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FERMENTATION KINETICS AND PROCESS ECONOMICS FOR THE PRODUCTION OF ETHANOL

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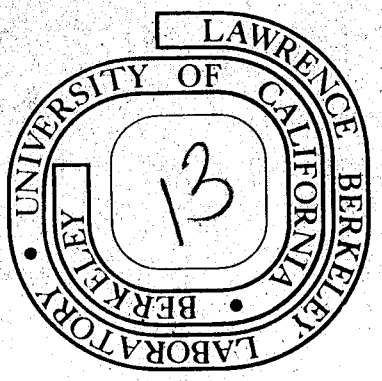

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Contents

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FERMENTATION KINETICS AND PROCESS ECONOMICS FOR THE PRODUCTION OF ETHANOL

Gerald Raymond Cysewski* and Charles R. Wilke

1. ABSTRACT

Although ethanol fermentation technology is extremely old, there have been no recent major technological advancements for the production of industrial ethanol by fermentation. Since 1929 industrial ethanol has been produced by the catalytic conversion of ethylene. Presently over 98% of industrial ethanol is produced by this method in the United States. However, with the impending petroleum storages there has been renewed interest in the fermentation of carbohydrates to ethanol. The overwhelming advantage of fermentation is that the raw materials are renewable. Any fermentable sugar can be used as the fermentation substrate. Recent developments in acid and enzymatic hydrolysis of cellulose to fermentable sugars may possibly allow the economic production of fermentation ethanol from the vast and renewable quantities of cellulose on the earth. The ethanol may then be used as a chemical feed stock and/or a liquid fuel.

The aim of this study was to develop and optimize fermentation technology for the production of ethanol. Using glucose as the fermentable substrate, optimal fermentation parameters of pH, temperature, oxygen tension and sugar concentration were determined in both batch and continuous culture. The experimental results indicate that although ethanol fermentation is an anaerobic process, trace amounts of oxygen are required for maximal ethanol production.

The ethanol productivity of the initial culture of Saccharomyces cerevisiae (ATCC #4126) was optimal at an oxygen tension of 0.7 mmHg and a temperature of 35°C. However, when long term continuous culture was maintained the yeast "adapted" after 3 weeks of operation, requiring an oxygen tension of only 0.07 mmHg for optimal ethanol production. As well, continuous ethanol production by the "adapted" yeast was found to be 43% higher than for the "unadapted" yeast under conditions of complete substrate utilization. The pH of the broth had only a slight affect on fermentation rates between 3.5 and 5.5. However, the sugar concentration did affect ethanol productivities in continuous culture, with the optimal concentration being 10 wt% sugar.

A cell recycle system employing an external settler to increase the biomass concentration in continuous fermentations was examined. The cell mass concentration was increased fourfold in the recycle system over conventional continuous operations. This produced a corresponding fourfold increase in ethanol productivity.

In addition, a novel vacuum fermentation scheme was developed where by ethanol is boiled away from the fermentation broth as it is produced. Vacuum operation is necessary to achieve boiling at temperatures compatible to the yeast. Since ethanol is removed from the fermenting broth as it is formed, ethanol inhibition is eliminated permitting solutions of high sugar concentration to be fermented. A sevenfold increase in ethanol productivity over conventional continuous operation was obtained in the vacuum system when a 33.4% glucose solution was fermented. By combining the vacuum fermentation with cell recycle, yeast cell densities of 124 g

dry wt/l were achieved resulting in a 14-fold increase in ethanol productivity over simple continuous operation.

From the experimental results obtained industrial size ethanol fermentation plants were designed and an economic evaluation conducted. The process design studies indicated that over a 50% reduction in capital expenditure may be obtained by continuous rather than batch operation. Further reductions in processing costs were achieved by both cell recycle and vacuum operation. However, the cost of fermentable substrate, either molasses are enzymatic hydrolysate sugars, dominates the economics of ethanol production.

2. INTRODUCTION

Ethanol fermentation technology is extremely old, dating back to prehistoric ages. During the Middle Ages the Arabs learned to distill fermented broth and produce "fire water", water which burns. The production and consumption of distilled spirits have influenced the history of mankind ever since. It was not until the Eighteenth Century that ethanol became an important industrial chemical. Up to 1929 practically all ethanol, industrial or beverage, was produced from fermentation. But with the advent of petrochemical technology and the availability of cheap petroleum, industrial ethyl alcohol was produced by the catalytic conversion of ethylene. This situation exists today. Over 98% of all industrial ethanol is currently manufactured from ethylene in this country. Beverage alcohol, however, by law is still produced by fermentation.

The recent petroleum shortages and variability of petroleum supplies in this country have renewed interest in the production of fermentation alcohol from renewable agriculture products and cellulosic wastes. The fermentation alcohol, ethanol, may be used as a liquid fuel or as a feed stock for the production of organic chemicals currently manufactured from petroleum. During the 1930's considerable research using ethanol as an automotive fuel indicated that a blend of 10 to 20% of absolute ethanol and gasoline could be used in automotive engines without any engine modifications.[1,2] These results have been supported by current research in Nebraska.[3] Fleet testing, consisting of over 700,000 vehicle miles, using a 10% ethanol-gasoline blend gave 5 to 7% better fuel economy than gasoline.

Engine wear and emission of pollutants were the same with the ethanol-gas blend as with gasoline.

Ethanol may also be used as a chemical feed stock. Currently some 900,000 tons of ethylene derived ethanol are consumed annually by industry.[4] Ethanol is used for the manufacture of ethyl acetate, acetic acid, glycols and ethylene dibromide, as well as an industrial solvent. Thus, there is a definite market for ethyl alcohol as a chemical feed stock and as a fuel. The potential also exists for saving petroleum by manufacturing ethanol from a renewable resource.

Ethanol can be produced by a yeast fermentation of a carbohydrate substrate. Strains of Saccharomyces cerevisiae are commonly used, but other yeasts, such as S. anamensis and Schizosaccharomyces pombe, may be employed under certain conditions.[5] Sugars from grain and "Blackstrap" molasses have been traditionally used as the fermentable substrate, however, any fermentable carbohydrate may be used. New developments in the enzymatic and acid hydrolysis of cellulose have re-opened the possibility of using cellulosic wastes to produce industrial ethanol.[6,7] The cellulose polymer of β-1-4 glucose is selectively degraded to the monomer glucose sugar by the catalytic action of either acid or enzymes. The glucose can then be fermented to ethanol by the yeast S. cerevisiae. The work of this study is directly connected with the enzymatic hydrolysis of cellulose by the enzyme system produced from the fungus Trichoderma viride. An engineering design of the enzymatic hydrolysis process indicates that a 4.0% sugar solution can be produced for 5 cents per pound of sugar from cellulosic wastes[7] exclusive of the raw material cost. This

compares favorably with the current selling price of 20 cent/lb for glucose.

In the United States each year, over 2.9×10^8 tons of residential, institutional and commercial solid waste are produced. This waste contains approximately 50% cellulosic materials. In addition, 4.73×10^8 tons of agricultural waste having a 40% cellulose content are available.[9] If these cellulosic wastes were enzymatically hydrolyzed to glucose and subsequently fermented to ethanol, the amount of fuel generated would be equivalent to 1/5 the current United States gasoline consumption. In addition, the possibility of cellulose farming exists. Here the sun's energy would be stored by the production of plant biomass. The biomass would then be hydrolyzed to glucose and fermented to a liquid fuel, ethyl alcohol.

Because industrial ethanol has been produced from ethylene for the past 40 years, there have been no recent important advances in ethanol fermentation technology applicable to the rapid and bulk production of industrial ethanol. There have, of course, been developments in beverage alcohol production, but the beverage industry is concerned with flavor components and maintaining "old family recipes." The goal of this research then, was to develop new alcohol fermentation technology with emphasis on economic and engineering aspects.

REFERENCES

1. O. C. Bridgeman, Ind. Eng. Chem. 28, 1102 (1936).

2. P. B. Jacobs, H. P. Newton, U. S. Department of Agriculture Miscellaneous Publications No. 327 (1938).

3. Wm. A. Scheller, Agricultural Alcohol in Automotive Fuel, Nebraska Gasohol. Presented at the Eighth National Conference on Wheat Utilization Research (1973).

4. United States International Trade Commission, Synthetic Organic Chemicals (1974).

5. S. C. Prescott, C. G. Dunn, Industrial Microbiology (McGraw-Hill, NY, 1959).

6. H. E. Grethlein, The Acid Hydrolysis of Refuse in Cellulose as a Chemical and Energy Resource Symposium, C. R. Wilke, ed. (John Wiley and Sons, NY, 1975).

7. C. R. Wilke, R. D. Yang and U. Von Stockar, Preliminary Cost Analysis for Enzymatic Hydrolysis of Newsprint, presented at the Symposium on Enzymatic Conversion of Cellulosic Materials, Boston, MA, September 1975.

8. First Annual Report of the Council on Environmental Quality, United States Congress, 1970.

9. Chemical Week 109(13), 21 (1970).

3. BACKGROUND INFORMATION AND PREVIOUS WORK

3.1. Yeast Metabolism

The yeast Saccharomyces cerevisiae used in the alcohol fermentation studies is a facultative anaerobe, able to grow in the presence or absence of oxygen. Under anaerobic conditions Saccharomyces ferments glucose to ethanol and carbon dioxide, but when oxygen is present only carbon dioxide and cell mass are produced through a respiratory metabolism. There is not a clean delineation as to whether the catabolic metabolism of Saccharomyces will be anaerobic or aerobic for a given fermentation. Many times a mixture of oxidative and fermentative metabolisms exists during an aerobic fermentation.[1] The type of metabolism depends on the oxygen, glucose and to some extent, inorganic salt concentrations in the fermentation broth.[2-4]

A simplified schematic of anaerobic and aerobic metabolisms is shown in Fig. 3.1. Under anaerobic conditions glucose is fermented to ethanol and carbon dioxide by glycolysis.[5] Glucose is first converted to pyruvate by ten enzyme catalyzed steps. The pyruvate is then further metabolized to acetaldehyde and carbon dioxide, and then to ethanol by two additional enzymatic reactions. The overall reaction, shown in Eq. (3.1), produces 2 moles of ethanol and carbon dioxide for every mole of glucose fermented.

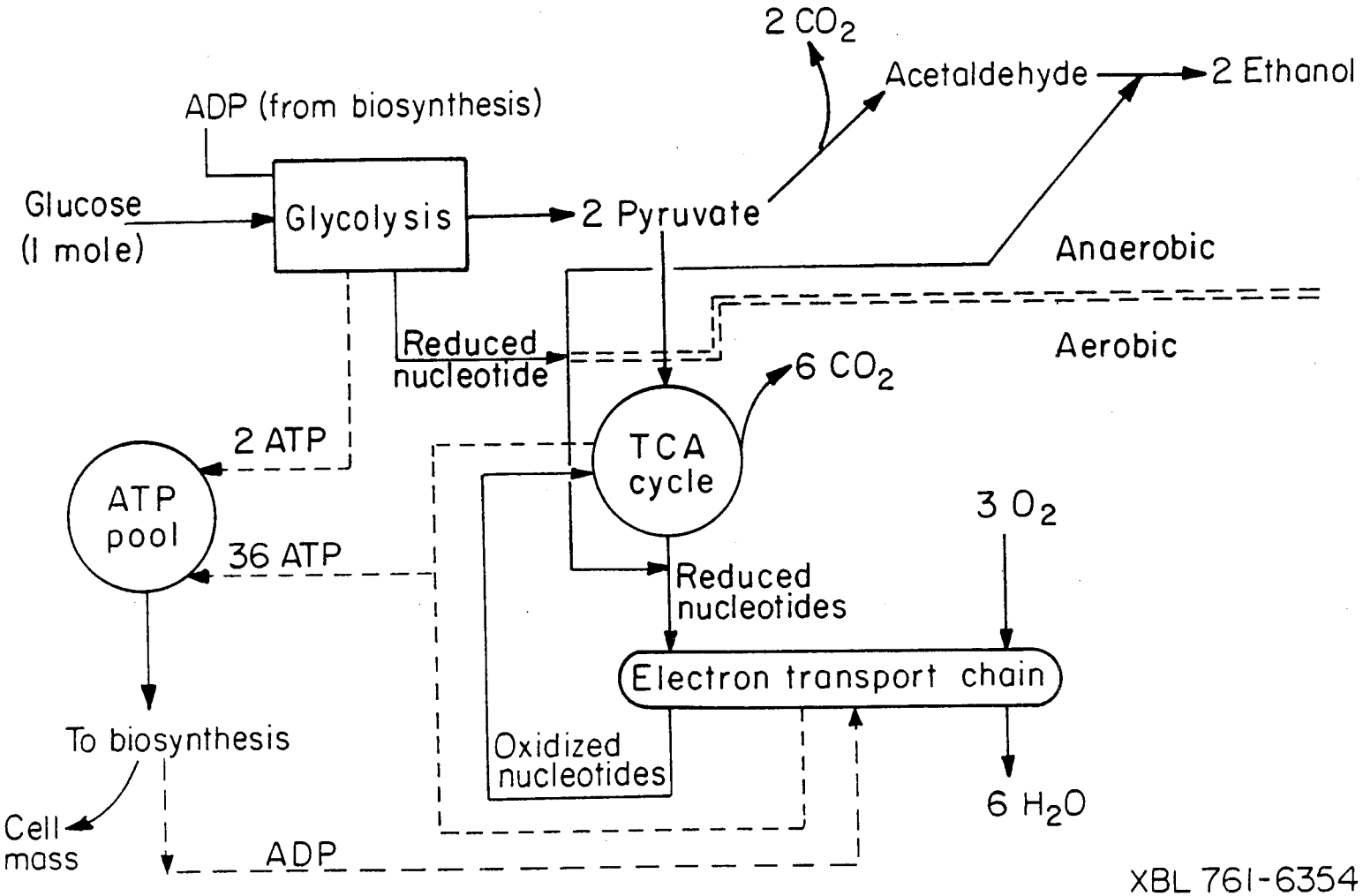
$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (3.1)$$

Glucose Ethanol Carbon dioxide

On a weight basis every gram of glucose can theoretically yield 0.511 grams of ethanol. In actual practice the ethanol yield is about 90 to 95% of the theoretical yield, since a portion of the

Fig. 3.1.

Simplified Chart of Anaerobic and Aerobic Catabolism of Saccharomyces cerevisiae



glucose is channeled into biosynthetic pathways.

The anaerobic fermentation of 1 mole of glucose to ethanol produces 2 moles of the energy translating compound adenosine triphosphate (ATP) during glycolysis by addition of phosphate to ADP formed in biosynthesis. The energy produced from the fermentation and stored in the ATP is consumed in biosynthesis to produce cell mass. This points out an interesting fact. If the yeast growth rate is high, the fermentation rate must also be high in order to supply the energy required for the production of cell mass. Conversely, if growth is suppressed the ethanol production rate will also be reduced.

During aerobic conditions glucose is metabolized to cell mass and carbon dioxide and no ethanol is formed.[5] Also, as in the anaerobic metabolism, the glucose is first converted to pyruvate by glycolysis. The pyruvate then enters the Tricarboxylic acid cycle (TCA cycle) and is oxidized to carbon dioxide by the action of oxidized nucleotides. As the pyruvate is oxidized, reduced nucleotides, NADH and FADH are formed. These nucleotides are reoxidized through an electron transport chain using oxygen as the final electron acceptor to produce water. The energy liberated by oxidation of the nucleotides is stored by the production of ATP and subsequently used in biosynthesis. The aerobic oxidation of 1 mole of glucose yields 38 moles of ATP as compared with 2 moles of ATP obtained from the strictly anaerobic pathway to ethanol. The consumption of glucose per unit of cell mass produced is lower under aerobic than under anaerobic conditions because the specific energy requirement, i.e., moles of ATP, for biosynthesis is approximately the same in either case, while the aerobic processes

makes 19 times more ATP available per mole of glucose consumed. Also, the yield of cell mass per gram of glucose consumed is higher in an aerobic metabolism because no glucose is used to produce ethanol. Typically 0.4 to 0.47 grams of cells are produced per gram of glucose aerobically metabolized while only 0.05 to 0.12 grams of cells are formed per gram of glucose in the anaerobic case.

3.1.1. Effect of Oxygen

Although the alcoholic fermentation has been classically thought of as totally anaerobic, numerous workers have shown that trace amounts of oxygen stimulate fermentation rates and that some strains of Saccharomyces cerevisiae actually require oxygen as a growth factor.[6-8] The amount of oxygen required to accelerate the fermentation rate is extremely small amounting to only 0.05 mmHg oxygen tension in the fermentation broth.[7] As a result, the determination of the absolute oxygen requirement is difficult and necessitates the purification of even high purity nitrogen to reduce the residual level of oxygen to less than 1 ppm.[9] For this reason the oxygen requirement of Saccharomyces has often been overlooked.

The stimulating effect of oxygen on fermentation rates is of an anabolic rather than catabolic nature. At the optimal oxygen tension of continuous culture, Haukeli et al.[4] have shown that over 99% in the catabolism was fermentative rather than oxidative. Oxygen is necessary for the biosynthesis of poly-unsaturated fats and lipids required in mitrochondrial and plasma membranes.[3,4,16] Without these important structural components many essential membrane functions are impaired.[10,11] Bloomfield and Black[12] demonstrated that

the synthesis of poly-unsaturated fatty acids in yeast is via thioester derivatives in the presence of oxygen and reduced pyridine nucleotides. Also, the production of the steroid, ergosterol in Saccharomyces has been shown to require oxygen.[3] Thus if the base fermentation medium is supplemented with erogsterol the oxygen requirement of S. cerevisiae may be eliminated.[13,16]

The history of the inoculum in batch fermentations also affects the oxygen demand.[13] If the inoculum is grown either aerobically or in an ergosterol supplemented media, the oxygen requirement of the subsequent fermentation is diminished. This illustrates the ability of Saccharomyces to store unsaturated compounds during aerobiosis for use when fermentation conditions become anaerobic. Ergosterol can constitute as much as 10% of the cell dry weight of S. cerevisiae during aerobic growth while no ergosterol is found if growth is anaerobic.[14]

High oxygen tension in the fermentation broth (above 200 mmHg) can inhibit fermentation rates.[15,16] Oura[1] found that if the gas sparged through his fermenter contained more than 30% oxygen, the metabolism of the yeast was disturbed and ethanol and acetaldehyde were produced even though the fermentation was highly aerobic. Cowland,[15] however, demonstrated a partial inhibition of ethanol fermentation at much lower oxygen tensions (2.3 mmHg), but was able to adapt the yeast to higher oxygen tensions, although no increase in ethanol productivity was reported.

There exists then an optimum oxygen concentration for the alcoholic fermentation. Indeed it may not be possible to maintain a continuous

ethanol fermentation without sparging some oxygen through the fermenter,[6] but if the oxygen tension is too high either the ethanol fermentation rate is depressed or the metabolism of the yeast becomes totally aerobic and no ethanol is produced.[1] However, the final metabolism of the yeast is dependent on the glucose concentration in the fermentation broth as well as the oxygen tension. The interrelation between glucose and oxygen concentrations is discussed in the next section.

3.1.2. Effect of Glucose

High concentrations of glucose (above 150 g/l) inhibit enzymes in both the fermentative and oxidative pathways.[17] This is commonly called substrate inhibition. At moderate concentrations, (3-100 g/l) glucose selectively inhibits respiration in Saccharomyces.[8] This catabolic repression of the respiratory metabolism permits alcoholic fermentation to continue even in the presence of high oxygen tension. The catabolism will be fermentative and independent of oxygen concentration as long as the glucose level is above 3 to 5 g/l.[18]

The mechanism of catabolic repression is not well understood. Richard et al.[19] have shown that glucose concentrations of 50 g/l strongly repress A-type cytochromes and to a lesser extent B- and C-type cytochromes of the electron transport chain. Therefore, an inhibition of the aerobic metabolism is expected. But Akbar[8] was not able to demonstrate a relation between the cessation of cytochrome synthesis and respiration activity during glucose catabolic repression in Saccharomyces. Akbar thus proposed that independent mechanisms repress the synthesis of cytochromes and other mitochondrial enzymes.

A theory of catabolic repression was also put forth by Sols[20] which relates the degree of repression to the increased level of the adenosine phosphate pool as the yeast begins to aerobically metabolize glucose. Although this theory supports the findings of Akbar, it does not explain the fact that cytochrome rich cells of S. cervisiae would make greater demands on the ATP pool for biosynthesis than actively fermenting cells.

When the glucose concentration drops below 2 g/l catabolic repression lifts and if oxygen is present, yeast metabolism switches from fermentative to oxidative.[8] This results in a decrease of the specific glucose consumption rate and is known as the Pasteur effect. The Pasteur effect stems from the fact that more energy (ATP) is derived from the aerobic than anaerobic catabolism of glucose while the energy requirement for production of yeast cell mass remains unchanged.

3.1.3. Effect of Ethanol

When S. cerevisiae ferments concentrated sugar solutions, ethanol accumulates in the broth to such an extend that the metabolic activity of the yeast is suppressed. The fermenting yeast pollutes its environment with the end products of its metabolism until it ceases to grow and eventually dies. This end product inhibition is the reason wine is typically only 12% alcohol.

The inhibition of ethanol and cell mass production by Saccharomyces in the presence of high ethanol concentrations has been extensively studied.[21-23] Particular reference is made to the work of Bazua[24] who characterized the alcohol inhibition kinetics of the

yeast used in this work. The mode of ethanol inhibition is of a noncompetitive type.[22] Zines and Rogers[25] using K. aerogenes demonstrated that the point of ethanol inhibition occurs after the catabolic metabolism and is probably located in the biosynthetic pathways. In addition, the permeability of the cell membrane was altered by high concentrations of ethanol.

There are numerous kinetics models relating alcohol inhibition to the fermentation rate of S. cerevisiae.[22,24] Most of these models are of a parabolic type showing negligible inhibition at low alcohol concentration with growth and fermentation stopping at an ethanol concentration of 9 to 12%. The exact form of the kinetic models differ somewhat depending on the yeast strain and experimental conditions used in the particular study.

Ethanol inhibition is of major importance in industrial fermentations because a high tolerance of ethanol permits concentrated sugar solutions to be fermented. This allows a more efficient use of fermentor volume and reduces the size of auxiliary equipment such as media sterilizers and pumps. Also the distillation cost of concentrating the ethanol is reduced if the fermented beer has a high alcohol concentration.

3.2. Present Ethanol Fermentation Technology

The major advances in ethanol fermentation technology have been made in the beverage industry, particularly for the manufacture of beer. But concern for flavor components has limited the development of rapid industrial fermentations. Ethanol fermentations have been traditionally operated batchwise, although there is much literature

dealing with the performance of continuous laboratory beer fermentations.[26-28] The commercial operation of one continuous beer fermentation, however, has been reported by Bishop.[29] The continuous system employs a two-stage fermentation to ferment the mixture of sugars present in the wort and a cooled settler to separate the yeast and fermented beer.

Portno,[30] as well as Pirt and Kurowski[31] have examined closed continuous fermentations in which the yeast cells remain in the fermentor by either sedimentation or filtration from the fermentation broth. Their findings indicate that a portion of yeast must be continually removed from the fermentor to maintain a viable and steady fermentation rate. If the fermentor system is operated totally closed with no yeast escaping in the fermented broth, the yeast viability declines with a corresponding decrease in fermentation rate. A deterioration of beer quality was also reported for the closed systems.

The current state of the art of alcohol fermentations leaves much to be desired when applied to industrial ethanol production.

REFERENCES

1. E. Oura, Biotechnol. Bioeng. 16, 1197 (1974).
2. H. Holzer, J. Witt, Biochem. Z. 330, 545 (1958).
3. P. J. Rogers, P. R. Stewart, J. Gen. Microbiol. 79, 205 (1973).
4. A. D. Haukeli, S. Lie, J. Inst. Brew. 79, 55 (1973).
5. R. Y. Stanier, M. Doudoroff and E. A. Adelberg, The Microbial World (Prentice Hall, Inc, NJ, 1970).
6. J. White, D. J. Munns, Vallestein Commun. 14, 199 (1951).
7. T. W. Cowland, D. R. Maule, J. Inst, Brew. 72, 480 (1966).
8. M. D. Akbar, P. D. Richard, F. J. Moss, Biotechnol. Bioeng. 16, 455 (1974).
9. A. A. Andreasen, J. J. B. Stier, J. Cell Comp. Phys. 41, 23 (1953).
10. S. Fleischer, B. Fleischer, Methods in Enzymology 10, 406 (1967).
11. L. E. Hokin, M. R. Hokin, Nature (London) 189, 836 (1971).
12. D. K. Bloomfield, K. J. Black, Bio. Chem. 235, 337 (1960).
13. A. D. Haukeli, S. Lie, J. Inst. Brew. 77, 253 (1971).
14. S. C. Prescott, C. G. Dunn, Industrial Microbiology (McGraw-Hill, NY, 1959).
15. T. W. Cowland, J. Inst. Brew. 73, 542 (1967).
16. M. H. David, B. H. Kirsap, J. Inst. Brew. 79, 20 (1973).
17. H. Holzer, Aspects of Yeast Metabolism, A. K. Mills, ed. (Oxford, Blackwell Scientific Publications, 1968).
18. F. J. Moss, P. A. D. Richard, F. E. Bush, Biotechnol. Bioeng. 13, 63 (1971).
19. P. A. D. Richard, F. J. Moss, D. Phillips, T. C. K. Mok, Biotechnol. Bioeng. 13, 164 (1971).

20. A. Sols, Aspects of Yeast Metabolism, A. K. Mills, ed. (Oxford, Blackwell Scientific Publications, 1968).

21. A. S. Aigar, R. Ludeking, Chem. Eng. Prog. Symp. 62(69), 57 (1969).

22. S. Aiba, M. Shoda, M. Nagatani, Biotechnol. Bioeng. 10, 845 (1968).

23. I. Holzbring, R. K. Finn, K. H. Steinkraus, Biotechnol. Bioeng. 9, 413 (1967).

24. C. Bazua, Effect of Alcohol Concentration on the Kinetics of Ethanol Production by Saccharomyaces cerevisiae (M. S. Thesis), University of California, Berkeley, 1975.

25. D. O. Zines, P. L. Rogers, Biotechnol. Bioeng. 13, 293 (1971).

26. A. D. Portno, J. Inst. Brew. 74, 55 (1968).

27. D. J. Millin, D. Phil, J. Inst. Brew. 72, 394 (1966).

28. T. G. Watson, J. S. Hough, J. Inst. Brew. 72, 547 (1966).

29. L. R. Bishop, J. Inst. Brew. 76, 172 (1970).

30. A. D. Portno, J. Inst. Brew. 73, 43 (1967).

31. S. J. Pirt, W. M. Kurowski, J. Gen. Microbio. 63, 357 (1970).

4. FERMENTATION KINETICS AND THEORY OF FERMENTER OPERATION

4.1. Batch Fermentation

Yeast cells of the Saccharomyces genus are oblate spheroids which reproduce asexually by a process called budding. A bud appears on the surface of a cell and grows until it reaches almost the size of the mother cell. The bud then separates from the mother cell and a new cell is formed. The budding process may be repeated 40 times before a cell stops reproducing.[1] The number of times a cell has budded can be found by counting the number of scars left on the cell surface. When budding scars cover the surface of the cell, the budding process stops, but the cell still metabolizes fermentable substrates.

Yeast growth is autocatalytic. One cell buds or divides to give two, two cells bud to give four and so on. The rate of cell mass production in batch culture is thus proportional to the cell mass concentration. The constant of proportionality is called the specific growth rate and designated by μ.

$$\frac{dx}{dt} = \mu x \tag{4.1}$$

where, x = cell mass concentration, g dry wt/l

t = time, hr

μ = specific growth rate, hr^{-1}

Rearranging Eq. (4.1) and solving for the cell mass as a function of time one obtains,

$$x = x_o e^{\mu t} \quad . \tag{4.2}$$

where, x_o = initial cell mass concentration at time $t = 0$, g dry wt/l

Equation (4.2) is a description of exponential growth which occurs during a batch fermentation. If the log of both sides of Eq. (4.2) is taken, Eq. (4.3) is produced.

$$\ell n\ x = \ell n\ x_o + \mu t \tag{4.3}$$

This illustrates that a plot of the log of cell mass vs time should yield a straight line with a slope equal to the specific growth rate. Depending on growth conditions such as pH, temperature and nutrient composition, the specific growth rate of yeast in batch culture varies between 0.2 and 0.6 hr^{-1}.

The specific growth rate has been related to the substrate concentration by Monod[2] as,

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{4.4}$$

where μ_{max} = maximum specific growth rate, hr^{-1}

K_s = saturation constant, g/l

S = concentration of growth-limiting substrate, g/l

The saturation constant, K_s, is typically quite small (0.1 to 0.3 g/l).[4] Therefore, the empirical model presented in Eq. (4.4) dictates that the growth rate will be maximal and independent of substrate concentrations at high substrate concentrations and a linear function of substrate concentration at low concentrations.

The production of ethanol is growth associated during exponential growth, but becomes non-growth associated during the latter stages of batch growth. It is also non-growth associated when the ethanol concentration is high (end product inhibition). With this in mind, Aigar and Luedeking[3] correlated the specific cell ethanol productivity in batch fermentation as,

$$\nu \equiv \frac{1}{X}\frac{dP}{dt} = \alpha\mu + \beta \tag{4.5}$$

where ν = specific ethanol productivity, g ethanol/g cell-hr

P = ethanol concentration, g/l

α = stoichiometric constant for conversion of substrate to ethanol

β = proportionality constant, hr^{-1}

According to Eq. (4.5) if ν is plotted against the specific growth rate, μ, one should get a straight line with a slope α and intercept β.

The efficiency of conversion of a substrate to ethanol and cell mass in both batch and continuous fermentations is often characterized by the following yield factors.

$$Y_{X/S} \equiv -\frac{\Delta X}{\Delta S} \tag{4.6}$$

where, $Y_{X/S}$ = yield factor relating grams of cells produced per gram of substrate consumed

ΔX = cell mass produced, g/l

ΔS = substrate consumed, g/l

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \tag{4.7}$$

where, $Y_{P/S}$ = yield factor relating grams of ethanol produced per gram of substrate consumed

ΔP = ethanol produced, g/l

ΔS = substrate consumed, g/l

$$Y_{P/X} = \frac{\Delta P}{\Delta X} \tag{4.8}$$

where, $Y_{P/X}$ = yield factor relating grams of ethanol produced per gram of cells produced.

4.2. Continuous Fermentation

In continuous fermentation a constant supply of fresh media is pumped to a well-mixed fermentation vessel while an appropriate amount of cells and fermented broth are continually withdrawn. At steady state the medium feed rate is equal to the fermented broth exit rate and there is no net accumulation of either cell mass or fermentation products in the fermentor. A cell mass balance may be written for the steady state operation of a continuous fermentor in a series of fermentation vessels.[4]

$$\mu_n X_n = D_n X_n - D_{n-1} X_{n-1} \tag{4.9}$$

where, μ = specific growth rate, hr^{-1}

X = cell mass concentration, g dry wt/l

D = dilution rate, hr^{-1}

n = fermenter number, $n = 1$ for first fermenter in series

The dilution rate, D, is defined as

$$D \equiv \frac{F}{V}$$

where, F = medium flow rate, l/hr

V = fermenter volume, l

By definition, D is the reciprocal of the mean residence time. If only one continuous fermentor is used, Eq. (4.9) reduces to

$$\mu = D \tag{4.10}$$

Therefore, at steady state the dilution rate, D, is equal to the specific growth rate, μ, for a single stage continuous fermentation. However, if the dilution rate is greater than the maximum specific growth rate of the cells, μ_{max}, washout will occur. Washout is a result of cells leaving the fermentor in the exit stream faster than they can reproduce within the fermentor.

Conditions of washout can be predicted by solving Eq. (4.4) for the exit substrate concentration, S, from the fermentor.

$$S = \frac{DK_s}{\mu_{max} - D} \tag{4.11}$$

Here use has been made of Eq. (4.10) to replace μ with D. From Eqs. (4.6) and (4.7) the cell mass and ethanol concentrations in the fermentor are found.

$$X = Y_{X/S}(S_o - S) \tag{4.12}$$

$$P = Y_{P/S}(S_o - S) \tag{4.13}$$

where, S_o = initial substrate concentration in the fresh medium, g/l. Equation (4.11) shows that at dilution rates much lower than μ_{max} the substrate concentration will be very low and proportional to D. The cell mass and ethanol concentrations calculated from Eqs. (4.12) and (4.13) will be maximal at low dilution rates, since S will be small. But as D approaches μ_{max}, Eq. (4.11) shows the exit substrate concentration approaching infinity. Actually, the substrate concentration can only rise to S_o since no substrate is being created. At this point, from Eqs. (4.12) and (4.13), the cell mass and ethanol

concentrations fall to zero and washout of the single stage fermentor occurs.

However, in a multi-vessel staged fermentation the dilution rate of the second and all succeeding fermentors may be greater than μ_{max} without washout taking place. This may be shown by solving for D_n in Eq. (4.9).

$$D_n = \mu_n + \frac{D_{n-1} X_{n-1}}{X_n} \tag{4.14}$$

Since, D_{n-1}, X_{n-1} and X_n are positive numbers, D_n can be larger than μ_{max} when the specific growth rate in the n^{th} fermentor, μ_n, is equal to μ_{max}. Equation (4.14) reflects that the feed to the second and latter fermentors contain cells which were produced in the previous fermentors. Therefore, to maintain a stable cell mass concentration, not as many cells need to be produced in these fermentors.

The overall ethanol productivity of any continuous system is defined as,

$$Q = \frac{F}{V_T} P = D_T P \tag{4.15}$$

where, Q = overall productivity, grams ethanol produced/(liter of total fermentation volume-hr)

F = medium flow rate, l/hr

V_T = total fermentation volume, l

P = ethanol concentration, g/l

D_T = system dilution rate based on total volume, hr^{-1}

The specific cell ethanol productivity can then be calculated.

$$\nu = \frac{Q}{X} \tag{4.16}$$

where ν = specific cell ethanol productivity, grams ethanol produced/(gram cells-hr)

X = cell mass, g/l

4.3. Cell Recycle

In a cell recycle fermentation system a portion of the cells is separated from the fermented broth and returned back to the main fermentor. The cell recycle increases the cell mass concentration in the fermentor and allows a single stage fermentor to be operated at a dilution rate higher than the specific growth rate of the cells. A schematic of a cell recycle system is shown in Fig. 4.1. F 1/hr of fresh medium is fed to the fermentor and WF 1/hr of cell concentrate is recycled back to the fermentor. The relationship between the specific growth rate, μ, of the cells and the fermentor dilution rate, D, is given by Ref. 4.

$$\mu = D\left[1 + W\left(1 - \frac{1 + W}{1 + W - \frac{F_e}{F}}\right)\right] \tag{4.17}$$

Provided the cell mass concentration of the recycle stream is much greater than the cell mass concentration in the fermentor.

where W = recycle ratio, $0 \leq W \leq 1.0$

F_e = flow rate of effluent from separator, 1/hr

F = flow rate of fresh medium, 1/hr.

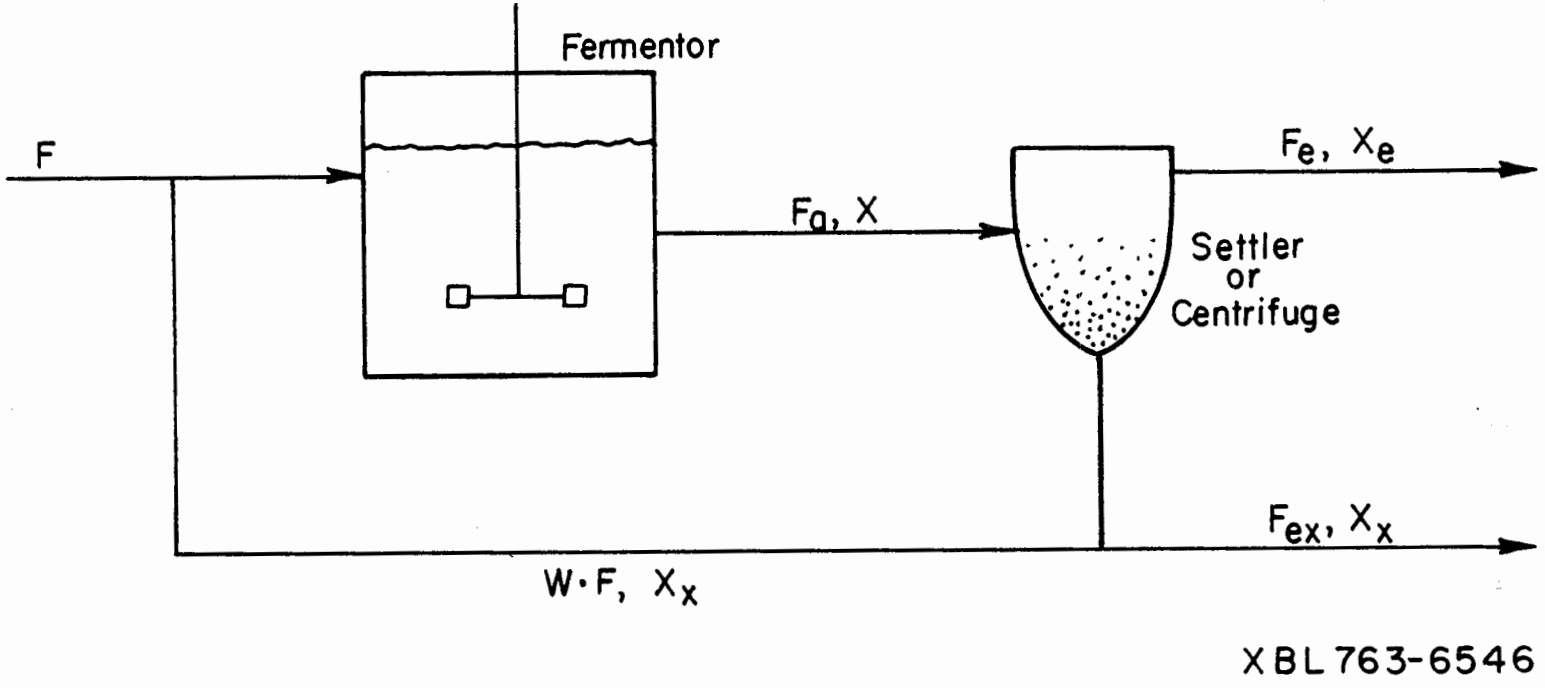


Fig. 4.1. Single vessel with cell recycle. F=flow of fresh medium, F_a=flow rate of cell suspension from fermentor, F_e=flow rate of effluent from separator, F_{ex}=flow rate of cell concentrate from separator, W=recycle ratio, X=cell mass concentration in fermentor, X_e=cell mass concentration in effluent from separator, X_x=cell mass concentration in recycled suspension.

Equation (4.17) indicates that steady state operation can be realized even if the value of D in single vessels is larger than μ, provided the cell recycle ratio, W, is selected appropriately. This implies that the ethanol productivity of a recycle system can be higher than schemes not employing cell recycle because the dilution rate in Eq. (4.15) can be increased. The rational of cell recycle operation may be viewed as increasing the cell mass concentration in the fermentor which produces a higher fermentation rate per unit volume of fermentor.

4.4. Vacuum Fermentation

The vacuum fermentation system takes advantage of the high volatility of ethanol. The ethanol is boiled away from the fermentation broth as it is formed, thus eliminating end product inhibition. The entire fermentation is carried out under a sufficient vacuum to cause boil off of the ethanol at a temperature compatible with the yeast. Since ethanol inhibition is eliminated with vacuum fermentation, high concentrations of sugar (50%) can be fermented. Also the cell mass in the vacuum fermentor may be maintained at a high level because only water and ethanol are vaporized and the cells remain in the fermentor. The ability of the vacuum system to use high sugar concentrations and to sustain high cell densities results in extremely high ethanol productivities.

The fermentation broth in the vacuum scheme may be idealized as a binary mixture of ethanol and water. The equilibrium vapor pressure of the solution is given by

$$P = \gamma_e X_e P_e^s + \gamma_w X_w P_w^s \tag{4.18}$$

where, P = equilibrium vapor pressure of solution, mmHg

X_e = mole fraction of ethanol

X_w = mole fraction of water

γ_e = activity coefficient of ethanol

γ_w = activity coefficient of water

P_e^s = saturation pressure of ethanol, mmHg

P_w^s = saturation pressure of water, mmHg.

The mole fraction of ethanol in the fermentor is held at a constant low value of 0.02 (5 wt%) to circumvent ethanol inhibition. Thus the activity coefficients in Eq. (4.18) may be assumed constant. The vapor pressure of ethanol and water are given by the Antoine Equation.[5]

$$\log P_e^s = 8.0449 - \frac{1554.3}{T - 50.5} \tag{4.19}$$

$$\log P_w^s = 8.1076 - \frac{1750.29}{T - 38.0} \tag{4.20}$$

where, T = temperature, °C.

The temperature is set by the optimum fermentation temperature of the yeast. With the temperature and composition set, the binary equilibrium is completely specified and the operating pressure of the fermentor is calculated from Eq. (4.18). In actual practice the inorganic salts in the fermentation broth decrease the fermentation pressure as calculated by Eq. (4.18). However, this was found to be a minor effect when compared to changes in ethanol concentration.

During steady state operation, water and the ethanol produced by fermentation are boiled away from the fermentor. The ethanol-water vapor is condensed and collected in a product receiving tank. The carbon dioxide formed during fermentation does not condense and must be compressed up to atmospheric pressure to maintain a vacuum in the fermentor. The ethanol mole fraction in the vapor will be higher than in the fermentation broth because of ethanol's high relative volatility. An equilibrium expression relating the mole fraction of ethanol in the vapor and broth is shown in Eq. (4.21).

$$Y_e = \frac{\alpha_{12}(X_e/X_w)}{1 + \alpha_{12}(X_e X_w)} \tag{4.21}$$

where, Y = vapor mole fraction

X = liquid mole fraction.

The subscript e refers to ethanol and w to water. The relative volatility, α_{12}, of the ethanol water system is calculated from a correlation due to Hala.[5] (Equation A.41 in Appendix 1.) If the fermenting broth contains 5 wt% of ethanol, Eq. (4.21) yields an ethanol composition of 32 wt% in the vapor. Thus only a portion of the initial feed must be vaporized to hold the ethanol mole fraction in the fermentor constant.

As ethanol and water are boiled away from the fermentor, fresh medium is fed to the fermentor to maintain the liquid level and supply substrate for fermentation. The boil up rate and hence the medium feed rate are controlled by the energy input to the fermentor. As more energy is transferred to the fermentation broth, the boil

up rate and feed rate are increased. The temperature, however, remains constant as is determined by the equilibrium relation set forth in Eqs. (4.18) through (4.20).

Because only ethanol and water are vaporized, unmetabolized inorganic salts and vitamin supplements accumulate in the fermentor. High concentrations of these constituents can have a deleterious effect on the yeast fermentation rate. Thus, a continuous bleed stream of fermented broth is required to keep the concentration of non-volatile components low enough so that they do not hinder fermentation. Cells also are removed with the bleed stream and this lowers the cell density in the fermentor. However, the cell concentration in the vacuum fermentor will still be larger than that experienced in conventional continuous culture. The cell mass concentration, as a function of fermentor bleed rate, is shown in Eq. (4.22) for the vacuum system.

$$X = \frac{F}{B} Y_{X/S}(S_o - S) \tag{4.22}$$

where, X = cell mass concentration, g dry wt/l

F = fresh medium feed rate, 1/hr

B = fermentation bleed rate, 1/hr

$Y_{X/S}$ = cell yield factor, g cell/g substrate

S_o = initial substrate concentration, g/l

S = exit substrate concentration, g/l

The cell mass increases in Eq. (4.22) as the bleed rate is decreased. Typically, a F/B ratio of 5 can be used in the vacuum system. This alone produces a fivefold increase in cell mass over conventional

continuous operations, provided $Y_{X/S}$ is constant. As mentioned previously, the initial substrate concentration, S_o, can also be increased in vacuum fermentations. Again from Eq. (4.22), an increase in substrate concentration raises the cell density in the fermentor.

The possibility of using the vacuum scheme with cell recycle also exists. If this is done, the cell density is independent of bleed rate. As shown in Chapter 6, very high cell concentrations are obtained with the vacuum-cell recycle combination, the end result being extremely high ethanol productivities.

REFERENCES

1. A. H. Cook, The Chemistry and Biology of Yeast (Academic Press, NY, 1958).

2. J. Monod, Ann. Inst. Pasteur 49, 333 (1913).

3. A. S. Aiyar, R. Luedeking, Chem. Eng. Progr. Symp. Ser. 62(69), 55 (1968).

4. S. Aiba, A. E. Hymphrey, N. F. Millis, Biochemical Engineering (Academic Press, NY, 1973).

5. E. Hala, T. Wichterle, J. Polak, T. Boublik, Vapor-Liquid Equilibrium Data at Normal Pressures (Pergamon Press, NY, 1958).

5. EXPERIMENTAL PROCEDURES

5.1. Yeast Strain and Culture Medium

The organism used in the alcohol fermentation studies was Saccharomyces cerevisiae, ATCC #4126. This yeast was chosen because it has been used for industrial ethanol production and ferments at high temperatures.[1] The yeast was stored at 4°C on agar slants of composition shown in Table 5.1. Every month new slants of the yeast were cultured to insure high viability and lower the chances of strain degradation.

Inocula for the fermentation experiments were prepared by transferring a small amount of yeast cells with an inoculating loop to a 250 ml shake flask containing 150 ml of sterile liquid medium shown in Table 5.2. The shake flasks were then incubated at 35°C in a reciprocating shaker water bath for 16 hr. A volume equal to 2% of the fermenter working volume of the resulting yeast suspension was added to the laboratory fermenter as the inoculum.

The medium listed in Table 5.2 is the standard media used in all batch and continuous experiments. When the sugar concentration was increased for the vacuum fermentation experiments, all other components were increased by the same ratio. The medium components were mixed and sterilized together at 121°C for 30 min for both batch and continuous fermentations. However, for the vacuum fermentation experiments, it was necessary to sterilize the glucose and minerals separately to avoid caramelization of the glucose, which, while not affecting ethanol or cell mass production, did interfere with the optical determination of cell mass concentrations in the vacuum fermentation system.

Table 5.1. Agar support composition.

Component*	g/l
Glucose (anhydrous)	20.0
Yeast extract (Difco)	2.2
NH_4Cl	0.33
$MgSO_4 \cdot 7H_2O$	0.03
$CaCl_2$	0.02
Agar (Difco)	15.0
Tap water	Make up to 1 liter

*All salts and glucose reagent grade.

Table 5.2. Base fermentation media.

Component*	g/l
Glucose (anhydrous)	100.0
Yeast extract (Difco)	8.5
NH_4Cl	1.32
$MgSO_4 \cdot 7H_2O$	0.11
$CaCl_2$	0.06
Anti-foam (General Electric AF60)	0.2 mls
Tap water	Make up to 1 liter

*All salts and glucose reagent grade.

Separate sterilization was accomplished by dissolving the glucose in water equivalent to 67% of the desired medium volume and the salts and yeast extract in the remaining 33% of the water. After sterilization in separate containers the solutions were allowed to cool to ambient temperature and mixed.

5.2. Experimental Apparatus and Operation

5.2.1. Batch Fermentations

A one liter "Mini Ferm" fermentor (New Brunswick Scientific) shown in Fig. 5.1 was used for the batch fermentation studies. The temperature was regulated by immersing the fermentor jar to 1/4 its height into a temperature controlled water bath. Agitation was supplied by a 1.5 in. Teflon coated magnetic stirring bar driven by a variable speed magnetic stirrer through the bottom of the water bath and fermentor jar.

The fermentor was charged with 800 ml of medium and sterilized. After securing the fermentor in the water bath, agitation was started and a flow of 20 ml/min of air was passed through the fermentor head space for 12 hr to insure the broth was initially saturated with oxygen at atmospheric conditions. The air was sterilized by filtration through a fiberous glass wool filter 5 cm in diameter and 10 cm long with a packed volume fraction of glass wool of 0.033.

The air flow was stopped and the fermentor inoculated through a silicone septum with a syringe. Thereafter, every hour for the duration of the experiment, 15 ml samples were withdrawn through the septum with a 20 ml syringe for analysis.

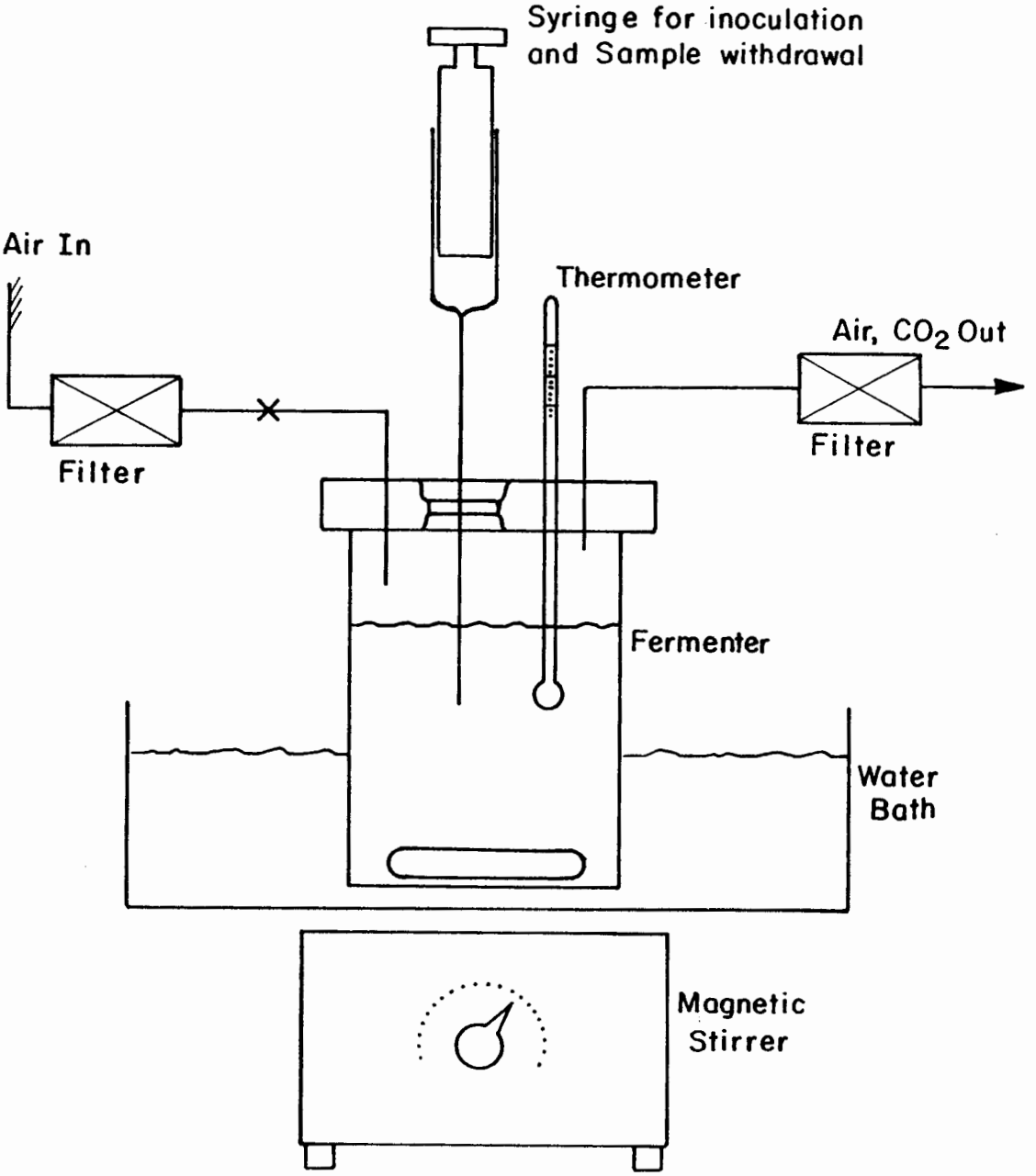


Fig. 5.1. Batch Fermentation Apparatus

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5.2.2. Continuous Fermentations

The continuous fermentations were conducted in a 5 liter "Micro Ferm" fermentor (Fermentation Design Model MA501). The continuous culture apparatus is shown in Fig. 5.2. The pH was controlled with a Fermentation Design Ph-RT recorder-controller module used in conjunction with an Ingold 761-351B combination pH electrode. The pH was held at the set point by automatic addition of either 6M H_2SO_4 or 6M NaOH to the fermenter. A continuous and constant flow of sterile medium was pumped by a kinetic clamp pump (Sigmamotors Model (TM-20-2)) to the fermentor from a 20 liter reservoir. The reservoir was replaced as needed through the term of an experiment. A fermentor working volume of 2 liters was maintained by an overflow port in the side of the fermentor jar. Air, sterilized by filtration, was sparged through the fermentor and the oxygen tension of the fermentation broth measured with a New Brunswick oxygen probe connected to a Leeds and Northrup Speedomax Type G recorder. The oxygen tension was controlled by changing the RPM of the agitator and/or the air flow to the fermentor.

The fermentor was first filled with 2 liters of medium and sterilized. With the fermentor in place, agitation and aeration were commenced and the system inoculated when the fermentor cooled to the fermentation temperature (35°C). At the end of batch growth, (usually 12 to 16 hr after inoculation) the medium feed pump was turned on. The medium flow rate was determined throughout the experiment by timing the filling of a graduate cylinder placed under the outlet of the fermentor.

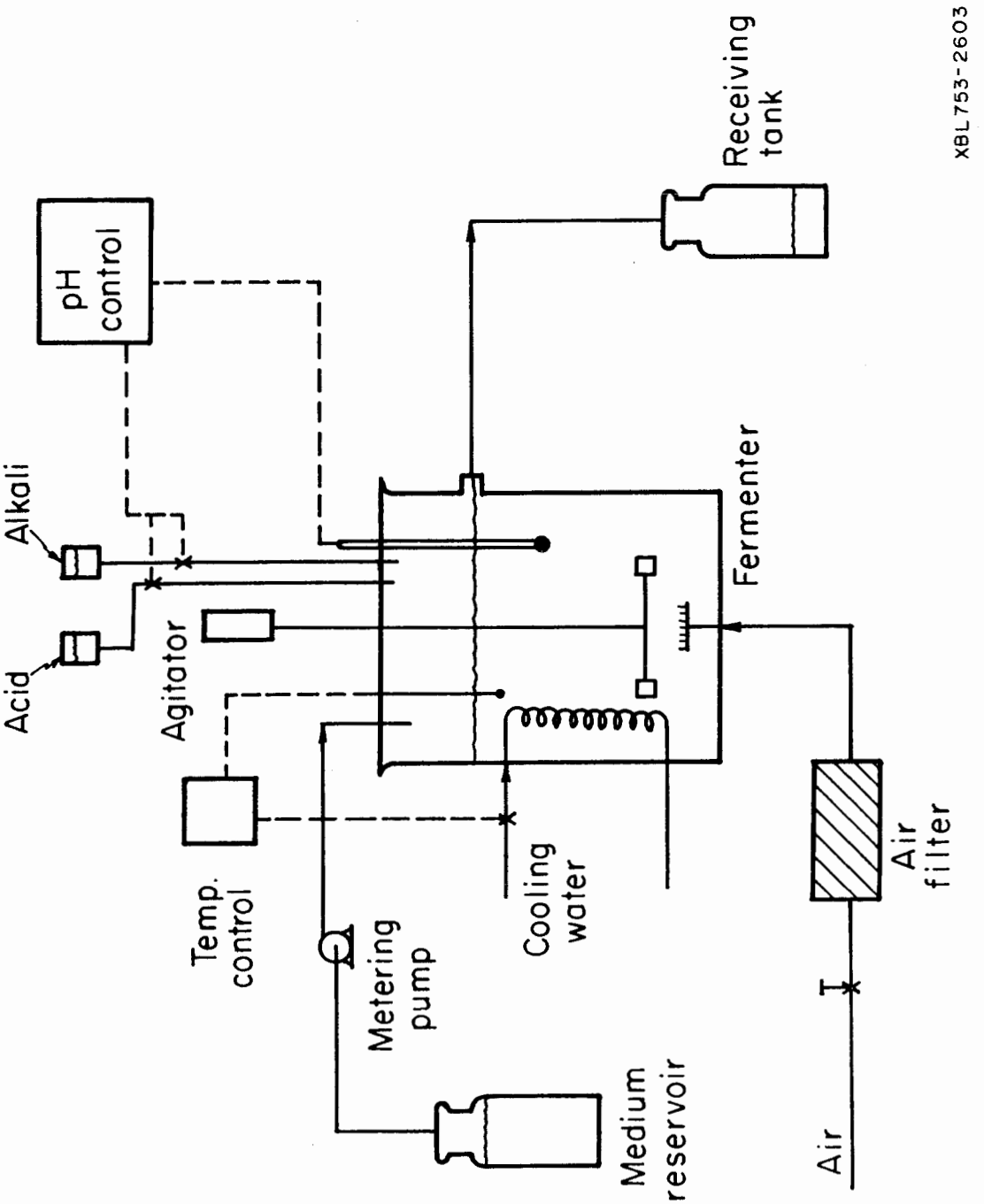


Fig. 5.2. Continuous Fermenter

After three fermentor volumes (i.e., 6 liters) of broth passed through the fermentor, 10 ml of samples of effluent were collected every 4 hr and the cell mass concentration measured. When the cell mass remained constant over an 8 hr period steady state operation was assumed and a 20 ml sample was aseptically withdrawn from the fermentor for analysis. Experimental conditions were then changed and a new steady state established.

5.2.3. Vacuum Fermentation

A 5 liter Fermentation Design fermentor used for continuous culture was adapted for the vacuum fermentation studies. A schematic of the complete vacuum system is shown in Fig. 5.3. In order to achieve the required boil up rate of ethanol and water a 1500 watt heater was added to the temperature control loop of the fermentor. The heater was constructed for four 10 in. diameter coils of 1/2 in. copper tubing wrapped with electrical heating tape. The heat input was controlled by adjusting either of two variable auto-transformers (Superior Electric Company Type 3PN1168). A 1 in. stainless steel pipe connected to the fermentor inoculation port led to two shell and tube condensers (American Standard No. 47M200-8A2) arranged in series. The vapor generated in the fermentor was condensed on the shell side of the exchangers by a 10% methanol-water solution chilled to 4.0°C by a Haws model HR4-24W water cooler. The condensate was then collected in a 40 liter stainless steel tank which was set in a dry ice bath. In addition, a 50 liter ballast tank was connected to the apparatus to dampen periodic pressure fluctuations.

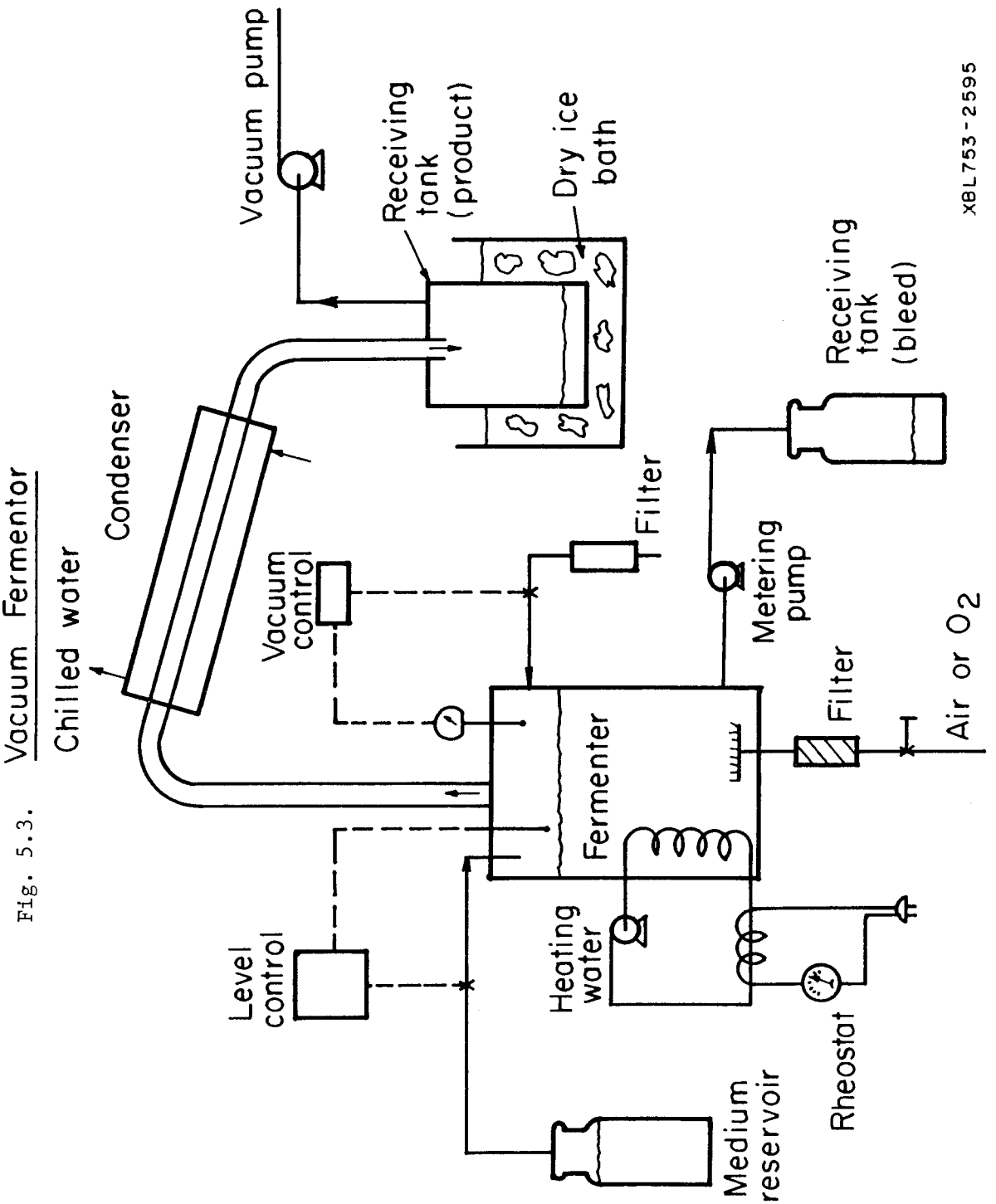

Vacuum Fermentor
Chilled water
Condenser
Vacuum pump
Receiving tank (product)
Dry ice bath
Receiving tank (bleed)
Vacuum control
Filter
Metering pump
Filter
Air or O_2
Fermenter
Level control
Heating water
Rheostat
Medium reservoir
XBL753-2595

Fig. 5.3.

The entire system was connected to a Kinney model K2-8 vacuum pump. The vacuum pump ran continuously and the pressure was controlled by a Manowatch model MW-1 controller (Instruments for Research and Industry, Inc.) which activated a solenoid valve that bled filtered air into the system when the pressure became too low. Although the fermentor pressure fluctuated 1-2 mmHg with this method of pressure control, it was found superior to placing the solenoid valve in line with the vacuum pump, as recommended by the manufacture, because the small pressure fluctuations helped to control foaming in the fermentor and allowed better liquid level control. This is discussed in more detail below. The absolute pressure in the fermentor was measured with a Zimmerli gauge.

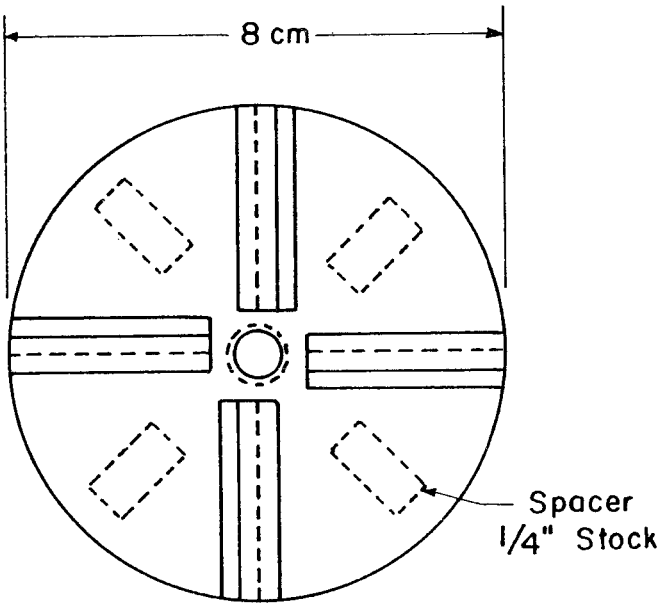
As the liquid level in the fermentor dropped due to boil off of vapor and the bleed off of the fermented broth, a liquid level controller (Cole Palmer Model 7186) opened a solenoid valve connected to the medium reservoir, and sterile broth was sucked into the fermentor. A liquid level probe was constructed of a 1/4 in. stainless steel rod which was forced down 1/2 in. teflon tubing so that both ends were exposed for electrical contacts. The Teflon coating was necessary because its high hydrophobic surface properties did not allow a condensate film to form on the probe. A liquid film (water) short circuits the probe and causes the controller to sense a high liquid level. However, during long term experiments the anti-foam and protein constituents of the medium adsorbed onto the Teflon, changing the surface properties and producing a short circuit. This was corrected by wrapping the length of probe above the heat plate

with heating tape to boil off any surface water.

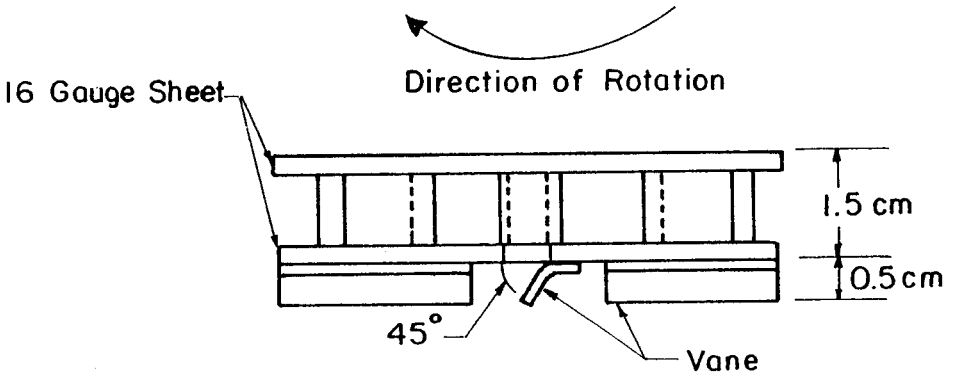
A bleed of fermented broth and cells was withdrawn from the fermentor by a kinetic clamp pump (Sigmamotors Model (TM-20-2)) into a 4 liter jar which was maintained at the same pressure as the fermentor. The cell bleed rate was adjusted by changing the speed of the pump, and measured by emptying the 4 liter jar at timed intervals and measuring the volume.

Although anti-foam was added to the fermentation broth, foaming proved to be a serious problem in the vacuum system. The foaming was overcome in part by arranging the pressure control system so that the pressure cycled 1-2 mmHg about the pressure set point of 50 mmHg. A complete pressure cycle required from 45 to 60 sec. As the pressure dropped below the set point, rapid boiling in the fermenter commenced and foaming became extensive. When the pressure reached 1 mmHg below the control point a solenoid valve was actuated and air was rapidly bled into the system until the pressure was a 1 mmHg above the set point. At this point, the boiling and foaming subsided somewhat, allowing the liquid level control system to sense the true liquid height and introduce the required volume of fresh medium into fermentor. With the solenoid valve closed, the pressure then began decreasing and the cycle repeated.

The extensive foaming experienced during the low pressure side of the pressure cycle was kept to a low level by the use of a mechanical foam breaker shown in Fig. 5.4. The foam breaker was connected to the agitator shaft 2-3 cm above the liquid level. When agitation levels were low (low impeller RPM) the foam breaker had little effect on collapsing the foam, however, foaming ceased to



Bottom View



Side View

All material 306 stainless steel

Fig. 5.4. Mechanical Foam Breaker

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be a serious problem at low agitation levels. But, as the agitation was increased and more foam was generated due to the greater dispersion of gas bubbles, the foam breaker became more effective. The net effect of the foam breaker and the fluctuating pressure produced very stable operation of the vacuum system over the wide range of experimental conditions.

The vacuum fermentor was sterilized in place by filling the fermenter with 300 ml of a 70 vol% ethanol-water solution and boiling the solution under 250 mmHg total pressure (house vacuum) for 8 hr. The system was then flushed with air (3 liter/min) for 4 hr to remove the last traces of the sterilizing solution. The fermentor was filled with three liters of 10% glucose medium shown in Table 5.2, brought to 35°C and inoculated. An air rate of 0.5 liters/min was maintained during batch growth. At the end of batch growth (12 to 16 hr) the air flow was stopped and 0.12 VVM (240 mls/min at STP) of oxygen was sparged through the fermentor. The pressure in the fermentor was slowly decreased, 25 mmHg/min, until the fermentation broth began boiling at 35°C. As the ethanol in the fermentation broth boiled off, the pressure was further lowered to 50 mmHg to maintain boiling.

When the volume of the fermentor dropped to 2 liters due to boil off of liquid, the liquid level controller began adding fresh sterile medium from a 40 liter reservoir, thus maintaining a 2 liter fermentor working volume.

The fermentation was run for 24 hr at these conditions; 35°C, 50 mmHg and no cell bleed, to build up the cell mass concentration to around 50 g dry wt/liter. At this point the cell bleed pump was

turned on and experimental conditions adjusted and the system allowed to come to steady state.

5.2.4. Cell Recycle

Cell recycle experiments were run with both the continuous and vacuum fermentation apparatus using a jacketed settler vessel. A diagram of the settler arrangement is shown in Fig. 5.5. The inner chamber was constructed of 100 mm Pyrex tubing. A 1 in. hole at the bottom of the chamber was plugged with a rubber stopper through which penetrations were made to remove clarified liquid and cell concentrate. The upper end was sealed with a 1 in. plexi-glass disk using a foam rubber gasket. The pressure in the settler and receiver flask was equilized enabling the clarified liquid to overflow by gravity to the receiver flask. The clarified liquid overflow rate was controlled by adjusting the difference between the pumping rate of the feed to the settler from the fermentor and the pumping rate of the cell concentrate recycle stream.

A solution of methanol and water chilled to -4.0°C was circulated through the jacket to slow fermentation in the settler. The settler system was operated at a total pressure of 250 mmHg in the vacuum system and at atmospheric pressure in the conventional continuous system. Both cooling the settler and operating at a pressure higher than the vacuum fermentation pressure of 50 mmHg was necessary to minimize mixing effects of CO_2 evolved during fermentation.

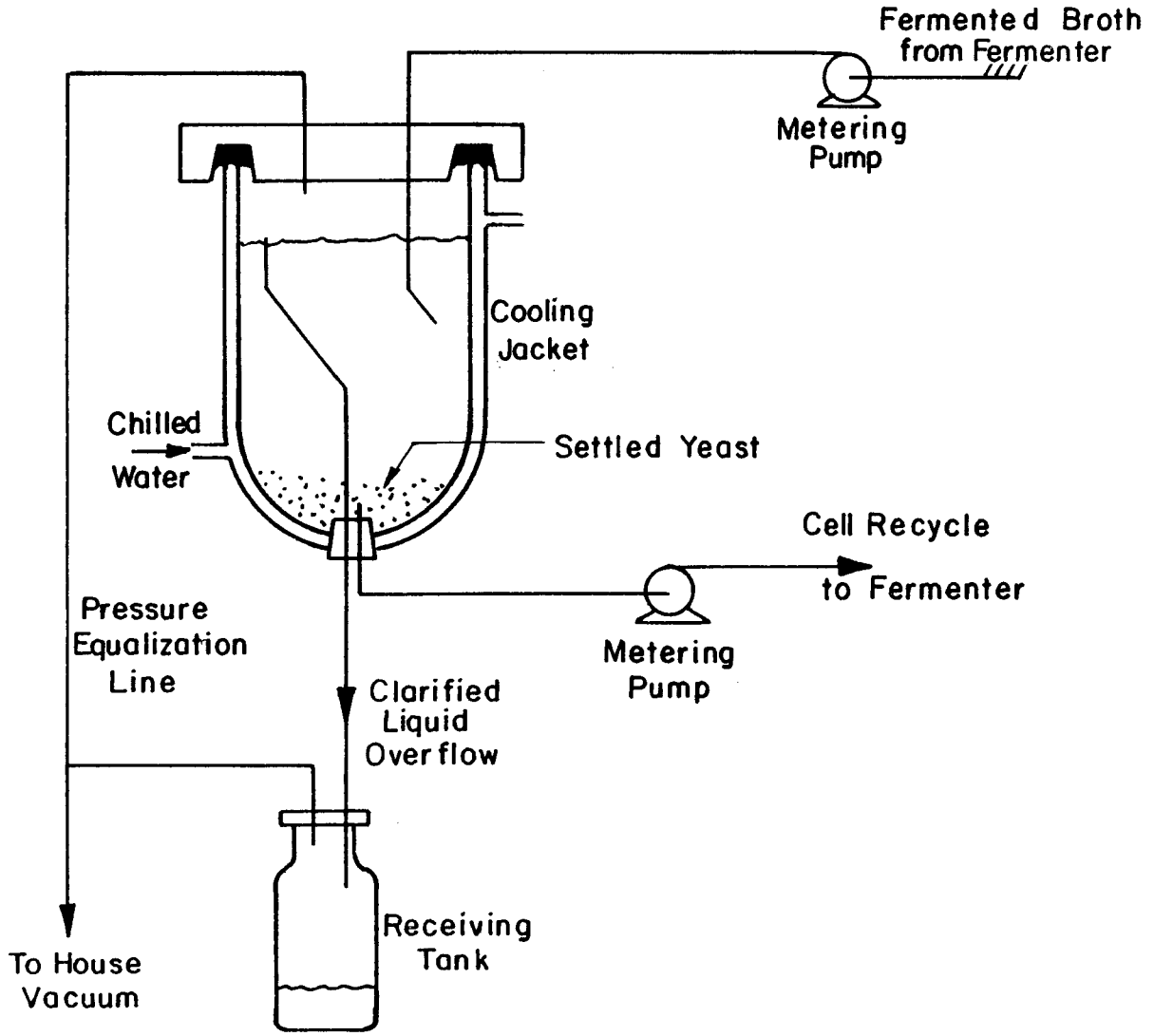

Fig. 5.5.

Settler Used for Cell Recycle Experiments

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5.3. Assay Procedures

5.3.1. Ethanol Concentrations

Ethanol was measured by gas chromatography using an Aerograph 1520 G-L Chromatograph. A 6 ft 1/4 in. column packed with chromasorb W-acid type 60-80 mesh was used with a flame ionization detector. The injector and detector temperatures were 175°C and the column oven operated isothermally at 105°C. A calibration curve of chromatogram areas vs ethanol concentration was determined. The ethanol compositions of the unknown samples was then read directly to ±2% of the total ethanol concentration once the sample's chromatogram area was found.

5.3.2. Cell Mass Concentrations

The cell mass concentrations were determined optically using a Fischer Electrophotometer with a 650 mμ filter. The standard curve shown in Fig. 5.6 was prepared by measuring the optical density (absorbance) of samples of varying cell concentrations. The cell mass (g dry wt/liter) of the samples was determined by centrifuging the cells, washing twice with distilled water and drying the cells at 105°C until no further weight change occurred. The cell mass of unknown samples was then found by measuring the optical density and reading the cell concentration directly from Fig. 5.6 after the proper dilution had been made so that the cell concentration of the sample was between 0.01 g/l and 0.35 g/l. The accuracy of the optical determination of yeast cell mass was consistantly found to be better than ±5%.

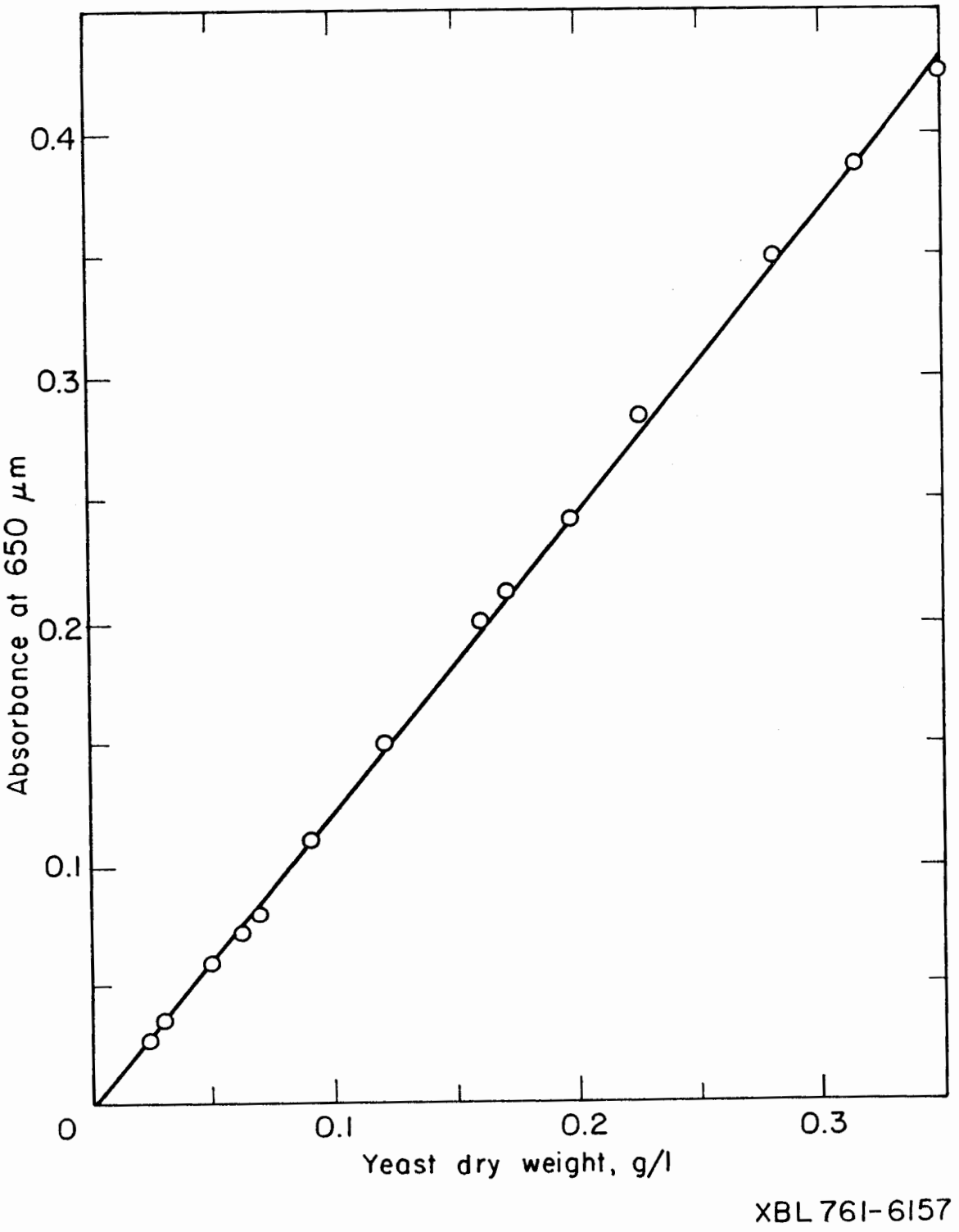

Fig. 5.6. Yeast dry weight, g/1, vs absorbance at 650 μm.

5.3.3. Glucose Concentration

Glucose was determined by the dinitrosalicylic acid (DNS) method.[2] The sample was clarified by centrifugation, and 1 ml added to 3 ml of DNS reagent and placed in a boiling water bath for 5 min. The reactants were cooled to room temperature and diluted with distilled water to a total volume of 24 ml. The absorbance of the diluted sample was measured at 600 mμ against an appropriate blank of DNS reagent and water with a Beckman DU-2 spectrophotometer. The absorbance reading was converted to glucose concentration from a standard calibration curve prepared with known concentrations of anhydrous glucose. With the DNS method, glucose concentrations down to 0.1 g/l could be determined with an accuracy of ±10%. However, the accuracy of this method was better than ±3% when the glucose concentration was above 1.0 g/l.

5.3.4. Oxygen Concentration

The oxygen concentration of the fermentation broth was measured with a galvanic type electrode (New Brunswick Scientific Co.). The current from the probe was passed through a variable resistor and the voltage drop recorded with a Leeds and Northrup Speedomax Type G recorder. In this system the current developed by the probe was proportional to the dissolved oxygen concentration in the fermentor. The oxygen probe was calibrated before each fermentation by placing it in a sulphite solution of zero oxygen concentration and an oxygen saturated solution. A 0.5 M Na_2SO_3 solution containing 0.004 M $CuSO_4$ was sparged with nitrogen for 1 hr to obtain the current at zero oxygen concentration. The saturation current was determined using

uninoculated fermentation broth sparged with air. The temperatures of these calibrations were carefully controlled at the temperature to be used in the subsequent fermentation. Once the calibrations had been made very low oxygen tensions (0.1 mmHg) could be measured ±0.05 mmHg by increasing the external resistance and switching the recorder from a 10.0 mV full scale reading to a 1.0 mV full scale reading. Of course the saturation calibration point was determined with a low external resistance and the 10.0 mV scale, while zero point calibration used a high external resistance and the 1.0 mV scale.

5.3.5. Yeast Viability

The percentage of viable yeast cells was determined using a methylene blue stain as described by Townsend.[3] Twenty ml of 0.2% methylene blue stain was mixed with approximately 0.5 g dry wt of washed cells. After the solution sat for 5 min, the stained dead cells and unstained viable cells were counted using a Betraff-Hausser cell counter.

REFERENCES

1. American Type Culture Collection Catalogue (Rockvill, Maryland, 1974), 11th edition.

2. J. B. Summer, G. E. Somers, Laboratory Experiments in Biological Chemistry (Academic Press, NY, 1944).

3. G. F. Townsend, C. C. Lindgren, Cytologia 18, 183 (1953).

6. RESULTS AND DISCUSSION

6.1. Batch Fermentation

Batch fermentations were used to determine the formulation of the fermentation medium and examine the effects of oxygen on fermentation rates. In all batch experiments the initial glucose concentration was 100 g/l and the initial pH of the broth was 5.5. During the fermentation the pH fell to a value between 4.0 and 3.5. As shown below, this low pH did not affect the fermentation rate of Saccharomyces cerevisiae. A 2 vol% inoculum prepared from either anaerobic or aerobic growth in shake flasks was used in the batch experiments.

Figure 6.1 summarizes the results of a typical batch fermentation. These data were obtained by initially air saturating the broth with oxygen before inoculation, but air was not sparged through the fermentor during fermentation. The composition of the medium is listed in Table 5.2. The fermentation temperature was held at 35°C.

As shown in Fig. 6.1, the yeast exhibits exponential growth for the first 8 hr of fermentation. During this period the specific growth rate of the yeast is 0.46 hr^{-1}. The fermentation requires 14 hr to run to completion, at which point, 5.4 grams of cells and 47 grams of ethanol were produced from 100 grams of glucose ($Y_{X/S} = 0.054$ and $Y_{P/S} = 0.47$). The ethanol yield is 92% of the theoretical yield and, hence, indicates near optimum fermentation conditions. The yield of cells is lower than reported by Aiba[1] during ethanol fermentations, but as discussed below, this does not effect the overall fermentation rate. Indeed, a low cell yield allows

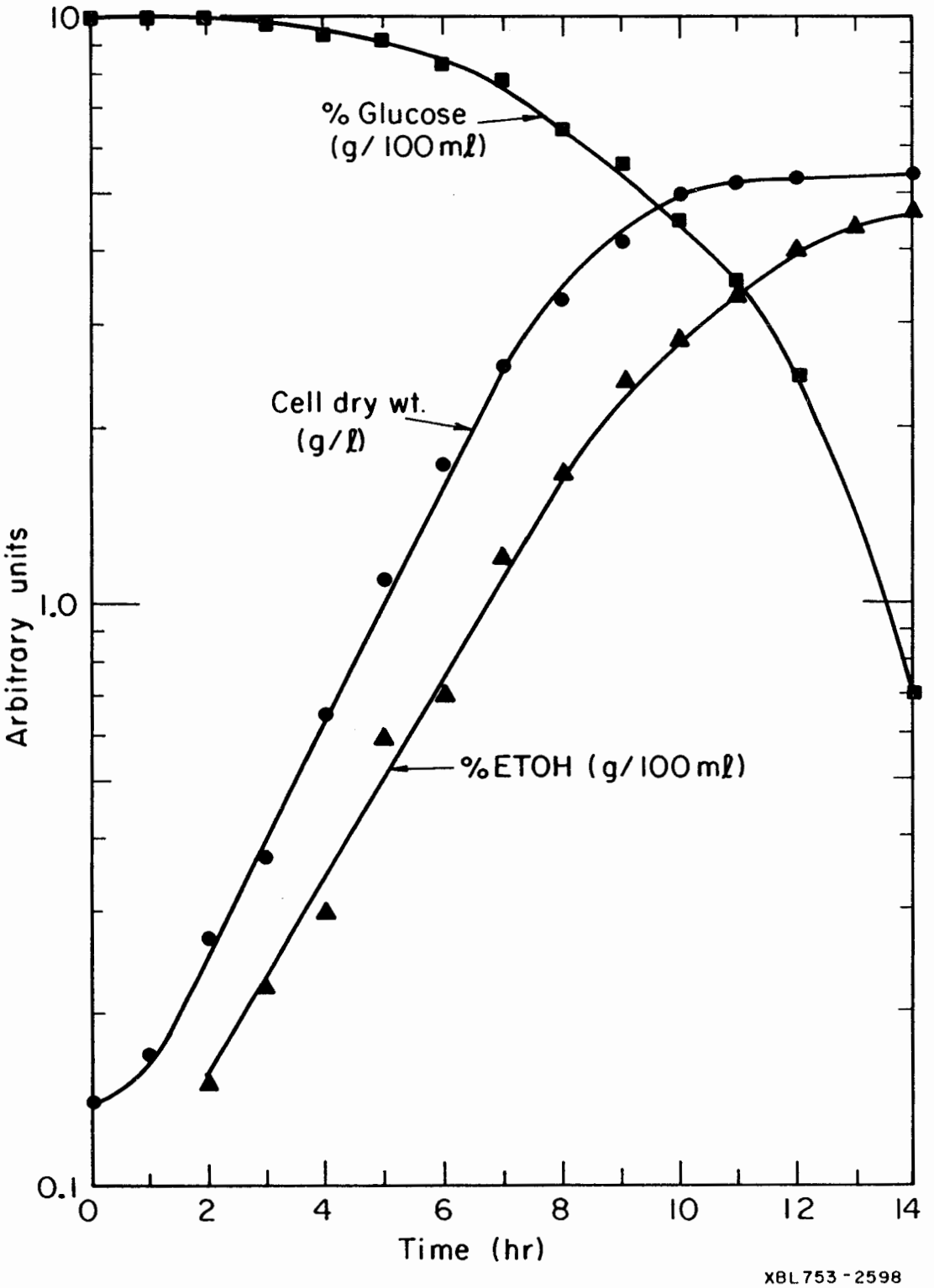

Fig. 6.1. Batch fermentation of initially air saturated medium using a 2% aerobically grown inoculum.

more glucose to be used for the production of ethanol rather than cell mass.

The ethanol production in Fig. 6.1 is growth associated. This is to be expected since the energy derived from the fermentation of glucose is used for the production of cell mass. The justification of the fermentation conditions in Fig 6.1 is now discussed.

6.1.1. Medium Formulation

The concentration of inorganic salts in the medium was initially based on the elemental composition of the yeast.[2] Yeast extract was added to the medium as a source of vitamins and amino acids. However, it was not possible to calculate the yeast extract requirement a priori because the exact vitamin and amino acid requirement of the yeast was not known. Figure 6.2 and Fig. 6.3 show the results of changing the yeast extract concentration in the fermentation broth. Again, the broth was initially saturated with air and a temperature of 35°C was used in these experiments.

The effect of yeast extract concentration on cell mass production, shown in Fig. 6.2, is relatively small. The yeast growth rate was independent of yeast extract concentration up to the seventh hour of fermentation. After the seventh hour the cell mass production began to level off when either 6.0 or 4.0 g/l of yeast extract was used. This indicates the yeast was starved for nutrients. There was no discernible difference in cell mass production if either 8.5 or 14 g/l of yeast extract was added to the medium. After 13 hr of fermentation there was only a 32% difference in total cell mass production between the 4.0 g/l curve and the 8.5 g/l curve. Beyond 13 hr the cell mass

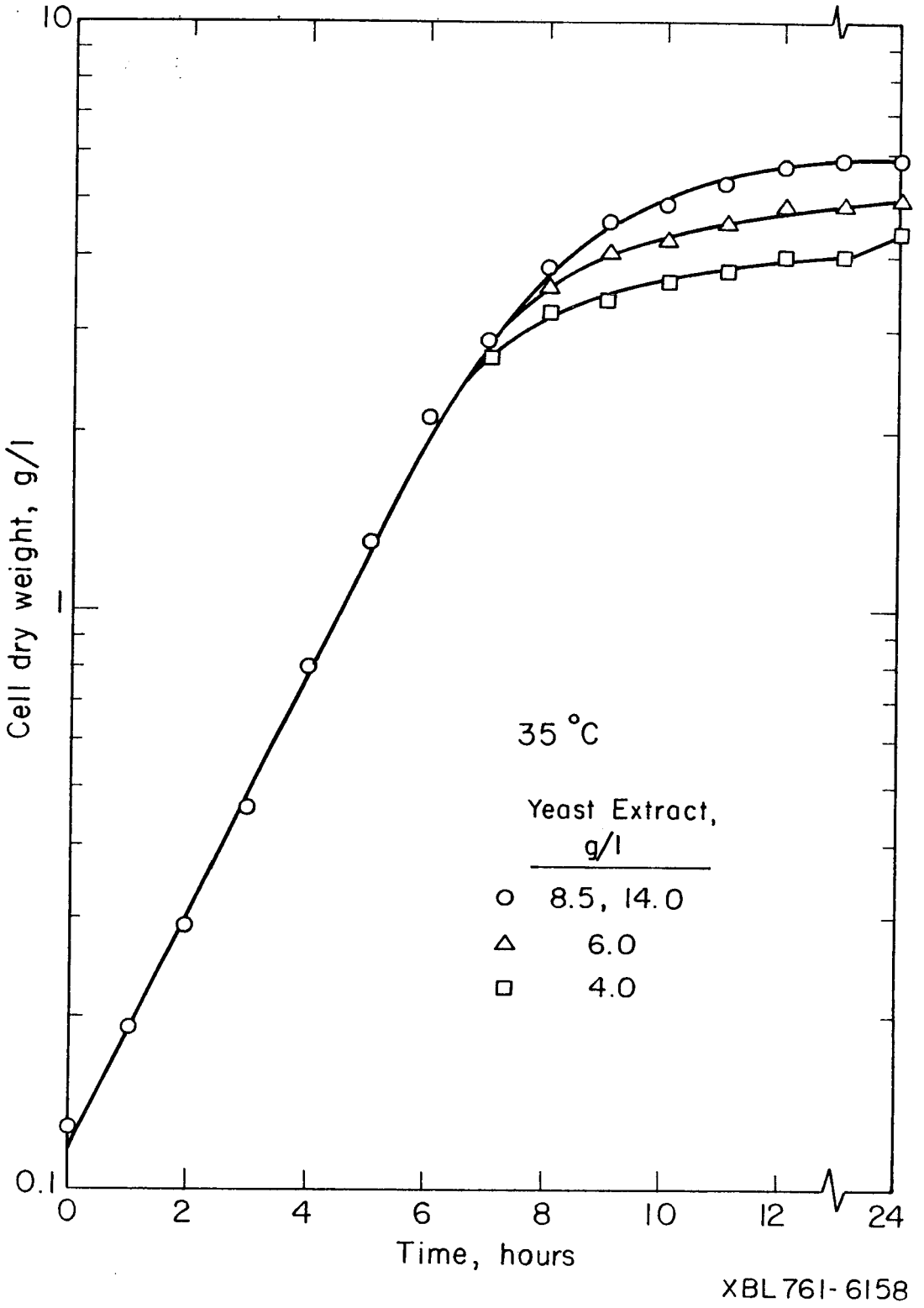

Fig. 6.2. The effect of yeast extract on cell mass production in batch fermentation.

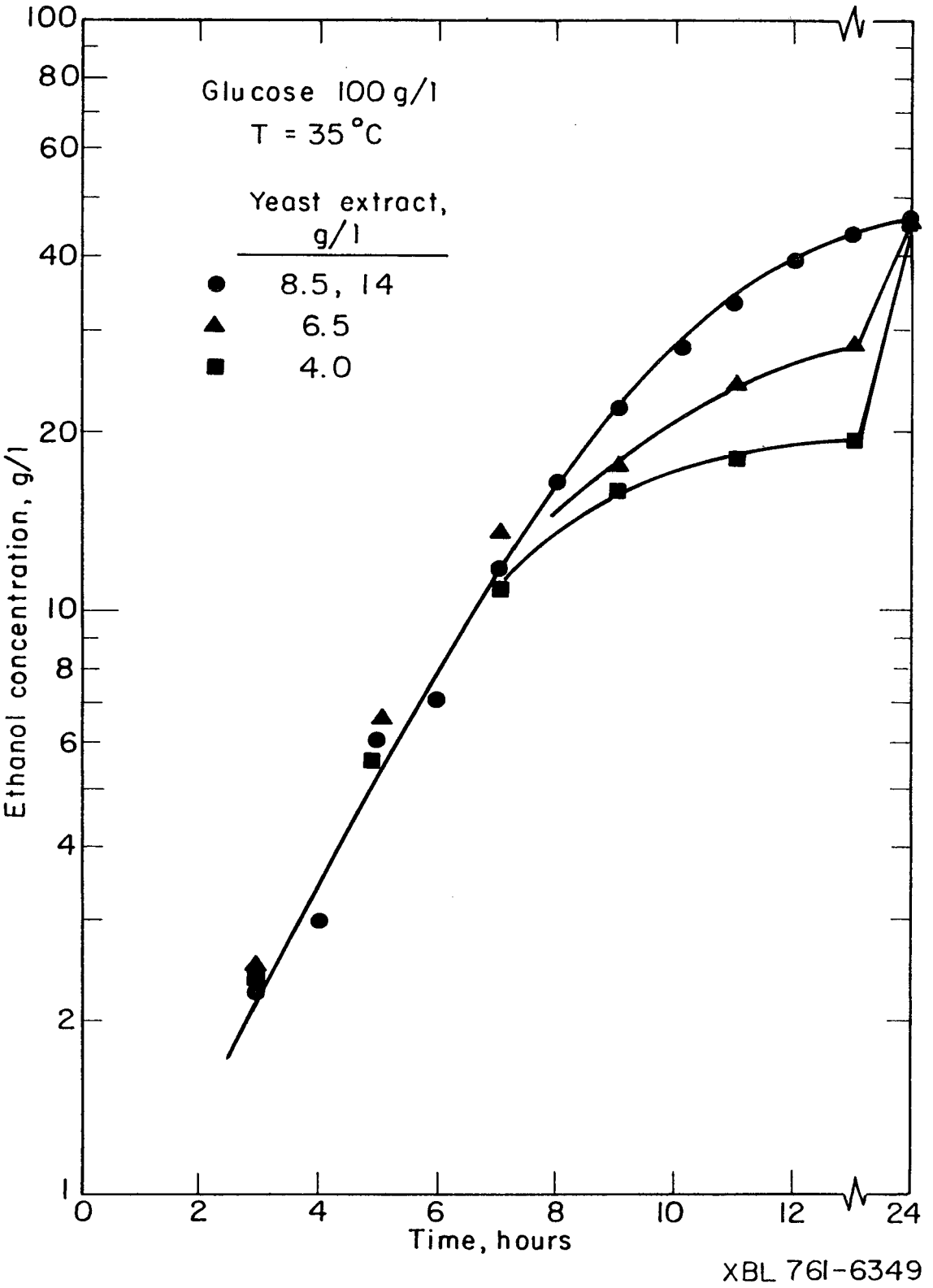

Fig. 6.3. The effect of yeast extract on ethanol production in batch fermentation.

concentration remained almost constant.

However, the effect of yeast extract concentration on ethanol production was much more pronounced. Figure 6.3 shows ethanol production was severaly restricted if the concentration of yeast extract was 6.0 or 4.0 g/1. Although not specifically shown in Fig. 6.3, 23 hr were required to reach the end of batch fermentation when the yeast extract concentration was 4.0 g/1 and 19 hr were required when 6.0 g/1 of yeast extract was used. But when either 8.5 or 14.0 g/1 of yeast extract was added the batch fermentation was complete in only 14 hr.

Comparison of Figs. 6.2 and 6.3 shows that at low concentrations of yeast extract ethanol production continued even though cell growth had stopped. This is indicative of growth limitation by the exhaustion of an essential growth factor. The growth factor is depleted before all the glucose has been fermented to ethanol. Growth ceases and glucose is only slowly fermented to supply energy for the maintenance of cellular integrity. This again points out that active yeast growth is necessary to obtain high fermentation rates.

From the above experiments the yeast extract requirement was set at 8.5 g per 100 g of glucose to be fermented. Yeast extract contains inorganic salts in addition to vitamins and amino acids. Thus, the amount of additional inorganic salts added to the medium could be lowered to less than initially predicted from the elemental composition of the yeast. Quantitative analysis of inorganic salts were made before and after fermentation using 8.5 g/1 of yeast in the fermentation broth. The inorganic salt concentrations listed in Table 5.2 are a

result of these analyses. The salt concentrations have been calculated to give a 10% excess over the absolute requirement.

The medium recipe of Table 5.2 will produce from 5 to 10 grams of cells per 100 grams of glucose fermented. Also the fermentation rate of Saccharomyces cerevisiae will not be increased by raising the concentration of the salts or yeast extract in Table 5.2. However, the fermentation rate may be decreased if the concentration of these components is lowered.

Enough trace minerals were present in the tap water and as impurities in chemicals so that the separate addition of trace elements was not required. If 1 ml of the trace element solution shown in Table 6.1 was added per liter of broth no difference in ethanol production or yeast growth was detected, as shown in Fig. 6.4.

General Electric Anti-Foam (AF-60) was added to prevent foaming during fermentation. Foam was produced as a result of carbon dioxide evolution and sparging the fermentor with air. Figure 6.4 shows the anti-foam had no effect on the fermentation.

The medium composition of Table 5.2 was used in all further ethanol fermentation studies. When the glucose concentration of the broth was altered all other components were changed by the same ratio.

6.1.2. Fermentation Temperature

Figure 6.5 shows the effect of temperature on the yeast growth rate and the maximum specific ethanol productivity in batch culture. A glucose concentration of 100 g/l was used and 15 mg/l of ergosterol was added to the fermentation broth. A flow of 20 ml/min of nitrogen

Table 6.1. Trace mineral solutions.

$ZnSO_4 \cdot 7H_2O$	50 mg
$MnSO_4 \cdot H_2O$	50 mg
$CuSO_4$	10 mg
$Co(NO_3)_2 \cdot 6H_2O$	10 mg
$Na_2B_4O_7 \cdot 10H_2O$	10 mg
$Na_2MoO_4 \cdot 2H_2O$	200 mg
Ferric-EDTA	100 mg
Distilled water to make	100 ml

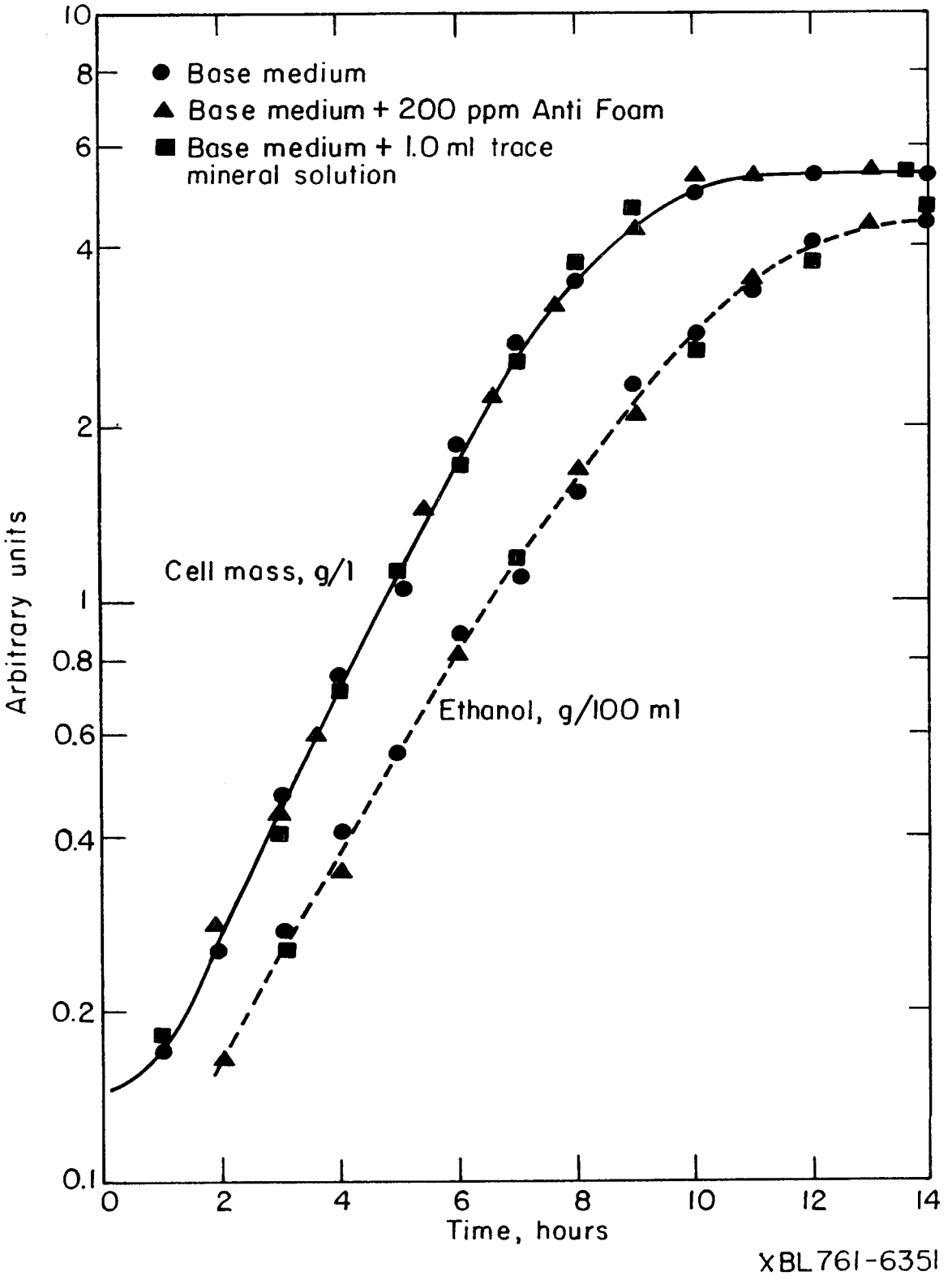

Fig. 6.4. The effect of Anti-foam and trace minerals on batch fermentation rates.

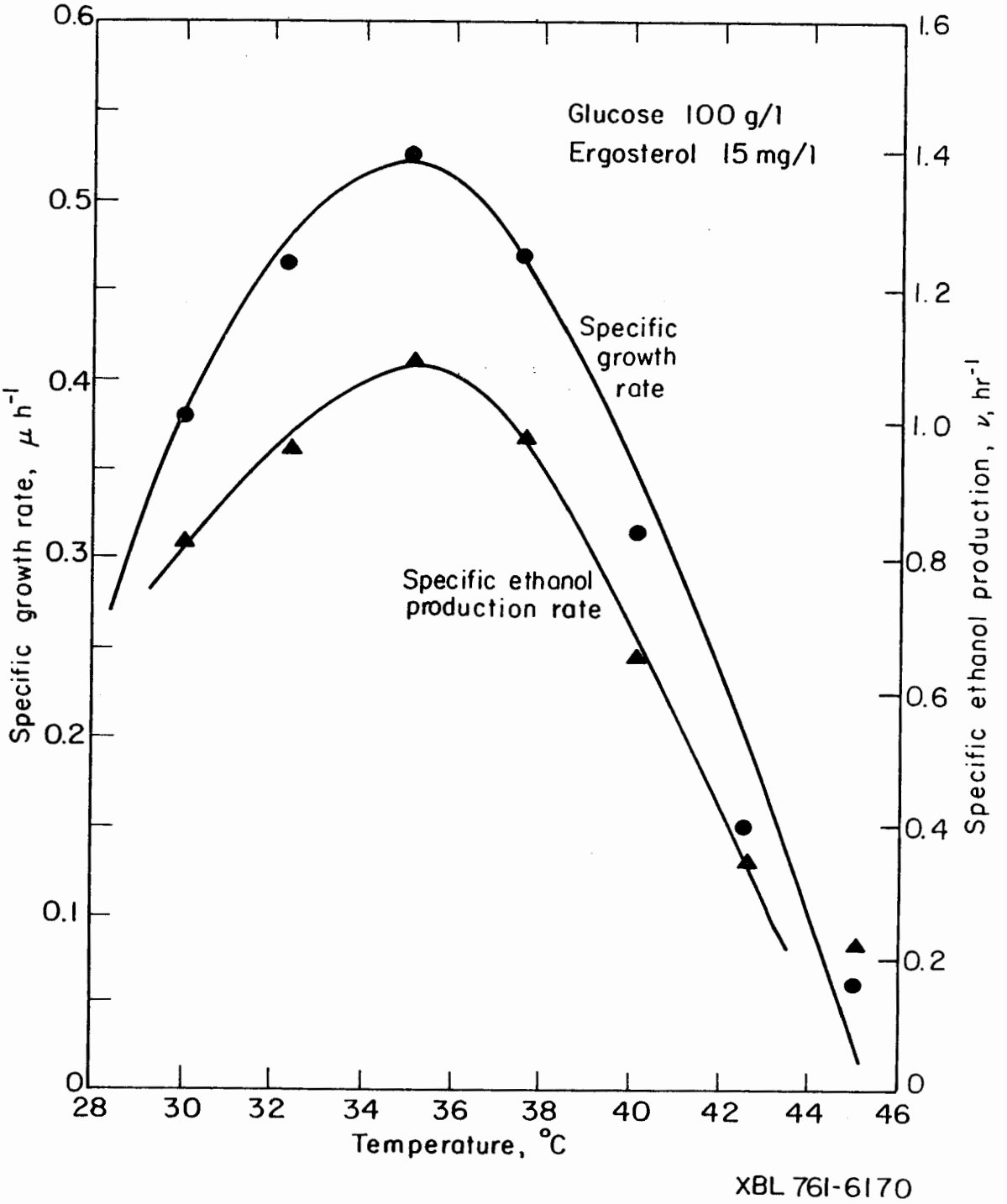

Fig. 6.5. Specific growth rate and specific ethanol production rate as a function of temperature in batch fermentation.

was continually sparged through the head space of the fermentor to maintain anaerobic conditions.

As discussed below, erogsterol was added to eliminate the oxygen requirement of the yeast. The fermentations could then be run under totally anaerobic conditions. This insured that the metabolism of the yeast was completely fermentative and that the experiments were not influenced by trace amounts of oxygen. While trace amounts of oxygen are beneficial to fermentation rates (see the next section) these trace concentrations are not easily reproduced in a series of batch experiments. Also, when oxygen is present the catabolism of the yeast may be partially aerobic,[3] and it was desired to obtain the optimum temperature for a strictly fermentative metabolism.

The optimum temperature for both cell growth and ethanol production is 35°C. The occurrence of the optimum specific growth and ethanol production rates at the same temperature reaffirms the fact that ethanol production is growth associated. High cell growth rates are necessary to achieve high ethanol production rates.

The specific growth rate is plotted against the specific ethanol productivity in Fig. 6.6 for the various fermentation temperatures. The linear relation between these two rates agrees with the kinetic model presented by Aigar and Ludiking[4] for ethanol production. It also shows the direct relation between growth and ethanol production. Ethanol production, however, becomes non-growth associated if yeast growth is limited by such factors as high concentration of ethanol and depletion of nutrients (i.e., when low concentrations of yeast extract were used). But optimal ethanol production is always associated

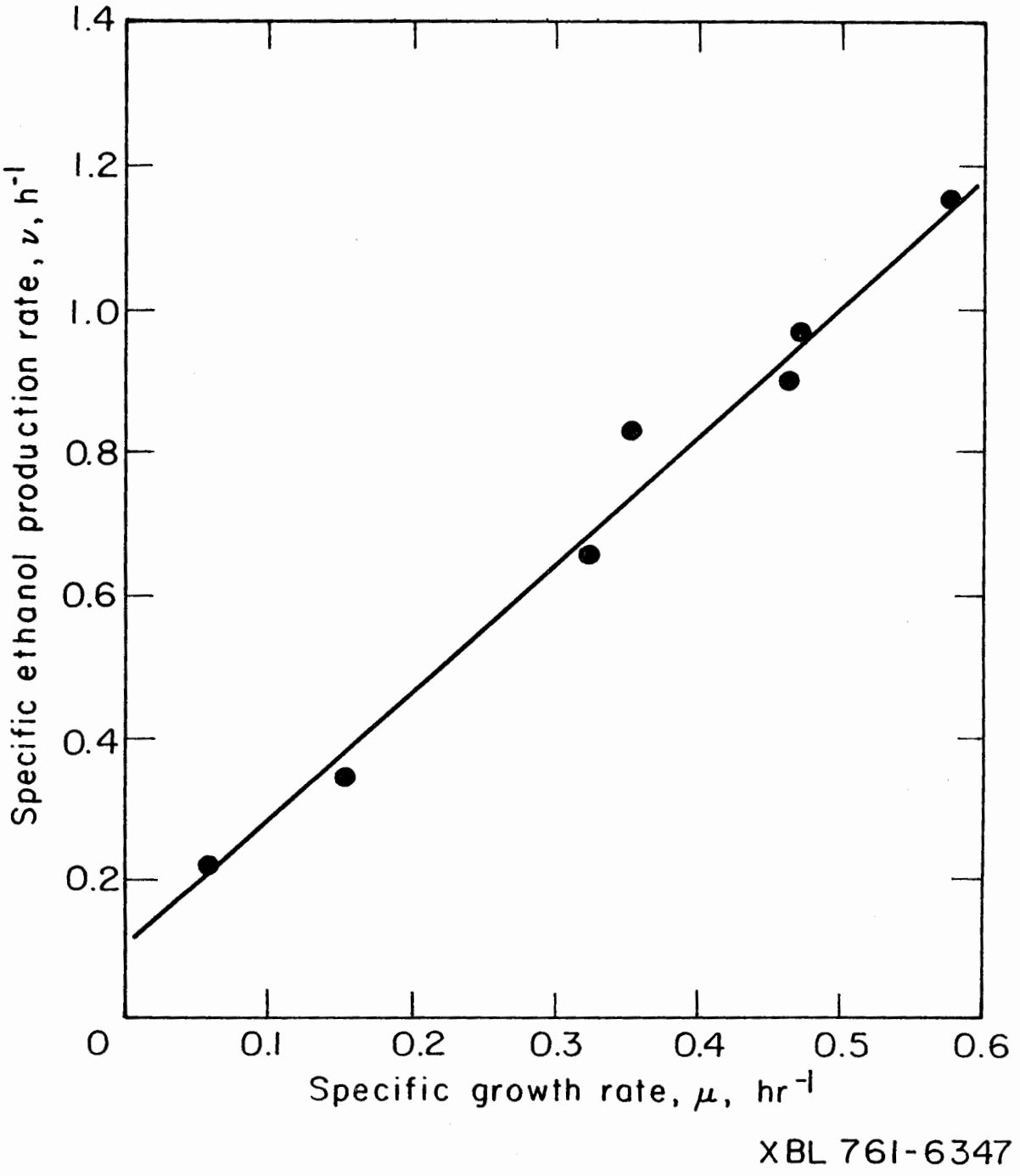

Fig. 6.6. Specific ethanol production rate as a function of specific growth rate in batch fermentation.

with high growth rates. Further illustrations of this fact are shown when the effect of oxygen on fermentation rates is examined.

The optimum fermentation temperature of 35°C is used in the remaining experiments. This is a higher temperature than normally used in fermentation work (25-30°C)[5,6] and certainly higher than used in the brewing industry (8-15°C).[7] However, temperature optimum experiments are not reported for the yeast strains employed in many studies. The beverage industry is forced to use low fermentation temperatures to reduce the production of fusel oils, which alter flavor.[8] But this is unimportant in the production of industrial ethanol. The fusel oil concentration, at most, is only 0.5% of the ethanol concentration.[9] This percentage of fusel oil has disastrous effects on the taste of the ethanol[10] but is an acceptable purity for the industrial use of ethanol, especially if the ethanol is used as a fuel.

A high fermentation temperature is advantageous in industrial ethanol fermentation processes. It allows easier temperature control of the fermentors in both batch and continuous operation because the temperature difference between the broth and cooling water is increased. Also, a high fermentation temperature in the vacuum system permits a higher pressure to be employed and still maintain boiling in the fermentor. This reduces the cost of compression equipment.

6.1.3. Effect of Oxygen

The effect of oxygen in batch fermentation is demonstrated in Fig. 6.7. A fermentation temperature of 35°C was used in these experiments and the initial glucose level was 100 g/l. The data presented in the upper curves were obtained by initially air saturating the fermentation broth and using an aerobically grown inoculum. Air was not sparged through the fermentor during fermentation. An expanded view of the upper curves is shown in Fig. 6.1. Anaerobic conditions were maintained in the lower set of curves by continually sparging with nitrogen. The fermentor was first sparged with nitrogen for 12 hr to deoxygenate the broth and then inoculated with an anaerobically grown culture.

The specific growth rate of the air saturated culture was 0.46 hr^{-1} and ethanol production paralleled growth. The fermentation reached completion in 14 hr. But under anaerobic conditions, the specific growth rate was only 0.15 hr^{-1} and the fermentation required over 80 hr to reach completion. The ethanol production of the anaerobic experiment paralleled growth initially. Toward the end of the experiment, however, growth stopped and ethanol production slowly continued until all the glucose was fermented.

Comparison of the two ethanol production curves again shows the necessity of achieving high growth rates to produce rapid ethanol fermentation rates. It should be pointed out that the oxygen in the initially air saturated culture was used for anabolic purposes and did not enter into the catabolism or energy yielding pathways. This may be seen by the identical yield of ethanol obtained in the air

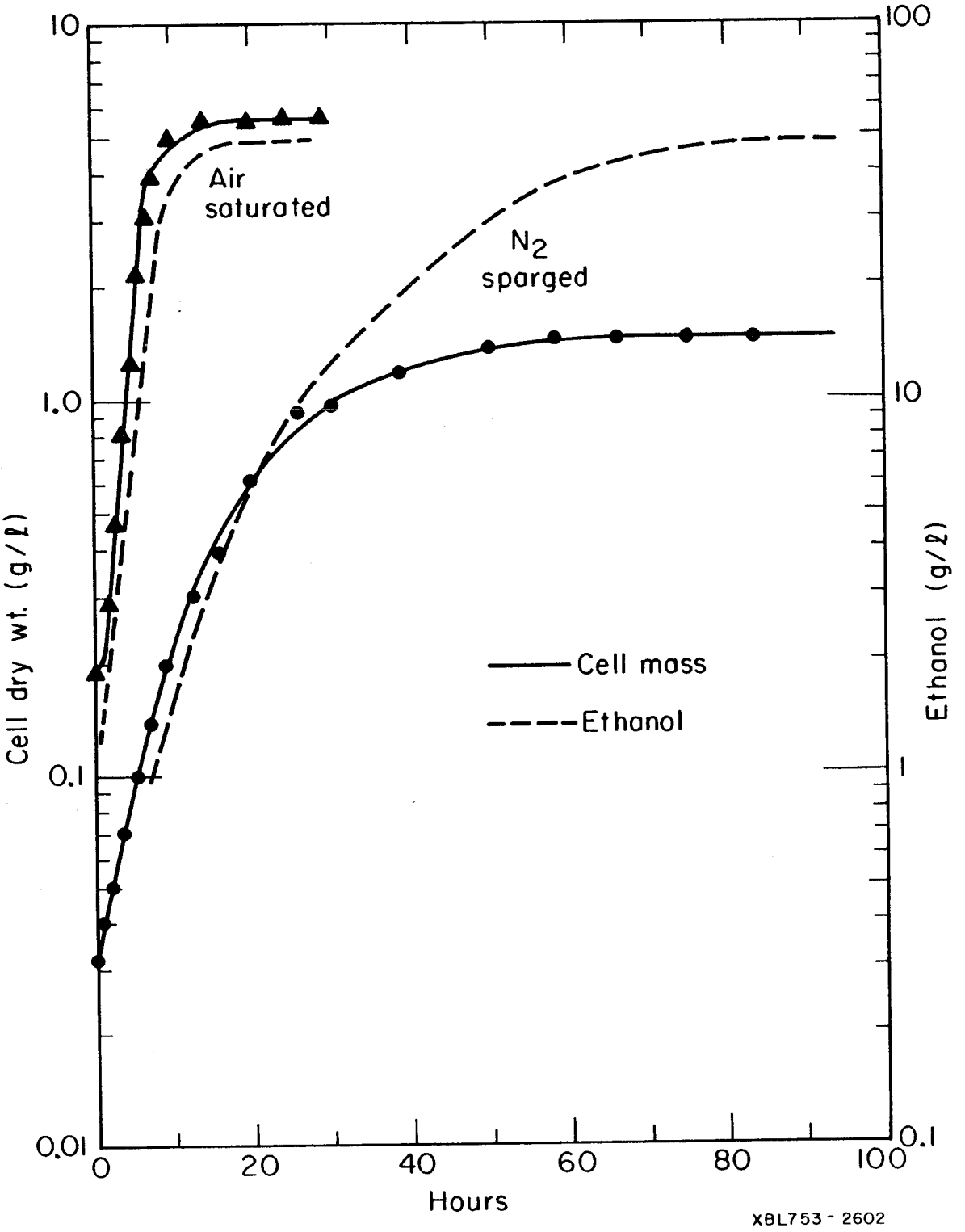

Fig. 6.7. Batch fermentation rates when the medium is initially air saturated and when the medium is sparged with nitrogen.

saturated and anaerobic experiments. In both cases $Y_{P/S}$ was 0.47.

The negligible production of cell mass under anaerobic conditions after the fiftieth hour is due to oxygen limitation. As previously discussed, oxygen is required as a growth factor by Saccharomyces, enabling the synthesis of unsaturated fatty acids and lipids.[6,11] When the supply of these unsaturated compounds, which was either carried over by the inoculum or initially present in the medium, has been exhausted anaerobic growth stops. Because growth has been limited by the lack of an essential nutrient (oxygen) the fermentation need only supply enough energy for the maintenance of cellular integrity. As a result, the fermentation proceeds at a very slow pace.

The limited yeast growth under anaerobic conditions may be due to trace amounts of oxygen in the nitrogen gas sparged through the fermentor. Even high purity commercial nitrogen has been shown to contain enough oxygen to support limited yeast growth.[12,13] If the nitrogen was further purified to remove the last traces of oxygen, no yeast growth was reported. No attempt was made to purify the nitrogen in this work and determine if the yeast had an absolute oxygen requirement. Since components in the medium can affect the oxygen requirement (see below), this experimentation would have been little use because semi-complex media was used in this work.

The growth history of the inoculum can affect fermentation under anaerobic conditions. This is why the inoculum for the nitrogen sparged fermentation was grown anaerobically. If an inoculum is grown under aerobic conditions, the yeast has the ability to produce unsaturated fatty-acids and lipids and store these components for use

during anaerobic growth.[5,6] This affected the fermentation in the air saturated culture even though only a 2% aerobic inoculum was used. This is illustrated in Fig. 6.8 in which the broth was initially saturated with air and inoculated with anaerobically grown yeast. Yeast growth began at the same rate as if an aerobic inoculum had been used ($\mu = 0.46\ hr^{-1}$). After 8 hr the growth rate decreased and yeast growth stopped after 14 hr. However, ethanol production continued until the eighteenth hour.

The fact that yeast growth stopped while ethanol production slowly continued is analogous to the anaerobic growth in Fig. 6.7. This indicates that fermentation of initially air saturated broth is partially oxygen limited if an anaerobic inoculum is used. Whereas, this is not the case if an aerobic inoculum is employed, as shown in the upper curves of Fig. 6.7 and in Fig. 6.1.

It is surprising that the inoculum can have such a large affect on fermentation rates. Saccharomyces, however, has been shown to produce and store ergosterol, an unsaturated lipid, to as much as 10% of the cells dry weight during aerobic growth.[9] But no ergosterol is produced under anaerobic conditions and, as shown in Fig. 6.7, yeast growth is very slow. However, if only 0.3% of the final yeast dry weight in ergosterol is added to the medium, rapid growth is obtained under anaerobic conditions (see the next section). There is a factor of 33 in difference between the ergosterol content of aerobic cells and that actually required during anaerobic growth. The aerobically grown cells can use this surplus of ergosterol for growth during anaerobic conditions, and if the above reasoning is correct, produce

33 times more cells than initially present in the inoculum. This explains why the growth history of even a 2% inoculum can have a pronounced effect on fermentation rates.

The oxygen concentration as a function of time is also shown in Fig. 6.8 for a batch fermentation. The oxygen concentration rapidly decreases to zero in the first 2 hr of fermentation. During this time very little cell mass is produced. This agrees with the observation of Maule et al.,[15] who examined the batch fermentation of brewers wort. These findings indicate that rapid fermentation commences only after all the oxygen has been adsorbed by the yeast.

The oxygen was not used for energy production. Rapid growth continued for 6 hr after the oxygen concentration had dropped to zero. If oxygen were used for energy production, the growth rate should have decreased as soon as the oxygen was depleted. However, the effect of the initial amount of oxygen in the saturated broth was evident well after the oxygen had been consumed.

One explanation of this phenomena is that the yeast first consumed the oxygen for the production of unsaturated compounds or their precursors. Only a minor amount of cell mass was produced during this time. When all the oxygen had been depleted growth proceeded using the pool of unsaturated lipids thus formed. This implies that unsaturated components are transferred from mother to daughter cells during the budding process.

The growth and ethanol production curves obtained when the oxygen tension in the fermentor was maintained at 0.7 mmHg are shown in Fig. 6.9. (This oxygen tension was found to be optimal for continuous fermentations.)

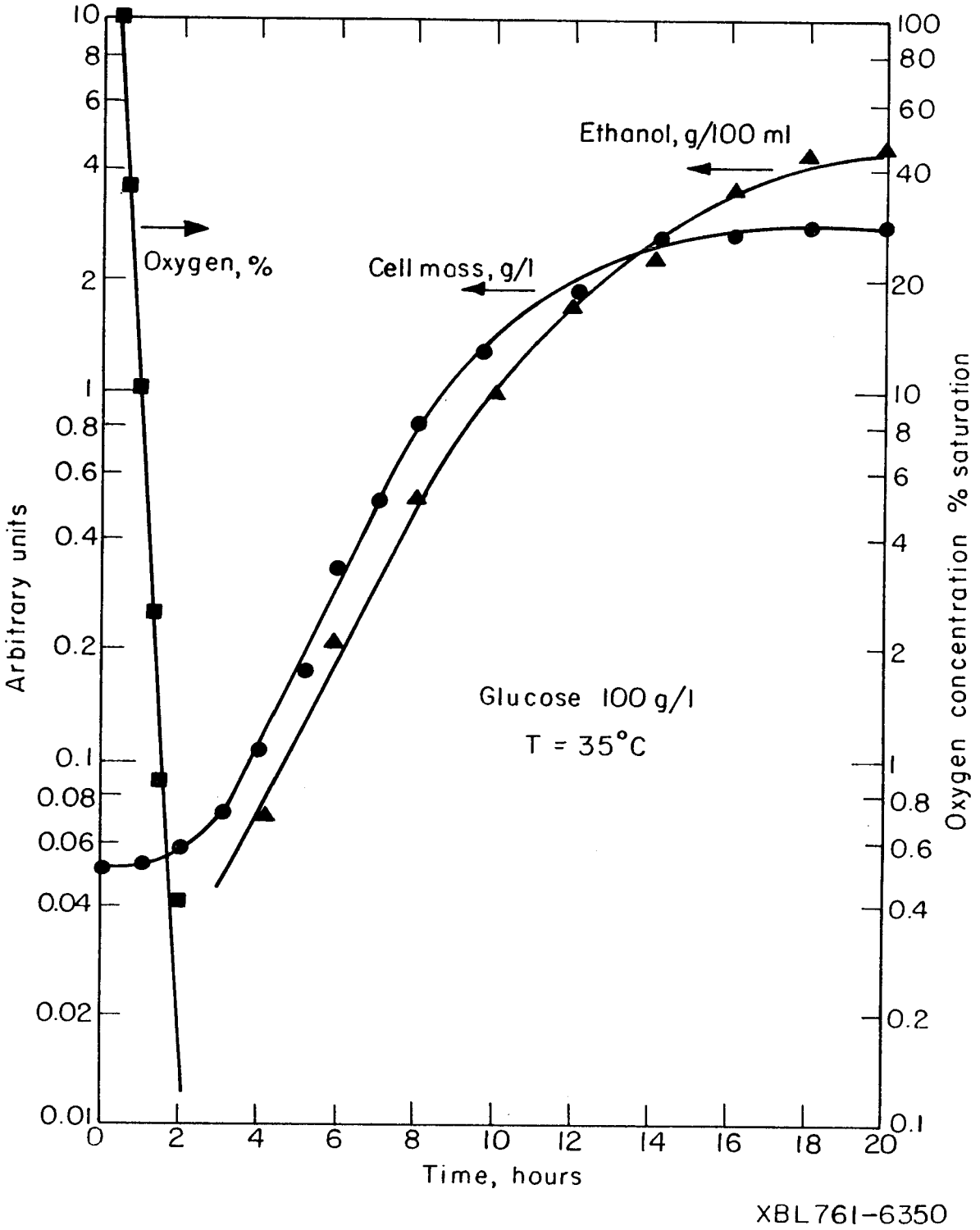

Fig. 6.8. Batch fermentation of initially air saturated medium employing an anaerobically grown inoculum.

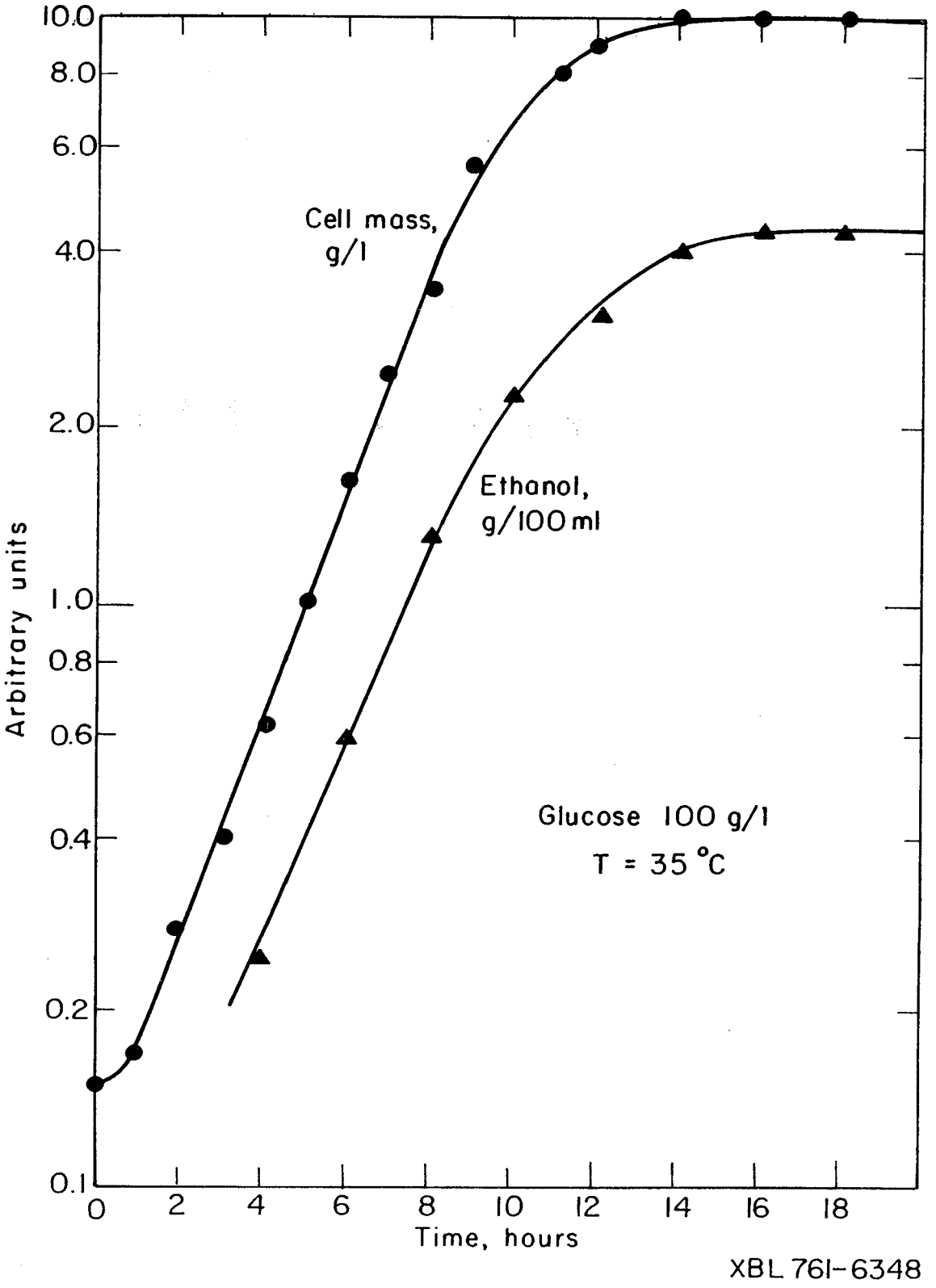

Fig. 6.9. Batch fermentation when the oxygen tension of the broth is maintained at 0.7 mmHg.

The oxygen tension was held constant by continually adjusting the air sparging rate during the fermentation. An aerobic inoculum was used.

When the oxygen tension was held at this level the total cell yield, $Y_{X/S}$, was 0.1 grams cells/gram glucose consumed. This is higher than the cell yield of 0.056 obtained if the medium was only initially saturated with air and air was not sparged through the fermentor.

The increased cell mass was produced at the expense of ethanol production. The ethanol yield, $Y_{P/S}$, was only 0.44 in this experiment, whereas, it was 0.46 if an initially air saturated broth was used and the concentration of oxygen was allowed to drop to zero.

The cell yield factor of 0.1 obtained when the oxygen concentration was held at 0.7 mmHg indicates that conditions for cell growth are near optimum (see continuous fermentation section). However, the fermentation time was not reduced and 16 hr were required to complete the fermentation. This was 2 hr longer than required if the broth was initially air saturated and the concentration of oxygen permitted to fall to zero (Fig. 6.1).

Thus the optimal batch fermentation procedure was to first air saturate the broth and use an aerobically grown inoculum. No air sparging of the fermentor was required. With this arrangement the fermentation of a 10% glucose solution required just 14 hr. Only 5.6 grams of cells were produced and the ethanol yield was 92% of the theoretical yield.

6.1.4. Effect of Ergosterol

Ergosterol was first shown to stimulate anaerobic yeast fermentations by Andreasen and Stier.[12] The effect of ergosterol on yeast growth and ethanol production is shown in Figs. 6.10 and 6.11, respectively. In these experiments anaerobic conditions were maintained by continually sparging nitrogen through the fermentor. The use of an anaerobic inoculum minimized the possibility of initial yeast cells containing a pool of unsaturated compounds. A stock solution of ergosterol was prepared as described by Andreasen[12] and an appropriate volume was added to the medium to give the desired concentration.

The increase in growth and ethanol production rates when the ergosterol concentration was above 7 mg/l is striking. When more than 7 mg/l of ergosterol was used the specific growth rate of the yeast was 0.56 hr^{-1} and the total fermentation time was 16 hr. Whereas, under anaerobic conditions without ergosterol, the specific growth rate was only 0.15 hr^{-1} and fermentation proceeds for 80 hr. Addition of more than 7 mg/l of ergosterol had little affect on the fermentation rate. Although, more cell mass was produced when the concentration was increased to 10 mg/l.

The data in Figs. 6.10 and 6.11 show that a concentration of 7 mg/l of ergosterol completely eliminated the oxygen requirement of the yeast. This is why numerous workers[14,16] have hypothesized that the role of oxygen during fermentation is for the production of unsaturated compounds, specifically ergosterol. However, the possibility exists that ergosterol is partially metabolized by the yeast to produce other unsaturated lipids and fatty acids.

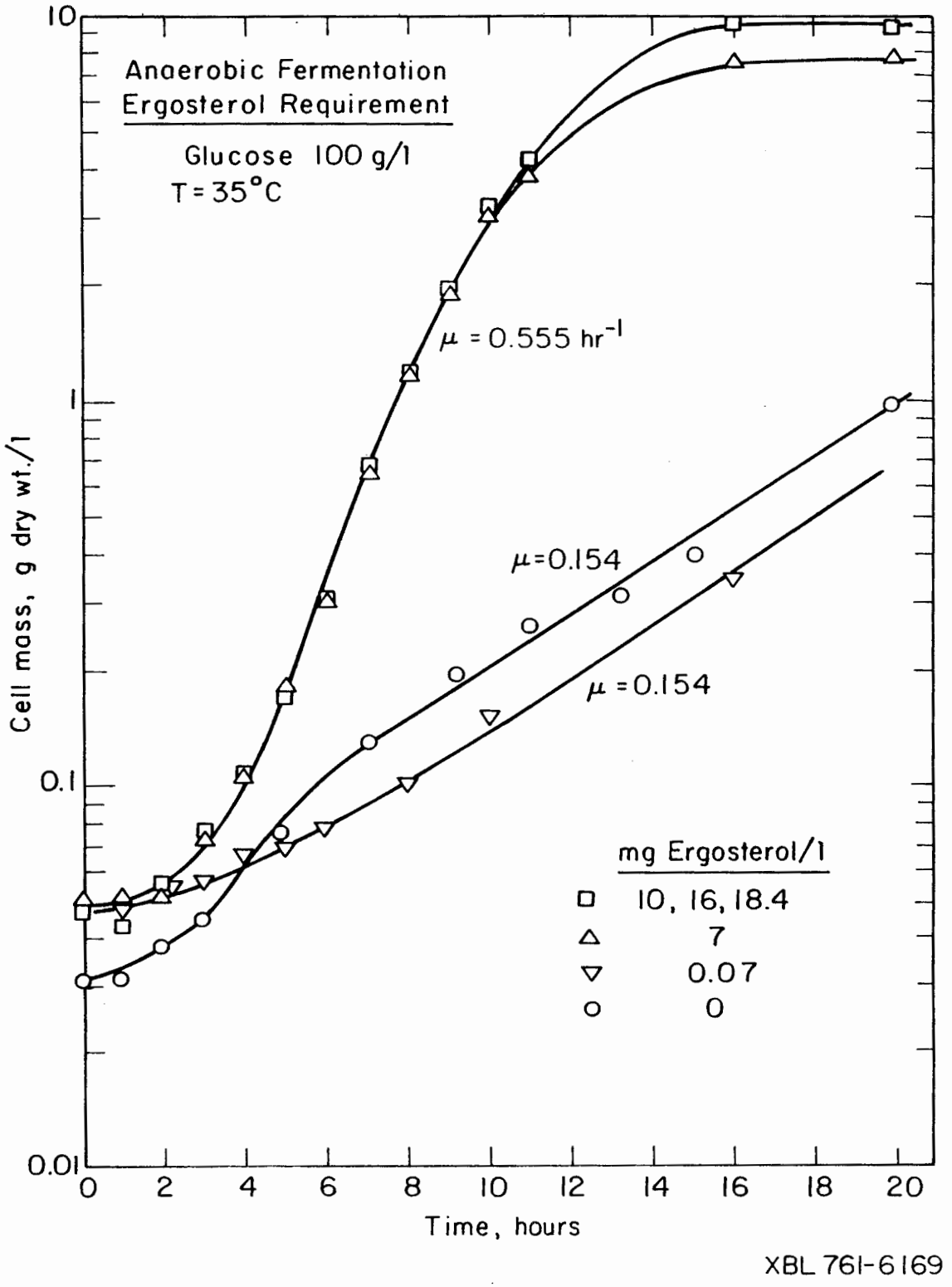

Fig. 6.10. Effect of ergosterol on cell mass production in anaerobic batch fermentations.

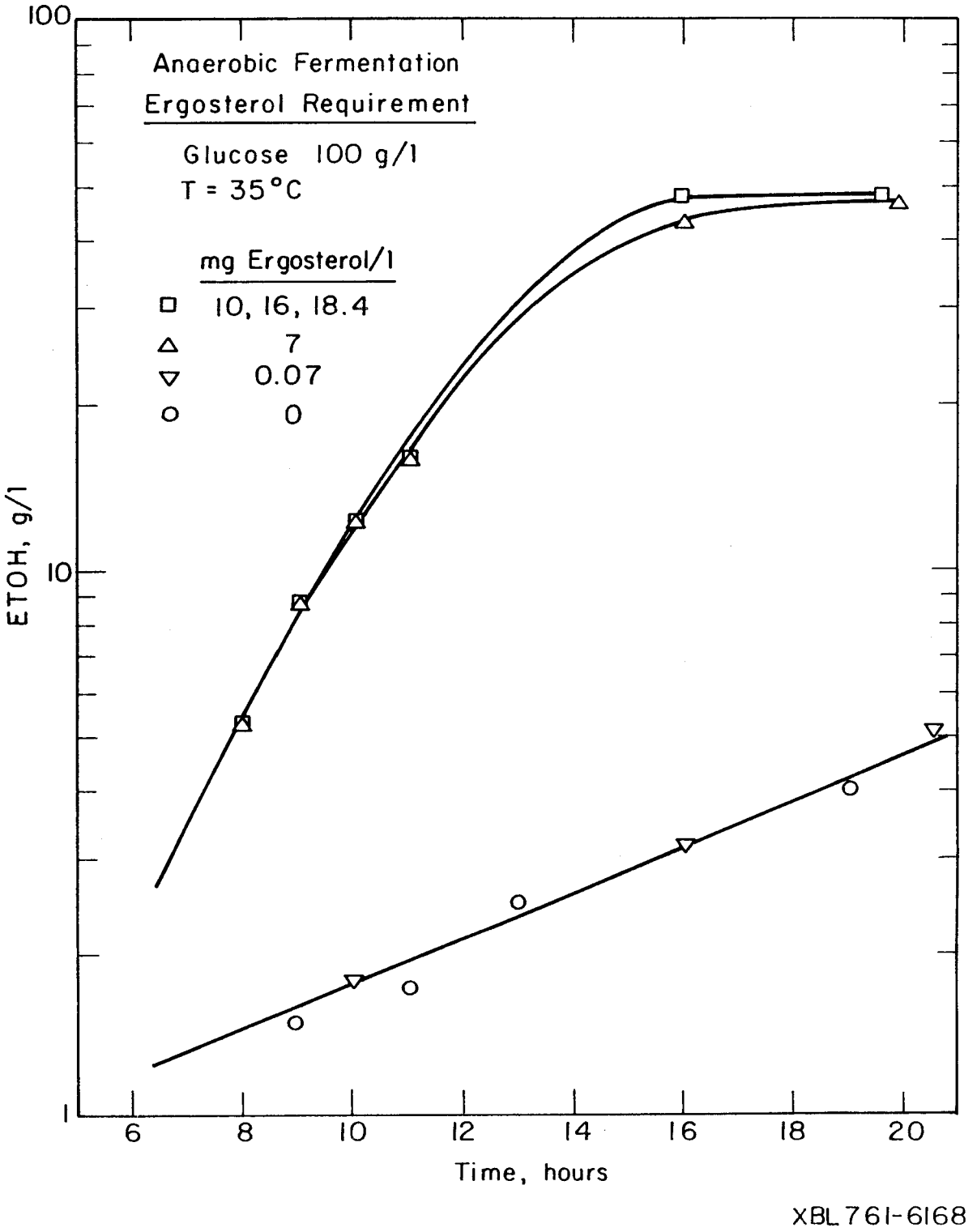

Fig. 6.11. Effect of ergosterol on ethanol production in anaerobic batch fermentations.

The result of adding ergosterol to air saturated medium is shown in Fig. 6.12. An aerobic inoculum was used in these experiments. The growth curves of the anaerobic and air saturated fermentations are almost identical. Ten percent more cell mass, although, is produced in the air saturated fermentation. This may be due to the catabolism of the small amount of oxygen initially present. The lack of an appreciable difference between the anaerobic and air saturated curves again points out the ability of ergosterol to eliminate the oxygen requirement during fermentation.

Although the ethanol production rates are the same whether or not ergosterol is added to an air saturated medium, the cell yield, $Y_{X/S}$, and the specific growth rate of the yeast differed (see Figs. 6.12 and 6.1). $Y_{X/S}$ and the specific growth rate are 0.09 and 0.53 hr^{-1}, respectively, when ergosterol is used. But when an air saturated broth without ergosterol is employed, $Y_{X/S}$ and the specific growth rate are only 0.056 and 0.46 hr^{-1}, respectively.

The higher cell yield and growth rate in an ergosterol medium is probably due to the easier incorporation of the ergosterol into cell mass. If only oxygen is present the yeast must produce ergosterol or other unsaturated lipids from simple carbon skeletons. This process requires more time and energy than if the ergosterol is already present in the medium.

Ergosterol is much too expensive to use in an industrial process (30 cent/g). Instead, the medium would be supplemented with oxygen. Ergosterol, however, does provide a useful research tool by eliminating the yeast oxygen requirement during fermentation. This permits

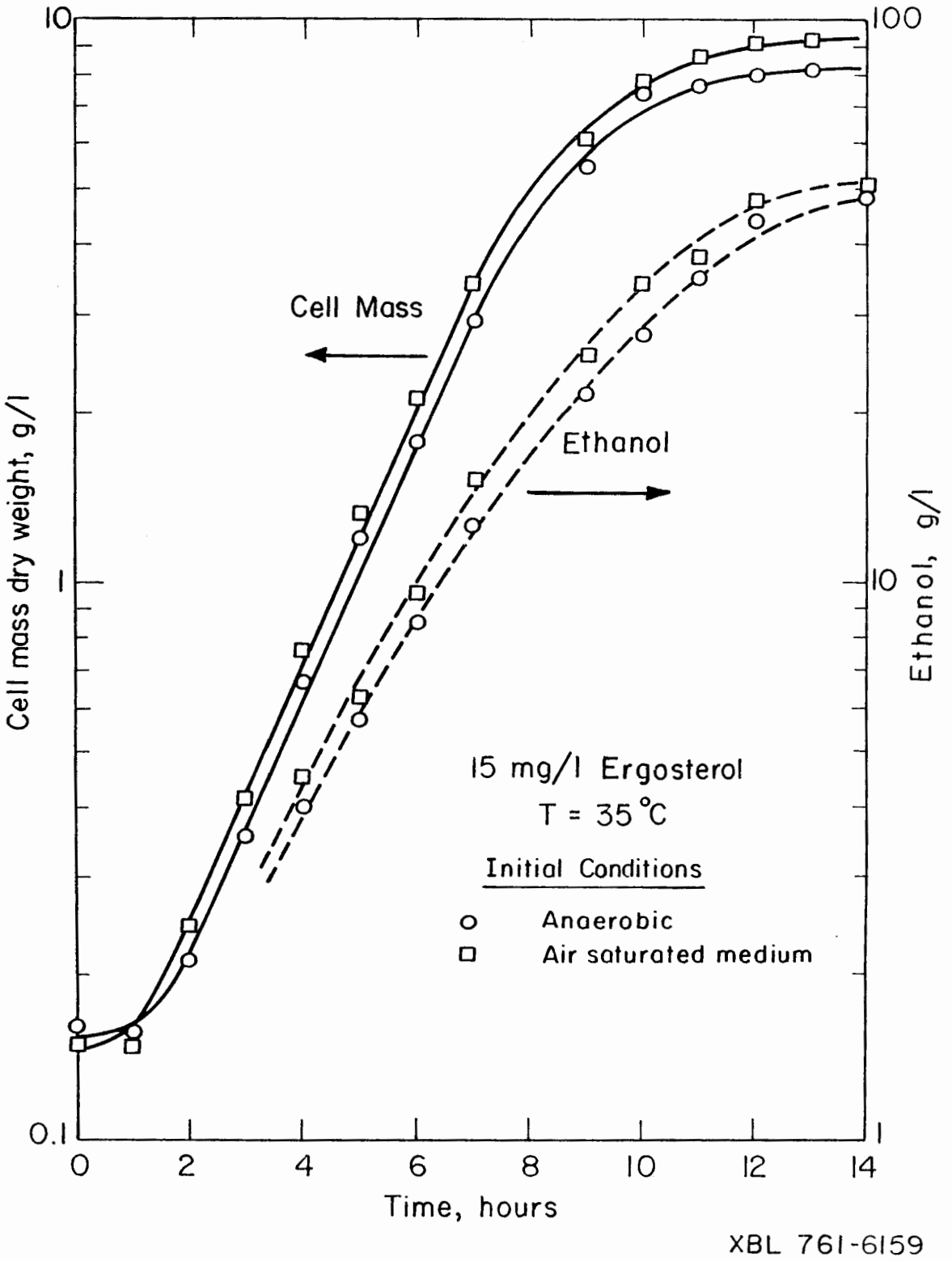

Fig. 6.12. Result of adding ergosterol to an initially air saturated medium and to an initially anaerobic medium when an aerobically grown inoculum is used.

fermentations to be run under totally anaerobic conditions so that just the fermentative metabolism may be examined. Possible spurious effects of oxygen can thus be eliminated. The use of ergosterol also provides some insight into yeast metabolism and the role of oxygen during fermentation.

6.2. Continuous Fermentation

Continuous fermentations were used to examine the effect of oxygen and feed sugar concentration on the production of ethanol. The goal of this work was to maximize the ethanol productivity in continuous culture and demonstrate the practicability of using continuous fermentations for the production of ethanol.

The optimum fermentation temperature of 35°C determined in the batch fermentations was used for all continuous experiments. To decrease the chances of contamination, a low pH of 4.0 was maintained in the continuous fermentations. However, the effect of pH on ethanol productivity was examined. The medium listed in Table 5.2 was used for all experiments when a 10% glucose feed was fermented. But when the glucose concentration was changed, all other compounds in Table 5.2 were adjusted by the same ratio.

6.2.1. Effect of Oxygen and Ergosterol

Figure 6.13 shows the results of a continuous fermentation with a 10% glucose feed. The feed to the fermentor was air saturated and no air was sparged into the fermentor. The glucose, ethanol and cell mass concentrations are plotted against the fermentor dilution rate. The fermentor ethanol productivity is also shown as a function of dilution rate.

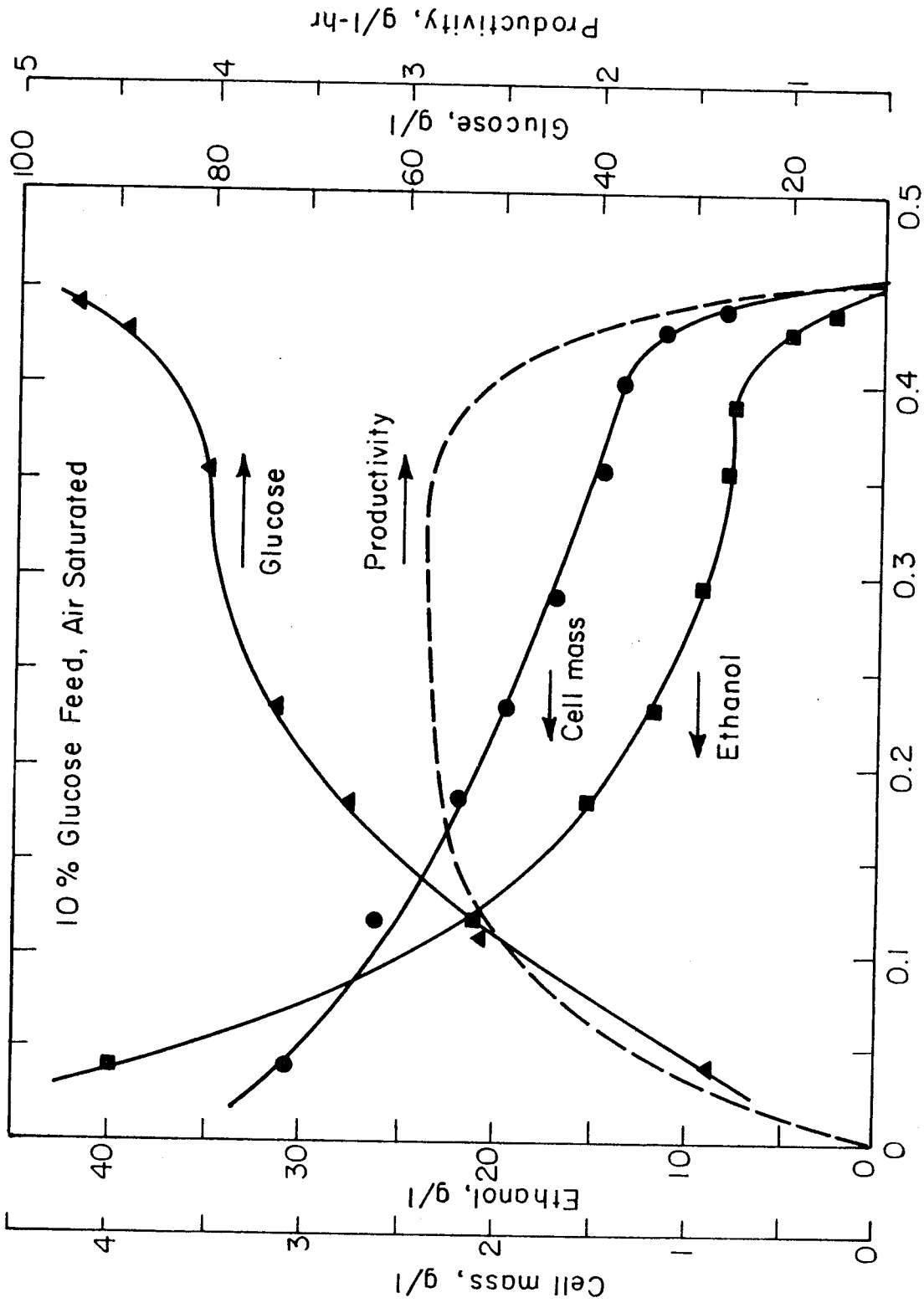

Fig. 6.13. Continuous fermentation using an air saturated feed and not sparging the fermentor with air.

As the dilution rate increased the cell mass concentration dropped. Since there were fewer cells to carry out the fermentation at high dilution rates, the sugar concentration rose with a corresponding decrease in ethanol concentration.

The ethanol productivity (grams ethanol produced in 1 hr per liter of fermentor volume) is calculated as the dilution rate times the ethanol concentration in the effluent from the fermentor. As the dilution rate was increased the productivity initially rose even though the ethanol concentration dropped. At a dilution rate of about 0.2 hr^{-1} further increases in dilution rate were counter balanced by the decrease in ethanol concentration and the productivity remained constant. Finally, at a dilution rate of 0.37 hr^{-1} the ethanol concentration rapidly dropped and the productivity followed suit.

The continuous fermentation results shown in Fig. 6.13 are very poor. Even at a low dilution rate of 0.05 hr^{-1} only 70% of the initial sugar was fermented. Extrapolation of the glucose curve shows that a zero dilution rate was necessary to ferment all the glucose. In other words, it was not possible to completely ferment a 10% solution of glucose in continuous culture if the medium was only air saturated. This was due to oxygen limitation of yeast growth. There was not enough oxygen in the incoming medium to sustain the required amount of yeast growth necessary to ferment all the glucose.

Figure 6.14 illustrates this fact. Here the cell yield factor, $Y_{X/S}$, specific cell ethanol productivity, ν, and oxygen tension are plotted against dilution rate. At dilution rates below 0.35 hr^{-1} the oxygen concentration in the fermentor was essentially zero. But

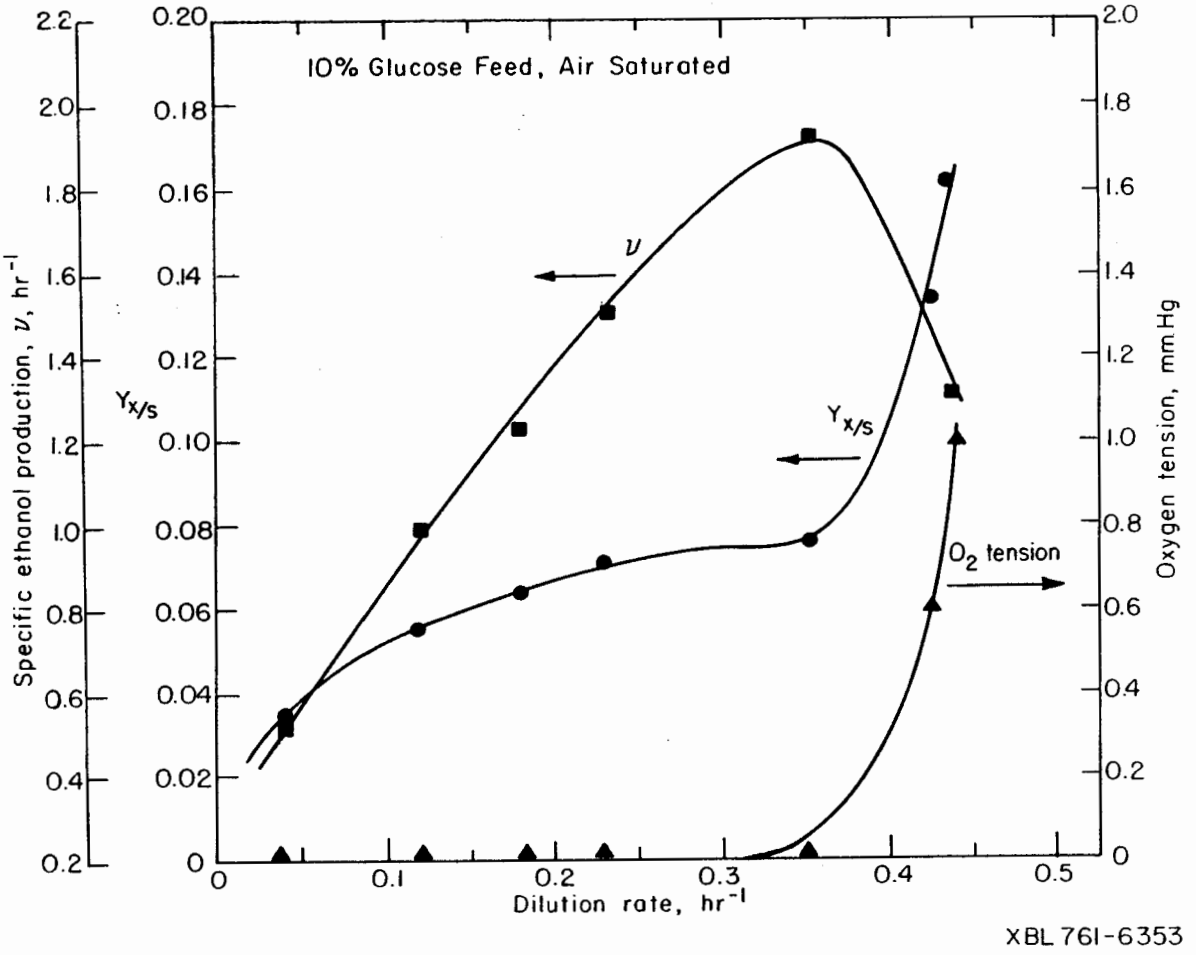

Fig. 6.14. Specific ethanol production, ν, cell yield factor, $Y_{x/s}$, and oxygen tension as a function of dilution rate in continuous culture when the feed medium is air saturated.

as the dilution rate was increased above 0.35 hr^{-1} the oxygen concentration began to rise. This is typical behavior of a growth limiting substrate in continuous culture. Also, note that as the oxygen concentration increased the cell yield factor, $Y_{X/S}$, rapidly rose indicating more optimal conditions for cell growth. The specific ethanol productivity, however, decreases as the oxygen tension increased. This was offset somewhat by the increase in $Y_{X/S}$, so that the total ethanol productivity in Fig. 6.13 did not drop as fast as did ν with increased dilution rate.

To eliminate the oxygen requirement of the yeast, ergosterol was added to the medium. From the batch experiments an ergosterol concentration of 10 mg/l was found to accomplish this task (see Figs. 6.10 and 6.11). The results of a continuous fermentation with 10 mg/l of ergosterol added to the medium are shown in Fig. 6.15. The maximum ethanol productivity was 5.95 g/l-hr. This was a twofold increase over the productivity obtained in Fig. 6.13 when ergosterol was not added to the medium. Also, the glucose was completely fermented at a dilution rate of 0.075 hr^{-1}. The higher fermentation rates in Fig. 6.15, as compared to Fig. 6.13, were due to an increase in cell mass. When the medium was supplemented with ergosterol 8.5 g dry wt/l of cell mass was produced at a dilution rate of 0.075 hr^{-1} while only 3 g dry wt/l of cell mass was obtained at the same dilution rate if no ergosterol was added.

This illustrates that yeast growth was indeed oxygen limited in continuous fermentation when the medium was only saturated with air. It also points out, as was discussed in the batch fermentation section,

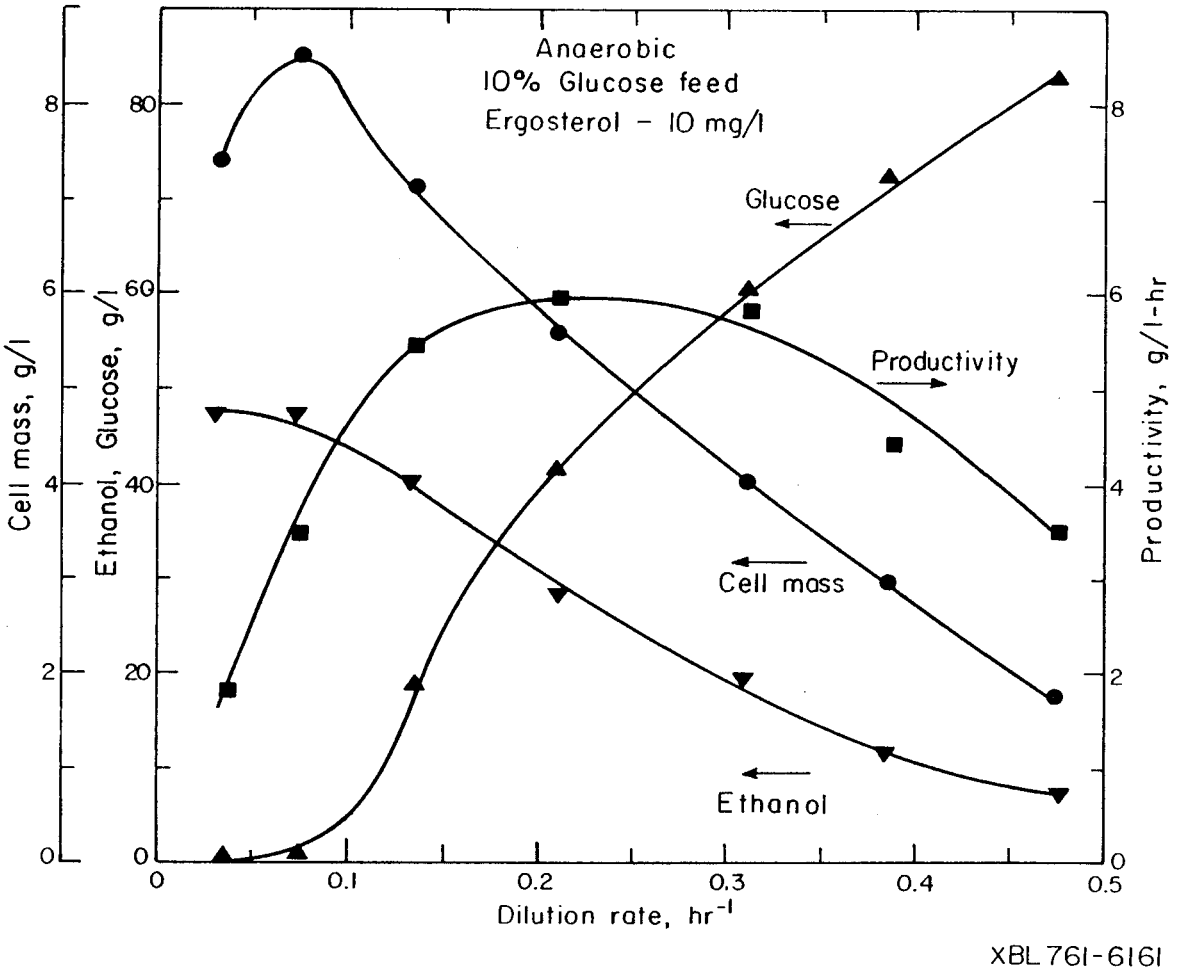

Fig. 6.15. Continuous fermentation when the medium is supplemented with ergosterol.

that substantial yeast growth was necessary to maintain high ethanol fermentation rates.

Figure 6.16 shows the effect of maintaining an oxygen tension of 0.07 mmHg in the fermentor. This experiment was run using a 8.9% glucose feed and a yeast that had been adapted to the oxygen concentration. (The adaption of the yeast to high oxygen tension is discussed below.) A constant oxygen tension of 0.07 mmHg was achieved by adjusting the air sparging rate and/or the agitation rate.

Comparison of Fig. 6.15 with Fig. 6.13 shows that both the cell mass concentration and ethanol productivity were substantially increased when additional oxygen was supplied to the yeast by sparging air into the fermentor. At a dilution rate of 0.2 hr^{-1}, the yeast concentration was 12.0 g/l when the oxygen tension was maintained at 0.07 mmHg. But when an air saturated feed was used and the oxygen tension allowed to drop to zero, as shown in Fig. 6.13, the yeast concentration was only 2.1 g/l at this dilution rate. This was reflected by an increase of ethanol productivity from 2.75 g/l-hr to 7.1 g/l-hr when the oxygen tension was held at 0.07 mmHg. A very important point to be made is that all the glucose in a 10% solution could be continuously fermented at a dilution rate of 0.19 hr^{-1} (see Fig. 6.24) if the oxygen tension was maintained at 0.07 mmHg. Whereas, if just an air saturated feed was used and the oxygen tension permitted to fall to zero, the complete fermentation of a 10% glucose solution was not possible.

It is also interesting to note that higher cell mass concentrations and ethanol productivities were obtained by supplying the yeast with

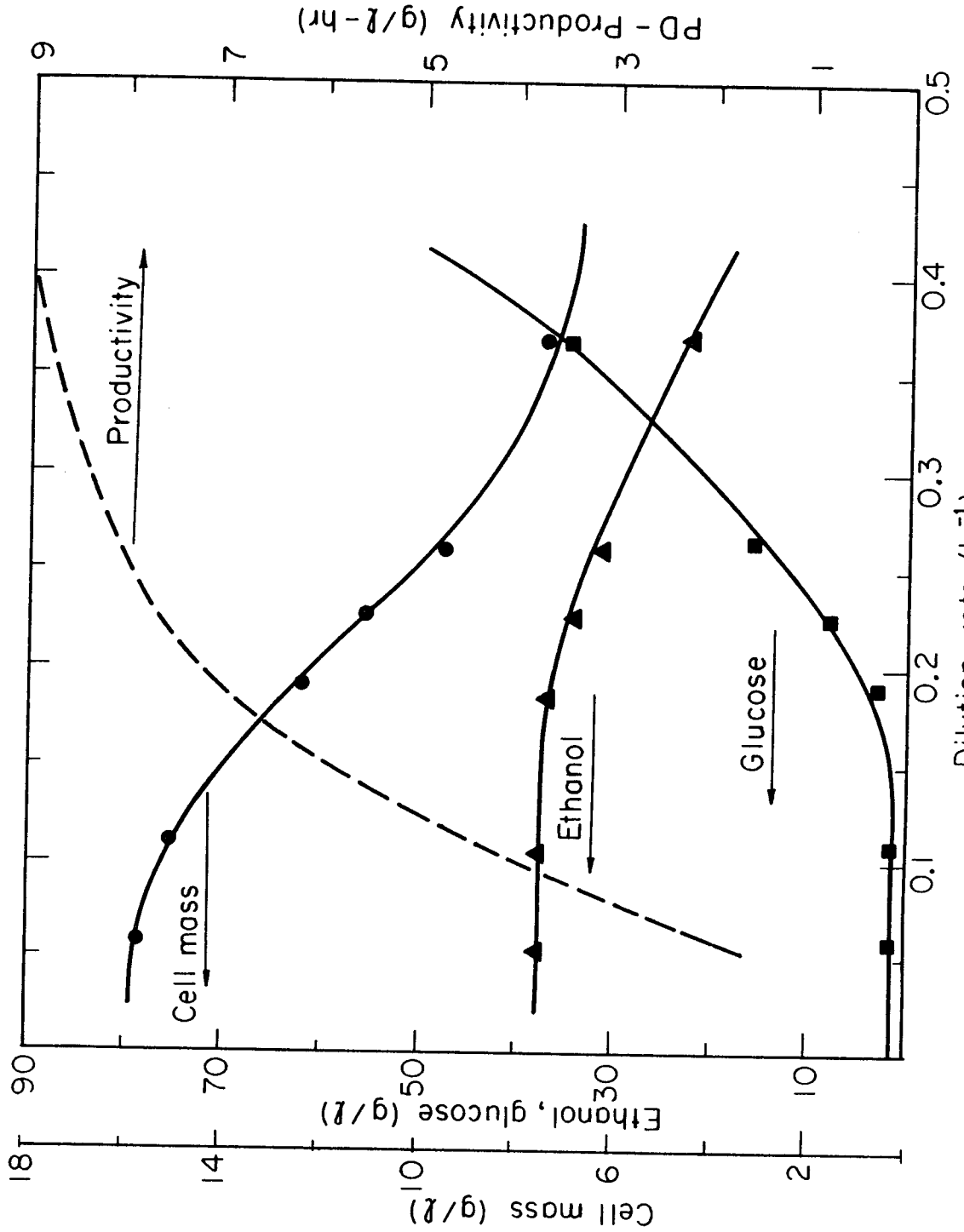

Fig. 6.16. Continuous fermentation of a 8.9% glucose feed when the oxygen tension was maintained at 0.07 mmHg.

oxygen than with ergosterol. For example, at a dilution rate of 0.3 hr^{-1} the productivity was 8.5 g/l-hr and cell concentration was 8.5 g/l when the oxygen tension was held at 0.07 mmHg. But when an anaerobic fermentation was supplemented with ergosterol, Fig. 6.15 shows the productivity and cell concentration was only 5.9 g/l-hr and 4.25 g/l, respectively, at a dilution rate of 0.3 hr^{-1}. If the ergosterol concentration was increased from 10 mg/l to 50 mg/l there was no increase in cell mass or ethanol productivity over that shown in Fig. 6.14. The cells were thus, not limited by ergosterol.

This seems to imply that when oxygen was present the yeast was respiring some glucose and producing added cell mass. This additional cell mass per unit volume then produced a higher fermentation rate per unit volume (higher ethanol productivity).

The increased cell mass was, of course, produced at the expense of a lower ethanol yield. $Y_{P/S}$ was 0.42 in the presence of 0.07 mmHg oxygen tension and 0.46 in the anaerobic experiment supplemented with ergosterol. This was only a 9% decrease in ethanol yield when oxygen was present. However, a 56% increase in productivity was experienced at conditions of complete substrate utilization when oxygen rather than ergosterol was employed.

A more quantitative description of the effect of oxygen on continuous ethanol fermentation is shown in Figs. 6.17 through 6.19. The solid curves in these figures show the initial behavior of the yeast to varying oxygen tensions. The broken curves represent the behavior of the yeast after it had adapted to high oxygen tensions. The initial response of the yeast to oxygen tension is first discussed.

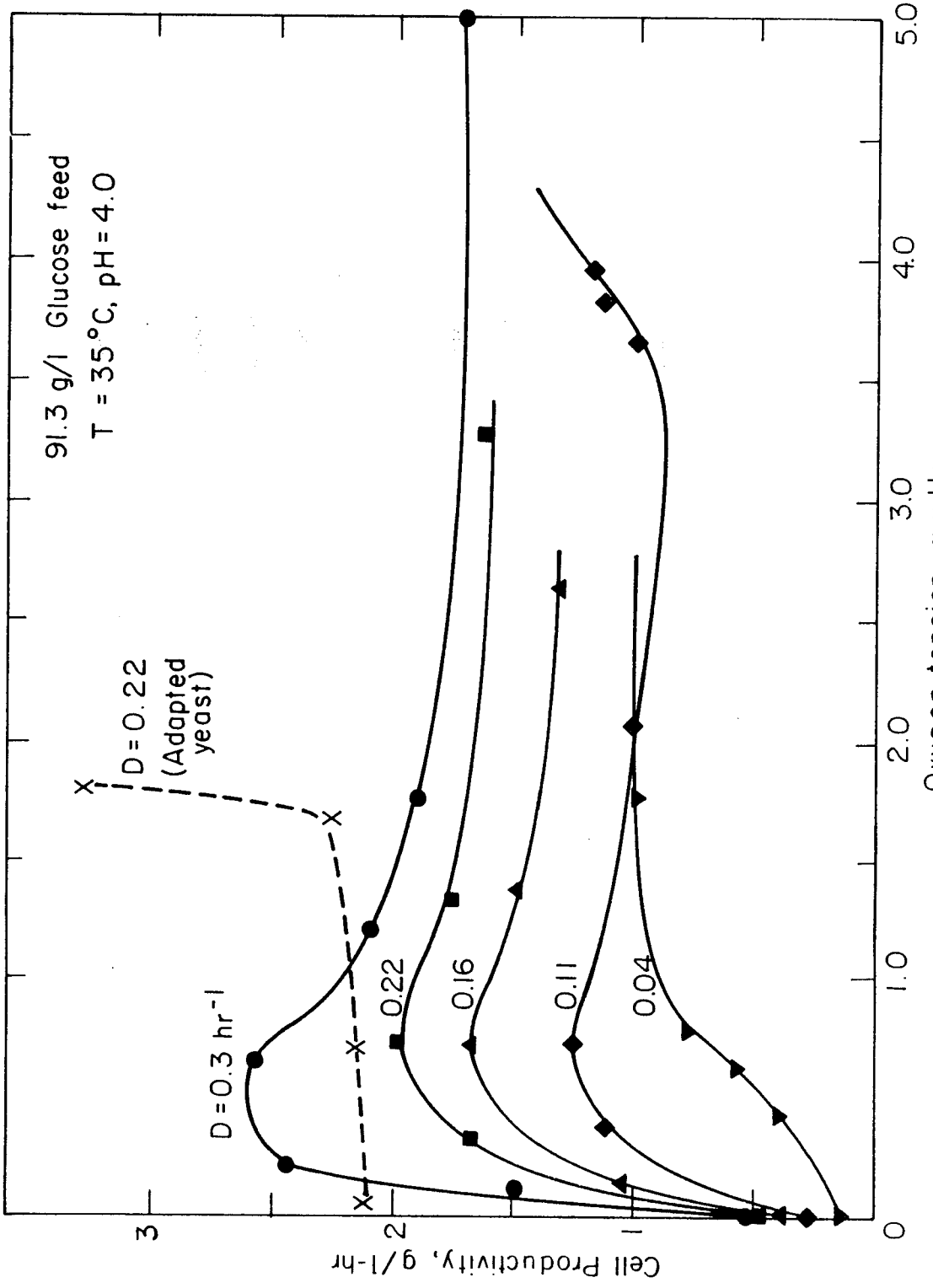

Fig. 6.17. Cell productivity in continuous fermentation as a function of oxygen tension at various dilution rates.

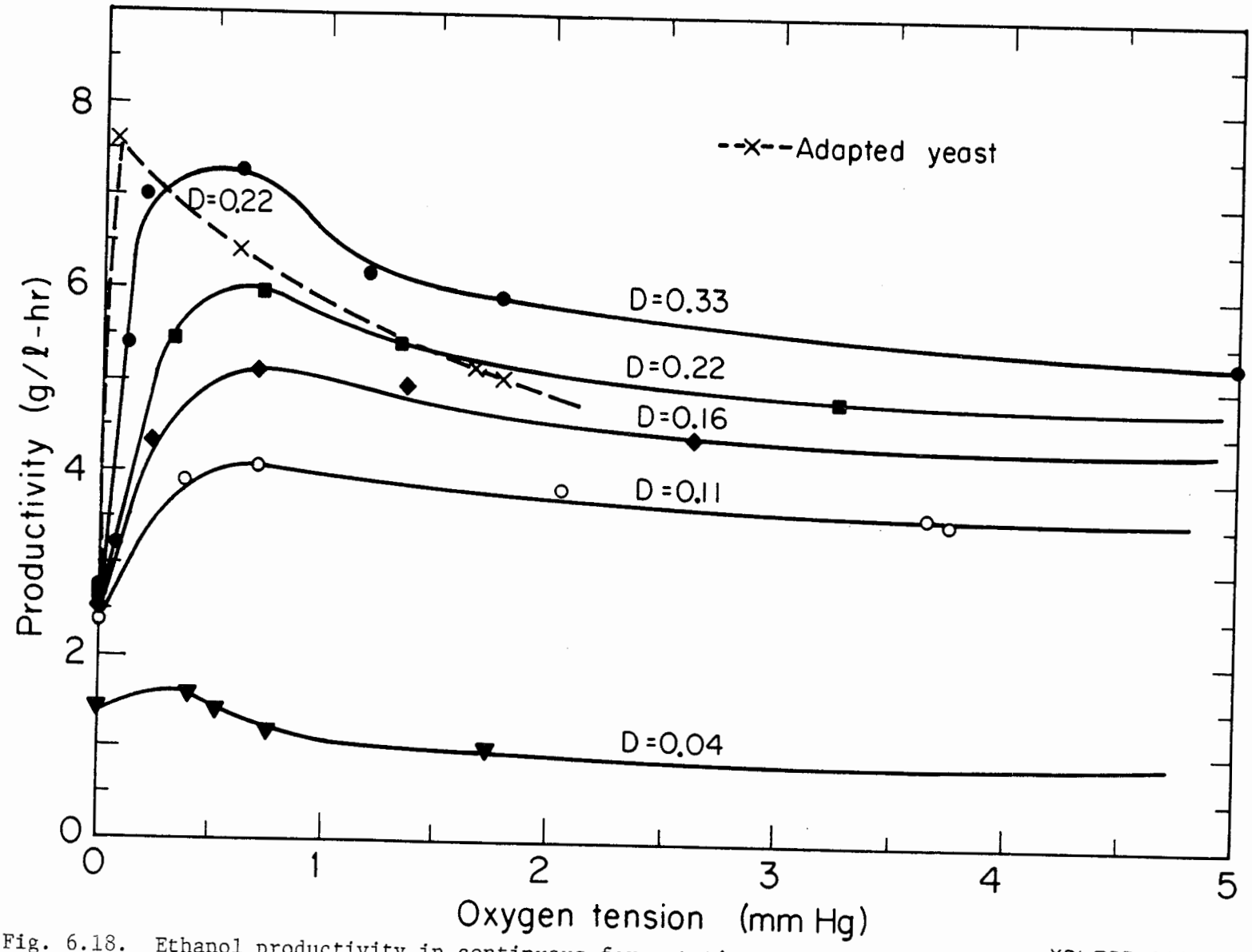

Fig. 6.18. Ethanol productivity in continuous fermentation as a function of oxygen tension at various dilution rates.

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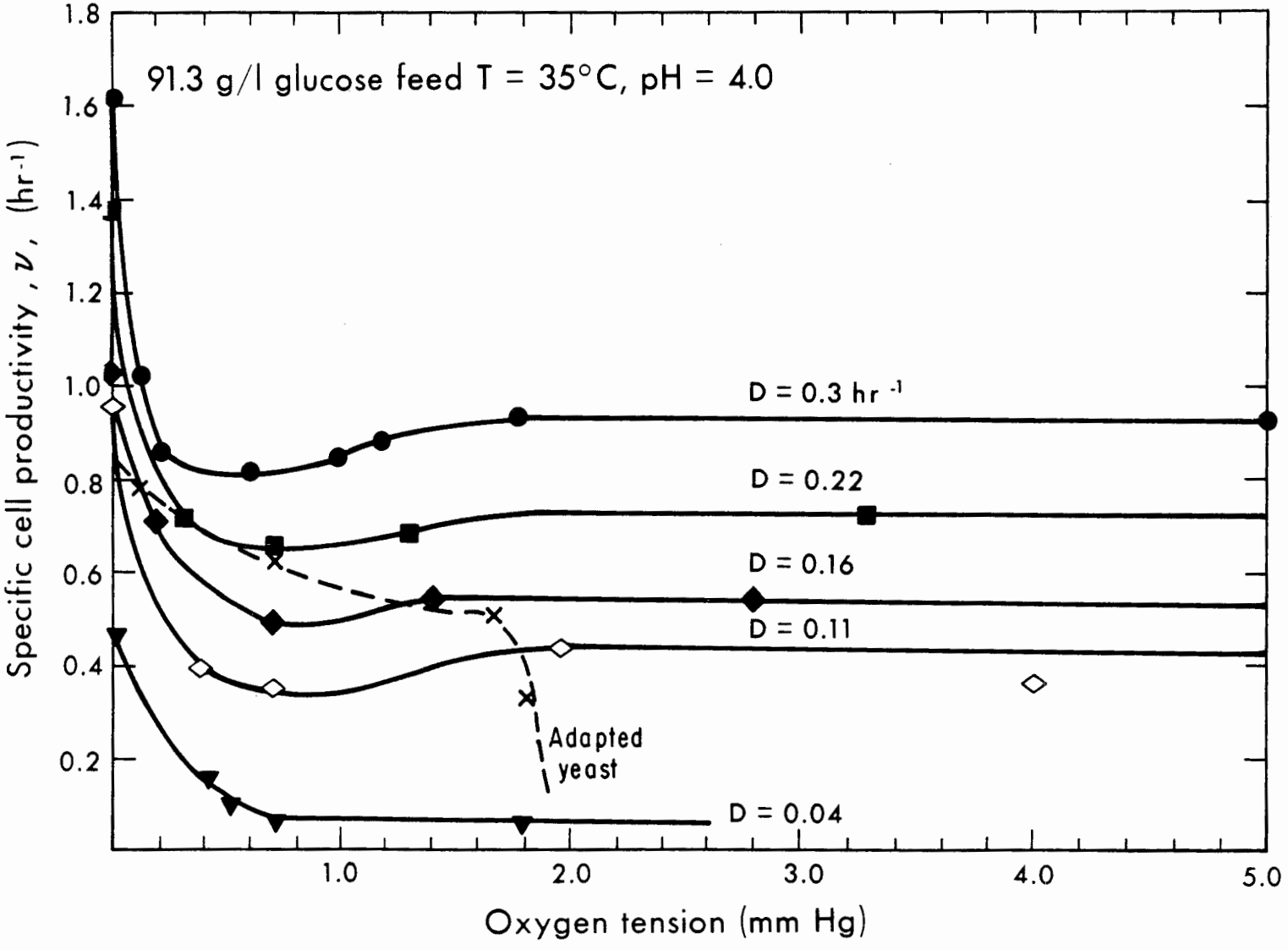

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Fig. 6.19. Specific ethanol productivity in continuous fermentation as a function of oxygen tension at various dilution rates.

The cell and ethanol productivities are plotted in Figs. 6.17 and 6.18, respectively, as a function of oxygen tension for various dilution rates. As shown in these figures, the unadapted yeast had a definite optimum for both cell mass and ethanol productivity at 0.7 mmHg oxygen tension. The occurrence of optimal cell mass and ethanol production at the same oxygen tension was to be expected. Since the energy requirement for growth was derived from the fermentation of glucose to ethanol, high cell mass production rates must imply high fermentation rates. This same relation between ethanol production and cell growth was also found in the preceeding batch culture experiments.

It is also worth noting the low productivities obtained at zero oxygen tension. These data at zero tension were obtained using an air saturated feed and not sparging air through the fermenter. Again this points out the very poor continuous ethanol fermentation results obtained if the fermentor was naively operated in an anaerobic mode. The ethanol productivities are from 1.5 to 3 times higher if the oxygen tension was held at 0.7 mmHg by sparging air into the fermentor.

Figure 6.19 plots the specific cell ethanol productivity (fermentor productivity in Fig. 6.17 divided by the cell mass concentration) against oxygen tension at various dilution rates. Here a minimum specific productivity occurred at the same oxygen tension observed for the maximum fermentor productivities. This, of course, was consistent because the increase in cell mass concentration at the optimum oxygen tension more than offset the decrease in specific productivity. In going from zero oxygen tension to 0.7 mmHg at a dilution rate of 0.22 hr^{-1} the specific ethanol productivity

decreased by 54%, but the cell concentration increased sixfold. The net effect was a factor of 2.2 increase in total fermentor productivity at 0.7 mmHg oxygen tension.

Since the ethanol concentration in these experiments was less than 5%, product inhibition by ethanol may be neglected.[17] The minimum specific productivity may then be viewed as indicative of optimal cell growth conditions. The maximum amount of cell mass was being produced per unit of glucose fermented to ethanol and the yeast was making the most efficient use of the energy derived from fermentation.

The decrease in ethanol and cell productivities at high oxygen tensions shown in Figs. 6.17 and 6.18 has also been reported by Haukeli.[19] Haukeli was able to adapt Saccharomyces cervisiae to high oxygen tensions and eliminate the oxygen inhibition of cell mass production. The ethanol production characteristics of the adapted yeast, however, was not reported.

The same phenomenon was observed in this work. A continuous fermentation was run at a dilution rate of 0.3 hr^{-1} and at an oxygen tension of 3.0 mmHg. After 7 days of continuous operation, an increase in cell mass concentration with a corresponding decrease in glucose concentration was detected. With 3 more days of continuous operation a new steady state was obtained and yeast growth was no longer inhibited by high oxygen concentrations.

The response of the adapted yeast to oxygen tension is shown by the broken lines in Figs. 6.17 through 6.19. Figure 6.17 shows the ethanol productivity of the adapted yeast as a function of oxygen tension at a dilution rate of 0.22 hr^{-1}. Two things are immediately

obvious. First, ethanol production by the adapted yeast was much more sensitive to oxygen tension than was ethanol production by the unadapted yeast. The optimum oxygen tension was only 0.07 mmHg. This was 10 times lower than for the unadapted yeast. Also the adapted yeast had a 25% higher total ethanol productivity than did the unadapted yeast at the same dilution rate. This increase in ethanol productivity was a result of the increased cell mass produced by the adapted yeast. (As will be shown later, the cost of producing somewhat more cell mass is more than offset by reduced fermentor costs resulting from the increased productivities.)

This can be seen in Fig. 6.18, which plots cell mass productivity against oxygen tension. The cell mass production of the adapted yeast was not inhibited by oxygen. Indeed, at an oxygen tension of 1.75 mmHg the cell mass production of adapted yeast drastically increased while the unadapted yeast productivity declined.

It appears that the adaption process had partially eliminated the catabolic repression mechanism of glucose in the yeast. As previously discussed, catabolic repression by glucose inhibits the respiratory metabolism of Saccharomyces when the glucose concentration is above 1 g/l.[21] (As was the case in the experiments of Figs. 6.17 to 6.19.) This forces the metabolism of S. cerevisiae to be fermentative even at high oxygen tensions. The unadapted yeast exhibited catabolic repression as was evident by the continued ethanol production at high oxygen tension. The specific ethanol production of the unadapted yeast actually increased with oxygen tension above 0.7 mmHg as shown in Fig. 6.19. In contrast, the specific ethanol production of the

adapted yeast continually decreased with increasing oxygen tension while the cell mass productivity increased. At an oxygen tension of 1.8 mmHg the cell yield factor $Y_{X/S}$, for the adapted yeast was 0.24, or 71% higher than for the unadapted yeast. But at this same oxygen tension the ethanol yield factor $Y_{P/S}$, was 26% lower for the adapted yeast than for the unadapted yeast.

Thus, the adapted yeast is producing substantial amounts of cell mass instead of ethanol at this oxygen tension. Catabolic repression had lifted in the adapted yeast and additional cell mass was produced via the respiration of glucose.

However, as shown by Oura,[3] there is an antigonistic effect between glucose and oxygen concentrations with regard to catabolic repression. High oxygen tension can eliminate catabolic repression when the glucose concentration is high, whereas, low oxygen concentrations will eliminate catabolic repression when the glucose concentration is low. Thus, the adapted yeast may be used for ethanol production by maintaining a very low oxygen tension in the fermentor so that the residual unfermented glucose will produce catabolic repression. The metabolism of the yeast will then be fermentative and produce ethanol. In the experiment shown in Fig. 6.18 an oxygen tension up to 1.6 mmHg could be used. Above this oxygen tension catabolic repression lifted and increased cell mass was produced at the expense of ethanol production.

Once the yeast had been adapted to high oxygen concentrations it could be deadapted by running at a dilution rate of 0.04 for 72 hr. But as shown in Fig. 6.17, the optimum ethanol productivity of the adapted yeast was 25% higher than the optimum productivity of the

unadapted yeast at a dilution rate of 0.22 hr^{-1}. Also, the ethanol yield factor of the adapted yeast was only 6% lower than the unadapted yeast at the optimum oxygen tensions. For these reasons the remainder of continuous fermentation experiments were run using the adapted yeast and controlling the oxygen tension at 0.07 mmHg. Further examples of increased ethanol productivities of the adapted yeast over the unadapted yeast are illustrated below.

In order to establish that the adapted yeast cells represented a stable population in continuous ethanol fermentations, a long term continuous fermentation was conducted. The results are shown in Fig. 6.20. A constant dilution rate of 0.19 hr^{-1} was maintained throughout the experiment. A feed of 8.9% glucose was used and the oxygen tension set at 0.07 mmHg.

The data of Fig. 6.20 shows a very stable continuous fermentation for over 480 hr or 20 days. Over 97% of the glucose in the feed was fermented to produce 13.4 g/l of cells and 40 g/l of ethanol. The ethanol yield, $Y_{P/S}$, was 0.46 representing 91% of the theoretical yield of ethanol.

The small graph in the center of Fig. 6.20 shows the results obtained by Portno[20] for the continuous fermentation of brewers wort. Portno was not able to maintain a stable continuous fermentation and the effluent sugar concentration cycled between 5% and 10%. The percentage of viable yeast cells was also reported to cycle between 90% and 50% of the total yeast population. Portno thus hypothesized that if a low concentration of fermentable sugars was maintained in continuous culture for an extended period of time the yeast loses the

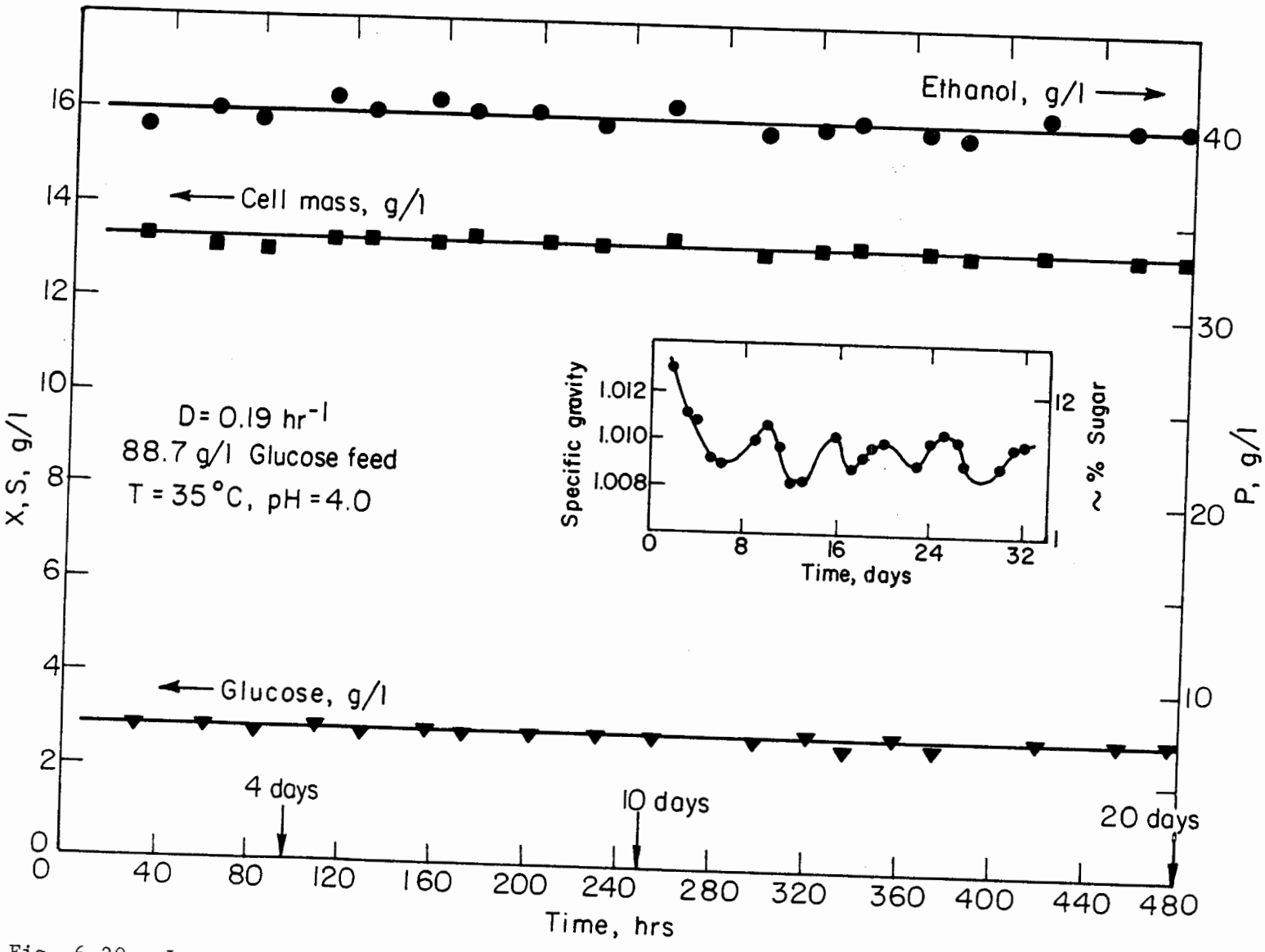

Fig. 6.20. Long term continuous fermentation with adapted yeast.

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ability to adsorb and ferment the sugars. Further, he points out that in order to maintain a stable continuous culture the yeast must be periodically exposed to high sugar concentrations.

However, this phenomenon was not found in the present investigation. Figure 6.20 shows a stable fermentation was maintained for over 20 days with a residual glucose concentration of less than 0.29% (2.93 g/l) without cycling of cell mass or fermentation rate. These results agree with Oura[3] who maintained stable long term continuous yeast fermentations once an optimum medium formulation was determined. The cycling observed by Portno in continuous ethanol fermentations was possibly due to a deficiency of a required yeast growth factor in the medium. Also, the fact that no oxygen was supplied to the yeast could have contributed to the cycling in Portno's fermentations.

The data then presented in Fig. 6.20 demonstrate the practicality of continuous ethanol production. A stable ethanol fermentation was maintained at various dilution rates for 90 days in this work without problems of cycling or contamination.

A residual glucose level of 2.9 g/l was sustained throughout the experiment shown in Fig. 6.20. As shown in Fig. 6.16, the residual glucose concentration was independent of dilution rates below 0.19 hr^{-1} and could not be reduced below 2.9 g/l even at a dilution rate of 0.06 hr^{-1}. This residual glucose level is higher than has been previously reported in ethanol fermentations[19,25] but is a direct consequence of the Pastuer effect discussed in Chapter 3.

When the concentration of glucose was high, catabolic repression takes place which inhibits the respiratory metabolism and the

energetic arguments of the Pasture effect do not hold. However, when the concentration of glucose was at this low value of 2.9 g/l catabolic repression was lessened. The specific glucose consumption rate decreased due to the increase in available energy that was obtained through the respiration of glucose (the Pastuer effect). A dynamic equilibrium was then struck between the stimulating effect of oxygen on fermentation rates and the Pastuer effect. As a result, there was always a small amount of unfermented sugar present in a continuous ethanol fermentation if oxygen was sparged into the fermentor. To overcome this problem a second stage anaerobic fermentor is needed. Since no oxygen would be present in the second stage fermentor, the Pastuer effect would be eliminated and the residual glucose would be fermented.

Figure 6.21 shows the results of a two stage fermentation using a 1 liter "mini ferm" (Fermentation Design, Inc.) as the second stage fermentor. The first stage was continually sparged with air to maintain the oxygen tension at 0.7 mmHg, while in the second stage, no air was sparged and the oxygen tension was essentially zero. With this arrangement the residual glucose level was reduced to less than 0.2 g/l at a total system dilution rate of 0.13 hr^{-1} (Total system dilution rate = medium flow/(sum of volumes of both stages).) This unfermented glucose concentration was 15 times lower than achieved in the one stage fermentation.

Two stages were necessary because if a one stage anaerobic fermentor was used the yeast became starved for oxygen as shown in Fig. 6.13. The use of an aerobic fermentor followed by an anaerobic

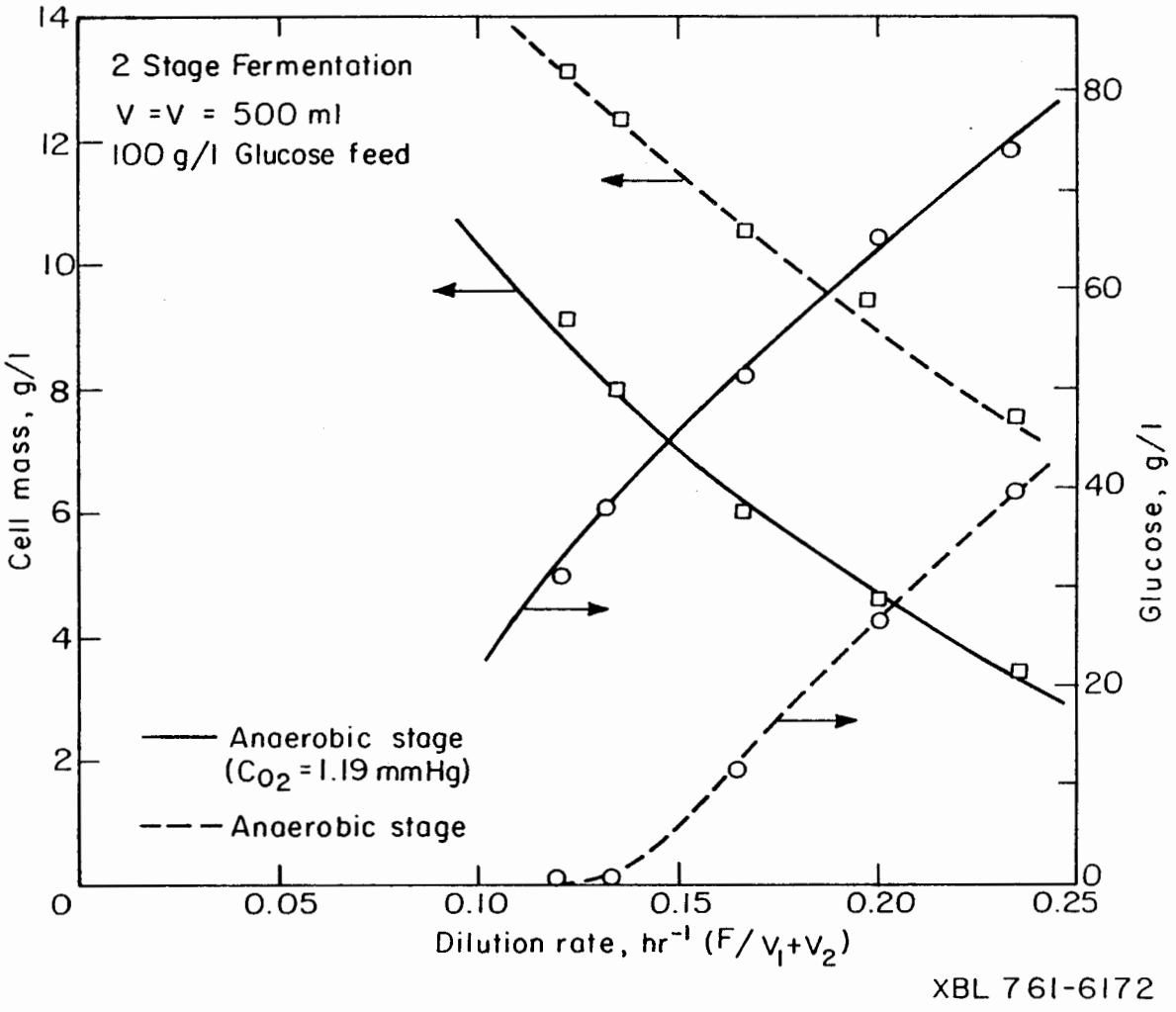

Fig. 6.21. Two stage continuous fermentation.

fermentor allowed the production of from 12 to 6 g/l of cell mass in the aerobic stage which was then fed to the anaerobic stage. The yeast during anaerobic conditions in the second stage could then use the pool of unsaturated compounds stored during aerobic growth to ferment the residual glucose and were not oxygen limited.

6.2.2. Effect of Feed Sugar Concentration

Figures 6.22 through 6.24 illustrate the effect of feed sugar concentration on continuous ethanol production. In these experiments the oxygen tension was held at 0.12 mmHg because the 0.07 mmHg optimum had not yet been determined. However, the behavior does not change significantly over this range. Figure 6.22 plots the effluent ethanol concentration against dilution rate for various sugar feed concentrations. As the dilution rate increased the ethanol concentration dropped due to incomplete utilization of the glucose. At high glucose concentrations the ethanol concentration dropped more rapidly with dilution rate because ethanol inhibition became an important factor, as shown by Bazua and Wilke.[17] An explanation of the continued steep decrease in alcohol concentration at high dilution rates for the 12.1% glucose feed is not clear but may have been due to a combination of ethanol and glucose inhibition.

Note also the poor performance of the unadapted yeast. The ethanol concentration began to drop at a dilution rate of 0.11 hr^{-1} if the unadapted yeast was used for the fermentation of a 8.9% glucose feed. However, if the adapted yeast was employed under the same conditions, the ethanol concentrations remained constant up to a dilution rate of 0.19 hr^{-1}. The premature drop in ethanol concentration with

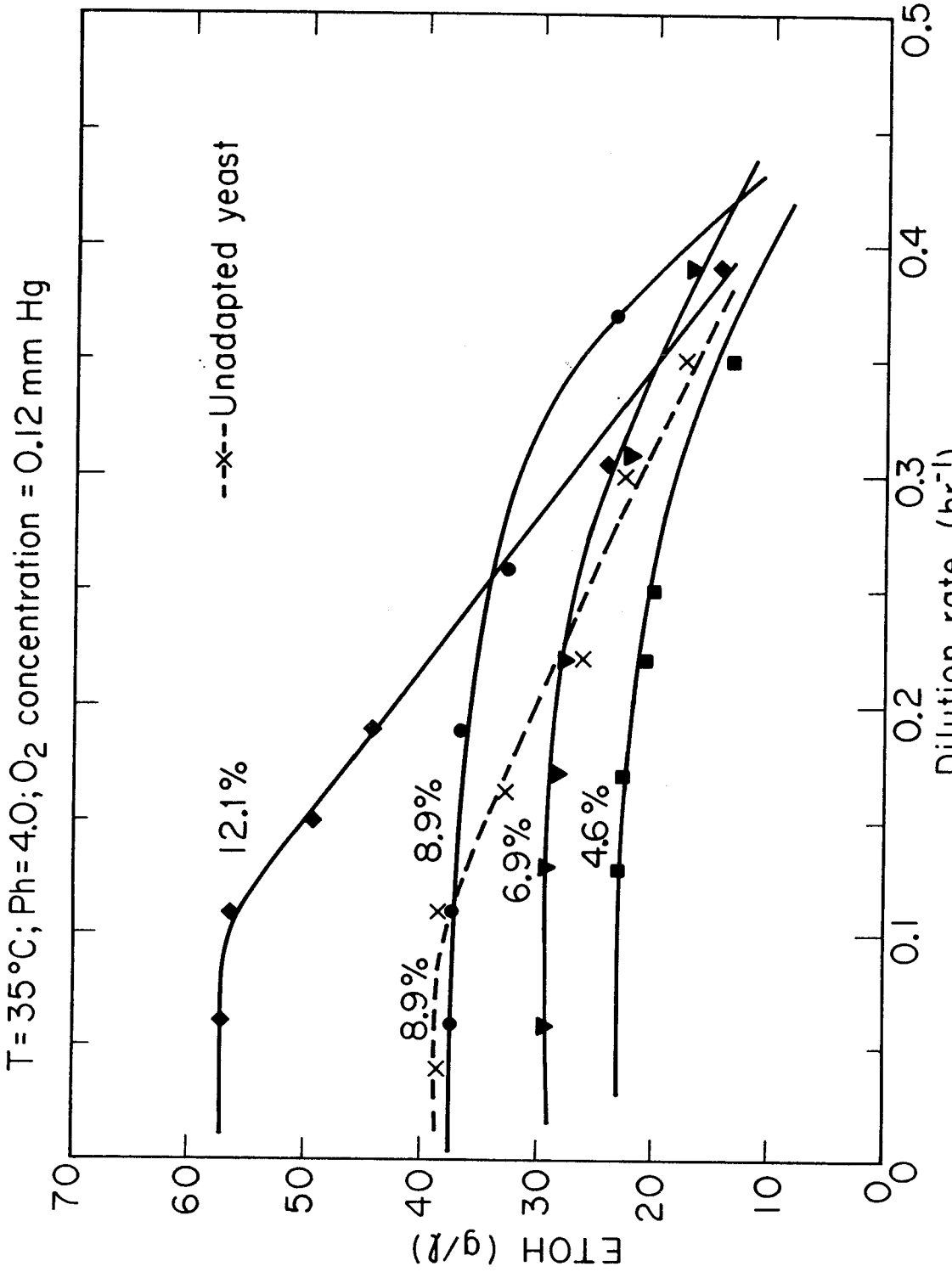

Fig. 6.22. Effluent ethanol concentration as a function of dilution rate for various glucose feed concentrations.

the unadapted yeast shows that the fermentation became incomplete at a lower dilution rate than for the adapted yeast.

The dilution rate required to ferment essentially all the glucose in the feed is shown as a function of feed sugar concentration in Fig. 6.23. The required dilution rate had a linear relation to feed glucose concentration at low concentrations, but as the sugar concentration increased the curve bent toward lower dilution rates. This was due to alcohol inhibition since higher sugar concentrations implied higher ethanol concentrations when the sugar was totally fermented. For the 16% sugar feed, 7.6% ethanol was present in the fermented broth and the specific cell ethanol productivity was reduced by 74% compared to conditions of negligible alcohol inhibition. This agrees with the work of Bazua,[17] who used ethanol enriched feeds rather than producing the ethanol by fermentation of concentrated sugar solutions.

As shown in Fig. 6.23, a 42% decrease in dilution rate was required for the unadapted yeast to completely ferment a 8.9% sugar feed. This directly corresponds to a 43% decrease in fermentor productivity if unadapted yeast is used.

Figure 6.24 is a combination of data presented in Figs. 6.22 and 6.23. Defining "complete" substrate utilization as only 0.3% sugar unfermented, Fig. 6.24 relates the specific ethanol productivity to initial feed sugar concentrations. It is interesting to note that an optimum fermentor productivity existed at a 10% sugar feed. Above this value, the specific ethanol productivity decreased due to ethanol inhibition. But if the sugar concentration was lowered, the cell mass

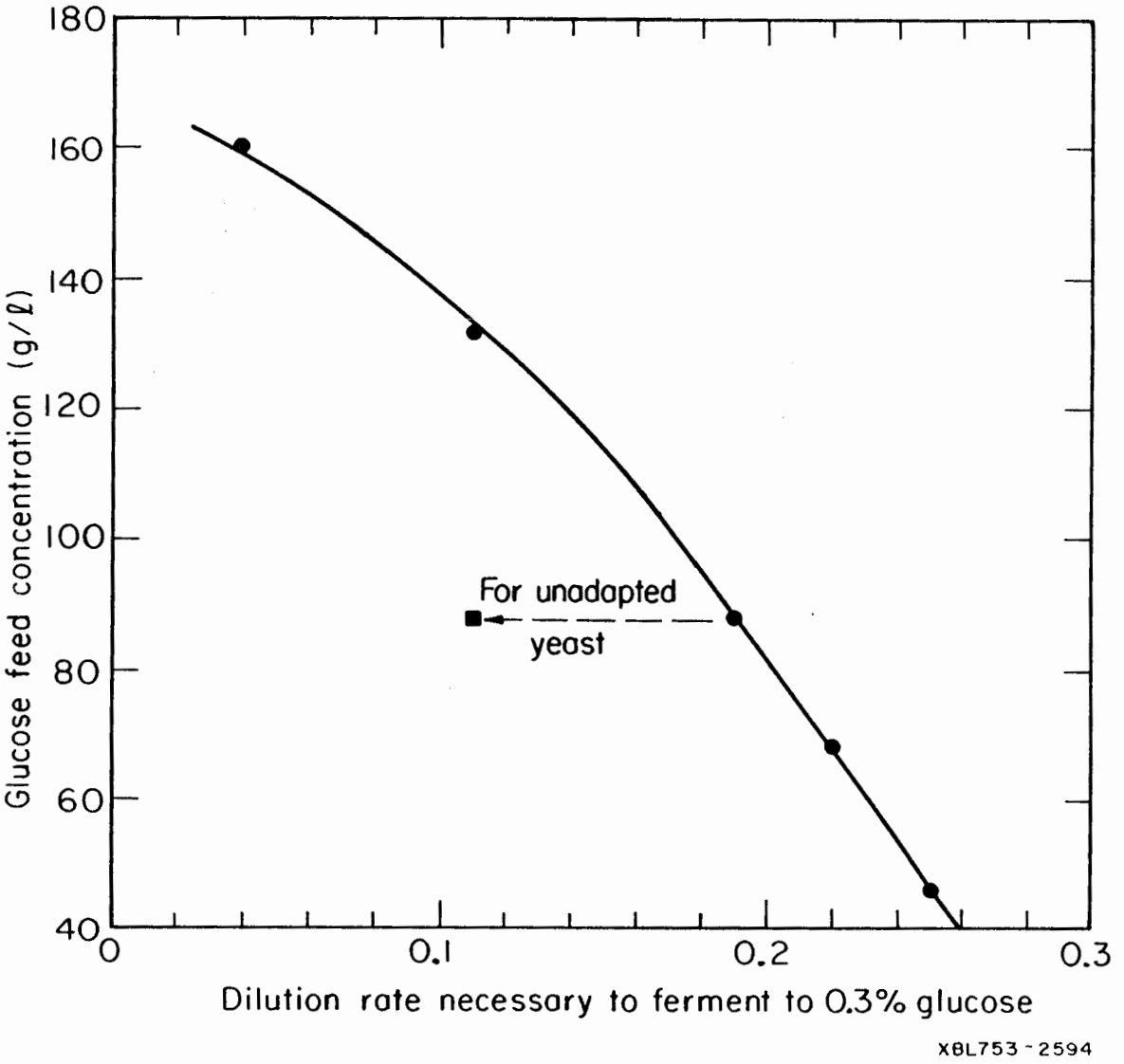

Fig. 6.23. Dilution rate required to ferment a given glucose feed concentration to 0.3% residual sugar.

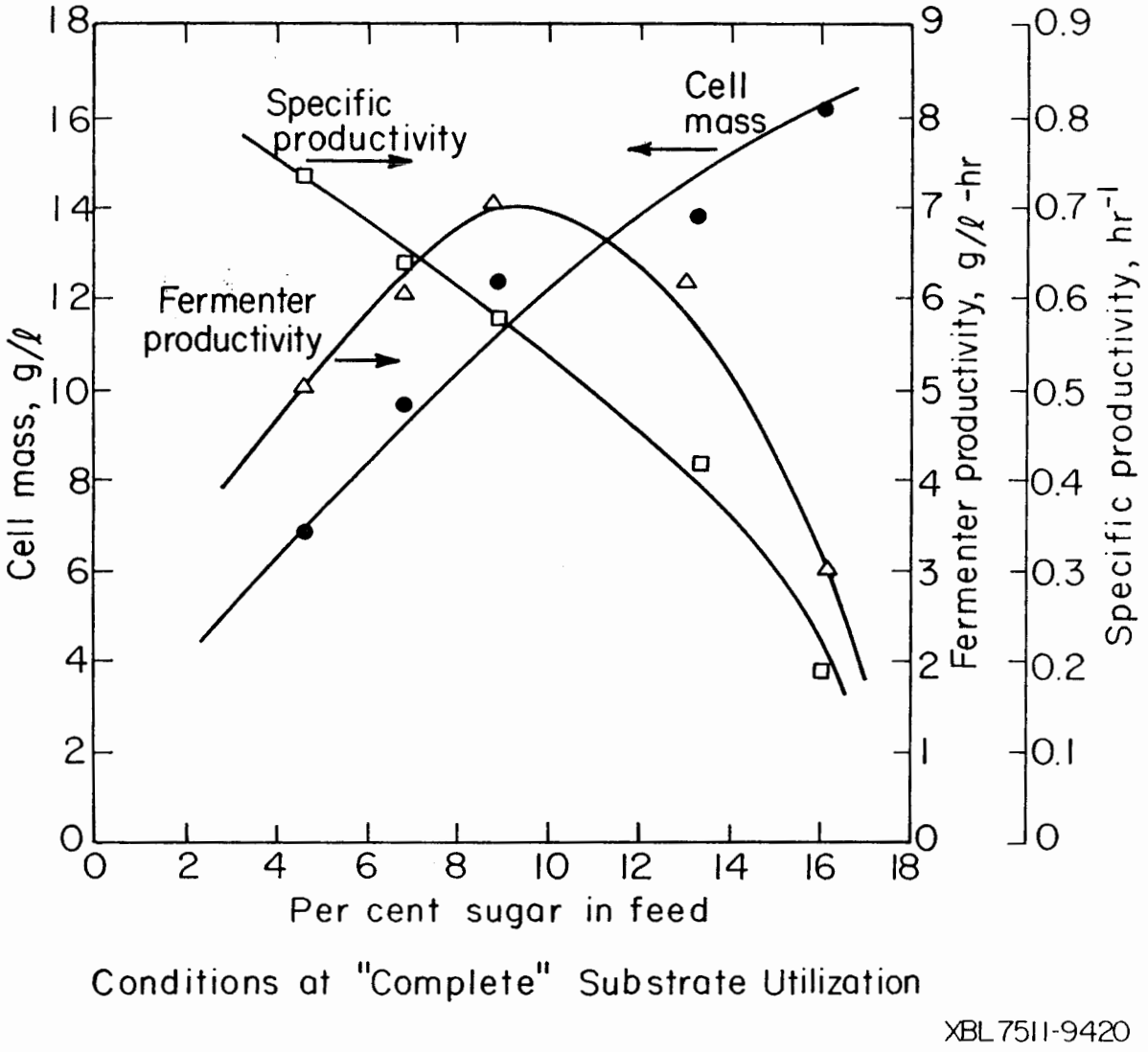

Fig. 6.24. Effect of glucose concentration on continuous fermentation.

dropped resulting in a lower fermenter productivity even though the specific productivity increased. It should be pointed out that the productivities shown in Fig. 6.24 are those at complete substrate utilization and are not the maximum productivity obtainble for a given substrate concentration.

6.2.3. Effect of pH

The effect of pH on fermentor productivities is shown in Fig. 6.25. The dilution rate and oxygen tension were held at 0.3 hr^{-1} and 0.7 mmHg, respectively, during the experiment. As shown, Saccharomyces cerevisiae had a broad pH optimum between 3.5 and 5.5. Within this range the cell mass and ethanol productivities were constant. Since the dilution rate was held at 0.3 hr^{-1}, this also implies that the cell mass and ethanol concentrations were unaffected between a pH of 5.5 and 3.5. A pH of 4.0 was chosen for the majority of fermentations to decrease the changes of contamination.

6.2.4. Cell Recycle

The results of a continuous fermentation employing cell recycle are shown in Fig. 6.26. A settler, as described in Chapter 5, was connected to the effluent of the continuous fermentor. The concentrated solution of settled cells was pumped back to the fermentor, and clarified product overflow from the settler was collected. The dilution rate to the fermentor was set and the recycle system allowed to operate until a steady cell mass concentration was achieved. A bleed of cells was not required because the settler was not 100% efficient and some cells were lost in the overflow of clarified product. At steady state and amount of cells lost in the overflow was equal to the

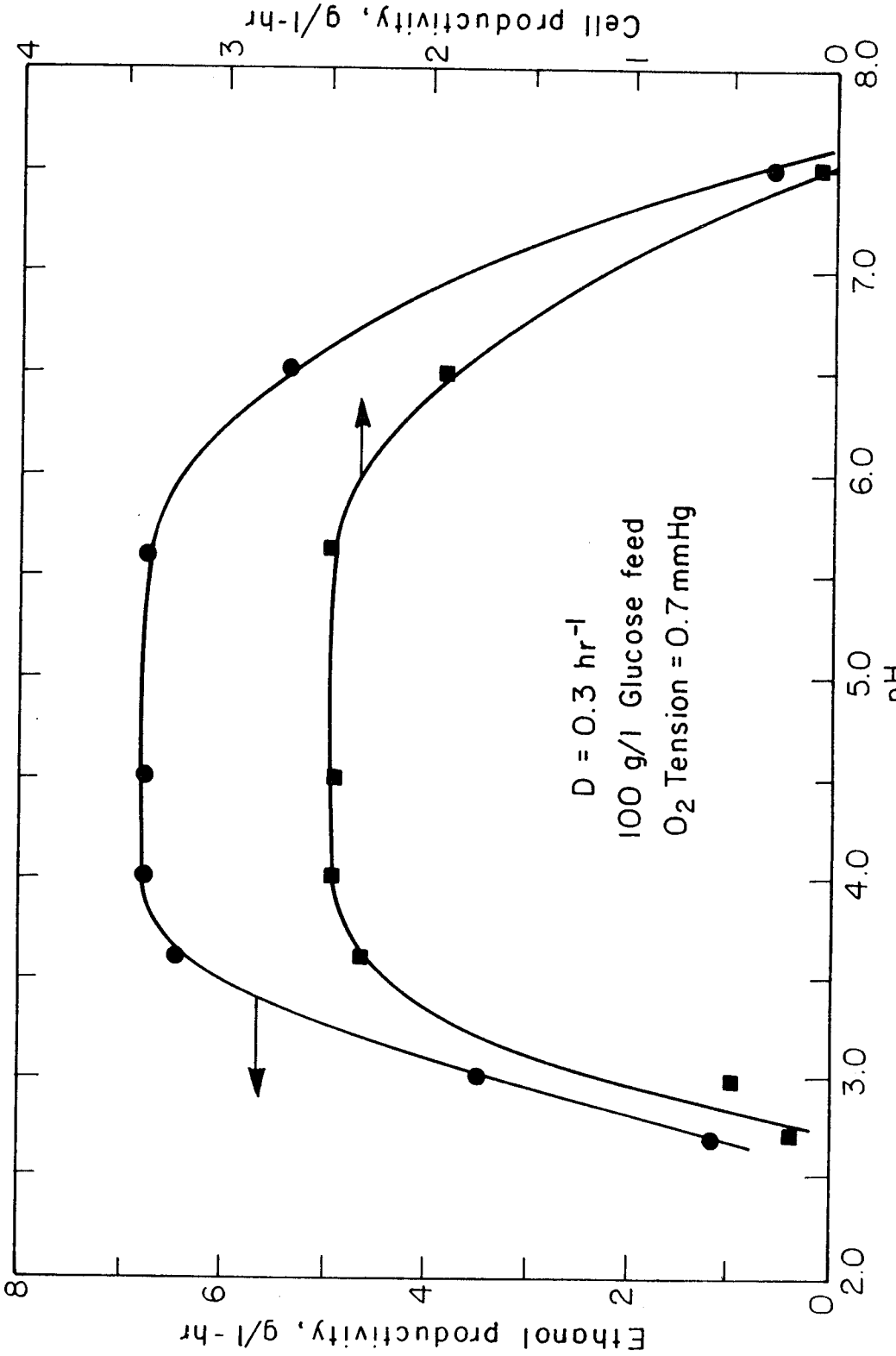

Fig. 6.25. Effect of pH on cell and ethanol productivities in continuous fermentation.

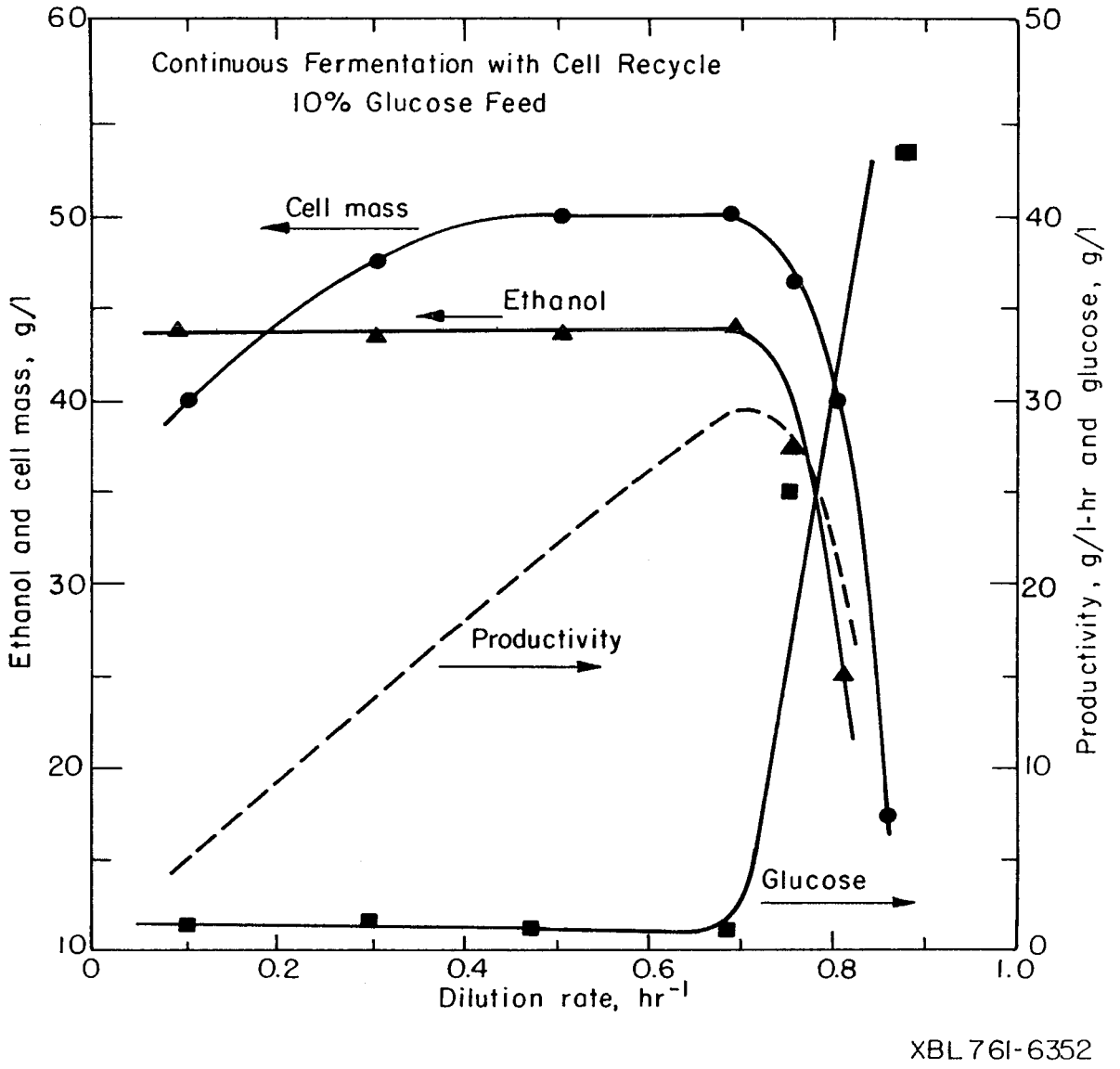

Fig. 6.26. Effect of increasing cell density by use of cell recycle in continuous fermentation.

amount of cells produced during fermentation. The cell concentration was adjusted by changing the pumping rate of the recycle stream. The fermentation was run at an oxygen tension of 0.12 mmHg and with a 10% glucose feed. To stop fermentation in the settler it was cooled to -4°C. The productivities were thus based only on fermentor volume and did not include the added volume of the settler.

The data presented in Fig. 6.26 definitely show an increase in ethanol productivity was realized by increasing the cell mass concentration in the fermentor with a recycle system. The maximum specific productivity of the yeast in the recycle system was identical to the specific productivity obtained with conventional continuous operation, 0.58 hr^{-1}, at conditions of complete substrate utilization. However, a cell mass concentration of 50 g/l or 4 times higher than without cell recycle was achieved. The net effect was a fourfold increase in fermentor ethanol productivity in the recycle system over conventional continuous operation.

The yeast did not degenerate or lose viability in the recycle system. This is evident by the same specific productivities obtained with or without cell recycle. Also, yeast viability, as determined by methylene blue strain, remained over 96% for the duration of the 14 day experiment.

The steep decrease in cell mass and ethanol productivities above a dilution rate of 0.75 hr^{-1} was due to exceeding the capacity of the settler and not because of a loss in yeast viability. When the dilution rate was increased above 0.75 hr^{-1} the flow velocity in the settler became higher than the settling velocity of the yeast. As a

result, more cells were lost in the overflow stream than generated in fermentation and the cell mass concentration in the fermentor rapidly decreased. This reduced the fermentation rate and the ethanol concentration dropped with a corresponding increase in unfermented glucose concentration.

These recycle experiments were conducted to demonstrate the feasibility and advantages of cell recycle operation for continuous ethanol production. The use of a settler was for experimental convenience only. In an industrial operation a continuous centrifuge would most probably be employed. A centrifuge is not as sensitive to changing flow rates as is a settler and would produce a more stable operation. Also a higher cell mass concentration can be obtained in the recycle stream with a centrifuge. Thus, it may be possible to achieve higher ethanol productivities than shown in Fig. 6.26 with the use of a centrifuge.

6.3. Vacuum Fermentation

A vacuum fermentation system was operated as described in Chapter 5. In all experiments the total pressure was 50 mmHg. At this pressure the boiling point of the fermentation broth containing 1% ethanol was 35°C, the optimum fermentation temperature of the yeast. A pH of 4.0 was used in the vacuum experiments. Unless otherwise stated, pure oxygen was sparged into the vacuum fermenter at a rate of 0.12 vvm at S.T.P. and an agitation rate of 500 rpm was used to supply adequate oxygen to the yeast. As discussed in Chapter 5, a liquid level control system maintained a constant fermentor volume. As liquid was removed from the fermentor through

either the vaporization of ethanol and water or by bleeding off fermented broth, fresh medium was introduced into the fermentor. The feed rate of fresh medium was then determined by the boil up rate and/or the bleed rate of fermented broth. The ethanol and water vaporized during fermentation was condensed and collected for analysis.

6.3.1. Semi-Continuous Vacuum Operation

Figures 6.27 through 6.29 illustrates the performance of the vacuum system during semi-continuous operation. Fresh medium was continually fed to the fermentor to maintain a constant volume as ethanol and water were boiled away. A bleed stream of fermented broth was not removed from the fermentor. This allowed the rapid accumulation of cell mass within the fermentor. However, components in the medium which were not metabolized by the yeast also accumulated in the fermentor under this method of operation.

The step like appearance of the ethanol productivity curves in Figs. 6.27 through 6.29 reflect that the productivity (boil up rate times the ethanol concentration in condensed product) was increased by manually increasing the biol up rate and hence the feed rate to the fermentor. The boil up rate, and hence the feed rate, was always adjusted so that the yeast was able to ferment almost all the glucose in the feed. By this means the glucose concentration in the fermentor was held between 2 to 5 g/l.

The results of a vacuum fermentation using a 10% glucose feed are shown in Fig. 6.27. The cell concentration and ethanol productivity steadily increased with time for 62 hr. A maximum ethanol productivity and cell mass of 42.5 g/l-hr and 47 g/l, respectively, were obtained.

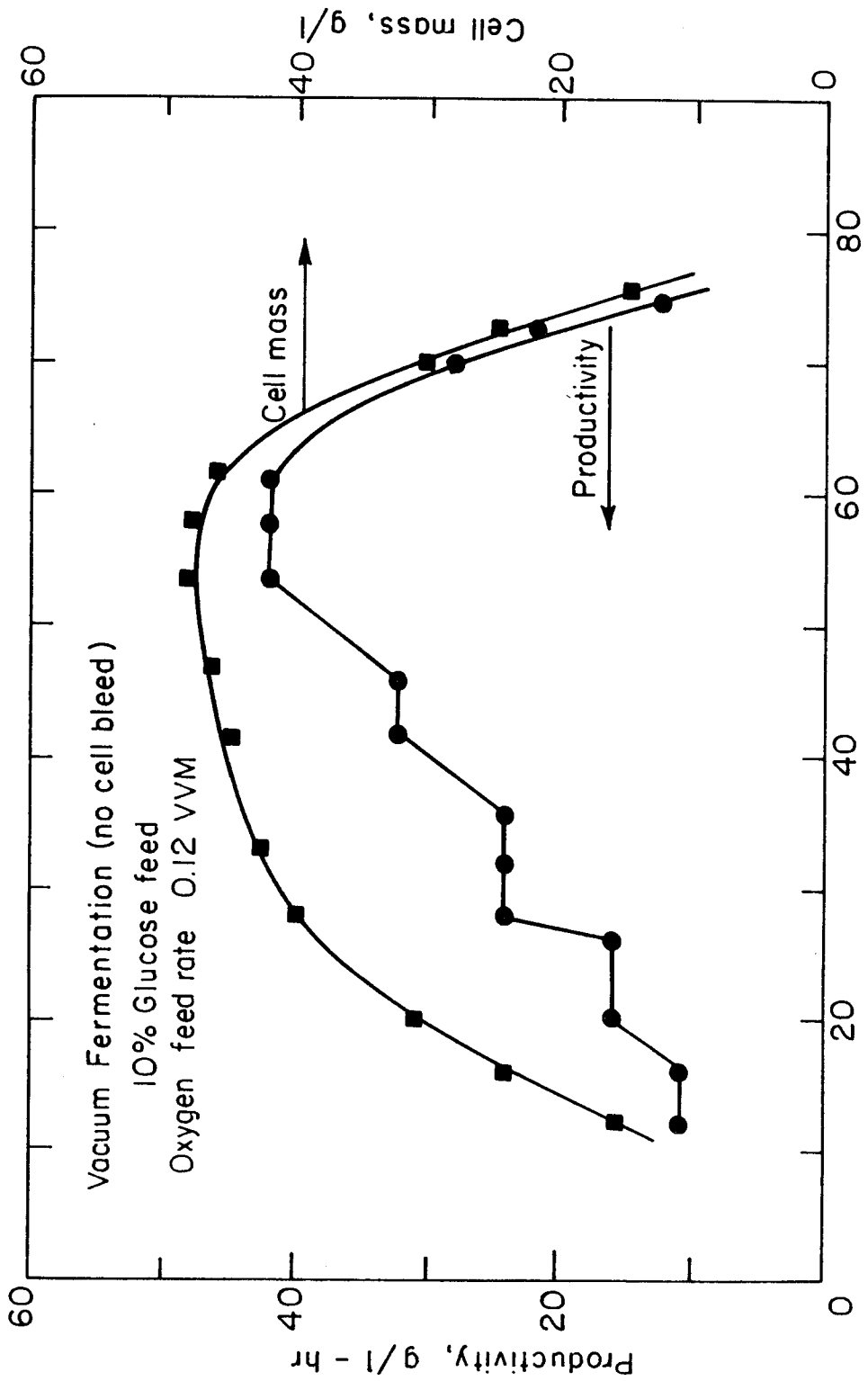

Fig. 6.27. Vacuum fermentation without cell bleed of a 10% glucose feed.

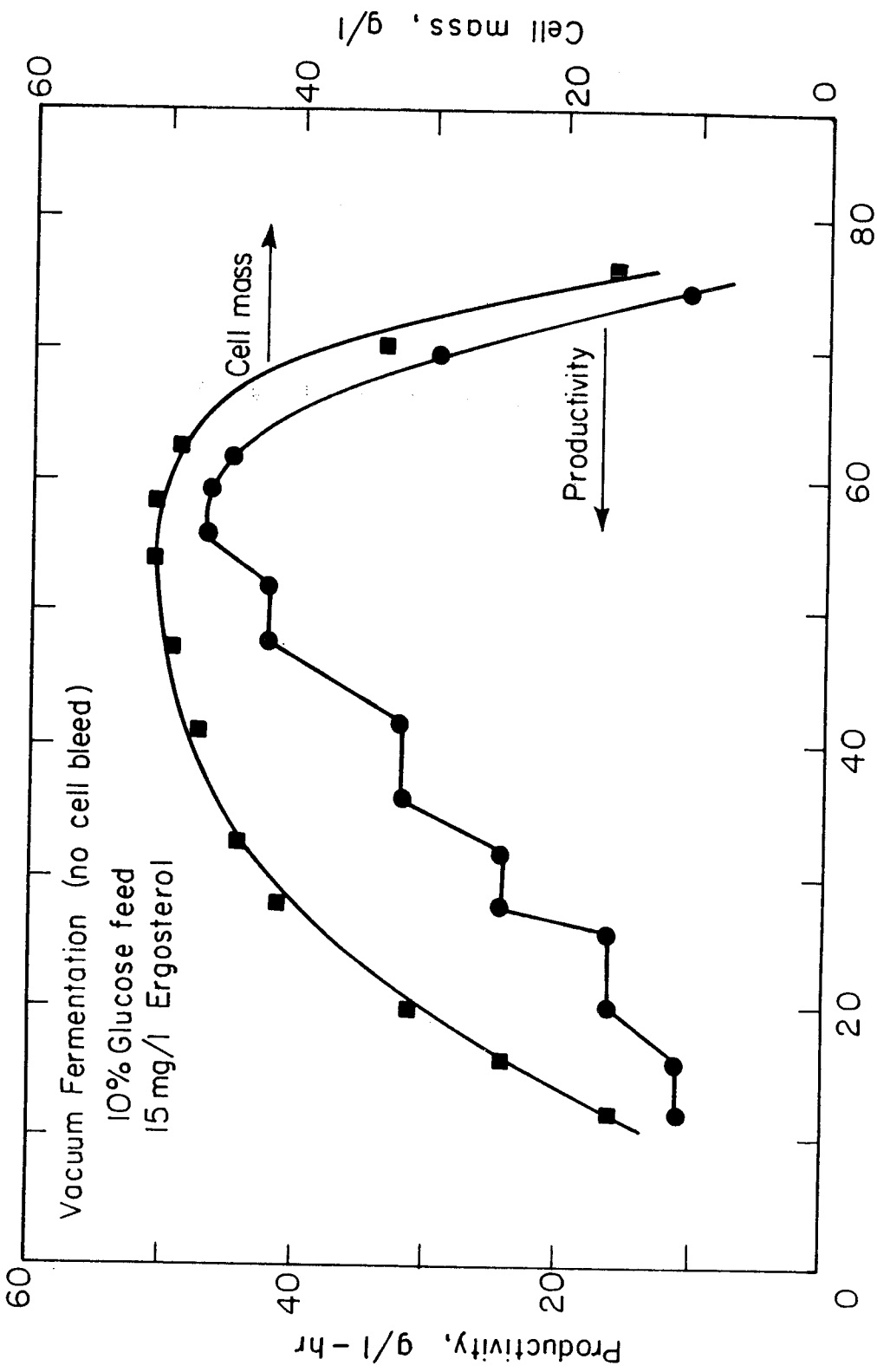

Fig. 6.28. Vacuum fermentation without cell bleed of a 10% glucose feed supplemented with 15 mg/l of ergosterol.

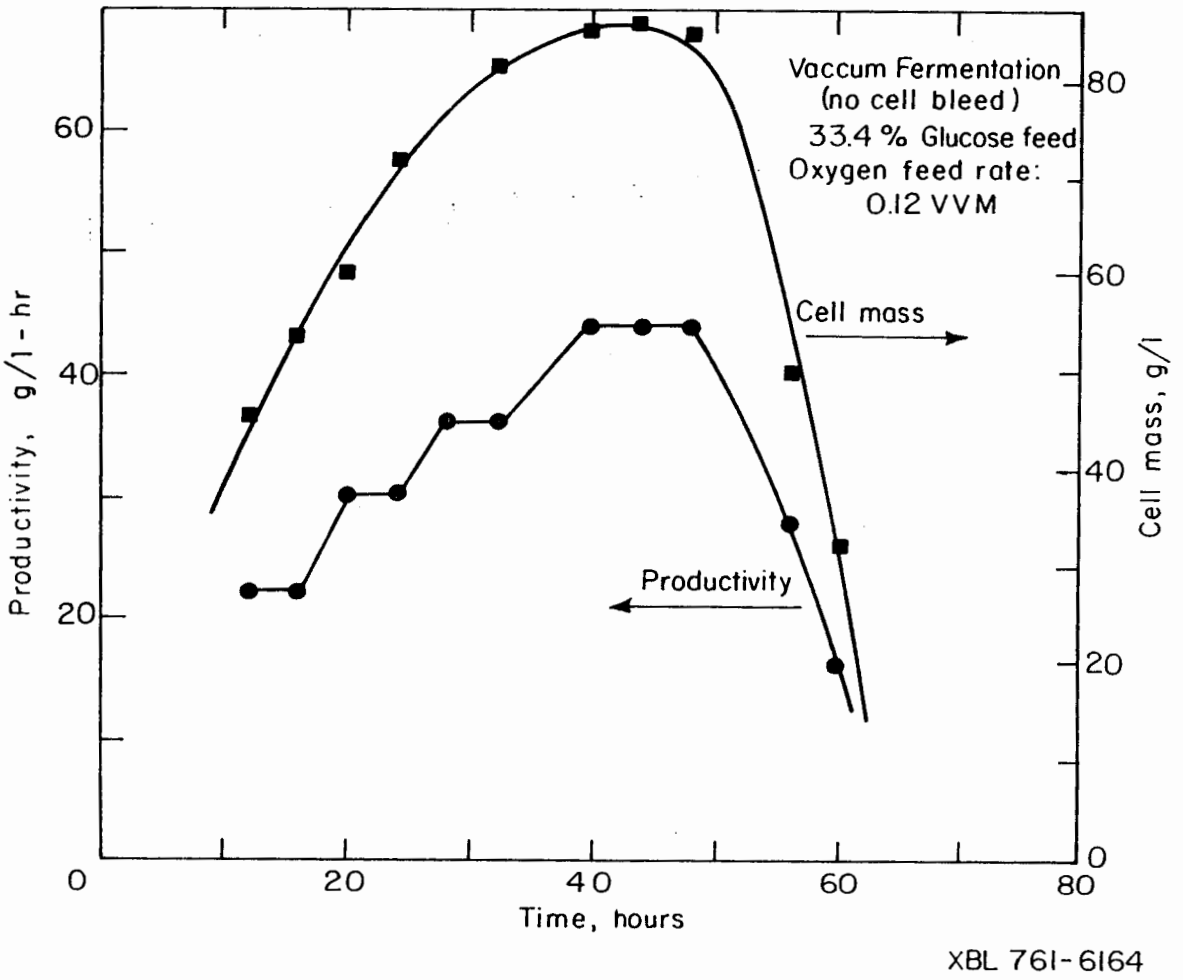

Fig. 6.29. Vacuum fermentation without cell bleed of a 33.4% glucose feed.

However, after 62 hr of fermentation the yeast cell mass concentration began to decline and the feed rate, or boil up rate, had to be sharply reduced to obtain complete fermentation of the glucose, and, as shown, the ethanol productivity correspondingly decreased.

Figures 6.28 and 6.29 show similar behavior for the semi-continuous operation of the vacuum system. The cell mass concentration initially increased permitting a higher fermentation rate per unit volume and the ethanol productivity increased. After 2 to 2.5 days of operation the cell mass and productivity declined. In Fig. 6.28 the medium was supplemented with ergosterol to eliminate the oxygen requirement during fermentation. The similar results obtained for a 10% glucose feed in Figs. 6.27 and 6.28 indicate that oxygen limitation was not affecting the fermentation and did not contribute to the decline in cell mass.

Figure 6.29 shows the results of fermenting a 33.4% glucose feed. No ethanol inhibition was detected and the ethanol productivities and cell mass rose to 44.0 g/l-hr and 68 g/l, respectively. However, as was the case with a 10% glucose feed, the cell mass and productivity sharply declined after 48 hr of fermentation.

The sharp decrease in cell mass after 2 to 2.5 days of semi-continuous operation indicated that non-volatile components were accumulating in the fermentor and killing the yeast. This required a bleed stream of fermented broth be continually withdrawn from the fermentor to keep the concentration of non-volatiles at a level that was compatable with the yeast.

6.3.2. Continuous Vacuum Operation

Figure 6.30 illustrates the effect of removing a bleed of fermented broth. The data in Fig. 6.30 were taken at steady state operation of the vacuum fermentor using a 33.4% glucose feed. The cell yield factor, $Y_{X/S}$, and cell concentration are plotted against a concentration factor. The concentration factor, C, is defined as

$$C = \frac{F}{B} (S_o)/100$$

where, F = volumetric feed rate, 1/hr

B = volumetric bleed rate, 1/hr

S_o = initial glucose concentration, g/l

A decrease in bleed rate, holding the feed rate constant, increases the concentration factor and also increases the concentration of non-volatiles in the fermentor. The concentration factor in Fig. 6.30 was increased by lowering the bleed rate. Thus, as the concentration factor increased the cell mass concentration rose because fewer cells were removed in the bleed stream. But when the concentration factor reached 8.5 the cell concentration and cell yield factor dropped. At this concentration factor the bleed stream was not sufficient and the concentration of non-volatiles reached a critical level which began to inhibit yeast growth. Further increases in the concentration factor had a deleterious affect on yeast growth. The results of Fig. 6.30 show that to sustain stable operation of the continuous vacuum fermentation a bleed of fermented broth had to be removed so that the concentration factor did not rise above 8.5.

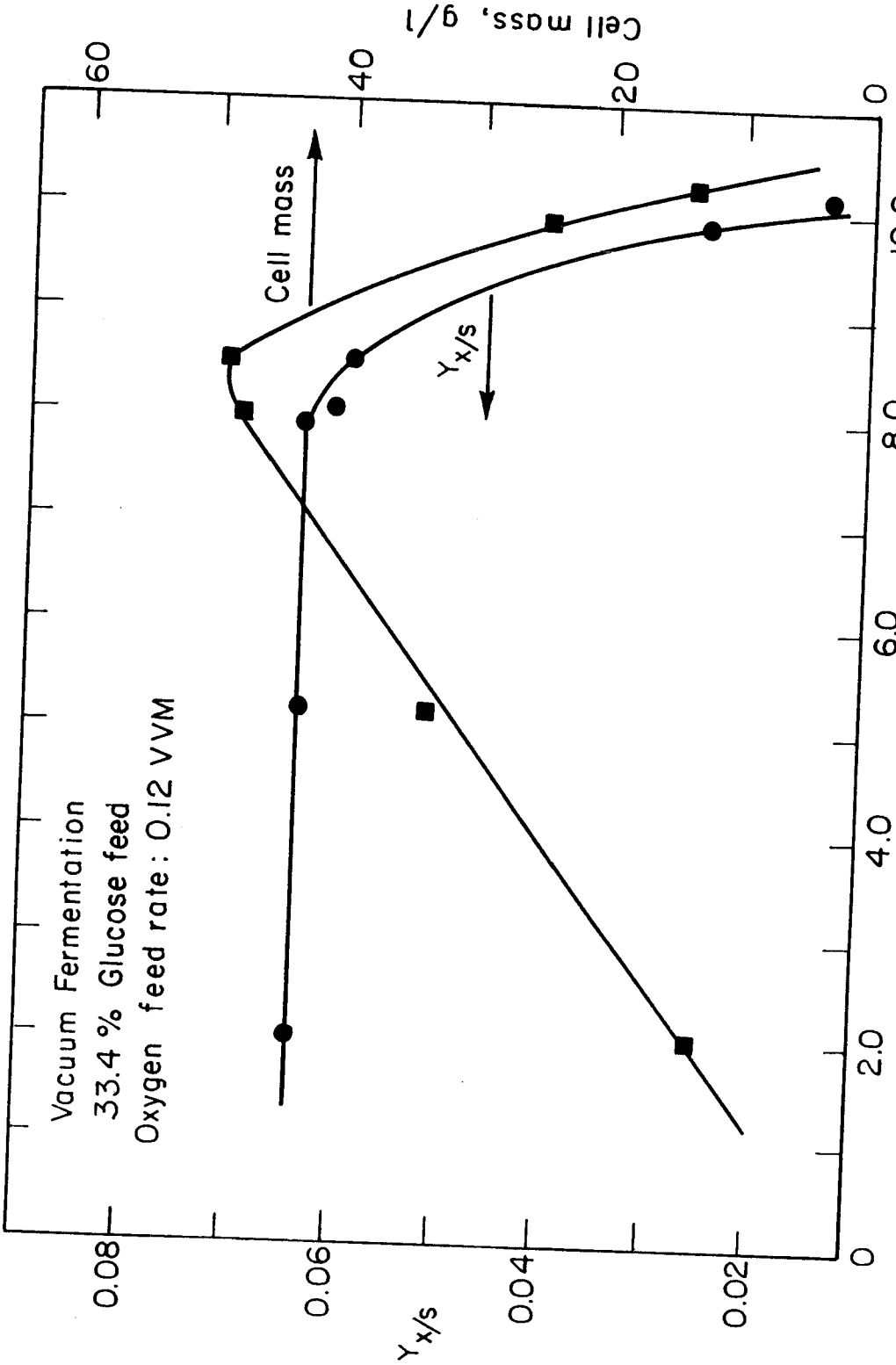

Fig. 6.30. Cell yield factor, $Y_{x/s}$, and cell mass concentration as a function of concentration factor in vacuum fermentation.

The results of a long term continuous vacuum fermentation are shown in Fig. 6.31 for a 33.4% glucose feed. A constant bleed of fermented broth was withdrawn to maintain a concentration factor of 7.7. The cell mass concentration remained stable at 50 g dry wt/l for over 13 days of continuous operation, at which point the experiment was terminated. With this concentration of yeast the 33.4% glucose feed was fermented to less than 0.4% residual sugars in a mean fermentor residence time of 3.8 hr. This corresponded to an ethanol productivity of 40 g/l-hr. Compared to conventional continuous operation, the vacuum system produced a sevenfold increase in ethanol productivity.

The specific ethanol productivity in the vacuum fermentor was 0.8 hr^{-1}. This is 33% higher than obtained for conventional continuous fermentations at optimal conditions (see Figs. 6.24). The increase in specific productivity experienced in the vacuum system seems to be a direct result of eliminating ethanol inhibition. The ethanol concentration in the fermentor was always below 10 g/l, however, during atmospheric continuous operation the ethanol concentration was 46 g/l for the optimal feed sugar concentration. As shown in Fig. 6.24, if the effluent ethanol concentration was reduced from 46 g/l to 10 g/l (representing a 10% and 2.2% sugar feed respectively in Fig. 6.24), the specific productivity was increased from 0.6 hr^{-1} to 0.8 hr^{-1} in the atmospheric fermentation. This is in direct support of the finding in the vacuum system.

The increase in ethanol productivity shown in Fig. 6.31, after 100 hr of fermentation, was achieved by simultaneously increasing

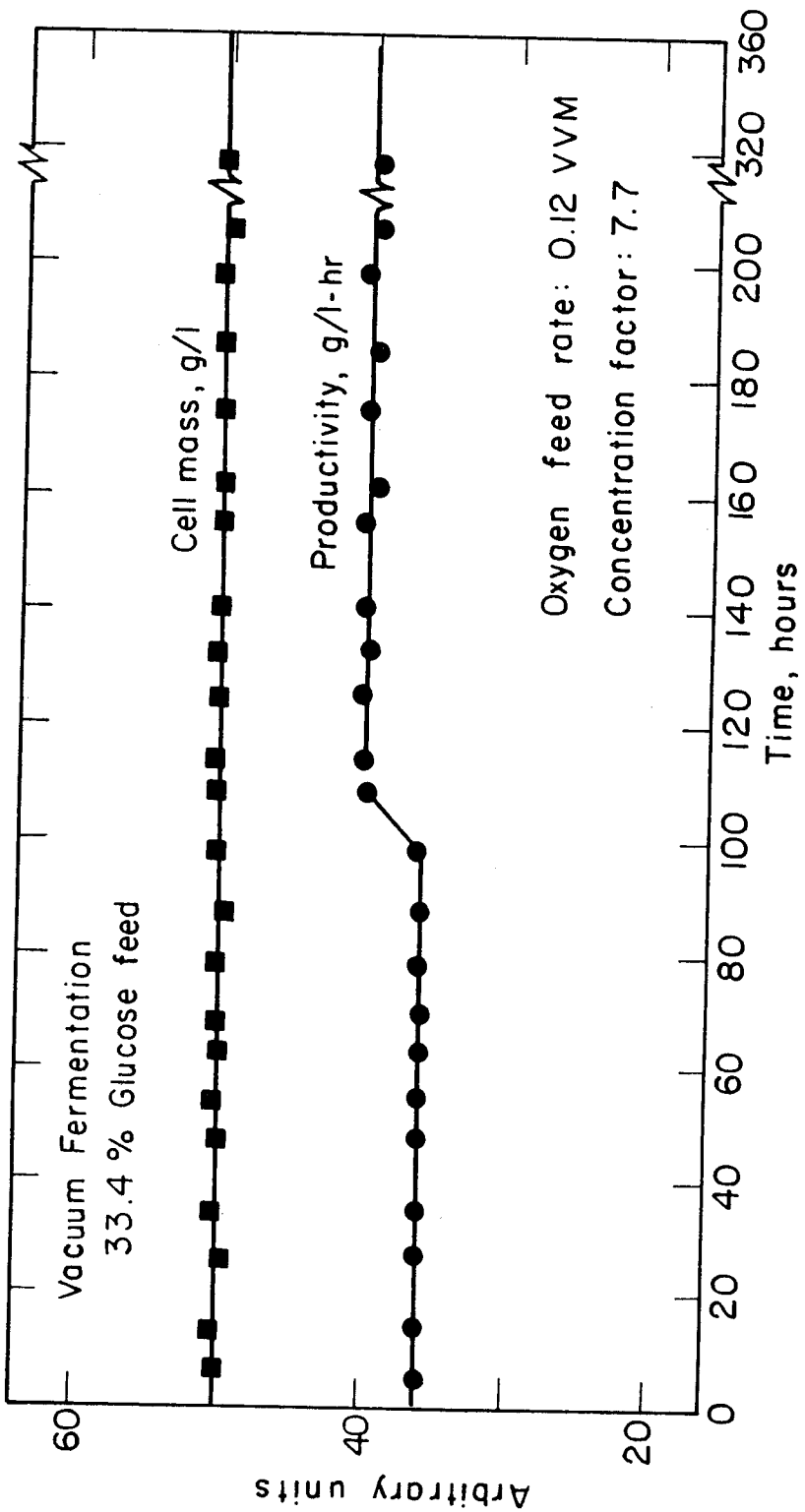

Fig. 6.31. Long term continuous vacuum fermentation.

the fermentor bleed and feed rate, thus keeping the concentration factor at 7.7. The productivity could not be increased above 40 g/l-hr and still maintain stable operation. To further increase the productivity an increase in feed rate was necessary. But from the above discussion, a corresponding increase in bleed rate had to be made to keep the concentration of non-volatile components at a level compatable with the yeast. However, as the bleed rate was increased more cells were lost in the bleed stream than produced during fermentation. As a result, the cell mass concentration rapidly dropped and conditions analogous to washout of a continuous fermentor were experienced.

6.3.3. Cell Recycle in Vacuum Fermentation

In order to remove inhibitory substances and still retain the cells a settler was used in conjunction with the vacuum system. The bleed stream from the fermentor was passed through the settler and the settled cells returned to the fermentor. In this manner, the a high concentration of cells was maintained in the fermentor at high bleed rates and conditions of washout did not occur.

The results of the settler-vacuum system are shown in Fig. 6.32. Once again a 33.4% glucose feed was used. A final cell mass of 124 g/l was achieved resulting in an ethanol productivity of 82 g/l-hr. This is almost a fourteenfold increase in productivity over that obtained in conventional continuous operation. The specific productivity of the yeast decreased from 0.8 hr^{-1} to 0.66 hr^{-1} when cell recycle was used in the vacuum system. This, no doubt, reflects that some of the yeast died during the extensive recycling. The mean residence

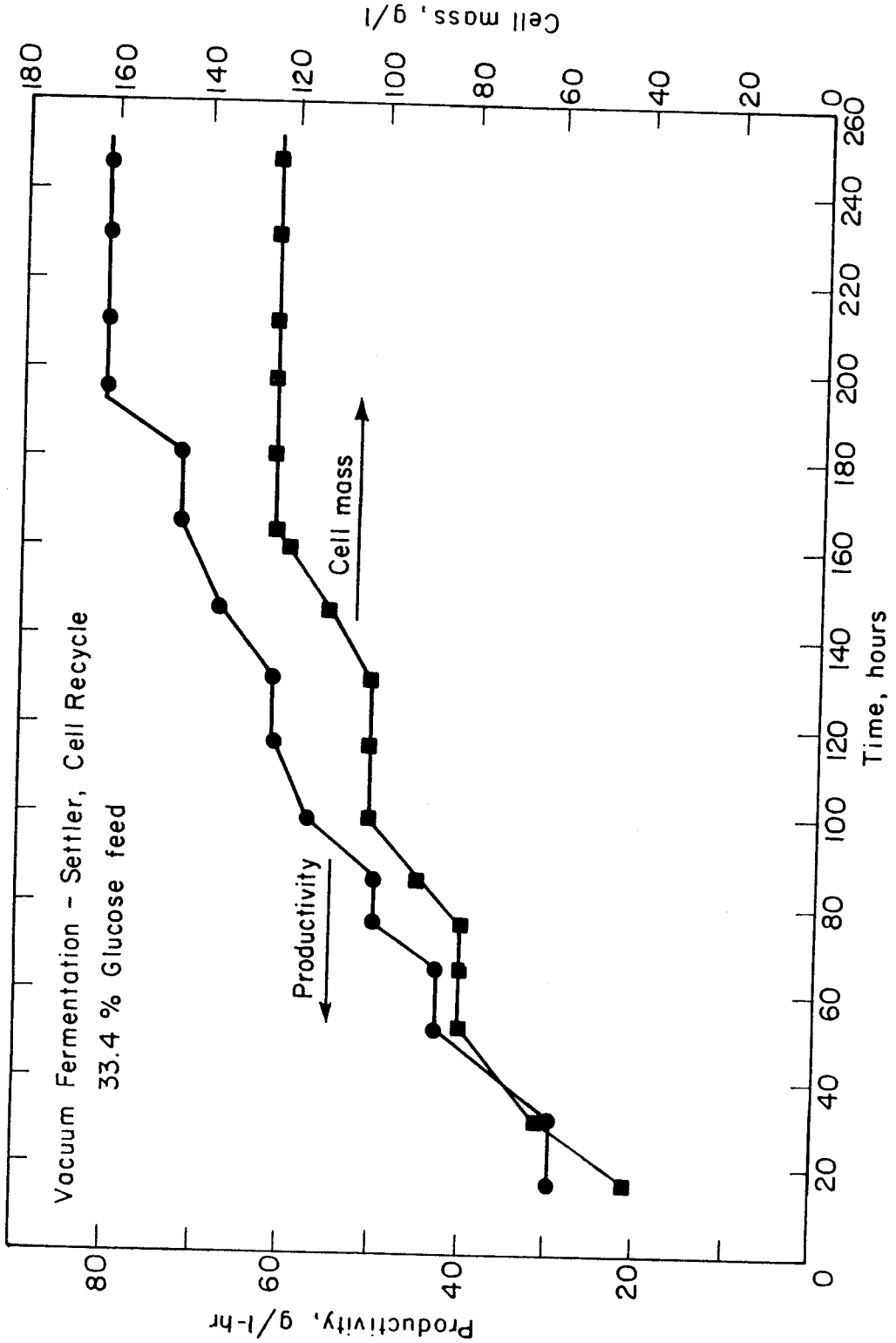

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Fig. 6.32. Effect of increasing cell density by use of cell recycle in vacuum fermentation.

time of the yeast in the fermentor was 10 times that in conventional continuous operation.

The increased productivities obtained in the vacuum system agrees with the recent work of Finn[22] on vacuum fermentations. However, Finn used an ergosterol supplemented growth medium and did not employ cell recycle. The ethanol productivity reported by Finn was 12.5 g/l-hr. This is much lower than the productivities reported here of 40 g/l-hr and 82 g/l-hr for the vacuum system with and without cell recycle, respectively. The low productivity reported by Finn may be a result of not pushing the vacuum system to its limit. The main emphasis of his work to demonstrate that a 50% sugar feed could be fermented in a vacuum fermentor.

6.3.4. Effect of Oxygen on Vacuum Fermentation

As mentioned above, Finn added ergosterol to the medium to eliminate the oxygen requirement of the yeast. However, this is not economically feasible on an industrial scale. The required amount of ergosterol would add 30 cents to the cost of producing one gallon of ethanol. For this reason, oxygen was used in this work instead of ergosterol. Although it was necessary to use pure oxygen rather than air to sustain the fermentation, the cost of oxygen adds only 0.5 cent/gal to the ethanol production cost.

Figure 6.33 illustrates the effect of oxygen on yeast viability in the vacuum system. If oxygen was not sparged into the fermentor the viability of the yeast continually dropped. Whereas, an oxygen feed rate of 0.12 vvm at S.T.P. maintained yeast viability above 95%.

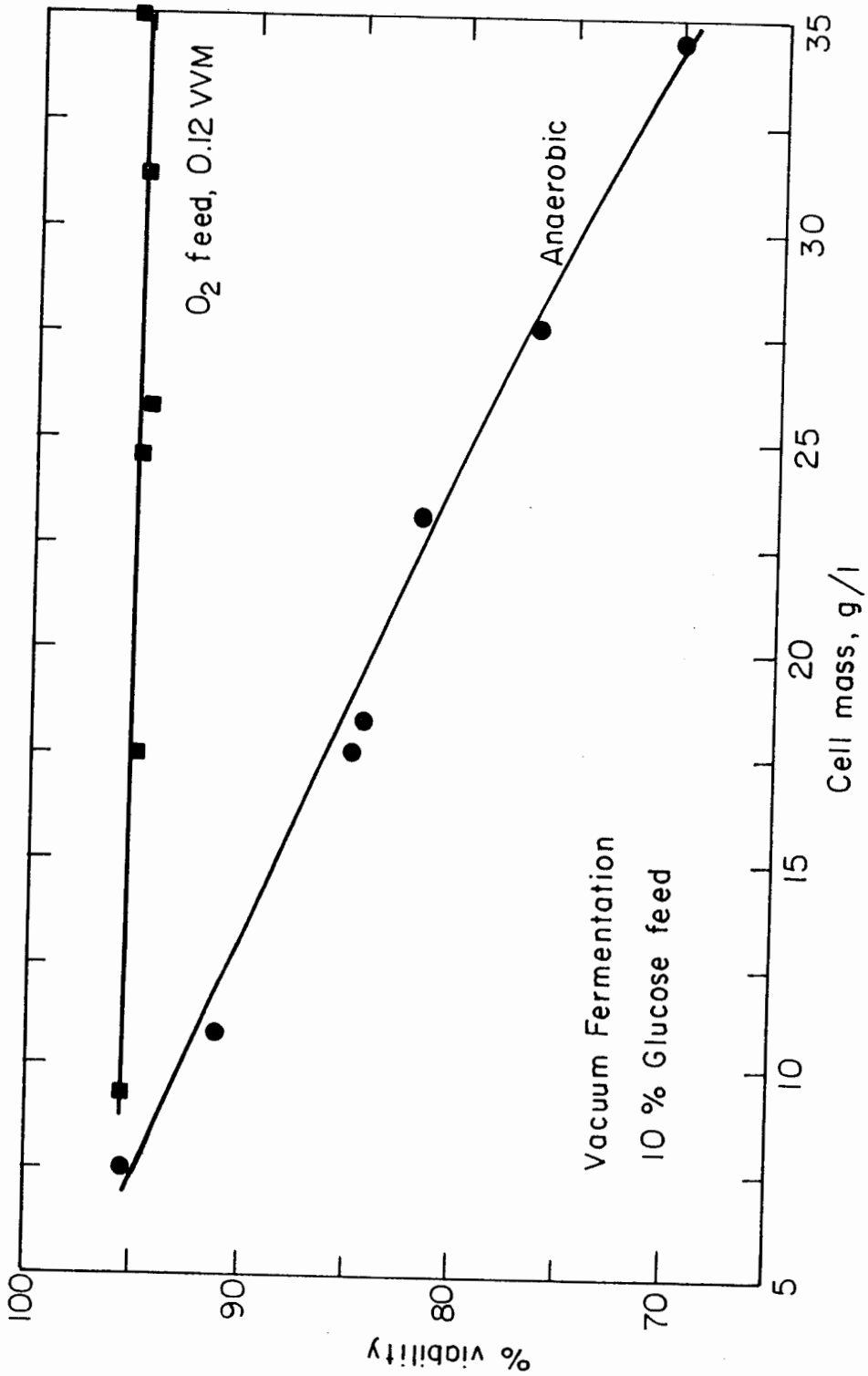

Fig. 6.33. Effect of an oxygen feed on yeast viability during vacuum fermentation.

The cell yield factor $Y_{X/S}$, was also dependent on oxygen as shown in Fig. 6.34. Under anaerobic conditions $Y_{X/S}$ was 0.016 which agrees with the anaerobic batch data shown in Fig. 6.7. When the oxygen sparge rate was increased to 0.1 vvm, $Y_{X/S}$ increased to 0.058. Further increases in the sparging rate did not effect $Y_{X/S}$. This indicated that the yeast oxygen requirement had been met at a sparging rate of 0.1 vvm.

The maximum cell yield factor of 0.058 in the vacuum system was lower than for atmospheric fermentations sparged with air. No clear explanation is evident for the low yield factor obtained in the vacuum system but may be a direct consequence of the vacuum on yeast metabolism.

It was not possible to measure the oxygen tension of the medium in the vacuum system, although this would have been very desirable. When an oxygen probe was put in the vacuum fermentor a stable reading could not be obtained because of the intense boiling taking place.

The optimal oxygen sparging rate was, however, determined for the vacuum system. The results are shown in Fig. 6.35 for the fermentation of a 33.4% glucose feed. The data were obtained at a concentration factor of 7.7. The ethanol productivity is plotted against the oxygen feed rate to the fermentor for various agitation rpm's. The highest oxygen feed rate used was 0.37 vvm at S.T.P. This corresponded to 5.6 vvm in the fermentor because of gas expansion under vacuum. Above this oxygen feed rate foaming was extensive and interferred with the liquid level control system. As a result a constant fermentor volume could not be maintained if more than 0.37 vvm

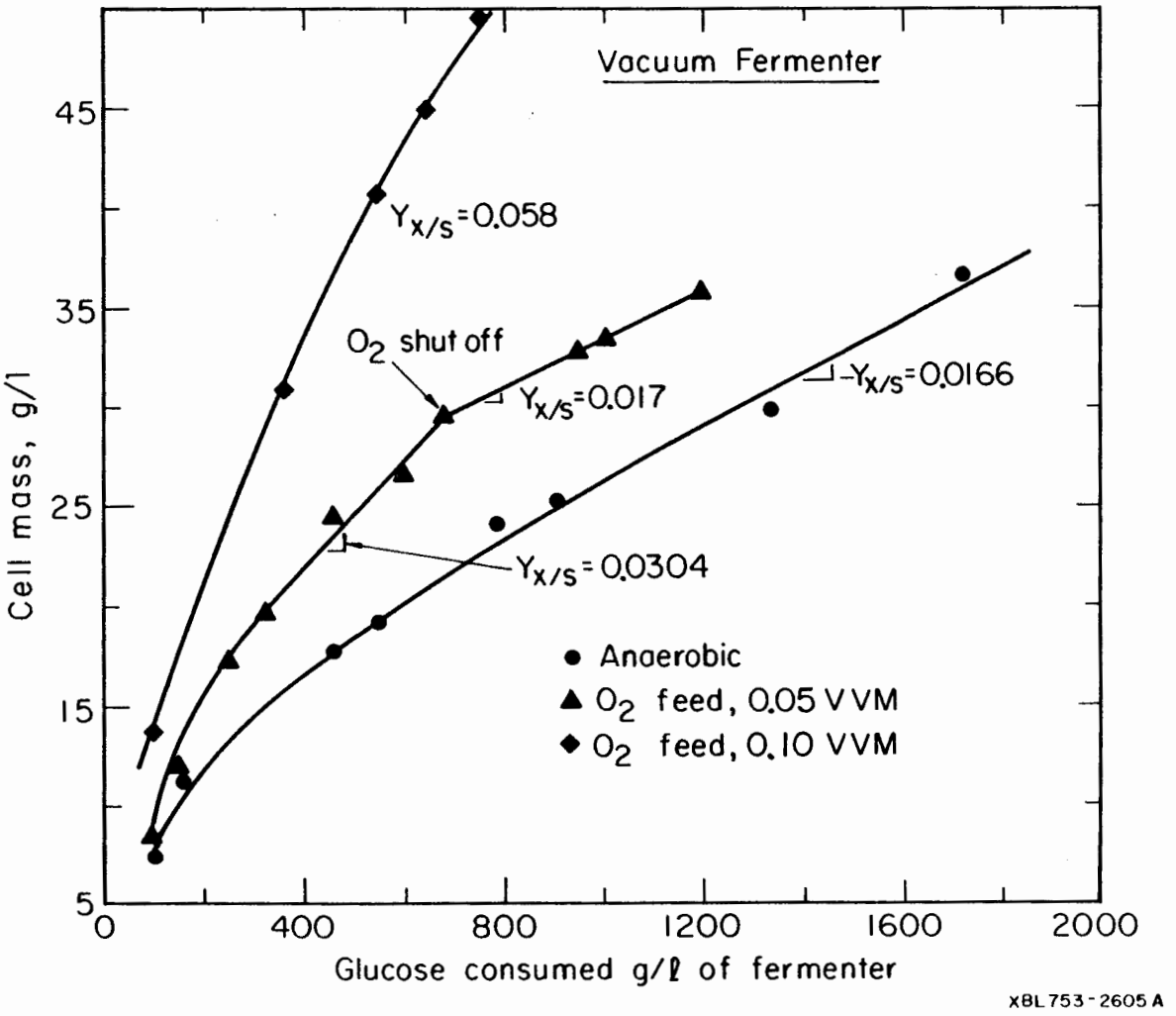

Fig. 6.34. Cell yield for various oxygen feed rates during vacuum fermentation.

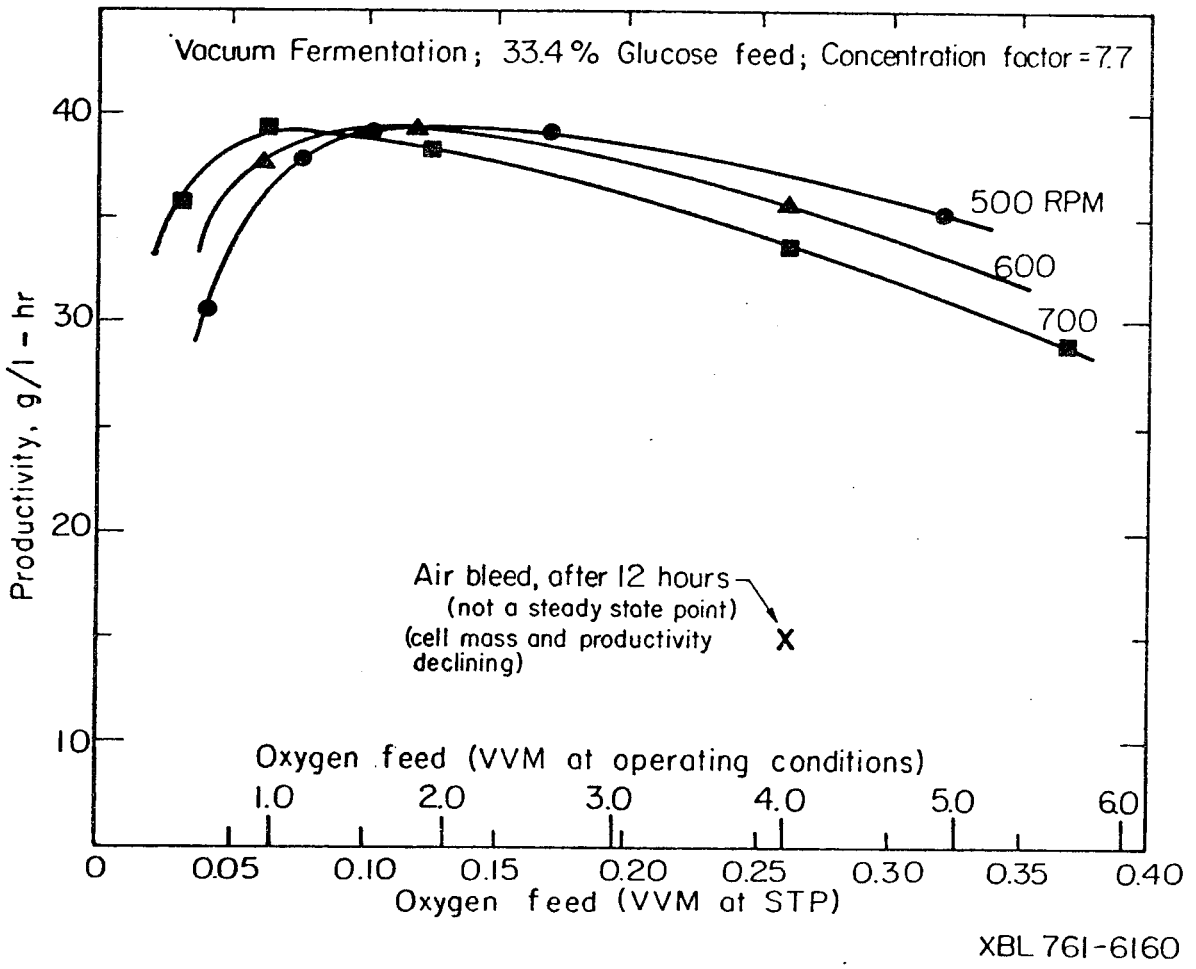

Fig. 6.35. Ethanol productivity as a function of oxygen feed rate at various agitation rates.

of gas was sparged into the fermentor.

The optimum oxygen feed rate for ethanol productivity was between 0.08 to 0.14 vvm at S.T.P. At high agitation rates the ethanol productivity declined more rapidly as the oxygen sparging rate was increased. Both increasing the agitation and oxygen feed rate increased the mass transfer rate of oxygen into the medium. This undoubtedly increased the oxygen tension in the fermentor. The productivity curves in Fig. 6.35 may then be viewed as analogous to the ethanol productivities obtained for atmospheric operation shown in Fig. 6.17. Trace amounts of oxygen stimulated ethanol production but if the oxygen concentration was too high the ethanol productivity decreased.

The result of using an air feed rather than oxygen in the vacuum system is also shown in Fig. 6.35. When air was sparged into the fermentor at a rate of 0.26 vvm at S.T.P., or 4.0 vvm at operating conditions, the ethanol productivity substantially decreased after only 12 hr of operation. The datum at 12 hr shown in Fig. 6.30 does not represent a steady state point. The productivity and cell mass concentration were declining. An oxygen feed was resumed because conditions of fermentor "washout" were feared. This points out the necessity of using pure oxygen instead of air to maintain a high enough oxygen transfer rate under vacuum to support yeast growth.

6.4. Conclusions

A major conclusion of the experimental work presented is that to obtain high ethanol production rates yeast growth is mandatory. Repeatedly, the optimal conditions for alcohol production were found to correspond to the optimal conditions for cell growth in both batch

and continuous fermentations. In this light, it was not surprising to find that oxygen had a stimulating affect on both yeast growth and ethanol productivities. If oxygen was excluded from the fermentations ethanol production rates were from 2 to 6 times lower. An otpimum oxygen concentration existed above which ethanol and cell mass production were suppressed. It was, however, possible to adapt the yeast to high oxygen concentrations and eliminate the inhibitory effect of oxygen on cell growth. But ethanol production by the adapted yeast was extremely sensitive to oxygen concentrations, requiring 1/10 the oxygen concentration for optimal ethanol production than did unadapted yeast.

The oxygen requirement of the yeast could be eliminated by the addition of 10 mg/l of ergosterol, an unsaturated lipid, to the medium. In batch fermentations the oxygen requirement could be met by initially air saturating the broth and using a 2% aerobically grown inoculum. Although the amount of oxygen in the saturated broth was limited, adequate yeast growth was obtained because the yeast utilized the pool of unsaturated fatty acids and lipids carried over in the aerobic inoculum. But in continuous operations the fermentor had to be sparged with air to achieve optimum ethanol production. If air was not sparged into the fermenter and a finite oxygen concentration maintained, continuous fermentation was not possible.

Under optimal fermentation conditions continuous culture proved to be a stable and workable means for the production of ethanol. Continuous ethanol production was maintained for over 90 days without problems of yeast mutation or contamination. When the cell mass

concentration in the fermenter was increased a factor of 4 by use of cell recycle the ethanol productivity correspondingly increased 4 times.

Further increases in ethanol productivities were obtained in a vacuum fermentor in which ethanol was boiled away from the broth as it was produced. Since ethanol inhibition was eliminated, concentrated sugar solutions (33.4%) could be fermented. Ethanol productivities of 82 g/l-hr and 40 g/l-hr were achieved in the vacuum fermentation scheme with and without cell recycle, respectively. The vacuum fermentor was thus able to increase ethanol productivities by as much as 14 times over conventional continuous culture. The economic implications of these increased productivities are discussed in the next chapter.

REFERENCES

1. S. Aiba, A. E. Humphrey, N. F. Mills, Biochemical Engineering (Academic Press, NY, 1973).
2. A. H. Cook, The Chemistry and Biology of Yeasts (Academic Press, NY, 1958).
3. E. Oura, Biotechnol. Bioeng. 16, 1197 (1974).
4. A. S. Aiyar, R. Luedeking, Chem. Eng. Prog. Symp. Ser. 62(69) 55 (1968).
5. S. Aiba, M. Shoda, M. Nagatani, Biotechnol. Bioeng. 10, 845 (1968).
6. P. J. Robers, P. R. Stewart, J. Gen. Microbio. 69, 205 (1973).
7. L. R. Bishop, J. Inst. Brew. 76, 172 (1970).
8. T. Ayrapaa, Brauwissinschafat 48 (Feb. 1970).
9. S. C. Prescott, C. G. Dunn, Industrial Microbiology (McGraw Hill, NY, 1959).
10. Organoleptic Evaluation by Author.
11. J. B. M. Rattray, S. Angelo, D. K. Kidby, Bact. Rev. 197 (Sept. 1975).
12. A. A. Andreasen, T. J. B. Stier, Cell Comp. Phys. 41, 23 (1953).
13. M. Kahler, J. Hospodka, Z. Caslavsky, Proc. A. M. Amer. Soc. Brew. Chem. 112 (1965).
14. A. D. Haukeli, S. Lie, J. Inst. Brew. 77, 253 (1971).
15. D. R. Maule, M. A. Pinnegar, A. D. Portno, A. L. Whitear, J. Inst. Brew. 72, 488 (1966).
16. D. K. Bloomfield, K. Block, J. Biol. Chem. 234, 337 (1960).
17. C. Bazua (M. S. Thesis), Dept. of Chem. Eng., Univ. of California, Berkeley (1975).

18. B. Magasanik, Cold Spring Harbor Symp. Quant. Biol. 26, 249 (1961).

19. A. D. Haukeli, S. Lie, J. Inst. Brew. 77, 253 (1971).

20. A. D. Protno, J. Inst. Brew. 74, 55 (1968).

21. P. Peringer, H. Blachere, G. Corieu, A. G. Lane, Biotechnol. Bioeng. 16, 431 (1974).

22. R. K. Finn, A. Ramalingam, The Vacuferm Process, A New Approach to Fermentation Alcohol, presented at the annual A. C. S. Meeting, Atlantic City, NJ, Sept. 9, 1974.

7. PROCESS DESIGN AND ECONOMIC EVALUATION

Based on the experimental observations in Chapter 6, ethanol fermentation plants have been designed and an economic evaluation conducted to compare four modes of operation; batch fermentation, continuous fermentation with and without cell recycle, and vacuum fermentation with cell recycle. In each design the optimum fermentation conditions of temperature, pH, and oxygen tension have been assumed. The major design variables examined are the feed sugar concentration and plant capacity. Each design is the result of a computer process model and represents an optimum design. The basic design equations and assumptions along with the cost estimation methods are shown in Appendix A.

Two sources of sugar for the ethanol fermentation process were considered; the sugar produced from the enzymatic hydrolysis of cellulosic waste as proposed by Wilke[1] and "Blackstrap" molasses. The hydrolysate sugars were assumed delivered to the ethanol fermentation plant in a 4.0% solution and 70% fermentable by S. cerevisiae. The effect of concentrating the hydrolysate sugars before fermentation was examined. But when hydrolysate sugars were used, the energy for the evaporative concentration of sugars, as well as additional energy required to run the fermentation plants, was assumed produced from the combustion of spent cellulosics in the hydrolysis process.[1] As a result, unit energy costs are substantially reduced by self-generation of power and steam when the ethanol plant is part of an enzymatic hydrolysis complex. The base energy costs, both self-generated and purchased, are shown in Table A.4.

Molasses was assumed delivered to the fermentation plant in a 50% solution, of which 100% was fermentable by S. cerevisiae.[3] For the fermentation of molasses, power was assumed purchased from a public utility. The cost of steam was calculated based on self-generation using No. 5 low sulfur fuel oil.

The costs reported below are the total ethanol production costs excluding the cost of sugar and medium nutrient supplements. The effect of sugar and nutrient costs on ethanol production is, however, examined at the end of this section.

7.1. Fermentation of Hydrolysate Sugars

The process design of each mode of operation is now discussed. The following designs were based on the fermentation of cellulose hydrolysate sugars to produce 78,000 gal/day of 95 wt% ethanol. The basis of these designs are justified following their description. In all cases the 4.0% hydrolysate sugar solution was concentrated by evaporation to 14.3% for atmospheric operation and to 50% for vacuum operation.

The ethanol produced during fermentation was concentrated to 95 wt% in each process by a single distillation step. Since only one distillation was performed, the distillate ethanol product will contain approximately 0.5% fusel oil.[3] The fusel oils have no affect on the product value if the ethanol is used as a liquid fuel. Also, the obnoxious taste imparted to the ethanol by fusel oils may eliminate the need for addition of denaturants.

7.1.1. Continuous Fermentation of Hydrolysate Sugars

The design basis of the continuous fermentation process is shown in Table 7.1. Figure 7.1 shows a schematic process flow diagram of the continuous fermentation process to produce 78,000 gal/day of 95% ethanol. The principal items of equipment corresponding to the flow sheet are listed in Table 7.2. The evaporator which concentrates the hydrolysate sugar solution is not shown, although it has been included in the process cost analysis.

After the sugars have been evaporatively concentrated from a 4.0% to a 14.3% solution (10% fermentable sugars), protein and mineral supplements are mixed with the sugars. Sterilized by steam injection, the fermentation broth is distributed to eight 1.89×10^{5} liter continuous fermentors, each operating at a dilution rate of 0.17 hr^{-1}. A low flow of air (8.0×10^{-4} vvm) is sparged through each fermentor to maintain the oxygen tension at the optimum level of 0.07 mmHg. The fermented beer then passes to four continuous centrifuges and the yeast is removed. (Only two centrifuges are shown in Fig. 7.1.) The yeast is subsequently dried and stored for sale as a protein feed supplement. The clarified beer from the centrifuges is next distilled to concentrate the ethanol to 95 wt%. An absorber, using the distillate bottoms as the absorbing liquid, is employed to recover ethanol lost in the exit gases (air and CO_2) from the fermentor. The ethanol rich stream from the absorber is also feed to the main distillation unit for final ethanol recovery.

Table 7.1. Continuous fermentation design basis.

Sugar concentration	14.3%, 70% fermentable
Dilution rate	0.17 hr^{-1}
Temperature	35°C
Cell yield factor, $Y_{X/S}$	0.12
Ethanol yield factor, $Y_{P/S}$	0.46
Cell concentration in fermentor	12.0 g dry wt/l

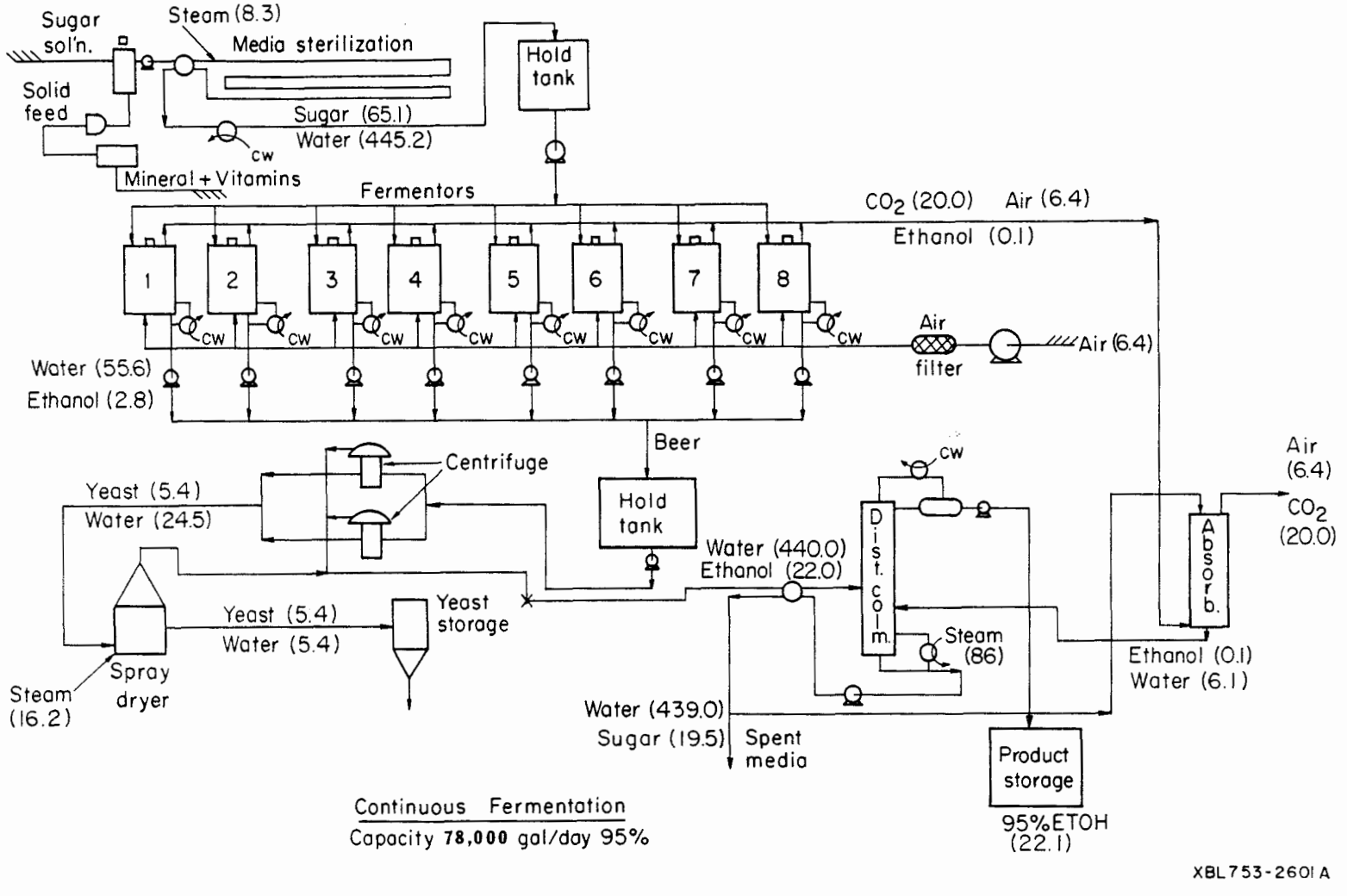

Fig. 7.1. Flow diagram and mass balance for continuous fermentation of hydrolysate sugars. Capacity 78,000 gal/day of 95% ethanol (flows in 10^3 lb/hr) cw = cooling water.

Table 7.2. Major itens of equipment for continuous ethanol fermentation plant (Capacity 78,000 gal/day of 95% ethanol).

Item	Unit Specification	Number of Units	Cost/Unit $*
Sugar Evaporator	7 effect, 4.4×10^4 ft^2 total area	1	231,300
Ethanol Fermentation		Total	1,856,700
Fermentor	Vol 1.89×10^5 liters, stainless steel Construction	8	90,500
Agitator	14 HP, stainless steel construction	8	6,500
Air Compressor	91 HP, centrifuger type, 30 psig	1	38,000
Air Filter	0.4×0.3 meters, glass fiber	8	210
Media Sterilizer	8.7×1.2 meters, insulated stainless pipe	1	11,700
Preheat Exchanger Coupled with Sterilizer	10,000 ft^2, stainless steel construction	1	112,500
Cooler Exchanger Coupled with Sterilizer	4,100 ft^2, stainless steel construction	1	64,000
Heat Removal Exchanger Coupled with Fermenter	410 ft^2, stainless steel construction	8	14,500
Solid Feeders	Screw conveyor, 4 ton/day	4	1,600
Nutrient Mixing Tank and Agitator	Vol 1.03×10^5 liters, stainless steel construction	1	45,300

Table 7.2. Continued.

Item	Unit Specification	Number of Units	Cost/Unit $*
Sugar Solution Storage Tank	Vol 2.48×10^6 liters, stainless steel construction	1	270,000
In Plant Beer Storate	Vol 4.14×10^5 liters, stainless steel construction	1	26,700
Centrifuge	Nozzle type bowl, 40 HP	4	62,000
Yeast Spray Dryer	18 ft dia stainless steel construction	2	31,100
Product Alcohol Storage Tank	Vol 5.9×10^5 liters, carbon steel construction	1	21,600
Yeast Storage Tank	Vol 1.0×10^4 liters, stainless steel construction	1	7,900
Pumps and Drivers		10	4,800
Ethanol Recovery		Total	295,600
Distillation Column	11.2 ft dia 45 sieve trays, carbon steel construction	1	76,200
Condenser	4,700 ft^2 carbon steel construction	1	69,900
Reboiler	2,600 ft^2 carbon steel construction	1	47,800
Preheat Exchanger	4,200 ft^2 carbon steel construction	1	64,500

Table 7.2. Continued.

Item	Unit Specification	Number of Units	Cost/Unit $*
Reflex Tank	Vol 1.13×10^3 carbon steel construction	1	3,800
Ethanol Absorber	7.2 ft dia, 26 ft high, 1 in. reasching rings	1	25,700
Pumps and Drivers		5	2,300

*Costs are estimated for the second quarter 1975, Marshell Stevens Index = 445.6.

7.1.2. Continuous Fermentation of Hydrolysate Sugars with Cell Recycle

The cell recycle process is identical to the continuous process described above except that a portion of cell concentrate from the centrifuges is returned to the fermentors. This increases the cell mass concentration in the fermentors permitting a higher volumetric ethanol productivity. As a result, the total fermentation volume is reduced in the cell recycle process requiring only 3 fermentors of 1.45×10^5 liters in volume to produce 78,000 gal/day of 95% ethanol. The reduction in fermentation volume is offset somewhat by an increased load on the centrifuges. Seven centrifuges are needed in the cell recycle fermentation to maintain a yeast cell concentration of 50 g dry wt/l in the fermentors. All other process equipment is the same as that listed in Table 7.2 for conventional continuous operation. A summary of the design basis of the cell recycle process is listed in Table 7.3.

7.1.3. Batch Fermentation of Hydrolysate Sugars

The batch fermentation process parallels the continuous process shown in Fig. 7.1, however, the fermentors are operated batchwise instead of continuously. A 14 hr fermentation time was assumed with an additional 6 hr required to fill, drain, and sterilize the fermentor. The net result is that 28 fermentors of 1.84×10^5 liters volume are required for the batch production of 78,000 gal/day of 95% ethanol. In addition, two 4.9×10^4 liter seed fermentors are required to produce a 2% inoculum for the main fermentors. All other processing equipment is identical to that listed in Table 7.2 for continuous operation.

Table 7.3. Continuous fermentation with cell recycle design basis.

Sugar concentration	14.3%, 70% fermentable
Dilution rate	0.7 hr^{-1}
Temperature	35°C
Cell yield factor, $Y_{X/S}$	0.12
Ethanol yield factor, $Y_{P/S}$	0.46
Cell concentration in fermentor	50.0 g dry wt/l

The design basis of the batch fermentation is shown in Table 7.4.

7.1.4. Vacuum Fermentation of Hydrolysate Sugars with Cell Recycle

Figure 7.2 shows a schematic flow diagram of a vacuum fermentation process to produce 78,000 gal/day of 95% ethanol. The design basis of the vacuum process is listed in Table 7.5. The principal items of equipment corresponding to Fig. 7.2 are shown in Table 7.6.

The hydrolysate sugars are concentrated by evaporation to 50 wt% (evaporator is not shown) and mineral and protein supplements are mixed with the sugar solution. The medium is sterilized by steam injection and fed to a single 1.89×10^5 liter vacuum fermentor operating at a total pressure of 50 mmHg and 35°C. As the fermentation proceeds, ethanol and water are boiled away from the fermentation broth. The vapor from the fermentor is compressed to 340 mmHg and condensed in the fermenter reboiler to supply the energy for the vaporization of ethanol and water in the fermentor. After the vapor recompression cycle, the uncondensable gases (carbon dioxide and oxygen) are compressed to 760 mmHg and cooled to 35°C to condense additional ethanol and water. The fermentation gases are finally fed to an absorber where the last traces of ethanol are removed. The fermented beer is pumped to atmospheric pressure and fed to two continuous centrifuges where the yeast concentrate is removed. A portion of the yeast concentrate is returned to the fermentor maintaining a yeast concentration of 125 g dry wt/l in the fermentor. The remaining yeast is spray dried and packaged for sale. The clarified beer from the centrifuges and the condensation products are fed to a

Table 7.4. Batch fermentation design basis.

Sugar concentration	14.3%, 70% fermentable
Fermentation time	14 hr
Fermentor down time per cycle	6 hr
Temperature	35°C
Cell yield factor, $Y_{X/S}$	0.056
Ethanol yield factor, $Y_{P/S}$	0.48

Table 7.5. Vacuum-recycle fermentation design basis.

Sugar concentration	50%, 70% fermentable
Dilution rate	0.26 hr^{-1}
Concentration factor*	3.0
Temperature	35°C
Pressure	50 mmHg
Cell yield factor, $Y_{X/S}$	0.058
Ethanol yield factor, $Y_{P/S}$	0.47

*See Eq. (6.1).

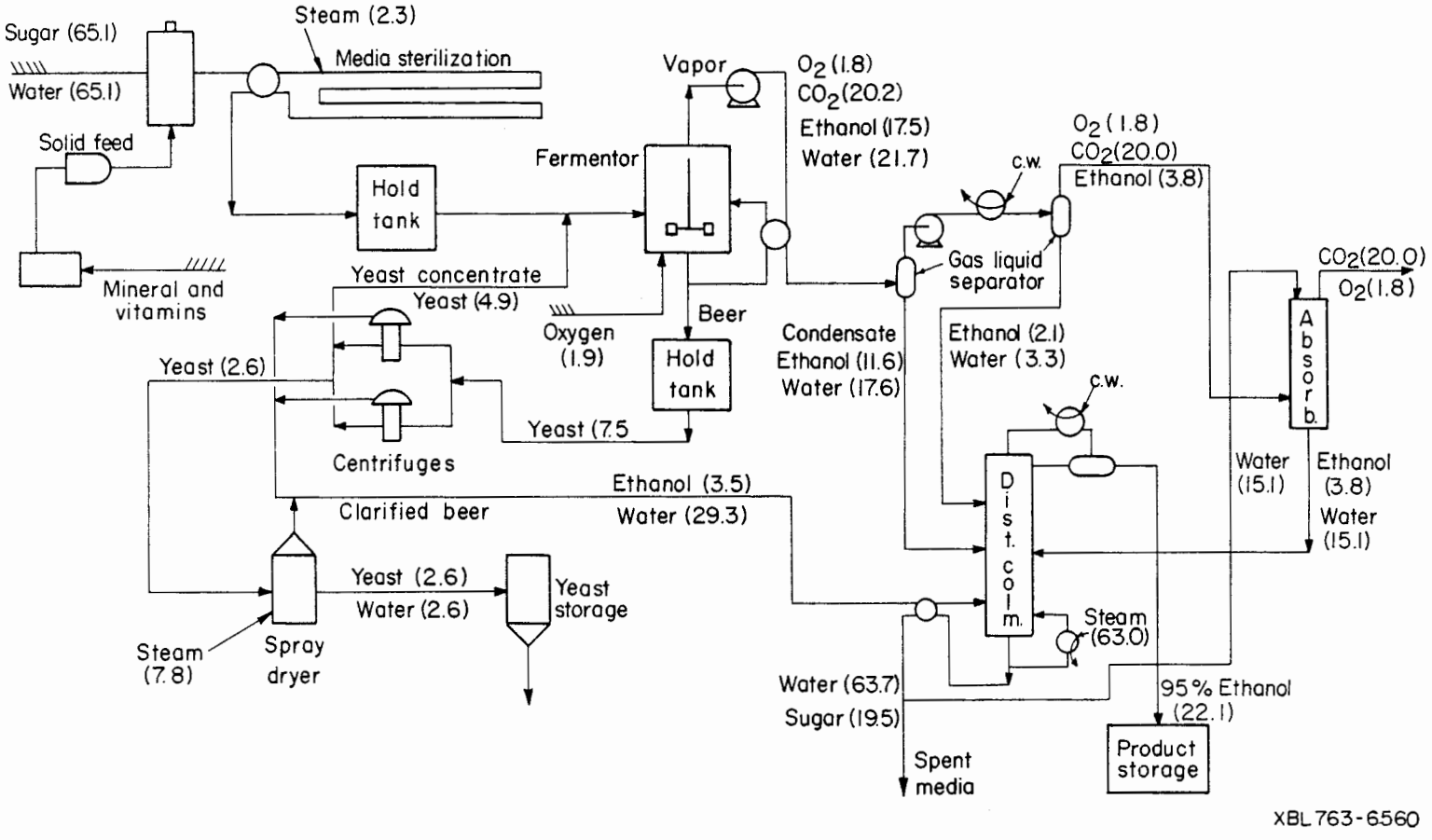

Fig. 7.2. Flow diagram and mass balance for continuous vacuum fermentation with cell recycle of hydrolysate sugars. Capacity 78,000 gal/day of 95% ethanol (flows in 10^3 lb/hr) cw = cooling water.

Table 7.6. Major items of equipment for vacuum ethanol fermentation plant (capacity 78,000 gal/day of 95% ethanol).

Item	Unit Specification	Number of Units	Cost/Unit $*
Sugar Evaporator	7 effect, 5.6×10^4 ft^2 total area	1	294,300
Ethanol Fermentation		Total	1,202,400
Fermentor	Vol 1.89×10^5 liters, stainless steel construction	1	95,500
Agitator	110 HP, stainless steel construction	1	16,400
Compressor for Vapor Recompression	2,200 HP, centrifuger type	1	385,900
Compressor for CO_2 Compression	326 HP, centrifuger type	1	80,100
Fermentor Reboiler	4,400 ft^2, stainless steel construction	1	66,500
Oxygen Filter	0.5×0.3 meters, glass fiber	1	360
Media Sterilizer	8.7×0.64 meters, insulated stainless steel pipe	1	5,500
Preheat Exchanger Coupled with Sterilizer	2,800 ft^2, stainless steel construction	1	50,500
Cooler Exchanger Coupled with Sterilizer	1,200 ft^2, stainless steel construction	1	28,900
Solid Feeders	Screw conveyors, 4 ton/day	4	1,600
Nutrient Mixing Tank with Agitator	Vol 2.9×10^5 liters, stainless steel construction	1	27,200

Table 7.6. Continued.

Item	Unit Specification	Number of Units	Cost/Unit $*
Sugar Solution Storage Tank	Vol 7.09×10^5 liters, stainless steel construction	1	121,000
In Plant Beer Storage Tank	Vol 1.18×10^5 liters, carbon steel construction	1	11,900
Gas Liquid Separators	Vol 1.5×10^3 liters, carbon steel construction	2	3,600
Secondary Vapor Condenser	100 ft^2 stainless steel construction	1	25,800
Centrifuge	Nozzle type bowl, 40 HP	2	62,000
Yeast Spray Dryer	18 ft dia, stainless steel construction	2	31,100
Yeast Storage Tank	Vol 1.0×10^4 liters, stainless steel construction	1	7,900
Product Alcohol Storage Tank	Vol 5.9×10^5 liters, carbon steel construction	1	21,600
Pumps and Drivers		12	4,800
Ethanol Recovery		Total	265,800
Distillation Column	10.2 ft dia 51 sieve trays, carbon steel construction	1	74,300
Condenser	3,700 ft^2, carbon steel construction	1	59,700
Reboiler	1,900 ft^2, carbon steel construction	1	39,400
Preheat Exchangers	200 ft^2, carbon steel construction	2	9,100

Table 7.6. Continued.

Item	Unit Specification	Number of Units	Cost/Unit $*
Reflex Tank	Vol 1.13×10^3 liters, carbon steel construction	1	3,800
Ethanol Absorber	9.5 ft dia, 70 ft high, 1 in. rasching rings	1	58,900
Pumps and Drivers		5	2,300

*Costs are estimated for the second quarter 1975, Marshell Stevens Index = 445.6.

distillation column where the ethanol is concentrated to 95%. A portion of the bottom product from the distillation column is cooled and fed to the absorber.

7.2. Process Economics

7.2.1. Fermentation of Hydrolysate Sugars

Table 7.7 compares the fixed capital investment required for the various fermentation processes. The figures in Table 7.7, as well as in Tables 7.8 and 7.9, are based on the production of 78,000 gal/day of 95% ethanol from enzymatic hydrolysate sugars. As shown in Table 7.7, a substantial decrease in capital investment is experienced in going from batch to continuous processing. The batch fermentation process requires 200 dollars of capital per gallon of ethanol produced each day. This figure is cut in half for continuous fermentation requiring only 96 dollar/gal/day. The vacuum process requires the lowest capital expenditure of only 5.46 million dollars or 70 dollar/gal/day.

The continuous-cell recycle process requires the lowest capital for the fermentation equipment, but this is offset somewhat by an increased expenditure for centrifuges for yeast recovery and to maintain a high cell density in the fermenters.

Although the vacuum system requires only one fermentor, a higher capital expenditure is needed for the fermentation equipment in the vacuum system than in the cell recycle fermentation. This is due to the large compressors and fermenter reboiler needed in the vacuum process. The increased fermentation equipment cost is, however, balanced by a reduction in the size of auxiliary equipment (media

Table 7.7. Required fixed capital investment for different modes of operation. Plant capacity 78,000 gal/day of 95% ethanol from 4.0% hydrolysate sugar solution.

	Fixed Capital Investment, 10^3 Dollars			
	Batch	Continuous	Continuous Cell Recycle	Vacuum Cell Recycle
Sugar Concentration	717	717	717	912
Fermentation	14,047	4,283	2,515	2,746
Ethanol Recovery	916	916	916	824
Yeast Recovery	986	986	1,362	794
Storage	839	839	839	183
Total	15,505	7,741	6,349	5,459

Table 7.8. Ethanol production costs for different modes of operation. Plant capacity 78,000 gal/day of 95% ethanol from 4.0% hydrolysate sugar solution.

	Production Cost Cent/Gal			
	Batch	Continuous	Continuous Cell Recycle	Vacuum Cell Recycle
Investment Related Costs	10.8	5.4	4.4	3.8
Operating Labor	3.3	1.0	0.6	0.4
Supervision and Clerical	0.2	0.1	0.1	0.1
Utilities				
Water	0.5	0.5	0.5	0.4
Power	0.4	0.2	0.3	0.2
Steam	3.7	3.6	3.6	3.8
Oxygen	-	-	-	0.5
Laboratory Changes	0.1	0.1	0.1	0.1
Plant Overhead	1.8	0.6	0.4	0.3
Total	20.8	11.5	10.0	9.6

Table 7.9. Ethanol production costs for different modes of operation. Plant capacity 78,000 gal/day of 95% ethanol from 4.0% hydrolysate sugar solution.

	Production Cost Cent/Gal			
	Batch	Continuous	Continuous Cell Recycle	Vacuum Cell Recycle
Sugar Concentration	2.9	2.9	2.9	3.8
Fermentation	13.9	4.6	2.8	2.7
Ethanol Recovery	2.5	2.5	2.5	2.2
Yeast Recovery	0.8	0.8	1.1	0.5
Storage	0.7	0.7	0.7	0.4
Total	20.8	11.5	10.0	9.6

sterilizer, storage and mixing tanks, centrifuges and distillation column) because a more concentrated sugar solution (50%) is used in the vacuum process. There is of course a slight increase in sugar concentration costs for the vacuum system.

The reduction of capital investment is reflected in the ethanol production costs shown in Tables 7.8 and 7.9. The largest decrease in production cost is achieved by using continuous operation rather than batch. Batch ethanol production costs are 20.8 cent/gal while only 11.5 cent/gal is required for continuous fermentation. A further decrease of production cost to 10.0 cent/gal and 9.6 cent/gal is obtained in the continuous-cell recycle and vacuum fermentations, respectively. As shown in Table 7.9 the low fermentation cost of the continuous-cell recycle process is partially offset by an increased cost for yeast recovery. Whereas, the low fermentation cost in the vacuum process is offset by an increased cost to concentrate the sugar to 50%.

7.2.2. Fermentation of Molasses

Ethanol production costs from "Blackstrap" molasses are shown in Table 7.10. The molasses is delivered to the fermentation plant in a 50% sugar solution. Thus, no sugar concentration step is required. Rather, the sugar must be diluted to a 10% solution before fermentation in all processes except the vacuum system. Also, as discussed above, the energy for the molasses fermentation processes was assumed purchased from a public utility rather than self-generated. As a result, the unit energy costs are much higher for the molasses fermentations (see Table A.4 in Appendix A).

Table 7.10. Ethanol production costs for different modes of operation. Plant capacity 78,000 gal/day of 95% ethanol from 50% "Blackstrap" molasses sugar solution.

	Production Cost Cent/Gal			
	Batch	Continuous	Continuous Cell Recycle	Vacuum Cell Recycle
Investment Related Costs	10.3	4.9	4.0	3.2
Operating Labor	3.2	0.9	0.5	0.4
Supervision and Clerical	0.2	0.1	0.1	0.1
Utilities				
Water	0.6	0.6	0.6	0.4
Power	1.2	0.6	0.9	0.6
Steam	10.1	9.5	9.5	6.8
Oxygen	-	-	-	0.5
Laboratory Changes	0.1	0.1	0.1	0.1
Plant Overhead	1.8	0.6	0.4	0.3
Total	27.5	17.3	16.1	12.4

Analogous to the hydrolysate sugar fermentations, as shown in Table 7.10 a reduction of almost 10 cent/gal in production costs results if continuous rather than batch fermentation is employed. However, the vacuum process appears much more attractive relative to the other processes for the fermentation of molasses than for the fermentation of hydrolysate sugars. Almost 5 cent/gal can be saved with the vacuum system compared to conventional continuous fermentation.

The advantage of the vacuum process are twofold. The 50% molasses solution must not be diluted and the ethanol distillation cost (as reflected by the steam cost) is reduced

The reduced distillation cost in the vacuum process is due to the preliminary concentration of ethanol achieved in the vapor recompression cycle used to maintain the fermentor vacuum. The ethanol concentration is increased from a mole fraction of 0.03 in the broth to 0.24 in the condensed vapor. The resulting increased ethanol concentration in the feed to the distillation column allows the use of a lower reflux ratio for the final concentration of ethanol to 95%. By decreasing the required reflux ratio (i.e., moles reflux per mole reflux plus product) from 0.88 for atmospheric fermentations to 0.85 for vacuum fermentation an overall steam savings of 17% is obtained. This includes the steam required for compressor operation in the vacuum system.

A reduced distillation cost was also experienced in the vacuum process for the fermentation of hydrolysate sugars, as shown in Table 7.9. But the steam requirement to concentrate the sugar to 50% offsets the steam savings in the distillation. Thus, as shown

in Table 7.8, the vacuum system actually requires more steam than conventional atmospheric pressure operation for the hydrolysate sugar fermentation.

As discussed above, another advantage of the vacuum fermentation scheme is elimination of end product inhibition by boiling off ethanol as it is produced. However, by changing the fermentation pressure, the equilibrium ethanol concentration is altered and the ethanol concentration in the fermenting broth may be adjusted to any desired level. Figure 7.3 shows the effect of fermentor ethanol concentration on production costs for the vacuum system. The production costs do not include sugar concentration costs. When the ethanol concentration of the broth is low the equilibrium vapor concentration is also low. A high boil up rate is thus necessary to remove the required amount of ethanol. This increases the vapor compression costs in the vapor recompression cycle and production costs increase. At high ethanol concentrations the compression costs are reduced but fermentation costs increase because the yeast becomes inhibited by the ethanol. As shown in Fig. 7.3 these two competing effects produce a rather flat production cost curve between ethanol concentrations of 5.0% and 8.0%.

The production cost of vacuum fermentation without cell recycle is also shown in Fig. 7.3. Production costs without cell recycle rise more rapidly with increased ethanol concentration than when cell recycle is employed. This stems from the overall mass balances. When the ethanol concentration is high the boil up rate necessary to remove the required amount of ethanol is low and, from the mass balance, a large bleed rate from the fermentor is necessary. But,

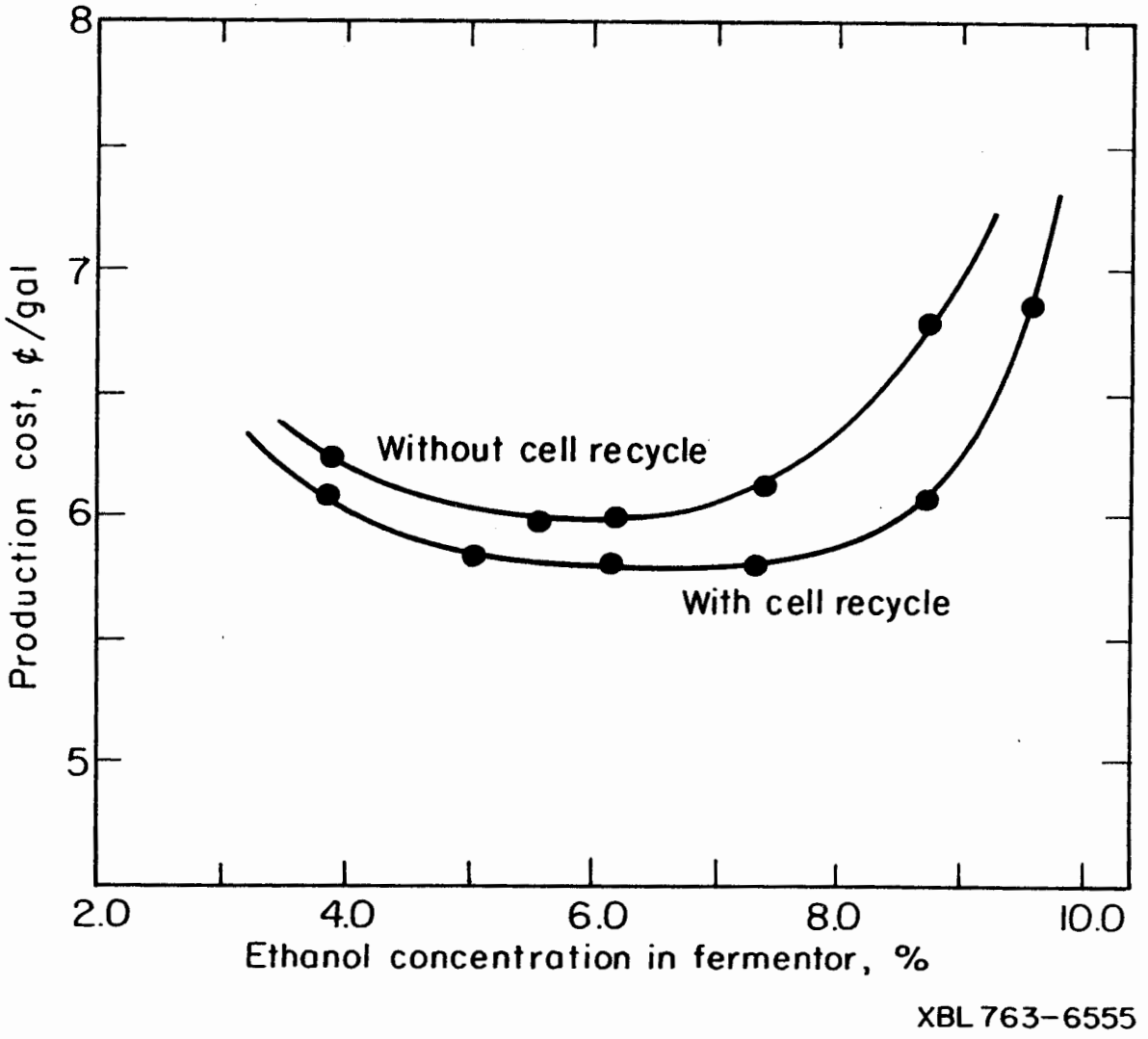

Fig. 7.3. Effect of ethanol concentration in vacuum fermentor on ethanol production costs.

a substantial amount of cell mass is removed with the bleed stream and the yeast concentration in the fermentor decreases. Since the cell mass concentration decreases, the fermentation rate per unit volume decreases and the fermentation costs increase. This of course is not the case when cell recycle is employed because the biomass concentration in the fermenter is maintained at a high level by returning a portion of the yeast to the fermenter.

7.3. Effect of Feed Sugar Concentration on Ethanol Production Costs

Ethanol production costs, excluding sugar concentration charges, are plotted against the feed concentration of fermentable sugar in Fig. 7.4 for conventional continuous fermentation. There is a definite optimum production cost at 10% sugar feed. This coincides with the optimum fermentor productivity at a 10% sugar concentration shown in Fig. 6.24. As discussed in Chapter 6, above a 10% sugar feed ethanol inhibition slows the fermentation rate. As a result, a larger fermentor volume must be used and as shown in Fig. 7.4 the fermentation costs increase. Below 10% sugar feed, the cell mass concentration decreases lowering the fermentor ethanol productivity which again increases the fermentation costs. Also, at low feed sugar concentrations dilute solutions of ethanol are produced. This increases the distillation cost because more energy is required to concentrate these dilute solutions to 95% ethanol.

Since 70% of the hydrolysate sugars are fermented by Saccharomyces[2] the 14.3% sugar feed used in the above continuous fermentation processes corresponds to the optimum sugar feed concentration shown in Fig. 7.4.

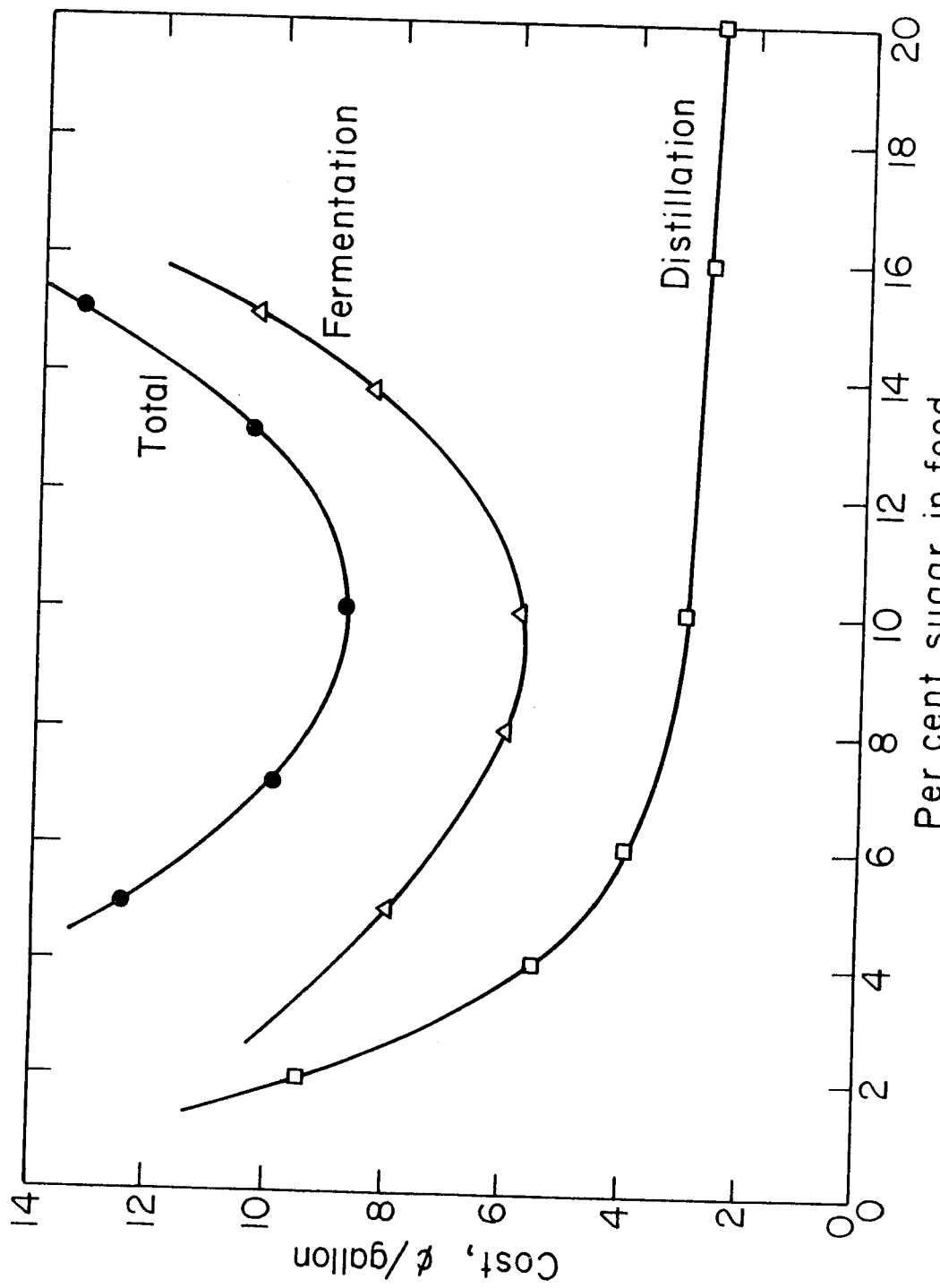

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Fig. 7.4. Effect of fermentable sugar concentration on ethanol production costs in continuous culture.

The effect of sugar concentration on ethanol production costs for the vacuum fermentation process is illustrated in Fig. 7.5. The production costs shown are exclusive of sugar concentration costs. As the sugar concentration increases, the fermentation costs steadily declines since ethanol inhibition is not a problem in the vacuum fermentation and high sugar concentrations allow a reduction in the size of process equipment. However, there is a practical limit to the sugar concentration which may be employed. If the solution becomes too concentrated it is extremely difficult to pump because of high viscosity. Also as the sugar is concentrated, so are non-volatile constituents in the medium such as minerals and salts. At high concentrations these components may become toxic to the yeast. In absence of knowledge of the exact optimum, a total sugar concentration of 50% was set in the above designs. This is a typical concentration for "Blackstrap" molasses which has been shown readily fermentable by Saccharomyces.[3]

The cost of concentrating the hydrolysate sugars is shown in Fig. 7.6. An initial hydrolysate sugar concentration of 4.0% was taken.[1] The sugar was assumed concentrated in a 7 effect evaporator having a total steam efficiency of 5.0.[4] If low cost steam is available (as was the case when the ethanol fermentation was part of an enzymatic hydrolysis facility) it is economical to concentrate the sugar to 14.3% and 50% for continuous and vacuum fermentation, respectively. Comparison of Fig. 7.6 with Fig. 7.5 shows that 1.1 and 2.0 cents per gallon may be saved by concentrating the sugar in the continuous and vacuum fermentations respectively if the cost of

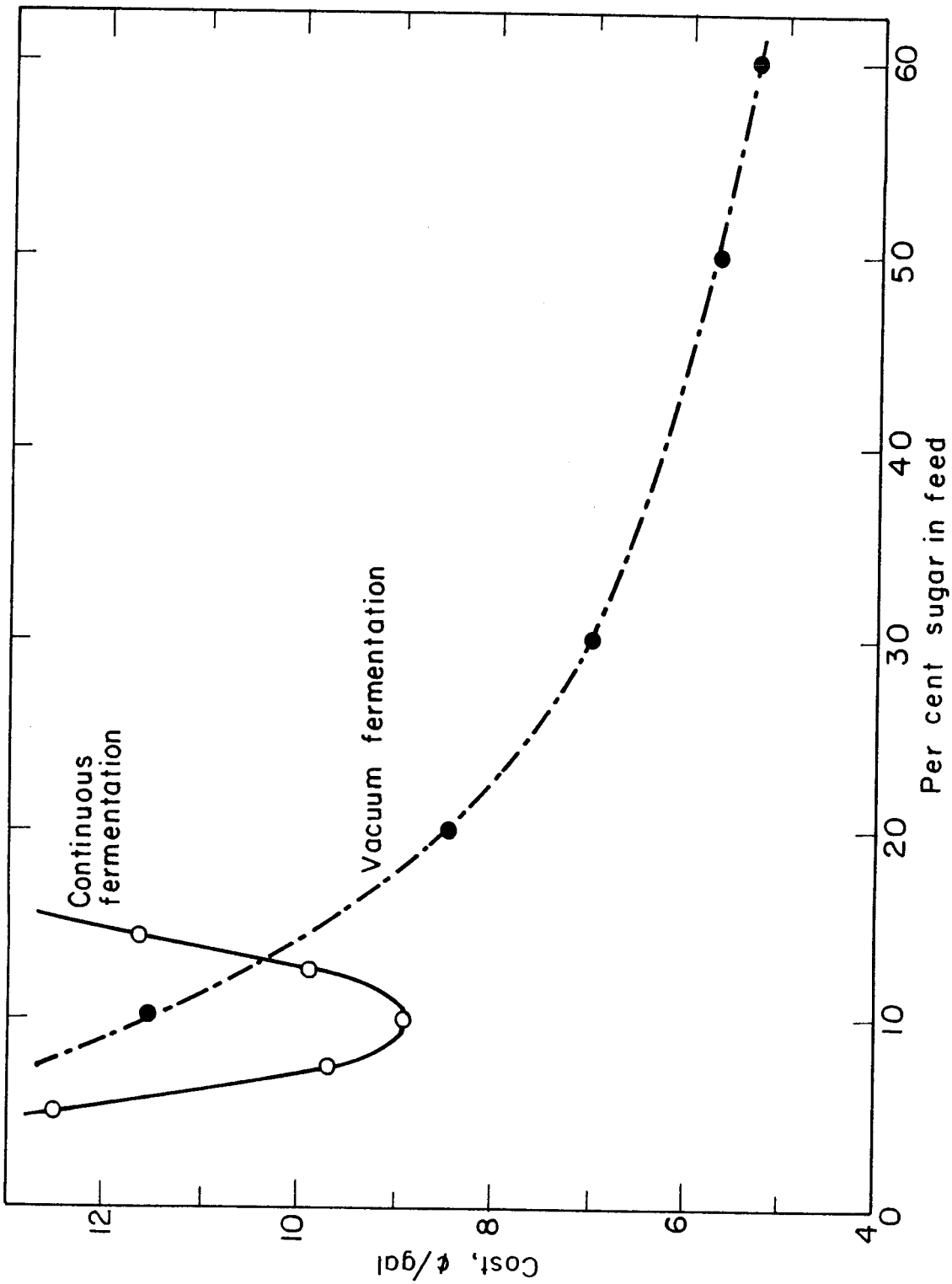


Fig. 7.5. Comparison of ethanol production cost in vacuum and atmospheric pressure continuous fermentations as a function of sugar concentration.

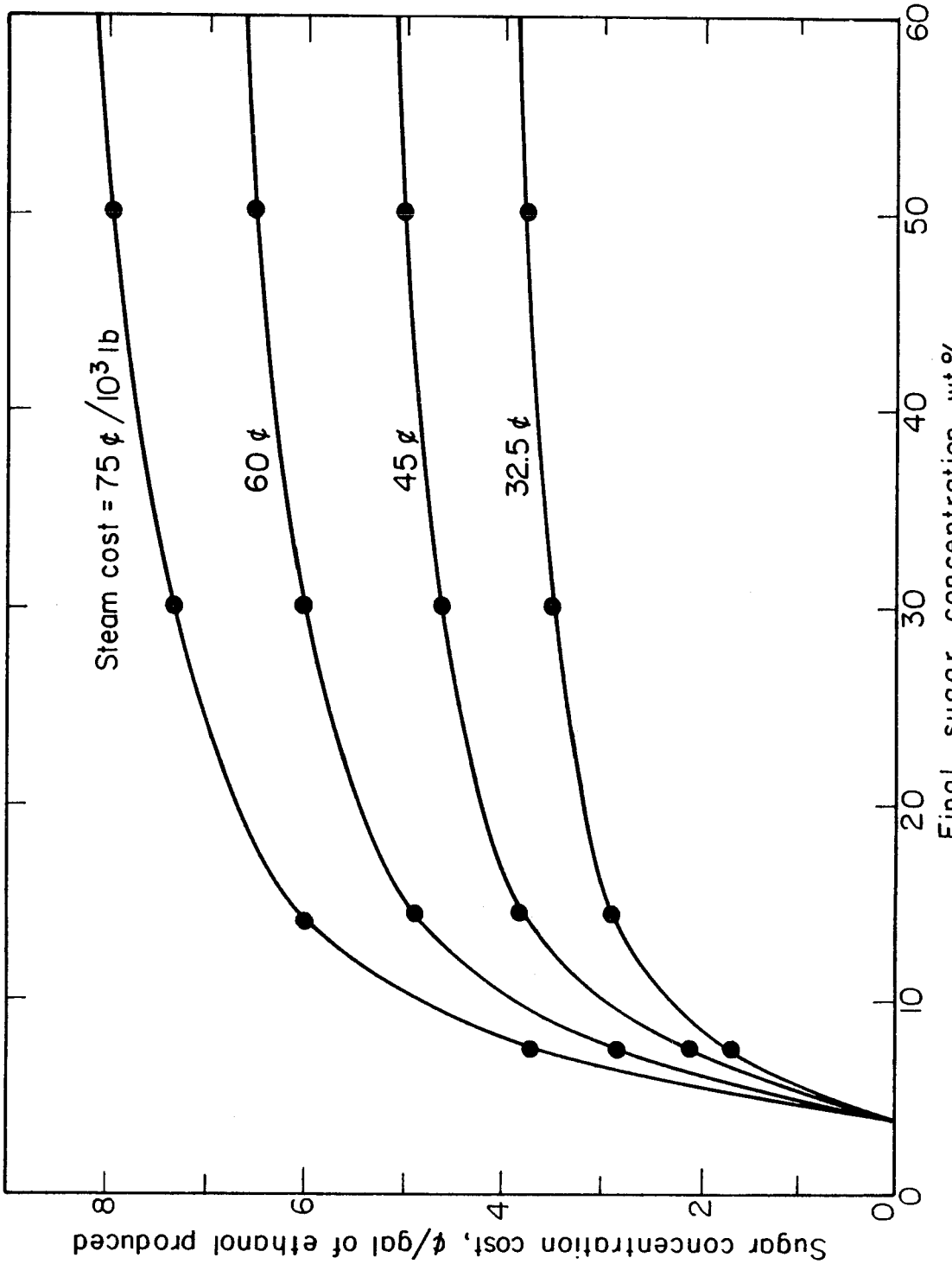


Fig. 7.6. Cost of concentrating the 4.0% hydrolysate sugar solution.

steam is $\$0.325/10^3$ lb. However, when the cost of process steam is above $\$0.45/10^3$ lb, sugar concentration is uneconomical.

As illustrated in Fig. 7.5 the vacuum fermentation process reduces fermentation costs over conventional continuous operation only when the fermentable feed sugar concentration is above 13%. Thus, in order for the vacuum fermentation to be economically attractive, either a concentrated sugar feed (molasses) must be available or low cost steam must be available to concentrate a dilute sugar solution.

7.4. Effect of Fermentation Plant Capacity

Figures 7.7 and 7.8 show ethanol production costs vs ethanol production rate for the four modes of fermentation employing molasses and hydrolysate sugars respectively, as the fermentation substrate. The production cost of batch fermentation, although high, is a weak function of production rate. This is because a large number of fermentors are needed for the batch fermentation. As the plant capacity is decreased the number of fermentors drops but the cost per unit of fermentation volume remains the same.

This is not the case for the vacuum fermentation, for which a production rate of over 70,000 gal/day is necessary to achieve maximum economic efficiency. The vacuum system requires only one 1.89×10^5 liter fermentor to produce 78,000 gallons of ethanol per day. Thus, as the production rate is decreased the fermentor volume drops but the cost per volume increases (economy of scale). As a result, the economic advantage of the vacuum system over conventional continuous operation decreases as the production rate is lowered. The production cost of continuous operation both with and without

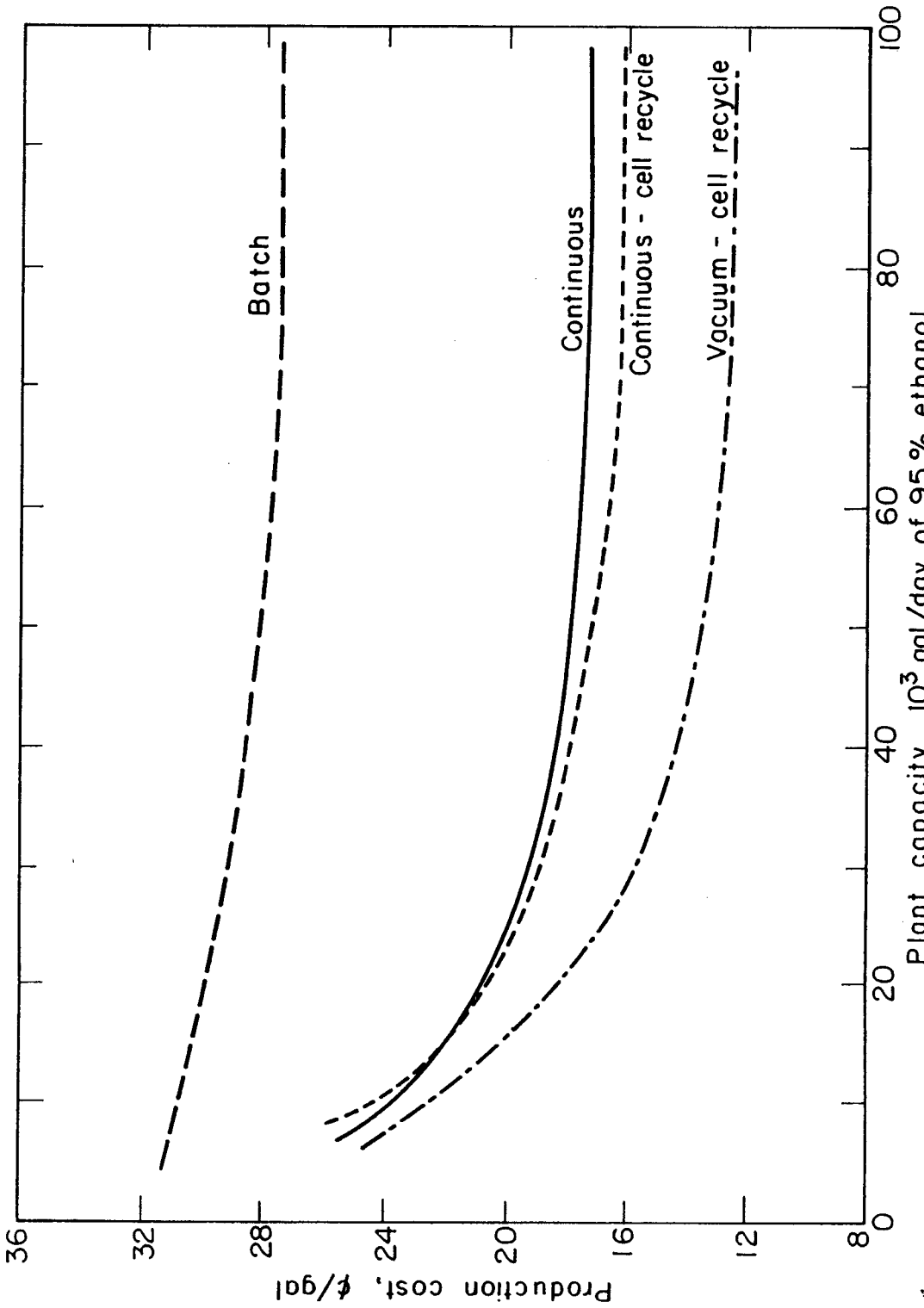


Fig. 7.7. Ethanol production costs from molasses as a function of plant capacity for different modes of operation.

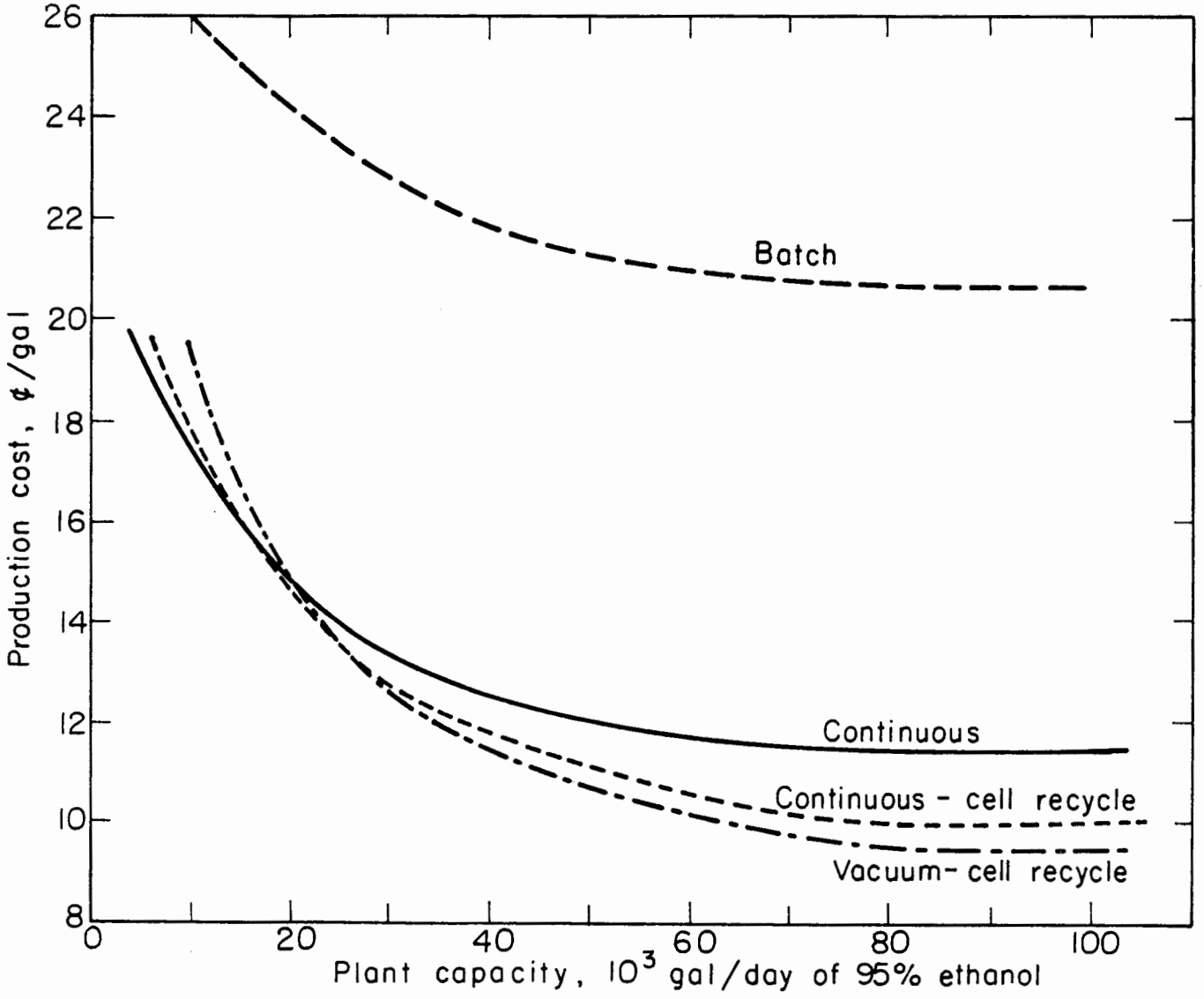


Fig. 7.8. Ethanol production costs from hydrolysate sugars as a function of plant capacity for different modes of operation.

cell recycle is not as sensitive to plant capacity as vacuum operation because multiple fermentors are needed for high production rates. However, cell recycle has no benefit in continuous fermentations if less than 15,000 gal/day of ethanol is produced.

The main conclusion that can be drawn from Figs. 7.7 and 7.8 is that a production rate of over 60,000 gal/day is necessary to achieve the most efficient use of capital in ethanol fermentation processes.

7.5. Vapor Recompression Distillation

Because the distillation of fermented broth is the most energy intensive step for the production of 95% ethanol, vapor recompression distillation was examined as a means of reducing the energy requirement, and hence the cost of the distillation step. In this method the overhead vapors are compressed and condensed in the reboiler to supply the heat for distillation. The overhead vapors contain a substantial amount of energy in the form of latent heat. This energy can be recovered by adiabatically compressing the vapors so that they will condense in the reboiler. The energy for compression is a small fraction of the energy required for vaporization in the reboiler. Thus, vapor recompression reduces the energy cost of distillation, but at the expense of a higher capital cost because a compressor is needed.

Figure 7.9 compares the ethanol separation cost for a distillation column using a conventional direct steam heated reboiler with a vapor recompression heated reboiler. A constant overall heat transfer coefficient of 487 Btu/hr-ft^2-°F was assumed for the steam-heated reboiler. However, when vapor recompression was employed, the heat transfer coefficient was calculated as a function of temperature

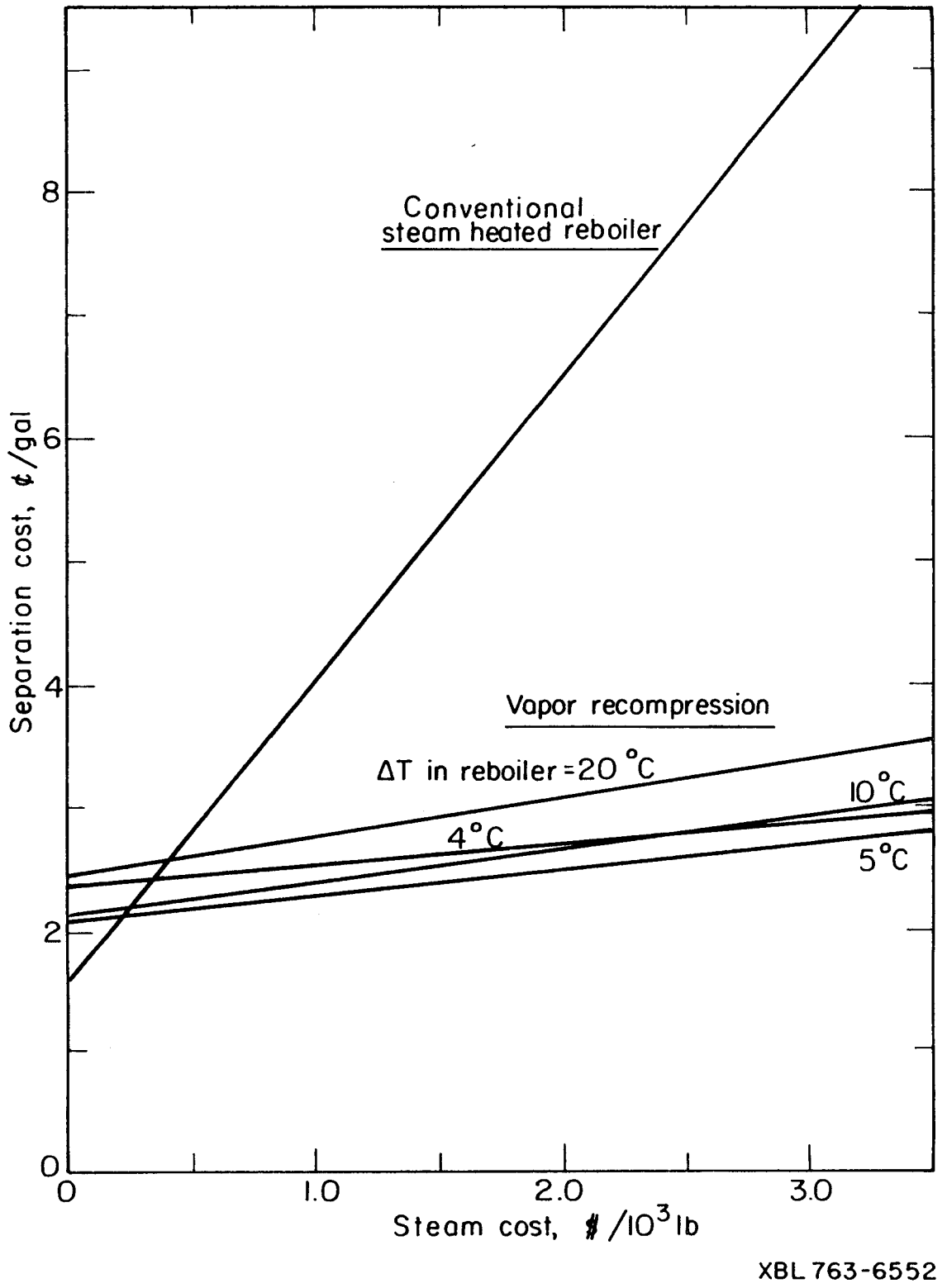


Fig. 7.9. Comparison of conventional steam fired and vapor recompression distillation to concentrate ethanol from 5 to 95%.

difference, ΔT, in the reboiler (see Eq. (A.32) in Appendix A).

It is obvious from Fig. 7.9 that as the cost of steam is increased the vapor recompression distillation produces substantial savings. Also, a temperature difference of 5.0°C in the reboiler was found to be optimal for the ethanol-water distillation using vapor recompression. At ΔT greater than 5°C, the compression cost increases due to the higher pressure required for condensation, while at ΔT less than 5°C, the reboiler cost increases due to the large exchanger area required.

If a current open market price of $\$2.80/10^3$ lb for steam is assumed, the separation cost is reduced from 8.5 to 2.5 cent/gal when vapor recompression distillation is employed. This decreases the ethanol production costs from Blackstrap molasses shown in Table 7.10 to 12.3 cent/gal and 8.2 cent/gal for continuous and vacuum fermentation, respectively. Thus, the economic advantage of the vacuum system is partially reduced by using vapor recompression rather than distillation with direct steam.

7.6. Total Ethanol Production Costs

The total ethanol production cost includes the cost of sugar and any medium supplements that are required. Based on simple stoichiometry, every cent per pound of fermentable sugar costs adds 14.0 cents to the manufacturing cost of 1 gallon of ethanol. Thus it is easily seen, if sugar costs are above 2 to 3 cent/lb the sugar cost will dominate the economics of ethanol production. Unfortunately, this is the present situation. Both the price of molasses and of enzymatic hydrolysate sugars are around 5 cent/lb.

A break down of the total ethanol production costs is shown in Table 7.11 for vacuum fermentation of both molasses and hydrolysate sugars. As mentioned previously, reduced energy costs have been assumed for the hydrolysate fermentation.

The cost of molasses was taken at 5.0 cent/lb of sugar[5] and that of enzymatic hydrolysate at 5.3 cent/lb of sugar.[1] Since the hydrolysate sugars are only 70% fermentable, the actual cost of fermentable sugar would be 5.3/0.7 or 7.57 cent/lb. However, the residual unfermented sugars would not be discarded, but rather, fed to a second fermentation process. The residual sugars could be used for the production of single cell protein (SCP) by a Torula yeast or for the production of methane by anaerobic digestion[8] and thus have an inherent value. A recent economic analysis for the production of Torula yeast from the residual sugars left after ethanol fermentation indicated that the net processing cost was equal to the market value of the yeast, 30 cent/lb.[9] The residual sugar cost was taken at the total sugar cost of 5.3 cent/lb in the above study. Thus, the production of Torula yeast from the residual sugars defrays the cost of these unfermented sugars and the cost of fermentable hydrolysate sugars may be taken at the total sugar cost of 5.3 cent/lb.

The medium supplement cost of 10 cent/gal of ethanol produced was calculated based on the assumption that half of the mineral and vitamin yeast growth factors are present in the raw sugar solutions. A yeast credit of 30 cent/lb[6] of yeast produced was subtracted from the total production costs.

Table 7.11. Total ethanol production costs.

	Molasses cent/gal	Hydrolysate Sugars cent/gal
Sugar Concentration	-	3.8
Fermentation	4.6	2.7
Ethanol Recovery	6.7	2.2
Yeast Recovery	0.7	0.5
Storage	0.4	0.4
Sugar	70.0	74.2
Medium Supplements	10.0	10.0
Sub Total	92.4	93.8
Yeast Credit	39.9	39.9
Total	52.5	53.9

As shown in Table 7.11 the cost of sugar does indeed dominate the ethanol production cost, representing almost 80% of the total manufacturing cost. However, the net production costs of 53.9 and 52.5 cent/gal, after the yeast credit has been substracted, compares favorably with the current selling price for 95% ethanol of $1.04/gal.[6]

Since the processing costs represent only 12% of the total ethanol production costs, the effect of taxes, interest and labor rates on the final ethanol cost is minor. This is shown in Table 7.12 along with the effect of sugar costs.

As mentioned above, the production of Torula yeast from the residual hydrolysate sugars offsets the cost of these unfermentable sugars when the total sugar cost is 5.3 cent/lb. However, if the cost of hydrolysate sugar increases above 5.3 cent/lb the residual sugar cost is no longer offset by the production of SCP and the ethanol production cost must reflect the added cost of the residual sugars. This results in a 20 cent/gal increase in ethanol production cost for every cent the hydrolysate sugar cost is increased above 5.3 cent/lb. However, in the case of molasses in which the sugars are completely fermented each cent per pound increase in sugar cost increases the ethanol cost by only 14 cent/gal.

Table 7.12. Incremental effect of variables on ethanol production cost.

	Cent/gal Increase of Ethanol Cost
$5.60/hr → $10.0/hr Increase in Labor Costs	0.5
4.0% → 12% Increase in Taxes	3.6
6.0% → 12% Increase in Interest	1.0
5.0 cent/lb → 8.0 cent/lb Increase in Molasses Sugar Costs	42.0
5.3 cent/lb → 8.3 cent/lb Increase in Hydrolysate Sugar Costs	60.0

8. CONCLUSIONS AND RECOMMENDATIONS

Although careful control of oxygen tension and use of optimum feed sugar concentrations in alcoholic fermentations may reduce capital expenditures by as much as 50%, sugar costs overwhelm the ecomonics of ethanol production. Every cent/lb of fermentable sugar adds 14 cent/gal to the ethanol production costs while the basic fermentation charges excluding sugar are only 10 cents to 26 cents per gallon. Thus before ethanol fermentation process improvements and optimization become of equal economic importance to sugar costs, the base cost for sugar must be reduced to 2 cents to 3 cents per pound.

The experimental work presented in this study does demonstrate the practicality of continuous ethanol fermentations. Under optimal fermentation conditions, continuous alcohol production in a laboratory fermentor was maintained for 90 days without problems of contamination or of unfavorable mutation. Vacuum fermentation has been shown to eliminate the problem of ethanol inhibition. With a 33.4% glucose feed the ethanol productivity of the vacuum system was 7 times greater then that for conventional continuous operation. However, a bleed of fermented broth had to be continually withdrawn from the vacuum fermentor to keep the concentration of nonvolatile components at a level compatible to the yeast. Fermentor productivities were also increased by using cell recycle in both vacuum and atmospheric pressure fermentations. The increased fermentor cell mass concentrations achieved with cell recycle produced a corresponding increase in ethanol productivity. Extensive cell recycling had little effect on yeast viability, even when the mean residence time of a cell was

increased 10 times over simple continuous operation.

The fermentation studies of this work were conducted using a pure glucose sugar feed. Further definitive studies are required to assess the ethanol fermentation of commercially available substrates. The most promising fermentation substrate appears to be sugars produced from the enzymatic hydrolysis of waste cellulose. The hydrolysate sugars produced from newsprint have been shown to contain 70% glucose, 26% cellobiose and 4.0% xylose and are 70% fermentable by S. cerevasiae.[2] Thus, the need exists to find other strains of yeast capable of fermenting the remaining 30% of the hydrolysate sugars. This becomes extremely important in light of the dominating cost of the hydrolysate sugars and could increase the ethanol yield per ton of cellulosic waste by as much as 15%. This same situation exists if the hydrolysate sugars are produced from agricultural wastes. However, the hemicellulose fraction of agricultural wastes offer an additional source of sugars (xylose, manose and arabinose). Organisms must be found which ferment these sugars to useful products. Yeast strains of Kloeckera and Hansen iaspora appear to be likely candidates.

Cost studies of the various fermentation methods show that the sugar cost dominate the economics at present. However, even at a sugar cost of 5 cent/lb, the net manufacturing cost of 95% ethanol is 52.5 cent/gal with reasonable by-product credits. If the cost of sugar were reduced to 3 cent/lb the manufacturing cost would be lowered to 26 cent/gal, making ethanol competetive with petrolum as a motor fuel. Current efforts to produce hydrolysate sugars and ethanol from celluolse, therefore, appear to be quite promising and well justified by the foregoing economic analysis.

REFERENCES FOR CHAPTERS 7 AND 8

1. C. R. Wilke, R. D. Yang, U. Von Stockar, Preliminary Cost Analyses for Enzymatic Hydrolysis of Newsprint, presented at the Symposium on Enzymatic Conversion of Cellulosic Materials: Technology and Applications, Boston, MA, Sept. 10, 1975.

2. T. Batter (M. S. Thesis), University of California, Berkeley. To be completed December 1976.

3. S. C. Prescott, C. G. Dunn, Industrial Microbiology (McGraw-Hill, NY, 1959).

4. G. L. Spencer, G. P. Meade, Cane Sugar Handbook (John Wiley and Sons, NY, 1945).

5. Wall Street Journal, May 22, 1975.

6. Chemical Marketing Reporter, January 12, 1976.

7. H. E. Grethlein, The Acid Hydrolysis of Refuse, presented at NSF Seminar, Cellulose as a Chemical and Energy Resource, Berkeley, CA, June 1974.

8. M. Mandels, J. Weber, Cellulases and Their Applications, G. J. Hajny and E. T. Reese, eds., Advances in Chemistry Series 95, A.C.S publications (1969).

9. G. R. Cysewski, C. R. Wilke, Biotech. Bioeng., to be published.

APPENDIX A. DETAILED DESCRIPTION OF PROCESS DESIGN AND COST ESTIMATION PROCEDURES

Computer programs were used to design and optimize the ethanol fermentation plants. Three main programs were written to optimize the major subsystems within the alcohol plant so as to keep the programs of a general nature and applicable to other fermentation processes. The principal program designs the fermentors and the auxiliary equipment (heat exchanges, sterilizers, and compressors, etc). Another program is used for the product recovery, i.e., distillation, and a third used for vapor recompression cycles which are employed as an alternative to conventional reboiler operation in distillation and in the vacuum fermentation system. The design equations used in programs are first explained along with the general computational procedures employed. Next, the cost estimation methods are outlined. Finally, a description and flow chart of the programs are shown and the complete Fortran program listed.

A.1. Design Equations

A.1.1. Fermentor Volume

Batch Fermentation. The fermentation cycle time, ϕ, in batch fermentation is taken as:

$$\phi = T_f + T_h + T_c + T_s \quad . \tag{A.1}$$

where, T_f = actual fermentation time

T_h = time required to harvest or drain the fermenter (assumed to be 1 hr)

T_c = time required to charge the fermentor with fresh medium (assumed to be 1 hr)

T_s = time required to clean and sterilize the fermenter (assumed to be 4 hr).

Then,

$$\phi = T_f + 6 \quad . \tag{A.2}$$

Since the feed rate of broth to the fermentation plant is continuous, the total fermentation volume, V_f, must be,

$$V_t = \phi \cdot F$$

or the number of fermentors, N, is,

$$N = V_t/(V_m)(0.8) \quad . \tag{A.3}$$

where, F = broth flow rate, liters/hr

V_m = maximum fermentor volume, liters (assumed to be 1.89×10^5 liters or 5×10^4 gallons).

An 80% working volume has been assumed in Eq. (A.3) for the alcoholic fermentation. The actual number of fermentors, N', is taken as the next highest intergral number of N. The individual fermentor volume, V_f, is then calculated by,

$$V_f = V_t/N'$$

For continuous fermentation the total fermentation volume was found as,

$$V_t = F/D$$

where, D = dilution rate, hr^{-1}

And again the number of fermentors and individual fermentor volume was found as,

$$\begin{aligned} N &= V_t/(V_m)(0.8) \\ V_f &= V_t/N' \end{aligned} \tag{A.4}$$

A.1.2. Agitation and Aeration

In the design of the aeration and agitation systems standard geometry fermentors using flat bladed turbines have been assumed. A summary of the dimensional ratios of the standard geometry fermentor is given in Fig. A-1.

From the oxygen demand of the yeast, Q_{O_2}, and the cell mass concentration, X, the required volumetric oxygen-transfer coefficient, $K_L a$, can be found.

$$K_L a = Q_{O_2} \cdot X/(C^* - C_c) \tag{A.5}$$

where, Q_{O_2} = microbial oxygen demand, moles O_2/hr-gm cells

X = cell concentration, gm dry wt/liter

C^* = saturation concentration of oxygen at the fermentation conditions, moles/liter

C_c = oxygen concentration which is to be maintained in the fermenter, moles/liter

In order to take into account the consumption of oxygen by the yeast, C^* was calculated from an oxygen balance around the fermentor. The partial pressure of oxygen leaving the fermentor, $\overline{P^O}$, is found from,

$$\overline{P^O} = P_f Y_i - \frac{RTQ_{O_2} XV_w}{Q} (1 - Y_i) \tag{A.6}$$

where, Y_i = mole fraction of oxygen in inlet gas (for air Y_i = 0.209)

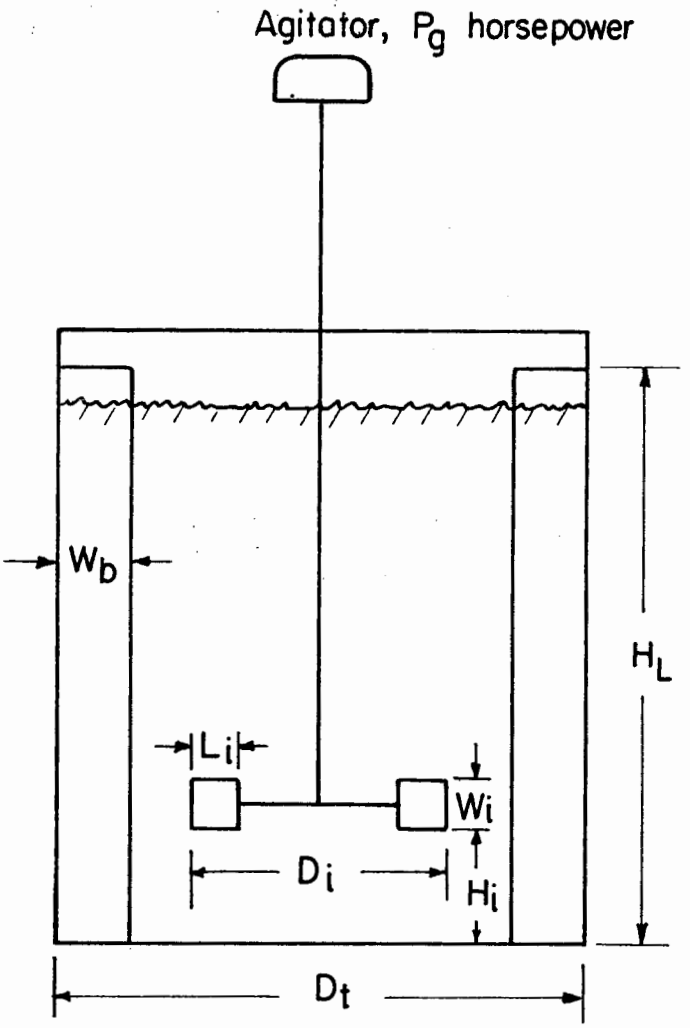
P_f = total pressure in fermentor head space, atm

R = the gas constant, 8.21×10^{-5} atm-m^3/°K-mole

T = temperature, °K

V_w = working volume of fermentor, m^3

Q = volumetric gas flow rate, m^3/hr



$$H_L = D_t$$

$$D_t/D_i = 3.0$$

$$W_b/D_t = 0.1$$

$$W_i/D_i = 0.2$$

$$L_i/D_i = 0.25$$

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Fig. A.1. Standard geometry fermentor using flat bladed turbine.

The last term in Eq. (A.6) is due to the oxygen consumption by the yeast. Assuming that both the gas and liquid phases are well mixed, the liquid saturation concentration is then,

$$C^* = \overline{P^O}/H \tag{A.6a}$$

where, H = Henery's law constant in atm/mole-liter

The assumption of a back mixed gas phase made in Eq. (A.6a) results in a lower mass transfer driving force than if plug flow of the gas is assumed. This increases the required $K_L a$ for oxygen transfer as calculated in Eq. (A.5) and increases the agitator power (see below). In actual practice the gas flow will be neither completely plug flow or completely back mixed, but exists in a regime somewhere between these two limits. However, the assumption of a well back mixed gas produces a conservative design of the aeration and agitation system, and is thus made because there is insufficient data to calculate the actual flow conditions of the gas phase.

Once C^* is found from Eq. (A.6a), the required $K_L a$ is determined from Eq. (A.5) and the agitator power calculated from,[24]

$$P_g/V = \left[26.3(K_L a/H)(1/v_s)^{0.67}\right]^{1.89} \tag{A.7}$$

where, P_g/V = agitator power, HP/m^3 of fermentor volume

v_s = superfacial air velocity through the fermentor, m/hr

Equation (A.7) is due to a correlation presented by Cooper et al.[24] There are many similar correlations for the calculation of fermentor agitator power,[3,25] however, the correlation presented in Eq. (A.7) results in a higher agitator power requirement compared to other

available correlations. Due to the discrepancies between various correlations and uncertainty in microbial oxygen demands, the Cooper correlation was chosen because it leads to a conservative agitator design.

As can be seen from Eq. (A.7), once $K_L a$ has been determined many values of v_s will give a reasonable agitator power. As discussed below, an optimum air compressor and agitator cost is used to determine v_s and hence P_g. However, a minimum power is required to keep the cells in suspension and to insure a well mixed system. This minimum power is calculated according to Calderbank and Moo-Young.[3]

$$P_g/V = 4.33\times10^{-3} \frac{(g\Delta\rho)^{4/3}\mu_c^{1/3}}{\rho_c^{2/3}} \qquad (A.8)$$

where, g = acceleration due to gravity, cm/sec^2

$\Delta\rho$ = density difference of dispersed and continuous phases, gm/cm^3

μ_c = continuous phase viscosity, gm/cm-sec

ρ_c = continuous phase density, gm/cm^3

Substituting representative values for yeast and the fermentation broth into Eq. (A.8).

$\Delta\rho = 0.024$ gm/cm^3 (Ref. 2)

$\mu_c = 0.0072$ gm/cm-sec (water at 35°C)

$\rho_c = 1.091$ gm/cm^3

$g = 980.7$ cm/sec^2

one obtains, $P_g/V = 0.06$ HP/m^3.

This sets the lower limit of P_g/V in Eq. (A.7). The upper limit of P_g/V is set by the largest commercially available agitator of 400 horsepower,[4] or for a 5×10^4 gallon fermentor, a P_g/V ratio of 2.1 HP/m^3. It should be pointed out that for the main alcoholic fermentation, where the oxygen demand is very small, the optimum solution inevitably lies at the lower limit of P_g/V. However, it is necessary to keep the aeration and agitation equations of a general nature so that one can design either growth stage fermentors or seed fermentors, both of which require high aeration and agitation levels.

The design of the aeration and agitation system requires that the superficial air velocity through the fermentor, v_s, and the pressure in the fermentor, P_f, be specified. If P_f is increased the saturation concentration of oxygen, C^*, rises and the required K_La will decrease (Eq. (A.5)) which leads to a lower agitator power as shown in Eq. (A.7). The same situation exists for v_s; a higher air rate allows a lower agitator power in Eq. (A.7), but the decrease in agitator power achieved by raising the pressure and air flow are offset by a higher air compressor power requirement. Thus an optimum must be struck between the agitator and compressor costs using v_s and P_f as optimizing parameters. However, another parameter must be introduced since the air is sterilized by filtration and the pressure drop through the air filter enters into the compressor power calculations. As discussed in the air sterilization section, the degree of sterilization is set a priori and the filter length and pressure drop are calculated once the filter diameter is determined. As a result the filter diameter is used as the third parameter necessary to optimize the agitation and aeration system.

The RPM of the agitator impeller is calculated from a correlation due to Michel and Miller[5] which relates power consumption in gassed and ungassed fermentors.

$$P_g = 0.7\left(\frac{P^2 n D_i^3}{Q^{0.56}}\right)^{0.45} \tag{A.9}$$

where, P_g = power consumed in the gassed liquid, HP

P = power consumed in the ungassed liquid, HP

n = rotational speed of impeller, rev/min

Q = volumetric flow rate of gas, m^3/hr

D_i = impeller diameter, m.

An additional equation can be written for the power number, N_p, when there is turbulent agitation in the fermentor.[6] For flate bladed turbine impellers,

$$N_p = \frac{P_{g_c}}{n^3 D_i^5 \rho_i} = 6.0 \tag{A.10}$$

Conditions of turbulence are insured by Eq. (A.8). Rearrangement of Eq. (A.10) and substitution for the density of the fermentation broth gives,

$$P = 9.66\ n^3 D_i^5 \tag{A.11}$$

Substitution of Eq. (A.11) into Eq. (A.9) for the ungassed power and solving for the rotational speed of the impeller, n, gives,

$$n = 0.643\left(\frac{P_g^{2.22} Q^{0.56}}{D_i^{13}}\right)^{1/7} . \tag{A.12}$$

Thus, once P_g and Q are known the rotational speed may be solved for directly.

The gas hold-up within the fermentation broth is then calculated to insure that the expanded volume of the broth due to gas entrainment does not exceed a 90% working volume in the fermentor. A 10% volume head space is taken to allow complete disengagment of the gas and liquid. The per cent gas hold-up has been correlated by Richards[12] as,

$$H_o = 17.5 \ln\left[(P_g/V)^{0.4}(v_s)^{0.5}\right] - 29.5$$

where, H_o = per cent hold-up of gas in the liquid.

If the hold-up is greater than 10%* the volume of the fermentor is recalculated so that the total volume of entrained gas and liquid is 90% of the total fermentor volume.

A.1.3. Fermentor Temperature Control

The heat of fermentation must be removed in the batch and continuous fermentations to maintain a constant temperature in the fermentor. The temperature control system for batch fermentations must be designed for the highest heat load which occurs at the end of the exponential growth phase. The maximum heat load is calculated from,

$$(H_f)_{max} = 3.97(\nu X)_{max}\left(\frac{H_s^c}{Y_{p/s}} - Y_{x/p}\, H_x^c - H_e^c\right) V_w + 2540\, P_g \qquad (A.13)$$

*The ungassed liquid working volume has been previously set to 80%. If H_o is greater than 10%, 80% + H_o will be greater than 90%.

where: $(H_f)_{max}$ = maximum heat generation rate, Btu/hr

ν = specific ethanol productivity, hr^{-1}

X = cell mass concentration, gm dry wt/liter

H_s^c = heat of combustion of substrate, for glucose 3.74 kcal/gm

H_x^c = heat of combustion of cell mass, for yeast 3.3 kcal/gm[20]

H_e^c = heat of combustion of ethanol, 7.1 kcal/gm

$Y_{p/s}$ = product yield, gm ethanol produced per gm substrate consumed

$Y_{x/p}$ = cell yield, gm cell mass produced per gm ethanol produced

The last term in Eq. (A.13) is due to the power dissipation of the agitator in the fermentation broth with P_g the agitator power in horsepower. The similar relation is written for continuous fermentation systems

$$H_f = (S_o - S)\left(H_s^c - Y_{x/s} H_x^c - Y_{p/s} H_c^e\right) V_W D + 2540\ P_g \qquad (A.14)$$

with, S_o = inlet substrate concentration, gm/liter

S = outlet substrate concentration, gm/liter

To assure a constant fermentor temperature the heat of fermentation as calculated in Eq. (A.13) or Eq. (A.14) is removed through an external heat exchanger. Details of the exchanger design are shown below in the heat exchange equipment section.

The flow rate of fermentation broth, w_f, and cooling water, w_c, through the external exchanger are calculated from an energy balance around the exchanger,

$$H_f = w_f c_p (T_{h2} - T_{h1}) = w_f c_p (T_{c1} - T_{c2}) \qquad (A.14a)$$

when, w_f = flow rate of fermentation broth, lb/hr

w_c = flow rate of cooling water, lb/hr

c_p = heat capacity of broth and water, 1.0 Btu/lb-°F

T_{h2} = inlet temperature of broth, °F

T_{h1} = exit temperature of broth, °F

T_{c1} = inlet temperature of cooling water, 77°F

T_{c2} = exit temperature of cooling water, °F.

The inlet temperature of broth, T_{h2}, is set by the fermentation temperature and the inlet cooling water temperature, T_{c1}, was taken at 77°F. Thus, T_{h1} and T_{c2} may be arbitrarily set and the flows calculated from Eq. (A.14a) provided,

$$T_{h1} > T_{c1} = 77°F$$

$$T_{c2} < T_{h2} = \text{fermentation temperature}$$

However, as T_{h1} approaches T_{c1}; and T_{c2} approaches T_{h2} the required flow of broth and cooling water diminishes, but the exchanger area as calculated in Eq. (A.25) increases due to the smaller ΔT's. The trade off between heat exchanger costs, and the cost of cooling water and pumping is optimized using T_{h1} and T_{c2} as the optimization parameters.

A.1.4. Gas Compression

For both the air supply to the fermentors and the vapor recompression in the vacuum fermentation, adiabatic compression is used. The compression power requirement is calculated as,

$$HP = \left(\frac{1}{0.8}\right)\left(\frac{0.376\ K}{1 - K}\right) P_1 Q \left[\left(\frac{P_2}{P_1}\right)^{\frac{K-1}{K}} - 1\right] \qquad (A.15)$$

where: HP = horsepower required for compression

K = ratio of specific heats of gas at constant pressure to specific heat of gas at constant volume

P_1 = intake pressure, atm

P_2 = final delivery pressure, atm

Q = volumetric flow rate of gas at inlet conditions, m^3/hr

A compressor efficiency of 80% has been assumed in Eq. (A.15) for the adiabatic compression.[4]

A.1.5. Air Filtration and Sterilization

The air feed to the fermentors is sterilized by filtration through fibrous glass wool having a mean fiber diameter of 19.0 microns. The single fiber collection efficiency, ζ_o, is determined from a correlation presented by Aiba.[2]

$$\zeta_o = 2.75\left(N_{Re}^{0.092} N_{Pe}^{-0.45} N_R^{0.66}\right) \qquad (A.16)$$

when,

$$N_R N_{Pe}^{1/3} N_{Re}^{1/18} \leq 1.0$$

and,

$$\zeta_o = 2.75\left(N_{Re}^{0.199} N_{Pe}^{0.198} N_R^{2.59}\right) \qquad (A.17)$$

when,

$$N_R N_{Pe}^{1/3} N_{Re}^{1/18} > 1$$

where, $N_R = d_p/d_f$

$N_{Pe} = vd_f/D_{BM}$

$N_{Re} = d_f v\rho/\mu$

$v = v_s/(1 - \alpha)$

with, d_p = diameter of particle which is retained by filter, assumed to be 10^{-4} cm (diameter of bacterial spore)

d_f = fiber diameter, 1.9×10^{-3} cm

v = air velocity through filter, cm/sec

α = packed volume fraction of filter material, 0.033 for industrial filters[2]

D_{BM} = diffusivity of particles, cm^2/sec

μ = viscosity of air, gm/cm-sec

ρ = density of air, gm/cm^3.

The Brownian diffusion coefficient, D_{BM}, is calculated as,[7]

$$D_{BM} = \frac{CKT}{3\pi\mu d_p}$$

With the Cunningham correction factor for slip flow, C, being found from,

$$C = 1 + \frac{2\bar{L}}{d_p}\left(1.23 + 0.41 \exp\left(-\frac{1.44\ d_p}{L}\right)\right)$$

$\bar{L}$ is the mean free path of gas molecules in centimeters,

$$\bar{L} = \frac{2\mu}{\rho\bar{v}}$$

where;

$$\bar{v} = \frac{8\ g_c P}{\pi\rho} \quad ;$$

the average molecular velocity of gas molecules in cm/sec.

P = total gas pressure, dynes/cm^2

K = Boltzmann constant

T = absolute temperature, °K.

Throughout these calculations the air velocity through the filter, v, is held less than 30 cm/sec to avoid inertial impaction collection mechanisms to insure strict adherance to the correlation as presented in Aiba.

Once the single fiber efficiency, ζ_o, has been found from Eqs. (A.16) or (A.17) the collection efficiency of fibers in a filter bed, ζ_α, of volume fraction α is calculated as,

$$\zeta_\alpha = \zeta_o(1 + 4.5\alpha) \quad . \tag{A.18}$$

Then the length of the air filter, L, is found from a log penetration theory.

$$L = \frac{\pi d_f(1 - \alpha) \ln\left(\frac{N_o}{N}\right)}{4\zeta_\alpha \alpha} \tag{A.19}$$

where, L = filter length, cm

N_o = level of contamination in inlet air (assumed to be 10^4 organisms/m^3)

N = level of contamination in air after filtration.

The contamination level of the filtered air, N, is set so that only one contaminating organism passes the filter every 100 days.

The pressure drop through the air filter is calculated by means of a modified drag coefficient, C_{DB}, which was introduced by Kimura.[8]

$$C_{DM} = 52/N_{Re}$$

and,

$$\Delta P = \frac{1.96\times10^{-6}\ LC_{DM}\rho v^2 \alpha^{1.35}}{\pi d_f} \qquad (A.20)$$

where, ΔP = pressure drop through the air filter, atm.

A.1.6. Media Sterilization

A continuous media sterilizer is used for both batch and continuous fermentations. A schematic of the sterilizer is shown in Fig. A.2. The incoming medium is first preheated by the hot sterile medium leaving the sterilizer. This also cools the exiting media saving on cooling water costs in the cooler exchanger which cools the sterile medium to the fermentation temperature. Direct steam injection is used to heat the broth to the sterilization temperature. The sterilization temperature is maintained through the holding section by insulated pipe. Although direct steam injection dilutes the medium with condensate, only a 1%-2% dilution was found for all the design cases. This small dilution has a negligible effect on the subsequent fermentation and saves the cost of a heat exchanger.

An effective sterilization level, N/N_o, for the media sterilizer is obtained from an exact solution of the material balance and death rate equations.[2]

$$N/N_o = \frac{4\zeta \exp\left(\frac{LPe}{2d}\right)}{(1+\zeta)^2 \exp\left(\frac{\zeta LPe}{2d}\right) - (1-\zeta)^2 \exp\left(-\frac{\zeta LPe}{2d}\right)} \qquad (A.21)$$

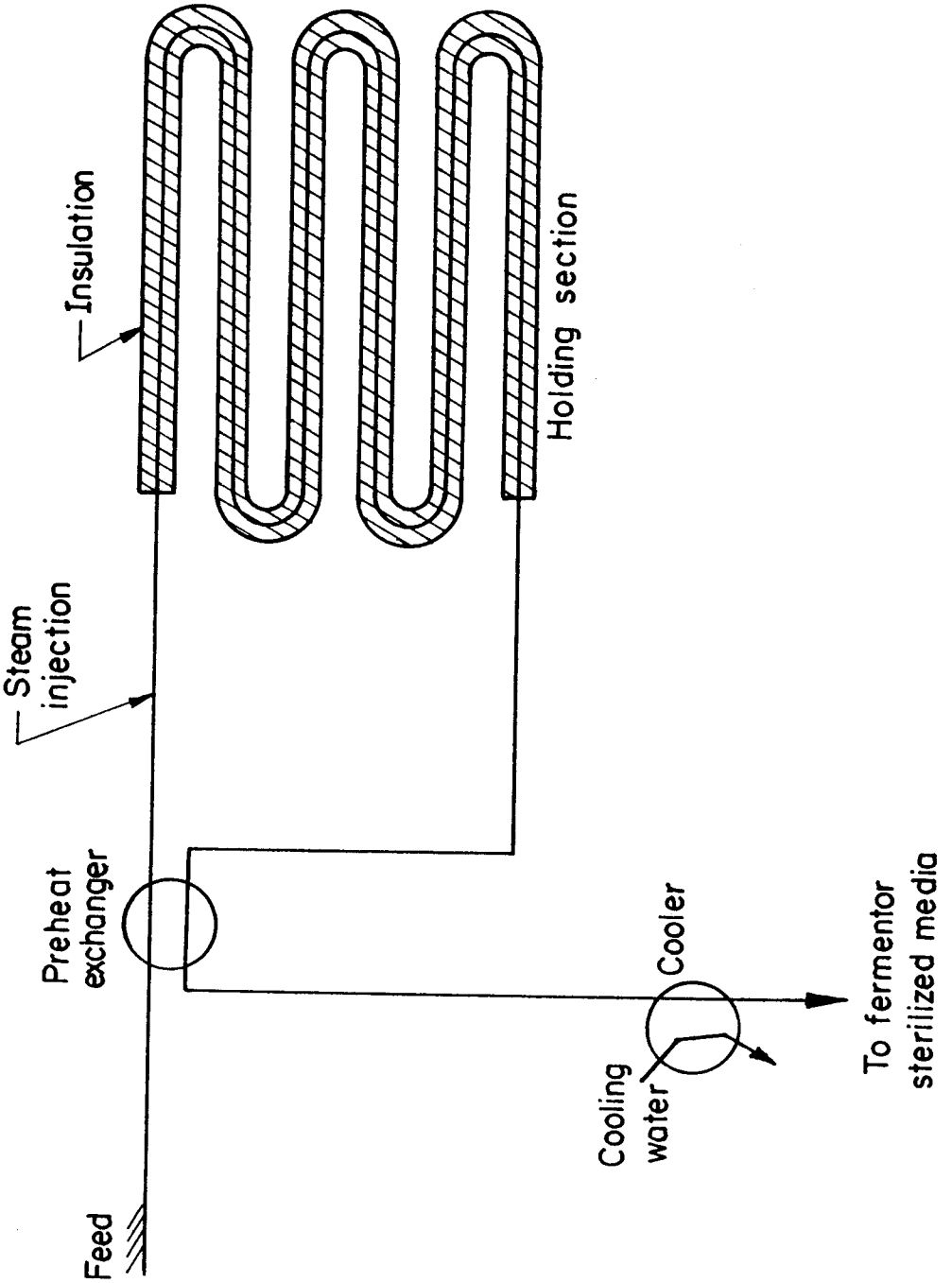

Fig. A.2. Continuous media sterilizer.

where,

$$\zeta = (1 + 4Kd/UPe)^{1/2}$$

with, L = length of the holding section, m

d = diameter of the holding section, m

U = mean velocity of fluid through the holding section, m/min

N_o = initial level of comtamination in media (assumed to be 10^4 organism/cm^3

N = level of contamination after sterilization

K = death rate constant of contaminating organisms

Pe = Peclet number ($Pe = Ud/E_z$)

E_z = axial diffusion coefficient, m^2/min

The death rate constant, K, of the contaminating organisms is assumed to be the same as the heat resistant spores of B. stearothermophilus to insure a conservative design. The death rate constant for this organism can be written as a function of temperature[9] as,

$$K = 7.94\times10^{38} \exp - \frac{6.87\times10^4}{RT} \qquad (A.22)$$

where, T = temperature, °K

R = the gas constant, 1.98 cal/mole-°K.

The Peclet number, Pe, is calculated according to Levenspiel.[10]

$$Pe = 0.27\sqrt{f}$$

with, f = fanning friction factor.

A standard relation for the friction factor may be combined with Eq. (A.22).[11]

$$Pe = \frac{0.27}{\left(0.046\ N_{Re}^{-1/5}\right)^{1/2}} = 1.26\ N_{Re}^{1/10} \qquad (A.23)$$

where, N_{Re} = Reynolds number ($dU\rho/\mu$).

The quanity, LPe/d, in the exponentials of Eq. (A.21) is a measure of the degree of back mixing in the sterilizer holding section. If conditions of extreme back mixing and a low sterilization temperature (i.e., LPe/d = 10, T = 120°C) are substituted into Eq. (A.21) the two terms in the denominator are found to be:

$$(1 + \zeta)^2 \exp \frac{\zeta LPe}{2d} = 7.17\times10^4$$

$$(1 - \zeta)^2 \exp - \frac{\zeta LPe}{2d} = 2.131\times10^{-5}$$

These conditions of back mixing and temperature represent a very poor sterilizer design and in the actual design the difference between these two terms will be larger. Thus the last term in the denominator of Eq. (A.21) may be neglected with respect to the first. Then rearranging Eq. (A.21) to solve directly for the sterilizer length one obtains:

$$L = \frac{2\ Pe\ d\ \ln\left[\left(\frac{N}{N_o}\right)\left(\frac{(1 + \zeta)^2}{4\zeta}\right)\right]}{1 - \zeta} \qquad (A.24)$$

Equation (A.24) is then used to solve directly for the length of the sterilizer after the temperature and diameter have been specified. The contamination level, N/N_o, was set to achieve 100 days of contamination free operation.

The steam requirement in the media sterilizer is calculated from a heat balance as,

$$Q_s = w_m c_p (T_s - T_1)/915 \quad . \tag{A.24a}$$

where, Q_s = stream requirement, lb/hr

w_m = flow of media, lb/hr

C_p = heat capacity of media, 1.0 Btu/lb-°F

T_s = sterilization temperature, °F

T_1 = temperature of media coming from preheat exchanger, °F

Here process steam saturated at 60 psia is used to heat the media. Details of the heat exchanger design are covered in the next section.

There are four parameters which must be specified to design the sterilizer complex shown in Fig. 2. The calculation of the sterilizer length requires that the sterilization temperature, T_s, and the diameter of the holding section, d, be known. It is evident from Eqs. (A.22) and (A.24) that as T_s increases the length and hence the cost of the sterilizer decrease. This advantage is off set by the higher steam requirement needed to obtain the higher sterilization temperature. The steam requirement is also affected by the design of the preheat exchanger. If more heat is transfered to the incoming media less steam is needed, but a larger exchanger is necessary. Also, the cooling water costs of the cooler are linked to the preheat exchanger operation since a higher medium exit temperature from the preheater dictates a higher cooling load in the cooler.

To optimize this situation four parameters were chosen: the diameter of the sterilizer, d, the sterilization temperature, T_s,

the degree of approach in the preheat exchanger, ΔT, and the exit cooling water temperature from the cooler exchanger, and an optimization preformed.

A.1.7. Heat Exchange Equipment

The process heat exchangers were sized using overall heat transfer coefficients and log mean temperature differences. The area of a heat exchanger was found from:

$$A = \frac{Q}{U\Delta T_{lm}} \tag{A.25}$$

where: A = heat exchanger area, ft^2

Q = heat load, Btu/hr

U = overall heat transfer coefficient, $Btu/hr\text{-}ft^2\text{-}°F$

ΔT_{lm} = log mean temperature difference, °F

Overall transfer coefficients were not calculated for each individual exchanger. Rather, a representative coefficient was calculated for each mode of heat transfer (lqiuid-liquid, liquid-condensing vapor, liquid-boiling liquid, boiling liquid-condensing vapor) used in the fermentation process. These heat transfer coefficients were then assumed to apply to all exchangers using that particular mode of heat transfer.

The overall heat transfer coefficient may be written as,

$$\frac{1}{U} = \frac{1}{h_t} + \frac{1}{h_f} + \frac{1}{h_s} + \frac{W}{K} \quad , \tag{A.26}$$

with, h_t = tube side film coefficient, Btu/hr-ft^2-°F

h_f = fouling coefficient, Btu/hr-ft^2-°F

h_s = shell side film coefficient, Btu/hr-ft^2-°F

W = tube wall thickness, ft

K = thermal conductivity of tube, Btu/hr-ft-°F

The shell side and tube side film coefficients were calculated for each type of heat transfer and substituted into Eq. (A.26) and a overall coefficient determined.

A 5,000 ft^2 heat exchanger was taken as a typical process exchanger. The exchanger specifications are listed below.

Area = 5,000 ft^2

Shell diameter = 4 ft

Shell length = 14 ft

Type of tubes = BWG 14, 3/4 in. o.d., 0.584 in. I.D.

Number of tubes = 1810

Tube spacing = 1 in. triangular pitch

Baffle spacing = 5.0 ft

One pass design

The properties of the heat exchange liquid were taken to be the same as those of water at 150°F, and the condensing vapor the same as saturated steam at 266°F unless otherwise specified.

For liquid-liquid heat exchange the tube side film coefficient is calculated as:[4]

$$h_t = 0.023\left(\frac{K}{D_i}\right)\left(\frac{D_i G}{\mu}\right)^{0.8}\left(\frac{C_p \mu}{K}\right)^{1/3} \qquad (A.27)$$

where, K = thermal conductivity of the liquid, Btu/hr-ft-°F

D_i = tube inside diameter, ft

G = mass velocity of liquid, lb/hr-ft^2

μ = viscosity of liquid, lb/hr-ft

C_p = heat capacity of fluid

Using a mass velocity of 1.5×10^6 lb/hr-ft^2 (this will be justified below by pure drop calculations) one obtains:

$$h_t = 0.23\left(\frac{0.381}{0.0491}\right)\left(\frac{(0.0491)(1.5\times10^6)}{1.05}\right)^{0.8}\left(\frac{(1.0)(1.05)}{0.381}\right)^{1/3}$$

$$h_t = 1887 \text{ Btu/hr-ft}^2\text{-°F}$$

The shell side film coefficient is found from[4]

$$h_s = 0.26\left(\frac{K}{D_o F_s}\right)\left(\frac{D_o G_s}{\mu}\right)^{0.6}\left(\frac{C_p \mu}{K}\right)^{1/3} \qquad \text{(A.28)}$$

with: F_s = safety factor to account for bypassing effects, from Peters[4] $F_s = 1.8$

G_s = shell side mass velocity based on free area between baffles and shell axis, lb/hr-ft^2

D_o = tube outside diameter, ft.

Using the same total mass flow rate as on the tube side (5.0×10^6 lb/hr G_s is calculated as:

$$\text{No. of tubes normal to flow} = \frac{\text{48 in. shell i.d.}}{\text{0.75 in. tube o.d. + 0.25 in. clearance}} = 48$$

$$\text{free area for flow} = \frac{(48)(0.25)(5.0)}{12} = 5.0 \text{ ft}^2$$

$$G_s = 5.0\times10^6/5.0 = 1.0\times10^6 \text{ lb/hr-ft}^2$$

Substitution into Eq. (A.28) yields,

$$h_s = 903 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

Using a fouling coefficient of 2000 Btu/hr-ft^2-°F,[4] the overall coefficient for liquid-liquid heat exchange is found from Eq. (A.26).

$$\frac{1}{U} = \frac{1}{1887} + \frac{1}{2000} + \frac{1}{903} + \frac{0.0069}{26}$$

$$U = 416 \text{ Btu/hr-ft}^2\text{-°F}$$

The pressure drop through the exchanger is next calculated to assure the assumed flow rates are reasonable. The tube side pressure drop is correlated as,[4]

$$\Delta P = \frac{2B_i f G^2 n_p}{g_c \rho D_i} \qquad \text{(A.29)}$$

with, n_p = number of tube passes

f = Fanning friction factor

G = mass velocity in tube

B_i = correction for sudden contraction and expansion at headers

g_c = 4.17 ft-lbm/hr-lbf

ρ = liquid density, lb/ft^3.

For the existing flow conditions, B_i and f as calculated according to Peters[4] are,

$$f = 0.005$$

$$B_i = 1.24$$

Substitution into Eq. (A.29) gives,

$$\Delta P = 328 \text{ lbf/ft}^2 = 2.28 \text{ psi}$$

In a similar manner the shell side pressure drop is found as,

$$\Delta P = \frac{2B_o f' N_r G_s^2}{g_c \rho} \tag{A.30}$$

where, f' = special friction factor for shell side flow

N_r = number of tube rows

B_o = correction for flow reversal

Once again flowing Peters[4] for the shell side flow,

$$f' = 0.084$$

$$N_r = 24$$

$$B_o = 15$$

and,

$$\Delta P = 554 \text{ lbf/ft}^2 = 3.84 \text{ psi}$$

These pressure drops are certainly reasonable and justify the assumed flow rates.

Condensation and Boiling. For the condensation of vapors on the shell side of a horizontal exchanger the film coefficient has been correlated for steam as,[13]

$$h_s = \frac{3100}{(N_V D_o)^{1/4} (\Delta T_f)^{1/3}} \tag{A.31}$$

where: N_v = number of tube rows in a vertical tier, 48

D_o = tube outside diameter, ft

ΔT_f = temperature difference across film, °F

Letting ΔT_f be 10% of the average total ΔT between saturated steam at 266°F and boiling water at atmospheric pressure,

$$\Delta T_f = 0.1(266-212) = 5.4°F \quad ,$$

one obtains,

$$h_s = 1343 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

To calculate the tube side coefficient, forced convection with nucleate boiling is assumed. According to Ibele,[14] the film coefficient may be found from,

$$h_t = h_{fc} + h_{pb} \quad .$$

h_{fc} is the film coefficient due just to the forced movement of fluid as calculated above for liquid-liquid heat exchange. The pool boiling coefficient, h_{pb}, is given as,

$$h_{pb} = \left(\frac{4.55\times10^5}{\Delta T}\right)\left(\frac{C_p \Delta T}{\lambda}\right)^3\left(\frac{g(\rho_L - \rho_v)}{\sigma g_c}\right)^{1/2}\left(\frac{K}{C_p \mu}\right)^{5.1} \qquad (A.32)$$

with, λ = latent heat of vaporization, Btu/lb

σ = surface tension, lbf/ft

ΔT = temperature difference between tube wall and saturation temperature

Using the properties of saturated water at 1 atmosphere and a ΔT of 30°F gives,

$$h_{pb} = 975 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

or,

$$h_t = 975 + 1887 = 2862 \text{ Btu/hr-ft}^2\text{-°F}$$

Combining the shell side and tube side film coefficients with a fouling coefficient of again 2000 one obtains the overall heat transfer coefficient for a reboiler arrangement,

$$U = 487 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

This reboiler heat transfer coefficient must be recalculated for the vacuum fermentation system to take account of the non-condensable gases (carbon dioxide and oxygen) in the vapor stream used in the vapor recompression cycle. The shell side coefficient may by estimated from a two film model of heat transfer which takes into account heat conduction both through a noncondensable gas film adjacent to the condensate film on the cooling surface and through the condensate film.[19]

$$h_s = \frac{1}{(Q_s/Q_t)\,\frac{1}{h_g} + \frac{1}{h_c}} \qquad \text{(A.33)}$$

where, Q_s = sensible heat for cooling non-condensable gas, Btu/hr

Q_t = total heat load, Btu/hr

h_g = gas film coefficient, Btu/hr-ft^2-°F

h_c = condensate film coefficient, Btu/hr-ft^2-°F

To estimate the gas film coefficient a representative vapor composition from the vacuum fermentor is taken as,*

mole fraction of water = 0.657

mole fraction of ethanol = 0.139

mole fraction of CO_2 = 0.151

mole fraction of O_2 = 0.053

*This represents a 40% sugar feed and a oxygen flow of 0.13 VVM at STP.

Using a 100 mole basis and a condensation temperature of 40°C, the heat loads can be calculated assuming a compressor discharge temperature of 200°C.*

$$Q_s = [(15.1)(18.05) + (5.3)(6.73)]\ (220-40)$$

$$Q_s = 4.93\times10^4$$

$$Q_t = 4.93\times10^4 + (65.7 + 13.9)(1.75\times10^4)$$

$$Q_t = 1.56\times10^6$$

$$Q_s/Q_t = 0.0316$$

The gas film coefficient is found from the shell side film coefficient correlation given in Eq. (A.28) as,

$$h_g = 20.1 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

Substitution of these values into Eq. (A.33) along with the condensate film coefficient previously calculated, the shell side coefficient for the fermentor reboiler is:

$$h_s = \frac{1}{\frac{0.316}{20.1} + \frac{1}{1066}} = 398 \text{ Btu/hr-ft}^2\text{-°F}$$

and the overall transfer coefficient for the fermentor reboiler in the vacuum system becomes,

$$U = 237 \text{ Btu/hr-ft}^2\text{-°F} \quad .$$

Again a fouling coefficient of 2000 Btu/hr-ft^2-°F has been assumed.

---

*This discharge temperature corresponds to a discharge pressure of 500 mmHg and will condense 99% of the ethanol and water.

The various film coefficients thus far calculated may now be combined with use of Eq. (A.26) to determine the overall heat transfer coefficients for the other modes of heat exchange. The results are summarized in Table A.1. It should be remembered that for the coefficients listed in Table 1, all boiling which takes place is on the tube side with condensation taking place on the shell side.

A.1.8. Vapor Recompression

A vapor recompression cycle is used in the vacuum fermentation system and as an alternative to conventional reboiler operation in the distillation to 95 wt% ethanol. A general schematic of a vapor recompression cycle is shown in Fig. A.3. The recompression cycle adiabatically compresses saturated vapor coming from a boiling environment to a pressure such that the vapor will condense at a higher temperature than the boiling point of the solution. The latent heat of condensation of the vapors then supplies the energy necessary for boiling.

The basic approach was to calculate the required compressor power given the composition and flow rate of vapor. Other parameters specified by the process conditions are:

T_1 = inlet temperature of vapor to compressor, °K

T_2 = condensation temperature of vapor in reboiler, °K

P_1 = suction pressure of compressor, mmHg.

The compressor discharge pressure, P_2, necessary for condensation of the water-ethanol vapor in the reboiler is calculated from:

Table A.1. Summary of typical overall heat transfer coefficients.

Mode of Exchange Tube-Shell	U Btu/hr-ft^2-°F	Process Application
Boiling liquid-condensing vapor	487	Distillation reboiler
Liquid-condensing vapor	448	Condenser
Liquid-Liquid	416	Media sterilization, heat recovery fermentor temperature control
Boiling liquid-condensing vapor with noncondensable gas present	237	Fermentor reboiler in vacuum system

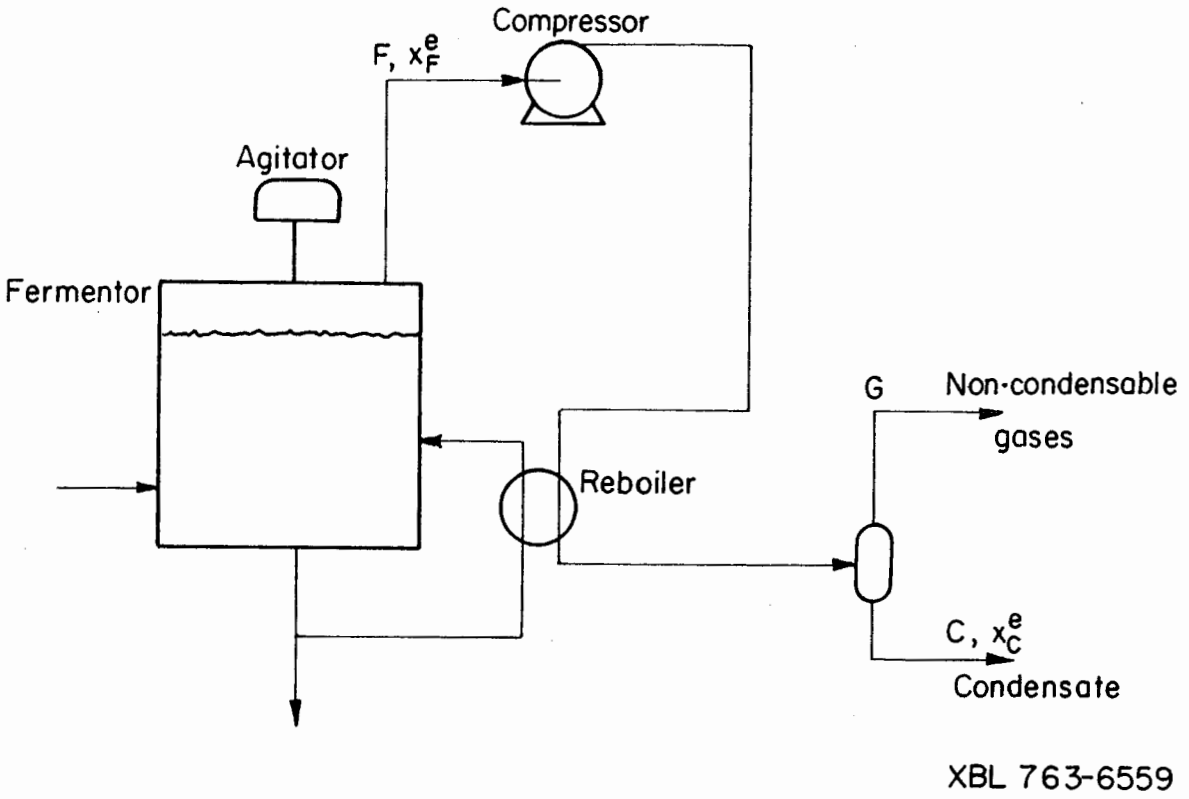

Fig. A.3. Vapor recompression cycle for vacuum fermentation. Symbols explained in text.

$$P_2 = \left(\gamma^e X^e P_e^s + \gamma^w X^w P_w^s\right)/(1 - y^i) \tag{A.34}$$

where, X^e = mole fraction of ethanol in vapor

X^w = mole fraction of water in vapor

y^i = mole fraction of non-condensable gases

The liquid phase activity coefficients for water, γ^w, and ethanol, γ^e, are given by[15]

$$\log \gamma^e = \frac{0.7715(X^w)^2}{(2.00\ X^e + X^w)^2}$$

$$\log \gamma^w = \frac{0.3848(X^e)^2}{(0.5\ X^w + X^e)^2} \tag{A.35}$$

The saturation pressures are obtained from the Antoine equation, for ethanol,

$$\log P_e^s = 8.0449 - \frac{1554.3}{T_2 - 50.35}$$

and water,

$$\log P_w^s = 8.1076 - \frac{1750.29}{T_2 - 38.0}$$

The presence of carbon dioxide and oxygen in the vacuum system must be taken into account since some ethanol and water will leave with these non-condensable gases. An ethanol balance is first written around the compressor and reboiler.

$$X_F^e F = X_c^e C + \left(X_c^e \gamma^e P_e^s\right)/P_2 \tag{A.36}$$

where: F = vapor feed to compressor, moles/hr

C = condensate flow from reboiler, moles/hr

G = uncondensed gas flow from reboiler, moles/hr

Dividing Eq. (A.36) by F and solving for the mole fraction of ethanol in the condensate, x_c^e, gives,

$$x_c^e = x_F^e\left[(1 - \theta)\ \gamma^e P_e^s/P_2 + \theta\right]^{-1} \qquad (A.37)$$

where: $\theta = C/F$

Then knowing θ from process specifications Eq. (A.37) must be solved by trial and error for x_c^e since the activity coefficient, γ^e, is a function of x_c^e as shown in Eq. (A.35). After x_c^e is known the compressor discharge pressure is found from Eq. (A.34) and the compressor power calculated from Eq. (A.15).

A.1.9. Distillation

The alcohol in the fermentation broth is concentrated to 87.5 mole% (95 wt%) by distillation. A McCabe-Thiele type computer model was used to determine the number of plates required to accomplish the concentration. The McCabe-Theile approach is applicable since the molar heats of vaporization of ethanol and water differ by only 5%. Also as shown in Fig. A.4 the heat of mixing of the water-ethanol system is minor. The net affect is only a 6% change in the molar flow rates through out the column.

A general schematic of a distillation column with multiple feed streams is shown in Fig. A.5. For the design calculations, the operating parameters specified by the process conditions are:

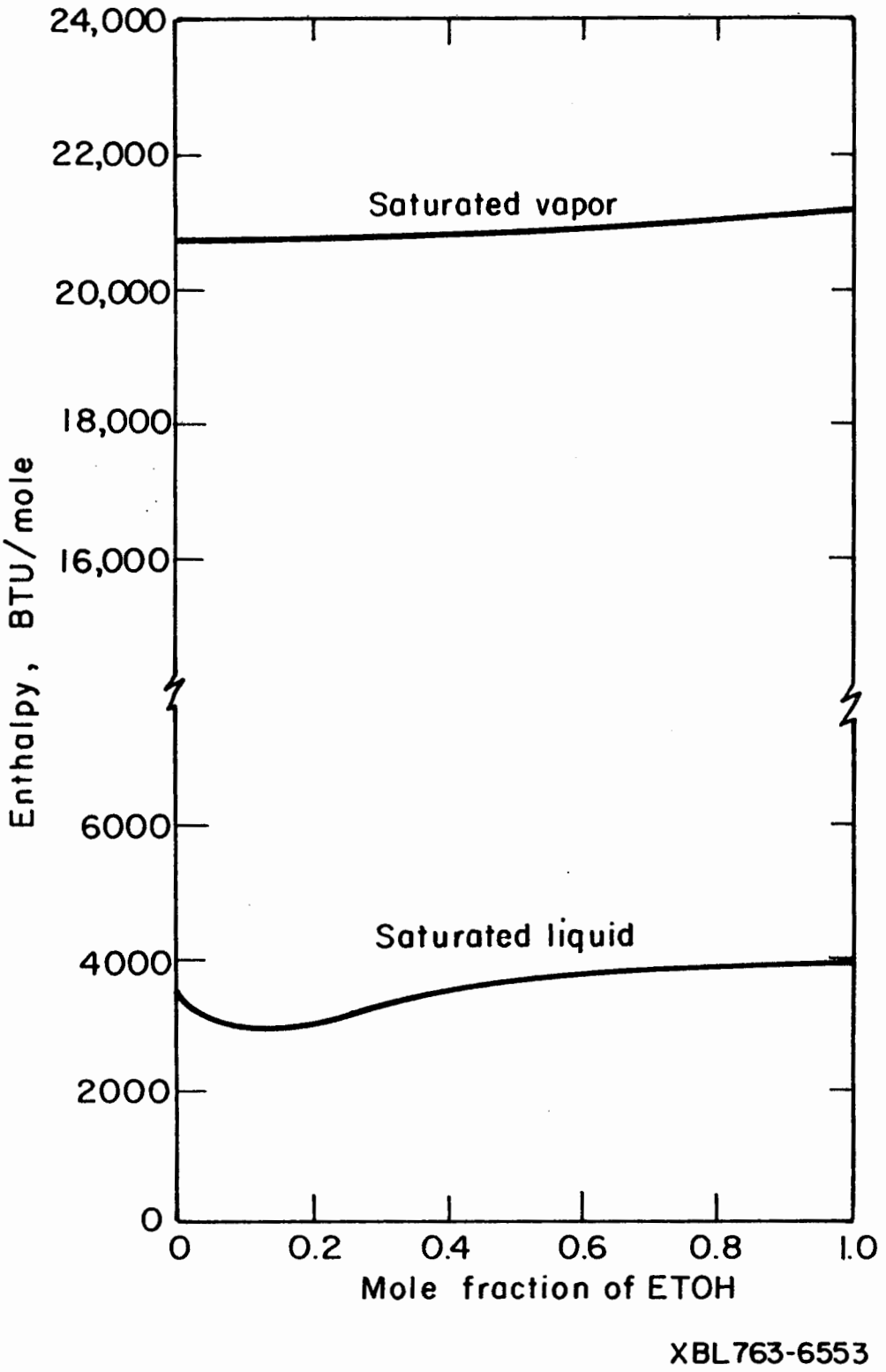

Fig. A.4. Enthalpy-composition diagram for ethanol water binary system.

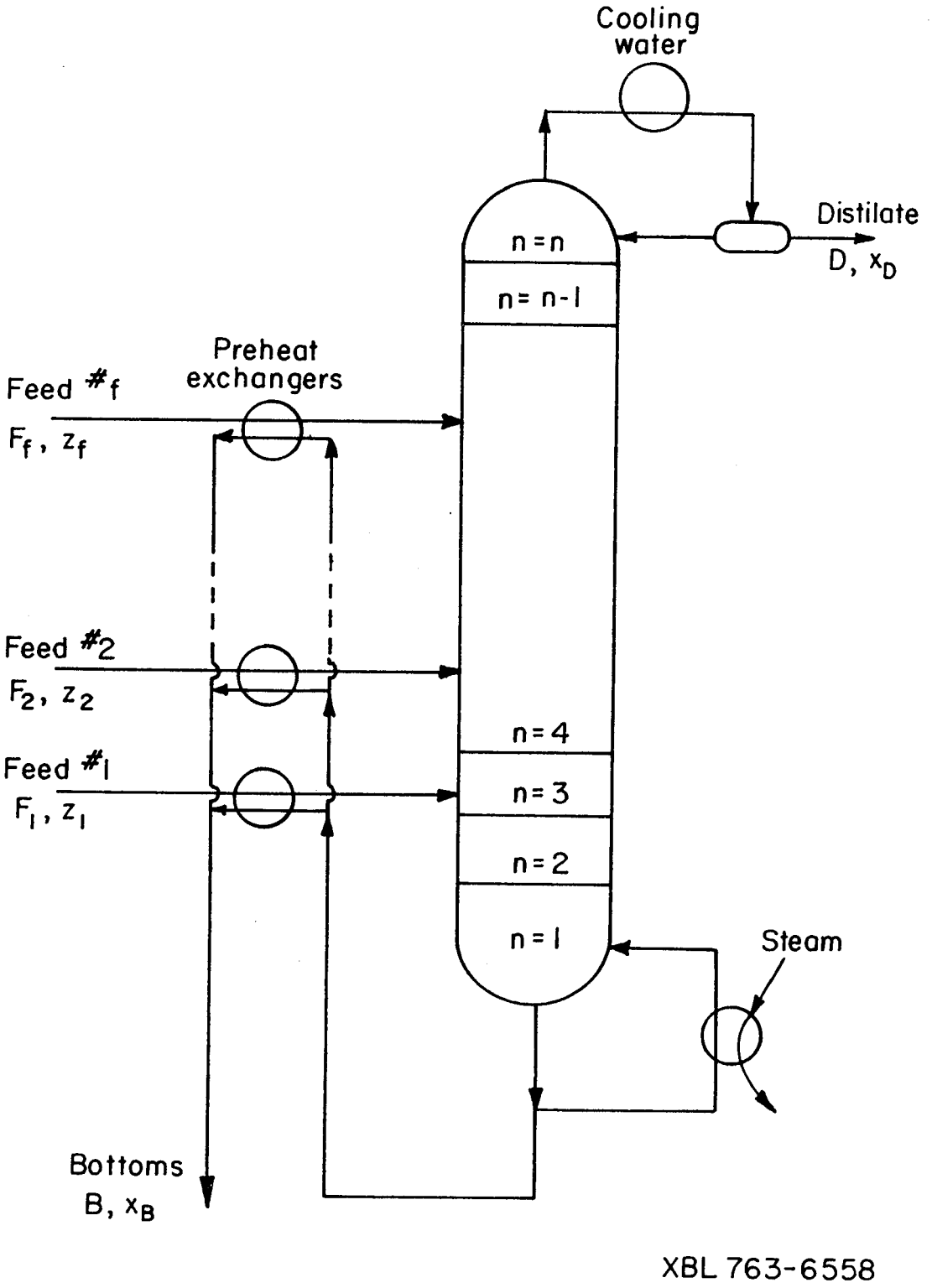

Fig. A.5. Distillation column with multiple feeds. Symbols explained in text.

x_D, mole fraction of ethanol in distilate, 0.875

$F_1, F_2, \ldots F_f$, molar feed rates, moles/hr

$z_1, z_2, \ldots z_f$, mole fraction of ethanol in feeds

P_r, per cent recovery of ethanol in distilate

f, number of feeds

In addition, the reflux ratio, r, must be set to totally specify the distillation. The affect of the reflux ratio on the seperation costs is examined below.

From an overall ethanol balance the distillate and bottoms flows can be found as:

$$D = \frac{P_r(z_1F_1 + z_2F_2 + \ldots z_fF_f)}{x_D}$$

$$B = F_1 + F_2 + \ldots F_f - D$$

And the ethanol concentration in the bottoms stream, x_B is

$$x_B = \frac{(z_1F_1 + z_2F_2 + \ldots z_fF_f) - x_DD}{B} \qquad (A.38)$$

where, B = bottoms flow rate, moles/hr

D = distillate flow rate, moles hr,

Once x_B is known, the composition on each plate of the column can be calculated from a mass balance in conjunction with vapor-liquid equilibrium data. For the stripping section the mass balance yields,

$$x^e_{n+1} = \frac{(R + D)\, y^e_n + x_BB}{(R + F_1 + F_2 + \ldots F_f)} \qquad (A.39)$$

with, $R = \frac{D\ r}{1 - r}$ (the reflux flow rate, moles/hr).

where, x^e_{n+1} = mole fraction of ethanol in down flowing liquid from the $n + 1^{th}$ plate

y^e_n = mole fraction of ethanol in rising vapor from the n^{th} plate

n = plate number, n = 1 for reboiler

In order to start the calculation the vapor phase mole fraction coming from the reboiler, $y^e_{n=1}$, must be found. From the data reduction correlation of Hala et al.[15]

$$y^{*e}_n = \frac{\alpha_{12}\left(x^e_n/x^w_n\right)}{1 + \alpha_{12}\left(x^e_n/x^w_n\right)} \tag{A.40}$$

where the superscript e refers to ethanol and w refers to water. y^{*e}_n is the equilibrium vapor phase mole fraction from the n^{th} plate. The relative volitility, α_{12}, is calculated from an empirical equation presented by Hala.[15]

$$\alpha_{12} = \frac{1 + a_{12}x^w_n + a_{122}\left(x^w_n\right)^2}{1 + a_{21}x^e_n + a_{211}\left(x^e_n\right)^2} \tag{A.41}$$

For the water-ethanol system Hala[15] has reported;

$$a_{12} = 6.5784$$

$$a_{21} = 7.4661$$

$$a_{122} = 5.9449$$

$$a_{211} = -7.2566$$

Thus once x_B is found from Eq. (A.38),

$$x^e_{n=1} = x_B$$

and

$$x^w_{n=1} = 1 - x_B$$

are substituted into Eq. (A.41) to find α_{12}. Then assuming equilibrium conditions exist in the reboiler (i.e., $y^{*e}_{n=1} = y^e_{n=1}$), $y^e_{n=1}$ is calculated from Eq. (A.40). Knowing $y^e_{n=1}$, the liquid mole fraction of ethanol, $x^e_{n+1=2}$, coming from the first plate is found from Eq. (A.39). This process is then repeated to calculate the composition on each plate of the stripping section by adjusting the subscripts in Eqs. (A.39) through (A.41). However, the assumption of equilibrium which was made for the reboiler is not valid for the contacting plates. To correct for this a Murphee-V-phase efficiency is used.

$$E_G = \frac{y^e_n - y^e_{n-1}}{y^{*e}_n - y^e_{n-1}} = 0.70$$

where again y^{*e}_n refers to equilibrium conditions and E_G is the Murphee plate efficiency. (A 70% efficiency is assumed for the water-ethanol distillation.) Solving for y^e_n,

$$y^e_n = E_G\left(y^{*e}_n - y^e_{n-1}\right) + y^e_{n-1} \quad . \tag{A.42}$$

Then after y^{*e}_n is found from Eq. (A.40), Eq. (A.42) is used to determine y^e_n to be used in Eq. (A.39). When a feed point is reached, Eq. (A.39) must be replaced by a new mass balance for the intermediate feed sections:

$$x_{n+1} = \frac{(R + D)\, y_n^e + x_B B - \sum_{m=1}^{P} (z_m F_m)}{R + \sum_{m=P+1}^{f} F_m} \qquad (A.43)$$

where, f = total number of feeds

p = number of feed points already reached

When all the feed points have been reached, i.e., p = f, a mass balance for the rectifying section is used in place of Eq. (A.43).

$$x_{n+1}^e = \frac{(R + D)\, y_n^e - x_D D}{R} \qquad (A.44)$$

The feed points are determined by simultaneously evaluating Eqs. (A.38), (A.43) and (A.44) for all plates. Whenever the mass balance equation of a lower section gives a lower liquid phase mole fraction, x_{n+1}^e, than the next higher section, a feed point is determined and the mass balance equation for this next section is used. As shown in Fig. 5 the feed streams are preheated with the exiting bottoms stream. As well as producing a steam saving which more than offsets the cost of the heat exchangers, the preheat exchangers heat the feeds to essentially saturation conditions.

To determine the column diameter, the temperature on each plate must be evaluated in order to calculate the liquid and vapor velocities. The Antoine equation for the vapor pressure of ethanol is first rearranged to give:

$$T = \frac{1623.22}{8.1629 - \log P_e^s} \qquad (A.45)$$

where, T = temperature, °C

p_e^s = saturation pressure of ethanol, mmHg.

Then assuming an ideal gas phase, the partial pressure of ethanol, p_e, in the vapor phase may be written as:

$$P_e = P_T y^e = p_e^s \gamma^e s^e$$

or,

$$p_e^s = \frac{P_T y^e}{\gamma^e x^e} \tag{A.46}$$

where, P_T = total pressure, mmHg

γ^e = ethanol liquid phase activity coefficient

From Hala,[15]

$$\log \gamma^e = \frac{0.7715\ (x^w)^2}{(2.00\ x^e + x^w)^2} \tag{A.47}$$

After the liquid and vapor phase compositions on each plate have been found from Eqs. (A.39) through (A.44), these values are substituted into Eqs. (A.45) through (A.47) to determine the temperature on each plate.

The densities of the vapor and liquid phases are next calculated.

$$\rho_v = \frac{\bar{M}_v (0.7604)}{273 + T}$$

$$\rho_L = \frac{\bar{M}_L}{x^e\ 46/\rho_e + x^w\ 18/\rho_w}$$

where, ρ_v = vapor density, lb/ft^3

ρ_L = liquid density, lb/ft^3

$\bar{M}_v$ = average molecular weight of vapor

$\bar{M}_L$ = average molecular weight of liquid

ρ_e = density of ethanol, lb/ft^3

ρ_w = density of water, lb/ft^3

The densities of water and ethanol have been correlated by France[17] as:

$$\rho_e = 62.38\ (0.87 - 6.2\times10^{-4}\ T - 19/(300 - T))$$

$$\rho_w = 62.38\ (1.0064 - 2.5\times10^{-4}\ T - 2.3\times10^{-6}\ T^2)$$

Finally, the volumetric flow rates are determined,

$$Q_v = 3.65\times10^{-4}\ V(273 + T) \tag{A.48}$$

$$Q_L = \frac{L\bar{M}_L}{3600\ \rho_L} \tag{A.49}$$

where, Q_v = volumetric flow of vapor, ft^3/sec

Q_L = volumetric flow of liquid, ft^3/sec

V = molar flow rate of vapor, (V = R + D), lb moles/hr

L = molar flow rate of liquid, lb moles/hr.

The gas flooding velocity can then be calculated at each plate following Treybal.[18]

$$V_F = C_F \left(\frac{\rho_L - \rho_v}{\rho_v}\right)^{1/2} \tag{A.50}$$

where, V_F = superficial gas flooding velocity

C_F = empirical constant

For sieve trays C_F has been correlated as,

$$C_F = \left[a \log\left(\left(\frac{Q_V}{Q_L}\right)\left(\frac{\rho_v}{\rho_L}\right)^{0.5}\right) + b\right]\left(\frac{\sigma}{20}\right)^{0.2}\left(5\,\frac{A_h}{A_a} + 0.5\right) \qquad \text{(A.51)}$$

with the constants a and b given by

$$a = 0.0062 \cdot t + 0.0385$$

$$b = 0.0025 \cdot t + 0.05$$

where, t = tray spacing, in.

A_h = total hole area per tray, ft^2

A_a = active area per tray, ft^2

σ = surface tension of solution on plate, dynes/cm

The surface tension was estimated by a parachor contribution method.[19] The average parachor of the water-ethanol solution, $\bar{P}$, is,

$$\bar{P} = 130.8\ x^e + 50.8\ x^w$$

and the surface tension is found as,

$$\sigma = \left(\frac{\bar{P}(\rho_L - \rho_v)}{62.4\ \bar{M}_L}\right)^4 .$$

to evaluate C_F the geometric configuration of the distillation column was assumed to be:

Tray spacing, t = 24 in.

Ratio of hole area to active area per tray, $A_h/A_a = 0.128$

Ratio of total tower cross sectional area to area available to gas flow = 0.92.

After the flooding velocity has been calculated from Eqs. (A.48) through (A.51) the column diameter D_C is found,

$$D_C = \left[\left(\frac{Q_V}{(V_F)(0.8)(0.92)}\right)\left(\frac{4}{\pi}\right)\right]^{1/2} \qquad (A.52)$$

Here the superficial gas velocity has been taken at 80% of the flooding velocity. With this constraint the liquid entrainment was found to be less than 4% for all column designs and the gas velocity through the perforated tray was high enough to stop incipient weeping.[18] Using Eq. (A.52) a column diameter corresponding to 80% of flooding was calculated for each plate. The largest diameter was then taken as the actual diameter for the design. A uniform column diameter was used since the high reflux ratios required to achieve the separation minimize the effect of the increased liquid flow in the stripping section. As a result the exact column diameters in the stripping and rectifying section differ by only 10% and do not justify the use of a multiple diameter column.

Reflux Ratio. Examination of the ethanol-water equilibrium diagram shows three possible areas which could determine the minimum reflux ratio: (1) the rectifying section, (2) the last feed point, and (3) the first feed point. A minimum reflux ratio corresponding to each of these points was calculated and the largest ratio was taken to be the minimum reflux ratio for the particular distillation.

The minimum reflux in the rectifying section was determined graphically from the intersection of the operating and equilibrium curves. For all the water-ethanol distillations this occurs at,

$$r_{min} = R(R + D) = 0.76$$

or,

$$R_{min} = 0.76\ D/(1 - 0.76) \quad , \tag{A.53}$$

because the conditions of the feeds do not affect the shape of the equilibrium curve. However, more severe limitations on the reflux may take place at the feed points. Since minimum reflux occurs when the operating and equilibrium curves intersect, the equations for these curves can be equated and the minimum reflux solved directly. For the final feed point:

$$y_n^{*e} = \frac{\alpha_{12}\left(x_n^e/x_n^w\right)}{1 + \alpha_{12}\left(x_n^e/x_n^w\right)} = \frac{x_{n+1}R + x_D D}{R + D} \tag{A.54}$$

Because conditions of constant composition exist at the pinch point, i.e., $x_n^e = x_{n+1}^e$, Eq. (A.54) may be rearranged to find the minimum reflux.

$$R_{min} = \frac{D\left(y_n^{*e} - x_D\right)}{\left(x_n^e - x_n^{*e}\right)} \tag{A.55}$$

Substitution of, $x_n^e = z_f$ and $x_n^w = 1 - z_f$ into Eq. (A.55) specifies the minimum reflux as determined from the last feed point. In the same manner the minimum reflux set by the first feed point is found as:

$$R_{min} = \frac{x_n^e(F_1 + F_2 + \ldots F_f) - x_B B - D y_n^{*e}}{\left(y_n^{*e} - x_n^e\right)} \tag{A.56}$$

with, $x_n^e = z_1$ and $x_n^w = 1 - z_1$.

By use of Eqs. (A.53) through (A.56) a minimum reflux is evaluated at the three possible pinch points. The largest R_{min}

calculated is then set as the minimum reflux required for the separation. It should be pointed out that only the first and last feed points need be examined because the slope of any intermediate operating line between these two points will be greater than the rectifying operating line but less than the stripping operating line.

Once the minimum reflux rate has been specified the operating reflux rate is set at 1.25 times the minimum, i.e., $R = 1.25(R_{min})$. This procedure was used rather than determining the optimum reflux rate because of the extreme sensitivity the distillation exhibits at the optimum reflux ratio and a rather flat cost curve in the region of higher reflux. This is shown in Fig. A.6 which plots reflux ratio against separation costs for a 5 wt% ethanol feed. The optimum reflux ratio is 0.8 requiring 110 contacting plates to accomplish the separation. If, however, the reflux ratio is decreased a small amount to 0.793 the number of plates required jumps to 200 and the overall separation costs also drastically increases. Because of possible uncertainties in equilibrium data, plate efficiencies and stability of operation; this optimum reflux ratio does not represent a rational design. Hence, the reflux ratio is increased to 1.25 times the minimum. Although this raises the separation costs by 2% it produces a much more plausible and realistic design.

Since the reflux ratio is set, the optimization of the distillation column is centered around the heat exchange equipment. An analogous equation to Eq. (A.14a) may be written for the overhead condenser.

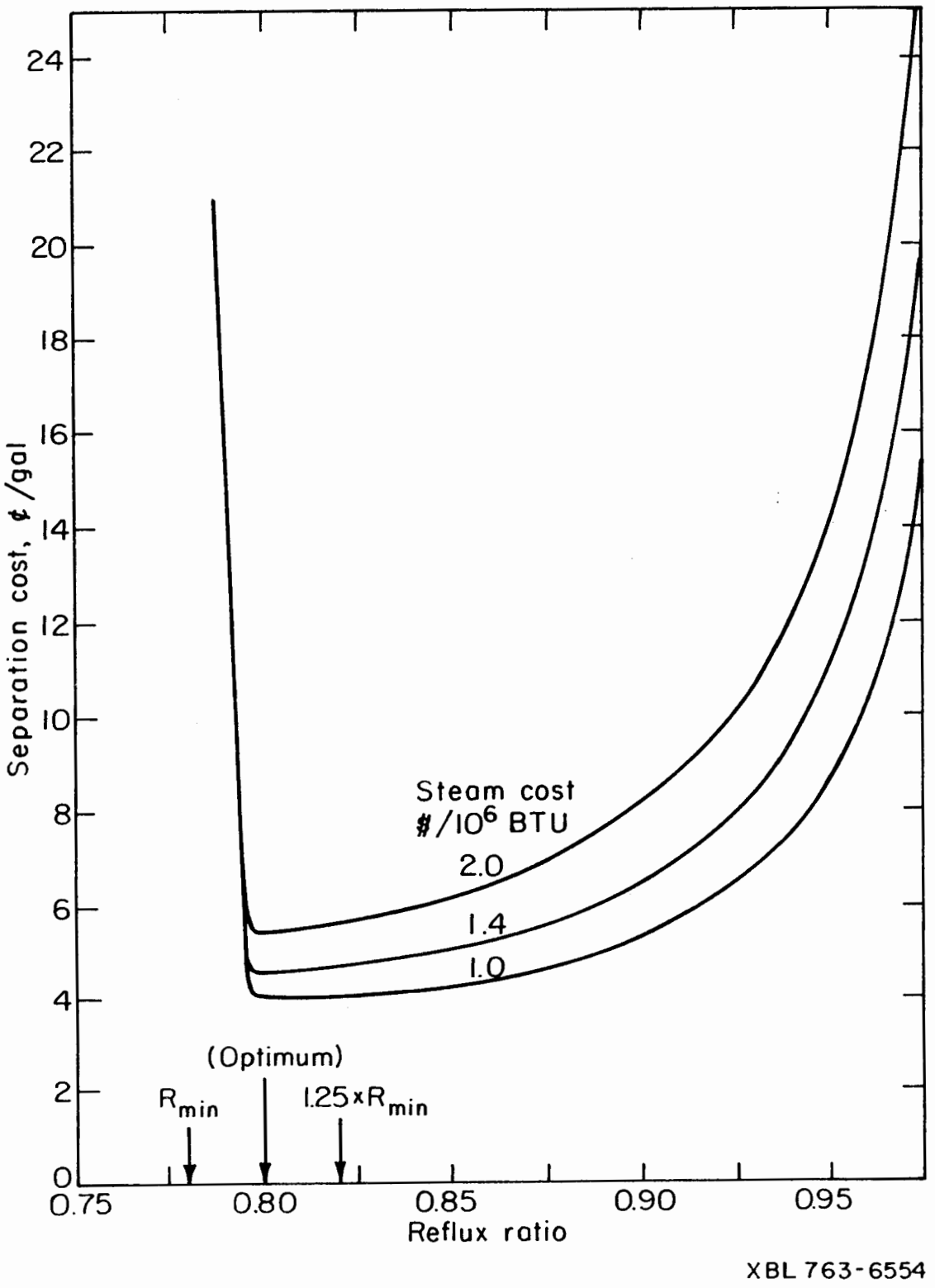

Fig. A.6. Effect of reflux ratio on separation costs to produce 64,700 gal/day of 95% ethanol from a 5% ethanol feed.

$$Q_c = (R + D)\ \Delta H^V = W_c C_p (T_{c1} - T_{c2}) \qquad (A.57)$$

where, Q_c = condenser duty, Btu/hr

R = reflux flow, lb moles/hr

D = distillate flow, lb moles/hr

ΔH^V = molar heat of vaporization Btu/lb mole

W = cooling water flow rate, lb/hr

C_p = heat capacity of water 1 Btu/lb-°F

T_{c1} = exit temperature of cooling water, °F

T_{c2} = inlet temperature of cooling water, 25°F

The ethanol vapor condenses on the shell side of the condenser at 78°C, thus once T_{c1} is set the cooling water flow and condenser area may be found from Eqs. (A.57) and (A.25) respectively. However, as T_{c1} approaches the vapor condensation temperature (78°C), Eq. (A.57) dictates a lower cooling water requirement, but from Eq. (A.25), the condenser area must be increased. An optimum then exists and is determined using T_{c1} as the optimization parameter.

Likewise, in the preheat exchangers, if there is a small ΔT between the bottoms and feed streams, the feed enters the column at saturation conditions and the reboiler duty is lowered but large preheat exchangers are needed. The energy requirement of the reboiler may be related to the operation of the preheat exchangers by,

$$Q_B = Q_C + Q_F \qquad (A.58)$$

where,

$$Q_F = \sum_{m}^{f} C_p F_m \left(T_s^m - T_F\right)$$

with, Q_B = reboiler duty, Btu/hr

Q_C = condenser duty, Btu/hr

T_s^m = saturation temperature of m^{th} feed, °F

T_F = inlet temperature of feeds, °F

C_p = molar heat capacity of feeds, Btu/lb mole °F

F_m = molar flow of m^{th} feed, lb moles/hr

Here the inlet temperature of all feeds has been assumed the same, i.e., the same degree of approach in all preheat exchangers. The exiting bottoms temperature is set by the boiling point in the reboiler (212°F) and the feeds enter the preheat exchangers at the fermentation temperature. With these temperatures known, the preheat exchanger area is calculated from Eq. (A.25) and the boiler duty from Eq. (A.58) once T_F is specified. The trade off between boiler heat load, or steam cost, and preheat exchanger cost is then optimized using T_F as the variable parameter.

It is evident from Eq. (A.58) that if the optimum T_F is appreciably lower than T_s^m the energy required to heat the feeds to saturation will be substantial. This energy is derived from condensing vapor on the feed plates and will cause a corresponding increase in liquid flow. The net result being a change in the mass balances equations in the stripping and intermediate sections of the column (Eqs. (A.39) and (A.43)). Thus after the preheat exchangers have been optimized new molar flows of liquid and vapor are calculated and the

column design repeated until the molar flows of succeeding designs differ by only 2%. The new molar flows are found from,

$$L_m = R + \sum_{m=p+1}^{p} F_m + V \qquad (A.59)$$

$$V_m = R + D + V$$

where,

$$V = \frac{C_p}{\Delta H^v} \sum_{m=p+1}^{f} F_m \left(T_s^m - T_F^* \right) \qquad (A.60)$$

with, T_F^* = optimum valve of T_F

f = total number of feeds

P = number of feed points reached.

To obtain the design based on corrected internal flows L_m is substituted for the denominator in Eqs. (A.39) and (A.43) and V_m substituted for the (R + D) term in the numerator of the same equations.

A.1.10. Auxiliary Equipment

Tanks. In addition to the fermentor vessels mixing and storage tanks are required. The three areas within the process which require storage facilities are: feed sugar solution storage, fermented beer storage and product ethanol storage. The storage capacity of both the sugar feed solution and product alcohol was set to accommodate a 2 day supply.

The in plant storage of the fermented beer was designed to allow 2 hr operation of the distillation column in the event of a process interruption. One additional vessel is used as a mixing tank to

dissolve mineral and vitamin supplements in the sugar feed solution. This vessel was sized assuming a 30 min residence time necessary for dissolution of the solids.

The solids are fed to the mixing tanks by screw conveyors; one conveyor for each solid supplement. The conveyor designs were based on the tonage of material to be handled and a 70 ft conveyor length was assumed in each case. Solid storage bins to accommodate a 20 day supply were also included in the design and cost estimation.

Pumps. The centrifugal pumps used to pump the fermentation broth through the media sterilizer, from the fermentor to the beer storage and to and from the distillation column were assumed to pump against a 30 psi pressure differential. A 10 psi pressure differential was used to size the pumps linked to heat exchangers.

Centrifuges. The centrifuges used to separate the yeast and fermentation broth were designed using actual performance data for de Laval yeast separators.[23]

Ethanol Absorption. The ethanol vapor in the carbon dioxide and air stream leaving the fermentors is recovered by absorption into water. A portion of the distillation bottoms is used as the absorbing liquid which then returns to the distillation column and is stripped of ethanol. The ethanol adsorption column was designed using a computer program as described in Sherwood, Pigford and Wilke[22] (Chapter 9, Appendix A).

The ethanol absorber represents a minor piece of equipment amounting to only 0.45% of the total capital investment, and its operation increases the feed rate to the distillation column by

only 1.0%. Hence, no attempt was made to optimize the absorber. However, it was found economically advantageous to recover the ethanol in the exiting gas stream by absorption. The total absorption recovery cost amounted to 4.5 cents per gallon of ethanol recovered while total conversion costs varied from 8 to 20 cents per gallon.

A.2. Cost Estimation

Fixed Capital Investment. The process equipment costs were estimated using two main sources, Peters[4] and Guthrie.[21] The graphical cost data presented in these two references was fitted to exponential equations relating the F.O.B. equipment cost to the equipment size. A summary of these cost equations is listed in Table A.2. The equipment costs have been generalized by dividing by the Marshell Steven cost index of the year of the reference. To obtain the current F.O.B. cost, the current Marshell Stevens index is used in the cost equations shown in Table A.2. Also listed in Table A.2 is the maximum unit capacity for each piece of equipment. When the process design dictated a larger total equipment capacity than the listed maximum, an integral number of equally sized units was used.

The materials of construction for the ethanol fermentation process were taken to be stainless steel for any piece of process equipment which came in contact with the fermentation broth before or during the fermentation, and carbon steel for all other process equipment. The fermentor costs were estimated using stainless steel mixing tanks rated at an operating pressure of 100 psi. The cost includes nozzles, manways and all supports. The agitator cost was then added to obtain the price of the basic fermentor unit. The cost of the media sterilizer

Table A.2. Summary of capital cost equations; MSI=Current Marshall Stevens Cost Index.

Item	Unit Cost, $ FOB	Size Unit	Maximum Size per Unit	Construction	Ref.
Fermentors Mixing Tanks	MSI $0.323\ (Size)^{0.53}$	Liters	1.89×10^{5}	Stainless Steel	4
Agitators	MSI $3.33\ (Size)^{0.56}$	Horsepower	400	Stainless Steel	4
Heat Exchangers	MSI $0.696\ (Size)^{0.64}$	Ft^2	10^4	S.S. Tubes Carbon Steel Shell	21
Compressors	MSI $2.90\ (Size)^{0.75}$	Horsepower	10^4	Centrifugal Carbon Steel	21
Media Sterilizer	MSI $(0.674\ S_d^{0.93} + 0.0139\ S_d^{0.5})\ 3.82\ S_L$	S_d=Dia, m S_L=Length, m	2.0 200	S.S. Pipe 2 in. Insulation	4
Columns					
Shell	MSI $0.308\ S_h^{0.812} S_d^{0.879}$	S_h=Height, ft	150	Carbon Steel	21
Plates	MSI $0.0157\ S_h^{0.976} S_d^{1.63}$	S_d=Dia, ft	10	Carbon Steel	21
Packing	MSI $0.0125\ S_v$	S_v=Volume, ft^3		1 in. Rashing Rings	4
Storage Tanks	MSI $0.112\ (Size)^{0.642}$	Gallons	10^7	Stainless Steel	21
Storage Tanks	MSI $0.035\ (Size)^{0.642}$	Gallons	10^7	Carbon Steel	21

Table A.2. Continued.

Item	Unit Cost, $ FOB	Size Unit	Maximum Size per Unit	Construction	Ref.
Spray Dryers	MSI $8.06\ (\text{Size})^{0.181}$	lb/hr Water Removed	9000	14 ft dia	4
Pumps	MSI $(2.64 + 0.0068\ \text{Size}^{0.718})$	gpm×psi	300,000	Stainless Steel	21
Centrifuges	See Text				
Solid Feeders	MSI $1.10\ (\text{Size})^{0.72}$	Ton/hr	30	70 ft Screw Conveyor	4

includes the stainless steel pipe and 85% magnesia insulation.

The cost of centrifuges has been left out of Table A.2 because the design, performance and cost of centrifuges differ greatly depending on manufacture. Many commercial centrifuges are not able to separate a yeast suspension. As a result, performance and cost data for de Laval yeast separators[23] was used throughout the design calculations.

After the F.O.B. equipment costs have been calculated, a ratio factor of 3.09 is applied to estimate the total fixed capitol investment. A breakdown of the factor is shown below. The multiplier factor for fermentors was increased to 4.24 to reflect the additional instrumentation, piping, and installation costs associated with fermentors as compared with mixing tanks used to determine the F.O.B. fermentor costs.

Operating Costs. The plant operating costs were divided into two main catagories: fixed charges and direct production costs. A summary of the fixed charges are shown in Table A.4. Here a 10 year straight line depreciation was assumed and local taxes have been included. The total fixed charges amount to 19.0% of the fixed capital investiment per year. The direct production costs include labor, utilities and overhead. The labor and utility charges are often subject to debate and are difficult to estimate in today's inflating economy. As a result, a sensitivity analysis of the effect of labor and energy costs on ethanol production costs was made. The base labor and utilities rates are discussed below.

Table A.3. Ratio factor for estimation of fixed capital investment from F.O.B. equipment cost.

	Process Equipment	Fermenters
1. Direct costs (D)		
(a) Purchased equipment (E)	1.0	1.0
(b) Equipment Installation	0.3	0.5
(c) Piping	0.2	0.4
(d) Instrumentation	0.1	0.3
(e) Insulation	0.05	0.05
(f) Electrical	0.1	0.2
(g) Building/Facilities	0.3	0.4
(h) Land/Yard Improvement	0.1	0.1
	2.15	2.95
2. Indirect cost (I)		
(a) Engineering + Construction 0.25D		
(b) Contractor's Fee + Contingency 0.15 (D + 0.25D)		
3. Fixed capital investment (D + I)		
Process Equipment (1.15)(1.25)(2.15E) = 3.09E		
Fermentors (1.15)(1.25)(2.95E) = 4.24E		

Table A.4. Fixed operating costs.

	Per Cent of Fixed Capital Investment per Year
Depreciation	10.0%
Taxes	4.0%
Insurance	1.0%
Maintenance and Repair	3.0%
Operating Supplies	1.0%
Total	19.0%

The labor requirement was taken as 0.25 men per fermentor plus one man to run auxillary fermentation equipment. The distillation column and ethanol absorber were assumed to require one man each. Throughout the cost calculations a 8,500 hr year was assumed and the base labor rate was set at $5.60 per man hour. Laboratory changes were taken at 12% of labor costs, and plant overheat at 50% of labor and maintenance cost. Supervision and clerical costs were estimated at 12% of labor.

The base utility rates are shown in Table 4. These costs are assumed to include the capital amortization, raw material and fuel costs to supply the particular utility.

Electric power requirements were calculated assuming an 80% efficiency in electric to mechanical power converson. Also, an 80% efficiency was taken for adiabatic compression and pumps.

A.3. Computer Programs

General Optimization Procedures. The computer programs used to design both the fermentation equipment, CONFER, and the distillation equipment, DISTL, employ a pattern search subroutine, PATERN,* to determine the optimum values of design variables. The independent design variables required for the design of a piece of process equipment, or complex of process equipment, are initially specified in the main programs. Each step size by which the design parameters are initially adjusted is also set in the main programs. The subroutine PATERN is then called and an array of values for the design variables is generated, each value being equally spaced around the initial variable

*The pattern search subroutine was not written by the author and is of unknown origin.

Table 4. Base utility rates.

Cooling Water	$\$0.128/10^3$ gal
Electric Power	1 cent/kw,* 3 cent/kw†
Steam	$\$0.325/10^3$ lb,* $\$2.81/10^3$ lb**

*Cost based on fermentation plant coupled to cellulose hydrolysis facility which burns waste ligno-celulosics for power and steam.

**Bought from public utility.

†Self generated from low sulfur fuel oil.

value by the step size set in the main program.

The physical limit of each variable is set in a subroutine BOUND. As a new value of a variable is generated in PATERN, BOUND is called to verify that the value is within specified limits. With the multi-dimentional array of parameter values set, subroutine PROC is called from PATERN. In PROC the equipment is designed and costed for each set of parameter values generated in PATERN. Control is then returned from PROC to PATERN and the cost at each set of variable values compared. The set of parameter values that produced the lowest operating cost is selected and another search conducted around these parameter values. This process is continued until no further decrease in cost is determined. The step size is then reduced by a factor of ten and the search repeated around the above optimum parameter values. The number of times the pattern search is repeated is set by specifying the number of step size reductions to be performed in the variable list of PATERN. The optimum values of the design variables are returned to the main program.

As with most optmization techniques, the pattern search does not guarantee that a universal rather than local optimum will be determined. However, by setting a large initial step size the pattern search is conducted over the entire specified range of each variable. The fact that the pattern search method does result in a optimum design and that the optimum design parameters are correct was demonstrated by hand calculation of fermentation plant designs. Comparison of 2 weeks of hand calculations, making the most judicious choice of design parameters, with a computer design showed a 30% reduction in

ethanol production cost was obtained by the computer design. It should be pointed out that the hand calculations were done before the computer programs were developed and the tediousness of the calculations was a main impetus for the development of the programs.

A.3.1. Optimization of Fermentor and Auxiliary Equipment. Computer Program CONFER

The computer program CONFER designs and optimizes the fermentation system shown in Fig. A.7; this includes the media sterilizer, fermentors, agitators, temperature control and air delivery systems. The program has been arranged so that up to four simultaneous fermentation products and cell mass may be produced. Although a single stage fermentation is shown in Fig. 7, CONFER may be used for multiple stage fermentations by entering the succeeding stage fermentation conditions and rerunning the program.

There are three areas within the fermentation system which are independently optimized; (1) the media sterilizer, (2) the fermentor agitator and air delivery system, and (3) the fermentor temperature control system. The general structure of the computer program and the interrelationship between the various subroutines is illustrated in Fig. A.8.

The optimization of the media sterilizer complex was performed over four variables:

DS = diameter of sterilizer holding section, m

TS = sterilization temperature, °C

DTH = degree of approach in sterilizer pre-heat exchanger, °C

TC2 = exiting cooling water temperature from cooler, °C

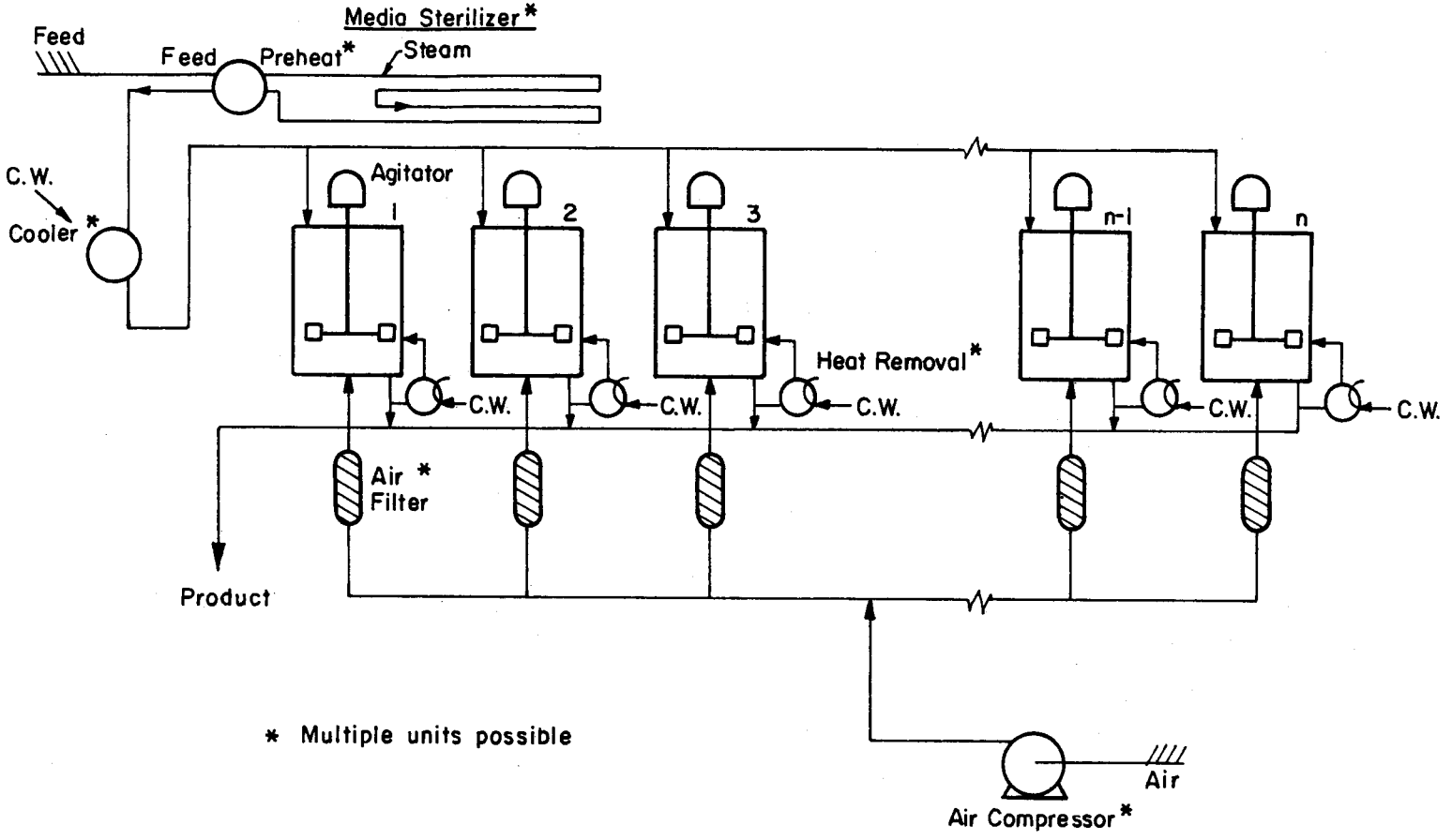

Fig. A.7. Fermentation scheme for optimum design. Maximum unit fermentor volume = 50,000 gal, maximum unit heat exchanger area = 10,000 ft^2, maximum agitator power = 200 HP.

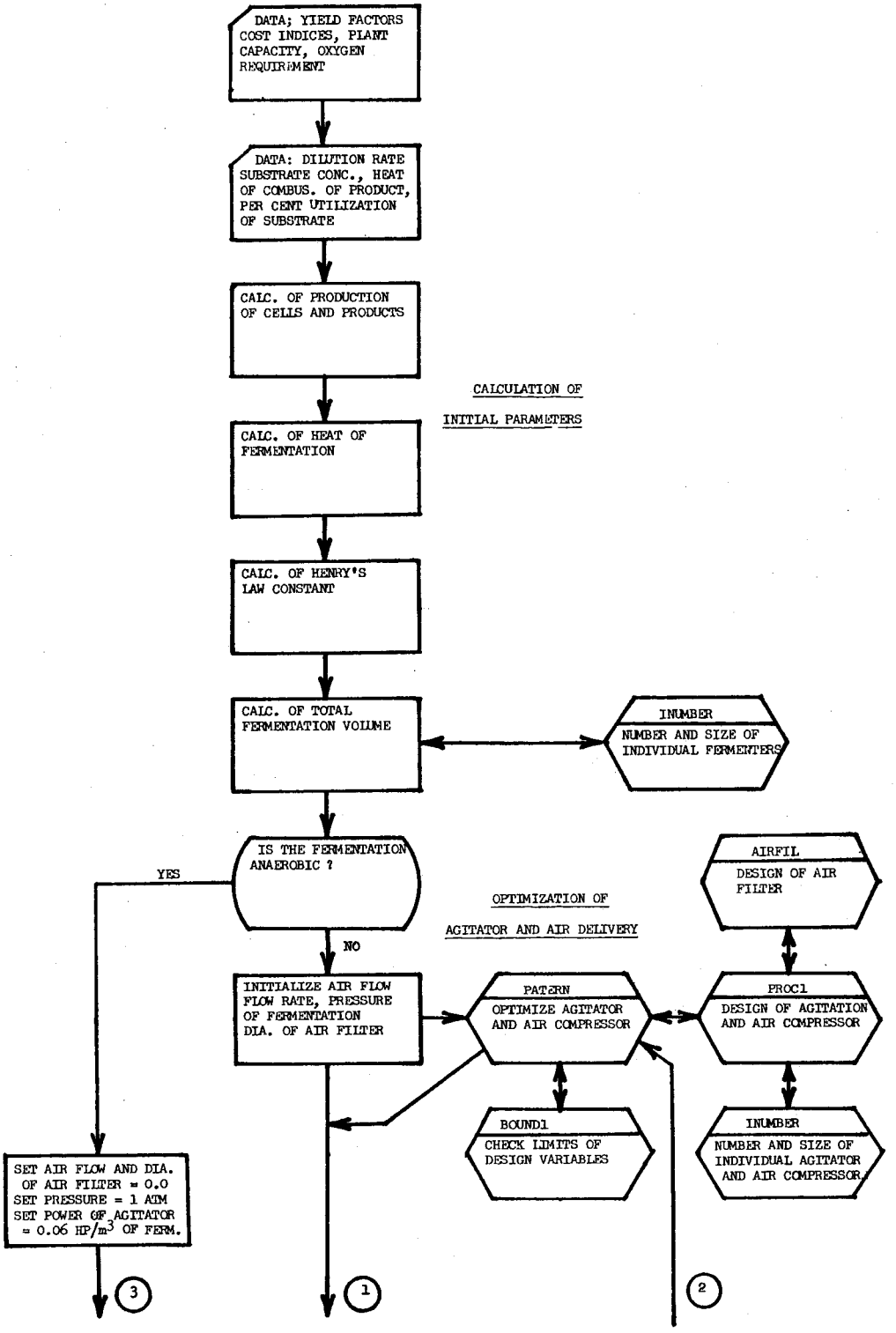

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Fig. A.8. Flow diagram for computer program CONFER.

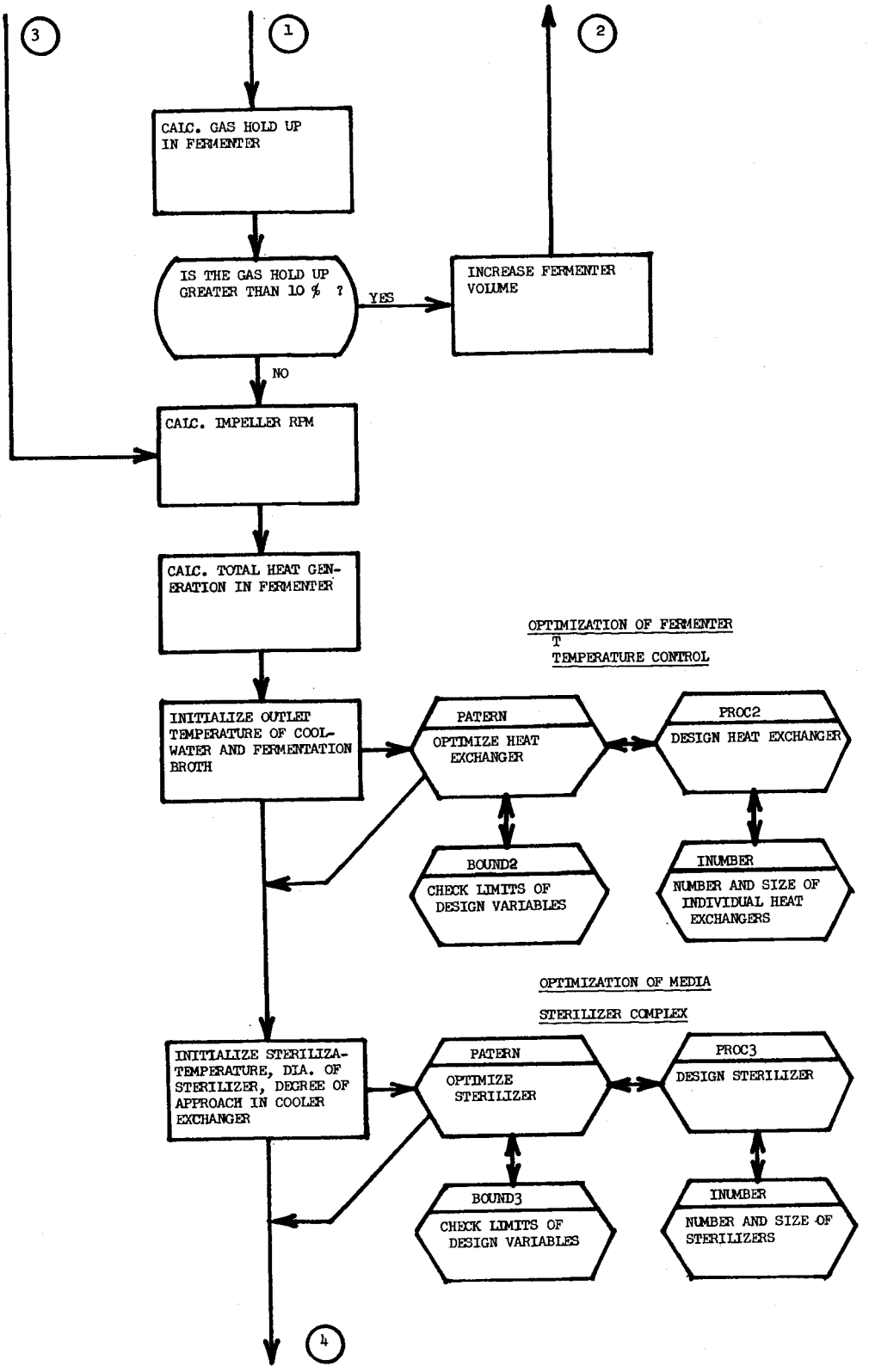

Fig. A.8. Continued.

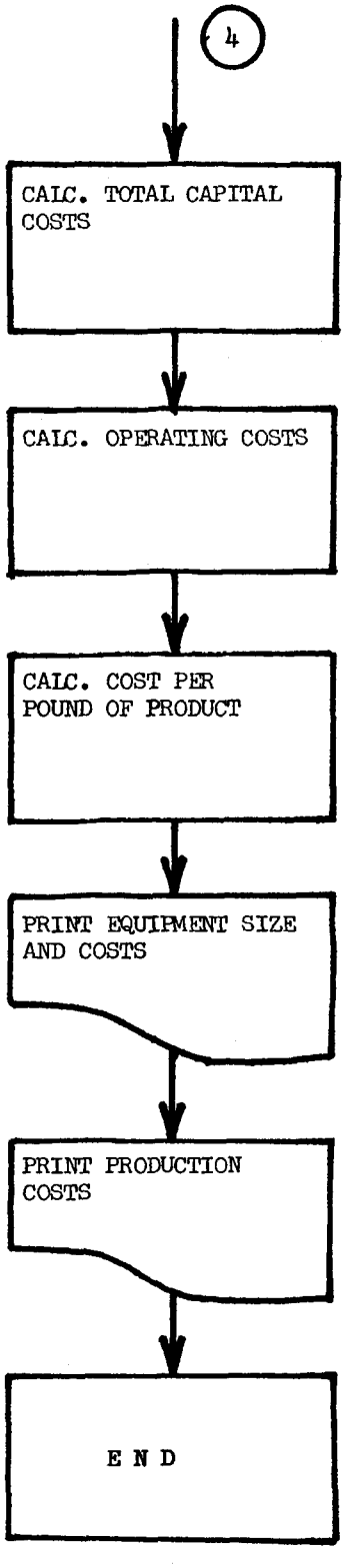

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Fig. A.8. Continued.

These variables are initiallized in the main program and then a pattern search subroutine, PATERN, is called to locate the optimum values with the aid of subroutine PROC3. The PATERN subroutine determines the direction and magnitude which the variables are changed while the actual cost and design calculations are done in PROC3 with the adjusted variables fed to PROC3 by PATERN. PROC3 calculates the length of the sterilizer holding section from Eq. (A.24) and uses Eq. (A.25) in conjuction with the heat transfer coefficients listed in Table A.1 to size the heat exchangers. The steam requirement is found from the energy balance of Eq. (A.24a). With the equipment sized, the cost of the sterilizer complex is calculated using the cost parameters listed in the cost estimation section. The total operation cost of the sterilizer (dollars per year) is returned to PATERN and compared with previously calculated cost figures and the variables adjusted until the optimum valves are determined and returned to the main program. Throughout these calculations the value of the adjusted variables generated in PATERN are checked in subroutine BOUND3 to insure that they are within specified limits and represent a reasonable design.

The fermentor agitator and air delivery system are optimized in the same manner. The optimized parameters initiallized in the main program are:

P = pressure of the fermentation, ATM

VS = superficial air velocity through fermentor, m/hr

CSAFT = cross sectional area of air filter, m^2

The subroutines PROC1 and BOUND1 have the same functional relationship to PATERN as enumerated above for PROC3 and BOUND3. Within PROC1 the agitator power is calculated from Eqs. (A.6) and (A.7). Subroutine AIRFIL is then called from PROC1 to determine the length of the air filter from Eqs. (A.16) through (A.19), and the pressure drop through the air filter from Eq. (A.20). These values are returned to PROC1 and the compressor power calculated from Eq. (A.15). Next the overall operation cost of the agitation, air filter, and compressor is found and control is returned to PATERN until an optimum is reached.

Similarly, for the fermentor temperature control, two parameters are initiallized in CONFER.

TH1 = temperature of broth returning to fermentor from external heat exchanger, °C

TC1 = exit temperature of cooling water, °C

The size of the heat exchanger from Eq. (A.25), and the total cost from the cost equations are calculated in PROC2. The values are returned to PATERN and the parameters TH1 and TC1 adjusted until an optimum is found within the specified limits set in subroutine BOUND2. The optimum values are then returned to the main program, CONFER. Throughout the program, subroutine INUMBER is called from the PROC subroutines to determine the size and number of individual pieces of equipment. The subroutine INUMBER calculates the integral number of equally sized units to meet the design specifications generated in the PROC subroutines when the total capacity is greater than the maximum unit capacity set in the cost estimation section.

A FORTRAN listing of CONFER and summary of variables are given next.

Explanation of Variables Used in Fermentation Plant Optimization, Computer Program CONFER (in order of appearance)

MM	Number of total different fermentation processes
XMSI	Current Marshall-Stevens Index
QH	Dummy variable
YXS	Cell yield factor (gram/gram)
YPS1-4	Product yield factor of products 1 to 4 (gram/gram)
HCS	Heat of compustion of substrate (kilocalories per gram)
HCX	Heat of combustion of cell mass (kilocalories per gram)
HC1-4	Heat of combustion of up to four products (kilocalories per gram)
DUMY	Dummy variable (reads in 0)
MN	= 1 if producing product or = 0 if producing cell mass (integer)
NN	Number of the case for a given fermentation (can vary capacity, S_o, dilution rate, temperature, Q_{O_2}, critical liquid oxygen concentration, and per cent utilization of substrate)
CAP	Capacity (pounds per day)
SO	Limiting substrate concentration inlet (gram/liter)
DI	Dilution rate (hr^{-1})
T	Temperature (°K)
QO2	Specific respiratory rate (millimoles O_2/(gram dry weight-hour)
CLC	Critical liquid oxygen concentration (10^{-4} gram moles per liter)
PPT	Per cent utilization of substrate

Calculation of Product and Cell Yields

FM	Medium flow rate (liters per hour)
P	Product steady-state concentration in fermentor (gram/liter)
X	Cell mass steady-state concentration in fermentor (gram/liter)

Calculation of Heat of Fermentation

HFERM	Heat of fermentation (kilocalories per liter)
QH	Heat of fermentation (Btu per hour per liter)

Calculation of Henry's Law Constant "H"

TK	Temperature in °K
A-E	Parameters
BT	1000/T
HL	Logarithm of Henry's Law constant
H	Henry's Law constant (atm/mole fraction)
TC	Temperature in °C
DSF	Density of water in grams per cubic centimeter
Q	Total oxygen demand (moles/liter-hour)

Calculation of Number and Size of Fermentors
(Working volume = 0.8* Total Volume)

VW	Working volume of fermentor (liters) (total required for all fermentors)
V	Total volume of all fermentors
VM	Maximum allowable size of fermentor (1.89×10^5 liters=5×10^5 gal)
INUMBER	Subroutine calculates integral number of equal capacity units.

Parameter list:

V	total required capacity
VM	Maximum unit capacity
VF	Calculated unit capacity
FN	Number of units

VWW	Working volume per fermentor (liters)
DF	Diameter of each fermentor (meters)

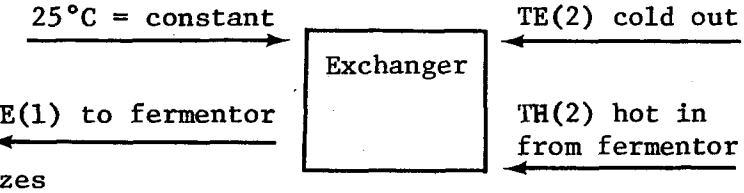
Optimization of Agitation, Air Compressor, and Air Filter

STP1	Parameters of PATERN subroutine, initial step sizes
OP	Parameters of PATERN subroutine, initial guesses
	OP(1) Pressure (atm)
	OP(2) Superficial gas velocity, V_s (meter/hour)
	OP(3) Cross-sectional area of air filter (square meter)
PATERN	Optimizes over pressure in fermentor, superficial air velocity, air filter cross-sectional area based on total of compressor capital cost plus power cost, agitator capital cost plus power cost, and cost of air filter
PROC	External function to calculate cases and costs for PATERN
BOUND	External function to insure that the guesses PATERN makes are within stated limits
P	Pressure (atmospheres)
VS	Superficial air velocity (meter/hour)
CSAFT	Cross-sectional area of air filter (square meter)
HO	Per cent hold-up of gas in fermentor
PV	Power per unit volume (hoursepower per cubic meter)
VCK1	Total gassed volume
VCK2	90% of total fermentor volume (liter)
PA	Agitator power (horsepower)
VVM	Volume of gas per volume of fermentor liquid per minute (liter/liter-min)
DAF	Diameter of air filter (meter)
XLAF	Length of air filter (meter)

CAF	Cost of air filter (dollars)
AFN	Number of air filters (integer)
PC	Power of compressor (horsepower)
CC	Cost of compressor (dollars)
CN	Number of compressors (integer)
CA	Cost of agitator (dollars)
RPM	Revolutions per minute of impellor
CF	Cost of fermentor (dollars)
PSWV	Per cent working volume of fermentor
FL	Fermentor height (meter)

Optimization of Fermentor Heat Exchangers

QH	Total heat generated in fermentor by all sources (Btu/hr) (previous QH plus heat produced by agitator)
TE(1)	See diagram
TE(2)	See diagram
STP2	Initial step sizes
TH2	Temperature of fermentor stream fed into fermentor heat exchanger to remove heat of fermentation (°C)
WC	Cooling water flow rate (gallons per hour)
DT21	Degree of approach in exchanger (TH2-TE2) (°F)
AHE	Area of heat exchanger (ft^2)
HEN	Number of heat exchangers (integer)
CHE	Cost of heat exchanger (dollars)
CCW	Cost of cooling water (dollars)



Optimization of Medium Sterilizer

SP(1)	Temperature of sterilizer (TS) (°C)
SP(2)	Diameter of sterilizer holding section (DS) (meter)
SP(3)	Degree of approach in feed pre-heat economizing heat exchanger (DTH) (°C)
SP(4)	Exiting cooling water temperature from medium heat exchanger (TC2) (°C)
STP3	Step sizes for optimization iteration
TH2	Temperature of medium coming out of economizer (°C)
TS	Temperature of sterilizer (°C)
DS	Diameter of sterilizer holding section (meter)
DTH	Degree of approach in feed pre-heat in economizing heat exchanger (°C)

Accounting of Final Cost Items

AFNT	Total number of air filters (integer)
AFN	Number of air filters per fermentor (integer)
FN	Number of fermentors (integer)
CC	Cost of all compressors (dollars)
CN	Number of compressors (integer)
TPEC	Total purchased equipment cost (dollars)
CF	Cost of a fermentor (dollars)
CA	Cost of an agitator (dollars)
CSTP	Cost of medium sterilizer (dollars)
SHEN	Number of exchangers for economizer (integer)
CMSHE	Cost per unit exchanger for economizer (dollars)
TFCI	Total fixed capital investment (dollars)

FOC	Fixed operating charges (dollars)
CWCT	Cooling water cost (dollars)
STCT	Steam cost (dollars)
PWCT	Electrical power cost (dollars)
CSTLS	Labor and Supervision cost (dollars)
PlOCT	Plant overhead cost (dollars)
TOCT	Total cost (dollars)
TPPO	Yearly production rate of cell mass (pounds per year)
TPP1	Yearly production rate of Product 1 (pounds per year)
TPP2	Yearly production rate of Product 2 (pounds per year)
TPP3	Yearly production rate of Product 3 (pounds per year)
TPP4	Yearly production rate of Product 4 (pounds per year)
TPCP	Total cost per pound of product (dollars per pound)

Computer Program CONFER

```
      PROGRAM CC(TAPETTY=700,OUTPUT=TAPETTY,TAPE1=TAPETTY,INPUT=TAPETTY)
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      COMMON/AGIP/ H,DSF,G,CLC,PA,CA,AFN,XLAF,CAF,PC,CN,PV,CC ,DAF
      COMMON/HEOP/ QH,AHE,CHE,WC,CCW,DT2,HEN,TH2,PUMPC1
      COMMON/STMOP/ Z,AHMS,SHEN,CMSHE,CSTP,XLBS,CSTS,SMN
      DIMENSION YPS(4),STP1(3),OP(3),TE(2),SP(4),STP2(2),STP3(4)
      DIMENSION IFET(8)
      EXTERNAL PROC1, PROC2, PROC3, BOUND1, BOUND2, BOUND3
      CALL FET(5LTAPE1,IFET,8)
      IFET(2)=IFET(2).OR.0000 0010 0000 0000 0000B
      IFET(8)=IFET(8).OR.4000 0000 0000 0000 0000B
      CALL FET(5LTAPE1,IFET,-8)
  308 PRINT 301
  301 FORMAT (1X,*HOW MANY DESIGNS ARE YOU GOING TO DO*)
   41 READ 300,MM
  300 FORMAT (I1)
      IF(MM.LE.0)GO TO 40
      PRINT 302
  302 FORMAT (1X,*ENTER XMSI,YXS,YPS(1),YPS(2),YPS(3),YPS(4) ONE PER LIN
     1E*)
      READ 303,XMSI,YXS,YPS(1),YPS(2),YPS(3),YPS(4)
      FACTR =4.91
      STEM =1.20
      POWRCT =0.01
      WATER =.128
      LABRCT =5.80
  303 FORMAT (F10.3)
      PRINT 304
  304 FORMAT (1X,*ENTER HCS,HCX,HC1,HC2,HC3,HC4 ONE PER LINE*)
      READ 303, HCS,HCX,HC1,HC2,HC3,HC4
      PRINT 305
  305 FORMAT (1X,*ARE YOU PRODUCING CELL MASS=0 OR A PRODUCT=1*)
      READ 300,MN
      DO 60 L=1,MM
      NN=L
      PRINT 307
  307 FORMAT (1X,*ENTER CAP,SO,DI,T,002,CCL,PPT ONE PER LINE*)
      READ 303, CAP,SO,DI,T,002,CLC,PPT
C-------------CALCULATION OF PRODUCT AND CELL YEILDS-------------------
      IF(MN.EQ.1)GO TO 100
      FM=CAP*453.6/(85.*YXS*SO*PPT)*354.
      GO TO 101
  100 FM=CAP*453.6/(85.*YPS(1)*SO*PPT)*354.
  101 S=SO*(1.-PPT/100.)
      X=YXS*(SO-S)
      P1=YPS(1)*(SO-S)
      P2=YPS(2)*(SO-S)
      P3=YPS(3)*(SO-S)
      P4=YPS(4)*(SO-S)
```

```
C------------------CALCULATION OF HEAT OF FERMENTATION------------
      HFERM = (SO-S)*(HCS - YXS*HCX - YPS(1)*HC1 - YPS(2)*HC2 - YPS(3)*
     1HC3 - YPS(4)*HC4)
      QH = HFERM*3.968
C--------------CALCULATION OF HENRYS LAW CONSTANT H--------------------
      TK=T
      A=0.0005943
      B=.147
      C=0.0512
      D=.1076
      E=.8447
      BT=1.0/TK*1000.
      HL=-(C*BT+D-SQRT((C*BT+D)**2-4.*A*(B*BT**2-E*BT+1.)))/(2.*A)
      H=EXP(2.3026*HL)*10000.
      TC=T-273.15
      DSF=1.0-.0000048*TC**2
      Q=Q02*X*.001
C--------------CALCULATION OF NUMBER AND SIZE OF FERMENTERS------------
      VW=FM/DI
      V=VW/.8
      VM=189000.
      CALL INUMBR(V,VM,VF,FN)
      VWW=VF*.8
      DF=(VWW*.001273)**.333
C--------------OPTIMIZATION OF AGITATION, AIR COMPRESSOR AND AIR FILTER
      IF(Q.EQ.0.0)GO TO 6
      STP1(1)=(4.33-1.)/4.
      STP1(2)=150./4.
      OP(1)=2.
      PLL=0.1*DF*.209/ALOG((.1*DF+OP(1))/OP(1))
      TOP=.08206*T*Q*DF
      BOT=PLL-H*CLC/(DSF*5.56E5)
      OP(2)=1.+TOP/BOT
      OP(3)= OP(2)*3.14159*DF**2/2880.
      STP1(3)= OP(2)*3.14159*DF**2/1440.
    4 CALL PATERN(3,OP,STP1,3,0,CST1,PROC1,BOUND1)
      CALL PROC1(OP,CST1)
      P=OP(1)
      VS=OP(2)
      CSAFT=OP(3)
      HO=17.15*ALOG(PV**.4*VS**.5)-29.5
      VCK1=VW*(1.+HO/100.)
      VCK2=V*.9
      IF(VCK1.LT.VCK2)GO TO 3
      V=VCK1/.8
      CALL INUMBR(V,VM,VF,FN)
      VWW=FM/(DI*FN)
      DF=(VWW*.001273)**.333
      GO TO 4
    6 PV=.06
```

```
      PA=PV*VWW/1000.
      VS=0.0
      P=1.0
      VVM=0.0
      DAF=0.0
      XLAF=0.0
      CAF=0.0
      AFN=0.0
      PC=0.0
      CC=0.0
      CN=0.0
      CA=XMSI*3.335*PA**.56
      RPM=175.5*(PA/DF**5)**.333
      GO TO 7
    3 RPM=18.9*((PA**2.22*(VS*DF**2)**.56)/DF**13)**.1429*5.948
    7 CF=XMSI*.204*VF**.53
      PSWV=VWW/VF*100.
      VVM=VS*DF*13.03/VF
      FL=.001273*VF/DF**2
C-------------OPTIMIZATION OF FERMENTER HEAT EXCHANGERS--------------
      QH=GH*VWW*DI+PA*2540.
      TE(1)=T-276.15
      TE(2)=28.0
      STP2(2)=(T-25.2)/4.
      STP2(1)=(T-25.2)/4.
      TH2=T-273.15
      CALL PATERN(2,TE,STP2,3,0,CST2,PROC2,BOUND2)
      CALL PROC2(TE,CST2)
      WC1=WC
      DT21=DT2
      AHE1=AHE
      HEN1=HEN
      CHE1=CHE
      CCW1=CCW
C-------------OPTIMIZATION OF MEDIA STERILIZER-----------------------
      SP(1)=140.
      SP(2)=0.1
      SP(3)=3.
      SP(4)=35.
      STP3(1)=(160.-110.)/4.
      STP3(2)=.5
      STP3(3)=2.
      STP3(4)=7.85
      TH2=50.+SP(3)
      CALL PATERN(4,SP,STP3,3,0,CST3,PROC3,BOUND3)
      CALL PROC3(SP,CST3)
      TS=SP(1)
      DS=SP(2)
      DTH=SP(3)
      AFNT=AFN*FN
```

```
      CC=CC*CN
      TPEC= FN*CF+FN*CA+HEN1*CHE1+CC+AFNT*CAF+CSTP+SHEN*CMSHE+HEN*CHE
      TFCI=TPEC*FACTR
      FOC=TFCI*.19
      CWCT=CCW1+CCW
      STCT=CSTS
      PUMPCT=PUMPC1+FM*.2034*3.*POWRCT
      PWCT=(PA*FN+PC*CN)*POWRCT*6339./.8+PUMPCT
      CSTLS=(FN*.25+1.)*LABRCT*8500.
      PLOCT=.5*(CSTLS+.03*TFCI)
      TOCT=FOC+STCT+PWCT+CWCT+PLOCT+CSTLS
      TPP0=FM*X*85./4.536
      TPP1=FM*P1*85./4.536
      TPP2=FM*P2*85./4.536
      TPP3=FM*P3*85./4.536
      TPP4=FM*P4*85./4.536
      TPCP=TOCT/(CAP*354.)*6.728
      PRINT 33
   33 FORMAT  (//,1X,10(1H*),*INTERNAL DESIGN PARAMETERS*,10(1H*)/)
      PRINT 34, VWW, PSWV, DF, FL, P
      PRINT 34, VS, VVM, RPM, WC1, DT21
      PRINT 34, DTH, WC, DT2,TS, DI
      PRINT 34, X, P1, P2, P3, P4
   34 FORMAT (1X,5F14.4/)
      PRINT 10,NN
   10 FORMAT (1H1,1X,10(1H*),*OPTIMUM DESIGN OF RUN NUMBER*,I2/)
      PRINT 11
   11 FORMAT (3X,*ITEM*,17X,*CAPACITY*,7X,*NO. OF UNITS*,4X,*COST/UNIT F
     10B*,1H$)
      PRINT 15
   15 FORMAT (1X,70(1H*))
      PRINT 12, VF, FN, CF
   12 FORMAT (1X,*FERMENTOR*,11X,F8.1,* LITERS*,6X,F5.1,10X,F10.1/)
      PRINT 13, PA, FN, CA
   13 FORMAT (1X,*AGITATOR*,12X,F8.1,* HP.*,9X,F5.1,10X,F10.1/)
      PRINT 14, AHE1, HEN1, CHE1
   14 FORMAT (1X,*HEAT REMOVAL EXCHR*,2X,F8.1,* SQ.FT.*,6X,F4.1,10X,F10.
     11/)
      PRINT 16, PC, CN, CC
   16 FORMAT (1X,*AIR COMPRESSOR*,6X,F8.1,* HP.*,10X,F4.1,10X,F10.1/)
      PRINT 17, XLAF, DAF, AFNT, CAF
   17 FORMAT (1X,*AIR FILTER*,10X,F4.1,*M X *,F4.1,*M*,9X,F5.1,10X,F10.1
     1/)
      PRINT 18, Z, DS,SMN, CSTP
    E FORMAT (1X,*MEDIA STERILIZER*,4X,F5.1,*M X *,F5.3,*M*,7X,F4.1,10X,
     1F10.1/)
      PRINT 19, AHMS, SHEN, CMSHE
   19 FORMAT (1X,*PRE HEAT EXCHANGER*,2X,F8.1,* SQ.FT.*,7X,F4.1,10X,F10.
     11/)
      PRINT 20, AHE, HEN, CHE
```

```
20 FORMAT (1X,*COOLER EXCHANGER*,4X,F8.1,* S0.FT.*,7X,F4.1,10X,F10.1)
   PRINT 15
   PRINT 21, TPEC
21 FORMAT (1X,*TOTAL PURCHASED EQUIPMENT COST*,E11.4,1H$/)
   PRINT 22, FACTR
22 FORMAT(1X,*MULTIPLICATION FACTOR IS *,F5.2/)
   PRINT 23, TFCI
23 FORMAT(1X,*TOTAL FIXED CAPITAL INVESTMENT IS*,E11.4,1H$/)
   PRINT 24
24 FORMAT(1X,*TEN YEAR STRAIGHT LINE DEPRECIATION ASSUMED*/)
   PRINT 25
25 FORMAT(1X,10(1H*),*OPERATING COSTS DOLLARS/YEAR*,10(1H*)/)
   PRINT 15
   PRINT 26, FOC
26 FORMAT(1X,*FIXED CHARGES (.19/YR OF TCI)*,10X,E12.3/)
   PRINT 27,STCT
27 FORMAT(1X,*STEAM*,34X,E12.3/)
   PRINT 28, PWCT
28 FORMAT(1X,*POWER*,34X,E12.3/)
   PRINT 29, CWCT
29 FORMAT(1X,*COOLING WATER*,26X,E12.3/)
   PRINT 30, CSTLS
30 FORMAT(1X,*LABOR AND SUPERVISION*,18X,E12.3/)
   PRINT 31,PLOCT
31 FORMAT(1X,*PLANT OVERHEAD*,25X,E12.3)
   PRINT 15
   PRINT 32,TOCT
32 FORMAT(24X,*TOTAL*,9X,E12.3/)
   PRINT 36, MN, TPCP
36 FORMAT (1X,*PRODUCTION COST OF PRODUCT *,I1,4X,F8.4,4H$/GAL/)
   PRINT 37
37 FORMAT (1X,*PRODUCTIONS RATES IN LBS/YR*)
   PRINT 38, TPP0, TPP1, TPP2
38 FORMAT (1X,*CELL MASS *,E11.3,3X,*PRODUCT 1 *,E11.3,3X,*PRODUCT 2
  1*,E11.3)
   PRINT 39, TPP3, TPP4
39 FORMAT (1X,*PRODUCT 3 *,E11.3,3X,*PROUDCT 4 *,E11.3)
60 CONTINUE
   GO TO 308
40 PRINT 43
43 FORMAT (1X,*THU THU THU THU THAT#S ALL FOLKS*)
   CALL EXIT
   END
```

```
      SUBROUTINE PATERN(NP,P,STEP,NRD,IO,COST,PROC,BOUNDS)
C-----THE SIZE OF B1,B2,T,AND S NEED ONLY BE EQUAL TO THE NUMBER OF PAR
      DIMENSION P(NP),STEP(NP),B1(10),B2(10),T(10),S(10)
C-----STARTING POINT
      L=1
      ICK=2
      ITTER=0
      DO 5 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) GO TO 10
      PRINT 1005
      PRINT 1000,(J,P(J),J=1,NP)
 6    RETURN
 10   CALL PROC(P,C1)
      IF(IO.LE.1) GO TO 11
      PRINT  1001, ITTER,C1
      PRINT 1000,(J,P(J),J=1,NP)
 11   DO 99 INRD=1,NRD
      DO 12 I=1,NP
 12   S(I)=S(I)/10.
      IF(IO.LE.1)GO TO 20
      PRINT 1003
      PRINT 1000,(J,S(J),J=1,NP)
 20   IFAIL=0
      DO 30 I=1,NP
      IC=0
 21   P(I)=T(I)+S(I)
      IC=IC+1
      CALL BOUNDS(P,IOUT)
      IF(IOUT.GT.0) GO TO 23
      CALL PROC(P,C2)
      L=L+1
      IF(IO.LT.3) GO TO 22
      PRINT 1002,L,C2
      PRINT 1000,(J,P(J),J=1,NP)
 600  CONTINUE
 22   IF(ABS(C1-C2).LT.1.0E-08) GO TO 23
      IF(C1-C2)23,23,25
 23   IF (IC.GE.2) GO TO 24
      S(I)=-S(I)
      GO TO 21
 24   IFAIL=IFAIL+1
      P(I)=T(I)
      GO TO 30
 25   T(I)=P(I)
```

```
      C1=C2
30    CONTINUE
      IF(IFAIL.LT.NP) GO TO 35
      IF(ICK.EQ.2) GO TO 90
      IF(ICK.EQ.1) GO TO 35
      CALL PROC(P,C2)
      L=L+1
      IF(IO.LT.3) GO TO 31
      PRINT 1002, L,C2
      PRINT 1000,(J,P(J),J=1,NP)
 31   IF(C1-C2)32,34,34
 32   ICK=1
      DO 33 I=1,NP
      B1(I)=B2(I)
      P(I)=B2(I)
 33   T(I)=B2(I)
      GO TO 20
 34   C1=C2
 35   IB1=0
      DO 39 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) GO TO 90
      ICK=0
      ITTER=ITTER+1
      IF(IO.LT.2) GO TO 40
      PRINT 1001, ITTER,C1
      PRINT 1000,(J,P(J),J=1,NP)
 40   CONTINUE
      DO 45 II=1,11
      SJ=11-II
      SJ=SJ/10.
      DO 42 I=1,NP
      T(I)=B2(I)+SJ*(B2(I)-B1(I))
 42   P(I)=T(I)
      SJ=SJ-.1
      CALL BOUNDS(P,IOUT)
      IF(IOUT.LT.1) GO TO 46
      IF(II.EQ.11) ICK=1
45    CONTINUE
 46   DO 47 I=1,NP
 47   B1(I)=B2(I)
      GO TO 20
 90   DO 911 I=1,NP
 911  T(I)=B2(I)
 99   CONTINUE
      DO 100 I=1,NP
100   P(I)=T(I)
      COST=C1
```

```
      IF(IO.LE.0) RETURN
      PRINT 1004, L,C1
      PRINT 1006, (J,P(J),S(J),J=1,NP)
 1000 FORMAT(1X,I7,E13.6)
 1001 FORMAT(//1X,*ITERATION*,I10,3X,*ERROR=*,E13.6)
 1002 FORMAT(/,1X,*NO.*,I7,3X,*ERROR=*,E13.6)
 1003 FORMAT(/,*STEP SIZE*)
 1004 FORMAT(///,1X,*FINAL ANSWER*,I7,*EVAL.*,3X,*ERROR=*,E13.6)
 1005 FORMAT(1X,*INITIAL PARAMETERS OUT OF BOUNDS*)
 1006 FORMAT(1X,I7,E13.6,*PLUS OR MINUS*,2X,E13.6)
      RETURN
      END
```

```
      SUBROUTINE PROC1(OP,CST1)
      COMMON/AGIP/ H,DSF,Q,CLC,PA,CA,AFN,XLAF,CAF,PC,CN,PV,CC ,DAF
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      DIMENSION OP(3)
      P=OP(1)
      VS=OP(2)
      CSAF1=OP(3)
      POUT=P*.209-0.06491*1*Q*DF/VS
      Y=POUT/H
      CLS=Y*DSF*55.556*10000.
      XKV=Q/((CLS-CLC)*H)*55.556*10000.
      PV=(XKV/(.0635*VS**.67))**1.053
      IF(PV.LT.0.06)PV=0.06
C-------------P/V=0.06 IS NESSACARY TO KEEP CELLS IN SUSPENSION
      PA=PV*VWW/1000.
      CALL AIRFIL(VS,PDAF,CSAF1,XLAF,CAF,AFN,P,DAF)
      DP=.1*DF+PDAF+P+.07
      PCT=0.1333*P*VS*DF**2*(DP**.2832-1.)/.8*FN
      PCM=10000.
      CALL INUMBR(PCT,PCM,PC,CN)
      CA=XMSI*3.335*PA**.56
      CC=XMSI*2.90*PC**.75
      CST1=FACTR*.19*(CA*FN+CC*CN+CAF*AFN*FN)+(PCT+PA*FN)*POWRCT*6339./.
     18
   11 RETURN
      END
      SUBROUTINE BOUND1(OP,IOUT)
      COMMON/AGIP/ H,DSF,Q,CLC,PA,CA,AFN,XLAF,CAF,PC,CN,PV,CC ,DAF
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      DIMENSION OP(3)
      IOUT=0
      IF(OP(1).LT.1.0)IOUT=1
      IF(OP(1).GT.4.33)IOUT=1
      PLL=0.1*DF*.209/ALOG((.1*DF+OP(1))/OP(1))
      TOP=.08206*T*Q*DF
      BOT=PLL-H*CLC/(DSF*5.56E5)
      IF(OP(2).LT.TOP/BOT)IOUT=1
      IF(OP(2).GT.600.)IOUT=1
      IF(OP(3).LT.OP(2)*3.14159*DF**2/2880.0)IOUT=1
      IF(OP(3).GT.31.4)IOUT=1
      RETURN
      END
```

```
!
      SUBROUTINE PROC2(TE,CST2)
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      COMMON/HEOP/ QH,AHE,CHE,WC,CCW,DT2,HEN,TH2,PUMPC1
      DIMENSION TE(2)
      TH1=TE(1)
      TC2=TE(2)
      TC1=25.0
      DT1=TH1-TC1
      DT2=TH2-TC2
      IF(ABS(DT1-DT2).LT.0.0001)GO TO 1
      DTLM=(DT2-DT1)/ALOG(DT2/DT1)*1.8
      AHET=QH/(400.*DTLM)
      GO TO 2
    1 AHET=QH/(400.*DT1*1.8)
    2 WC=QH/((TC2-TC1)*8.34*1.8)
      AHEM=10000.
      CALL INUMBR(AHET,AHEM,AHE,HEN)
      WH=QH/((TH1-TH2)*8.34*1.8)
      CHE=XMSI*.696*AHE**.64
      PUMPC1=WH*9.72E-5*6339./.8*POWRCT
      CCW=WC*WATER*8.5
      CST2=FACTR*.19*CHE*HEN+CCW+PUMPC1
      RETURN
      END
      SUBROUTINE BOUND2(TE,IOUT)
      COMMON/HEOP/ QH,AHE,CHE,WC,CCW,DT2,HEN,TH2,PUMPC1
      DIMENSION TE(2)
      IOUT=0
      IF(TE(1).GT.TH2-.3)IOUT=1
      IF(TE(1).LT.25.3)IOUT=1
      IF(TE(2).LT.25.3)IOUT=1
      IF(TE(2).GT.TH2-.3)IOUT=1
      RETURN
      END
```

```
      SUBROUTINE PROC3(SP,CST3)
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      COMMON/STMOP/ Z,AHMS,SHEN,CMSHE,CSTP,XLBS,CSTS,SMN
      COMMON/HEOP/ QH,AHE,CHE,WC,CCW,DT2,HEN,TH2,PUMPC1
      DIMENSION SP(4)
      TS=SP(1)
      DS=SP(2)
      DTH=SP(3)
      Y=4.165E-12/FM
      G=7.98E38/EXP(6870./(.198*(TS+273.15)))
      S=.7854*DS**2
      U=FM/(6000.*S)
      RE=U*DS*20000.
      PE=3.57*SQRT(.046/RE**.2)
      ZTA=SQRT(1.+4.*G*PE*DS/U)
      Z=PE*DS*2.*ALOG(Y*(1.+ZTA)**2/(4.*ZTA))/(1.-ZTA)
      TM1=50.0
      TM4=50.0+DTH
      TM3=TS
      TM2=TM3-DTH
      QHM=FM*(TM3-TM4)/.252
      AHME=QHM/(400.*DTH*1.8)
      AHMEN=10000.
      CALL INUMBR(AHME,AHMEN,AHMS,SHEN)
      CSAT=.785*DS**2
      CSAM=5.1
      CALL INUMBR (CSAT,CSAM,CSA,SMN)
      DS=SQRT(CSA/.785)
      CMSHE=XMSI*.696*AHMS**.64
      CSTP=(5.85*(DS/.0245)**.93*XMSI/273.1 +1.7*(DS/.2286)**.5*(XMSI/
     1256.))*Z*3.28*SMN
      XLBS=FM*(TS-TM2)/(.252*901.7)
      CSTS=XLBS*STEM*8.5
      QH=FM*(50.+DTH-T+273.15)/.252
      TC2=SP(4)
      TH1=35.
      TC1=25.0
      DT1=TH1-TC1
      DT2=TH2-TC2
      IF(ABS(DT1-DT2).LT.0.0001)GO TO 1
      DTLM=(DT2-DT1)/ALOG(DT2/DT1)*1.8
      AHET=QH/(400.*DTLM)
      GO TO 2
    1 AHET=QH/(400.*DT1*1.8)
    2 WC=QH/((TC2-TC1)*8.34*1.8)
      AHEM=10000.
      CALL INUMBR(AHET,AHEM,AHE,HEN)
      WH=QH/((TH1-TH2)*8.34*1.8)
      CHE=XMSI*.696*AHE**.64
      CCW=WC*WATER*8.5
      CST3=(CSTP+CMSHE*SHEN)*FACTR*.19+CSTS+CCW+CHE*HEN*FACTR*.19
      RETURN
      END
```

```
      SUBROUTINE BOUND3(SP,IOUT)
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      COMMON/HEOP/ QH,AHE,CHE,WC,CCW,DT2,HEN,TH2,PUMPCI
      DIMENSION SP(4)
      IOUT=0
      IF(SP(1).LT.110.0)IOUT=1
      IF(SP(1).GT.160.0)IOUT=1
      IF(SP(2).LT.0.076)IOUT=1
      IF(SP(2).GT.FM*2.12E-5)IOUT=1
      IF(SP(3).LT.0.3)IOUT=1
      IF(SP(3).GT.20.)IOUT=1
      IF(SP(4).LT.25.1)IOUT=1
      IF(SP(4).GT.TH2-.3)IOUT=1
      RETURN
      END
```

```
      SUBROUTINE AIRFIL(VS,PDAF,CSAFT,XLAF,CAF,AFN,P,DAFU)
      COMMON/UNIVR/ T,FM,DF,FN,VF,XMSI,VWW,FACTR,STEM,POWRCT,WATER,
     1LABRCT
      FD=1.6E-03
      PD=1.0E-04
      XIN=1.0E+04
      FREQ=100.
      TAC=20.
      ALPHA=.033
      TAK=TAC+273.15
      RHO=P/(2.82966*TAK)
      TR=TAK/132.5
      B=(0.9/2.302585)*ALOG(1.9*TR)
      FUNK=1.058*(TR**0.645)-0.261/((1.9*TR)**B)
      VIS=(1.0941E-04)*FUNK
      PI=3.14159
      VBAR=SQRT((P*8.104E+06)/(PI*RHO))
      XMFP=2.*VIS/(RHO*VBAR)
      RAT=2.*XMFP/PD
   12 C=1.0 + RAT*(1.23+0.41/EXP(0.88/RAT))
      DBM=C*(1.38E-16)*TAK/(3.0*PI*VIS*PD)
      ENR=PD/FD
      EPS=1.0-ALPHA
      VC=1.125*VIS*FD/(C*PD**2)
      FLOW=VS*PI*DF**2/4.
      FLS=FLOW/3600.
      VSF=100.*FLS/CSAFT
      VAF=VSF/EPS
   20 RE=RHO*FD*VAF/VIS
   25 VOL=FLOW*24.*FREQ
      XOUT= 1.0/VOL
      ARG=XIN/XOUT
      PE=FD*VAF/DBM
      XAXIS=(ENR)*(PE**(1./3.))*(RE**(1./18.))
      IF(XAXIS-10.) 15,15,16
   16 PRINT 99, XAXIS
   99 FORMAT(1X,*WARNING... XAXIS = *,E10.5,*WHICH IS OFF GRAPH*)
   15 IF(XAXIS-1.) 21,21,22
   21 YAXIS=10.**(.43912 + 1.66096*ALOG10(XAXIS))
      GO TO 23
   22 YAXIS=10.**(.43912 + 3.59258*ALOG10(XAXIS))
   23 ADA0=YAXIS/(ENR*PE)
      ADAA=ADA0*(1.0 + 4.5*ALPHA)
      Z = (PI*FD*EPS/(4.*ADAA*ALPHA))*ALOG(ARG)
      CDM=52./RE
      PDAF= CDM*2.0*RHO*Z*(VAF**2)*(ALPHA**1.35)/(PI*FD*1.025E+06)
      XLAF=Z/100.
      CSAFM=PI
      CALL INUMBR(CSAFT,CSAFM,CSAF,AFN)
      DAFU=SQRT(CSAF*4./PI)
```

```
      VUAF=CSAF*XLAF
      VTAF=VUAF*AFN*FN
      CFIB=(VTAF*211.87)*(XMSI/332.)*10.
      CUFS=572.5*(VUAF**.557)*XMSI/263.
      CTFS=CUFS*AFN*FN
      CAF=(CFIB + CTFS)/(AFN*FN)
      RETURN
      END
      SUBROUTINE INUMBR(TC,UMC,UC,XY)
C-------------TC IS THE TOTAL CAPACITY REQUIRED
C-------------UCM IS THE MAXIMUM UNIT CAPACITY
C----------------UC IS THE ACTUAL UNIT CAPACITY BASED ON XY INTERGRAL
C-------------NUMBER OF EQUAL CAPACITY UNITS
      IF(TC.LE.UMC)GO TO 1
      XX=TC/UMC
      LL=XX
      XY=LL+1
      UC=TC/XY
      GO TO 2
    1 UC=TC
      XY=1.0
    2 RETURN
      END
```

A.3.2. Optimization of Distillation Column Computer Program DISTL

The computer program DISTL optimizes the distillation column shown in Fig. A.5 for two feed streams. Since, as discussed above, the reflux ratio is set a priori at 1.25 times the minimum, the optimization is made around the condenser and feed preheat exchangers. A flow chart of the computer program DISTL is shown in Fig. A.9.

First, the minimum reflux is calculated, and then the bottoms ethanol concentration, XB, is found from the overall mass balance, Eq. (A.38). Beginning at the bottom of the column, subroutine EQUIB is called to calculate the gas phase ethanol mole fraction, Y, and the tower cross sectional area, AN, at each plate in the stripping section. When the mass balance equations (Eqs. (A.39) and (A.43)) indicate a lower liquid concentration in the stripping section, XS, than would be present in the intermediate section, XI, a feed point is determined. This process is repeated to determine the second feed point; the ethanol liquid phase mole fractions are calculated in the main program and then EQUILB is called to determine the gas phase mole fraction from Eqs. (A.40) to (A.42), and a tower cross sectional area at each plate from Eq. (A.52). When the top of the tower is reached (i.e., Y = 0.875), the column diameter is found from the largest required cross sectional area.

The condenser is then optimized using the temperature difference, DTC, between the condensing ethanol vapor and exiting cooling water as the optimized variable. DTC is initiallized in DISTL and a patern search subroutine, PATERN, is called to perform the optimization. From

the PATERN subroutine PROC4 is called which designs and costs the condenser using the adjusted value of DTC generated in PATERN. The total condenser operating cost is then fed back to PATERN for comparison with previously calculated costs and further adjustment of DTC until the minimum cost is found. The subroutine BOUND4 is called from PATERN each time the valve of DTC is altered to check that the adjusted valve is within physical limits.

With the condenser costs minimized, the feed preheat exchangers are optimized using the degree of approach between the column bottoms product and the feeds, DTPH, as the parameter. The subroutines PROC5 and BOUND5 have the same relation to PATERN as PROC4 and BOUND4. The preheat exchangers are sized and operating costs determined in PROC5 every time PATERN feeds a new value of DTPH. In both the condenser and preheat optimization subroutine INUMBER is called from the PROC routines to determine if the design specifications exceed the maximum unit capacity set in the cost estimation section. If so, INUMBER sizes an integral number of equally sized units to meet the design requirement.

As discussed previously the operation of the preheat exchangers affect the vapor and liquid flow rates in the lower sections of the column. Thus once the preheat exchangers have been optimized, new molar flows are found from Eqs. (A.59) and (A.60) and the entire design repeated until the flow converges to within 2.0% between successive designs.

A FORTRAN listing of DISTL and a summary of variables is shown below. A listing of subroutine PATERN used with DISTL is not shown,

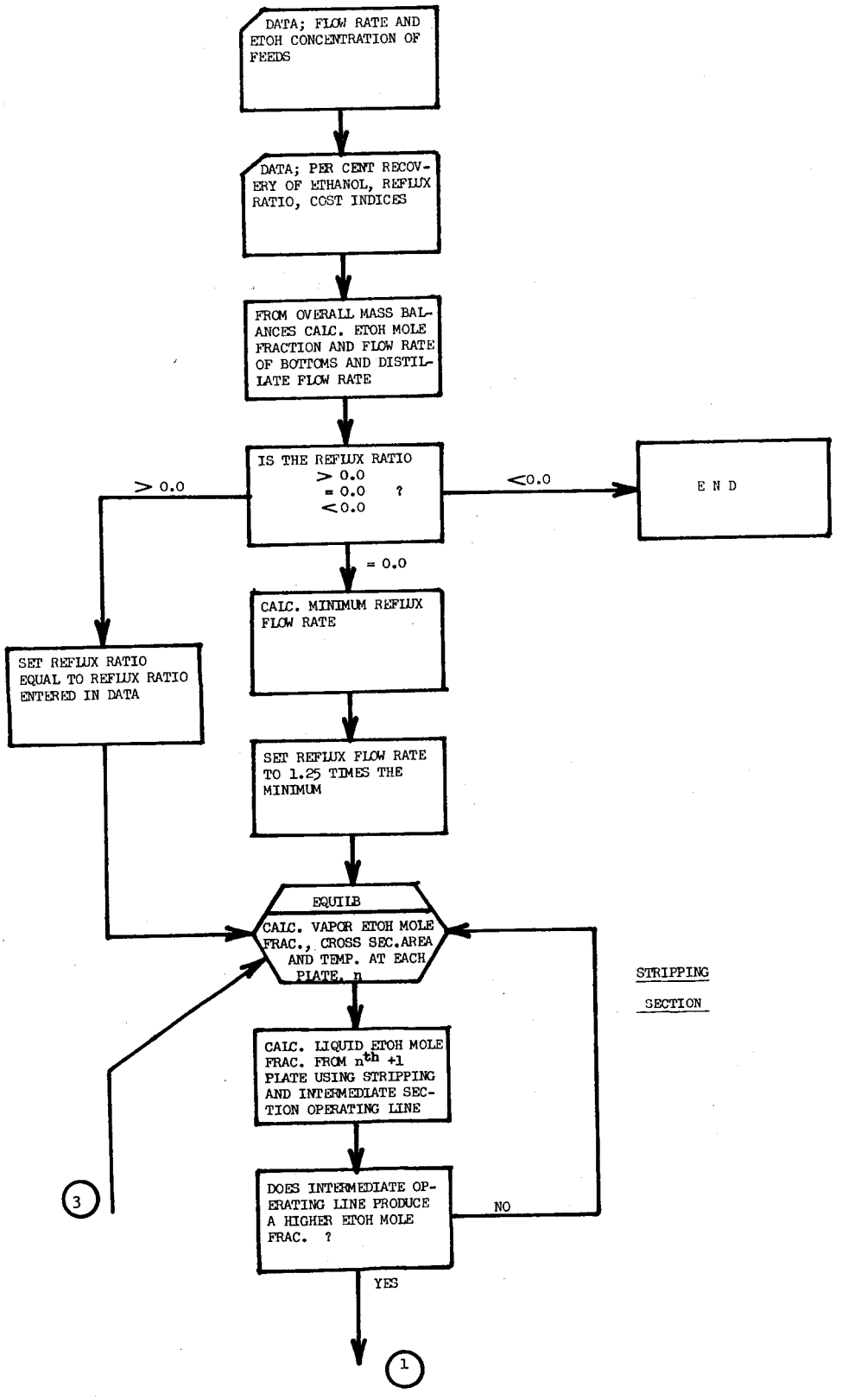


Fig. A.9. Flow diagram for computer program DISTL.

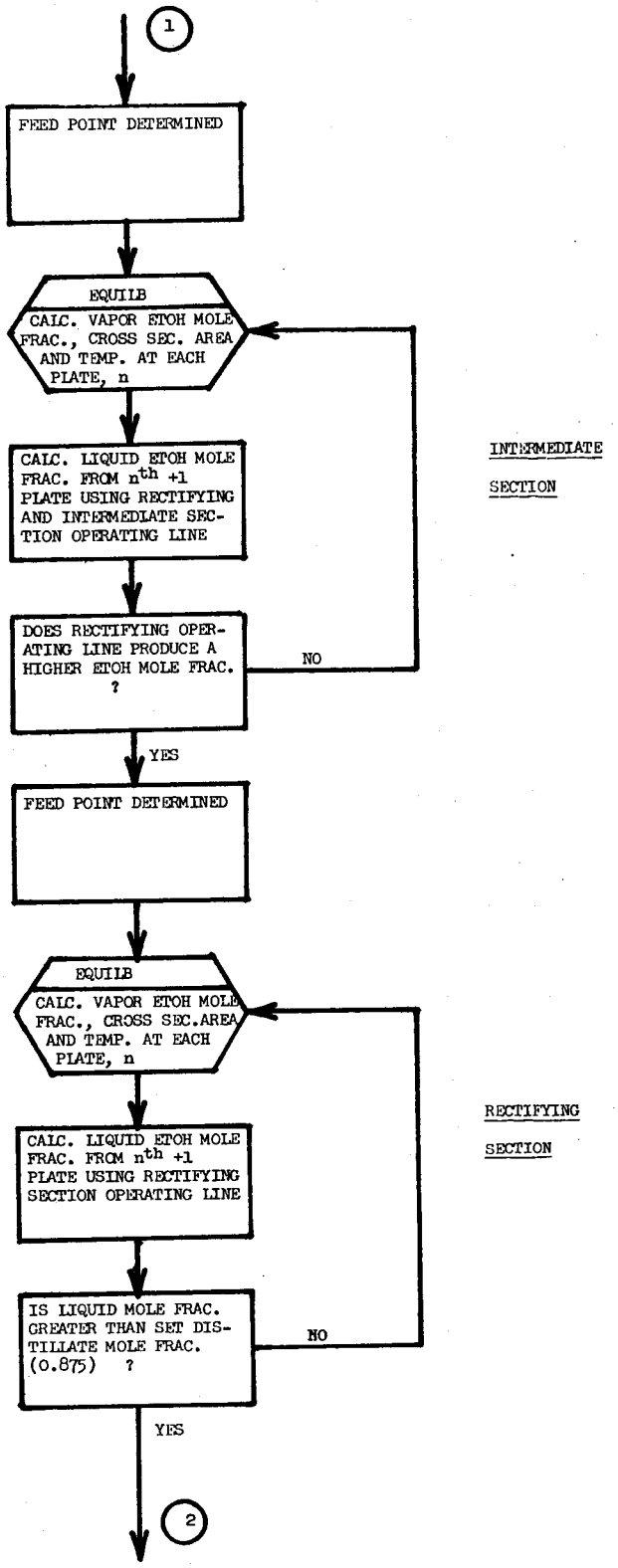


Fig. A.9. Continued.

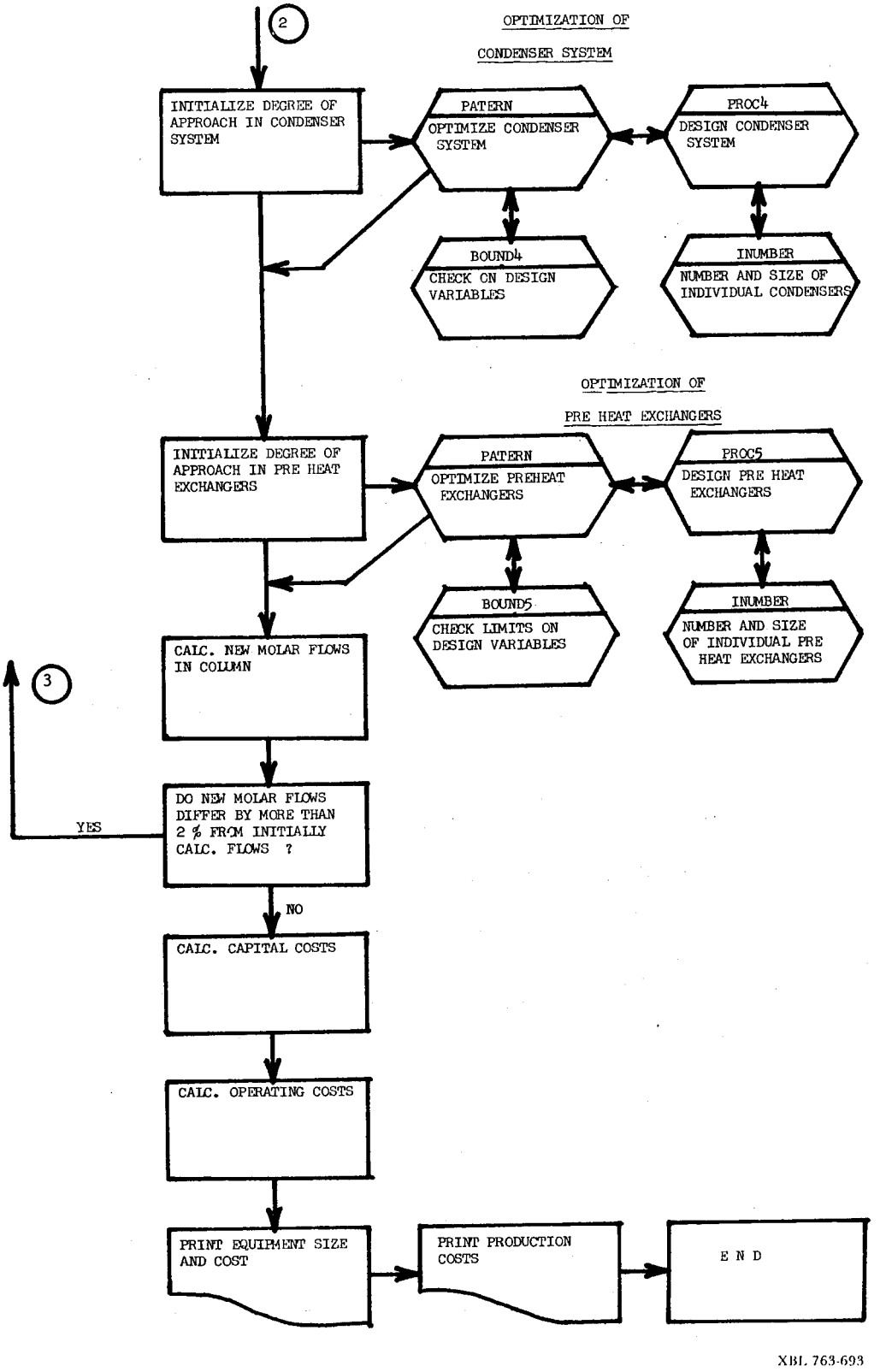


Fig. A.9. Continued.

but is identical to the PATERN subroutine used with the computer program CONFER.

Explanation of Variables used in Distillation Column Optimization, Computer Program DISTL (in order of appearance)

XMSI	Current Marshall Stevens cost index
ZI	Mole fraction of ethanol in feed number one
Z2	Mole fraction of ethanol in feed number two
Fl	Feed rate of first feed (moles/hr)
F2	Feed rate of second feed (moles/hr)
PR	Per cent recovery of ethanol in distillate
RR	Reflux ratio (RR = R/(D + R))
EM	Murphee V-phase plate efficiency
AM	Maximum cross sectional area required in column (ft^2)
XD	Distillate mole fraction
D	Distillate flow (mole/hr)
B	Bottoms flow (mole/hr)
XB	Ethanol mole fraction of bottoms product
R	Reflux flow (mole/hr)
X(I)	Ethanol mole fraction of liquid on I^{Th} plate
Y(I)	Ethanol mole fraction of vapor coming from I^{Th} plate
RS	Liquid flow in stripping section (mole/hr)
VS	Vapor flow in stripping section (mole/hr)
RI	Liquid flow in intermediate section (mole/hr)
VI	Vapor flow in intermediate section (mole/hr)
AN	Tower cross sectional area at I^{TH} plate (ft^2)
T	Temperature (°C)

DI	Diameter of tower (ft)
H	Height of tower (ft)
CT	Cost of tower (dollars)
CST	Cost of trays (dollars)
DTI	Degree of approach in condenser (°F)
DT2	Degree of approach in feed preheat exchangers (°F)
QC	Condenser heat load (Btu/hr)
AC	Heat exchange area in condenser (ft^2)
CDC	Cost of condenser (dollars)
QB	Reboiler heat load (Btu/hr)
AB	Heat exchange area in reboiler (ft^2)
CDB	Cost of reboiler (dollars)
QPH	Heat load of feed preheat exchangers (Btu/hr)
APH	Area of preheat exchangers (ft^2)
CPH	Cost of preheat exchangers (dollars)
TPEC	Total purchased equipment cost (dollars)
CSTLS	Labor and supervision operating cost (dollars)
PLOCT	Overheat cost (dollars)
GPD	Production rate (gal/day)
TOCT	Total operating cost (dollars)
CPG	Production cost (cent/gallon)
STCT	Cost of steam (dollars)

Computer Program DISTL

```
      PROGRAM DI(TAPETTY=700,OUTPUT=TAPETTY,TAPE1=TAPETTY,INPUT=TAPETTY)
      COMMON/DIST/ A12,A21,A122,A211,PW,PE,EM,V,TL
      COMMON/COST/XMSI,FACTR,STEM,POWRCT,WATER,LABRCT
      COMMON/HEXOP/AUC,WC,CDC,CWCT,XYC,QC
      COMMON/PHEXC/CB,F1,F2,ABU,CDB,CPH,QPH,STCT,XYB,XYPH,XYPH1,XYPH2,
     1CPH1,CPH2
      DIMENSION X(310), Y(310), DT1(1),DT2(1),ST1(1),ST2(1),CK(60)
      DIMENSION IFET(8)
      EXTERNAL PROC4,PROC5,BOUND4,BOUND5
      CALL FET(5LTAPE1,IFET,8)
      IFET(2)=IFET(2).OR.0000 0010 0000 0000 0000B
      IFET(8)=IFET(8).OR.4000 0000 0000 0000 0000B
      CALL FET(5LTAPE1,IFET,-8)
C     FLOWS IN POUND MOLES PER HOUR ************************************
      PRINT 200
  200 FORMAT(1X,* ENTER MARSHALL STEVENS COST INDEX.*)
      READ 201,XMSI
  201 FORMAT(F10.3)
      PRINT 202
  202 FORMAT(1X,* ENTER Z1, Z2, F1, F2, PR,  ONE ENTRY PER LINE*)
      READ 201,Z1,Z2,F1,F2,PR
  103 PRINT 203
  203 FORMAT(1X,* ENTER REFLUX RATIO.*)
      PRINT 505
  505 FORMAT(1X,*IF R<0.0 STOP, R=0.0 1.25 RMIN FOUND, R>0.0 R=R*)
      READ 201,RR
      PRINT 204
  204 FORMAT(1X,* DO YOU WANT A LISTING OF CONC. AND TEMP. ON EACH PLATE
     1?  01=YES, 00=NO*)
      PRINT 504
  504 FORMAT(1X,*-1 ONLY SEPERATION COST IS PRINTED*)
      READ 205,K
  205 FORMAT(I2)
      II=2
      EM=0.70
      AM=0.0
      FACTR=4.91
      STEM=1.2
      POWRCT=0.01
      WATER=0.128
      LABRCT=5.6
      XD=.875
       A=1.0
      A12=7.7105
      A21=9.9817
      A122=6.7599
      A211=-10.0836
      PW=50.8
      PE=130.8
      R2=0.0
```

```
C     MASS BALANCES ******************************************************
      IF(RR.LT.0.0) GO TO 12
      IF(K.LE.0) GO TO 303
      PRINT 100
  100 FORMAT (1H1,12X,*X*,13X,*Y*,12X,*AREA*,12X,*T*//)
  303 D=PR*(Z1*F1+Z2*F2)/XD
      B=F1+F2-D
      XB=(Z1*F1+Z2*F2-XD*D)/B
      IF(RR.GT.0.0)GO TO 700
      XW1=1.-Z1
      AL1=(1.+A12*XW1+A122*XW1**2)/(1.+A21*Z1+A211*Z1**2)
      GP1=(AL1*Z1/XW1)/(1.+AL1*Z1/XW1)
      R1=D*(GP1-XD)/(Z1-GP1)
      IF(Z2.LE.0.0)GO TO 702
      XW2=1.-Z2
      AL2=(1.+A12*XW2+A122*XW2**2)/(1.+A21*Z2+A211*Z2**2)
      GP2=(AL2*Z2/XW2)/(1.+AL2*Z2/XW2)
      R2=(Z2*(F1+F2)-XB*B-D*GP2)/(GP2-Z2)
  702 R3=D*.74/(1.-.74)
      R=R3*1.25
      IF(R1*1.25.GT.R)R=R1*1.25
      IF(R2*1.25.GT.R)R=R2*1.25
      RR=R/(R+D)
      GO TO 701
  700 R=D*RR/(1.-RR)
  701 RS=R+F1+F2
      RI=R+F1
      V=D+R
      VS=V
      VI=V
  703 I=2
C     STRIPPING SECTION **************************************************
      X(2)=XB
      Y(1)=XB
    3 X2=1.-X(I)
      TL=RS
      CALL EQUIL(X,Y,AN,I,X2,T)
      XS=(VS*Y(I)+XB*B)/RS
      XI=(VI*Y(I)-Z2*F2+XB*B)/RI
      XR=(V*Y(I)-D*XD)/R
      IF(XS.LT.XI)GO TO 1
      IF(XS.LT.XR)GO TO 1
      N=I-2
      IF(AN.GT.AM)AM=AN
      IF(K.LE.0) GO TO 302
      PRINT 2, X(I), Y(I), AN, T, N
    2 FORMAT (1X,2E15.4,2F15.4,I5)
  302 IF(N.GT.300)GO TO 9
      X(I+1)=XS
      I=I+1
```

```
      GO TO 3
    1 IF(F2.LE.0.0)GO TO 4
      IF(K.LE.0) GO TO 6
      PRINT 5
    5 FORMAT (1X,*ABOVE PLATE IS FEED PLATE*)
C     INTERMEDIATE SECTION ********************************************
    6 X2=1.-X(I)
      TL =RI
      CALL EQUIL(X,Y,AN,I,X2,T)
      XI=(VI*Y(I)-Z2*F2+XB*B)/RI
      XR=(V*Y(I)-D*XD)/R
      IF(XI.LT.XR)GO TO 4
      N=I-2
      IF(AN.GT.AM)AM=AN
      IF(K.LE.0) GO TO 301
      PRINT 2, X(I), Y(I), AN, T, N
  301 IF(N.GT.300)GO TO 9
      X(I+1)=XI
      I=I+1
      GO TO 6
    4 IF(K.LE.0) GO TO 7
      PRINT 5
C     RECTIFYING SECTION **********************************************
    7 X2=1.-X(I)
      TL=R
      CALL EQUIL(X,Y,AN,I,X2,T)
      XR=(V*Y(I)-D*XD)/R
      N=I-2
      IF(AN.GT.AM)AM=AN
      IF(K.LE.0) GO TO 310
      PRINT 2, X(I), Y(I), AN, T, N
  310 IF(N.GT.300)GO TO 9
      IF(Y(I).GE.XD)GO TO 8
      X(I+1)=XR
      I=I+1
      GO TO 7
    9 PRINT 10
      ENDFILE 1
   10 FORMAT (1X,*PINCH POINT POSSIBLE---300 PLATES WILL NOT ACCOMPLISH
     1 SEPERATION*)
      PRINT 206
  206 FORMAT (1X,*I ASKED FOR REFLUX RATIO NOT YOUR I. Q.*)
      GO TO 103
C     SEPERATION COSTS ************************************************
    8 AT=AM/(1.-.088)
      DI=(1.27*AT)**.5
      H=N*2+4
      CT=XMSI*0.3089*H**.8118*DI**.8787
      CST=XMSI*0.0156*H**.9758*DI**1.634
      CTT=CT+CST
```

```
C     HEAT EXCHANGER EQUIPMENT AND HEAT LOAD
C     30 PSI STEAM USED IN REBOILER T=135 C
      DT1(1)=3.0
      ST1(1)=2.0
      DT2(1)=3.0
      ST2(1)=2.0
      CALL PATERN(1,DT1,ST1,3,0,CST4,PROC4,BOUND4)
      CALL PROC4(DT1,CST4)
      CALL PATERN(1,DT2,ST2,3,0,CST5,PROC5,BOUND5)
      CALL PROC5(DT2,CST5)
      CK(II)=VS
      CK(1)=D+R
      VSC=DT2(1)*(F1+F2)*18./17510. + CK(II-1)
      IF(ABS((VSC-VS)/VS).LT.0.02)GO TO 704
      II=II+1
      IF(II.GT.50)GO TO 506
      VS=VS+DT2(1)*(F1+F2)*18./17510.
      VI=VI+DT2(1)*F1*18./17510.
      RS=RS+(F1+F2)*(DT2(1)*18./17510.)
      RI=RI+F1*(DT2(1)*18./17510.)
      GO TO 703
  704 TPEC=CTT+CDC*XYC+CDB*XYB+CPH1*XYPH1+CPH2*XYPH2
      TFCI=TPEC*FACTR
      FOC=TFCI*.19
      CSTLS=LABRCT*8500.
      PLOCT=.5*(48384.+0.03*TFCI)
      GPD=D*(XD*46.+(1.-XD)*18.)*24./6.728
      TOCT=FOC+CSTLS+PLOCT+CWCT+STCT
      CPG=TOCT/(360.*GPD)*100.
      CPH=CPH1+CPH2
      IF(K.LT.0)GO TO 503
      PRINT 38
   38 FORMAT (1H1,1X,*CAPTIOL COSTS*,10(1H*)/)
      PRINT 101, RR
  101 FORMAT (1X,*REFLUX RATIO = *,F7.3/)
      PRINT 11
   11 FORMAT (3X,*ITEM*,17X,*CAPACITY*,7X,*NO. OF UNITS*,4X,*COST/UNIT F
     10B*,1H$)
      PRINT 15
   15 FORMAT (1X,70(1H*))
      PRINT 13, DI, N, A, CTT
   13 FORMAT (1X,*DIST COLUMN*,5X,F6.2,*FT.DIA.*,I4,*PLATES*,4X,F3.1,
     111X,F9.2/)
      PRINT 14, QC, XYC, CDC
   14 FORMAT (1X,*CONDENSER*, 8X,E13.3,*BTU/HR*,6X,F3.1,11X,F9.2/)
      PRINT 16, QB, XYB, CDB
   16 FORMAT (1X,*REBOILER*,12X,E10.3,*BTU/HR*,6X,F3.1,11X,F9.2/)
      PRINT 17, QPH, XYPH, CPH
   17 FORMAT (1X,*PREHEAT EXCHR*,8X,E10.3,*BTU/HR*,6X,F3.1,11X,F9.2/)
      PRINT 15
```

```
      PRINT 21, TPEC
   21 FORMAT (1X,*TOTAL PURCHASED EQUIPMENT COST*,E11.4,1H$/)
      PRINT 22, FACTR
   22 FORMAT(1X,*MULTIPLICATION FACTOR IS *,F5.2/)
      PRINT 23, TFCI
   23 FORMAT(1X,*TOTAL FIXED CAPITAL INVESTMENT IS*,E11.4,1H$/)
      PRINT 24
   24 FORMAT(1X,*TEN YEAR STRAIGHT LINE DEPRECIATION ASSUMED*/)
      PRINT 25
   25 FORMAT(1X,10(1H*),*OPERATING COSTS DOLLARS/YEAR*,10(1H*)/)
      PRINT 15
      PRINT 26, FOC
   26 FORMAT(1X,*FIXED CHARGES (.19/YR OF TCI)*,10X,E12.3/)
      PRINT 27,STCT
   27 FORMAT(1X,*STEAM*,34X,E12.3/)
      PRINT 29, CWCT
   29 FORMAT(1X,*COOLING WATER*,26X,E12.3/)
      PRINT 30, CSTLS
   30 FORMAT(1X,*LABOR AND SUPERVISION*,18X,E12.3/)
      PRINT 31,PLOCT
   31 FORMAT(1X,*PLANT OVERHEAD*,25X,E12.3)
      PRINT 15
      PRINT 32,TOCT
   32 FORMAT(24X,*TOTAL*,9X,E12.3/)
  503 PRINT 36, GPD
   36 FORMAT (1X,*PRODUCTION RATE*,E11.3,3X,*GAL/DAY*/)
      PRINT 37, CPG
   37 FORMAT (1X,*SEPERATION COST*,3X,F7.4,*CENTS/GAL*/)
      GO TO 103
  506 PRINT 507
  507 FORMAT(1X,*ITERATION OF VAPOR FLOWS EXCEED LIMITS--STOP*)
   12 STOP
      END
```

```
      SUBROUTINE EQUIL(X,Y,AN,I,X2,T)
      COMMON/DIST/ A12,A21,A122,A211,PW,PE,EM,V,TL
      DIMENSION X(310),Y(310)
C     EQUILBRIUM CALCULATIONS ****************************************
      AL=(1.+A12*X2+A122*X2**2)/(1.+A21*X(I)+A211*X(I)**2)
      YEQ=AL*X(I)/(X2+AL*X(I))
      Y(I)=EM*(YEQ-Y(I-1))+Y(I-1)
      GAL=.7715*X2**2/(X(I)*.7715/.3848+X2)**2
      T=1623.22/(8.1629+ALOG10(X(I)/(YEQ*760.))+GAL)-228.98
C     TOWER CORSS SECTIONAL AREA *************************************
      WM=46.*X(I)+18.*X2
      P=130.8*X(I)+50.8*X2
      DV=WM*.7604/(273.+T)
      DE=62.38*(0.87-.00062*T-19./(300.-T))
      DW=62.38*(1.0064-.00025*T-.0000023*T**2)
      DA=WM/(X(I)*46./DE+X2*18./DW)
      ST=(P*(DA-DV)/(62.38*WM))**4
      QV=V*3.653E-4*(273.+T)
      QL=TL*WM/(DA*3600.)
      CLV=QL/QV*(DA/DV)**.5
      IF(CLV.GT.0.1)GO TO 1
      CF=.1862*ST**.2
      GO TO 2
    1 CF=(.1107-.1873*ALOG10(CLV))*.6248*ST**.2
    2 VF=CF*((DA-DV)/DV)**.5*.8
      AN=QV/VF
      RETURN
      END
      SUBROUTINE INUMBR(TC,UMC,UC,XY)
C--------------TC IS THE TOTAL CAPACITY REQUIRED
C--------------UCM IS THE MAXIMUM UNIT CAPACITY
C----------------UC IS THE ACTUAL UNIT CAPACITY BASED ON XY INTERGRAL
C--------------NUMBER OF EQUAL CAPACITY UNITS
      IF(TC.LE.UMC)GO TO 1
      XX=TC/UMC
      LL=XX
      XY=LL+1
      UC=TC/XY
      GO TO 2
    1 UC=TC
      XY=1.0
    2 RETURN
      END
```

```
      SUBROUTINE PROC4(DT1,CST4)
      COMMON/HEXOP/ AUC,WC,CDC,CWCT,XYC,QC
      COMMON/DIST/A12,A21,A122,A211,PW,PE,EM,V,TL
      COMMON/COST/ XMSI,FACTR,STEM,POWRCT,WATER,LABRCT
      DIMENSION DT1(1)
      DTC=DT1(1)
      QC=16611.*V
      DTLMC=((53.-DTC)/(ALOG(53./DTC)))*1.8
      AC=QC/(448.*DTLMC)
      CALL INUMBR(AC,10000.,ACU,XYC)
      WC=QC/((53.-DTC)*1.8*8.34)
      CWCT=WC*WATER*8.5
      CDC=XMSI*.696*ACU**.64
      CST4=CWCT+CDC*FACTR*.19*XYC
      RETURN
      END
      SUBROUTINE BOUND4(DT1,IOUT)
      DIMENSION DT1(1)
      IOUT=0
      IF(DT1(1).LT.0.5)IOUT=1
      IF(DT1(1).GT.20.)IOUT=1
      RETURN
      END
      SUBROUTINE PROC5(DT2,CST5)
      COMMON/PHEXC/ QB,F1,F2,ABU,CDB,CPH,QPH,STCT,XYB,XYPH,XYPH1,XYPH2,
     1CPH1,CPH2
      COMMON/HEXOP/AUC,WC,CDC,CWCT,XYC,QC
      COMMON/COST/XMSI,FACTR,STEM,POWRCT,WATER,LABRCT
      DIMENSION DT2(1)
      DTPH=DT2(1)
      QB=QC+(F1+F2)*18.*DTPH*1.8
      AB=QB/30680.
      CALL INUMBR(AB,10000.,ABU,XYB)
      CDB=XMSI*.696*ABU**.64
      STCT=QB/945.3*STEM*8.5
      QPH1=F1*32.4*(65.-DTPH)
      QPH2=F2*32.4*(65.-DTPH)
      QPH=QPH1+QPH2
      APH1=QPH1/((416.*DTPH)*1.8)
      APH2=QPH2/((416.*DTPH)*1.8)
      CALL INUMBR(APH1,10000.,APH1U,XYPH1)
      CALL INUMBR(APH2,10000.,APH2U,XYPH2)
      IF(F2.EQ.0.0)XYPH2=0.0
      XYPH=XYPH1+XYPH2
      CPH1=XMSI*.696*APH1U**.64
      CPH2=XMSI*.696*APH2U**.64
      CPH=CPH1+CPH2
      CST5=STCT+(CDB*XYB+CPH*XYPH)*FACTR*.19
      RETURN
      END
```

```
SUBROUTINE BOUND5(DT2,IOUT)
DIMENSION DT2(1)
IOUT=0
IF(DT2(1).LT.0.5)IOUT=1
IF(DT2(1).GT.20.)IOUT=1
RETURN
END
```

*** The pattern search subroutine PATERN is listed with program CONFER.

A.3.3. Design of Vapor Recompression Cycle. Computer Program VAPRC

The computer program VAPRC designs the recompression cycle shown in Fig. A.3 used in the vacuum fermentation system and as an alternative to conventional steam driven reboilers used in the final alcohol distillation. The composition, temperature, and pressure of the feed to the compressor are entered, and the compressor discharge pressure and temperature are calculated from Eqs. (A.34) through (A.36) to obtain complete condensation of the vapor at a specified condenser (or reboiler) temperature. If non-condensable gased are present, Eq. (A.37) is used to determine the ethanol concentration in the condensate for a set of condensation ratios (condensate rate/feed rate). As explained previously, Eqs. (A.34) through (A.37) are solved by trial and error when noncondensables are present.

With the molar flows established, the required compressor power is found from Eq. (A.15).

Explanation of Variables Used in Computer Program VAPRC

F	Feed to compressor (moles/hr)
T	Uncondensed vapor (moles/hr)
B	Condensed vapor (moles/hr)
T1	Temperature of feed (°K)
T2	Condensation temperature (°K)
T3	Exit temperature from compressor (°K)
EF	Ethanol in feed (moles/hr)
WF	Water in feed (moles/hr)
CO2F	CO_2 in feed (moles/hr)
O2F	Oxygen in feed (moles/hr)

PES	Saturation pressure of ethanol at condensation temperature (mmHg)
PWS	Saturation pressure of water at condensation temperature (mmHg)
P1	Compressor intake pressure (mmHg)
P2	Compressor discharge pressure (mmHg)
PHI	Ratio of condensed vapor to condensable vapor in feed (B = PHI(F) - CO2F - O2F)
ET	Ethanol which is not condensed (moles/hr)
HP	Compressor power (horsepower)

Computer Program VAPRC

```
* FLOWS ARE IN MOLES PER HOUR
* E REFERS TO ETHANOL,W REFERS TO WATER
* PRESSURES P1,P2, ARE IN MM HG
* F IS FEED,T TOPS, B BOTTOMS
* T1, T2, ARE TEMPERATURE IN DEGREES KILVEN
      READ,T1,P1,EF,WF,CO2F,O2F
4     READ,T2
      PHI=0.9
      I=1
      F=EF+WF+CO2F+O2F
      XEF=EF/F
      YI=(CO2F+O2F)/F
      XEB=EF/(EF+WF)
3     XWB=1.-XEB
      T2C=T2-273.0
      PES=EXP(2.303*(8.04494-1554.3/(222.65+T2C)))
      PWS=EXP(2.303*(8.10765-1750.286/(235.+T2C)))
   5  GME=EXP(2.303*.7236/(1.+1.90*XEB/XWB)**2)
      GMW=EXP(2.303*.3818/(1.+.526*XWB/XWB)**2)
      P2=(GME*XEB*PES+GMW*PWS-GMW*PWS*XEB)/(1.0-YI)
      IF(CO2F+O2F.EQ.0.0)GO TO 7
      XEB1=XEF/((1.-PHI)*GME*PES/P2+PHI)
      B=PHI*F-CO2F-O2F
      T=F-B
      YI=(CO2F+O2F)/T
      IF(ABS(XEB1-XEB).LE.0.0001)GO TO 8
      XEB=XEB1
      I=I+1
      IF(I.GT.100)GO TO 4
      GO TO 5
   7  B=EF+WF
      PHI=1.0
      I=CO2F+O2F
8     PRINT,PHI
      E1=EF-XEB*B
      W1=WF-(1.0-XEB)*B
      PRINT,E1,W1
      PRINT 8,F,T,B,XEF,XEB
      PRINT 9,P2
      QF=F*16.65*T1/P1
      PC1=P1*2.76
      HP=1.818E-4*PC1*QF*((P2/P1)**.166-1.)
      T3=T1*(P2/P1)**.166
      PRINT 6,HP,T3
      IF(PHI.GE.0.999)GO TO 4
      PHI=PHI+0.02
      I=1
      GO TO 5
      STOP
```

REFERENCES

1. W. Maxon and M. Johnson, IEC 45, 2554 (1953).
2. S. Aiba, A. Humphrey and N. Mills, Biochemical Engineering (Academic Press, NY, 1973).
3. P. Calderbank and M. Moo Young, Chem. Eng. Sci. 16, 39 (1961).
4. M. Peters and K. Timmerhaus, Plant Design and Ecomonics for Chemical Engineers (McGraw Hill, NY, 1968).
5. B. Michell and S. Miller, A.I.Ch.E.J. 8, 263 (1962).
6. H. Rushton, W. Costick and J. Everett, Chem. Eng. Prog. 46, 467 (1950).
7. E. Ranz and J. Wong, IEC 49, 1371 (1952).
8. N. Kimura and G. Iinaga, Chem. Eng. (Japan) 23, 792 (1959).
9. S. Aiba, and K. Toda, J. Ferm Tech. 43, 257 (1965).
10. O. Livenspiel, IEC 50, 343 (1958).
11. C. Bennett and J. Myers, Momentum, Heat and Mass Transfer (McGraw Hill, NY, 1962).
12. J. Richards, Prog. Ind. Microb. 3, 143 (1961).
13. McAdams, Heat Transmission (McGraw Hill, NY, 1954).
14. W. Ibele, Modern Developments in Heat Transfer (Academic Press, NY, 1963).
15. E. Hala, T. Wichterle, J. Polak and T. Boublik, Vapor Liquid Equilibrium Data at Normal Pressures (Pergamon Press, NY, 1958).
16. D. Himmelblau, Basic Principles and Calculations in Chemical Engineering (Prentice Hall, NJ, 1967).
17. A. Fransic, Chem. Eng. Sci. 10 37 (1958).

18. R. Treybal, Mass Transfer Operations (McGraw Hill, NY, 1968).

19. R. Perry, C. Chilton, and S. Kirkpatrick, Chemical Engineers Handbook (McGraw Hill, NY, 1968).

20. H. Spoehr and Milner, Plant Phys. 24, 120 (1949).

21. K. Guthrie, Chem. Eng. 114 March 24 (1969).

22. T. Sherwood, R. Pigford and C. Wilke, Mass Transfer (McGraw Hill, NY, 1975).

23. Alfa-Laval Yeast Separators, Technical Bulletin TB-054/E

24. C. M. Cooper, G. A. Fernstrom, S. A. Miller, Ind. Eng. Chem. 36, 504 (1944).

25. C. W. Robinson, C. R. Wilke, LBL-20472, Lawrence Berkeley Laboratory, University of California, 1971.

APPENDIX B. EXAMPLE DESIGN USING COMPUTER PROGRAMS

In order to use the computer programs to design an ethanol fermentation plant the ethanol production rate must be specified as well as certain metabolic parameters of the yeast. For the design example a continuous fermentation plant producing 78,000 gal/day of 95% ethanol from enzymatic hydrolysate sugars was taken. Unless otherwise indicated, the fermentation parameters used in the design represent optimal conditions as determined in Chapter 6.

First using the computer program CONFER, the basic fermentation process was designed. The input data required by CONFER are shown in Table B.1. The data are entered in the order shown below, one datum input per line when the program is run on a time sharing basis using a teletype. Minor modifications of input format statements are required if the data is entered on cards. As mentioned in Appendix A, the computer program CONFER was written to be applicable to general fermentation processes and capable of accounting for the simultaneous production of four fermentation products and cell mass. Thus for the ethanol fermentation only two product yield factors and product heats of combustion were specified. The remaining yield factors and heats of combustion were set equal to zero.

The optimum design of the fermentation process specified in Table B.1 was obtained from the output of CONFER shown in Table B.2. The internal design parameters of the fermentation process which were generated in CONFER are first listed. An explanation of these numbers is shown in Table B.3. The internal design parameters are followed by an equipment list for the fermentation process. The

Table B.1. Input data to CONFER.

Variable Name in CONFER	Explanation	Value for Design Example
XMSI	Current Marshall Stevens Cost Index	445.6
YXS	Cell yield factor, g cells/g substrate fermented	0.12
YPS(1)	Yield Factor of product 1 (ethanol), g product/g substrate fermented	0.46
YPS(2)	Yield factor of product 2 (CO_2), g product/g substrate fermented	0.47
YPS(3)	Yield factor of product 3, g product/g substrate fermented	0.0
YPS(4)	Yield factor of product 4, g product/g substrate fermented	0.0
HCX	Heat of combustion of cell mass, kcal/g	3.0(1)
HC1	Heat of combustion of product 1 (ethanol), kcal/g	7.1
HC2	Heat of combustion of product 2 (CO_2), kcal/g	0.0
HC3	Heat of combustion of product 3, kcal/g	0.0
HC4	Heat of combustion of product 4, kcal/g	0.0
CAP	Plant capacity, lb/day	500000.0
SO	Limiting substrate concentration, g/l	143.0
DI	Fermentor dilution rate, hr^{-1}	0.17
T	Fermentation temperature, °K	308.0
QO2	Specific respiratory rate of yeast, mM O_2/g cell-hr	0.3(2)
CCL	Critical liquid oxygen concentration, moles/l$\times 10^{-4}$	0.004
PPT	Per cent utilization of limiting substrate	70.0

Table B.2. Computer output of CONFER.

```
**********INTERNAL DESIGN PARAMETERS**********

   150833.0694        80.0000         5.7590         7.2367         2.5161
        2.1036          .0008        21.1653      9517.3446         1.4768
        9.0520     54994.6923         4.1369       139.0750          .1700
       12.0120        46.0460         0.             0.             0.

1 **********OPTIMUM DESIGN OF RUN NUMBER 1

  ITEM                  CAPACITY        NO. OF UNITS    COST/UNIT FOB$
**********************************************************************
FERMENTOR            188541.3 LITERS         8.0              90530.3

AGITATOR                13.9 HP.             8.0               6482.7

HEAT REMOVAL EXCHR     408.3 SQ.FT.          1.0              14540.6

AIR COMPRESSOR          90.7 HP.             1.0              37974.1

AIR FILTER             .4M X   .3M           8.0                206.9

MEDIA STERILIZER      5.7M X 1.378M          1.0              11749.0

PRE HEAT EXCHANGER    9994.8 SQ.FT.          1.0             112566.4

COOLER EXCHANGER      4119.2 SQ.FT.          1.0              63831.1
**********************************************************************
TOTAL PURCHASED EQUIPMENT COST 1.0184E+06$

MULTIPLICATION FACTOR IS  3.09

TOTAL FIXED CAPITAL INVESTMENT IS 3.1469E+06$

TEN YEAR STRAIGHT LINE DEPRECIATION ASSUMED

**********OPERATING COSTS DOLLARS/YEAR**********

**********************************************************************
FIXED CHARGES (.19/YR OF TCI)              5.979E+05

STEAM                                      2.257E+04

POWER                                      1.776E+04

COOLING WATER                              7.019E+04

LABOR AND SUPERVISION                      1.275E+05

PLANT OVERHEAD                             1.110E+05
**********************************************************************
                     TOTAL                 9.469E+05
```

Table B.3. Explanation of internal design parameters in output of computer program CONFER.

Fermentor working volume, l	% Working volume of total fermentor volume	Diameter of fermentor, m	Height of fermentor, m	Fermentation pressure, atm
Superficial gas velocity through fermentor, m/hr	VVM of gas flow	Agitator RPM	Cooling water flow to fermentor, gal/hr	Degree of approach in fermentor exchanger, °C
Degree of approach in sterilizer preheat exchanger, °C	Cooling water flow to sterilizer, gal/hr	Degree of approach in cooler exchanger of sterilizer, °C	Temperature of sterilization, °C	Fermentor dilution rate, hr^{-1}
Cell mass concentration in fermentor, g/l	Steady-state concentrations of products 1-4 in fermentor, g/l			

capacity, number of units and cost per unit is listed for each item of equipment. Finally, the operating cost for the basic fermentation is shown.

Once the basic fermentation process is designed the computer program DISTL was used to design and cost the distillation step. The ethanol concentration and total flow rate of fermented beer determined in CONFER were fed to DISTL. The input data required by DISTL to design the distillation equipment for the continuous production of 78,000 gal/day of 95% ethanol are shown in Table B.4. As shown in Table B.4, a zero reflux ratio was specified in the input data. When this is done 1.25 times the minimum reflux is calculated in DISTL and used for the column design. Justification of using 1.25 times the minimum reflux was discussed in Appendix A, Section A.1.9. However, if a reflux ratio greater than zero is entered that value will be used in the column design. If the reflux ratio is too low and a pinch point is reached an error message will be printed.

An optimum design of the distillation equipment for the example design is shown in the computer print out of DISTL in Table B.5. The equipment is first listed along with the capacity and cost per unit. This is followed by the operating cost for the distillation to produce 78,000 gal/day of 95% ethanol.

To obtain a complete ethanol fermentation plant design the auxiliary equipment specified in Appendix A, Section A.1.10 was next designed. The capacity of mixing and storage tanks were calculated from the required holding time and the volumetric flow of process streams (see Section A.1.10). The ethanol absorber was sized based on the

Table B.4. Input data to DISTL.

Variable Name in DISTL	Explanation	Value for Design Example
XMSI	Current Marshall Stevens cost index	445.6
Z1	Mole fraction in feed 1	0.0194
Z2	Mole fraction in feed 2	0.0064
F1	Flow rate of feed 1, lb moles/hr	23350.0
F2	Flow rate of feed 2, lb moles/hr	341.0
PR	Recovery fraction of ethanol in distillate	0.99
R	Reflux ratio	0.0

Table B.5. Computer output of DISTL.

```
1 CAPTIOL COSTS**********

 REFLUX RATIO =     .880

   ITEM                    CAPACITY        NO. OF UNITS     COST/UNIT FOB$
 ***************************************************************************
 DIST COLUMN       11.19FT.DIA.  45PLATES     1.0              76220.12

 CONDENSER              7.095E+07BTU/HR       1.0              69857.60

 REBOILER               8.051E+07BTU/HR       1.0              47830.80

 PREHEAT EXCHR           3.961E+07BTU/HR      1.0              64484.35

 ***************************************************************************
TOTAL PURCHASED EQUIPMENT COST 2.5839E+05$

 MULTIPLICATION FACTOR IS  3.09

 TOTAL FIXED CAPITAL INVESTMENT IS 7.9843E+05$

 TEN YEAR STRAIGHT LINE DEPRECIATION ASSUMED

 **********OPERATING COSTS DOLLARS/YEAR**********

 ***************************************************************************
FIXED CHARGES (.19/YR OF TCI)              1.517E+05

 STEAM                                     2.353E+05

 COOLING WATER                             1.043E+05

 LABOR AND SUPERVISION                     4.250E+04

 PLANT OVERHEAD                            3.617E+04
 ***************************************************************************
                        TOTAL              5.700E+05
```

computer program presented by Sherwood, Pigford and Wilke[3] assuming a molar mass velocity of 13.6 and 12.1 lb moles/hr-ft^2 for gas and liquid respectively ($\frac{mG}{L} = 0.7$). With these mass velocities the average height of a gas phase transfer unit was 2.2 ft and that of a liquid phase transfer unit 0.77 ft when 1 in. rasching rings were employed. Also, the pressure drop through the column was only 4.0 in. of water at these flow conditions. For a more detailed description of the absorber design the reader is referred to the original reference.[3] The ethanol absorber was not optimized because, as discussed previously, it represents only 0.45% of the total capital investment and its operation has a negligible effect on the distillation column.

To complete the design of the enzymatic hydrolysate fermentation process an evaporative sugar concentrator must be designed. Based on the ethanol yield factor, 14.0 lb of fermentable sugar are required to produce 1 gallon of 95% ethanol. Since the hydrolysate sugars are 70% fermentable, the total amount of sugar needed is 14.0/0.7 or 20 lb. The sugar arrives at the fermentation plant in a 4.0% solution and is concentrated to 10% fermentable sugars or 10/0.7 = 14.3% total sugar solution. Thus, the amount of water which must be removed per gallon of ethanol produced is,

$$20.0\left(\frac{0.96}{0.04} - \frac{0.875}{0.143}\right) = 378 \text{ lb } H_2O/\text{gal of ethanol}$$

Assuming the heat of vaporization of the water to be 10^3 Btu/lb, the total heat load on the evaporator to concentrate enough sugar to produce 78,000 gal/day of 95% ethanol is,

$$\frac{78{,}000 \text{ gal}}{\text{day}} \cdot \frac{\text{day}}{24 \text{ hr}} \cdot \frac{378 \text{ lb}}{\text{gal}} \cdot \frac{10^3 \text{ Btu}}{\text{lb}} = 1.23\times10^9 \text{ Btu/hr}$$

If an overall heat transfer coefficient of 560 Btu/ft^2-hr-°F and a temperature driving force of 50°F are assumed[4] the total evaporator area is calculated as,

$$\frac{1.23\times10^9 \text{ Btu}}{\text{hr}} \cdot \frac{\text{ft}^2\text{-hr-°F}}{560 \text{ Btu}} \cdot \frac{1}{50°\text{F}} = 4.4\times10^4 \text{ ft}^2$$

The steam requirement for the evaporation may then be calculated for a 7 effect evaporator having a total steam efficiency of 5.[4]

$$\frac{378 \text{ lb water}}{\text{gal of ethanol}} \cdot \frac{1}{5} = 75.6 \text{ lb steam/gal of ethanol}$$

The process equipment designs generated in CONFER and DISTL as well as the auxiliary equipment are combined to produce a complete fermentation plant design. A complete equipment listing of the example design is shown in Table 7.2 and the operating costs are shown in Table 7.8.

The vacuum fermentation processes were designed in an analogous manner. CONFER and DISTL were used to design the fermentation and distillation equipment respectively. However, an additional computer program, VAPRC, was used to size the compression equipment necessary for the fermentor vapor compression cycle. The input data to VAPRC for the production of 78,000 gal/day of 95% ethanol by vacuum fermentation is shown in Table B.5. The numbers shown in Table B.5 correspond to maintaining a 7.3% liquid ethanol concentration in the fermentor. The effect of changing the fermentor liquid ethanol concentration was

discussed in Section 7.2.2 and illustrated in Fig. 7.3.

The output of VAPRC is shown in Table B.6. In order to condense 90% of the condensable vapor in the feed to the compressor (PHI = 0.9) at 313°K, (a 5°C ΔT in the fermentor reboiler) a compressor power of 2194 HP was required (HP = 2194). The remaining output of VAPRC corresponds to mass balances around the compressor-reboiler complex and was explained in Section A.3.3.

With the compressor power for the recompression cycle calculated, the auxiliary equipment was then designed as discussed above and combined with the process equipment specified in CONFER and DISTL for a complete ethanol fermentation plant design. An entire equipment list and operating cost for a vacuum fermentation is shown in Table 7.6 and 7.8 respectively.

Table B.6. Input data to VAPRC

Variable Name in VAPRC	Explanation	Value for Design Example
T1	Temperature of feed to compressor, °K	308.0
P1	Compressor intake pressure, mmHg	55.0
EF	Ethanol in feed to compressor, moles/hr	381.0
WF	Water in feed to compressor, moles/hr	1207.0
CO2F	CO_2 in feed to compressor, moles/hr	456.0
O2F	Oxygen in feed to compressor, moles/hr	70.0
T2	Condensation temperature in fermentor reboiler, °K	313.0

Table B.7. Computer output of VAPRC.

PHI = 0.9

ET = 125.200074 WT = 86.1999259

F = 2114.0 T = 737.40 B = 1376.6 XEF = 0.1802 XEB = 0.1858

P2 = 418.273

HP = 2194.0 T3 = 431.3

REFERENCES

1. H. A. Spoehr and H. W. Milner, Plant Physiology 24, 120 (1949).

2. A. D. Haukeli and S. Lie, J. Inst. Brew 79, 55 (1973).

3. T. Sherwood, R. Pigford and C. R. Wilke, Mass Transfer (McGraw Hill, NY, 1975).