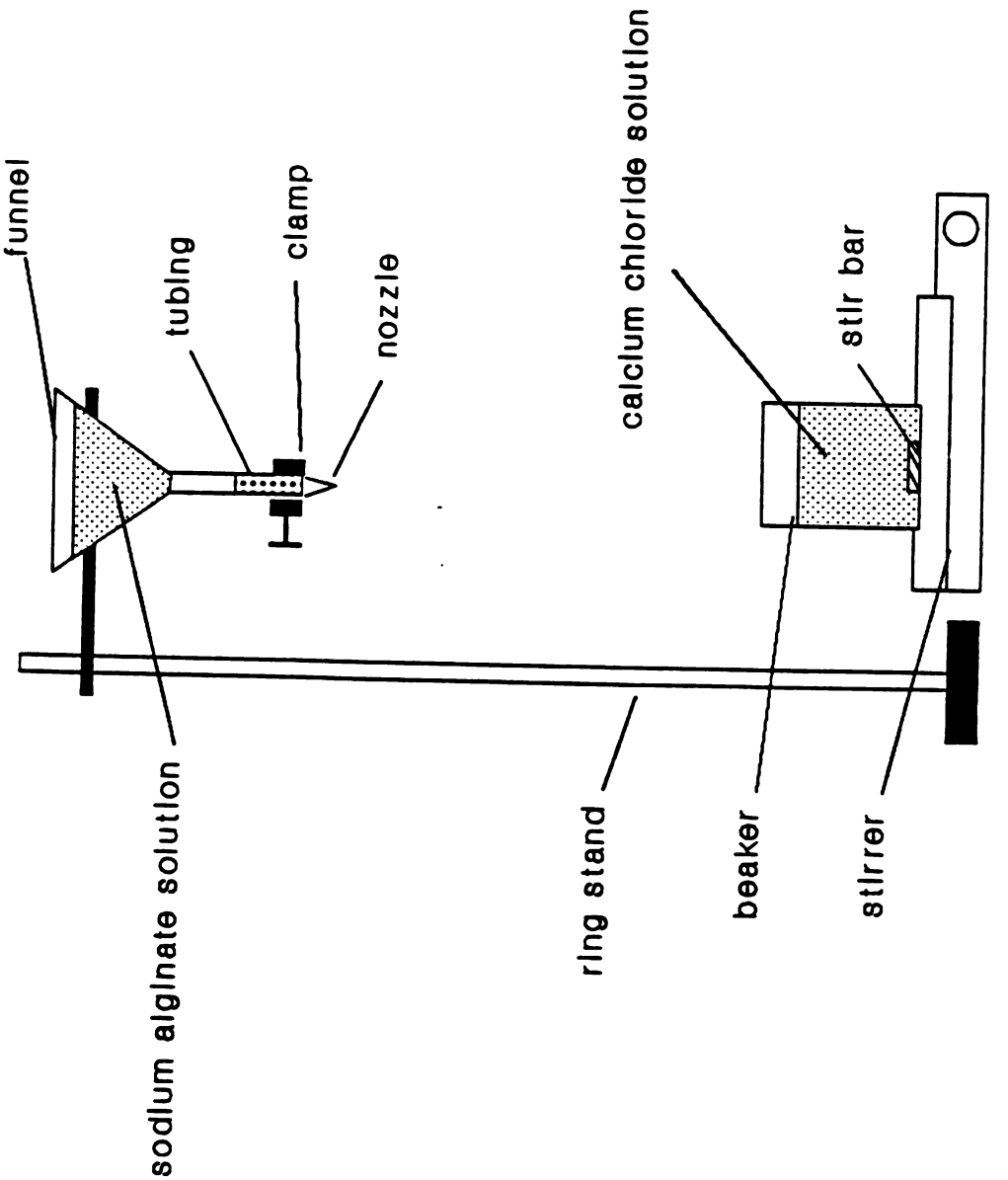

Figure 1. Apparatus for Forming Gel Beads

included a conductivity probe with meter, water bath shakers with a circular orbits of 0.5 and 1 inch, and the Camile Data Acquisition and Control System to record the data. The general setup of the equipment is shown in Figure 2.

Solutions of 2, 5, 8, and 10 g/L $CaCl_2$ were used to create a calibration curve. The voltage output from the conductivity meter was registered by the Camile System for each of the standards. The calibration curve for each of the standards at 30°C is shown in Figure 3.

The experimental data were analyzed using an existing Fortran program that fit Equation 7 to the data by changing the parameters D_e and α to minimize the deviation between the model and the experimental data. The program uses Newton's Method to find the roots (q_n) to Equation 8. The program utilizes the non-linear optimization subroutine PATERN. A copy of the program is given in Appendix II.

Results and Discussion

The results were obtained using both 0.5 and 1 inch orbit shaker operating at 400 rpm. Figures 4 and 5 show experimental data as well as theoretical curves. The deviation between the model and the data is greatest for the earliest time points. Periodic fluctuations in the meter output rate are primarily due to poor temperature control of the shaker. Figure 6 illustrates these characteristic trends of the larger 1" orbital shaker. The 1" orbital shaker had superior temperature control and more reproducible shaker speed settings. The use of larger beads also produced better results. Possible reasons for the improved results include increased inertia causing greater flow around the beads

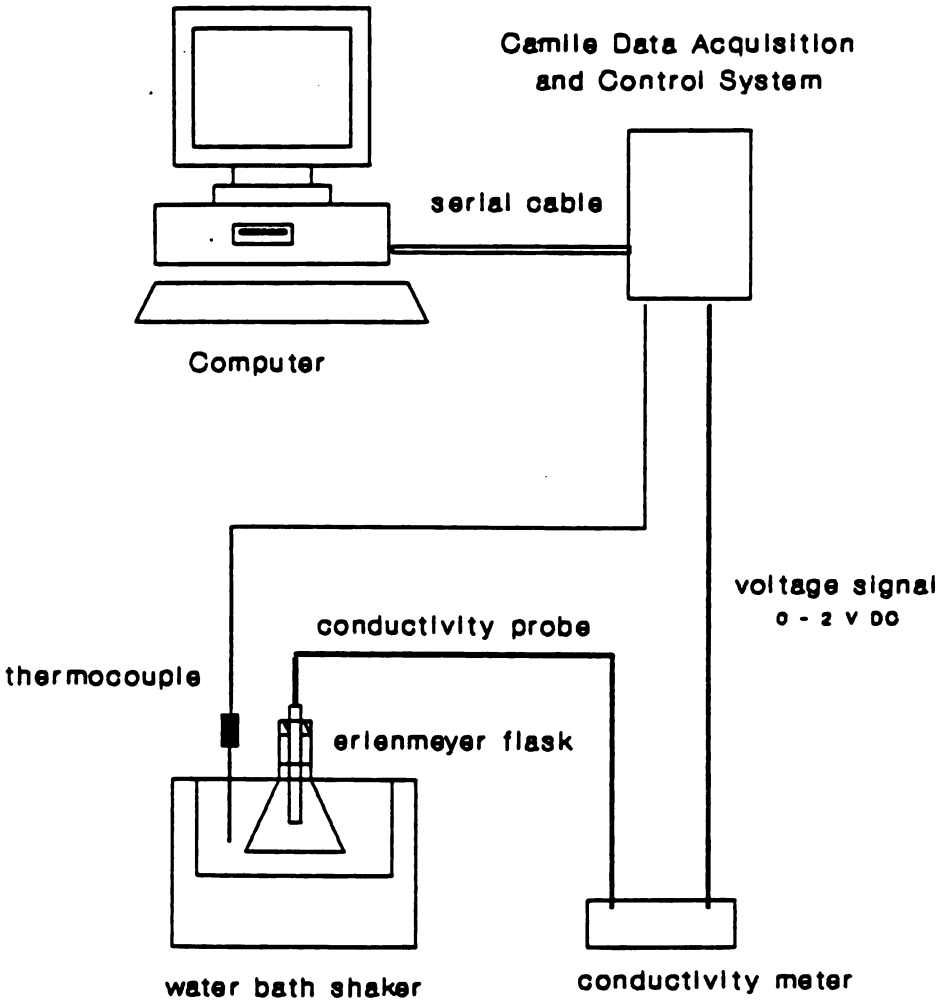

Figure 2. Experimental Setup for Determination of Effective Diffusion Coefficients

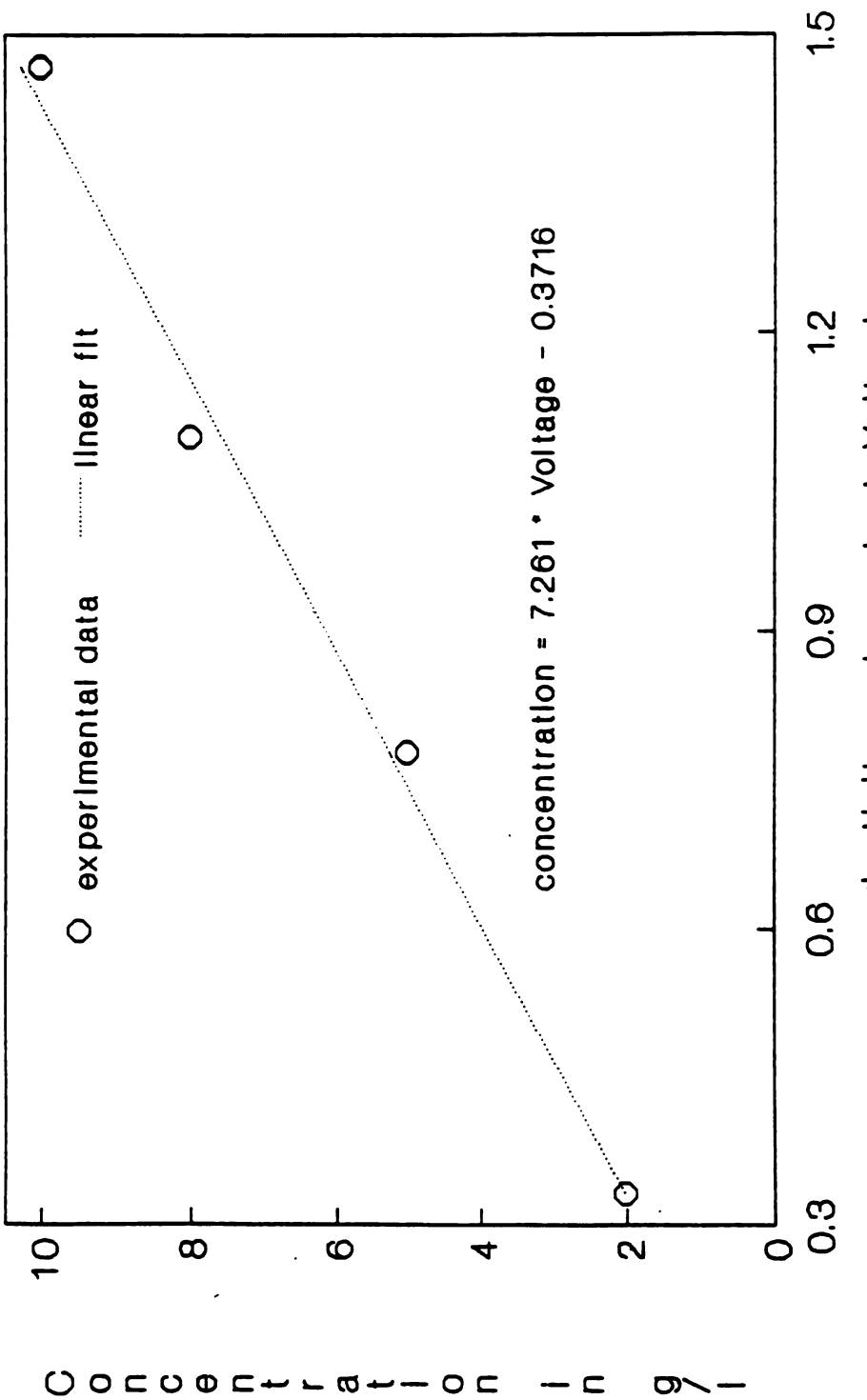

Figure 3. Calibration Curve for Conductance Measurements of Calcium chloride at 30°C

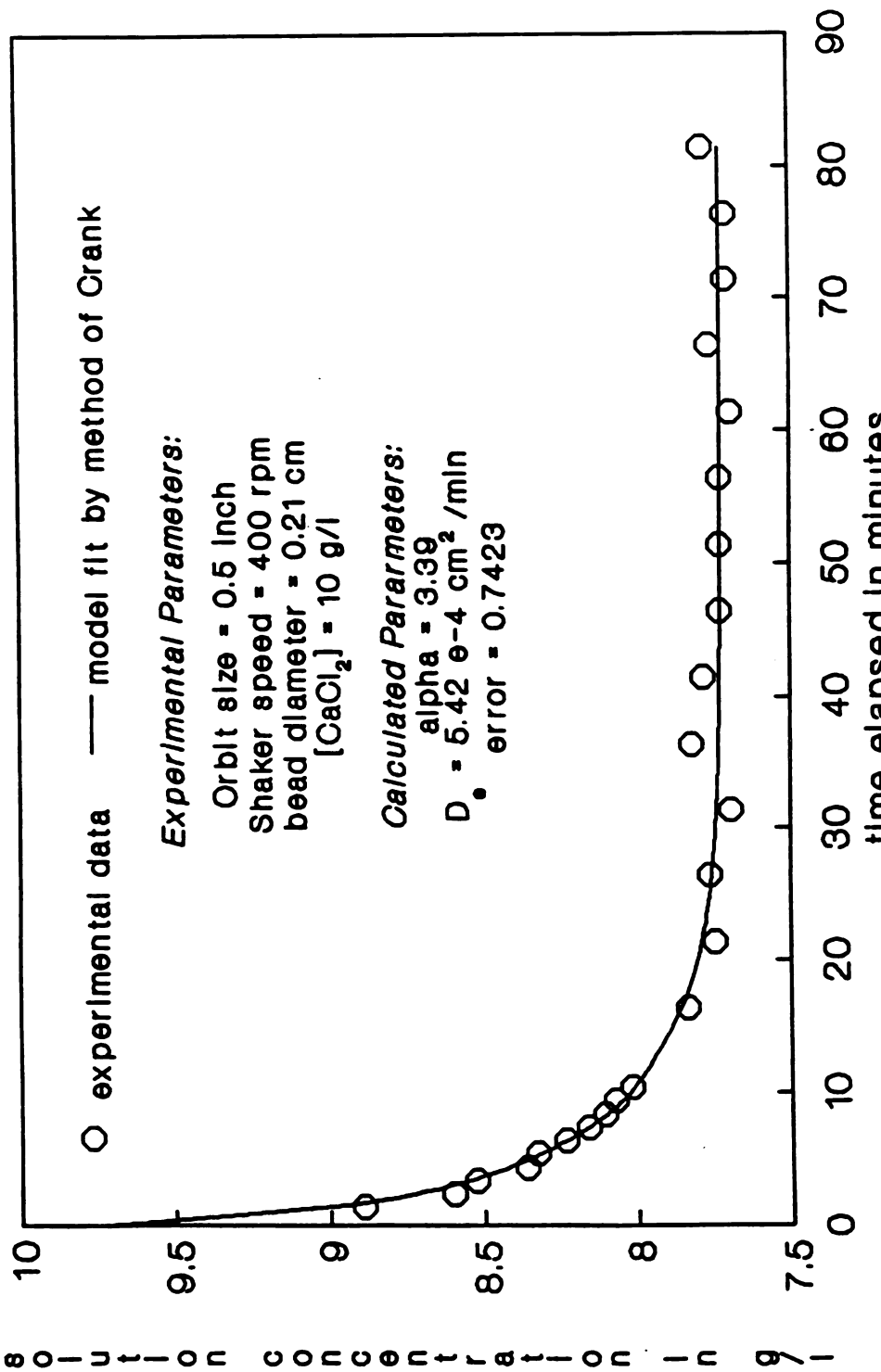

Figure 4. Disappearance of $CaCl_2$ from Solution versus Time

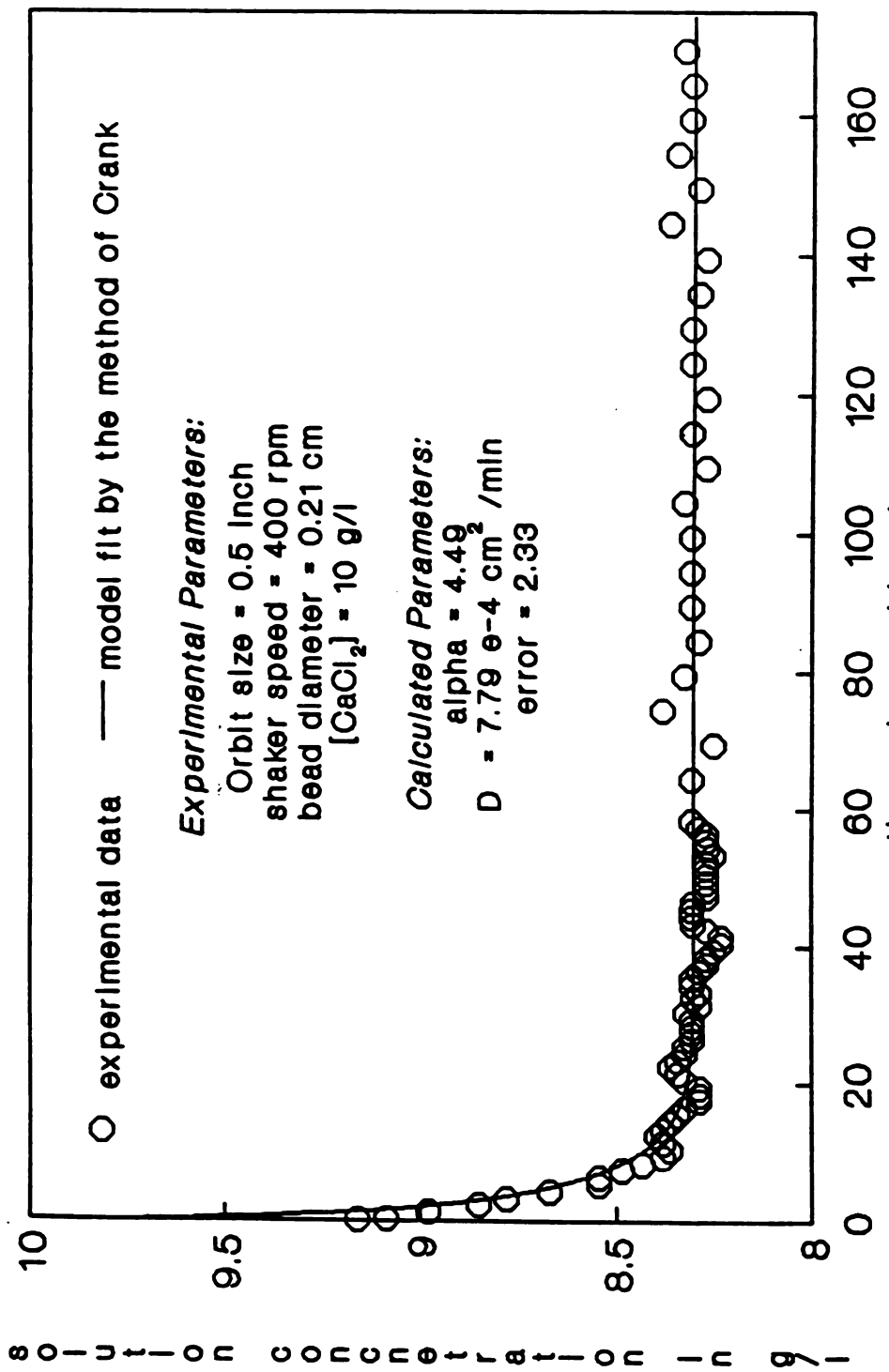

Figure 5. Disappearance of $CaCl_2$ from Solution versus Time

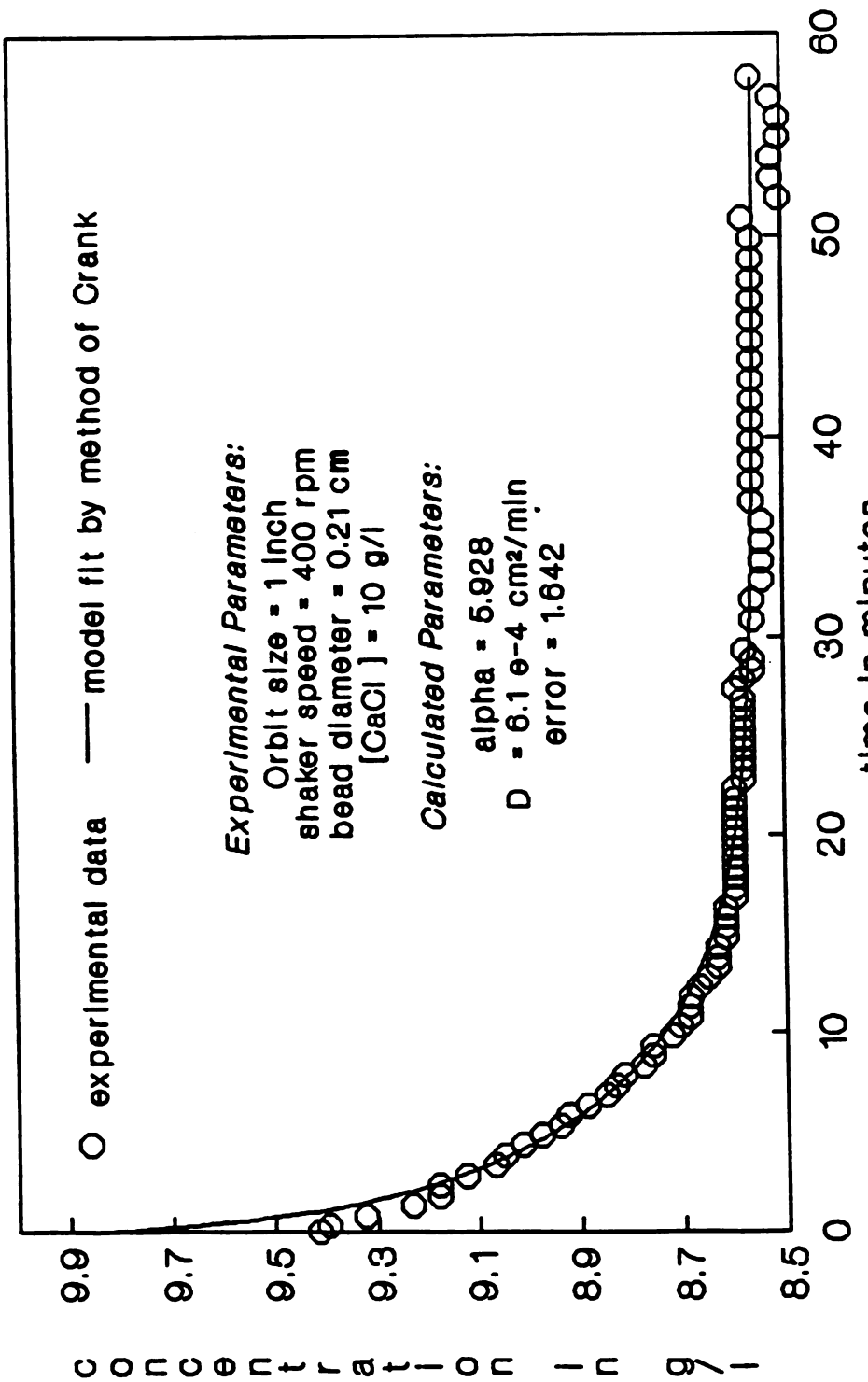

Figure 6. Disappearance of $CaCl_2$ from Solution versus Time

and an increase in the diffusion path of the experiment. Increasing the diffusion path of the experiment increases the diffusion time reducing the relative magnitude of the timing errors. Four experiments with identical amounts of beads and $CaCl_2$ solution were carried out using the 1" orbital shaker, and the results are presented in dimensionless form in Figure 7. Note that as the shaker speed increases up to 250 rpm the final concentration of the solution increases. Also, the data for 250 rpm fit the model more accurately than the data at 350 rpm. The better fit of the data taken at 250 rpm is explainable by the fact that forced convection within the bead may be occurring at 350 rpm, invalidating the assumption of no convective flux. Unfortunately correlations of the mass transfer coefficient for the case of an orbital shaking motion were not found in the literature. Therefore the mass transfer limitations can only be assumed to be overcome for these experiments.

The periodic fluctuations in the data can be attributed mostly to temperature fluctuations in the water bath. The water bath shaker temperature did fluctuate in sinusoidal fashion, as can be expected from the control system.

The experimental values of the effective diffusion coefficient, D_e, ranged from 5 x 10^{-4} to 8 x 10^{-4} cm^2/min and the literature values ranged between 2 x 10^{-4} and 8 x 10^{-4} cm^2/min (Itamunoala, 1987). This indicates that the experimental procedure produces results that are accurate enough to be consistent with the literature.

Conclusions and Recommendations

The experiment can be used to determine the effective diffusion coefficients for

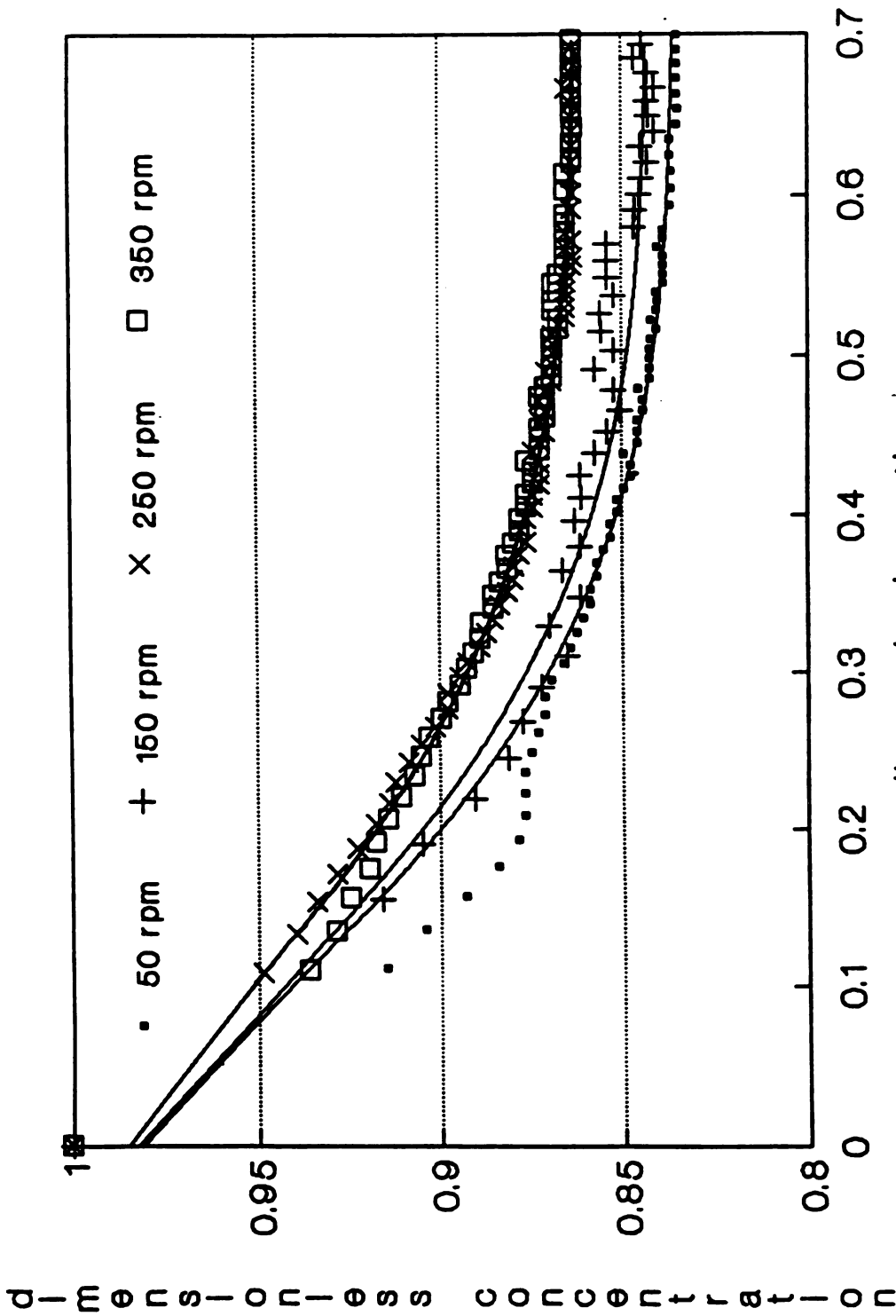

Figure 7. Effect of Shaker Speed on Diffusion Data at 30°C

diffusion into or out of porous spheres with sufficient accuracy to match literature values. The shaker speed and temperature control of the shaker are crucial for the data collection. High agitation rates are required to minimize mass-transfer resistance in the liquid film surrounding the spheres. Effective diffusion coefficients determined by this approach would be useful for mathematical models for packed- and fluidized-bed bioreactors.

Modification of the experimental apparatus to include temperature compensation at the level of the conductivity meter or at the level of calculations within the data acquisition system could greatly improve the quality of the data. The use of a baffled flask and experimentation at different rotational rates might provide interesting insight into the interphase mass transfer regimes of the shaker as well. Modification of the experimental apparatus to use a fluidized bed of the gel spheres in recirculated $CaCl_2$ solution might allow the mass-transfer properties of the beads to be studied.

Symbols

α = ratio of liquid volume to bead volume
δ = film thickness
μ = fluid viscosity
ρ = fluid density
A_s = surface area of the beads
C_i = the concentration of component i
C_L = concentration in the liquid phase
C_{Lo} = initial concentration in the liquid phase
C_s = solute concentration in the beads
d = bead diameter
D_e = effective diffusion coefficient
D_e = the effective diffusion coefficient
D_m = diffusivity into water
J_i = the diffusive flux of component i
k = external mass transfer coefficient

K_p = partition coefficient between liquid and beads
n = eigenvalue index
N_i = total flux of component i
q_n = eigenvalues of the solution for C_L/C_{Lo}
r = radial coordinate
R = radius of the beads
r = radial coordinate
Re = Reynolds number = $\rho ud/\mu$
Sc = Schmidt number = $\mu/\rho D_l$
Sh = Sherwood number = kd/D_l
t = time
u = liquid velocity relative to beads
V_L = volume of the liquid phase

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Appendix I. Experimental Equipment and Procedure

Equipment and Reagents Needed:
sodium alginate
calcium chloride
graduated cylinders
funnel
rubber tubing
screw clamps
conductivity meter
Reverse osmosis (RO) water
balance

I. Preparation of the Alginate Solution and Beads

1. Check to see if any alginate solution has been prepared for you. If not, weigh out sufficient alginic acid to prepare a 1-3 wt. % solution of alginic acid in RO water. Otherwise skip to step 4.

2. Slowly add alginic acid to 500-1000 ml of water while heating and stirring vigorously on a stirrer/hot plate. The recommended method is to slowly scrape enough alginate to barely cover the spatula into the vortex to prevent clumping.

3. Once the alginate solution has completely dissolved, allow the solution to stand and cool. Make sure that the air trapped within the alginate is allowed to completely escape.

4. Set up the apparatus as shown in Figure 1. Make sure to completely pinch off the tube with a screw clamp. For best results, the plastic nozzle chosen should be of the largest size available. Make sure that the ring holding the funnel is as high as possible on the stand. This allows the beads time to form a spherical shape as they fall and avoids splashing from the beaker onto the nozzle.

5. Prepare about 200 ml of a 15 g/l $CaCl_2$ in water solution in a 400 ml beaker. Place the beaker on the stir plate and add a stir bar to the solution.

6. Adjust the speed of the stirrer so that the surface of the fluid is just barely disturbed by the vortex. If the stirring rate is too high, the beads will deform due to shear before they cure.

7. Slowly open the clamp and allow the alginate solution to drip out. Open the clamp enough to allow the alginate to leave the nozzle as individual drops. Poor beads will be formed if the dripping rate is too fast. When the funnel containing the alginate is empty, let the beads cure in the calcium chloride solution for 30-60

minutes.

8. While the beads are curing, turn on the main power on the shaker and adjust the shaker to the desired temperature of operation. Use the conductivity meter to determine the conductivity of RO water.

9. Create several standard salt solutions in the range of 5-10 g/l $CaCl_2$.

10. Start up the Camile system by turning on the computer and typing "cd\camile" to get to the Camile directory. Then type "menu" and the Camile logo will appear. Press any key and the main menu will be displayed. Use the arrow keys to highlight "run" and hit ENTER. Then use the arrow keys to highlight the database called "HEATTRAN" and hit ENTER. The computer will then ask you if you wish to initialize the Camile control constants. Type "y" and hit enter. The computer will then prompt for the default log directory. "C:\camile\logs" should be present and highlighted. If not, backspace and type it in then hit enter. The log file screen should now be on the monitor. Use the arrows to move to log file 4 and type the name you have chosen for your log file. Check with the other lab groups using the computer to enter in their file names before continuing. Make sure that "BIN" and "APP" appear in the fields to the right of the log file name. When you have finished press END. If you have repeated any log file name currently on file, the computer will prompt you to change it. The computer will then ask you to enter a title. This and the comments it prompts for next are optional, but your names and a few brief words describing your experiment might save considerable confusion later. Leave a blank line to finish and hit ENTER. The computer will then inform you that the printer does not respond and will ask you if you wish to retry. Type N.

11. Press the F4 button and then PAGE UP or PAGE DOWN until you are on the screen that has the tag name "COND". Look in the lower right hand corner next to the bar graph. Check to see that the slope is equal to 1 and that the intercept and filter constant are both equal to zero. If they are not you may reset these values by typing "SL 1","IN 0","FL 0" to change the slope, intercept and filter constant, respectively.

12. Place the capped standard solutions in the shaker bath and allow them to come to the desired temperature (eg. 30°C). Always turn off the shaking on the shaker before adding glassware to or removing glassware from the platform. Place the conductivity probe into each solution and record the voltages and the corresponding concentrations in g/l. Use the on-line trending (F3) to make sure that the solution temperature is fully equilibrated before moving on to the next solution. It is important that a calibration be carried out for each temperature you will be experimenting at since the conductivity of $CaCl_2$ solutions is a fairly strong function of temperature.

13. Linearize the concentration versus voltage data to yield an equation for the calibration curve. Use a calibration curve of the form: concentration = slope x voltage + intercept.

14. On the F4 screen of the camile system you must change the slope and intercept values to the ones you determined using the commands in step 11, substituting your slope and intercept values for 1 and 0, respectively. Make sure the values for the constants you determined for your calibration curve appear on the screen in the lower right corner so you will have an on-line representation of the $CaCl_2$ concentration. Press F6 and type "S2 ON". This activates a question and answer sequence that will prompt you for the parameters needed to log the data. You may leave a question and answer screen at any time by pressing another one of the function keys. The F1 key is help and a list of the commands for the function keys is given by ALT-F1. You can then return to the question and answer screen by hitting ALT-F8 at any time.

15. Once the beads have cured, transfer the beads to a 250 ml erlenmeyer flask. Rinse the beads with RO water then fill the flask to about the 200 ml mark with RO water and place the flask in the shaker at 250 rpm with the conductivity probe capping the solution. Periodically drain off the water using a wire screen placed against the mouth of the flask. Repeat the process of replacing the water and putting the flask in the shaker until the water over the beads gives the same conductivity reading as pure RO water for 10 minutes.

16. Prepare a sufficient amount of 10 g/l $CaCl_2$ solution for 4 experiments and label the bottle with your group letter, names, and contents and place it in the refrigerator.

II. Determination of Gel Permeation

1. Set the water bath shaker at the desired temperature for the diffusion experiment. On the NBS G76D this can be accomplished by turning the MODE SELECT knob to °C and toggling the switch below it to "SET" and changing the displayed temperature using the switch with the arrows.

2. Fill a 250 ml graduated cylinder with somewhere between 100 and 130 ml of water and record the volume. Then drain the beads and add them to the graduated cylinder containing the water and record the final volume.

3. Drain the water off the beads using the wire screen and place the beads on a clean cloth or paper towel. Do not thoroughly dry the beads, use the towel to absorb the excess water only.

4. Measure out somewhere between 100 and 170 ml of your 10 g/l solution, record the volume, and place it in the water bath with the conductivity probe in the solution.

5. Securely mount the conductivity probe into the flask in the shaker. The speed of the shaker can be adjusted by turning the MODE SELECT switch to "RPM" and using the rocker switch with arrows. The shaking action can be turned on or off without changing the set point using the switch labeled shaker. Never attempt to add or remove anything to the bath while the shaker is running. Set the shaker at a speed that allows the probe to obtain an accurate reading by keeping the solution slightly above the level of the hole on the side of the probe.

6. Turn on the power to the conductivity meter and begin monitoring the concentration on the Camile system for your experiment. Make sure that the slope and intercept for the COND tag are set to your constants from the calibration curve. You should log your data in intervals of about 30 seconds for the first 1/2 hour of the experiment and about 1/min for next 1/2 hour after that.

7. Use a wide mouth funnel and ring stand to transfer the beads from the towel to an erlenmeyer flask and cover it with parafilm. Place the flask in the water bath to allow it to come to temperature.

8. Leave the $CaCl_2$ solution in the shaker until the Camile indicates that the concentration is equal to 10 g/l. This lets you know that the probe is responding properly and that the solution is at the proper temperature for your calibration curve.

9. Once the solution has reached the proper temperature, go to the question and answer screen that asks if you wish to begin logging for the conductivity experiment. Make sure that the logging period is correct and that it says "yes" after the question "do you wish to begin logging?". If it does not, use the arrows to move the cursor onto the box and type over the current answer and hit return. When you are ready to add the beads press END and logging will begin. Wait 30 seconds after you have pressed the END key and then remove the flask containing the $CaCl_2$ solution and the beads from the bath and add the $CaCl_2$ to the flask containing the beads. Place the probe into the flask containing the beads and the $CaCl_2$ into the bath.

10. Make sure that the stopper containing the conductivity probe is securely fastened and then set the shaker to 350 rpm. Carefully move the flask into the shaker. Avoid rough handling of the wire connected to the probe.

11. You may monitor the progress of you experiment by using the on-line trend screens (F3). You may explore the functions of the Camile at this time as most

functions that will harm your experiment are denied access without a password. Do not change your slope and intercept. An interesting feature of the on line trending screens of the Camile system is the history trend. The history trend appears as graph 9 in the on-line trending screens and can be reached by paging up or down after hitting F3. The history trend displays the data already logged into your file. To view a history trend type "Read" while above graph 9 and then the cursor will move to the middle of the screen next to the word tagname. Type in "cond" and then hit enter. Then the cursor will move to the next row and which will say log file. Enter 4 and press END. At this point the computer should begin reading your data from the log file and displaying it on the screen. If you or another lab group wishes to use the history trend later you must first type "Clear" and then re-enter the pertinent information.

12. After you have been logging data for the time you specified on the question and answer screens, the Camile will automatically stop logging data. To get your log file off the hard disk for analysis you will have to exit run and use the DATA subprogram to convert your binary log file into a comma separated value ASCII file. At this time ask the teaching assistant or the instructor for the password. Once it has been entered by pressing the F9 key and typing "LEVEL ENGINEER" and the password, you may type "BYE" and exit run.

13. While one of your group members converts the log file into a form suitable for analysis by the provided program, (directions are given below) at least 20 diameter measurements of the beads must be taken by the other members to obtain a statistically significant average value.

III. Data Analysis

1. Use the arrows to move the highlighting to DATA and press ENTER. The screen should say "output file type:ASCII" and "Report all time points:yes" then hit END. A whole list of options will then be provided for you to change. Highlight a particular selection and then press the space bar to view the various options and hit ENTER when the option you desire is shown. Make sure you include comments, tag values, elapsed time, date, and the time of day. The column delimiter must be a comma for easy retrieval of the log file into a spreadsheet program. When the appropriate options have been selected, press END. Highlight the two tag names (COND and CTEMP) and press the space bar. An arrow should be present on the left of the names and then press END.

2. The computer will then show the prompt "EDIT filename:" and your log file title with a ".prn" extension will be displayed. Hit END and you will exit DATA and then press escape to exit the Camile menu.

3. Import the log file into a spreadsheet as a comma separated value format (CSV).

(i.e. \\Import,CSV,\camile\logs\cond.prn). You may do this from SuperCalc4 from cd\bin\sc4 or SuperCalc5 (cd\bin\sc5).

4. You will then need to find the time data and convert it from seconds to minutes. Once the time data has been converted to minutes you must create a file that contains only the time data in the left hand column and the concentration data in the right hand column.

5. You can isolate the data by using the "/Replicate,Range,Values" command to copy the cells containing the time and concentration data.

6. At this point you may delete everything on the spreadsheet except the values of the time and concentration. Then you will need to move the time and concentration data to the upper left hand corner making sure that the time is in column A and the concentration is in column B using the move command (i.e. \Move,Block,Range). The alternative is to export only the range containing the copies of the time and concentration data. This can be done using the "\\Export,CSV,Part,Range" command. The time data and the concentration data must still be in adjacent columns with the time on the left for this to work.

7. Record the number of time data pairs in your file along with the filename.

8. Either obtain a copy of DIFFTR5 or use the copy on the Camile computer to analyze your data.

9. To run DIFFTR5 simply type DIFFTR5 or the path name (i.e. "cd\diff" then "difftr5" or "C:\diff\difftr5" for the lab computer).

10. The computer will then ask you whether you wish to solve case 1 or 2. Enter 1 since you wish to determine the diffusion coefficient from diffusion of solute into initially solute free spheres.

11. Then the computer will prompt you for the name of the ".csv" file where you stored your data and the number of data points. You must enter the name including the path in single quotes and then follow that with a comma and the number of points (i.e. 'a:\cond.csv',10)

12. The computer will then prompt you for the number of q_n values you wish to use. Six is usually sufficient though you may use more if desired.

13. The computer will then ask for the *radius* of the beads in cm. Enter that value and press ENTER. The screen will then flash many values across the screen.

14. When the screen stops scrolling and reports that the program is finished, record

the cost, parameter 1, parameter 2, and the 6 or so values that correspond to your q_n values. Parameter 1 is the effective diffusion coefficient in cm^2/min and parameter 2 is α.

15. To obtain a theoretical curve for the solute concentration it is necessary to use the q_n values calculated by the computer. The q_n values, α, and D_e can then be plugged into equation 4 to generate the curve.

16. Plot your experimental data points and the theoretical curve in a fashion similar to Figure 6.

Appendix II. Difftr5: Program for Evaluation of D and α

```
C      SUBROUTINE PROC FOR PROGRAM CALLED PATERN
C
C     PURPOSE:      TO ESTIMATE DIFFUSION COEFFICIENTS BY READING
IN DATA
C                   CONTAINING TIME -VS- CONCENTRATION  VALUES.

C
C      MAIN PROGRAM
C
C      PROGRAM CHE460
C
      IMPLICIT DOUBLE PRECISION(A-H,O-Z)
      INTEGER NDATA,NP,NPASS,IO, NED
      COMMON C(4000), T(4000), Q(10000), NDATA, X, NQ, R
      COMMON NTYPE, XB
      DIMENSION P(10), STEP(10)
      CHARACTER CONCEN*20
C
C      SEARCH INITIALIZATION
      P(1)=5.0E-4
      P(2)=4.00
      STEP(1)=1.0E-5
      STEP(2)=0.1
      NP=2
      NPASS=3
      IO=3
C
C      READ IN DATA
C
5     WRITE(*,*)'WHAT TYPE OF DIFFUSION PROBLEM DO YOU WISH TO'
      WRITE(*,*)'SOLVE?  ENTER THE NUMBER FOR THE DESIRED TYPE.'
      WRITE(*,*)
      WRITE(*,*)'1.  DIFFUSION FROM LIQUID INTO INITIALLY'
      WRITE(*,*)'    SOLUTE FREE SPHERES.'
      WRITE(*,*)'2.  DIFFUSION FROM SPHERES INTO INITIALLY'
      WRITE(*,*)'    SOLUTE FREE LIQUID.'
      READ(*,*) NTYPE
      WRITE(*,*)
C
      IF(NTYPE.EQ.1) THEN
        WRITE(*,*)'ENTER INITIAL LIQUID CONCENTRATION IN g/L'
        READ(*,*) X
        WRITE(*,*)
```

```
      ELSEIF(NTYPE.EQ.2) THEN
        WRITE(*,*)'ENTER INITIAL BEAD CONCENTRATION IN g/L'
        READ(*,*) XB
        WRITE(*,*)
      ELSE
        WRITE(*,*)'YOU DID NOT ENTER A 1 OR A 2. TRY AGAIN'
        WRITE(*,*)
        GOTO 5
      ENDIF
C
      WRITE(*,*)'ENTER THE NAME OF THE TIME-CONCENTRATION FILE'
      WRITE(*,*)'AND THE NUMBER OF DATA POINTS'
      READ(*,*) CONCEN, NDATA
      WRITE(*,*)
C
      OPEN(UNIT=20,FILE=CONCEN,STATUS='OLD')
C
C
      WRITE(*,*)'ENTER THE NUMBER OF Q VALUES DESIRED'
      READ(*,*) NQ
      WRITE(*,*)

      WRITE(*,*)'ENTER THE RADIUS OF THE BEADS'
      READ(*,*) R
      WRITE(*,*)
C
      WRITE(*,*)
      WRITE(*,*)'CONCENTRATION(g/L)          TIME(MIN)'
      DO 150 J=1,NDATA
        READ(20,*)t(J),c(J)
        WRITE(*,350)C(J),T(J)
350      FORMAT(6X,F6.2,18X,F6.2)
150    CONTINUE
C
C
C      START SEARCH
C
      CALL PATERN(NP,P,STEP,NPASS,IO,COST)
C
C      SEARCH COMPLETE, PRINT RESULTS
C      PRINT 300, P(1), P(2), COST
C300    FORMAT(F10.3,10X,F10.3)
      OPEN (UNIT=30,FILE='FOR30.DAT',STATUS='NEW')
      WRITE (30,300) P(1),COST
```

```
300    FORMAT(E10.3,5X,F6.3,5X,E10.3)
       DO 346 L=2,NQ+1
       WRITE (*,*)Q(L)
346    CONTINUE
       STOP
       END
C
C
C
C      THIS FILE IS A PAIR OF SUBROUTINES WRITTEN TO BE
C      COMPATIBLE WITH THE OPTIMIZATION SUBROUTINE PATERN.
C      THEY SIMULATE A PROCESS USING DISCRETE DIFFERENCE
C      EQUATIONS AND COMPARE THE SIMULATION OUTPUT WITH
C      THE ACTUAL OUTPUT (READ IN THROUGH A DATA FILE),
C      CALCULATING AN ERROR OR "COST" ASSOCIATED WITH THAT
C      SIMULATION.  PATERN USES THESE SUBROUTINES ITERATIVELY
C      IN ORDER TO FIND THE OPTIMUM SET OF TRANSFER FUNCTION
C      PARAMETERS TO FIT THE DATA.
C
C
       SUBROUTINE PROC(P,COST)
       IMPLICIT DOUBLE PRECISION (A-H,O-Z)
       COMMON C(4000), T(4000), Q(10000), NDATA, X, NQ, R
       COMMON NTYPE, XB
       DIMENSION P(10), STEP(10), CONC(100)
C
C      INITIALIZE ARRAYS AND DEFINE PARAMETERS
C
       D=P(1)
       ALPHA = P(2)
       ERROR=0.0
       TOTAL=0.0
C
C      PURPOSE: TO CALCULATE THE Q VALUES BASED ON THE ALPHA
GIVEN
C
C
* Program solver
*Solves for the first N solutions of the variable QN given alpha
*USING NEWTON'S METHOD
* DEFINE THE FUNCTION F WHICH MUST = 0 TO OBTAIN A QN VALUE
* FP = THE DERIVATIVE OF THE FUNCTION F
C
C
```

```
* TOL1 IS THE CONVERGENCE CRITERION
      TOL1 = .00000001
* QNOLD IS THE CURRENT VALUE OF QN
      QNOLD = 3
      NC = 1
* ALL OF THE IF STATEMENTS BELOW INVOLVING ALPHA GIVE THE
COMPUTER
* A FIRST GUESS CLOSE TO THE TABULATED VALUES FROM CRANK
      IF (ALPHA .LT. .6667) THEN
        QNOLD = 4
      ENDIF
      IF (ALPHA .LT. .4286) THEN
        QNOLD = 4.18
      ENDIF
* THIS IS THE MASTER LOOP IT IS CONTROLLED BY THE NUMBER OF QN
VALUES DESIRED
110     IF (NC .LE. NQ) THEN

        FPOLD = 1/((DCOS(QNOLD))**2)-(3*ALPHA*QNOLD**2
     +   -6*ALPHA*QNOLD+9)/((3+ALPHA*QNOLD**2)**2)
        FVAL = DTAN(QNOLD)- 3*QNOLD/(3+ALPHA*QNOLD**2)
         IF (FPOLD .EQ. 0) THEN
            WRITE (*,*) 'NEWTONS METHOD FAILS'
            NC=NQ+1
         ELSE
            QNNEW=QNOLD-(FVAL/FPOLD)
            QNOLD=QNNEW
            NED=NED+1
          ENDIF
         IF (ABS(FVAL) .LT. TOL1) THEN
            NC = NC + 1
            QNOLD=QNOLD+2
C             WRITE (*,120) 'QN','F(QN)','FP(QN)'
C120            FORMAT (15X,A6,15X,A6,25X,A6)
C             WRITE (*,*) QNNEW,FVAL,FPOLD
C
            Q(NC) = QNNEW
            WRITE (*,*) NC, Q(NC),NED,ALPHA
            NED = 0
C
          ENDIF
       GOTO 110
       ENDIF
C
```

```
C
C     CALCULATE COST
C
      IF(NTYPE.EQ.1) THEN
      DO 20 J=1,NDATA
            DO 10 I=2,NQ+1
                  TERM1=(6*(1+ALPHA)*EXP(-D*Q(I)**2*T(J)/R**2))
                  TERM=TERM1/(9+9*ALPHA+Q(I)**2*ALPHA**2)
                  TOTAL=TOTAL+TERM
10           CONTINUE
            CONC(J)=((X*ALPHA)/(1+ALPHA))*(1+TOTAL)
            ERR=ABS(C(J)-CONC(J))
            ERROR=ERROR+ERR
            COST=ERROR
            TOTAL=0.0
20     CONTINUE
      GOTO 300
      ELSE
      DO 30 J=1,NDATA
            DO 40 I=2,NQ+1
                  TERM1=(6*(1+ALPHA)*EXP(-D*Q(I)**2*T(J)/R**2))
                  TERM=TERM1/(9+9*ALPHA+Q(I)**2*ALPHA**2)
                  TOTAL=TOTAL+TERM
40           CONTINUE
            CONC(J)=(XB/(1+ALPHA))*(1-TOTAL)
            ERR=ABS(C(J)-CONC(J))
            ERROR=ERROR+ERR
            COST=ERROR
            TOTAL=0.0
30     CONTINUE
      ENDIF
300    RETURN
      END
C
C
C
C
      SUBROUTINE BOUNDS(P,IOUT)
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      COMMON C(4000),T(4000),Q(10),NDATA, X, NQ, R
      COMMON NTYPE, XB
      DIMENSION P(1), STEP(1)
      IOUT=0
      IF(P(1).LT.0.OR.P(2).LT.0) IOUT=1
```

```
      RETURN
      END
C
      SUBROUTINE PATERN(NP,P,STEP,NRD,IO,COST)
C-----THE SIZE OF B1,B2,T,AND S NEED ONLY BE EQUAL TO THE NUMBER
OF PAR
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
      DIMENSION P(1000),STEP(1000),B1(100),B2(100),T(100),S(100)
C-------------------------------------------------------
C       THE FOLLOWING COMMAND ALLOWS PATERN TO USE AN INTEGER
C       VARIABLE AS THE THIRD PARAMETER P(3).
C
      NSRC    =3
      P(NSRC) =       IDINT(P(NSRC))
C-------------------------------------------------------
C-----STARTING POINT
      L=1
      ICK=2
      ITTER=0
      DO5 I=1,NP
      B1(I)=P(I)
      B2(I)=P(I)
      T(I)=P(I)
 5    S(I)=STEP(I)*10.
C-----INITIAL BOUNDARY CHECK AND COST EVALUATION
      CALL BOUNDS(P,IOUT)
      IF(IOUT.LE.0)GOTO10
                IF(IO.LE.0)GOTO6
                WRITE(*,1005)
                WRITE(*,1000)(J,P(J),J=1,NP)
 6    RETURN
 10   CALL PROC(P,C1)
                IF(IO.LE.0)GOTO11
                WRITE(*,1001)ITTER,C1
                WRITE(*,1000)(J,P(J),J=1,NP)
C-------BEGINNING OF PATTERN SEARCH STRATEGY
 11   DO99 INRD=1,NRD
      DO12 I=1,NP
 12   S(I)=S(I)/10.
C-------------------------------------------------------
      S(NSRC) =       1.0001
C-------------------------------------------------------
                IF(IO.LE.0)GOTO20
                WRITE(*,1003)
```

```
                WRITE(*,1000)(J,S(J),J=1,NP)
 20   IFAIL=0.0
C-----PRETURBATION ABOUT T
      DO30 I=1,NP
      IC=0
 21   P(I)=T(I)+S(I)
      IC=IC+1
      CALL BOUNDS(P,IOUT)
      IF(IOUT.GT.0)GOTO23
      CALL PROC(P,C2)
                L=L+1
                IF(IO.LT.3)GOTO22
                WRITE(*,1002)L,C2
                WRITE(*,1000)(J,P(J),J=1,NP)
 22   IF(C1-C2)23,23,25
 23   IF(IC.GE.2)GOTO24
      S(I)=-S(I)
      GOTO21
 24   IFAIL=IFAIL+1
      P(I)=T(I)
      GOTO30
 25   T(I)=P(I)
      C1=C2
 30   CONTINUE
      IF(IFAIL.LT.NP)GOTO35
      IF(ICK.EQ.2)GOTO90
      IF(ICK.EQ.1)GOTO35
      CALL PROC(T,C2)
                L=L+1
                IF(IO.LT.2)GOTO31
                WRITE(*,1002)L,C2
                WRITE(*,1000)(J,T(J),J=1,NP)
 31   IF(C1-C2)32,34,34
 32   ICK=1
      DO33 I=1,NP
      B1(I)=B2(I)
      P(I)=B2(I)
 33   T(I)=B2(I)
      GOTO20
 34   C1=C2
 35   IB1=0
      DO39 I=1,NP
      B2(I)=T(I)
      IF(ABS(B1(I)-B2(I)).LT.1.0E-20)IB1=IB1+1
```

```
 39   CONTINUE
      IF(IB1.EQ.NP)GOTO90
      ICK=0
                  ITTER=ITTER+1
                  IF(IO.LT.2)GOTO40
                  WRITE(*,1001)ITTER,C1
                  WRITE(*,1000)(J,T(J),J=1,NP)
C-----ACCELERATION STEP
 40   SJ=1.0
      DO45 II=1,11
      DO42 I=1,NP
      T(I)=B2(I)+SJ*(B2(I)-B1(I))
C-------------------------------------------------------------
       IF(I.EQ.NSRC)T(I)=IDINT(T(I))
C-------------------------------------------------------------
 42   P(I)=T(I)
      SJ=SJ-.1
      CALL BOUNDS(T,IOUT)
      IF(IOUT.LT.1)GOTO46
      IF(II.EQ.11)ICK=1
 45   CONTINUE
 46   DO47 I=1,NP
 47   B1(I)=B2(I)
      GOTO20
 90   DO91 I=1,NP
 91   T(I)=B2(I)
 99   CONTINUE
      DO100 I=1,NP
100   P(I)=T(I)
      COST=C1
                  IF(IO.LE.0)RETURN
                  WRITE(*,1004)L,C1
                  WRITE(*,1000)(J,P(J),J=1,NP)
      RETURN
1000  FORMAT(3(35X,I7,5X,E13.6/))
1001  FORMAT(//1X13HITERATION NO.  ,I5/5X,5HCOST= ,E15.6,20X,
     1   10HPARAMETERS)
1002  FORMAT(10X3HNO.,I4, 8X5HCOST=,E15.6)
1003  FORMAT(/1X28HSTEP SIZE FOR EACH PARAMETER   )
1004        FORMAT(1H113HANSWERS    AFTER    ,I3,2X,23HFUNCTIONAL
EVALUATIONS   //
     1   5X5HCOST=,E15.6,20X,18HOPTIMAL PARAMETERS  )
1005  FORMAT(1H135HINITIAL PARAMETERS OUT OF BOUNDS        )
      END
```

Membrane Filtration

Summary

A hollow-fiber system with a molecular weight cutoff of 100,000 and membrane area of 0.3 m^2 was used to a concentrate cell suspension of *Z. mobilis* from 8 to 30 g/l. The recirculation rate for the hollow-fiber unit was 1000 ml/min with a pressure drop and total system operating pressure of 5 psig. The rejection coefficient of the cells was 0.744 at a flux of 31.9 $\pm$ 0.49 L/m^2 min. An aqueous blue dextran solution was concentrated from 0.5 to 1.4 g/l using a spiral ultrafiltration cartridge with a molecular weight cutoff of 30,000 and membrane area of 0.0929 m^2. The spiral cartridge was operated at a recirculation rate of 1000 ml/min at 30 psig with a 5 psig pressure drop across the cartridge. The blue dextran was concentrated, yielding a rejection coefficient of 0.872 and a flux of 14.66 l/m^2 min.

Introduction

Membrane separations are frequently used in the growing biotechnology industry. Polymer membranes separate dissolved and suspended material from a pressurized fluid streams based on molecular size and properties. Substances smaller than the pore size of the polymer membrane pass through the membrane with the solvent, and larger solutes are retained by the membrane (Tutunjian, 1985). Two types of filtration exist: dead-end and crossflow filtration. Dead-end filtration produces a cake that rapidly builds up on the membrane reducing the filtration rate, while, in crossflow filtration, the fluid moves

across the surface minimizing the build up of retained particles, as illustrated in Figure 1 (Murkes, 1988).

Crossflow filtration processes are categorized by the size of the particles retained by the membranes and the pore size. Ultrafiltration (UF) generally relates to species of molecular weights ranging from 10 to 100 Angstroms in size (500 to 1,000,000 daltons). Microfiltration refers to pore sizes ranging from 0.01 to 10 microns. Microfiltration is used to retain suspended particles such as cells, while ultrafiltration is used to remove macromolecules such as proteins, peptides, and viruses. Reverse osmosis uses even smaller pore sizes that retain salts and only allow water to pass through the membrane (Tutunjian, 1985).

Commercially available membrane geometries are usually classified into two categories: hollow fibers and flat sheets. Hollow fibers are self-supporting polymer tubes contained in cartridges. Hollow fibers are able to be backflushed, facilitating cleaning and product recovery, but are currently limited to pressures below 40 psi. Flat-sheet membranes exist in many forms, including conventional plate-and-frame filters and spiral cartridges. Flat sheet membranes may operate at higher pressures but can be difficult to clean (Tutunjian, 1985).

Membrane filtration can be used to concentrate, diafiltrate, or purify a mixture. Solutions are concentrated when processing is inconvenient or expensive for a dilute solution. Diafiltration removes low-molecular-weight solutes, such as salts, from solution by exchanging them for water through the membrane while retaining the higher-molecular-weight product.

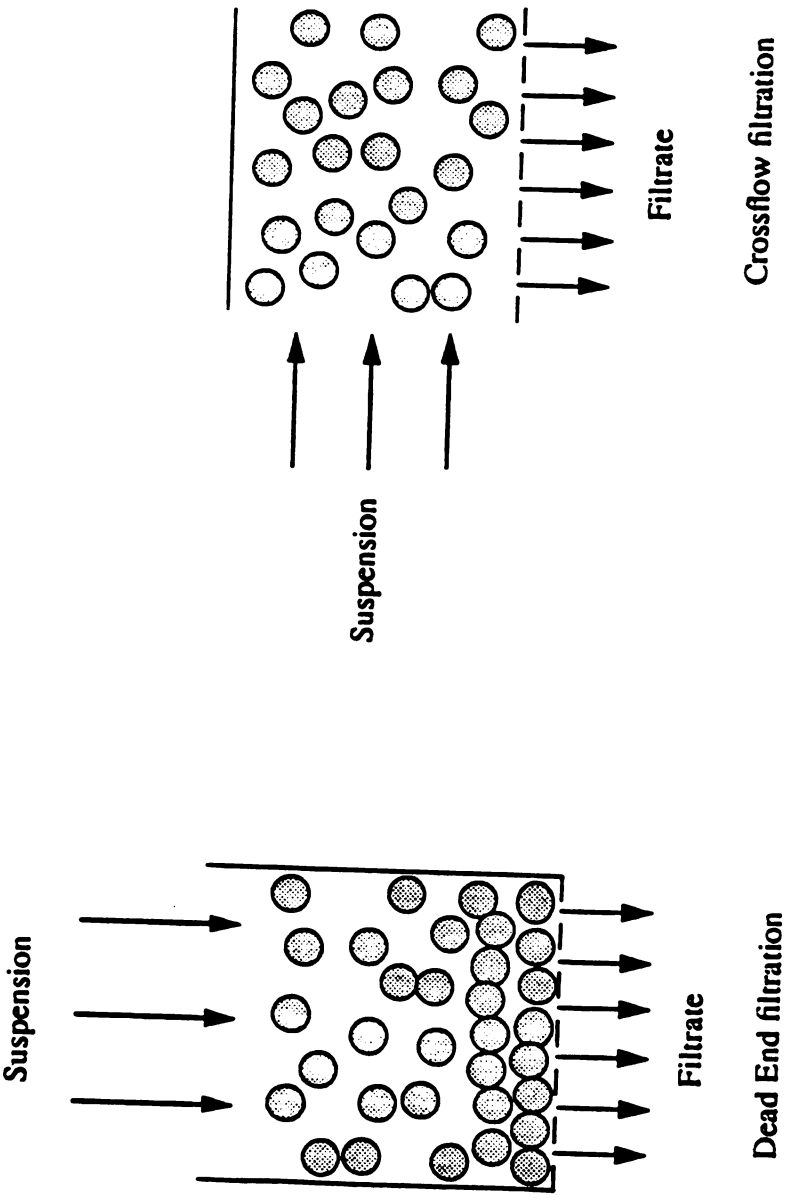

Figure 1. Schematic Comparison of Dead End and Crossflow Filtration

Purification is the isolation of the final product from other solutes (Tutunjian, 1985).

The advantages of using microfiltration and ultrafiltration for separations over conventional processes are numerous. Membrane filtration produces a high-purity filtrate, operates at low temperatures, allowing separation of heat-sensitive products and minimizing energy costs since no heating is required (Murkes, 1988). Membrane separations also facilitate other downstream-processing steps. The filtrate is essentially free of colloids and microorganisms, allowing other purification steps, such as ion-exchange or affinity chromatography. Since no other chemicals are required for membrane filtration, later recovery and disposal steps can be avoided (Beaton, 1980). If the products are retained by the membrane, they may be purified and concentrated in one step. Products permeable to the membrane are separated with minimal dilution. An example that uses all of the advantages of crossflow filtration is the concentration of a cell suspension. The performance of the membrane is not a strong function of cell size, and is not dependent upon a density difference between the cells and suspending media, eliminating the need for flocculating agents (Cheryan, 1986).

Despite its utility, membrane filtration also poses some disadvantages. Low membrane filtration rates are common, requiring relatively large membrane areas and therefore substantial capital investment. Technical or economic limitations of the membranes may exist for the maximum amount of solids that may be retained by the membrane (Beaton, 1980). Many common anti-foaming agents used in fermentations adhere to the membrane and rapidly foul it (Murkes, 1988). Crossflow filtration often damages shear sensitive products, such as mammalian cells or proteins. High viscosity

solutions, such as mycelial fungi fermentation broths require large pumping rates to minimize fouling and concentration polarization to maintain a suitable flux (Cheryan, 1986). Concentration polarization, the formation of a viscous and gelatinous boundary layer on the surface of the membrane, increases the resistance to flow, lowering the membrane flux. The membrane flux is maintained at high levels by operating the membrane using high flow rates that minimize the boundary-layer thickness (McGregor, 1986). In some documented cases, concentration polarization has reduced the flux to 5% of the maximum in 25 seconds (Cheryan, 1986).

Theoretical Analysis

The effect of transmembrane pressure difference on permeate flux varies with the properties of the membrane, the permeate and the solute. In general, the flux is directly proportional to the pressure difference across the membrane at low pressures and independent of the pressure difference at high pressures. Mathematical models have been proposed for both of these regimes.

At low operating pressures the membrane flux may be described by the Hagen-Poiseuille law for stream-line flow through channels. The form of this relation useful for modeling ultrafiltration is:

$$J = \frac{\varepsilon r^2 P}{8 \mu (\Delta x)} \qquad (1)$$

where J = membrane flux
r = channel (mean pore) radius
P = applied transmembrane pressure
μ = fluid viscosity

Δx = membrane thickness (or pore length)
ϵ = surface porosity of the membrane

If pure water is used as the feed to the filtration, $P = \Delta P_T$. Otherwise, the net driving force, P, is given by :

$$P = \Delta P_T - \Delta\pi = (P_F - P_p) - (\pi_F - \pi_p) \tag{2}$$

where ΔP_T = transmembrane pressure drop
$\Delta\pi$ = osmotic pressure gradient = $f(C_{p,m})$

The osmotic pressure gradient for macromolecules is usually assumed to be small compared to the transmembrane pressure drop, so P is equal to ΔP_T in most cases. The Hagen-Poiseuille model is based on assumptions of steady state; laminar flow through the pores; an incompressible, Newtonian fluid; and negligible end effects. It predicts that flux should be directly proportional to the applied pressure and inversely proportional to the viscosity. The viscosity at the membrane surface may differ from the bulk fluid viscosity due to concentration polarization. Flux is directly proportional to pressure only when concentration polarization is minimized, i.e. when the feed composition is low, the pressure is low, and at high crossflow velocities (Cheryan, 1986).

The high pressure, mass-transfer controlled regime may be described using film theory. Figure 2 shows two film resistances in series: the stagnant-liquid boundary layer and the concentration-polarization layer. The rate of solute transport to the membrane by convection, J_s, is given by the following equation (Cheryan, 1986):

$$J_s = JC_b \tag{3}$$

where J_s = rate of solute transport by convection, moles (or mass)/time
J = permeate flux, volume/time
C_b = bulk concentration of the retained (rejected) solute

The accumulation of high concentrations of solute at the membrane surface results in a concentration gradient that drives diffusion of the solute back into the bulk solution. Neglecting axial diffusion, the rate of diffusive transport back into the bulk phase is given by Fick's law:

$$J_s = D\frac{dC}{dx} \tag{4}$$

where D is the diffusion coefficient and dC/dx is the concentration gradient over a differential element in the boundary layer. At steady state, the two mechanisms of transport will be equal and equations (3) and (4) can be combined to eliminate J_s. The resulting equation may be integrated over the boundary layer to give the following result (Cheryan, 1986):

$$J = \frac{D}{\delta}\ln\left(\frac{C_g}{C_b}\right) = k\ln\left(\frac{C_g}{C_b}\right) \tag{5}$$

where k = mass transfer coefficient
δ = film (boundary layer) thickness
C_g = gel concentration at the membrane surface
C_b = concentration solute in the bulk fluid

This model is valid when flux is independent of transmembrane pressure drop. The flux is controlled by rate of solute transfer back into the bulk phase. The physicochemical properties of the fluid control C_g and C_b, so increasing the mass transfer coefficient, k

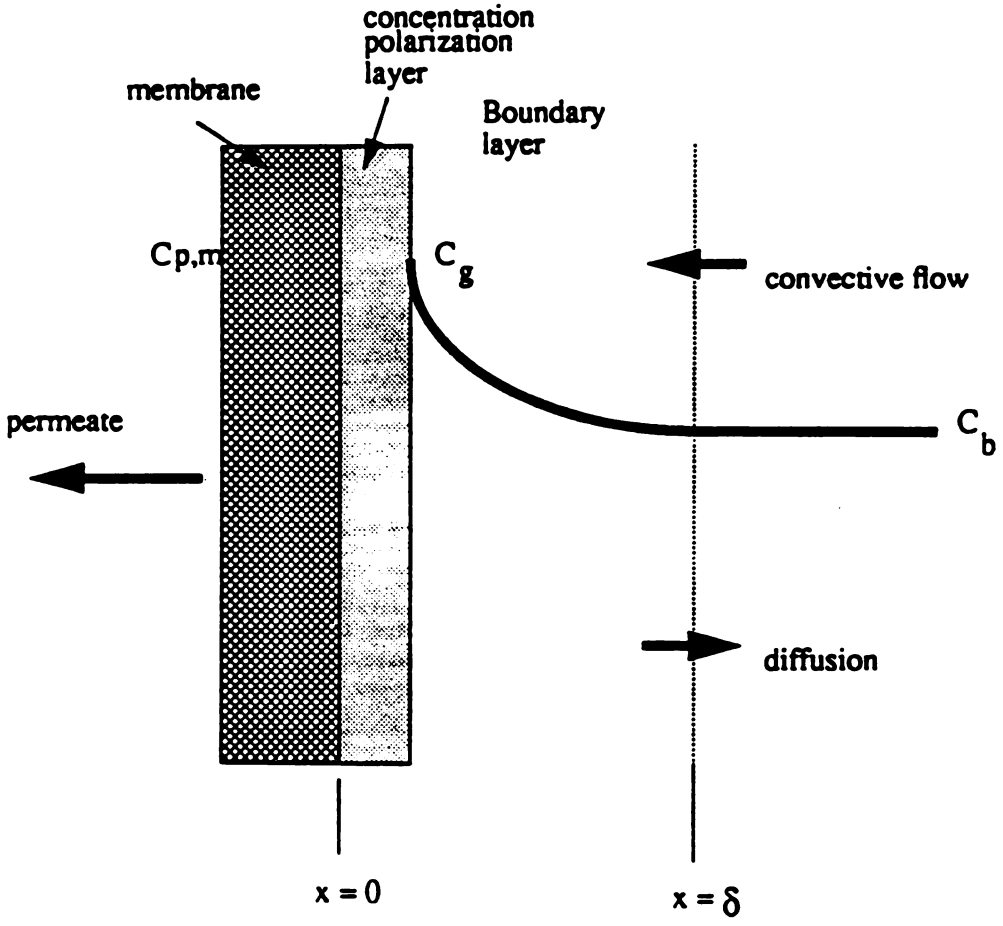

Figure 2. Schematic of Concentration Polarization and Boundary Layer

is the only way to increase the flux. The mass-transfer coefficient in increased by increasing the flowrate, which minimizes the boundary layer. Increasing the operating pressure of the membrane has only transient effects on the flux since the change in pressure ultimately affects the boundary layer and the mechanisms of transport into the bulk phase (Cheryan, 1986).

The mass-transfer coefficient can be correlated using dimensional analysis by a correlation of the general form (Cheryan, 1986):

$$Sh = A(Re)^{\alpha}(Sc)^{\beta}\left(\frac{d_h}{L}\right)^{\omega} \tag{6}$$

where d_h = hydraulic diameter = 4(cross sectional area)/(wetted perimeter)
L = channel length
Sh = Sherwood number = kd_h/D
Re = Reynolds number = $\rho u d_h/\mu$
Sc = Schmidt number = $\mu/\rho D$
D = diffusion coefficient
μ = fluid viscosity
ρ = fluid density
u = fluid velocity
α,β,ω,A = constants

For tubes and circular cross sections, d_h = diameter (d). For slits of width a and height b, d_h is of the form:

$$d_h = 4\left(\frac{ab}{2(a+b)}\right) \tag{7}$$

If a >> b for slits, d_h is equal to 2b. For laminar flow with fully developed velocity and concentration profiles, α and ß are zero. The correlation for laminar flow, when Re < 1800, $L_v < L$ and $L_e > L$:

$$Sh = 1.86 Re^{0.33} Sc^{0.33} (d_h/L)^{0.33} \quad (8)$$

where L_c = length required for a fully developed concentration profile
L_v = length required for a fully developed velocity profile

The correlation for laminar flow with $L_v > L$ and $L_c > L$:

$$Sh = 0.664 Re^{0.5} Sc^{0.33} (d_h/L)^{0.5} \quad (9)$$

For turbulent flow, Re > 4000 the correlation is given by (Cheryan, 1986):

$$Sh = 0.023 Re^{0.8} Sc^{0.33} \quad (10)$$

A third model to describe the pressure-flux behavior of ultrafiltration for both pressure regimes combines the two previous models. In the pressure-controlled regime the Hagen-Poiseuille equation shown in Equation (1) can be rewritten as:

$$J = \frac{B \Delta P_T}{\mu} = \frac{\Delta P_T}{R_m} \quad (11)$$

where B = membrane permeability coefficient
R_m = intrinsic membrane resistance using pure water as the feed
$= \mu/B$

The model predicts that the flux is directly proportional to the driving force and inversely proportional to the resistance. Since the resistances of both the membrane and concentration polarization are in series, they may be added in the equation as shown below:

$$J = \frac{\Delta P_T}{R_m' + R_p} \quad (12)$$

where R_m' = intrinsic membrane resistance including fouling

R_p = resistance due to concentration polarization

The resistance due to concentration polarization is a function of the applied pressure, such that:

$$R_p = \phi \Delta P_T \tag{13}$$

where ϕ is an empirical constant that reflects the degree of concentration polarization. Combination of the equations (11), (12) and (13) results in the following:

$$J = \frac{\Delta P_T}{R_m' + \phi \Delta P_T} \tag{14}$$

When $R_p > R_m'$ then the flux approaches the value of $1/\phi$ (Cheryan, 1986). The flux will increase linearly with the transmembrane pressure at relatively low fluxes and pressure drops. The filtration rate may be controlled by adjusting the operating pressure and recirculation rate is this regime. Upon the formation of a gel by concentration polarization, the flux becomes relatively insensitive to pressure. The filtration rate in this regime may be increased by increasing the flow velocity to reduce the thickness of the concentration polarization and boundary layers. Crossflow velocity has a strong effect on the flux of systems with particulates, such as bacteria (Tutunjian, 1985).

A batch concentration process removes a volume of permeate, V_p, from a suspension through the pores of the membrane, decreasing the retentate volume from the initial value, V_o, to its final volume, V. The retentate is the fluid that is retained by the membrane, and the permeate is the fluid that passes through the membrane. The fluid volumes are related by a simple material balance i.e. $V = V_o - V_p$. The membrane pores

may hold a significant volume of fluid, so the calculated value of V may not be exactly equal to the actual final volume (Cheryan, 1986).

Diafiltration removes low-molecular-weight solutes by exchanging them for water molecules across the membrane. Diafiltration removes the lower-molecular-weight solutes without affecting the concentration of the higher molecular weight solutes. Typically, the solutes removed are salts that were used to precipitate proteins out of aqueous solution (Tutunjian, 1985). The concentration of the removed solute decreases exponentially as the total volume of water added increases as described by the following equation:

$$C_s = C_{so}\exp(-V_d/V_o) \tag{15}$$

where C_s = concentration of removed solute
C_{so} = initial concentration of removed solute
V_d = total volume of water added
V_o = initial volume of solution

Purification operates similarly to diafiltration in that smaller molecules are separated from larger ones. The permeate usually contains the product in purification operations. The overall recovery, R, is given by the following equation:

$$R = \frac{V_p C_p}{V_o C_o} = 1 - \frac{VC}{V_o C_o} \tag{16}$$

where C_p = solute concentration in the permeate
C = retentate solute concentration
C_o = initial retentate solute concentration
V = volume of retentate
V_p = permeate volume

The rejection coefficient, σ, is an important variable that characterizes the membrane's

ability to retain the solute. The rejection coefficient may be determined using the following equation:

$$\sigma = 1 - \frac{C_p}{C} \tag{17}$$

where C = concentration of the solute in the retentate
C_p = solute concentration in the permeate

A rejection coefficient value of 1 indicates that the solute is completely retained, while $\sigma = 0$ implies that the solute freely passes through the membrane.

The filtration process can be represented in terms of the probability, P, of a solute passing through the membrane. The value of P is assumed to be constant for most filtration processes. Solutes that freely pass through the membrane i.e. those with $\sigma = 0$, have P = 1. Solutes with that are completely retained by the membrane have a P of zero. The rejection coefficient can be defined in terms of the probability, P, in the following form:

$$\sigma = 1 - P \tag{18}$$

The probability, P, can be defined as the ratio of C_p to C. The mathematical model developed below is derived from a material differential balance and is based on the volume-concentration ratio, VCR, which is defined below (Cheryan, 1986):

$$VCR = \frac{V_o}{V} \tag{19}$$

The concentration of the solute in the retentate is given by:

$$C = N/V \tag{20}$$

where C = concentration of the solute in the retentate
V = volume of the retentate during ultrafiltration
N = number of solute particles in the retentate

Each volume of liquid, ΔV, that passes through the membrane results in a corresponding change in the number of solute particles, N, which is equal to ΔN and is given by (Cheryan, 1986):

$$\Delta N = (C \Delta V) P \tag{21}$$

Dividing both sides by N, substituting in C = N/V, and rewriting it in differential form yields (Cheryan, 1986):

$$\frac{dN}{N} = P \frac{dV}{V} \tag{22}$$

Integrating and rearranging:

$$N = K V^P \tag{23}$$

Substituting this equation into the expression for C yields (Cheryan, 1986):

$$C = K V^{P-1} \tag{24}$$

Putting C in terms of VCR, and defining P in terms of the rejection coefficient yields:

$$C = C_o(VCR)^{\sigma} \tag{25}$$

Thus, a logarithmic plot of C versus VCR should yield straight lines with a slopes of σ. Deviation from the model suggests solute absorption by the membrane or that the rejection coefficient of the membrane varies with concentration. The yield, Y, is the mass of the solute present in the retentate divided by the mass of the solute that was initially present in the retentate and is given by the following equation (Cheryan, 1986):

$$Y = \frac{CV}{C_oV_o} = (VCR)^{\sigma-1} \tag{26}$$

Experimental Equipment and Procedure

The equipment used in this experiment included a hollow-fiber cartridge, a spiral cartridge, two peristaltic pumps, and a spectrophotometer. The hollow-fiber cartridge was an Amicon H1P100 cartridge (Danvers, MA) with a molecular weight cutoff of 100,000, and a membrane area of 0.3 m^2 which consisted of 55 1.1 mm inner diameter fibers. The spiral cartridge was an Amicon S1Y30 cartridge with a molecular-weight cutoff of 30,000 and a membrane area of 0.0929 m^2. The pumps used were both Masterflex Model 7523-00 peristaltic pumps (Chicago, IL). The spectrophotometer used for concentration determinations was a Perkin Elmer Lambda 3A (Norwalk, CT). The hollow-fiber and spiral cartridge were attached to the pumps as shown in Figures 3 and 4.

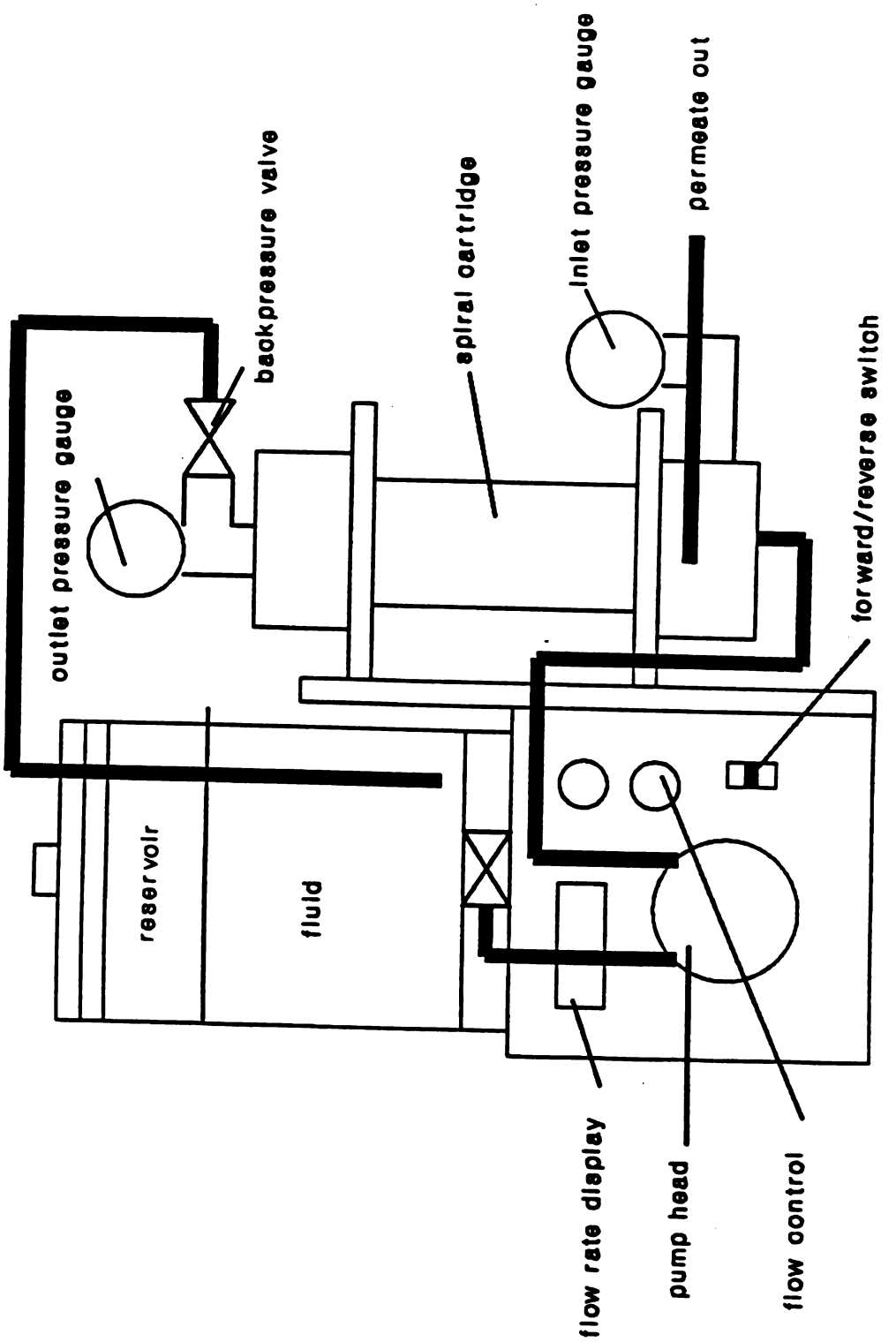

Figure 3. Diagram of Spiral Cartridge Apparatus

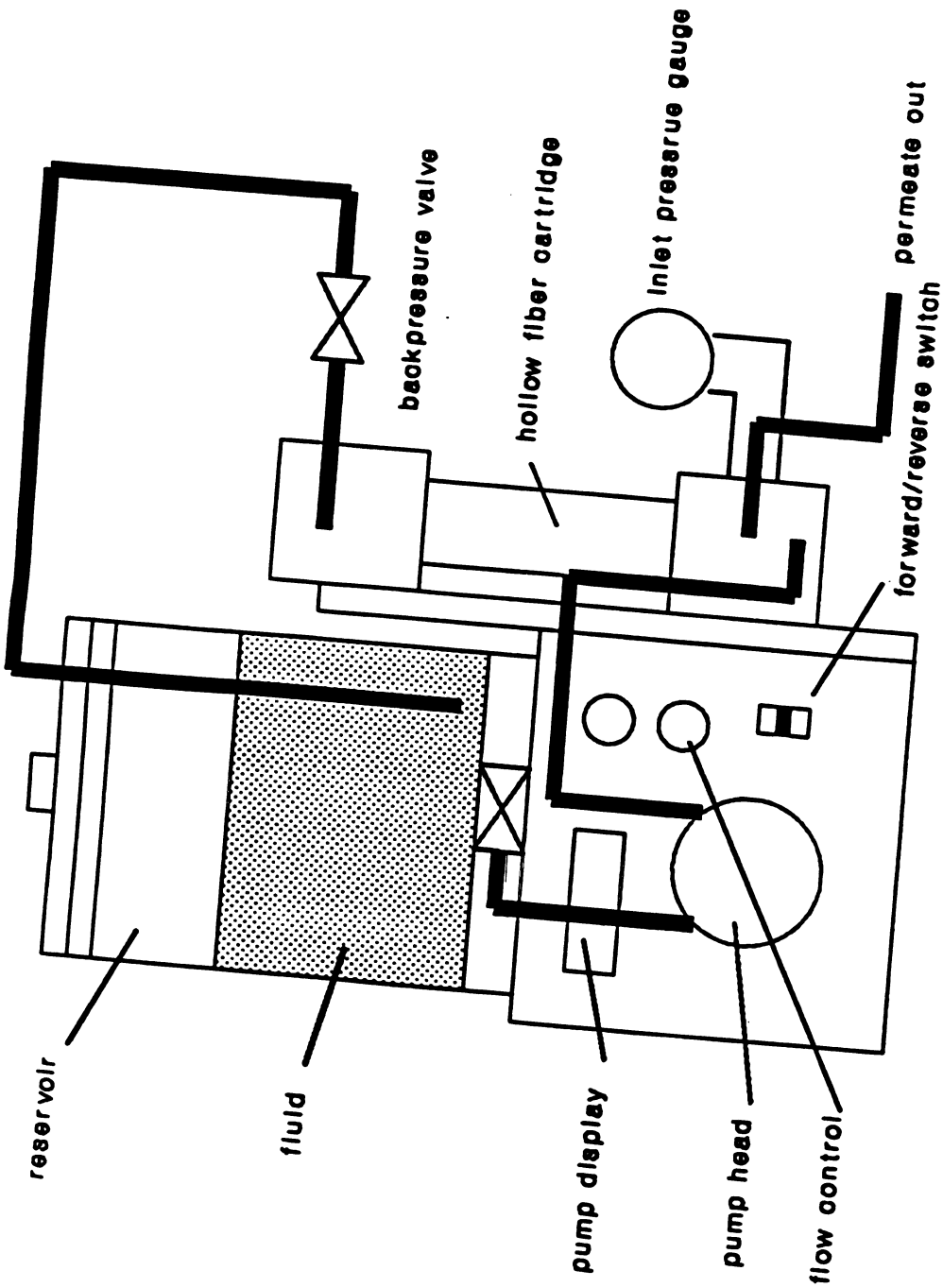

Figure 4. Diagram of Hollow Fiber Apparatus

The cell concentration was measured spectrophotometrically at 770 nm based upon the calibration curve for dry weight versus O.D. shown in Figure 5. The blue dextran concentration was also determined spectrophotometrically at 660 nm, and the calibration curve for blue dextran at 600 nm is shown in Figure 6. The time, volume of permeate, and the volume of retentate were recorded as the liquid level reached the volume gradations in the retentate reservoir. The permeate volume was monitored by collection in a graduated cylinder. Samples of about 3 ml were removed from both the permeate and the retentate whenever volumes were recorded.

Results and Discussion

The data were analyzed in terms of the volume-concentration-ratio theory and the flux. The fits for the concentration and yield data to the VCR theory for the hollow-fiber and spiral cartridges are shown in Figures 7 and 8 respectively. Linear regression yielded rejection coefficients of 0.873 ± 0.024 for the cells and 0.744 ± 0.019 for the blue dextran with correlation coefficients of 0.992 and 0.995, respectively. The fluxes were determined from the slope of a plot of permeate volume versus time divided by the membrane area as shown in Figures 9 and 10. After an initial adjustment period of about 6 seconds, the flux in both cases became constant, yielding lines with correlation coefficients of 0.999 for the hollow-fiber and 0.991 for the spiral cartridge. The flux for the hollow fiber was 31.9 ± 0.48 l/m^2 min, and the flux for the spiral cartridge was 14.66 ± 0.68 l/m^2 min.

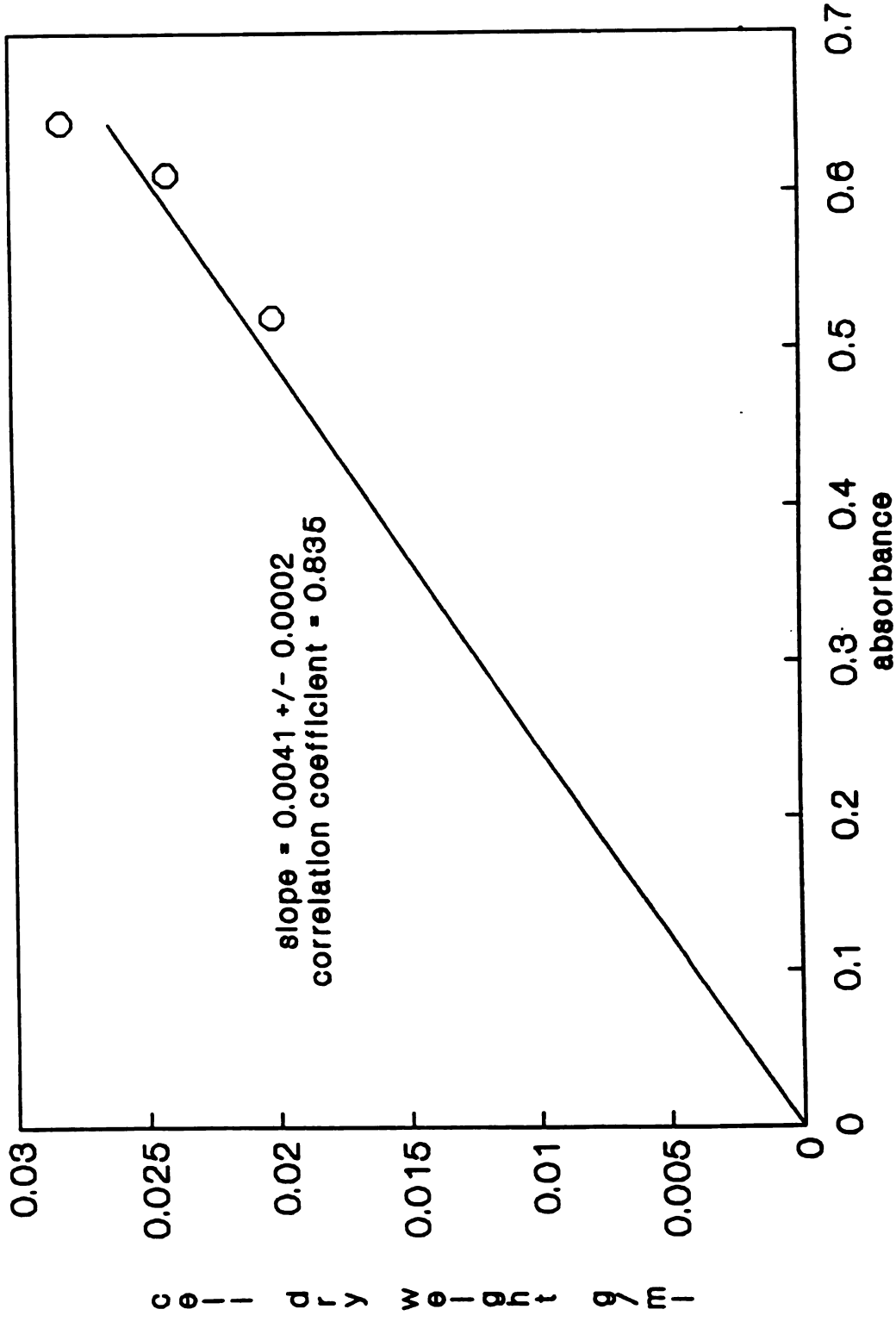

Figure 5. Cell Dry Weight versus Absorbance at 710 nm

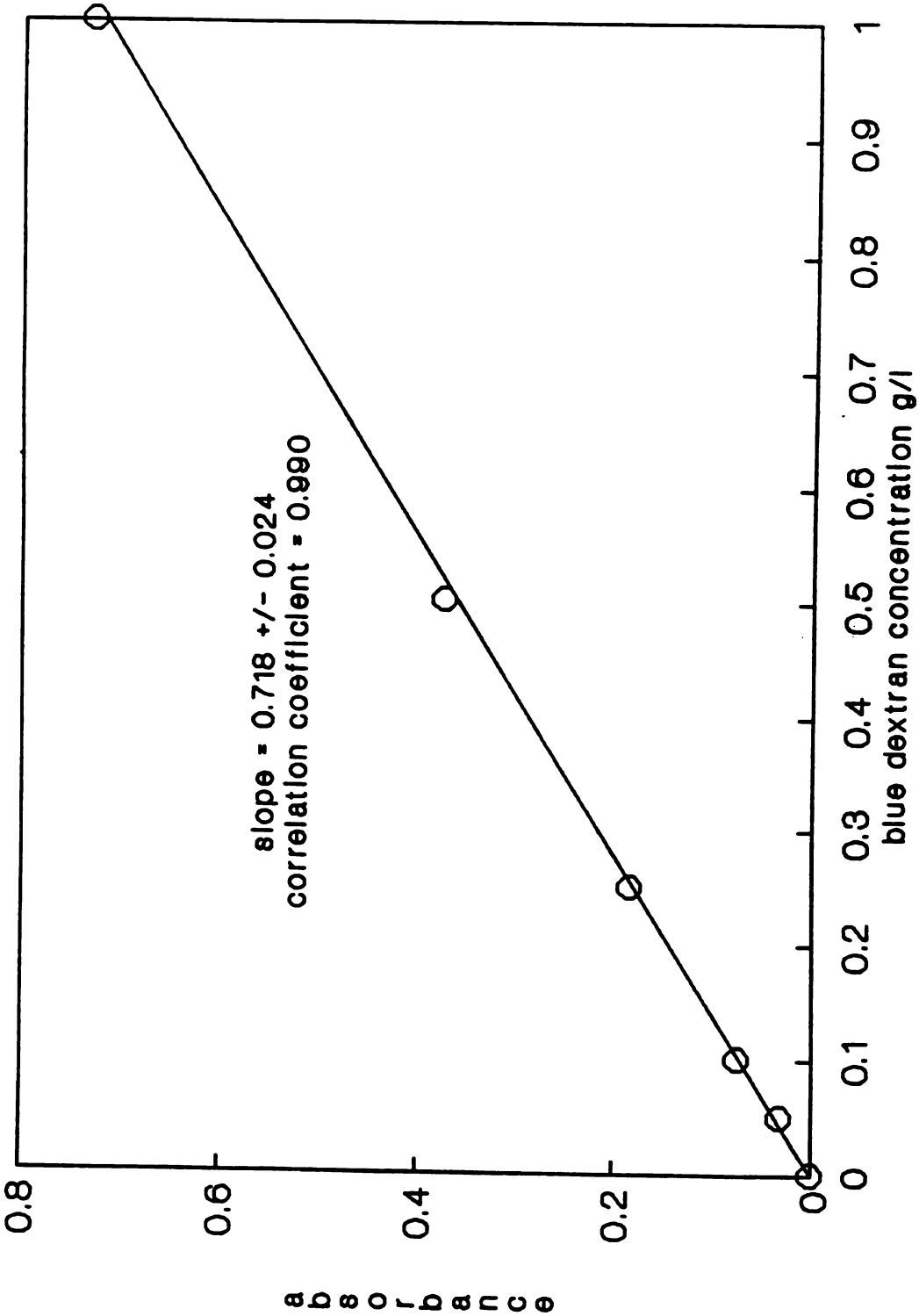

Figure 6. Calibration Curve for Blue Dextran Concentration versus Absorbance

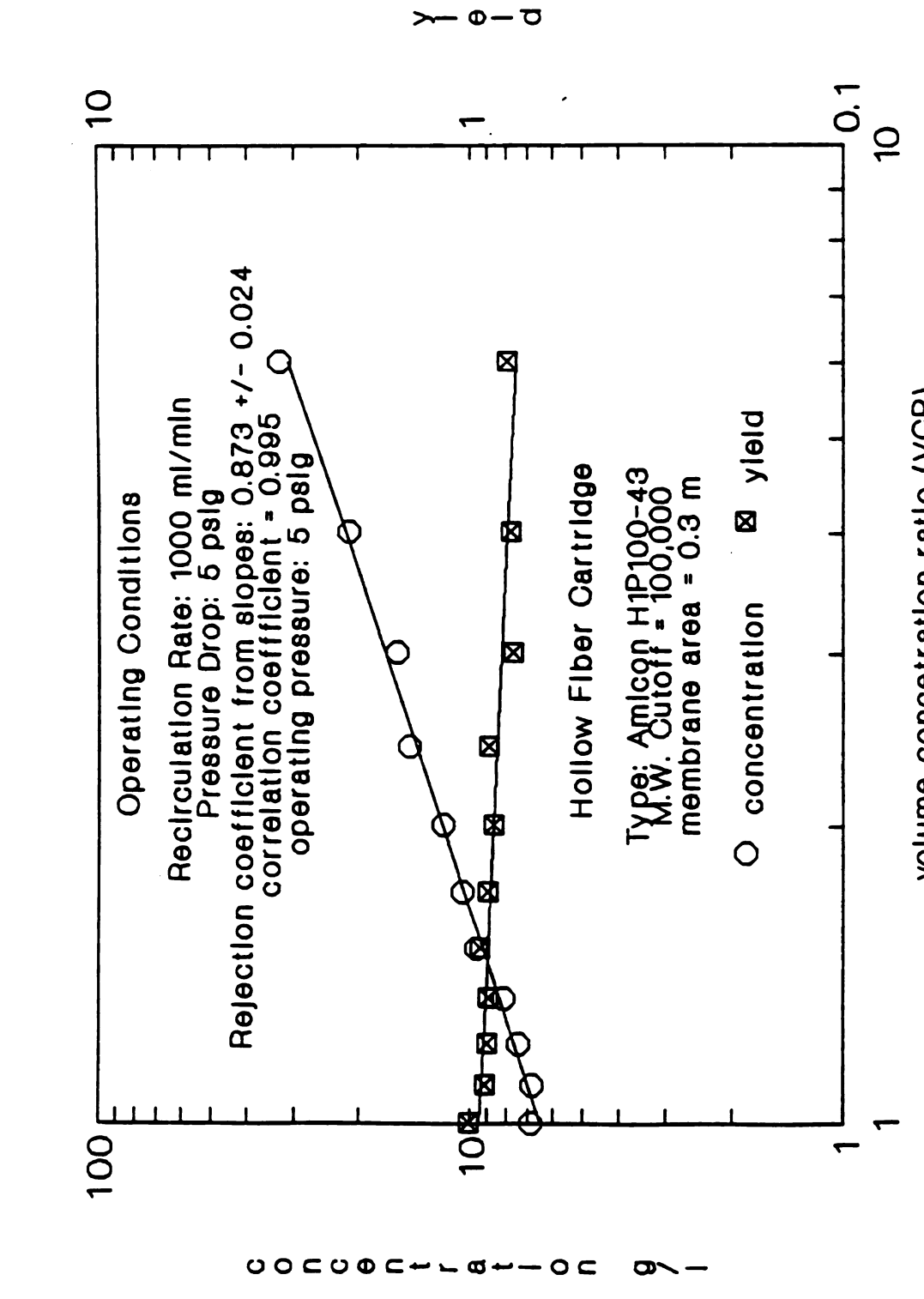

Figure 7. Concentration and Yield versus VCR for *Z. mobilis*

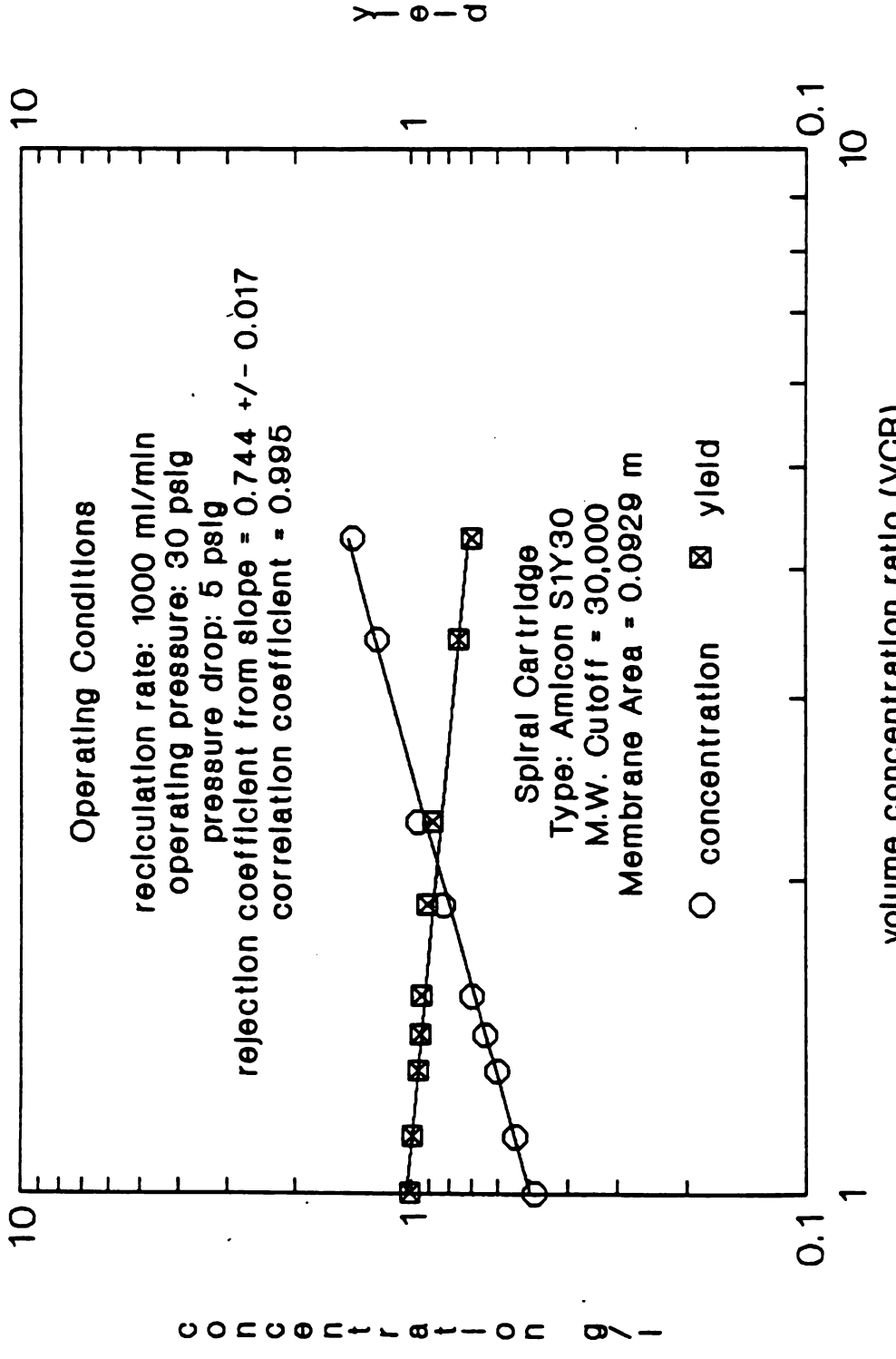

Figure 8. Concentration and Yield versus VCR for Blue Dextran

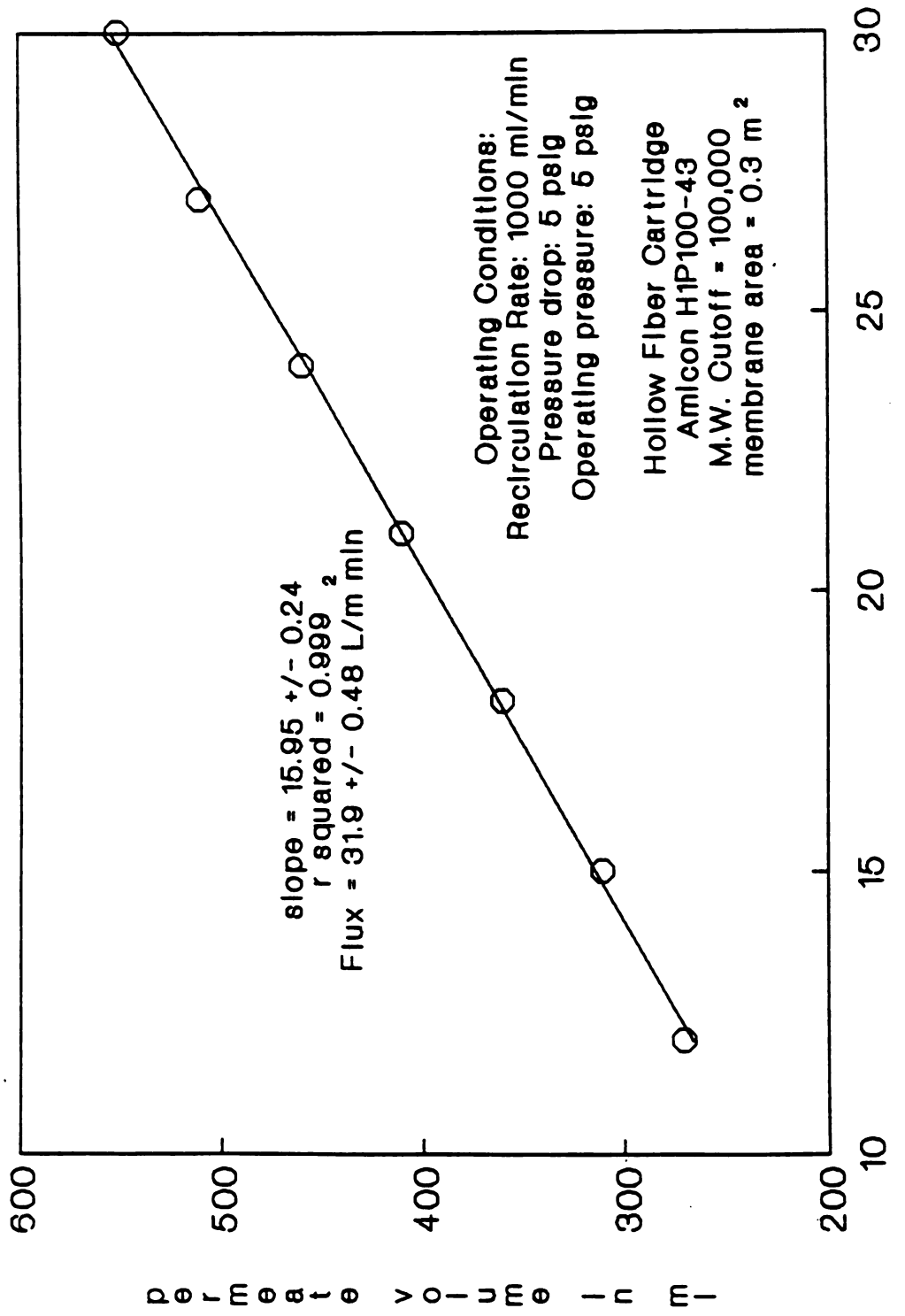

Figure 9. Hollow Fiber Flux Determination for *Z. mobilis*

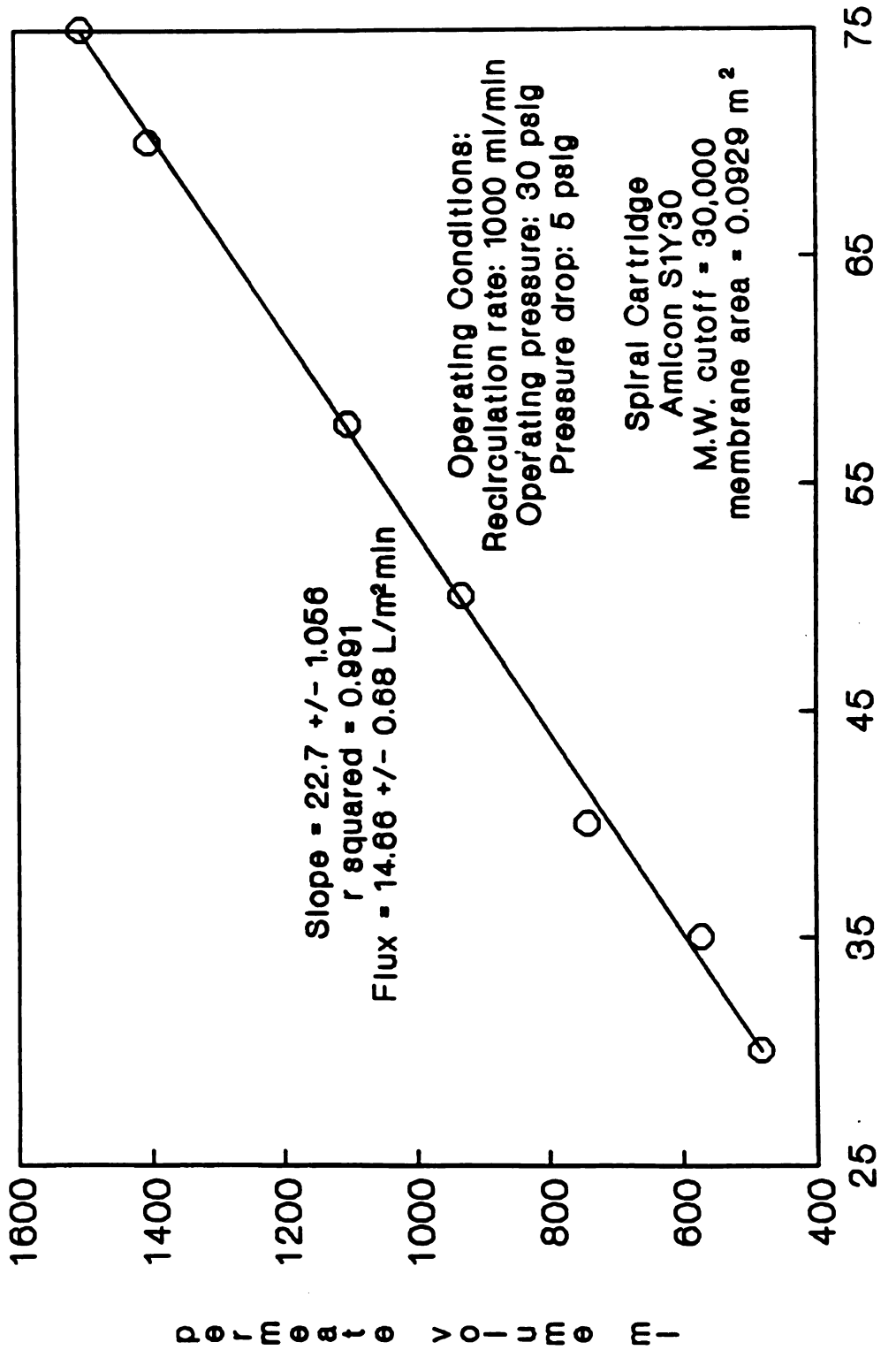

Figure 10. Spiral Cartridge Flux Determination for Blue Dextran

Conclusions and Recommendations

The experiment demonstrated the application of microfiltration for concentration of cell suspensions and ultrafiltration for concentration of blue dextran and other polymer solutions. The two systems studied were fit by the VCR model well. The high correlation coefficients determined for the concentration versus VCR data indicated that the rejection coefficients remained relatively constant for both the cells and the blue dextran, validating the use of the VCR model.

Further experiments using a series of recirculation rates and pressure drops should allow modeling of the dependence of the flux on the operating pressure and recirculation rate. The experiments are of relatively short duration, so several runs can be performed with different operating conditions.

Constants

δ = film (boundary layer) thickness
$\Delta\pi$ = osmotic pressure gradient
α,β,ω,A = constants
ΔP_T = transmembrane pressure drop
Δx = membrane thickness (or pore length)
ϵ = surface porosity of the membrane
μ = fluid viscosity
μ = fluid viscosity
ρ = fluid density
ϕ = empirical constant reflecting the degree of concentration polarization
B = membrane permeability coefficient
C = concentration of the solute in the retentate
C_b = bulk concentration of the retained (rejected) solute
C_g = gel concentration at the membrane surface
C_m = retentate concentration at the membrane surface
C_o = initial retentate solute concentration
C_p = solute concentration in the permeate
C_s = concentration of removed solute
C_{so} = initial concentration of removed solute

D = diffusion coefficient
dC/dx = concentration gradient over a differential element in the boundary layer
d_h = hydraulic diameter = 4(cross sectional area)/(wetted perimeter)
J = permeate flux, volume/time
J_s = rate of solute transport by convection, moles (or mass)/time
k = mass transfer coefficient
L = channel length
L_c = length required for a fully developed concentration profile
L_v = length required for a fully developed velocity profile
N = number of solute particles in the retentate
P = net driving force for an ideal membrane
r = channel (mean pore) radius
Re = Reynolds number = $\rho u d_h/\mu$
R_m = intrinsic membrane resistance using pure water as the feed = μ/B
R_m' = resistance of the membrane including fouling
R_p = resistance due to concentration polarization = $\phi \Delta P_T$
Sc = Schmidt number = $\mu/\rho D$
Sh = Sherwood number = kd_h/D
u = fluid velocity
V = volume of the retentate during ultrafiltration
V_d = total volume of water added
V_f = final volume of the permeate
V_o = initial volume of retentate solution
V_p = permeate volume

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Appendix
Equipment and Reagents Needed:
cells
hollow-fiber apparatus
$CoCl_2$
Blue Dextran (M.W. 100,000)
polymeric disposable membrane with 30,000 MW cutoff
spectrophotometer

I. Calibration of the Peristaltic Pump

1. Disconnect the pump from the filtration apparatus.

2. Turn the pump on to forward and adjust the flow rate until the display reads the desired flowrate.

3. Measure the flow rate using a graduated cylinder and a stopwatch for about 5 minutes to determine the flow rate.

4. If the measured flow rate does not match the flow rate on the display, adjust the calibration screw with a screwdriver until the display reads the measured flowrate.

II. Determination of Pure Water Flux

1. Loosen the knob at the top of the reservoir and pull off the lid. Flush the appropriate membrane with water and refill the reservoir.

2. Set the pump at the first flow rate and the appropriate pressure drop for each membrane type (see the instructions for each type below).

3. Place the permeate line in a large graduated vessel.

4. Record the time and permeate volume for the water.

III. Concentration of Blue Dextran from a Mixture

1. Develop a calibration curve by measuring the absorbance of blue dextran at 660 or 650 nm for several known concentrations ranging from 0 to 2 g/l.

2. Flush the membrane and determine the pure water flux for your first flow rate and pressure drop.

3. Loosen the knob at the top of the plexiglass reservoir for the to remove the lid.

Remove the lid and pour the blue dextran mixture into the tank.

4. Open the valve at the bottom of the tank by turning the handle so that is in line with the tank.

5. Place the end of the permeate line in a graduated vessel to collect the water that flows through the membrane.

6. Make sure that the backpressure valve at the top of the spiral filter cartridge is fully open (in line with the pipe).

7. Make sure that the pump is set for the appropriate tubing size (18), and turn on the pump by flipping the switch to 'forward'. Set the flowrate somewhere between 800 and 1,800 ml/min.

8. Slowly close the backpressure valve until the pressure on the top gauge rises to about 30 psi. Always make sure that the lower pressure gauge is no more than 4 psi higher in pressure that the upper gauge, or the filter may be damaged.

9. Record the time, pressures, absorbance, and volume of both the permeate and the retentate at regular intervals.

10. When the concentration is complete, drain the fluid from the cartridge. The tank can be drained by disconnecting the tubing which feeds the pump. Once the tank is empty, the pump can be used to move air through the cartridge to remove excess process fluid.

11. Refill the tank with reverse osmosis (RO) water and run the system with a low backpressure at a high pumping rate keeping the pressure below 8 psi.

12. Drain the water and refill with clean RO water and run the pump in reverse mode.

13. Determine the pure water flux, re-dilute the retentate, and then restart the experiment at a different flow rate or pressure drop.

14. After each flow rate flush the system with water and determine the pure water flux.

15. Flush the membrane thoroughly after the final run, and clamp off the filtrate tube and store wet. If the filter is to be stored for the weekend add a small amount of sodium azide solution or other bacteriostatic agent to the reservoir and recirculate it through the filter. Wear gloves when handling bacteriostatic agents since they are poisonous and label the filter and reservoir clearly after it is added to the

system. Do not pour sodium azide down the drain as an explosive reaction may take place.

IV. Concentration of Cells using Hollow Fibers.

1. Obtain the cell culture that was saved form the previous fermentation experiment or the culture that was prepared for this experiment. Filter sterilize 2-3 ml of fermentation broth to produce a cell free blank for absorbance readings. Swirl the suspension until it is uniform, remove 2-3 ml of cell suspension, place in a cuvette, and record the absorbance at 660 or 710 nm using the cell free broth as a blank.

2. Loosen the knob at the top of the plexiglass reservoir for the to remove the lid. Remove the lid and pour the cell suspension into the tank.

3. Open the valve at the bottom of the tank by turning the handle so that is in line with the tank.

4. Place the end of the permeate line in a large graduated collection vessel that has sufficient volume to collect the filtered broth that permeates the membrane.

5. Make sure that the backpressure valve at the top of the hollow-fiber cartridge is fully open (in line with the pipe).

6. Make sure that the pump is set for the appropriate tubing size (18), and turn on the pump by flipping the switch to 'forward'. Set the flow rate somewhere between 800 and 1,800 ml/min.

7. Slowly close the backpressure valve until the pressure on the gauge rises to about 8 psi.

8. Note the time and record the pressure, absorbance and volume of both the permeate and the retentate at regular intervals. Note that the pressure of the filtrate is atmospheric, so obtain a barometer reading from the lab downstairs (rm. 287).

9. When the concentration is complete, drain the fluid from the cartridge. The tank can be drained sliding the tubing that feeds the pump off the nozzle on the tank. Once the tank is empty, air can be pumped through the cartridge to remove excess process fluid by turning on the pump.

10. Refill the tank with reverse osmosis (RO) water, and run the system with a low backpressure at a high pumping rate but not in excess of 8 psi.

11. Re-dilute the retentate with the permeate and repeat the experiment at a different flowrate or pressure drop. Keep the pressure drop below 8 psig.

12. Repeat steps 10, 11, and 12 after each run.

14. Drain the water and refill with clean RO water and run the pump in reverse mode.

15. Clamp off the filtrate tube and store wet.

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