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ABSTRACT

A fermentation system was designed and constructed to study the growth characteristics of micro-organisms at low and high cell concentrations. The technique used to develop high cell densities utilized a rotating micro-filtration unit to permit the removal of cell-free product from the fermenter. The fermenter volume and the filter were contained in a single unit composed of a series of concentric cylinders. Annuli contained the fermenter volume while the second outermost cylinder supported a micro-filtration membrane. Feed to the system was pumped at constant rates, and the internal pressure built up to a value which would effect the required filtration rate. The system was operated batchwise and continuously with and without filtration.

The anaerobic growth characteristics of Steptococcus faecalus were determined at 37°C and pH 7.0 for batch, continuous and continuous with filtration modes of operation. The growth characteristics were unchanged when the cell density was increased. Changes in cell yield per mole of glucose consumed were clearly illustrated during the course of a single run by operating the fermenter in the unsteady state with filtration. No consumption of glucose for maintenance

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purposes was apparent for these organisms. The maximum cell density developed was 40% packed cell volume, a value 45 times larger than could be grown in simple batch culture.

I. INTRODUCTION

Investigators have, in recent years, switched much of their effort from studying the growth of micro-organisms in batch culture to investigating their growth in continuous culture. Batch culture by its very nature yields information on microbial processes which are in a state of flux and change. Only crude information as to the effect of various nutritional requirements on the physiology and metabolism of the cell can be determined since exhaustion of a necessary nutrient causes growth to cease. Continuous fermentation allows the investigator to study cells under a variety of growth conditions and nutrient limitations on a steady state basis, where the interference by transient behaviorial characteristics is absent. The dynamic response of cells when subjected to sudden or cyclical changes in environment can be studied, something which is impossible in batch culture.

One of the main disadvantages of studying continuous culture is the time involved in going from one steady state to the next, a time which may stretch into many hours or even days if the dilution rate in the fermenter is small. For this reason of efficiency in time utilization, the study of batch culture is very necessary for preliminary screening and testing of nutrient requirements and growth characteristics.

The study of continuous fermentation has been in progress for some time and the various interactions of microbe and medium have been investigated by many workers, but few have investigated the influence of cell density upon growth characteristics. The concentration of micro-organisms grown in batch and continuous culture is usually on the order of a few grams dry weight per liter of broth. If the bio-mass is considered to play the role of a catalyst in the

conversion of substrate to product, then major increases in productivity would result from techniques whereby cell concentration could be increased. Growth of micro-organisms is usually limited by one of two mechanisms: an essential nutrient is depleted in the medium or an inhibitory product of the metabolism is increased to a level which halts growth. To increase bacterial concentration beyond that possible in batch or continuous culture necessitates the continuous feeding of fresh medium and the removal of cell-free product from the fermentation system. There are a number of techniques to accomplish product removal. A possible method for large-scale operation would be the continuous centrifugation of broth coupled with the recycle of cells to the fermenter. The use of a centrifuge for small scale experimentation is not too practical because of the large volumes of broth associated with the external lines and the centrifuge.

Exchange of nutrients and products between a growing culture separated from an external reservoir by a dialysis membrane is another method of producing dense cultures. Gerhardt and Gallup[1] developed a dialysis flask to grow high density cultures batchwise with provision to exchange a cell-free product with a nutrient reservoir. Using this apparatus, Gerhardt and Gallup investigated a number of organisms and a variety of dialysis membranes. They found considerable increases in cell concentration when the growing culture was supplemented with nutrients derived from the reservoir. They investigated the effect of bacterial concentration on the yield of cells per unit of substrate by using different ratios of fermenter volume to reservoir volume. Using Serratia marcescens, they found that cell concentration increased directly with increasing reservoir to fermenter volume ratios up to 10:1. Beyond this point, little increase in cell concentration occurred.

These early high density batch culture investigations have been continued by Gallup and Gerhardt,[2] and Gerhardt et al.[3,4,5,6] Since dialysis was used to transfer material, nutrient concentrations were unknown since the mechanism of the transport was by diffusion and the driving force was concentration differences. To circumvent the problems of dialysis in the present study, a filtration device was proposed where a pressure driving force would cause the transfer of material. An experimental fermenter was designed and built in which a rotating cylindrical microfilter separated cells from the broth and allowed the removal of a cell-free effluent from the fermenter. Using this apparatus, an investigation of the anaerobic growth characteristics of Streptococcus faecalus at 37°C and 7.0 pH was undertaken.

S. faecalus converts glucose primarily to lactic acid via the Embden-Meyerhof pathway.[7,8] Some investigators[7] have shown that at high pH, diversion of glucose to other products (ethanol, acetic and formic acids) occurs, while others[9] have found no dependence on pH but instead a strong dependence on the specific growth rate with only formate and acetate being formed, and no ethanol. These latter experimenters employed a continuous fermenter for their studies and showed that in instances where growth was limited by nutrients other than glucose, dissociation of glucose catabolism and growth rate occurred. Bauchop and Elsden[10] used C^{14}-labelled glucose to show that almost none of the glucose was incorporated into cell material but served only as an energy source.

The nutritional requirements for S. faecalus are very strict. The presence of most amino acids together with a number of vitamins and minerals in the media are essential for growth.

II. EXPERIMENTAL APPARATUS AND PROCEDURES

Figure 1 shows a simplified flow diagram for the filtration-fermenter combination. The flow rates, v, and the concentrations of cells, N, and substrate (glucose), S, have been indicated. The fermenter was a 12-inch section of 3" ID pyrex pipe which contained a rotating filter designed to remove cell-free product. Positive displacement feed pumps and supply vessels for both sterile nutrient and NaOH addition, a recycle line to an external pH electrode holder and closed loop pH control and fermenter temperature control completed the system. This experimental set-up was used for all modes of operation: batch, continuous stirred tank and continuous stirred tank with filtration. A stirring shaft replaced the filter during batch and continuous, non-filtration experiments. When operating in the filtration mode, cell-containing effluent was removed in controlled amounts by means of a peristaltic pump in the bleed line and collected in a buret. Since the feed pumps were positive displacement, the difference in volume flow rate between the bleed and feed rates was forced through the filter membrane of the rotor and the pressure in the fermenter was allowed to fluctuate to supply the driving force needed to effect the filtration.

The filtration rotor was designed to fulfill several purposes: (1) to support the membrane which would separate the micro-organisms from the broth, (2) to remove the filtrate from the fermenter, (3) to serve as an agitator to completely mix the culture, and (4) to achieve as high a filter area to volume ratio as possible. The ultimate density of a bacterial culture which can be grown in this type of system is directly proportional to the ratio of filter area to culture volume since the limiting step is the filtration rate obtainable under conditions of moderate pressure drop through the membrane. For this reason, care was taken

to limit the volume of the fermenter in the end areas without making the assembly of the fermenter too difficult. Clearance at the top and bottom was restricted to 1/8" each. To accommodate the rotory seals, wells were machined into the heads so that as little culture volume as possible was added to the system. Another major fermenter volume was located in the annulus between the stationary outer cylinder and the rotating filter. There is a lower limit to the gap which can be tolerated because mixing of the broth becomes more difficult the smaller the gap becomes. In fact, special means had to be devised to assure that adequate mixing was achieved in the fermenter. The diameter of the filter rotor was chosen to be 2-1/2" which left a 1/4" annulus.

A Taylor Number of 5,600 was computed for this system using the fluid properties of water and rotational speed of 600 RPM. Turbulent conditions normally prevail beyond Taylor Numbers of 400. Experiments using dye tracers showed that little or no axial mixing occurred. To improve the mixing characteristics of the system, an internal pumping arrangement was devised.

A schematic diagram of the rotor is shown in Fig. 2. The rotor was composed essentially of a series of concentric cylinders with a central cylinder, a 1/2" diameter heavy-walled stainless steel tube, forming the power shaft connecting the motor to the rotor. On this shaft were attached the rotating portions of the Crane seals. The remaining two cylinders with their end plates formed the filtrate unit which was connected to the central shaft by two shear pins. The bottom shear pin was hollow and connected the interior of the filtrate unit to the hollow shaft and served as the exit line for the filtrate. The filtrate flowed down the shaft, through an external seal and then exited from the system.

The external surface of the filtration unit was a perforated plate support

for the filter membrane. The filter membrane was a rectangular sheet of type WH Millipore filter material which was wrapped around the support surface and glued along the seam with silicon rubber cement. After the cement had set, the ends were given a light coat of glue and sealed at the ends with "O" rings as shown in the diagram.

Attached to the bottom of the filtrate unit was a set of impeller blades which rotated with the filter. These impellers pumped the broth up the outside annulus and down through the inner annulus between the filtrate unit and the central shaft. This internal pumping action gave rise to excellent mixing. Dye tracer experiments showed that complete dispersion occurred within three to five seconds, while residence time distribution experiments demonstrated that the fermenter was uniformly mixed.

The area of the filter which was available for filtration was about 450 $cm.^2$ and the fermenter volume including recycle lines was 620 ml. When the system was used for batch and continuous stirred tank operation, the rotor was removed and the volume was 1360 ml.

Since lactic acid was produced by the organism studied, continuous pH control on a closed-loop basis was necessary. The control system consisted of a pH analyser, a recorder-controller and a piston pump driven by a DC motor for adding NaOH at controlled rates to the system. The RPM of the motor was measured by means of a tachometer-generator and a record of the motor speed as a function of time was used to determine the rate of base addition to the system.

The fiber junction of the pH reference electrode was easily plugged by the bacterial suspension and an insufficient flow of electrolyte resulted. To circumvent this problem, a remote reference electrode was constructed and connected via an aqueous KCl salt bridge to the fermentation system.

The pressure in the fermenter fluctuated depending upon the filtration rate. In order that electrolyte flowed into the system, the reference electrode was pressured with N_2 to a value above that in the fermenter. The pressure had no effect upon the measured pH.

Samples of the fermentation broth were removed through a serum cap in the recycle line using a sterile syringe and were immediately immersed in an ice bath to stop the cells from growing. The cell mass was determined indirectly by measuring the optical density (O.D.) of the culture. At a wave length of 720 mu where the medium background absorption was minimal, preliminary experiments showed that O.D. was linear with cell mass as long as the O.D. did not exceed 0.35. Since most of the samples had an O.D. much higher than 0.35, dilutions using a buffer-formalin solution were made. Samples from the steady state runs were analyzed for dry weight. One unit of O.D. corresponded to a dry weight of 0.347 mg/ml, with a standard deviation of 0.025 mg/ml.

Glucose was measured by two methods. Initially, the improved reducing sugar analysis developed by Kusmierek[11] was used. Difficulties were encountered in achieving reproducibility and considerable scatter occurred in the data. This method was used for all the batch and some of the continuous stirred tank runs. For the rest of the experiments, an enzymatic preparation for glucose analysis (Glucostat, Worthington Biochemical Corp.) was used.

Lactic acid was determined using a lactate dehydrogenase enzyme preparation (Worthington Biochemical Corp.) in conjunction with a hydrogen acceptor potassium ferrocyanide.

III. EXPERIMENTAL RESULTS

The results from the experimental program are presented in order of increasing operating complexity, beginning with batch culture, then continuous culture and finally the continuous operation with filtration. The mathematical framework describing each mode of operation is included at the most convenient position. The operating temperature and pH conditions were restricted to a single value each of 37°C and pH 7.0 respectively, and they remained constant throughout the experimental program.

A. Batch Fermentation

1. Experimental Results

Batch experiments were conducted to determine preliminary growth characteristics of S. faecalus and to develop a suitable nutrient medium for the continuous cultures. To simplify the interpretation of experimental results, the medium should be sufficient in all components except for one which is chosen to limit growth. Since the pathway of glucose metabolism was fairly well-known, it was desired to use glucose as the limiting substrate.

Preliminary experiments with a semi-defined medium published by Bauchop and Elsden[10] using hydrolysed casein to supply the amino acids required for growth, did not give reproducible growth curves or maximum specific growth rates. A complex nutrient recipe containing yeast extract was developed which did yield reproducible results. The nutrient medium had the following composition.

Difco yeast extract: 20 gm/l, anhydrous sodium acetate: 4 gm/l, ammonium sulfate: 2 gm/l, potassium monohydrogen phosphate $\cdot$ 3 H_2O: 6 gm/l, potassium dihydrogen phosphate: 1.5 gm/l, vitamin solution: 1.5 ml/l, mineral solution: 2 ml/l,

glucose: 7 gm/l. The vitamin solution had the following composition: nicotinamide: 400 mg/l, calcium patothanate: 400 mg/l, riboflavin: 400 mg/l, pyrodoxin · HCl: 800 mg/l, folic acid: 40 mg/l, and biotin: 40 mg/l. The mineral solution had the following composition: magnesium oxide: 10.75 gm/l, calcium carbonate: 2 gm/l, ferrous sulfate · 7 H_2O: 4.5 gm/l, zinc sulfate · 7 H_2O: 1.44 gm/l, manganese sulfate · H_2O: 0.80 gm/l, copper sulfate · 5 H_2O: 0.25 gm/l, cobalt sulfate · 7 H_2O: 0.28 gm/l, boric acid: 0.06 gm/l and concentrated hydrochloric acid: 51.3 ml/l.

The batch growth curves using this medium had a typical profile with a brief lag period followed by exponential growth. With 7 gm/l glucose present initially, the lag period abruptly entered the stationary phase with the exhaustion of the glucose. When more glucose was present, another component in the medium became limiting and a long transitionary phase occurred before growth terminated. The maximum doubling time for S. faecalus grown on this medium was approximately 24 minutes.

The conversion of glucose to lactic acid was almost stoichiometric for all of the batch experiments conducted. Plots of the glucose remaining in solution versus the lactic acid produced were linear with a slope very close to the stoichiometric value of -2.0. Plots of the lactic acid produced versus the NaOH consumed were also linear with a slope approaching 1.0 The relationship between glucose consumption and cell yield was more complicated and is illustrated in Fig. 3 where the glucose consumption (as measured by NaOH consumption) is plotted versus the bacterial cell concentration in optical density units. During the initial stages of growth, the amount of cell growth is directly proportional to the amount of energy released from the catabolism of glucose. However, this linearity ceases and is followed by a changing relationship where the amount of glucose

catabolised per unit of new cells produced increases greatly. Two possible models may account for this "dissociation" of energy production from cell growth. The first model assumes that the additional energy is used for cell maintenance while the second postulates that energy production and the biosynthetic pathways become uncoupled and the additional energy is wasted by some mechanism, possibly by means of an ATPase.

The presence of a maintenance energy requirement in bacteria has been shown by Marr *et al.*[12] and Dawes and Robbins[13] for *Escherichia coli*, Herbert[14] for *S. marsescens* and by Pirt[15] for several organisms. A discussion of the energy of maintenance concept for micro-organisms has been published by Dawes and Robbins.[16] Luedking and Piret[17] have shown that for *L. delbruckii*, a homofermentive lactic acid bacteria, the rate of lactate production (or glucose consumption) was proportional to the rate of growth plus a maintenance term. However, Rosenberger and Elsden[9] have shown that for *S. faecalus*, dissipation of energy may occur in the presence of an excess of glucose. The shape of the curve for lactate production versus cell concentration is similar for *S. faecalus* and for *L. delbruckii*, although the amount of lactate produced after the dissociation occurs is much larger for *S. faecalus*.

2. Mathematics of Batch Growth

Bacteria reproduce by binary fission, a process similar to autocatalytic chemical reaction. The differential equation describing this mechanism is:

$$\left(\frac{dN}{dt}\right)_G = \mu N \qquad (1)$$

where: N = cell concentration

t = time

μ = specific growth rate.

During the growth of a culture, glucose is catabolized to produce energy with the formation of lactate.

$$\frac{dP}{dt} = - Y_{P/S} \frac{dS}{dt} \tag{2}$$

where: P = product concentration-lactate

S = substrate concentration-glucose.

In this equation, $Y_{P/S}$ is the yield constant for this process. This yield constant approaches the stoichiometric value of 2.0 in the batch runs.

Luedking's model expressing the relation between substrate consumption (or lactate production) and its utilization for growth and maintenance is expressed in Eq. (3).

$$- \frac{dS}{dt} = \alpha\left(\frac{dN}{dt}\right)_G + \beta N \tag{3}$$

where α is a constant expressing the amount of glucose reuqired to produce new cells and β is the maintenance term. Substituting Eq. (1) into (3) and rearranging yields:

$$- \frac{1}{N} \frac{dS}{dt} = \alpha \mu + \beta \tag{4}$$

The left-hand side of Eq. (4) is the specific rate of glucose consumption and when plotted versus μ, the specific growth rate, should yield a linear curve with slope equal to α and the intercept equal to β.

As previously mentioned, stoichiometric conversion of glucose to lactate was approached and stoichiometric neutralization of lactic acid by NaOH was closely approximated. Since the rate of base consumption was more

precisely known than either glucose or lactate, Eq. (4) was expressed in terms of NaOH.

$$\frac{1}{N}\frac{d(NaOH)}{dt} = Y_{P/S}\,(\alpha\,\mu + \beta). \qquad (5)$$

The graphical expression of Eq. (5) is illustrated in the lower portion of Fig. (4). A linear relation between specific base consumption and specific growth rate is apparent, leading one to believe that Eq. (3) is an accurate representation of the fate of the energy produced during glucose catabolism. However, the values of α and β for a number of experimental runs were not constant with variations of 100% occurring. Also the maintenance term was quite large for these experiments when compared with the results of Luedking and Piret's data (17) for a similar organism, L. delbruckii. In fact for some of the runs, the specific consumption of glucose was almost independent of specific growth rate.

If the dissociation of energy production from growth resulted not from the maintenance term but rather from dissipation of energy, then the rate of glucose consumption would be related to the specific growth rate by the following equation:

$$-\frac{dS}{dt} = \frac{\alpha\,\mu N}{Y_{C/S}} \qquad (6)$$

where $Y_{C/S}$ is the fraction of the glucose consumed which is used to promote growth. The constant, α, has the dimensions mM glucose/ℓ/OD as in Eq. (3). During the initial stages of batch growth, the rate of substrate consumption, as measured by base consumption, was directly proportional to the rate of growth. If the value of $Y_{C/S}$ is taken to be 1.0 during this period, then α is equal to the initial slope of the curve in Fig. (3) divided by the stoichiometric constant 2.0.

The average value of α for five batch runs was 6.94 mM glucose/ℓ/OD with

a standard deviation of 5.9%. Using Eq. (6) and the value of α determined by the above procedure, $Y_{C/S}$ values were calculated and an example of the results is shown in the upper portion of Fig. 4. For Eq. (6) to be valid, $Y_{C/S}$ should approach zero as μ goes to zero. For the experiment shown, $Y_{C/S}$ does appear to approach the origin.

The values of $Y_{C/S}$ should be equal to 1.0 up to the point where dissociation occurs. Fair agreement resulted during the lag phases of growth and, for some runs, even well into the declining growth period. In all of the runs, the point of dissociation deduced from Eq. (6) was at higher growth rates than was indicated from the NaOH consumption versus OD plot, as shown in Fig. 3. This difference may result from errors introduced by the graphical differentiation necessary for the use of Eq. (6).

A comparison of the two models relating growth and glucose consumption shows that either model could be used. Further information taken from continuous fermentation experiments where glucose-limited growth occurred showed that little or no glucose was utilized for maintenance purposes and that the energy dissipation model appears to be more nearly correct.

B. Continuous Stirred Tank Fermentation

Following the development of a satisfactory growth medium, continuous fermentation experiments were undertaken to determine if the growth characteristics observed in batch culture remained unchanged during continuous growth. The pH of the sterile medium was slightly below 7.0 and adding NaOH at a constant rate resulted in a linear change of pH with time. The rate of change of pH per milliliter of NaOH was calculated and used to correct the base addition rates during continuous fermentation in order that the amount of acid produced during the fermentation could be determined from the titration.

1. Experimental Results

Figure (5) presents the optical density developed in the fermenter and the broth glucose concentration as a function of dilution rate. The data have been converted to a unit nutrient feed basis by correcting for the volume of base added. The curves have the typical form for continuous fermentation with OD being nearly constant at low dilution rates and then decreasing as the rate approaches cell washout. As the cell concentration began to decrease sharply, glucose appeared in the effluent broth. All of the glucose concentration data at high dilution rates fell on the same curve. Only one glucose concentration at the maximum dilution rate is presented although there was a slight variation in feed concentration from run to run, e.g., 36.5 mM/l for Run CG and 38.9 mM/l for Run CJ.

The runs were conducted at the same conditions so that the OD curves for the different runs should have fallen on top of each other. At the lower dilution rates where glucose concentration was zero, the data does not coincide while at the higher rates, good agreement was found.

Run CG was completed using one tank of medium and the results are quite consistent. During Run CJ, the nutrient supply became exhausted after sample CJ11 and had to be replenished. Whether some error in medium preparation caused the change in OD versus dilution rate curve is not known. The growth was glucose limited but the small differences in glucose concentration in the feed could not explain the change in the maximum cell concentration which occurred when the nutrient was replenished.

The yield of lactic acid produced during the continuous fermentation differs from that produced during batch culture. Figure (6) presents the lactate concentration per liter of nutrient medium as a function of dilution rate. At high

dilution rates, the decrease in lactate concentration is caused by washout of the culture and the presence of unconverted amounts of glucose. However, at dilution rates below 1.2 hr^{-1}, glucose concentration was essentially zero so that the decreasing lactate concentration was the result of partial diversion of the glucose to products other than lactate. Further evidence that such a diversion was occurring was shown by the rate of NaOH consumption per liter of nutrient medium which increased as the dilution rate decreased. At lower flow rates, the rate of NaOH consumption appeared to be a mirror image of lactate production. The conversion of the pyruvate to formate and acetate has been shown by Rosenberger and Elsden,[9] suggesting that the production of these acids required increased quantities of NaOH. The metabolic pathway can be summarized by the following equations:

$$1 \text{ mole glucose} \rightarrow 2 \text{ moles pyruvate}$$

$$x \text{ moles pyruvate} \rightarrow x \text{ moles lactate}$$

$$(2 - x) \text{ mole pyruvate} \rightarrow (2 - x) \text{ formate} + (2 - x) \text{ moles acetate.}$$

Since the base neutralized all of the acid produced, the amount of formate plus acetate was equal to the difference between the total base added and the lactate produced. Therefore, the fraction, F, of the initial glucose fed which can be accounted for is given by:

$$F = \frac{0.5P + 0.5\left[\frac{NaOH-P}{2}\right] + S}{S_0} \tag{7}$$

where P and S refer to the concentration of lactate and glucose in the broth and S_0 is the initial glucose concentration. The approximate validity of this material balance is shown in Fig. 7 over a range of specific growth rates. Three sets of data are presented; two are the steady state, low density data for

Runs CG and CJ while the third set is for the high density, steady state Run HDA. The other experimental results for HDA are presented and discussed in a following section.

The value of F was a slight function of specific growth rate. The shape of the curves for Run CG and CJ are similar but the data for CG was consistently about 7% higher. At high growth rates, all the glucose fed to the system could be accounted for and almost all of it was converted to lactate. The lactate yield constants as defined by Eq. (2) were computed for these runs and they are shown on Fig. 11. Discussion of these data is presented in a later section dealing specifically with lactate yield constants.

The fraction of glucose which can be accounted for by the proposed material balance, decreases to 0.9 as the specific growth rate approaches zero. The data for Runs CJ and HDA agree well except for one sample which was about 12% low. The continuous culture data of Rosenberger and Elsden[9] for S. faecalus agree well with the results obtained here. At low growth rates, 90% of the glucose consumed was converted to lactate, formate and acetate, while at higher growth rates, nearly 100% of the glucose was converted to these products.

2. Mathematics of Continuous Fermentation

For a continuous fermenter operated with no cells entering with the feed, Eq. (8) represents the material balance for the cells contained in the system.

$$\frac{dN}{dt} = \left(\frac{dN}{dt}\right)_G - \frac{v}{V} N \qquad (8)$$

$$= 0 \text{ at a steady state}$$

where:

v = rate of overflow

V = fermenter volume.

The substrate material balance is expressed in Eq. (9).

$$\frac{dS}{dt} = \frac{v_f}{V} S_0 - \left(\frac{dS}{dt}\right)_{consumed} - \frac{v}{V} S \qquad (9)$$

$$= 0 \text{ at steady state}$$

where:

v_f = feed rate

S_0 = initial substrate concentration.

During the discussion of the batch growth results, two different models for the rate of glucose were examined; the Luedking maintenance energy model and the energy dissipation model. In the continuous fermentation experiments, the initial glucose concentration was kept low in order that growth would be glucose-limited. If the Luedking model is assumed, then upon substituting Eq. (1) and (3) into Eq. (9) and re-arranging:

$$\alpha \frac{v}{V} + \beta = \left(\frac{v_f}{V} S_0 - \frac{v}{V} S\right) \frac{1}{N} . \qquad (10)$$

Equation (10) is plotted (for Runs CG and CJ) in Fig. 8 which shows that the intercept is equal to zero. Therefore, no apparent maintenance energy is required. Furthermore, the values of α calculated from the slopes of the linear curves agree quite well with those obtained from the batch experiments for the energy dissipation model. It can be concluded, then, that Eq. (6) is the correct representation of the glucose consumption mechanism with $Y_{C/S}$ = 1.0 for glucose limited growth.

The data for these continuous runs do not fall on a single curve. The differences in growth which were evident in Fig. 5 for the OD versus dilution rate appear in Fig. 8 as changes in the yield of cells per mole of glucose consumed. S. faecalus has very fastidious nutritional requirements and the medium used in

these experiments was undefined in composition because of the yeast extract. The same batch of yeast extract was used for all of the experiments but it is possible that alterations in cell metabolism may have occurred giving rise to slight changes in cell yield.

C. Continuous Fermentation With Filtration

Preliminary experiments with the filtration rotor in place demonstrated that successful operation without an excessive pressure drop across the membrane could be maintained for a period of days. Considerable difficulties were encountered during the preliminary runs in obtaining a cell-free filtrate. A number of sterilization methods were tried but most either did not achieve complete sterility or would cause the membrane to become brittle. A successful technique was developed using a preliminary steam sterilization without the membrane attached followed by a formaldehyde sterilization after the membrane was installed. Filtrate viable counts taken during Run TI gave counts of 2×10^3/ml in the early part of the run and 10^5 at the end of the run. These counts were so low that they could not be observed visually. The filtrate was perfectly clear, just as the nutrient feed.

Originally, experiments were planned for the study of dense cultures under steady state conditions. The time required to reach steady state was quite long and flow rates, particularly the bleed rate of cells, could not be maintained accurately without constant attention. The control of bleed flow rates was quite critical because slight changes had a large effect upon the final cell concentration. By measuring the flow continuously in a graduated buret, the amount of effluent could be accurately controlled as a function of time but constant attention was required. The poor control of bleed rate was caused by the periodic evolution of small quantities of gas from the fermentation and, since the bleed was

pumped out of the system in controlled volumes, the pumping rate had to be temporarily increased to compensate. Gas evolution was small , a few ml/hr, and was present during only a small part of the run. Reduction of liquid volume in the fermenter by collection of gas was not significant because of the location of the exit line at the extreme top of the fermenter and the small amounts of gas involved. Evolution of large quantities of gas would have caused a severe problem

1. Mathematics and Experimental Results

Equation (8) for the cell material balance in continuous fermentation can be expanded to account for cells lost due to leakage through the filter:

$$\frac{dN}{dt} = \mu N - \frac{v_B}{V} N - \frac{v_F}{V} N_F \qquad (11)$$

$$= 0 \text{ at steady state}$$

where:

v_B = bleed flow rate

v_F = filtrate flow rate

N_F = cell concentration in filtrate.

Equation (9) for the glucose material balance can also be expanded in a similar fashion to account for the filtrate.

$$\frac{dS}{dt} = \frac{v_f}{V} S_0 - \left(\frac{dS}{dt}\right)_{consumed} - \frac{v_B}{V} S - \frac{v_F}{V} S \qquad (12)$$

$$= 0 \text{ at steady state.}$$

Substituting Eqs. (6) and (11) into (12) results in:

$$N = \frac{v_f S_0 - (v_B + v_F)S}{\alpha\, v_B} - \frac{v_F}{v_B} N_F \,. \qquad (13)$$

The concentration of glucose was essentially zero for all samples of broth analyzed. Therefore:

$$N = \frac{v_f\ S_0}{\alpha\ v_B} - \frac{v_F}{v_B} N_F \quad . \tag{14}$$

Equation (14) can be re-arranged into a form more suitable for graphical presentation:

$$N + \frac{v_F}{v_B} N_F = \frac{v_f\ S_0}{v_B}\ \frac{1}{\alpha} \quad . \tag{15}$$

All of the variables contained in Eq. (15) were measured with the exception of α. One high density, steady state experiment, Run HDA, was conducted with cell concentrations ranging from two to eight times those obtained in batch culture using the same initial glucose concentration of 7 gm/l. When the data were plotted using Eq. (15), a linear curve resulted and the value of α calculated from the slope of the curve was 7.05 mM glucose/l/OD, a value very close to those determined from batch and continuous culture without filtration.

D. Transient Growth with Filtration

During the steady state runs, the concentration of glucose was negligible for growth rates below 1.2 hr^{-1}. This observation (together with the experimentally determined form of the cell yield constant) allowed the use of unsteady state experiments to investigate high density growth characteristics without assuming any specific kinetic model. The advantage of studying the transient regime lies in the greatly increased amounts of information obtained over a shorter period of time.

1. Mathematics and Experimental Results

The mathematical framework for transient behavior was developed using the cell and substrate material balance equations derived for the steady state case. Since S is equal to zero, then dS/dt = 0, and Eq. (12) can be solved for the specific growth rate:

$$\mu = \frac{v_f}{V} S_0/\alpha N \quad . \tag{16}$$

Substituting Eq. (16) into Eq. (11) results in a differential equation with N as the only dependent variable, assuming that the flow rates, feed concentrations and α, remain constant.

$$\frac{dN}{dt} = \frac{v_f S_0}{\alpha V} - \frac{v_B}{V} N - \frac{v_F}{V} N_F \quad . \tag{17}$$

This equation can be solved with the initial condition that $N = N_0$ when $t = 0$.

$$N = A - (A - N_0) \exp\left(- \frac{v_B}{V} t\right) \tag{18}$$

where:

$$A \equiv \frac{v_f S_0}{\alpha v_B} - \frac{v_F N_F}{v_B} \quad .$$

A plot of N versus $\exp(- v_B t/V)$ should be linear with slope equal to $A - N_0$ and intercept equal to A as time becomes very large. The value of A defines the maximum cell concentration which can be developed.

The experimental procedure for all the transient runs was very similar. The fermenter was operated at steady state with the feed and bleed rates adjusted so that an O.D. around 10 was established. At the start of the transient period, the bleed rate was adjusted to a pre-determined value and the flow rate of feed increased to approximately 1 liter/hr. This flow rate of nutrient was near the maximum which could be filtered for extended periods of time without excessive pressure buildup in the system. The flow rates of the filtrate and bleed streams were measured.

An example of the experimental results obtained is shown in Fig. 9 for Run TI. For this run, the bleed rate of cells was 28 ml/hr while the nutrient feed rate was 915 ml/hr. Curves are presented for the optical density, and for the lactate concentration. Several general observations were common to all of the transient experiments. It was quite clear that the linear relationship expressed by Eq. 18 was valid. However, it was found that during most of the runs, a single straight line did not represent the data and a series of linear curves was required. Since the slope of the curves was uniquely defined by the value of A in Eq. (18), then one of the variables incorporated in A was changing. Furthermore, it appeared as if an abrupt change occurred. All of the variables except α were measured and remained constant. Therefore, it must be concluded that the value of α was abruptly changing during the course of the run, indicating an alteration in the metabolic pathway scheme. Further proof that such pathway alterations (rather than measurement errors) were actually happening was given by the changes in lactate concentration at the point of change.

At the beginning of a run, the feed was increased stepwise from a low value to a much higher one. The cells had to increase their growth rate to utilize the additional substrate and their reaction was not instantaneous; a transition period was required. Adjustment to the increased feed rate was quite rapid and by the time that the first sample was taken after 30 minutes or an hour, the linear relation predicted by Eq. (18) was followed.

The yield of lactate from glucose catabolism was a function of the specific growth rate. The growth rate prior to the initiation of the transient run was much lower than the rate after the run began. Therefore during the first part of the experiment, the lactate concentration increased because of increased yield

of lactate per mole of glucose and began to decrease as the specific growth rate began to decrease. This is the reason for the maximum which appears in the lactate concentration during the initial period of the run.

The experimental results shown in Fig. 9 exhibit a complex spectrum. No fewer than five changes in α occur red during the experiment! The values of α given in the figure, begin initially at 6.77, increase to 10.2, decrease to 5.34 and then increase to 10.4 before decreasing again. The last value of α was not computed because too few data points were available although the value appeared to be between 7 and 7.5. The changes in α were paralleled by changes in lactate concentrations and seemed to be closely linked. While several changes in α were evident, the average value over the course of the run was near those obtained in the steady state experiments.

The run described above was terminated after 38 hours. The pressure in the system had increased gradually during the course of the run until 11 psi was reached. While the run could have been continued further, little added information would have been gathered since the cellular concentration was changing quite slowly with time. The bleed stream was then stopped and the nutrient feed rate reduced to 540 ml/hr in order to reduce the system pressure. Growth of cells continued. Very small samples of the culture were removed for optical density measurements. The filtrate was perfectly clear so that $N_F = 0$. The solution of Eq. (17) becomes straight-forward when v_B is zero.

$$N = N_0 + \frac{v_f S_0}{\alpha v} t \tag{19}$$

During the initial stages of the experiment, Run TJ, the linear dependence of cell concentration with time predicted by Eq. (19) held. However, as time and the cell concentration increased, the rate of growth decreased and a maximum

in cell concentration was approached. In other words, there appeared to be a maximum cell concentration which could be developed with the medium employed. A sample of the final broth, when centrifuged, did not give a clear supernatent as in samples taken from the other high density runs and microscopic examination revealed the presence of many "ghosts", the remnants of dead cells. Apparently, the fall-off in the rate of cell concentration increase was caused by cell death, although this has not been proven. The specific growth rate during the latter part of the experimental run was very low, approximately 0.007 hr^{-1}, and may have contributed to cell death.

The maximum cell concentration attained had an optical density of 270, a value 45 times that obtainable in batch culture using the same nutrient composition. A sample of the broth, centrifuged at 16,000 g's for an hour, had a packed cell volume of 40%.

The viscosity of the final broth exhibited pseudoplastic or "shear thinning" characteristics. As the shear rate increased from 5320 $seconds^{-1}$ to 12,600 $seconds^{-1}$, the viscosity decreased from 6.34 centipoise to 4.90 centipoise. From viable and total counts made during the course of the run, approximately 80% to 90% of the cells present were viable.

2. Lactate Yield Constant

The amount of glucose which was converted to lactate during the transient experiments could not be determined directly. The lactate concentration was time-dependent and was influenced by the previous concentration history. However, during any time interval, the average lactate yield constant could be evaluated.

The product material balance is given by:

$$\frac{dP}{dt} = \left(\frac{dP}{dt}\right)_{Produced} - \frac{v_F}{V} P - \frac{v_B}{V} P. \tag{20}$$

Substituting Eq. (2) and (12) into Eq. (20) with the assumption that S and dS/dt=0 yields, after re-arrangement:

$$\frac{dP}{dt} - P\left(\frac{v_F}{V} + \frac{v_B}{V}\right) = Y_{P/S}\,\frac{v_f}{V}\,S_0 \,. \tag{21}$$

For each time interval, the flow rates were known. If $Y_{P/S}$ is assumed to be constant for the time interval, $\Delta t_n = t_n - t_{n-1}$, then Eq. (21) can be integrated:

$$P_n = Y_{P/S}\,E_n - (Y_{P/S}\,E_n - P_{n-1})\,\exp(D'_n \Delta t_n) \tag{22}$$

where:

$$E \equiv \frac{v_f\,S_0}{v_F + v_B}$$

$$D' \equiv \frac{v_F + v_B}{V}$$

Since the values of P are known from experimental measurement, the average value of the yield constant can be computed.

To illustrate the results obtained the values of $Y_{P/S}$ for Run TI are presented in Fig. 10 as a function of time. Since the yield constants were average values, they were plotted at the median of the time interval when the time between samples was small. In Eq. (22), the exponential term for Δ t's larger than two hours was small so that the initial lactate concentration had little influence on the terminal value. For samples where the time interval was greater than two hours, the yield constants were plotted at the end of the time period.

The yield constant curve closely follows the lactate concentration profile. The yield constant for the system just prior to the start of the transient run was 0.54 and is plotted at t = 0. The yield constant data for the initial portion of the run when extrapolated back to the t = 0 axis, gives a

value for the initial yield very close to the stoichiometric one of 2.0. This observation was consistent for those runs where the initial cell concentration was small. A similar shape was obtained for those runs with a higher initial cell concentration but the t = 0 intercept was less than 2.0 due to a smaller initial specific growth rate. Apparently, just following the step change in feed rate, and therefore growth rate, an abrupt increase in yield occurs.

The points where the value of α changed have been indicated by arrows. Changes in α appear to correspond to alterations in the metabolism of the organisms, for after each change in α, the yield constant is quite drastically altered.

E. Lactate Yield Constant as a Function of Specific Growth Rate

For all of the data for continuous fermentation, the decrease in lactate yield appeared to correspond to a decreasing specific growth rate as long as α was constant. The yield data for all the continuous runs were plotted as a function of specific growth rate to see if a unique relation existed.

Fig. 11 contains the data for Runs CG, CJ and HDA. The values of $Y_{P/S}$ were calculated from the steady state concentrations. The same general form is displayed for these runs; the yield constant is close to the stoichiometric value at high growth rates and becomes smaller as the growth rate decreases. At low growth rates, the yield constant decreases very rapidly. The data for Run CJ is quite consistent and forms a smooth curve, linear at high growth rates with a slow change of yield with μ and then decreasing abruptly. More scatter is present for Run CG. Although both have the same form, the two curves do not coincide, particularly at the high growth rates. At low growth, they seem to approach a common asymptote. The high density run, HDA, had a much lower yield of lactate than the other two runs when compared at similar growth rates. The range of growth rates investigated was too small to see a distinct trend.

Also included in Fig. 11 are a few data points taken from the continuous fermentation studies of Rosenberger and Elsden[9] on S. faecalus. The range of dilution rates covered in their work was small but the same trend of decreasing lactate yield constant with decreasing dilution rate is evident.

The specific growth rates for the transient experiments were calculated from Eq. (16) and, as an example, the specific growth rate versus lactate yield data for Run TI is presented in Fig. 12. Two characteristics were immediately apparent: first, the general shape of the curves were similar to those shown in Fig. 11 for the steady state experiments, and during the period of any run where α was unchanged, the data fell on a smooth curve which became linear at low growth rates. Second, most of the data, when extrapolated to the ordinate, appeared to intersect at a common value of the specific growth rate.

The results for Run TI are plotted in Fig. 12, and the data for the different α regimes have been fitted with individual curves. For the last part of the experiment where $\alpha = 10.4$, the data lie below the intercept value for μ. It is possible that the extrapolations which have been made showing that $Y_{P/S} = 0$ when $\mu = 0.07\ hr^{-1}$, are not correct. Whether $Y_{P/S}$ goes to zero is not known but evidence to show that it does not is given by the lactate yield determined from the final broth of Run TJ. The specific growth rate was about $0.007\ hr^{-1}$ and the value of $Y_{P/S}$ was 0.25. It would appear that even at such low growth rates some lactate is produced.

IV. DISCUSSION

A. The Experimental Apparatus

The fermenter system for this study was developed primarily for the continuous growth of micro-organisms to concentrations many times larger than those which could be obtained by the usual techniques of stirred tank or batch fermentations. The fermenter accomplished this objective successfully with a maximum concentration factor of 45 over batch culture. The investigation of dense cultures at specific growth rates larger than those obtained was prevented by the upper limit on the filtration rate which could be achieved and maintained for long periods of time without development of excessive pressures. The equipment was designed to withstand 30 to 40 psi but the maximum pressure used did not exceed 20 to 25 psi and was usually much less.

A thorough study of the filtration as a function of time, rotational speed and cell concentration was not made but data taken during the experimental program revealed the general characteristics. Using a similar type of apparatus, Bhagat and Wilke[18] showed that, for small polystyrene spheres, the steady state rate of filtration was almost independent of pressure drop and was proportional to the square of the rotational speed. The dimensions of the apparatus, the particles and the mode of operation used by Bhagat were different from those encountered here. Bhagat employed a constant pressure drop through the membrane and filtration rate was the dependent variable. A cake of particles was very rapidly built up on the membrane until an equilibirum condition resulted where the rate of attrition of the particles from the cake due to shear and back diffusion equaled their rate of transport to the membrane by flow of filtrate. An increase in pressure resulted in a momentary increase in filtration rate until additional cake

was built up and equilibrium was again established.

In the present work, flow rate was fixed and pressure drop was the dependent variable. Cake was formed very slowly and the pressure increased gradually and a steady state pressure appeared to be reached so long as the cell density did not change. This was observed during the steady state, high density run where operation continued for many hours at constant cell concentrations with little pressure increase with time. The maximum filtration rate employed was approximately 3 $ml/hr/cm^2$ at a cell concentration of 43 OD and a pressure of 7 psi. At a filtration rate of about 1.8 $ml/hr/cm^2$ and an OD of 42, the pressure was approximately 4 psi. From these two examples, it would appear that the steady state filtration rate is a function of pressure drop for the present system rather than independent of it as observed in Bhagat's system.

A further deviation from the results of Bhagat was found in the filtration rate increase with rotational speed. He found that the filtration rate varied with the square of the rotational speed. However, in the bacterial filtration, increases in flow rate due to an increase in rotor speed were fairly small over the range employed of 600 to 1100 RPM. A possibly important difference between the present system and that used by Bhagat is that the organism layer formed during the filtration was slimy and not particulate like the layers formed by the plastic particles.

The bacterial filtration rate was somewhat time dependent as small particles penetrated the pores and gradually blinded the membrane. This effect was not very noticeable over time periods of several days.

The effect of cell concentration had a larger effect on the pressure required to effect a fixed filtration rate and was particularly noticeable when the packed cell volume exceeded 15 to 20%.

Uses of Filtration Fermenter Combinations

This type of apparatus has been shown by the experimental program described here to be uniquely suited to the investigation of microbial growth characteristics. The advantage of the high density device over continuous stirred tank fermentation is mainly the opportunity of operating a fermenter system with essentially zero concentration of limiting substrate while the cell concentration is changing in a predictable manner. Changes in cell yield are readily apparent. Furthermore, the amount of information obtained per unit time is much larger than in continuous culture where 4 to 6 residence times are required to change steady states.

Applications for the filtration-fermenter in the fermentation industry are probably very limited. Almost all of the major industrial fermentations are conducted batchwise with only limited use of continuous culture. Use of high density culture with its much more complex operating requirements, would necessitate the presence of special conditions. For example, the removal of a cell-free effluent could be advantageous in those instances where the cell material interferes with product recovery or, in those fermentations with low growth rates, the increased productivity per unit fermenter volume may offset the added cost involved.

The productivity of a continuous fermenter normally increases with dilution rate until the cell and product concentration begin to

decrease because of washout. For a continuous stirred tank fermenter (CST), the rate of cell formation per unit fermenter volume (Productivity, CST) can be expressed by the following relationship.

$$\text{Productivity, CST} = \frac{v}{V} N_{CST} = \mu N_{CST} \quad . \tag{23}$$

Similarly, for the high density system (HD),

$$\begin{aligned} \text{Productivity, HD} &= \frac{v_B}{V} N_{HD} \\ &= \mu N_{HD} \end{aligned} \tag{24}$$

assuming that no cells are lost in the filtrate. At the same specific growth rate, the ratio of the productivity for the high density with filtration to the productivity of the continuous stirred tank with no filtration is:

$$\text{Productivity Ratio (PR)} = \frac{N_{HD}}{N_{CST}} \quad . \tag{25}$$

The cell concentration for the CST is given by combining Eqs. (6) and (9) and is squal to the following expression assuming no substrate is present in the effluent.

$$N_{CST} = \frac{v_f}{V} \frac{S_0}{\alpha\mu} \quad . \tag{26}$$

The cell concentration for the high density case is given by Eq. (14).

Substituting these expressions for the cell concentrations into Eq. (25) and making use of the flow rate material balances, the productivity ratio reduces to the following form assuming there is no loss of cells in the filtrate.

$$PR = 1 + \frac{v_F}{v_B} = 1 + \frac{v_F}{V} \frac{1}{\mu} \quad . \tag{27}$$

The productivity ratio depends strongly on the filtrate rate per unit fermenter volume and on the specific growth rate at which the system is operated. For the present high density system, if operated at the flow rates such as in Run T1, the productivity ratio at steady state would be approximately 34.

For a continuous stirred tank fermenter, the maximum productivity occurs near washout where the increase due to increasing flow rates is counteracted by decreasing cell concentrations. For S. faecalus the optimum specific growth rate for maximum productivity in a continuous stirred tank was found to be near 1.25 hr^{-1}. Therefore, the productivity ratio was computed to be 2.3 for S. faecalus grown in the experimental apparatus at the optimum specific growth rate and at a filtration rate of one liter per hour.

S. faecalus grows much faster than most organisms and the improvement in productivity is not large. Very much larger increases are possible with slow-growing organisms for which this type of apparatus may be particularly useful.

An important factor is the power requirement for this type of device. In the present system power requirements estimated from existing correlations were on the order of 500 horsepower per thousand gallons, an excessive value for industrial practice. However, it seems likely that alternate designs might be possible for large scale systems which would reduce the volumetric power input. This remains an important variable for future investigation.

B. Experimental Results

1. Cell Yield

The rate of consumption of glucose to provide the energy for the increase in cellular mass was found to be directly proportional to the rate of cell growth as long as glucose was present in limiting amounts. Evidence to support this statement is contained in the steady state data for both normal and high density continuous experiments. There was no apparent effect of cell concentration on the specific rate of glucose consumption. During the batch experiments where an excess was present, glucose catabolism became uncoupled from cell growth and the energy derived therefrom was dissipated. The Luedking *et al.*[17] maintenance model fitted the batch data but gave widely differing maintenance energy terms from one experiment to the next.

The amount of glucose consumed which went to support new growth was subject to abrupt changes. These changes were not evident from the batch and steady state experiments for fluctuations in α from run to run were quite small. The only exception to this occurred during the last few data points for Run CJ. Without the transient growth experiments,

the conclusion must be that α is a constant for the average value was 7.05 mM glucose/l/hr with a standard deviation of 4.4% for five batch experiments, two continuous stirred tank runs and one high density, steady state run. The α 2 value for Run CJ was not included in the average and could be accepted as resulting from differences in medium composition following the preparation of more nutrient after sample CJ 11, for the organisms have complex requirements and the medium was undefined because of the presence of yeast extract. However, it became clear during the transient experiments that α could and did quite abruptly change. These changes were not caused by fluctuations in operating variables or nutrient composition for flow rates and the other system variables were monitered continuously and the nutrient was withdrawn from a single, well-agitated vessel. Furthermore, the lactate yield constant also indicated that a shift in metabolic pathway had been made.

2. Product Yield

The pathway alteration signified by the α changes seem to be tied to the product formation but the α - $Y_{P/S}$ variation is super-imposed on the μ - $Y_{P/S}$ relationship and complicates interpretation. The material balances for all of the batch experiments showed near stoichiometric conversion of glucose to lactate. In these runs, glucose was present in excess. When glucose was limiting, the conversion of glucose to lactic acid was a strong function of specific growth rate, particularly at low rates. The lactate yield constant curves appeared to extrapolate to a single specific growth rate value of 0.07 hr^{-1} and at high rates, the yield asymptotically approached the stoichiometric value of 2.0.

Glucose is converted via the glycolytic pathway in a series of enzymatically catalyzed steps to pyruvate with two moles of ATP and 2 moles of NADH produced per mole of glucose. During the conversion of pyruvate to lactate, the NADH is oxidized and recycled. If pyruvate is converted to formate plus acetate, one mole of ATP is formed, but no NADH is oxidized. Therefore, to ensure that the glycolytic sequence does not stop due to a lack of NAD^+, some other means of oxidizing NADH must occur. There are two possibilities. The first one involves the conversion of pyruvate to ethanol which would use a mole of NADH. Rosenberger and Elsden[9] found only formate and acetate when they grew S. faecalus in continuous culture. Almost identical results were found when the data of Rosenberger and Elsden were compared to those found here for the fraction of glucose which could be accounted for in the samples taken from the present steady state runs. Rosenberger and Elsden analyzed for formate and acetate directly while in the present study the titration for total acid production was used together with the measured lactate concentration to compute the other acids produced.

The second possibility assumes that the NADH is oxidized by a component in the nutrient medium. With 20 gm/l yeast extract in the medium, it is quite possible that sufficient material is available to act as a hydrogen acceptor. During the anaerobic fermentation of S. faecalus using glycerol as substrate, Gunsalus[19] has demonstrated that yeast extract does contain a hydrogen acceptor. Although no direct evidence for a similar regeneration of NAD^+ was obtained here, such a reaction sequence is quite possible.

Additional energy should be produced during the formation of acetate for formate from pyruvate. However, from the experimental data, no consumption of this ATP was evident, for the mass of new cells produced per glucose consumed remained unchanged during periods where the lactate yield constant was drastically altered. Although changes in cell-yield per mole of glucose did occur and they appeared to be somehow connected with alterations in lactate yield, no direct correlation between them was evident.

Since the changes in α do not appear to be caused by changes in the distribution of metabolic end products of glucose catabolism, a complex interaction of the cell with its environment may be responsible. The cell density did not appear to have an effect upon the metabolism of the cell, particularly in the steady state experiments. No consistent correlation could be developed to show that during the transient experiments, the high density of the culture had any influence upon the growth characteristics of S. faecalus.

V. SUMMARY

An experimental filtration-fermenter combination was designed and constructed for the investigation of microbial growth characteristics in batch, continuous and high cell density cultures. The micro-filtration technique for removal of cell-free product from the fermenter was successful. Filtration rates in excess of a liter per hour (2.2 ml/cm^2 hr) were maintained for several days with cell concentrations up to 20% packed cell volume. The maximum cell density obtained was 40% packed cell volume, a factor of 45 larger than could be obtained in the usual batch culture. Excellent filtration efficiency was achieved; the concentration of cell in the filtrate was six orders of magnitude smaller than in the fermenter.

The filtration-fermenter combination has a larger productivity per unit volume than a continuous stirred tank fermenter when both are operated at the same specific growth rate. The advantage of the filtration-fermenter over the continuous stirred tank increases with decreasing specific growth rate and with increasing filtration rate. Very large increases in productivity are possible at low specific growth rates. The application of high density fermentation to industrial processes will depend largely on the cost of filtration membranes which are expensive at present. The presence of a cell-free effluent is advantageous when product recovery systems require the separation of cells from the fermentation broth. The filtration equipment is more complex than a stirred tank but the added cost is offset by the smaller size required for the same throughout.

The anaerobic growth characteristics of S. faecalus were investigated at 37°C and pH 7.0. In batch culture, glucose was converted almost stoichiometrically to lactic acid. When an excess of glucose was present, dissociation of the rate of glucose consumption and growth rate occurred as the culture entered

stationary phase. In continuous culture with glucose limitation, the fraction of glucose going to lactate decreased with dilution rate. The consumption of glucose for cell maintenance was negligible. Results in terms of substrate conversion, yields, viability, etc., from steady state high density experiments were similar to those obtained from low density continuous culture.

The transient high density experiments showed that the yield of cells per mole of glucose changed quite abruptly as a run progressed. Complex interactions between the cell and its environment were probably responsible. No consistent correlation between these yield changes and cell density was evident. The changes in cell yield were usually accompanied by alteration in the lactate yield constant but no correlation could be developed.

The transient technique of operating the filtration-fermenter and the plotting methods employed for the data obtained, can be used for the rapid determination of changes in metabolic pathways which affect cell and product yield constants.

More detailed description of the experiments and tabulations of data are available in the original report.[20]

VI. NOMENCLATURE

Variables

N	bacterial density, optical density units
P	product concentration, mM/l.
PR	productivity ratio
S	substrate concentration, mM/l.
t	time, hr
V	fermenter volume, ml/hr
v	flow rate, mm/hr
Y	yield constant

Subscripts

B	bleed
f	feed
F	filtrate
G	growth
O	initial quantity
C/S	cells from substrate
P/S	product from substrate

Greek Letters

α	substrate consumed/yield of new cells, mM/l/OD
β	maintenance term, mM/l/OD.
μ	specific/growth rate, hr^{-1}

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FIGURE CAPTIONS

Fig. No.

1 A simplified flow schematic of the fermenter system.

2 A schematic diagram of the fermenter and the filtration rotor.

3 Glucose consumption (as measured by NaOH consumption) versus the bacterial cell concentration during a batch run.

4 Testing of the batch data by two different models. Upper graph: the energy dissipation model. The fraction of glucose consumed which supplies the energy for the production of new cells is plotted as a function of specific growth rate. Lower graph: the Luedeking et al. maintenance energy model. The specific glucose consumption is plotted as a function of specific growth rate. The value of the constants in Eq. (3) are:

α = 5.3 mM glucose/l/OD β = 3.0 mM glucose/l/OD·hr.

5 The cell concentration and the glucose concentration as a function of dilution rate for the continuous stirred tank experimental Runs CG and CJ. The data are presented on the basis of one liter of nutrient medium with corrections made for the volume of NaOH added.

6 The lactate concentration as a function of dilution rate for the continuous stirred tank experimental Runs CG and CJ. The data are presented on the basis of one liter of nutrient medium with corrections made for the volume of NaOH added.

7 The fraction of the glucose fed to the fermenter which can be accounted for in the effluent (using Eq. (7)) for stirred tank experimental Runs CG and CJ.

8 The dilution rate (equal to the specific growth rate at steady state) versus the specific glucose consumption for the continuous stirred tank experimental Runs CG and CJ. The data show that the maintenance energy term, β, in the Luedeking et al.[17] maintenance energy model is essentially zero.

9 The growth curve and the lactate concentration for the transient high density experimental Run TI.

10 The lactate yield constant calculated from Eq. 22 for the high density experimental Run TI.

11 The specific growth rate versus the lactate yield constant for the continuous stirred tank experimental Runs CG and CJ, the steady state high density Run HDA and the continuous stirred tank data of Rosenberger and Elsden[9] for S. faecalus.

12 The specific growth rate versus the lactate yield constant for the transient high density Run TI and for sample TJ 8, the final sample of Run TJ.

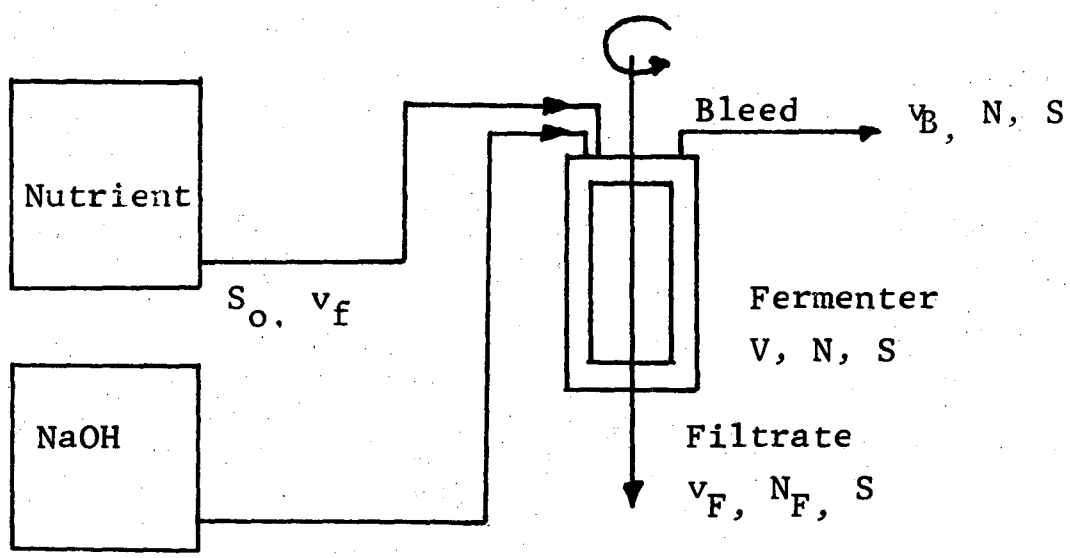

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Fig. 1

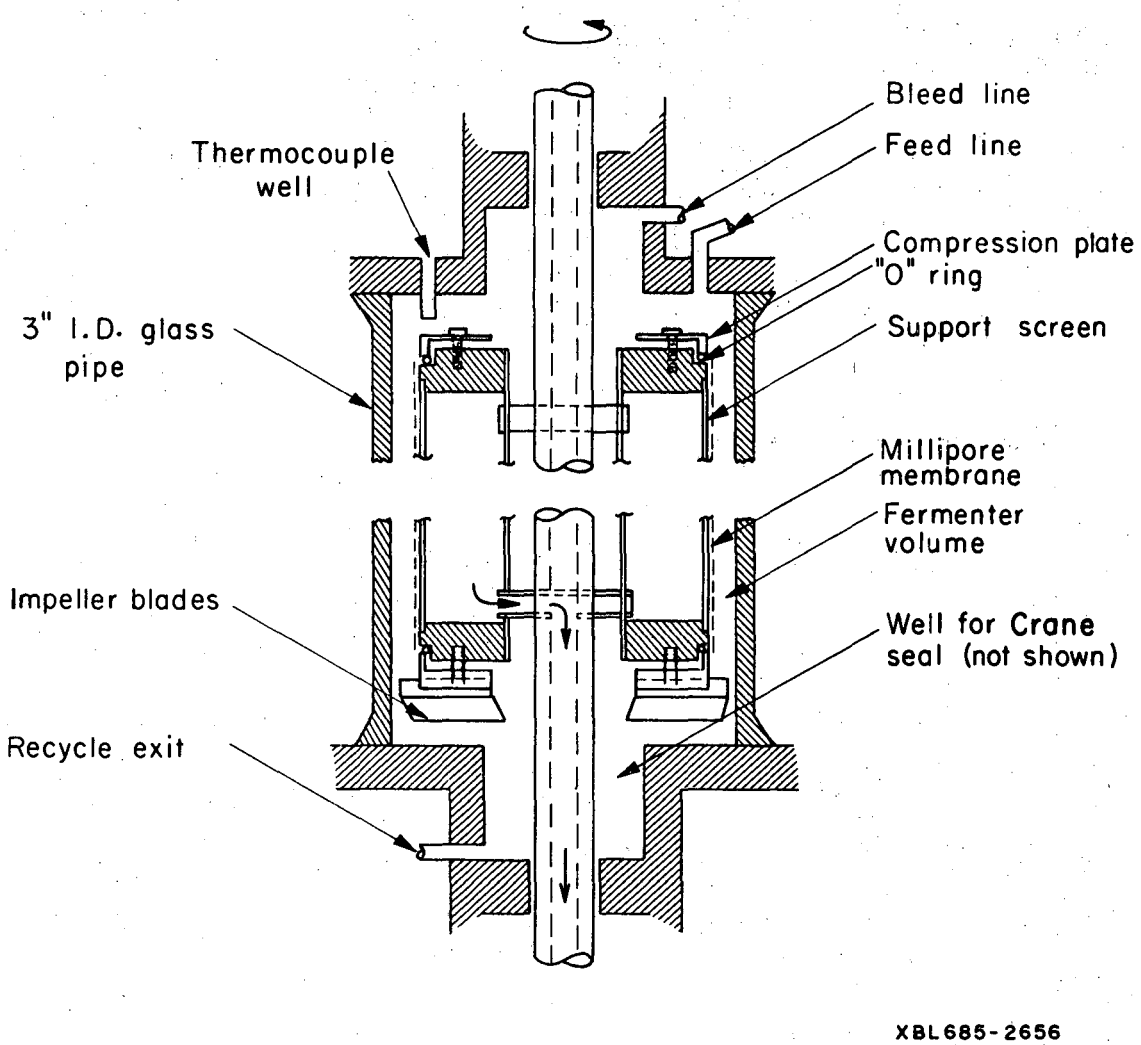
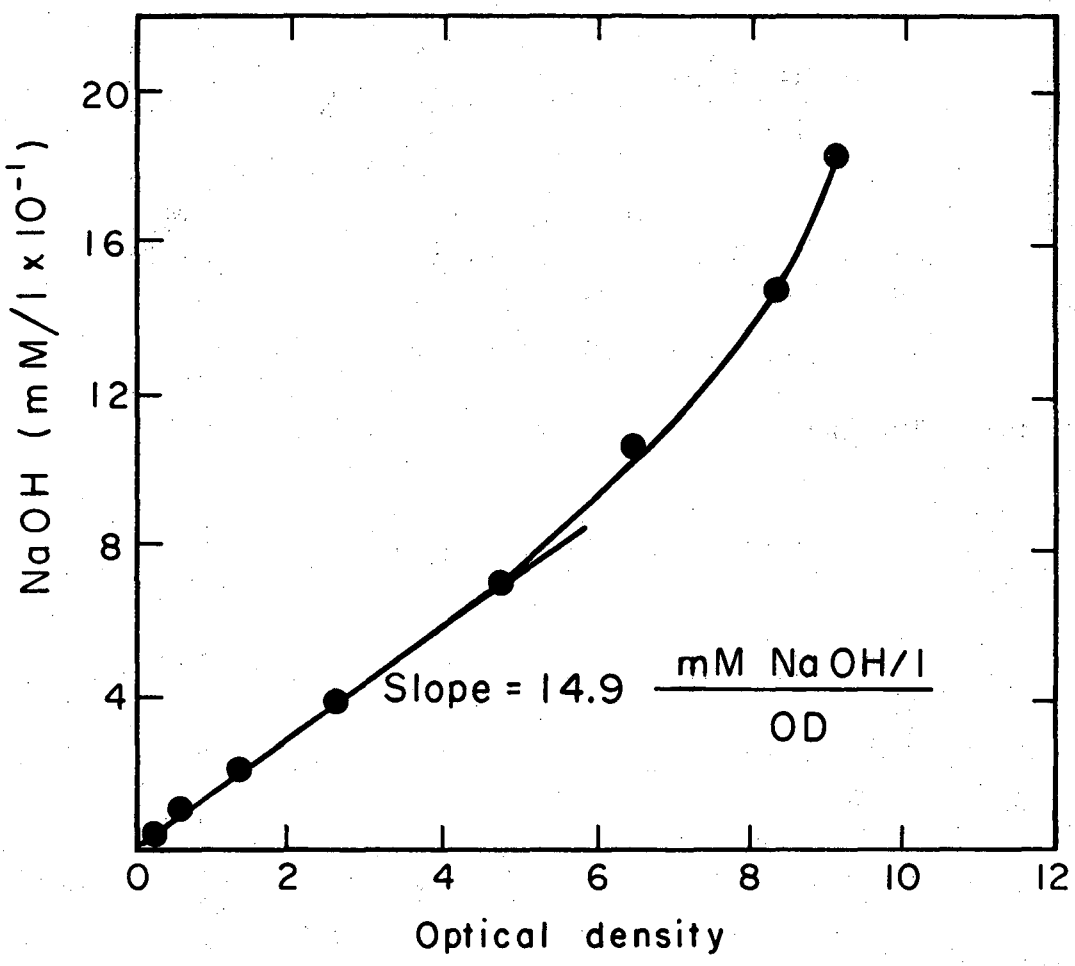

Bleed line
Feed line
Thermocouple well
Compression plate
"O" ring
3" I.D. glass pipe
Support screen
Millipore membrane
Fermenter volume
Impeller blades
Well for Crane seal (not shown)
Recycle exit
XBL685-2656

Fig. 2



XBL685-2827

Fig. 3

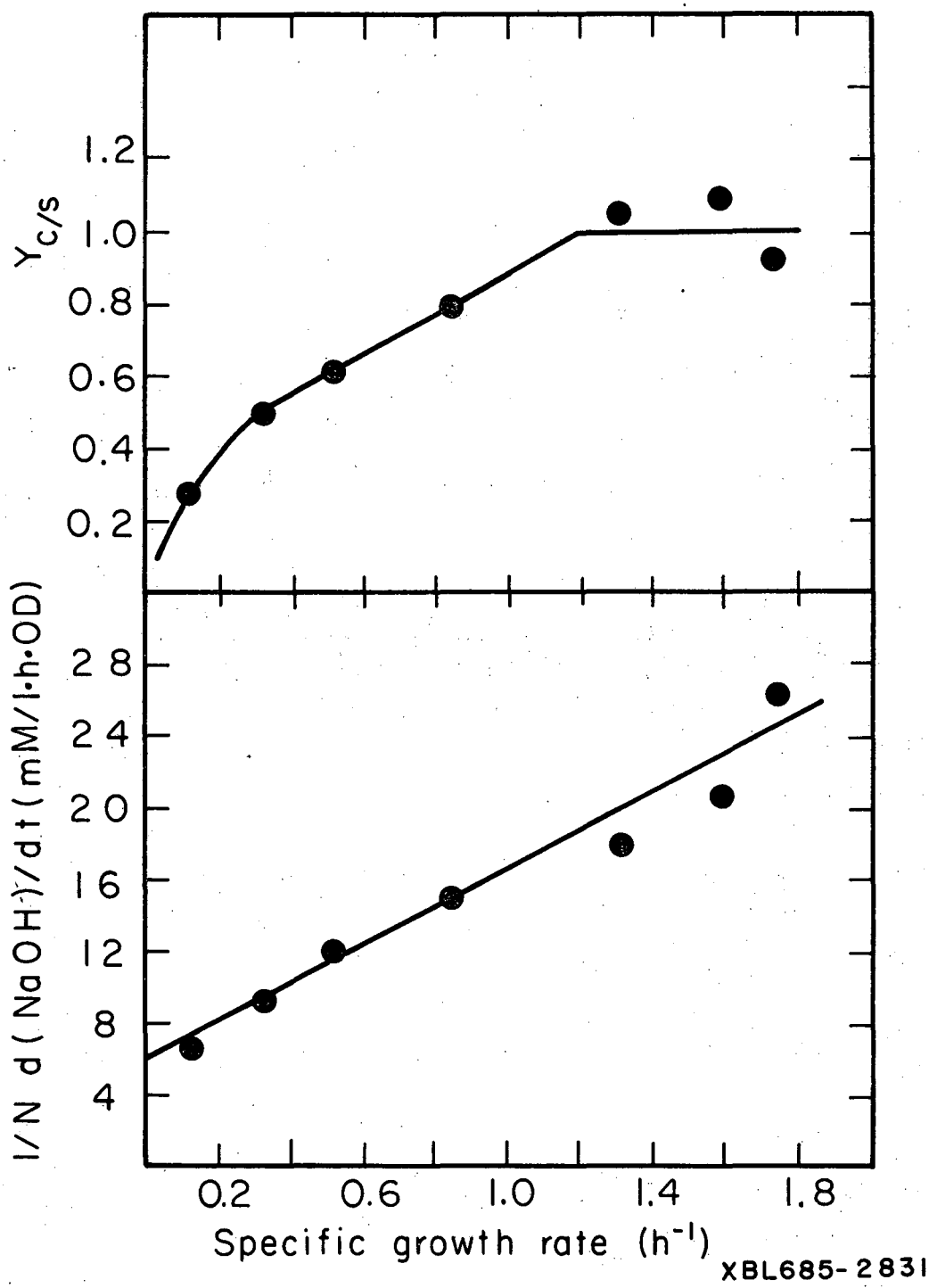
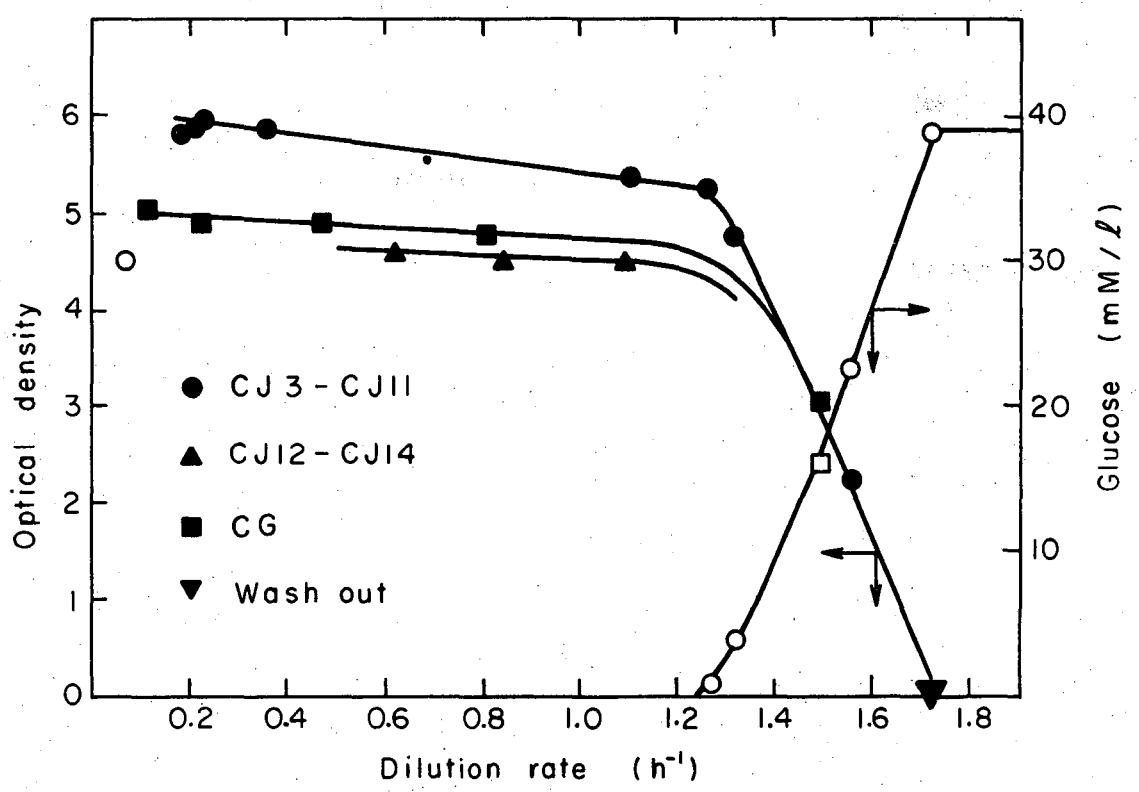

1.2
1.0
0.8
0.6
0.4
0.2
$Y_{c/s}$
28
24
20
16
12
8
4
1/N d(NaOH)/dt (mM/l·h·OD)
0.2
0.6
1.0
1.4
1.8
Specific growth rate (h^{-1})
XBL685-2831

Fig. 4



XBL685-2649

Fig. 5

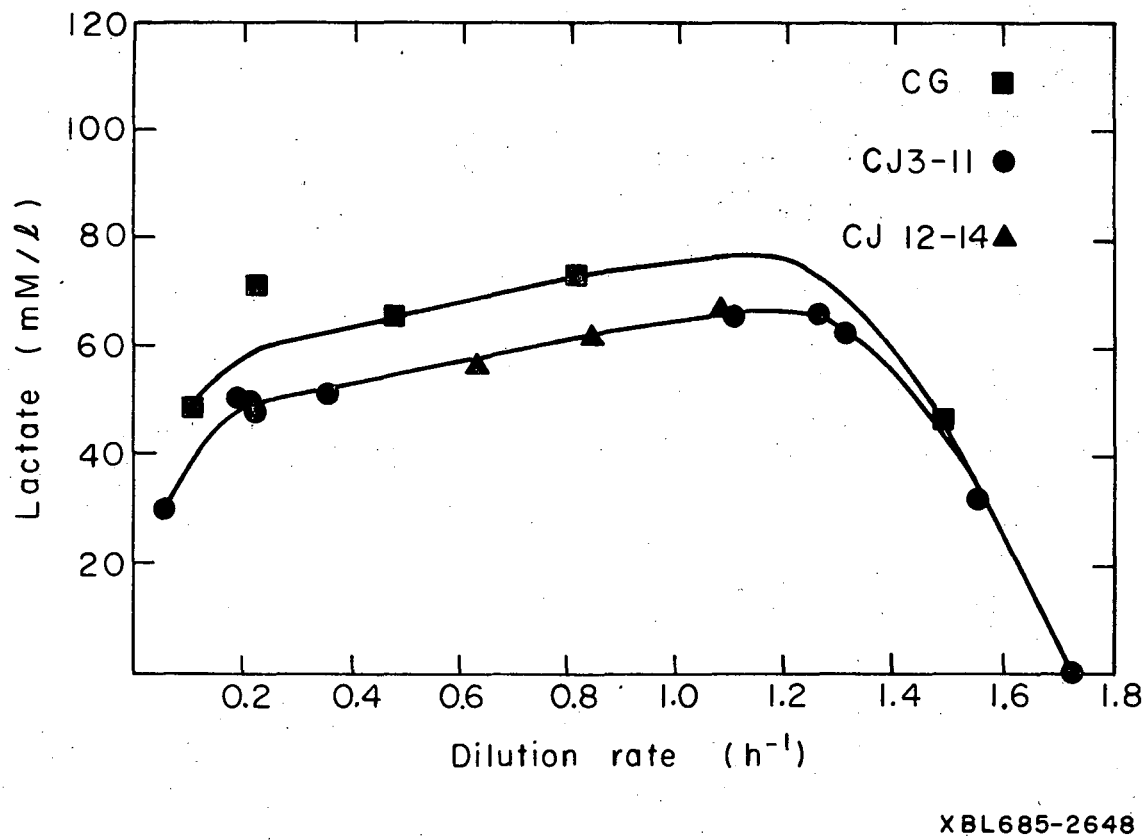

120
100
80
60
40
20
Lactate (mM/ℓ)
CG
CJ3-11
CJ 12-14
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Dilution rate (h⁻¹)
XBL685-2648

Fig. 6

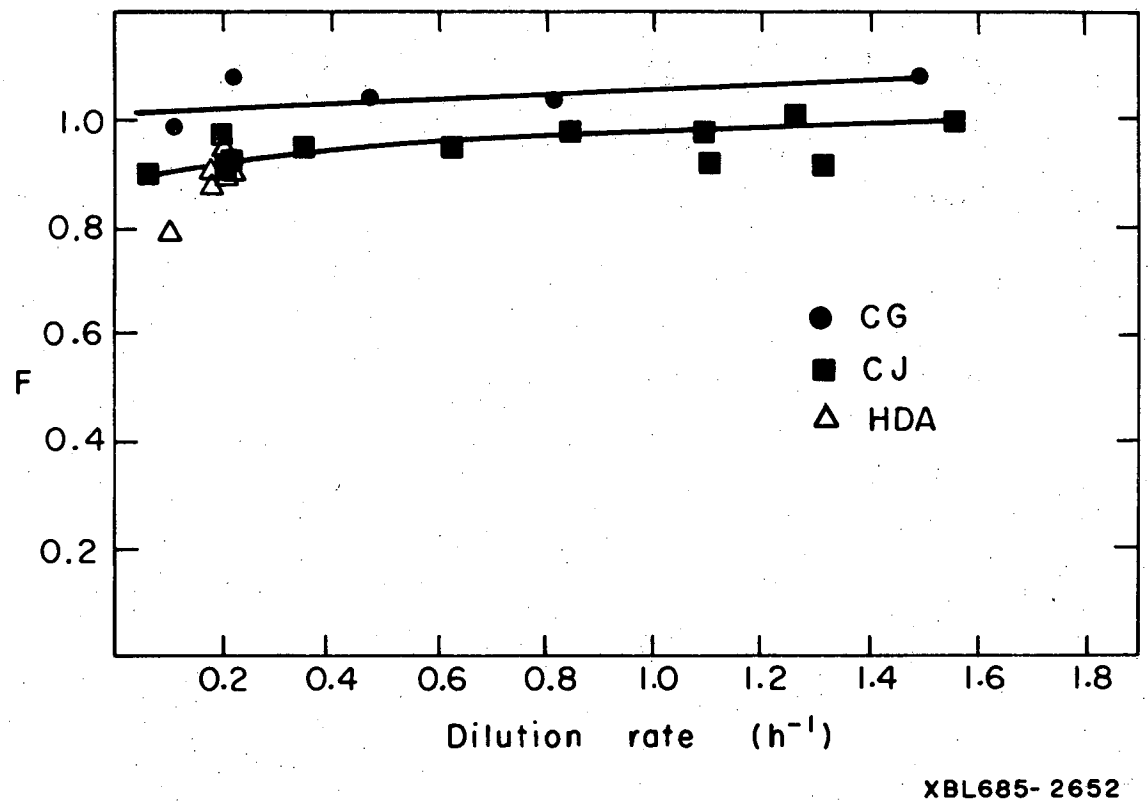
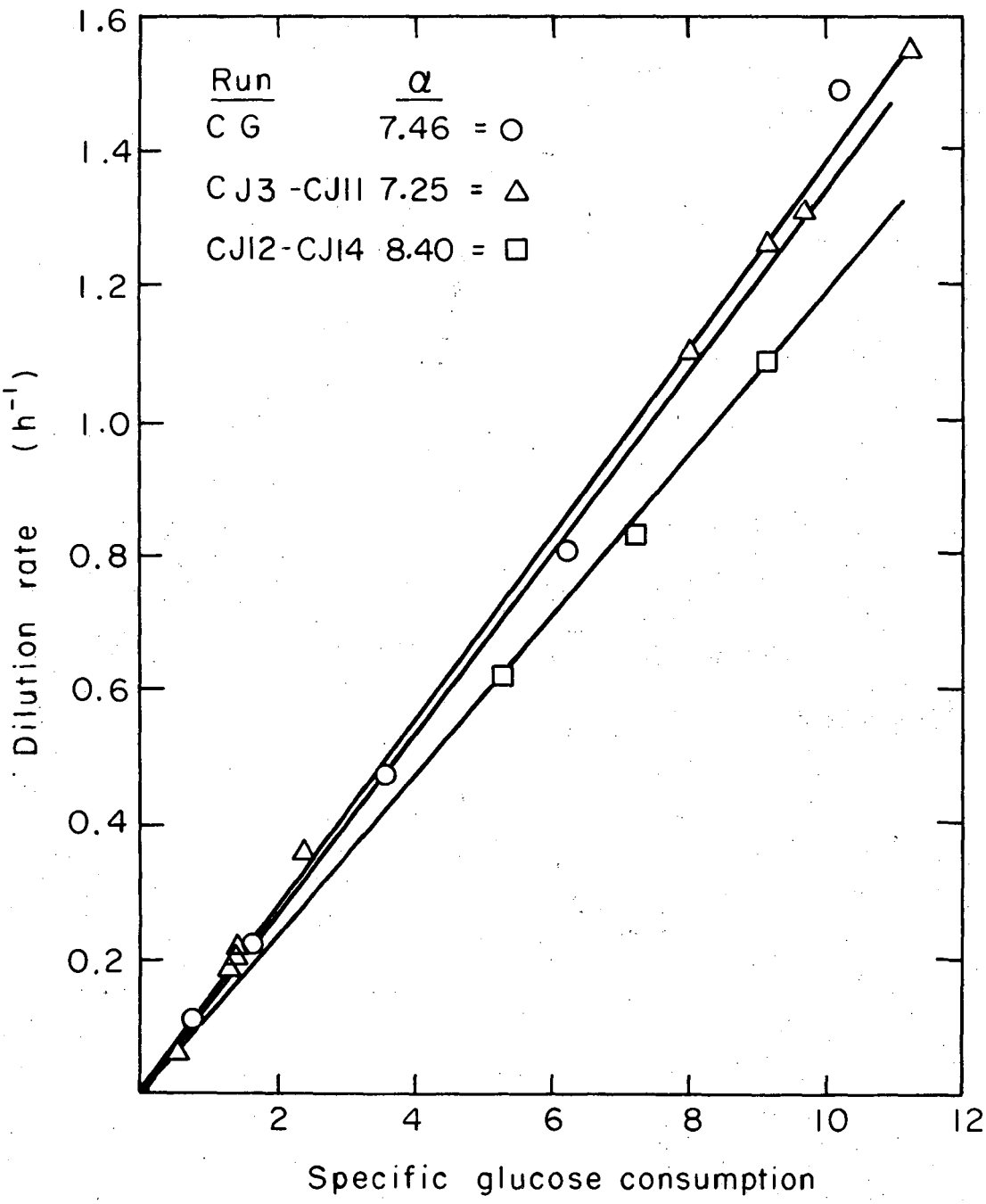
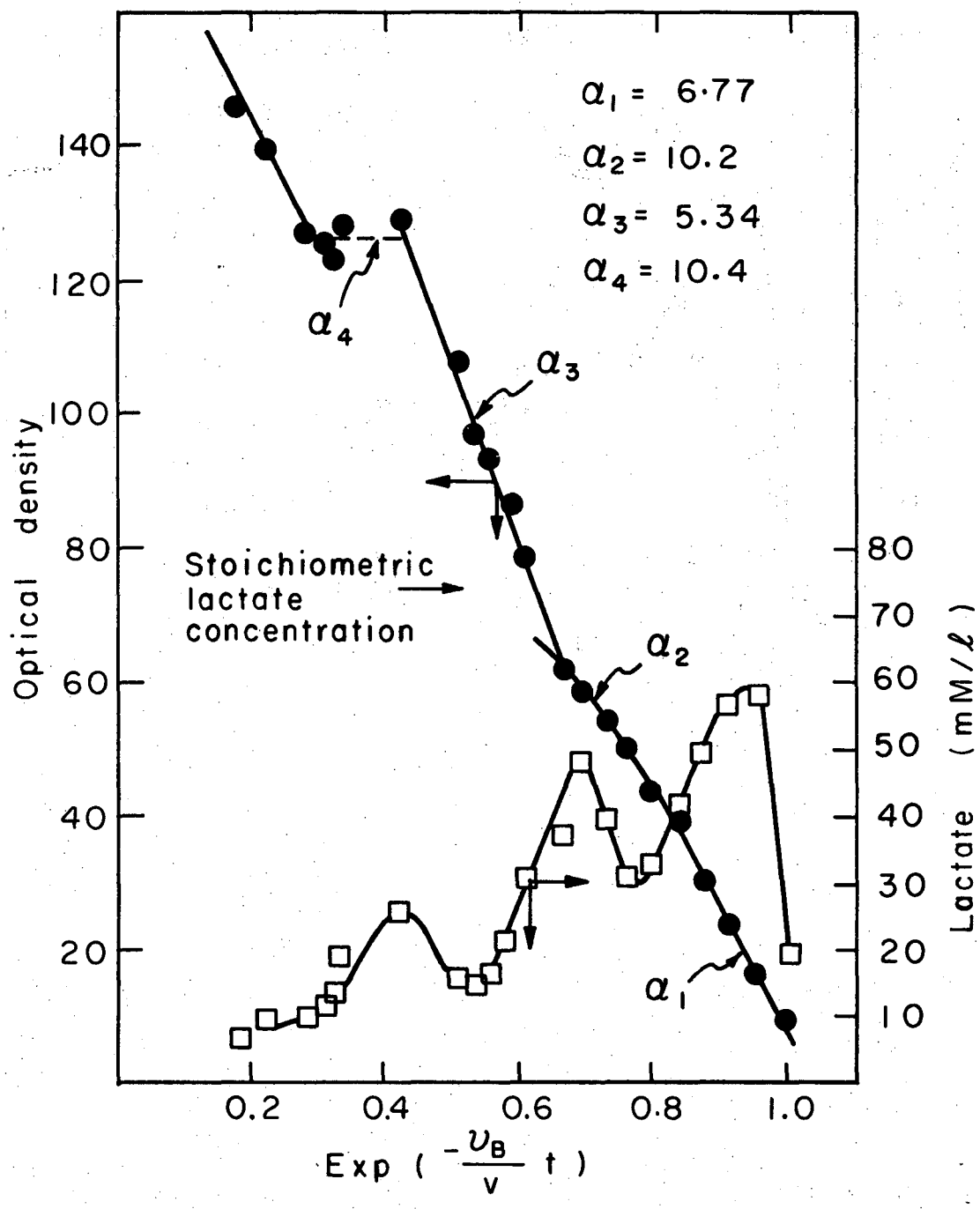

1.0
0.8
0.6
F
0.4
0.2
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
Dilution rate (h⁻¹)
CG
CJ
HDA
XBL685-2652

Fig. 7



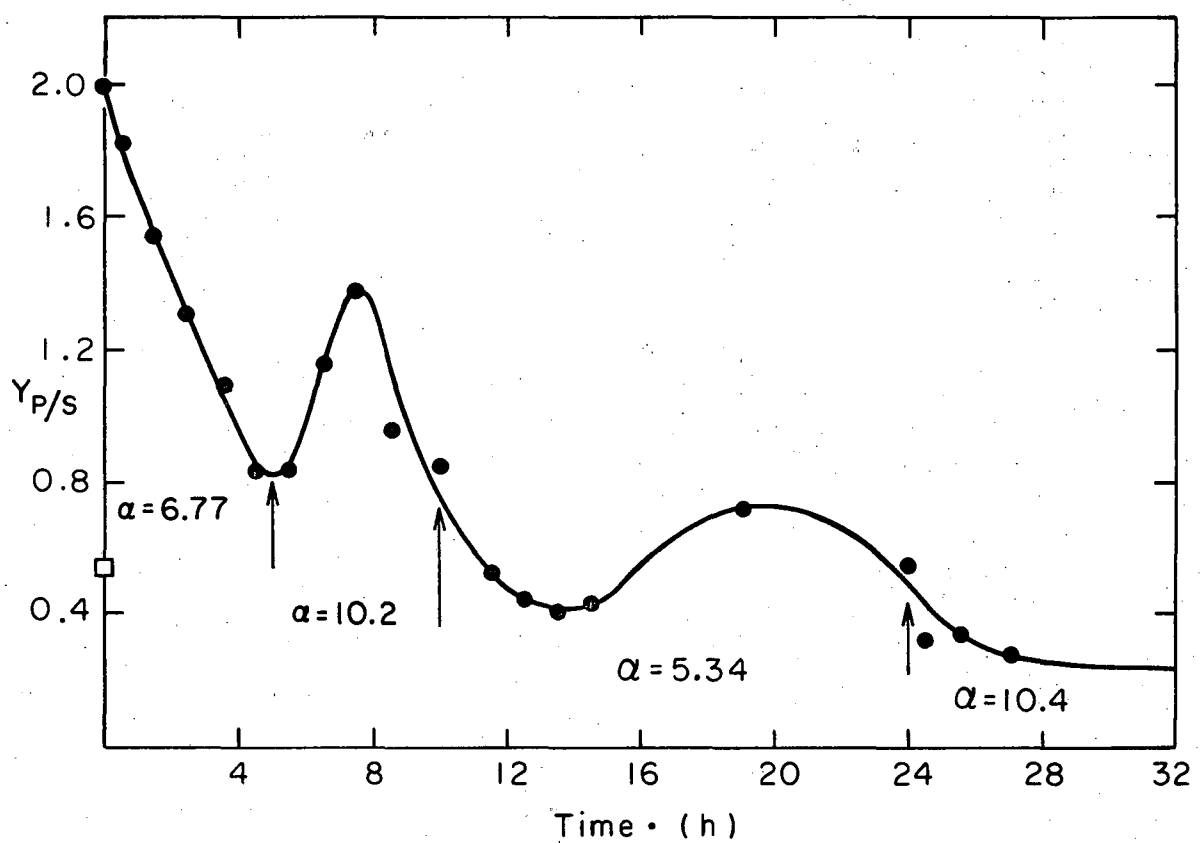
XBL685-2651

Fig. 8



XBL685-2654

Fig. 9



XBL685-2655

Fig. 10

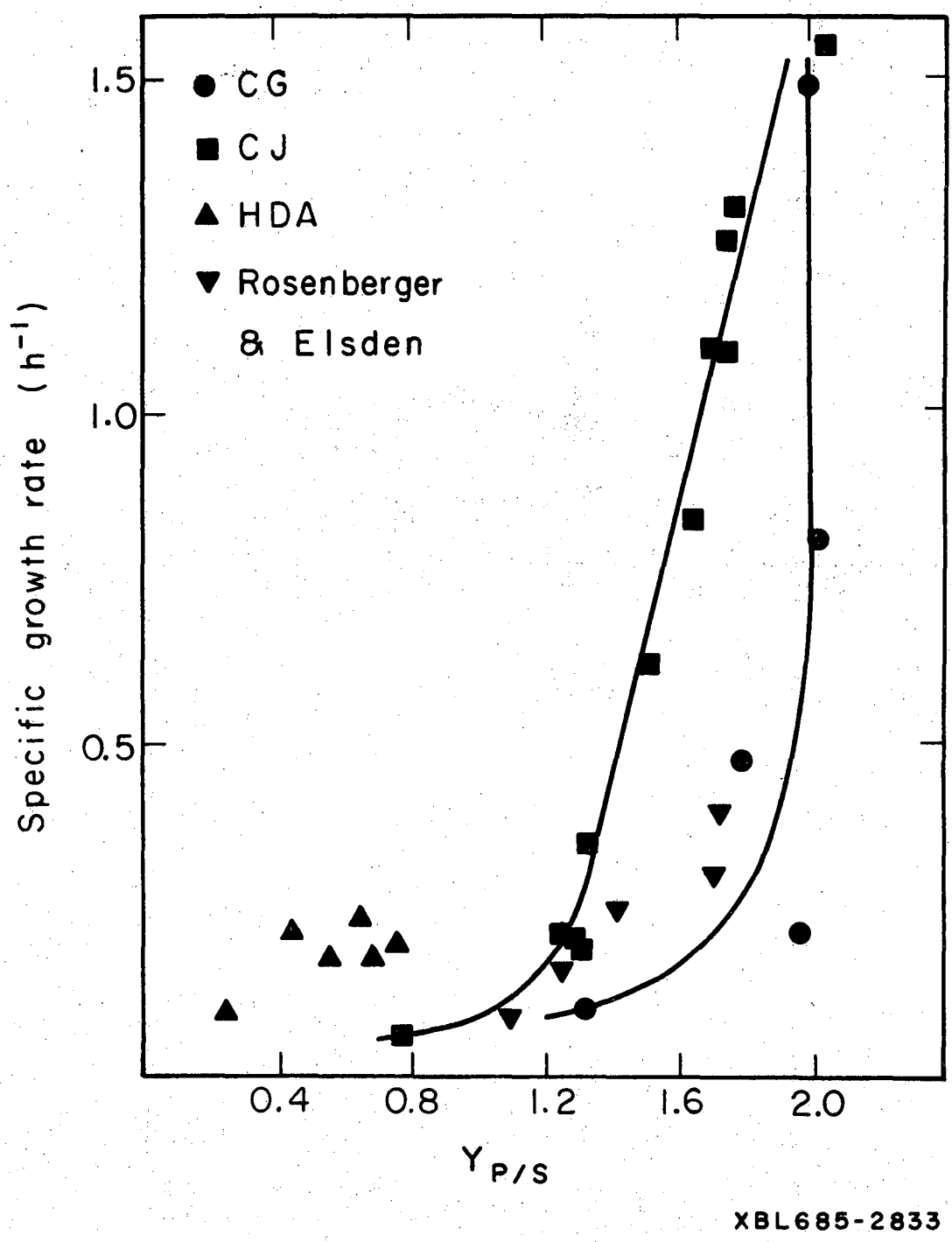
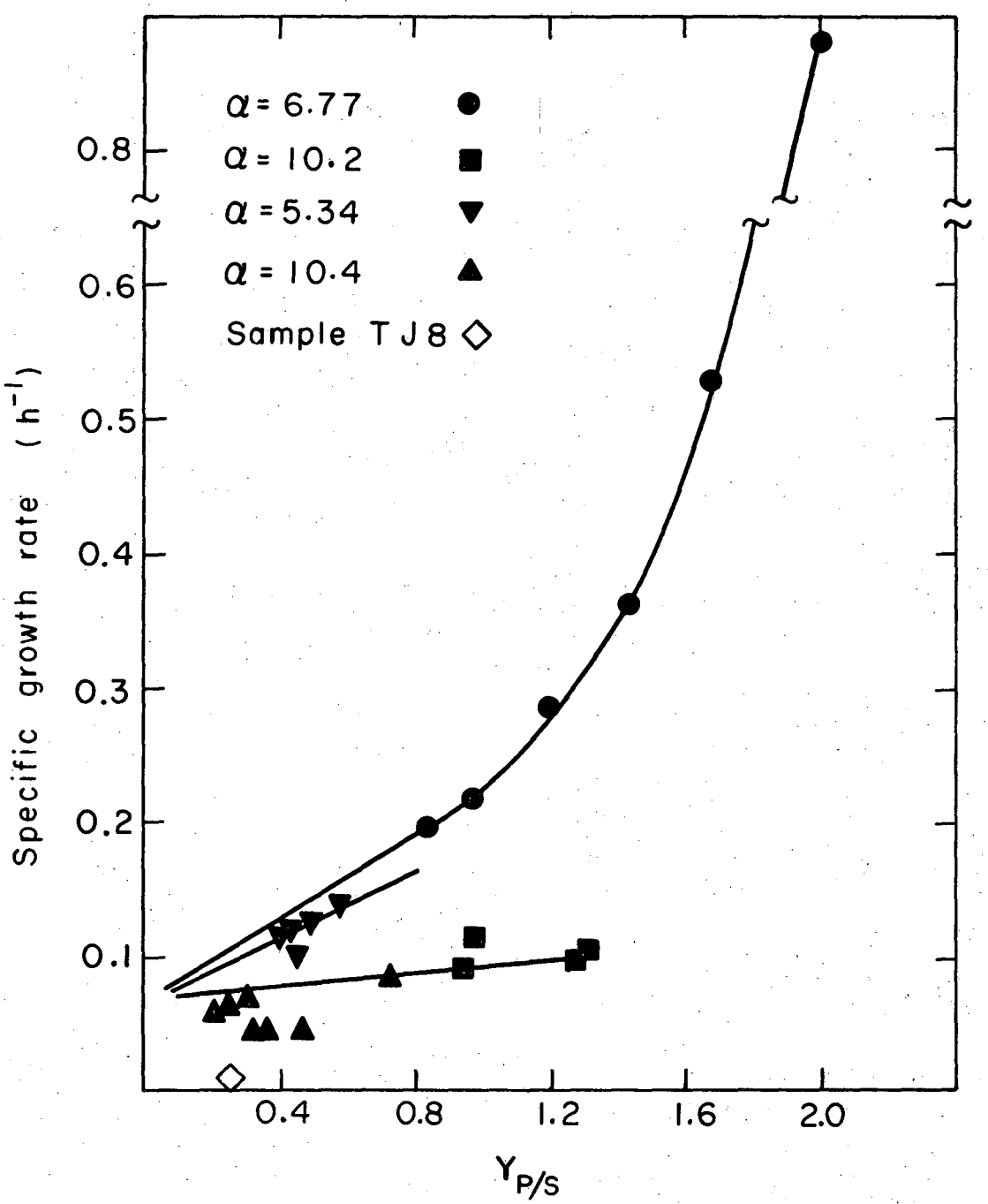

CG
CJ
HDA
Rosenberger & Elsden
Specific growth rate (h^{-1})
1.5
1.0
0.5
0.4
0.8
1.2
1.6
2.0
$Y_{P/S}$
XBL685-2833

Fig. 11



XBL685-2653

Fig. 12

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