USE OF MATERIAL AND ENERGY BALANCE REGULARITIES TO ESTIMATE GROWTH YIELDS AND MAINTENANCE COEFFICIENTS IN HYDROCARBON FERMENTATIONS

by

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B.S., Universidad del Zulia, Venezuela, 1975

A MASTER'S THESIS

Submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Chemical Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1979

Approved by:

Major Professor

ACKNOWLEDGMENT

The author dedicates this work to his mother's memory, to his wife for her support, assistance and help in the culmination of this work, and to his family.

The author wishes to express his gratitude to Dr. Larry E. Erickson for his guidance during the course of this work.

Financial support from CONICIT of Venezuela and encouragement from CIEPE of Venezuela are gratefully acknowledged.

The author also wishes to thank Dr. L. T. Fan and Dr. D. S. Chung for their useful suggestions in preparing this work.

The author wishes to express his gratitude to Mr. A. Ganesan, Mr. Y. Lee, Dr. P. McSwegin and Dr. W. B. Zuti for their invaluable contribution to this work.

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CHAPTER I

INTRODUCTION

This work is primarily concerned with the estimation of "true" growth yields and maintenance coefficients [1,2] of yeast grown on petroleum hydrocarbons in batch cultures and evaluation of the consistency of experimental data using available electron and carbon balances. This work is divided into chapters each of which is self contained. Tables and Figures appear at the end of each chapter.

Chapter II provides a review of the literature where enough experimental data were provided to estimate the consistency of the measurements using available electron and carbon balances and the mean values of the regularities for carbon weight fraction in biomass and reductance degree of biomass. "True" growth yields are estimated from both batch and continuous culture data and the results are compared.

Chapter III is an experimental study in which the progress of biomass concentration, substrate concentration, specific rate of oxygen consumption, specific rate of carbon dioxide evolution, and specific rate of substrate consumption are investigated in the batch cultivation of Candida lipolytica grown on n-hexadecane as the main source of carbon and energy. The consistency of the experimental data is examined. "True" biomass energetic yields and energetic maintenance coefficients are estimated and the results are compared to estimates from the literature in Chapter 2.

REFERENCES

1. Pirt, S. J., Proc. Royal Society of London, Series B 163, 224 (1965).

2. Pirt, S. J., Principles of Microbe and Cell Cultivation, Halsted Press, Wiley, N.Y. (1975).

CHAPTER II

EVALUATION OF DATA CONSISTENCY AND ESTIMATION OF YIELD PARAMETERS IN HYDROCARBON FERMENTATIONS

INTRODUCTION

The growth of microorganisms on petroleum hydrocarbons has received considerable attention [1-28] because of the possibility of producing single cell protein for feed and/or food uses. Since several reviews [8, 14, 27] covering various aspects of hydrocarbon fermentation are already available, this review will concentrate on those papers where sufficient experimental data are available to examine the consistency of the experimental results using the material and energy balance regularities which Minkevich and Eroshin have identified [6, 19].

One of the biggest challenges encountered in hydrocarbon fermentation processes is how to get accurate measurements of the main variables such as biomass production, substrate consumption, carbon dioxide evolution, oxygen and nitrogen consumption, etc. Hug et al. [12] discuss some of the analytical problems present in the biomass and substrate measurements.

Oxygen and substrate requirements, as well as biomass yield, which are some of the basic parameters that need to be considered in determining the feasibility of a fermentation process, may be only estimated properly if material and energy balances can be applied to the fermentation and the accuracy of the measurements can be tested. Oxygen balances were used in 1964 by Johnson [15]. He presented a balance equation which deals with oxygen requirements when there is no product formation, and this was later used by Nagai and Aiba [24]. The carbon balance has been used to check the accuracy of measurements by Hug and Fiechter [12], Camargo et al. [3] and Miller et al [18]. Carbon and oxygen balances have also been used by Herbert [11] to check the consistency of experimental data in continuous culture. Minkevich and Eroshin [10, 19, 20] have reported material and energy balances based on regularities found regarding the heat evolution in the fermentation, the weight fraction carbon

in biomass and the reductance degree of the biomass. Erickson et al. [5, 6, 7, 9] have applied these material and energy balance regularities [10, 19, 20] to specific conditions such as continuous fermentations, batch fermentations, and processes with extracellular products.

THEORY

Regularities and basic approach

The balance equation for microbial growth is [17]

$$CH_mO_\ell + aNH_3 + bO_2 = y_c\ CH_pO_nN_q + zCH_rO_sN_t \qquad (1)$$

$$+ cH_2O + dCO_2$$

where CH_mO_ℓ, $CH_pO_nN_q$ and $CH_rO_sN_t$ denote the elemental composition of organic substrate, biomass and product, respectively. The subscripts denote numbers of atoms of hydrogen, oxygen and nitrogen per carbon atom. The coefficients y_c, z and d are the fraction of substrate carbon converted to biomass, product and carbon dioxide, respectively.

The concept of reductance degree has been introduced and used in the material and energy balances [19]. The reductance degree, γ_b, of the biomass is the number of equivalents of available electrons in that quantity of biomass which contains one gram atom carbon; that is,

$$\gamma_b = 4 + p - 2n - 3q \qquad (2)$$

Similarly, the reductance degree, γ_s, of the organic substrate is

$$\gamma_s = 4 + m - 2\ell \qquad (3)$$

and, the reductance degree, γ_p, of the product is

$$\gamma_p = 4 + r - 2s - 3t \qquad (4)$$

where the number of equivalents of available electrons is taken as four for carbon, one for hydrogen, minus two for oxygen, and minus three for nitrogen. Therefore, there are no available electrons in CO_2, H_2O and NH_3.

Minkevich and Eroshin [19] have shown that the heat of reaction per electron transferred to oxygen, Q_0, is relatively constant for the organic

molecules used as substrates in fermentation, and equal to 27 kcal/ equivalent of available electrons transferred to oxygen. The coefficient of variation of this average value is 4%. This regularity enables one to make an energy balance as follows

$$Q_o\gamma_s + Q_o b(-4) = y_c Q_o \gamma_b + zQ_o\gamma_p \tag{5}$$

where $Q_o\gamma_s$ is the energy in substrate, $y_c Q_o \gamma_b$ is the energy in organic substrate which is transferred to biomass, $zQ_o\gamma_p$ is the energy in organic substrate which is transferred to product and

$$Q = 4Q_o b \tag{6}$$

is the heat evolved in the reaction. Dividing Equation (5) by $Q_o\gamma_s$ gives

$$1 = \varepsilon + \eta + \xi_p \tag{7}$$

where

$$\eta = \frac{y_c Q_o \gamma_b}{Q_o \gamma_s} = \frac{y_c \gamma_b}{\gamma_s} \tag{8}$$

$$\varepsilon = \frac{4b}{\gamma_s} \tag{9}$$

$$\xi_p = \frac{zQ_o\gamma_p}{Q_o\gamma_s} = \frac{z\gamma_p}{\gamma_s} \tag{10}$$

η, denotes the biomass energetic yield coefficient or the fraction of organic substrate energy transferred to biomass, ξ_p denotes the product energetic yield coefficient or the fraction of organic substrate energy transferred to product, and ε denotes the fraction of organic substrate energy evolved as heat.

If a balance is written based on the available electrons in Equation (1), it results in

$$\gamma_s + b(-4) = y_c\gamma_b + z\gamma_p \tag{11}$$

Dividing Equation (11) by γ_s, gives the fractional allocation of available electrons in the organic substrate

$$\frac{4b}{\gamma_s} + y_c \frac{\gamma_b}{\gamma_s} + z \frac{\gamma_p}{\gamma_s} = 1 \qquad (12)$$

and substituting Equations (8), (9) and (10) into this expression gives

$$\varepsilon + \eta + \xi_p = 1 \qquad (13)$$

Therefore, the constant value Q_o allows the mass and energy balances to be coupled together into a mass-energy balance. This equation is the same as that used by Johnson [15] but in different terms. It gives the oxygen requirement by using Equation (9).

Equations (2), (3) and (4) can be used to estimate the reductance degrees of biomass, substrate and product if the chemical compositions are known. However, Minkevich and Eroshin [19] have reported that the reductance degree of the biomass is relatively constant for any microorganism and equal to 4.291, with a coefficient of variation of 4%. The average value can be used when the chemical composition of the biomass is not known.

According to Equation (1), the overall carbon balance is given by

$$y_c + z + d = 1 \qquad (14).$$

The available electron and carbon balances, Equations (13) and (14), respectively, may be used to test the consistency of data.

True Yields and Maintenance

Using models containing separate terms for growth and maintenance can be useful to gain insight into the metabolism of the cell, and to test the consistency of the data if the values of true yields and maintenance coefficients can be estimated from different sources of data. The model of Pirt [25]

$$\frac{1}{Y_S} = \frac{1}{Y_S^{max}} + \frac{m_S}{\mu} \qquad (15)$$

is used to estimate the value of the maintenance coefficient, m_S, and the true growth yield, Y_S^{max}, based on organic substrate. Erickson et al. [7] illustrated the consistency of the model of Pirt in the analysis of continuous culture data. Erickson [5] also studied the energetic efficiency of biomass and product formation. Equations were developed [5, 7] to write the model of Pirt in terms of the biomass energetic yield coefficient, η, and other variables measured in fermentations such as biomass yields based on carbon dioxide evolution and oxygen consumption.

Maintenance coefficients can be expressed as the energetic maintenance coefficient, m_e, which is the rate of consumption of energy (or available electrons) in the organic substrate per unit of energy (or available electrons) in biomass (hour); oxygen maintenance coefficient, m_o, g moles O_2/g atom carbon in biomass (hour); carbon dioxide maintenance coefficient, m_d, g moles CO_2 evolved/ g atom carbon in biomass (hour); and organic substrate maintenance coefficient, m_s, g atom carbon of organic substrate/ g atom carbon in biomass (hour). The following equations [7] show how these maintenance coefficients are related.

$$m_e = \frac{4}{\gamma_b} m_o = \frac{48}{\sigma_b \gamma_b} m_O \tag{16}$$

$$m_e = \frac{\gamma_s}{\gamma_b} m_d = \frac{12\gamma_s}{\sigma_b \gamma_b} m_D \tag{17}$$

$$m_e = \frac{\gamma_s}{\gamma_b} m_s = \frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} m_S \tag{18}$$

Biomass true growth yields can be obtained based on organic substrate consumption, Y_S^{max}, oxygen consumption, y_o^{max} and carbon dioxide evolution, y_d^{max}. The true growth yields can be related to the true biomass energetic yield coefficient, η_{max} as follows [7]

$$\frac{1}{y_o^{max}} = \frac{\gamma_b}{4}\left(\frac{1}{\eta_{max}} - 1\right) \quad \text{or} \quad \frac{1}{Y_0^{max}} = \frac{\sigma_b \gamma_b}{48}\left(\frac{1}{\eta_{max}} - 1\right) \tag{19}$$

$$\frac{1}{y_d^{max}} = \frac{\gamma_b}{\gamma_s \eta_{max}} - 1 \quad \text{or} \quad \frac{1}{Y_D^{max}} = \frac{\sigma_b}{12}\left(\frac{\gamma_b}{\gamma_s \eta_{max}} - 1\right) \tag{20}$$

$$Y_S^{max} = \frac{\sigma_s \gamma_s}{\sigma_b \gamma_b} \eta_{max} \tag{21}$$

where σ_s and σ_b are the carbon weight fraction in the organic substrate and biomass, respectively. Minkevich and Eroshin [19] have found that the value of σ_b is relatively constant in biomass and equal to 0.462 with a coefficient of variation of 5%. This value can be used when the chemical composition of the biomass is not known. Herbert [11] has also found that σ_b is almost constant.

The values of maintenance coefficients and maximum yields can be obtained by using linear regression and Pirt's model in graphical form.

Pirt's model can also be written [7] as

$$Q_{O_2} = m_O + \frac{1}{Y_O^{max}} \mu \tag{22}$$

$$Q_{CO_2} = m_D + \frac{1}{Y_D^{max}} \mu \tag{23}$$

$$Q_S = m_S + \frac{1}{Y_S^{max}} \mu \tag{24}$$

$$\frac{1}{\eta} = \frac{1}{\eta_{max}} + \frac{m_e}{\mu} \tag{25}$$

from which the values of m_e and η_{max} can be estimated by using directly Equation (25), or by using Equations (22), (23) and (24) and Equations (16), (17), (18) for m_e, and (19), (20) and (21) for η_{max}.

METHODS OF DATA CONSISTENCY ANALYSIS

Available Electron and Carbon Balances

When extracellular products can be neglected, the available electron balance is

$$\eta + \varepsilon = 1 \tag{26}$$

and the carbon balance is

$$y_c + d = 1 \tag{27}$$

When $\eta + \varepsilon > 1$ or $y_c + d > 1$, experimental errors are probably present. When $\eta + \varepsilon < 1$ or $y_c + d < 1$, this could be due to either experimental errors or product formation. Small deviations of less than 5% may be present because of the use of the regularities.

Batch Cultures

It is possible to make balances at given times throughout the fermentation (instantaneous balances) or overall balances (integrated balances).

Instantaneous Balances. The available electron balance can be written as

$$\frac{\sigma_s \gamma_s}{12} Q_S = \frac{\sigma_b \gamma_b \mu}{12} + 4\, Q_{O_2} \tag{28}$$

By dividing Equation (28) by its lefthand side, Equation (26) is obtained where

$$\eta = \frac{\frac{\sigma_b \gamma_b \mu}{12}}{\frac{\sigma_s \gamma_s}{12} Q_S} \tag{29}$$

and

$$\varepsilon = \frac{4\, Q_{O_2}}{\frac{\sigma_s \gamma_s}{12} Q_S} \tag{30}$$

The carbon balance can also be written as

$$\frac{\sigma_s}{12} Q_S = \frac{\sigma_b \mu}{12} + Q_{CO_2} \tag{31}$$

By dividing Equation (31) by its lefthand side, Equation (27) is obtained, where

$$y_c = \frac{\frac{\sigma_b \mu}{12}}{\frac{\sigma_s}{12} Q_S} \tag{32}$$

and

$$d = \frac{Q_{CO_2}}{\frac{\sigma_s}{12} Q_S} \tag{33}$$

As shown by Equations (28) to (33), it is necessary to have the following data: Q_{O_2}, moles O_2/ g dry weight cells (hr), Q_{CO_2}, moles CO_2/ g dry weight cells (hr), X, g dry weight cells/ ℓ and S, g substrate/ ℓ.

The fact that Q_{O_2} and Q_{CO_2} are rates, while X and S are not must be considered in any analysis of errors. The values of X and S must be differentiated graphically to obtain values of the specific growth rate and the specific organic substrate consumption rate; however, it is difficult to obtain accurate results because of the errors associated with graphical differentiation. Moreover, the time constants associated with oxygen and CO_2 measurements are smaller than those associated with biomass and organic substrate measurements.

These balances have been applied to the data from Fig. 1 of Blanch and Einsele [2] and the data of Hug and Fiechter [12].

Integrated Balances. When substrate consumption data are not available and the assumption of complete consumption of organic substrate is valid ($S_f = 0$), the available electron balance [Equation (26)] can be applied, where

$$\eta = \frac{\frac{(X_f - X_o)}{12}\sigma_b\gamma_b}{\frac{(S_o - S_f)}{12}\sigma_s\gamma_s} \tag{34}$$

and

$$\varepsilon = \frac{4\int_0^{\theta_f} Q_{O_2} X d\theta}{\frac{(S_o - S_f)}{12}\sigma_s\gamma_s} \tag{35}$$

and the carbon balance [Equation (27)] is applied with

$$y_c = \frac{\frac{(X_f - X_o)}{12}\sigma_b}{\frac{(S_o - S_f)}{12}\sigma_s} \tag{36}$$

and

$$d = \frac{\int_0^{\theta_f} Q_{CO_2} X d\theta}{\frac{(S_o - S_f)}{12}\sigma_s} \tag{37}$$

where S_o and S_f are the initial and final substrate concentrations, g/ℓ, and X_f and X_o are the final and initial biomass concentrations, g/ℓ.

Equations (34) to (37) have been used with Blanch and Einsele data [2]. Direct gas measurements (O_2 and CO_2) could be used instead of Q_{O_2} and Q_{CO_2} as in data from Prokop et al. [26].

When the carbon dioxide yield is available, there is no need for integration in Equation (37) as in data from Miller et al. [18] and Ballerini [1].

Continuous Cultures

The consistency of data in continuous cultures is analyzed through available electron and carbon balances made at each dilution rate. In the following equations, it is assumed that the organic substrate is completely consumed in the fermentation. The available electron balance can be written as

$$\frac{DS_o}{X}\frac{\sigma_s\gamma_s}{12} = D\frac{\sigma_b\gamma_b}{12} + 4\,Q_{O_2} \tag{38}$$

Dividing Equation (38) by its left hand side, Equation (26) is obtained, where

$$\eta = \frac{\frac{D\sigma_b\gamma_b}{12}}{\frac{D\,S_o\sigma_s\gamma_s}{12X}} \tag{39}$$

and

$$\varepsilon = \frac{4Q_{O_2}}{\frac{D\,S_o\sigma_s\gamma_s}{12X}}$$

The carbon balance can be written as

$$\frac{D\,S_o}{X}\frac{\sigma_s}{12} = D\,\frac{\sigma_b}{12} + Q_{CO_2} \tag{40}$$

Dividing Equation (40) by its left hand side, Equation (27) is obtained, where

$$y_c = \frac{\frac{D\sigma_b}{12}}{\frac{D\,S_o\sigma_s}{12X}} \tag{41}$$

and

$$d = \frac{Q_{CO_2}}{\frac{D\,S_o\sigma_s}{12X}} \tag{42}$$

These balances are less subject to error than the balances for batch cultures since the constant dilution rate makes the biomass production and organic substrate consumption occur at constant rates so that the units are similar to those of Q_{O_2} and Q_{CO_2}. Errors due to differentiation are avoided.

These balances have been applied to data from Hug et al. [11] and Blanch and Einsele [2]. They were also applied to data from Kanazawa [16]. In this case, S_o was substituted by X/Y_S since S_o was not reported.

True Yields and Maintenance

The comparison of these parameters when they can be obtained from more than one source of data is useful to analyze whether or not there are experimental errors and extracellular products.

Batch Cultures

Estimate of specific growth rates by differentiation. The curve biomass, X, vs time can be differentiated and the specific growth rate evaluated as

$$\mu = \frac{1}{X}\frac{dX}{d\theta} \tag{43}$$

Using this estimate of μ, Equation (15) or (24), (22), (23) and (25) can be used to obtain the values of true yields and maintenance coefficients. True growth yields can be translated into any units by using Equations (19), (20) and (21), and the maintenance coefficients can be interrelated with Equations (16), (17) and (18).

Equation (15) has been used to analyze the data from Miura et al. [21], Moo-Young et al. [22] and Blanch and Einsele [2]. Equation (23) has been applied to data from Blanch and Einsele [2].

Results may be compared, graphically, when enough data are available to make more than one estimate of the parameters. For example, Q_{O_2}, Q_{CO_2} and Q_s data can be used to obtain values of η so that Equation (25) can be used in all the cases. The equations to find the values of η depending on the variables measured are:

$$\frac{1}{\eta} = \frac{48\, Q_{O_2}}{\sigma_b \gamma_b \mu} + 1 \tag{44}$$

$$\frac{1}{\eta} = \frac{12\gamma_s Q_{CO_2}}{\sigma_b \gamma_b \mu} + \frac{\gamma_s}{\gamma_b} \tag{45}$$

$$\frac{1}{\eta} = \frac{192\gamma_s Q_s}{\sigma_b \gamma_b \mu} \tag{46}$$

These equations have been applied to data from Hug and Fiechter [10].

Estimates of Specific Growth Rate from Oxygen, Carbon Dioxide and Substrate Data. True growth yields and maintenance coefficients may also be estimated without graphical differentiation of biomass data to estimate values of specific growth rate; however, these indirect estimates depend on the consistency of the experimental data. In each case, values of η and μ for use in Equation (25) are estimated from sets of measured values (see the Appendix for the derivation of these equations). When Q_{O_2} and Q_s data are available, η and μ can be found from the following expressions

$$\frac{1}{\eta} = \frac{4\gamma_s \frac{Q_s}{Q_{O_2}}}{4\gamma_s \frac{Q_s}{Q_{O_2}} - 1} \tag{47}$$

$$\frac{1}{\mu} = \frac{\sigma_b \gamma_b}{48(4\gamma_s Q_s - Q_{O_2})} \tag{48}$$

When Q_{CO_2} and Q_s data are available, η and μ are found as follows

$$\frac{1}{\eta} = \frac{\frac{Q_s}{Q_{CO_2}}}{\frac{Q_s}{Q_{CO_2}} \frac{\gamma_b}{\gamma_s} - \frac{\gamma_b}{16\gamma_s}} \tag{49}$$

$$\frac{1}{\mu} = \frac{\sigma_b}{12(16Q_s - Q_{CO_2})} \tag{50}$$

Equations (47), (48), (49) and (50) have been applied to data from Hug and Fiechter [12].

When Q_{O_2} and Q_{CO_2} data are available, the following equations can be applied

$$\eta = \frac{1 - \frac{\gamma_s}{4} \text{R.Q.}}{\frac{\gamma_s}{\gamma_b} - \frac{\gamma_s}{4} \text{R.Q.}} \tag{51}$$

$$\mu = \frac{48\, Q_{O_2}}{\sigma_b Y_b \left(\frac{1}{\eta} - 1\right)} \tag{52}$$

$$R.Q. = \frac{Q_{CO_2}}{Q_{O_2}} \tag{53}$$

Equations (51) and (52) were applied to data from Blanch and Einsele [2] (Fig. 2 in their paper).

Continuous Cultures

Equation (15) has been applied to data from Moo-Young et al. [22], Blanch and Einsele [2] and Katinger [17]. Equation (22) has been applied to data from Moo-Young et al. [22] and Blanch and Einsele [2]. Equations (15) and (22) enable one to find true growth yield and maintenance coefficients from biomass yield and oxygen data, respectively. In these equations, the dilution rate, D, is the specific growth rate, μ. Other maintenance coefficients besides those appearing in Equations (15) and (22) can be found from Equations (16), (17) and (18), and other true growth yields can be found from Equations (19), (20) and (21).

Endogenous Respiration

Experiments to determine the endogenous respiration, E.R., of a culture can be carried out in Warburg flasks. The endogenous respiration is usually expressed as $\mu\ell\ O_2$/ mg dry weight cell (hr). These units can be converted into those for the energetic maintenance coefficients as follows at standard conditions

$$m_e = \frac{4\ E.R.\ 12}{22{,}400\ \sigma_b Y_b} \tag{54}$$

m_e was estimated from data from Singh et al. [28] in this fashion.

RESULTS AND DISCUSSION

The consistency of available experimental data for growth on liquid hydrocarbons is examined in Tables 1-5 using available electron and carbon balances, Equations (26) and (27), respectively. When the data are consistent, similar values of η and other related yield parameters may be calculated from values of various sets of measured variables [6,7,9].

Various sets of data have been used to estimate the "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, which are reported in Tables 6 and 7. These values may be used to evaluate the consistency of experimental data. The results of linear regression analysis are shown in graphical form in Figs. 1-12 for those sets of data which were not previously reported in this form.

After examining the consistency of the results, the estimated values of η_{max} and m_e from several sets of data are examined and discussed taking into account available information on the consistency of the data. Values of yield and maintenance coefficients estimated from continuous and batch data are compared. Variations in liquid hydrocarbon composition, organism strain, agitation conditions, and initial substrate concentration are also considered in examining the variation of values of the yield and maintenance coefficients.

The average value of $\gamma_b = 4.291$ and $\sigma_b = 0.462$ were used to characterize the biomass. Since γ_b and σ_b have coefficients of variation of 4% and 5% respectively, small deviations of the results from the consistency conditions of Equations (26) and (27) are to be expected. The 95% confidence intervals corresponding to these coefficients of variation are $0.94 \leq \gamma_c + d \leq 1.06$ and $0.93 \leq \eta + \varepsilon \leq 1.07$.

Consistency of the data

Continuous Culture.

Table 1 shows the results of an examination of data consistency using available electron and carbon balances for growth of yeast on liquid hydrocarbon in continuous culture. It can be seen that the data of Kanazawa, Blanch and Einsele (available electron balance), and the data of Hug et al. (carbon balance) are quite consistent; that is, $(\eta + \varepsilon)$ and $(y_c + d)$ are very close to 1. Only some results of Kanazawa and those from Figure 9 of Blanch and Einsele show experimental errors where $\eta + \varepsilon > 1$. Values where $\eta + \varepsilon < 0.93$ may be due to measurement errors or product formation. This appears to be appreciable only in Figure 12 of Blanch and Einsele, and in the results of Hug et al. If $\eta + \varepsilon < 0.93$ because of product formation, these products were produced without any reduction in the values of the biomass energetic yield. The results in Table 1 appear to indicate that products are not produced in substantial quantities in hydrocarbon-limited continuous culture fermentations. The results for Kanazawa's data are more consistent in Table 1 of this manuscript than results in an earlier analysis [6] where the chemical composition of yeast of Kanazawa was used. Extraction of lipid may explain this because extraction changes the values of σ_b and γ_b. The results of Blanch and Einsele [2] and Hug et al. [13] indicate that the biomass energetic yield, η, is relatively constant and (except for two data points) ranges from 0.37 to 0.39 for Candida tropicalis in continuous culture. On the other hand, values of ε range from 0.34 to 0.82 with the extreme values occurring at the lowest dilution rates where accurate oxygen uptake measurements are most difficult.

Estimated values of the "true" biomass energetic yield coefficient, η_{max}, and the maintenance coefficient, m_e, are presented in Table 6. These

values have been obtained from biomass data and organic substrate data on the basis of the assumption of complete consumption of organic substrate and from oxygen and biomass data in Figures 9, 10, 11 and 12 of Blanch and Einsele's paper [2]. Values of η_{max} are similar except in the data from Figure 12. The estimates of η_{max} based on data from Figure 12 differ because $\eta + \varepsilon \leq 0.92$ (Table 1). If these results are due to oxygen uptake measurement errors, the estimated value of $\eta_{max} = 0.40$ from biomass and hydrocarbon measurements is the more accurate estimate. If extracellular products are present, other equations [5] which take this into account should be used to estimate η_{max} and m_e. Since the value $\eta_{max} = 0.40$ is in good agreement with the other estimates, oxygen uptake measurement errors are probably the reason why $\eta + \varepsilon \leq 0.92$ in the data from Figure 12.

Values of the maintenance coefficient, m_e, estimated from the biomass and hydrocarbon data and from the biomass and oxygen data of Blanch and Einsele [2] are also presented in Table 6. The value of m_e for Figure 9 from biomass and substrate data is much lower than the mean value of 0.012 hr^{-1}. This is probably due to experimental errors ($\eta + \varepsilon > 1$ and relatively few data; see Figure 3). If the assumption of the organic substrate being completely consumed were incorrect, $(\eta + \varepsilon)$ would be still higher. The straight lines fitted to the model of Pirt, as $\frac{1}{Y_s}$ vs. $\frac{1}{\mu}$, are shown in Figure 3. The plots of the oxygen and biomass data as Q_{O_2} vs. μ are not shown here since they are depicted in the original paper [2]. It is important to point out that the biomass and substrate data almost always appear to be more scattered than the oxygen and biomass data when Pirt's model is used. (See the values of the correlation coefficients) This is because the values of Q_{O_2} increase substantially in magnitude as μ increases and measurement errors are frequently small relative to the change in value of Q_{O_2} due to the change in μ.

On the other hand, small measurement errors have a large effect on the estimated value of m_e when biomass and hydrocarbon measurements are used. Therefore, care should be taken in analyzing the values of maintenance coefficients estimated from biomass and hydrocarbon measurements.

When the consistency of the data of Blanch and Einsele is examined considering the results in both Tables 1 and 6, it appears that they are quite consistent ($0.37 \leq \eta \leq 0.39$, $0.38 \leq \eta_{max} \leq 0.42$, and $0.01 \leq m_e \leq 0.02$ hr^{-1}).

Table 6 and Figures 1 and 2 show data from Moo-Young et al. [22]. It can be seen that very good agreement exists between the values of m_e, although the values of η_{max} are quite different. However, there was not enough data to make the available electron and carbon balances; therefore, this difference could be due to either experimental errors or product formation.

Batch Culture

Table 2 shows the results of an examination of data consistency using integrated available electron and carbon balances for growth of yeast on liquid hydrocarbons in batch culture. It can be seen that the data from Hug and Fiechter [12] are very consistent, the data from Fig. 2 of Blanch and Einsele [2] are quite consistent, and the rest of the data are not consistent [Ballerini, Prokop, Blanch and Einsele (Fig. 1), Einsele et al. and Miller and Johnson]. In general, $\eta + \varepsilon < 1$ and $y_c + d < 1$; thus, it appears that there are either more experimental errors or more extracellular products in batch cultures than in continuous culture.

Tables 3, 4, and 5 examine the consistency of batch data at points along the batch curve. Tables 3 and 5 report the results of making instantaneous available electron and carbon balances at several different points in time, during batch growth using the data from Fig. 1 of Blanch and Einsele and from Fig. 2 of Hug and Fiechter. In Table 4, values of γ_s

have been estimated using carbon and available electron balances for the data from Fig. 2 of Blanch and Einsele. In examining these tables, it should be kept in mind that accurate measurements are more difficult to obtain at the beginning and end of the fermentation where oxygen uptake, growth, and CO_2 evolution rates are small.

Tables 2 and 3 show that the data from Fig. 1 of Blanch and Einsele [2] are not consistent. The coefficients of variation in the instantaneous available electron and carbon balances are 41% and 40%, respectively. There are some measurement errors during the first five hours ($y_c + d > 1$ and $\eta + \varepsilon > 1$). During the rest of the fermentation, $y_c + d < 1$ and $\eta + \varepsilon < 1$. This could be due to either measurement errors or product formation. The estimates of y_c and η during the last five hours of the fermentation are consistent and in good agreement with values from other experiments (see Tables 1 and 2). The estimates of ε and d are much lower than one would expect. Thus, measurement errors in oxygen uptake and CO_2 evolution appear to be an important source of error in Table 3.

According to Table 2, relatively consistent results are obtained using integrated available electron and carbon balances for the data of Fig. 2 of Blanch and Einsele. Table 4 shows the estimated values of γ_s which were obtained from instantaneous balances using biomass production, oxygen uptake, and CO_2 evolution rate data. It can be seen that the values are reasonably close to the expected value of 6.125 except at the beginning where the estimates are low and at the end of the fermentation where the estimates are too large. Higher values than $\gamma_s = 6.125$ can be due to either over estimation of oxygen uptake rates or under estimation of CO_2 evolution rates. Product formation may also contribute to errors in the estimation

of γ_s. It can be also seen in Table 4 that estimates of η from oxygen and biomass data (Q_{O_2}, X) and carbon dioxide and biomass data (Q_{CO_2}, X) are very similar except at the beginning and end of the fermentation. This is in agreement with the values of γ_s. Estimates of η from oxygen and carbon dioxide data (Q_{CO_2}, Q_{O_2}) are considered to be very poor since there is more error propagation. The estimated value of the biomass energetic yield, η, of 0.31 is somewhat lower than the mean value of 0.38 in continuous culture for the same strain and substrate while the integrated balance results from Fig. 1 of Blanch and Einsele (η = 0.38) are in good agreement with the continuous culture results. The consistency of the results from Fig. 2 of Blanch and Einsele appear to be much better than those from Fig. 1 of Blanch and Einsele.

The consistency of the results in Fig. 2 of Blanch and Einsele has also been examined by using different sets of measurements to estimate the true biomass energetic yield, η_{max}, and the maintenance coefficient, m_e. As shown in Table 7, different values of η_{max} and m_e are obtained when using CO_2 and biomass data, oxygen and biomass data, and respiratory quotient and biomass data. These results can be compared to those in Table 6 for continuous culture of this strain by the same authors. The results obtained using CO_2 and biomass data are in reasonable agreement with the results from continuous culture while the estimates of η_{max} and m_e from O_2 and biomass data are somewhat larger than those from continuous culture data. This could be due to overestimation of oxygen uptake rates at low specific growth rates at the end of the fermentation. When respiratory quotient and biomass data are used, the estimated values of η_{max} and m_e are even larger and they are clearly outside the range of values estimated using data from continuous culture. The consistency of these three sets of estimates shows that the

data from Fig. 2 of Blanch and Einsele are not sufficiently consistent to obtain highly accurate estimates of true growth yields and maintenance parameters. The instantaneous balances and the estimation of yield and maintenance parameters from different sets of data provide much more severe tests of data consistency compared to the integrated balances. It appears that small measurement errors at the beginning and end of the fermentation do not greatly affect the integrated balances, but they may considerably affect the estimates of yield and maintenance parameters because of the important role of data at low specific growth rates in estimating yield and maintenance parameters.

Table 2 shows that the integrated balances for the data of Hug and Fiechter give very consistent results. Table 5 shows the results of making instantaneous available electron and carbon balances; the coefficients of variation are 23% and 21%, respectively. The largest errors are at the start and end of the fermentation where specific growth rates are smaller. The consistency of the results is relatively good during the rest of the fermentation where the rates are larger and, therefore, easier to measure accurately.

Table 8 shows several sets of estimates of the reciprocal values of μ and η using different sets of experimental data. Figures 10, 11, and 12 show these results in graphical form. Figures 10, 11 and 12 indicate that most of the data fall within a very short interval of specific growth rate, and that there are very few data at low values of specific growth rate. Data at very low values of the specific growth rate from the beginning and end of the fermentation have a greater effect on the values of the slope and intercept, and the estimated parameters, η_{max} and m_e. This effect is illustrated in Figures 11 and 12 where the least squares line is plotted with and without

the data point at the lowest estimated specific growth rate. Tables 5 and 8 show that the results at low specific growth rates are not very consistent. Large errors at low specific growth rates may be another reason why these values significantly alter the least squares curve. Table 7 shows the estimated values of the true biomass energetic yield, η_{max}, and the maintenance coefficient, m_e. Comparison of different estimates of these parameter shows that there are inconsistencies in the experimental data, but that the differences are not extremely large. The effect of neglecting the data at the lowest specific growth rate is shown in Table 7 and in Figures 11 and 12 (dashed lines).

The low values of $(y_c + d)$ and $(\eta + \varepsilon)$ in Table 2 for the data of Prokop et al., Miller and Johnson, Ballerini, and Einsele et al., may be due to either measurement errors and/or undetected products. The authors did not report product formation. Because the integrated balances are not consistent, these data were not examined further.

Yield and maintenance coefficients.

Tables 6 and 7 show the values of the "true" biomass energetic yield coefficient and the energy maintenance coefficient for growth of yeasts on liquid hydrocarbons in continuous and batch cultures, respectively. The correlation coefficient, r^2, is also shown to give an idea of the fit of straight lines by linear regression.

Continuous Cultures

Figures 1, 2, 3 and 4 show straight lines which have been fitted by linear regression to continuous culture data. Table 6 shows the values of

η_{max}, m_e, r^2, and type of microorganism and substrate for each set of data plotted in the Figures. Figures 1 and 2 correspond to data from Moo-Young et al. [22]. Figure 3 shows the biomass data (assumption of organic substrate completely consumed) from Blanch and Einsele [2] (using data from Figs. 9, 10, 11 and 12 in the original paper). The lines corresponding to the same experiments but using oxygen and biomass data are not shown since they are reported in the original paper as Q_{O_2} vs μ. The data from Katinger [17] from three different experiments appear as a pool in one straight line in Fig. 4. Candida sp was used in all the data, and n-hexadecane was used in all the experiments except in Moo-Young et al.'s data where n-dodecane was used.

According to data from Moo-Young et al. and Blanch and Einsele, low values of m_e appear to be characteristic of continuous culture. The agreement is quite good although they used different substrates. The average value is 0.014 hr^{-1}, if the value of m_e = 0.0006 hr^{-1} (Blanch and Einsele, Fig. 9, biomass data) is not considered. This value is much smaller, and there were appreciable errors in the experiments as discussed in the section on consistency of data.

The value of the biomass energetic yield, η_{max}, is higher in n-hexadecane than n-dodecane. It has been reported in the literature [23] that n-hexadecane shows a higher biomass yield per unit of organic substrate consumed. This is then also true for the value of η_{max}. However, different strains were also used and this could also explain the differences in the value of η_{max}. A lower value of η_{max} is estimated using the biomass and hydrocarbon data of Moo-Young et al. compared to the value obtained from their oxygen and biomass data. As was stated earlier, this could be due to product formation

and/or measurement errors. Comparison of Figures 1 and 3 with Fig. 2 shows that biomass and substrate data are more scattered than the oxygen and biomass data, and this influences the results.

The values of the maintenance coefficient estimated from the data of Katinger are the only ones that show relatively high values of m_e for continuous culture. Fig. 4 shows a straight line fitted to data from three different experiments (data from Figs. 8, 11 and 12 in Katinger's paper) and it gives a pool estimate of $m_e = 0.34$ which is high compared to 0.014 from the rest of the data. This could be due to measurement errors; however, there is not enough data to test for errors and the author did not say anything about the presence of products. For this reason and the fact that the data from Blanch and Einsele have been shown to be quite consistent, a 95% confidence interval for the maintenance coefficient, m_e, for the Blanch and Einsele continuous culture data was found to be $0 \leq m_e \leq 0.027$. Values from Moo-Young et al.'s data are also within these limits. The 95% confidence interval for the true biomass energetic yield, η_{max}, for this same data is $0.35 \leq \eta_{max} \leq 0.47$; the pool estimate of η_{max} from Katinger's data is within these limits.

Batch Cultures

Table 7 shows that for most of the data, the estimated values of the energy maintenance coefficient, m_e, are larger in batch fermentation than in continuous culture. This could be due to differences between batch and continuous culture. The organic substrate concentration is higher during most of the batch culture. This could influence the

maintenance energy requirement and/or the efficiency with which organic substrate energy is utilized for maintenance. The analysis of batch endogenous respiration data from Singh et al. [28] shows a low value of m_e similar to that for continuous culture. Since organic substrate concentrations are low in both of these cases, this agreement is reasonable.

Miura's data and the CO_2 and biomass data from Blanch and Einsele also show relatively low values of m_e (0.034 and 0.022 hr^{-1}, respectively). The plot of Miura's data appears in the original paper [21] while these results of Blanch and Einsele are shown in Figure 7.

Figures 5 and 6 show straight lines fitted to data from Moo-Young et al. [22] (Figs. 8 and 9 in their paper). The values of η_{max} as well as those of m_e are in very good agreement with each other although the experiments varied in agitation speed (500 rpm and 250 rpm, respectively). The data are scattered in both cases and show a hysteresis pattern. The consistency of the data of Miura and Moo-Young et al. could not be tested because there was not sufficient data.

Figures 7, 8 and 9 show the plots of Blanch and Einsele's data [2] and Figures 10, 11 and 12 show those for Hug and Fiechter's data [12]. Analysis of integrated and instantaneous available electron and carbon balances for these data, as well as comparison of estimates of η_{max} and m_e obtained from different sets of measurements, showed that these data are the most consistent batch data. Values of m_e appear to be quite high. The estimated 95% confidence interval values of the maintenance coefficient, m_e, based on these two experiments range from 0 to 0.11 hr^{-1}. The values in Table 7 appear to be about two to four times larger than in continuous culture. The results using data of Moo-Young et al. show that m_e is about 3 to 4 times

larger in batch culture. The variance of the estimated values of m_e is larger in batch culture than in continuous culture because of the difficulty of making precise measurements.

The 95% confidence interval values of the true biomass energetic yield, η_{max}, range from 0.32 to 0.62 for the batch cultures considered in Tables 4 and 5. In comparing estimated values, it is important to realize that in the linear regression process larger estimated values of m_e usually also result in larger estimated values of η_{max}.

Values of η_{max} and m_e from Blanch and Einsele's data and Hug and Fiechter's data are very similar. Ranges of values have been obtained from them since the data are the most consistent. In addition, both sets of experiments were carried out with the same microorganism. (Candida tropicalis). Moo-Young et al. used Candida lipolytica grown on n-dodecane and similar results are found with their data. Thus, it appears that operating conditions and strains of microorganisms do not alter greatly the values of η_{max} and m_e in batch hydrocarbon fermentations.

When the "t" test [29] was used with the estimates from the continuous culture data of Blanch and Einsele [2] and the batch culture data considered in Tables 4 and 5, the estimated mean values of η_{max} and m_e for batch culture were found to be significantly different from those from continuous culture. However, one would not expect η_{max} for batch culture to be higher than η_{max} for continuous culture. The 95% confidence interval for batch culture $0.32 \leq \eta_{max} \leq 0.62$ includes the mean value of $\eta_{max} = 0.41$ for continuous culture.

CONCLUSIONS

Available electron and carbon balances, as well as the comparison of estimated values of yield and maintenance parameters can be used to test the consistency of the data in fermentations and to gain insight about the possibility of product formation. Instantaneous balances in batch cultures and the comparison of estimates of true biomass energetic yield and maintenance coefficients when they can be estimated from more than one source of data can detect relatively small experimental errors.

The analysis of the data for consistency shows that either more experimental errors or extracellular products appear to be present in batch cultures than in continuous cultures.

Comparison of Tables 1 and 2 indicates that the biomass energetic yield is frequently slightly larger in continuous culture than it is in batch culture.

The estimated values of "true" biomass energetic yield appear to be larger for batch culture than for continuous culture. The estimated values of the maintenance coefficient are also larger (from two to four times) in batch culture than in continuous culture. Since one would expect n_{max} to be similar, it may be that the larger value of m_e and n_{max} in Table 7 are the results of measurement errors in batch cultures.

NOMENCLATURE

a	Moles of ammonia per quantity of organic substrate containing one g atom carbon, g mole/ g atom carbon
b	Moles of oxygen per quantity of organic substrate containing one g atom carbon, g mol/ g atom carbon
c	Moles of water per quantity of organic substrate containing one g atom carbon, g mole/ g atom carbon
D	Dilution rate, hr^{-1}
d	Moles of carbon dioxide per quantity of organic substrate containing one g atom carbon, g mole/ g atom carbon
E.R.	Endogenous respiration, ml O_2/ mg dry wt. (hr).
l	Atomic ratio of oxygen to carbon in organic substrate, dimensionless
m	Atomic ratio of hydrogen to carbon in organic substrate, dimensionless
m_d	Rate of carbon dioxide evolution for maintenance, g moles/ g atom biomass carbon (hr)
m_D	Rate of carbon dioxide evolution for maintenance, g moles/ g dry biomass (hr)
m_e	Rate of organic substrate consumption for maintenance, g equiv. of available electrons/ g equiv. of available electrons in biomass (hr) or kcal/kcal of biomass (hr)
m_o	Rate of oxygen uptake for maintenance, g moles/ g atom biomass carbon (hr)
m_O	Rate of oxygen uptake for maintenance, g moles/ g dry biomass (hr)
m_S	Rate of organic substrate consumption for maintenance, g/g of dry biomass (hr)
m_s	Rate of organic substrate consumption for maintenance, g atom carbon/ g atom biomass carbon (hr)
n	Atomic ratio of oxygen to carbon in biomass, dimensionless
p	Atomic ratio of hydrogen to carbon in biomass, dimensionless
Q	Heat evolution associated with Equation (1), kcal/g atom substrate carbon
Q_o	Heat evolution in fermentation per equivalent of oxygen uptake kcal/g equiv.

Q_{CO_2} Rate of evolution of carbon dioxide, g moles/ g dry wt (hr)

Q_{O_2} Rate of consumption of oxygen, g moles/ g dry wt (hr)

Q_S Rate of organic substrate consumption g/ g dry wt (hr)

Q_s Rate of organic substrate consumption, g moles hexadecane/ g dry wt. (hr)

q Atomic ratio of nitrogen to carbon in biomass, dimensionless

R.Q. Respiratory quotient, dimensionless

r Atomic ratio of hydrogen to carbon in products, dimensionless

r^2 Correlation coefficient

S Organic substrate concentration, g/liter

S_f Final organic substrate concentration, g/ℓ

S_o Initial organic substrate concentration, g/liter

s Atomic ratio of oxygen to carbon in products, dimensionless

t Atomic ratio of nitrogen to carbon in products, dimensionless

X Biomass concentration, g/liter

X_f Final biomass concentration, g/liter

Y_S Biomass yield on organic substrate, g dry weight/ g substrate

Y_D^{max} Maximum biomass yield based on CO_2 evolution, g dry biomass/ g mole CO_2 evolved

Y_O^{max} Maximum biomass yield based on oxygen, g dry biomass/ g mole O_2

Y_S^{max} Maximum biomass yield based on organic substrate, g dry weight/ g substrate

y_c Biomass carbon yield (fraction of organic substrate carbon in biomass), dimensionless

y_c^{max} Maximum biomass carbon yield, dimensionless

y_d Biomass yield based on CO_2 evolution, g atoms biomass carbon/ g mole CO_2 evolved

y_d^{max} Maximum biomass yield based on CO_2 evolution, g atoms biomass carbon/ g mole CO_2 evolved

y_o Biomass yield based on oxygen, g atoms biomass carbon/ g mole O_2

y_o^{max} Maximum biomass yield based on oxygen, g atoms biomass carbon/ g mole O_2

z Fraction of organic substrate carbon in products, dimensionless

γ_b Reductance degree of biomass as defined by Equation (2), equivalents of available electrons per g atom carbon

γ_p Reductance degree of products, equivalents of available electrons per g atom carbon

γ_s Reductance degree of organic substrate, equivalents of available electrons per g atom carbon

ε Fraction of energy in organic substrate which is evolved as heat, dimensionless

η Fraction of energy in organic substrate which is converted to biomass or biomass energetic yield, dimensionless

η_{max} Maximum biomass energetic yield, dimensionless

μ Specific growth rate, hr^{-1}

ξ_p Fraction of energy in organic substrate which is converted to products, dimensionless

σ_b Weight fraction carbon in biomass, dimensionless

σ_s Weight fraction carbon in organic substrate, dimensionless

θ Time, hr

θ_f' Final time, hr

REFERENCES

1. Ballerini, D., These, Université de Paris (1969).

2. Blanch, H. W. and A. Einsele, Biotechnol. and Bioeng. 15, 861-877 (1973).

3. Camargo, Rubio E., A. Ornelas Vale, G. Casarrubias Arcos and S. Nagai, J. Ferment. Technol., Vol. 55, No. 1, 56-62 (1977).

4. Einsele, A., A. Fiechter and H. P. Knöpfel, Arch. Mikrobiol. 82, 247-253 (1972).

5. Erickson, L. E., Biotechnol. and Bioeng., In Press (1978).

6. Erickson, L. E., I. G. Minkevich and V. K. Eroshin, Biotechnol. and Bioeng. 20, 1595-1621 (1978).

7. Erickson, L. E., I. G. Minkevich and V. K. Eroshin, Seventh International Symposium on Continuous Cultivation of Microorganisms, Prague, Czechoslovakia (July 1978).

8. Erickson, L. E., T. Nakahara and A. Prokop, Process Biochemistry 10, No. 5, 9 (June 1975).

9. Erickson, L. E., S. E. Selga and U. E. Viesturs, Biotechnol. and Bioeng. 20, 1623-1638 (1978).

10. Eroshin, V. K., Biotechnol. and Bioeng. Symposium No. 4, 995-1001 (1974).

11. Herbert, D., "Stoicheiometric Aspects of Microbial Growth" in Continuous Culture 6: Applications and New Fields, A.C.R. Dean et al. editors, p. 1-30, Halsted Press, New York (1976).

12. Hug, H. and A. Fiechter, Arch. Mikrobiol. 88, 77-86 (1973).

13. Hug, H., H. W. Blanch and A. Fiechter, Biotechnol. and Bioeng. 16, 965-985 (1974).

14. Humphrey, A. E., Biotechnol. and Bioeng. 9, 3-24 (1967).

15. Johnson, M. J., Chemistry and Industry, 1532-1537 (1964).

16. Kanazawa, M., "The production of yeast from n-paraffins," Single Cell Protein II, S. R. Tannenbaum & D. I. C. Wang, ed., p. 438, M.I.T. Press, Cambridge, Mass. (1975).

17. Katinger, H.W.D., Biotechnol. and Bioeng. Symposium No. 4, 485-505 (1973).

18. Miller, T. L. and M. J. Johnson, Biotechnol. and Bioeng. 8, 549-565 (1966).

19. Minkevich, I. G. and V. K. Eroshin, Folia Microbiol. 18, 376-385 (1973).

20. Minkevich, I. G. and V. K. Eroshin, Biotechnol. and Bioeng. Symposium No. 4, 21-25 (1973).

21. Miura, Y., M. Okazaki, S. Hamada, S. Murakawa & R. Yugen, Biotechnol. and Bioeng. 19, 701-710 (1977).

22. Moo-Young, M., T. Shimizu and D. A. Whitworth, Biotechnol. and Bioeng. 13, 741-760 (1971).

23. Munk, V., O. Volfová, M. Dostálek, J. Mostecký and K. Pecka, Folia Microbiologica 14, 334-344 (1969).

24. Nagai, S. and S. Aiba, J. of General Microbiology 73, 531-538 (1972).

25. Pirt, S. J., Principles of Microbe and Cell Cultivation, Halsted Press, Wiley, N.Y. (1975).

26. Prokop, A., M. Sobotka, J. Panos and R. Pecka, Folia Microbiol. 19, 125-132 (1974).

27. Shennan, J. L. and J. D. Levi, Progress in Industrial Microbiology, 13, 1-57 (1974).

28. Singh, H. D., P. K. Barua, K. R. Pillai, J. N. Baruah & M. S. Iyengar, Indian Journal of Biochemistry and Biophysics 9, 315-320 (1972).

29. Snedecor, G. W., and W. G. Cochran, Statistical Methods, Sixth Edition, Iowa State University Press, Ames, Iowa (1967).

Table 1. Examination of data consistency using available electron and carbon balances for growth of yeasts on liquid hydrocarbons in continuous cultures (γ_b = 4.291, ε_b = 0.462).

Source of data	Results			Comments
	n	e	n+e	
Kanazawa [16]	.43	.53	.96	O_2, biomass and hydrocarbon
	.43	.54	.97	yield data from two separate
	.40	.49	.89	tables. Experiments carried
	.43	.72	1.15	out in 100 m^3 fermentor
	.42	.64	1.06	
	.45	.58	1.03	
	.43	.48	.91	
	.40	.47	.87	
	.42	.55	.97	
	.43	.51	.99	Heat evolution, biomass and
	.39	.55	.94	hydrocarbon yield data
	.42	.56	.98	
Blanch and Zinsele [2]*				O_2 and biomass data. Hydrocarbon is assumed to be completely consumed. Candida tropicalis on $n\text{-}C_{16}H_{34}$
Fig. 9	.38	.82	1.20	
	.38	.74	1.12	
	.38	.66	1.04	S_o = 1.0% (w/v)
	.37	.63	1.00	FBT system · 3000 rpm
Fig. 10	.35	.34	.69	
	.38	.60	.98	S_o = 0.55% (w/v)
	.37	.59	.96	
	.37	.53	.90	FBT system · 3000 rpm
	.37	.50	.87	
Fig. 11	.37	.59	.96	
	.37	.60	.97	S_o = 1.0% (w/v)
	.38	.61	.99	
	.38	.55	.93	Circulation stirrer · 2500 rpm
	.39	.60	.99	
Fig. 12	.38	.54	.92	
	.39	.48	.87	S_o = 0.55% (w/v)
	.39	.50	.89	
	.39	.49	.88	Circulation stirrer · 2500 rpm
	.40	.50	.90	
	.38	.47	.85	

Table 1. Continued

Source of Data	Results						Comments
	y_c	d	y_c+d	n	ε	n+ε	
Hug et al. [13]							
Fig. 6	.56	.37	.93	.39	.46	.85	O_2, CO_2 and biomass data. It was assumed that substrate consumption in Fig. 6 was equal to that of Fig. 8.
Fig. 8	.56	.35	.91	.39	.46	.85	
							Candida tropicalis on n-hexadecane

*Mixing is better in conditions of Fig. 11 and 12 than in Fig. 9 and 10.

Table 2. Examination of data consistency using integrated available electron and carbon balances for growth of yeasts on liquid hydrocarbons in batch cultures (γ_b = 4.291, σ_b = 0.462).

Source of data	Results						Comments
	y_c	d	y_c+d	n	ε	n+ε	
Prokop et al [26]	.46	.33	.79	.32	.55	.87	O_2, CO_2 and biomass data. Assumed all substrate was consumed. Candida lipolytica on n-alkanes C_{11} - C_{18}
Hug and Fiechter [12] Fig. 2	.58	.43	1.01	.41	.60	1.01	O_2, CO_2, biomass and substrate data. Candida tropicalis on n-hexadecane
Blanch and Einsele [2]							
Fig. 1	.55	.23	.78	.38	.32	.70	O_2, CO_2 and biomass data. Hydrocarbon data in Fig. 1.
Fig. 2	.44	.52	.96	.31	.75	1.06	Assumed all substrate was consumed in Fig. 2. Candida tropicalis on n-hexadecane. Agitation conditions: Fig. 1 3500 rpm, Fig. 2 850 rpm.
Miller and Johnson [18] Table 2	.42	.31	.73				CO_2 and biomass yield data. Candida intermedia and C. lipolytica on n-hexadecane
Ballerini [1]	.53	.38	.91				CO_2 and biomass yield. Candida lipolytica on n-hexadecane.
Einsele et al. [4] Fig. 2,3 Table 2.	.51	.30	.81	.35	.42	.77	O_2, biomass, respiratory quotient and biomass yield data. Candida tropicalis on n-hexadecane.

Table 3. Examination of data consistency using instantaneous available electron and carbon balances for growth of yeasts on liquid hydrocarbons in batch cultures. Data from Blanch and Einsele, Figure 1 in original paper [2]. Candida tropicalis on n-$C_{16}H_{34}$ (σ_b = 0.462, γ_b = 4.291).

θ (hr)	μ (hr^{-1})	y_c	d	y_c+d	η	ε	$\eta+\varepsilon$
2	.094	.46	.56	1.02	.32	.73	1.05
5	.361	1.07	.43	1.50	.75	.51	1.26
7	.389	.32	.13	.45	.22	.15	.37
9	.438	.36	.13	.49	.25	.18	.43
10	.300	.26	.11	.37	.18	.16	.34
11	.302	.35	.13	.48	.25	.17	.42
12	.304	.40	.13	.53	.28	.19	.47
13	.308	.56	.18	.74	.39	.26	.65
14	.236	.56	.24	.80	.39	.33	.72
15	.195	.56	.29	.85	.40	.39	.79
16	.164	.56	.34	.90	.39	.51	.90
17	.142	.56	.35	.91	.39	.51	.90
Integrated Results		.55	.23	.78	.38	.32	.70

η, ε, y_c, d and μ are estimated from Equations (29), (30), (32), (33) and (43), respectively.

Table 4. Values of specific growth rate, biomass energetic yield coefficient and γ_s for growth of Candida tropicalis on n-hexadecane. Data from Figure 2 of Blanch and Einsele [2]. (σ_b = 0.462, γ_b = 4.291).

θ (hr)	μ (hr^{-1})	γ_s	$\eta_{Q_{CO_2},Q_{O_2}}$	$\eta_{Q_{O_2},X}$	$\eta_{Q_{CO_2},X}$
1	.069	5.14	-.03	.44	.37
2	.082	5.00	-.15	.46	.38
3	.090	5.17	.05	.45	.38
4	.092	5.50	.21	.41	.37
5	.107	5.92	.34	.39	.38
6	.116	6.09	.37	.37	.37
7	.124	6.24	.37	.35	.35
8	.109	6.68	.41	.30	.32
9	.110	6.60	.41	.31	.33
10	.114	6.34	.38	.33	.34
11	.101	6.41	.38	.32	.33
12	.085	6.44	.38	.31	.32
13	.072	6.32	.35	.30	.31
14	.057	6.30	.31	.28	.28
15	.045	6.00	.23	.27	.26
16	.035	6.18	.25	.25	.25
17	.033	7.39	.44	.22	.27
18	.029	7.86	.48	.22	.28
19	.026	8.54	.52	.21	.29
20	.023	11.59	.61	.19	.36
21	.020	11.94	.61	.17	.33
22	.017	13.54	.63	.16	.35
23	.006	20.00	.64	.06	.20

μ, $\eta_{Q_{O_2},X}$, $\eta_{Q_{CO_2},X}$ and $\eta_{Q_{CO_2},Q_{O_2}}$ are estimated from Equations (43), (44), (45) and (51), respectively.

γ_s is estimated by the ratio available electrons in consumed organic substrate/ g atom carbon in consumed organic substrate. The numerator and denominator are determined with Equations (28) and (31), respectively. The actual value of γ_s for n-hexadecane is 6.125.

Table 5. Examination of data consistency using instantaneous available electron and carbon balances for growth of yeasts on liquid hydrocarbons in batch cultures. Data from Hug and Fiechter [12], Fig. 2 in original paper. Candida tropicalis on n-$C_{16}H_{34}$. (σ_b = 0.462, γ_b = 4.291).

θ (hrs)	μ (hr^{-1})	y_c	d	y_c+d	η	ε	$\eta+\varepsilon$
1	.183	1.19	.46	1.65	.83	.79	1.62
2	.229	.82	.34	1.16	.58	.52	1.10
4	.278	.92	.41	1.33	.64	.59	1.23
5	.236	.63	.34	.97	.44	.51	.95
6	.238	.62	.37	.99	.43	.52	.95
7	.238	.58	.34	.92	.41	.49	.90
8	.274	.76	.38	1.14	.53	.58	1.11
9	.243	.72	.43	1.15	.51	.63	1.14
10	.176	.51	.42	.93	.36	.61	.97
11	.136	.46	.50	.96	.32	.72	1.04
13	.012	.24	.82	1.06	.17	.76	.93
14	.002	.07	.72	.79	.05	.54	.59
Integrated Results		.58	.43	1.01	.41	.60	1.01

η, ε, y_c, d, and μ are estimated from Equations (29), (30), (32), (33) and (43).

Table 6. Values of "true" biomass energetic yield coefficient, n_{max}, and maintenance coefficient, m_e, from growth of yeast on liquid hydrocarbons in continuous culture ($Y_b = 4.291$, $\sigma_b = 0.462$).

Source of data	Results			Comments
	n_{max}	m_e, hr^{-1}	r^2	
Moo-Young et al. [22]				Candida lipolytica on n-dodecane;
Fig. 6	.29	.013	.86	biomass yield data, Y_s; Equation (15)
Fig. 10	.37	.014	.97	O_2 and biomass data. Equation (22)
Blanch and Einsele [2]				Candida tropicalis on $n\text{-}C_{16}H_{34}$
Fig. 9	.38	.0006	.90	Biomass data. Assumption: substrate completely consumed. Equation (15).
Fig. 10	.39	.014	.64	
Fig. 11	.39	.010	.74	
Fig. 12	.40	.010	.80	
Fig. 9	.40	.020	.99	Oxygen and biomass data. Equation (22)
Fig. 10	.41	-	.94	
Fig. 11	.42	.015	.98	
Fig. 12	.46	.012	.99	
Katinger [17]				Candida sp on $n\text{-}C_{16}H_{34}$. Biomass yield data. Equation (15).
Fig. 8	.40	.034	.95	
Fig. 11	.49	.062	1.0	
Fig. 12	.37	.039	.91	
Blanch and Einsele [2]	.47	.033	.93	O_2 and biomass. Fig. 9, 10, 11 & 12. Equation (22)
Katinger [17]	.40	.036	.56	Biomass yield. Fig. 8, 11 & 12. Equation (15)

Table 7. Values of "true" biomass energetic yield coefficient, Y_{max}, and maintenance coefficient, m_e, from growth of yeast on liquid hydrocarbons in batch culture (Y_b = 4.291, r_b = 0.462).

Source of data	Results			Comments
	η_{max}	m_e hr^{-1}	r^2	
Singh et al. [28]		.016		Endomycopsis lipolytica on n-$C_{16}H_{34}$. Endogenous respiration in Warburg flasks. Equation (54).
Miura et al. [21]	.49	.034	.76	Candida intermedia on n-$C_{11}H_{30}$. Oxygen and biomass data. Equation (22).
Moo-Young et al. [22]				Candida lipolytica on n-$C_{12}H_{26}$. Oxygen and biomass data. Equation (22).
Fig. 8	.40	.048	.51	500 rpm.
Fig. 9	.41	.046	.51	250 rpm.
Blanch and Einsele [3]				Candida tropicalis on n-$C_{16}H_{34}$.
Fig. 2	.37	.022	.87	CO_2 and biomass data. Equation (23)
	.45	.064	.66	O_2 and biomass data. Equation (22)
	.51	.087	.96	Respiratory quotient and biomass data. Equations (31), (32) and (25).
Hug and Fiechter [12]				Candida tropicalis on n-$C_{16}H_{34}$. r_b is estimated from graphical differentiation of biomass data (Equation 43).
Fig. 2	.48	.044	.80	O_2 and biomass data. Equations (43) (44) and (25).
	.50	.057	.90	CO_2 and biomass data. Equations (43), (45) and (25).
	.53	.049	.83	Hydrocarbon and biomass data. Equation (43), (46) and (25)
a	.50	.050	.84	O_2, CO_2, hydrocarbon and biomass data. Equations (43), (44), (45), (46) and (25) (Pool of the data used in first three estimates).*

Source of data	Results			Comments
	μ_{max}	m_e (hr^{-1})	r^2	
				λ is estimated without graphical differentiation of biomass data.
b	.43	.042	.53	O_2, hydrocarbon and biomass data. Equations (47), (48) and (25).
c	.48	.059	.99	CO_2, hydrocarbon and biomass data. Equations (49), (50), and (25).
				Estimates of parameters without considering the point of lowest μ.
a	.56	.084	.45	O_2, CO_2, hydrocarbon and biomass data. Equation (43), (44), (45), (46) and (25).
b	.61	.153	.91	O_2, hydrocarbon and biomass data. Equations (47), (48) and (25).
c	.49	.060	.41	CO_2, hydrocarbon and biomass data. Equations (49), (50) and (25).

*95% Confidence intervals for μ_{max} and m_e

$$0.46 \leq \mu_{max} \leq 0.55 \; ; \quad 0.043 \leq m_e \leq 0.057$$

Estimates of parameters with the same letter belong to the same data and calculation technique.

Table 8. Data for growth of Candida tropicalis on n-$C_{16}H_{34}$ in batch culture. Hug and Fiechter [12] (Fig. 2 in original paper). $\gamma_b = 4.291$, $\sigma_b = 0.462$.

Data used	X	X,Q_s	X,Q_{O_2}	X,Q_{CO_2}	Q_{O_2}, Q_s		Q_{CO_2}, Q_s	
θ hrs	$1/\mu_{slope}$	$1/\eta$	$1/\eta$	$1/\eta$	$1/\mu$	$1/\eta$	$1/\mu$	$1/\eta$
0	18.52		2.61	2.25				
1	5.46	1.20	1.95	1.98	22.15	4.86	12.07	2.65
2	4.37	1.74	1.91	2.02	5.29	2.10	5.45	2.17
3	3.88		1.90	2.03				
4	3.60	1.56	1.92	2.07	5.59	2.42	5.60	2.42
5	4.24	2.26	2.16	2.20	3.84	2.05	4.07	2.18
6	4.20	2.32	2.20	2.28	3.77	2.08	4.09	2.26
7	4.20	2.47	2.222	2.26	3.35	1.97	3.67	2.15
8	3.65	1.88	2.08	2.15	4.57	2.36	4.49	2.32
9	4.12	1.98	2.24	2.29	5.61	2.70	5.26	2.53
10	5.68	2,80	2.72	2.62	5.26	2.59	5.04	2.48
11	7.35	3.10	3.22	2.96	8.40	3.54	6.73	2,84
12	20.00		4.09	4.10				
13	83.33	5.93	5.54	6.68	59.86	4.26	175.0	12.46

Blanks: there was not enough data

Figs. 10, 11 and 12 correspond to the graphical representation of Equation (25).

Data from Q_{O_2}, Q_s are depicted in Fig. 10 (Equations (47) and (48) were used.

Data from X,Q_{O_2}, X,Q_{CO_2}, X,Q_s and $1/\mu_{slope}$ are depicted in Fig. 12 (Equations (43), (44), (45) and (46) were used).

APPENDIX

The biomass energetic yield coefficient has been presented earlier [7] as a function of the biomass yield with respect to oxygen as

$$\frac{1}{\eta} = \frac{4}{\gamma_b \, y_o} + 1 \qquad \text{(A-1)}$$

as a function of the biomass yield with respect to carbon dioxide evolution as

$$\frac{1}{y_d} = \frac{\gamma_b}{\gamma_s \, \eta} - 1 \qquad \text{(A-2)}$$

and as a function of the biomass yield with respect to organic substrate as

$$\eta = \frac{\sigma_b \, \gamma_b}{\sigma_s \, \gamma_s} \, Y_s \qquad \text{(A-3)}$$

In addition, the biomass yield coefficients can be expressed by definition as

$$y_o = \frac{\mu \, \sigma_b}{12 \, Q_{O_2}} \qquad \text{(A-4)}$$

$$y_d = \frac{\mu \, \sigma_b}{12 \, Q_{CO_2}} \qquad \text{(A-5)}$$

$$Y_s = \frac{\mu}{226 \, Q_s} \qquad \text{(A-6)}$$

Substituting Equation (A-4) into Equation (A-1), Equation (A-5) into Equation (A-2), and Equation (A-6) into Equation (A-3) gives Equations (44), (45), and (46), respectively.

Equation (48) may be obtained by equating Equations (44) and (46) and solving for $1/u$. Substitution of Equation (48) into Equation (44) gives Equation (47). Combining Equations (45) and (46) and solving for $1/\mu$ gives Equation (50). Equation (49) is obtained by substituting Equation (50) into Equation (45). Equation (52) is obtained by solving Equations (44) for u. Substitution of Equation (52) into Equation (45) gives Equation (51).

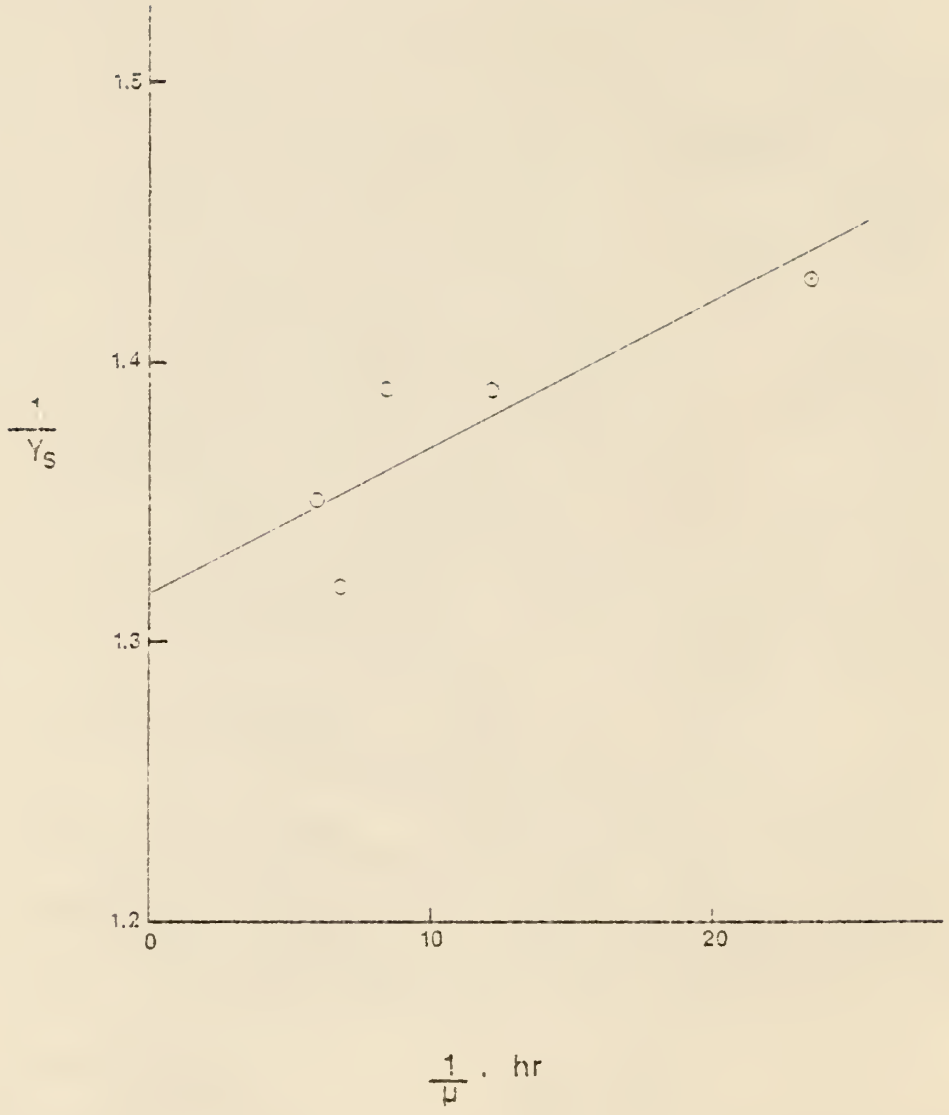

Fig. 1. Relationship between the biomass yield, Y_S, and the specific growth rate, μ, for Candida lipolytica grown on n-dodecane in continuous culture. Data from Fig. 6 of Moo-Young et al. [22]. Graphical representation of Equation (15).

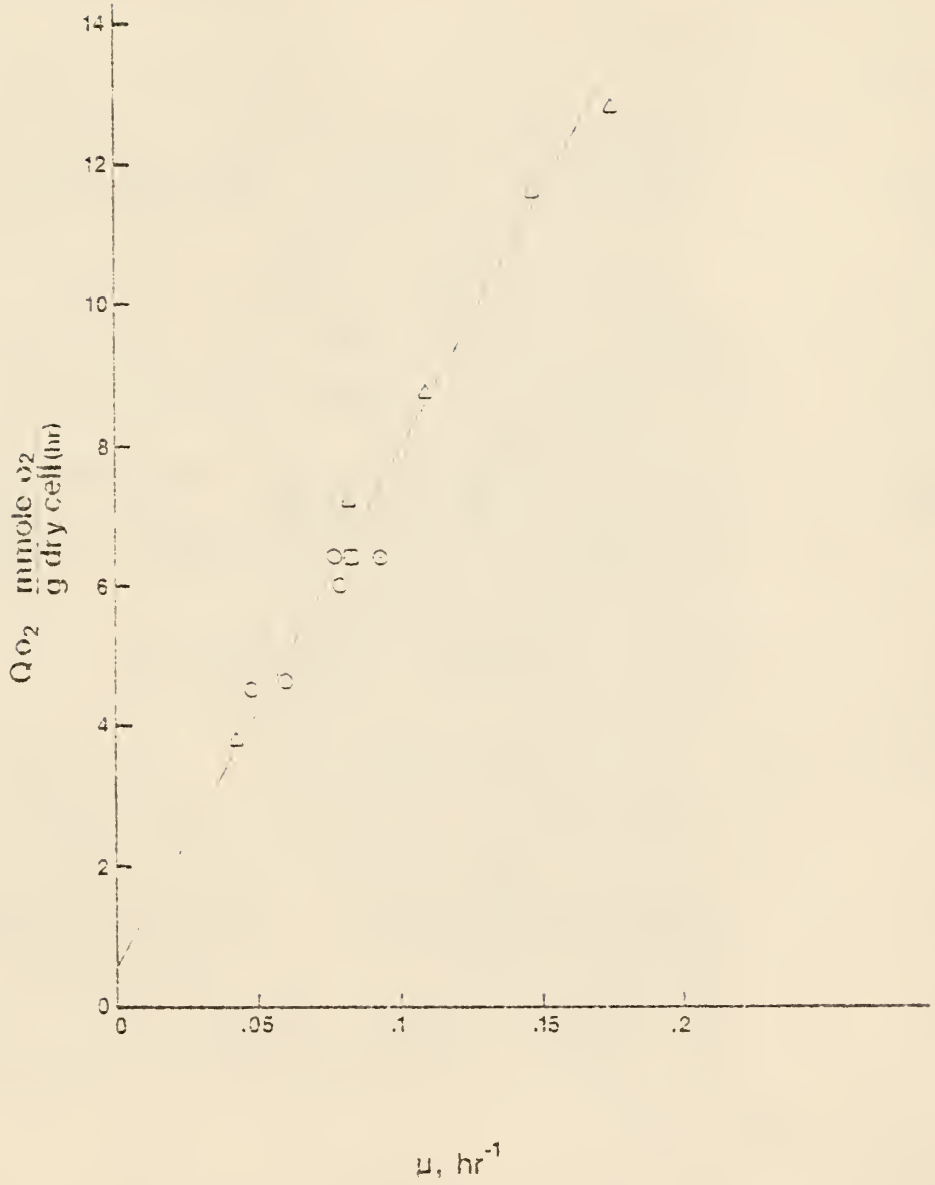

Fig. 2. Relationship between the oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ, for Candida lipolytica grown on n-dodecane in continuous culture. Data from Fig. 10 of Moo-Young et al. [22]. Graphical representation of Equation (22).

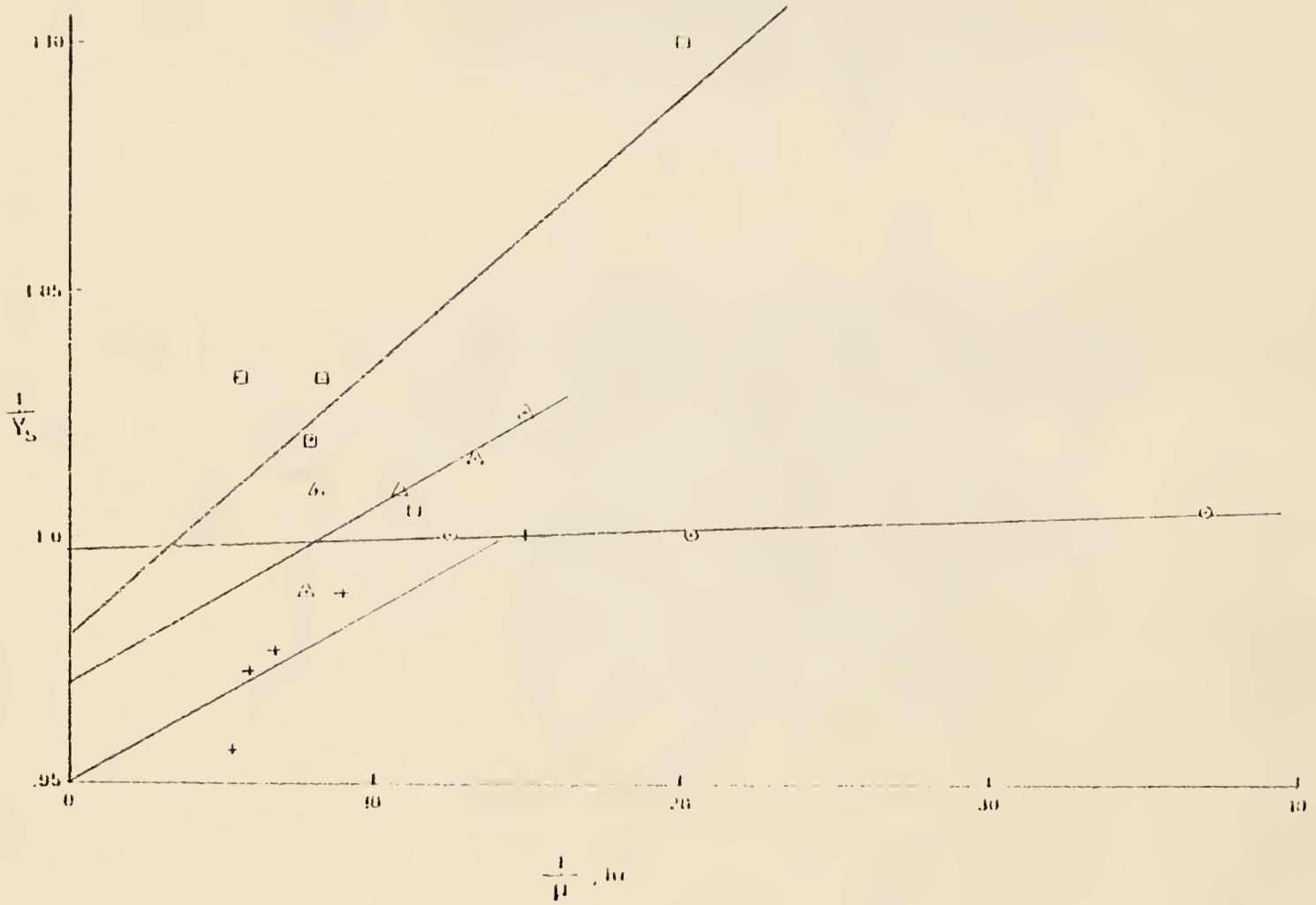

Fig. 3. Relationship between the biomass yield, Y_S, and the specific growth rate, μ, for Candida tropicalis grown on n-hexadecane in continuous culture. Data from Fig. 9 (●), Fig. 10 (□), Fig. 11 (△), and Fig. 12 (×), of Blanch and Einsele [2]. Graphical representation of Equation (15).

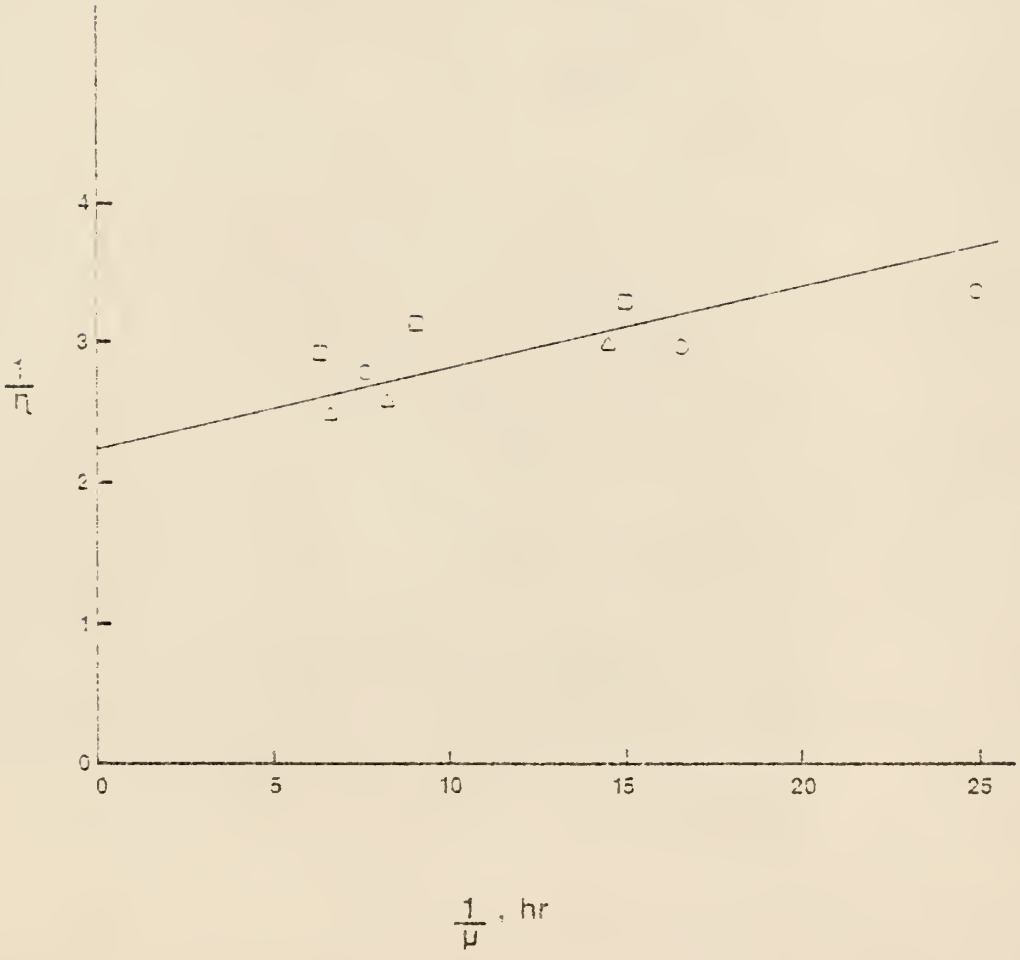

Fig. 4. Relationship between the biomass energetic yield coefficient, η, and the specific growth rate, μ, for Candida sp grown on n-hexadecane in continuous culture. Data from Fig. 8 (⊙), Fig. 11 (△), and Fig. 12 (□) of Katinger [7]. Graphical representation of Equation (15).

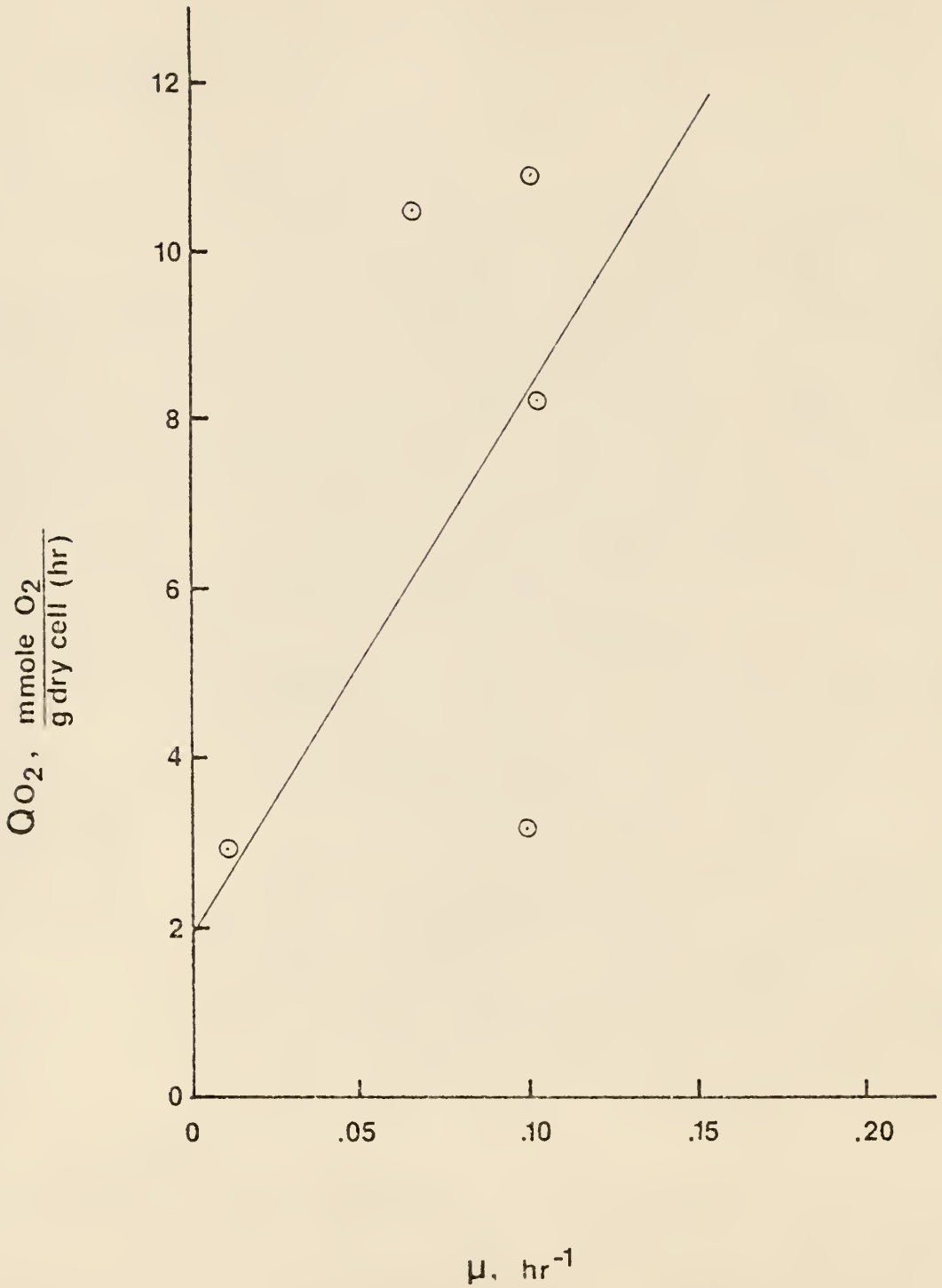

Fig. 5. Relationship between the oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ, for Candida lipolytica grown on n-dodecane in batch culture. Data from Fig. 8 of Moo-Young et al. [22]. Graphical representation of Equation (22). μ is estimated by graphical differentiation of biomass data (Equation (43).

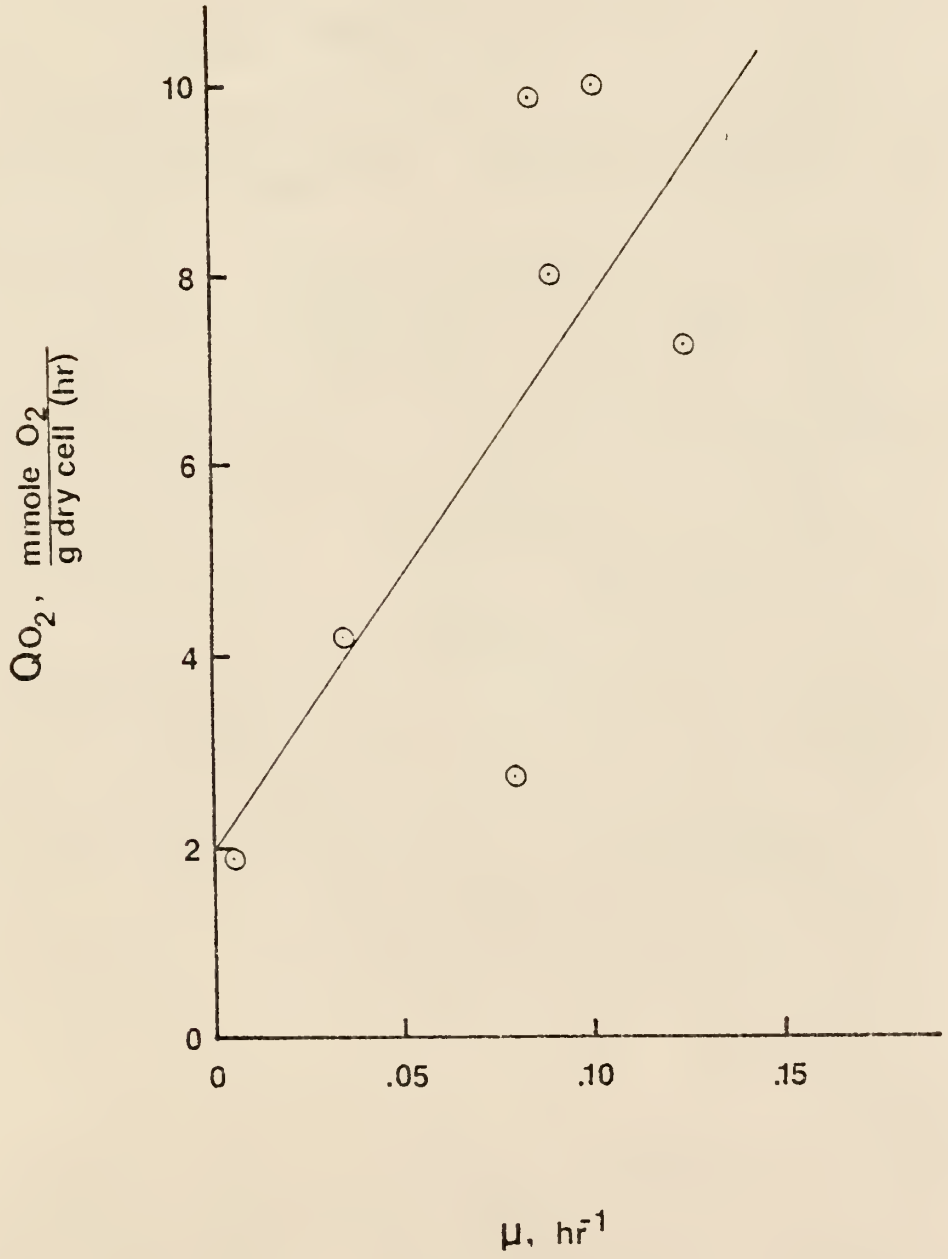

Fig. 6. Relationship between the oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ, for Candida lipolytica grown on n-dodecane in batch culture. Data from Fig. 9 of Moo-Young et al. [22]. Graphical representation of Equation (22). μ is estimated by graphical differentiation of biomass data (Equation 43).

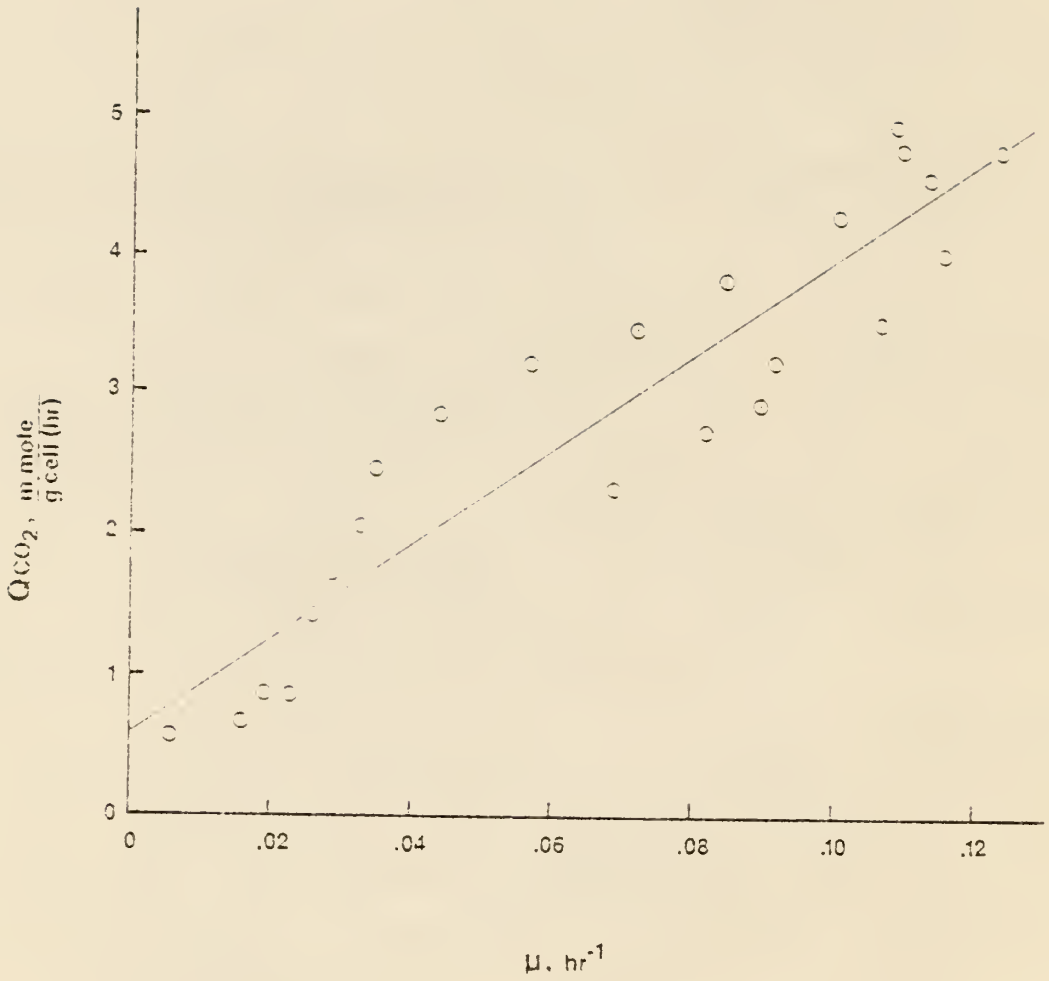

Fig. 7. Relationship between the carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Blanch and Einsele [2]. Graphical representation of Equation (23). μ is estimated by graphical differentiation of biomass data (Equation 43).

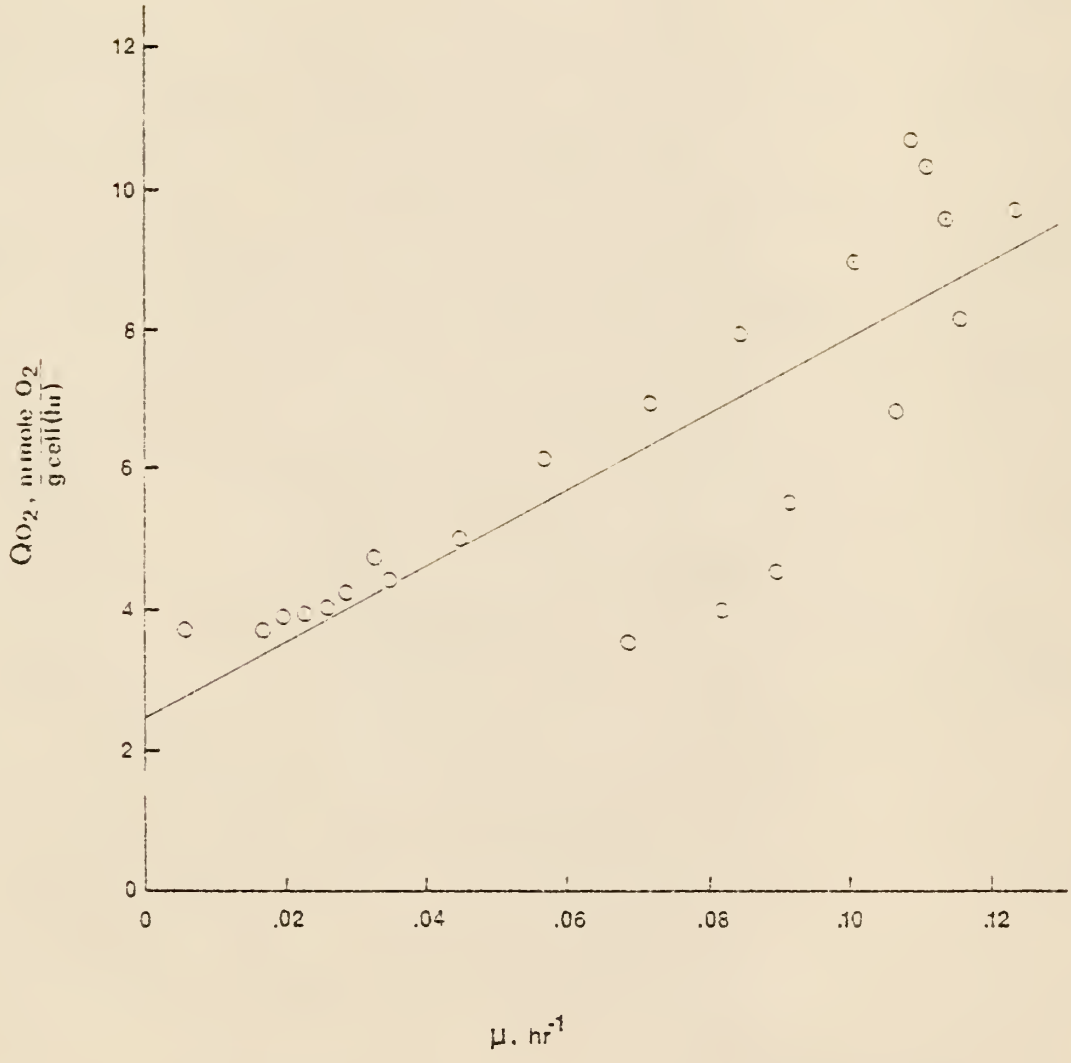

Fig. 8. Relationship between the oxygen uptake rate, Q_{O_2}, and the specific growth rate for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Blanch and Einsele [2]. Graphical representation of Equation (22). μ is estimated by graphical differentiation of biomass data (Equation 43).

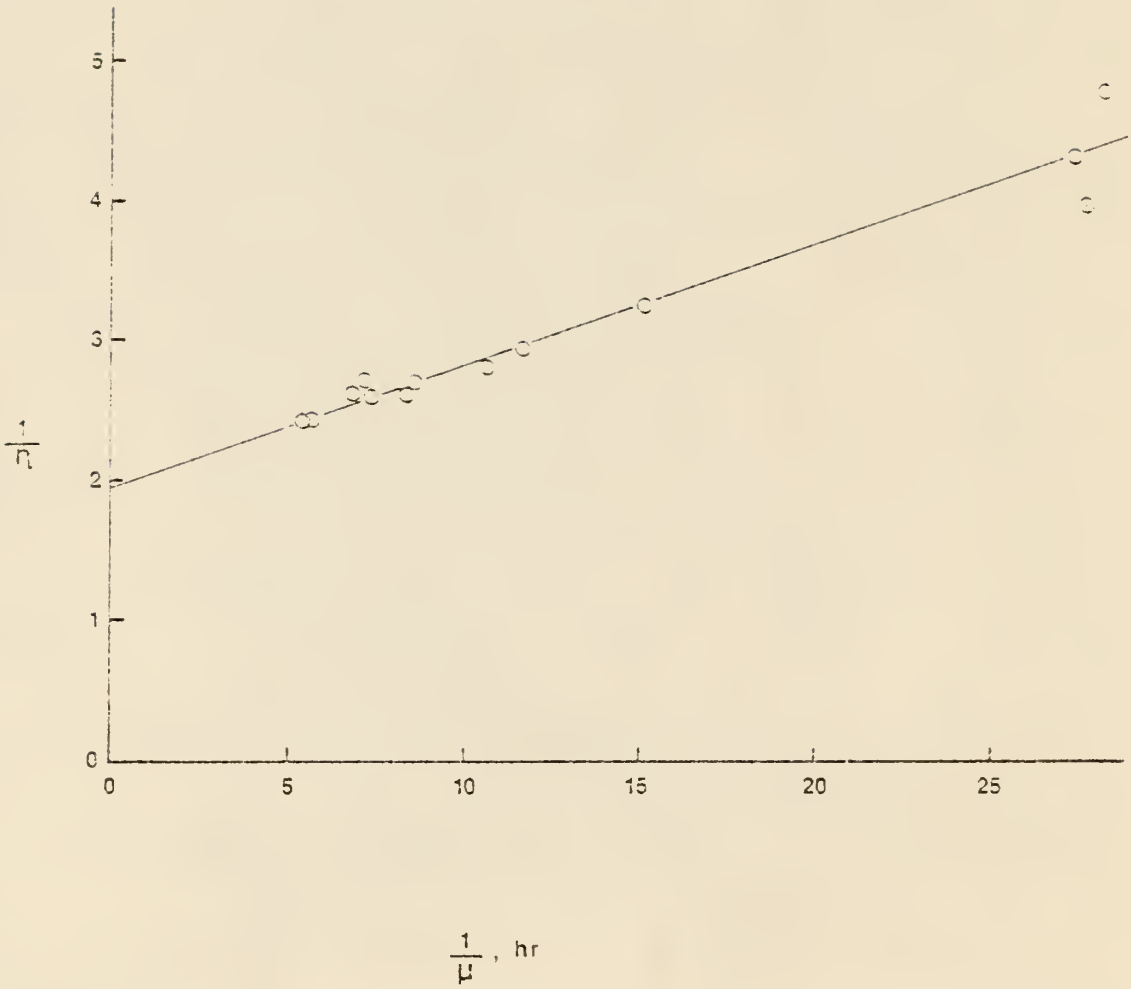

Fig. 9. Relationship between the biomass energetic yield coefficient, η, and the specific growth rate, μ, for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Blanch and Einsele [2]. Graphical representation of Equation (25). η and μ are estimated from respiratory quotient data (Equations 51 and 52).

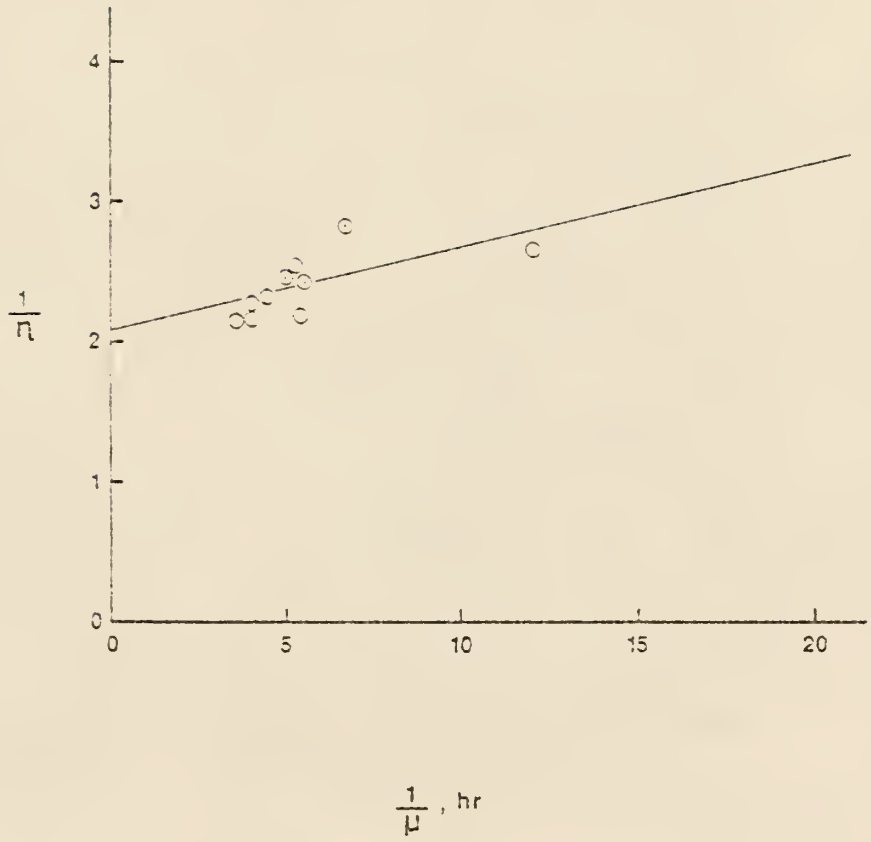

Fig. 10. Relationship between the biomass energetic yield coefficient, η, and the specific growth rate, μ, for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Hug and Fiechter [12]. Graphical representation of Equation (25). η and μ are obtained from Q_{CO_2} and Q_s data with Equations (49) and (50).

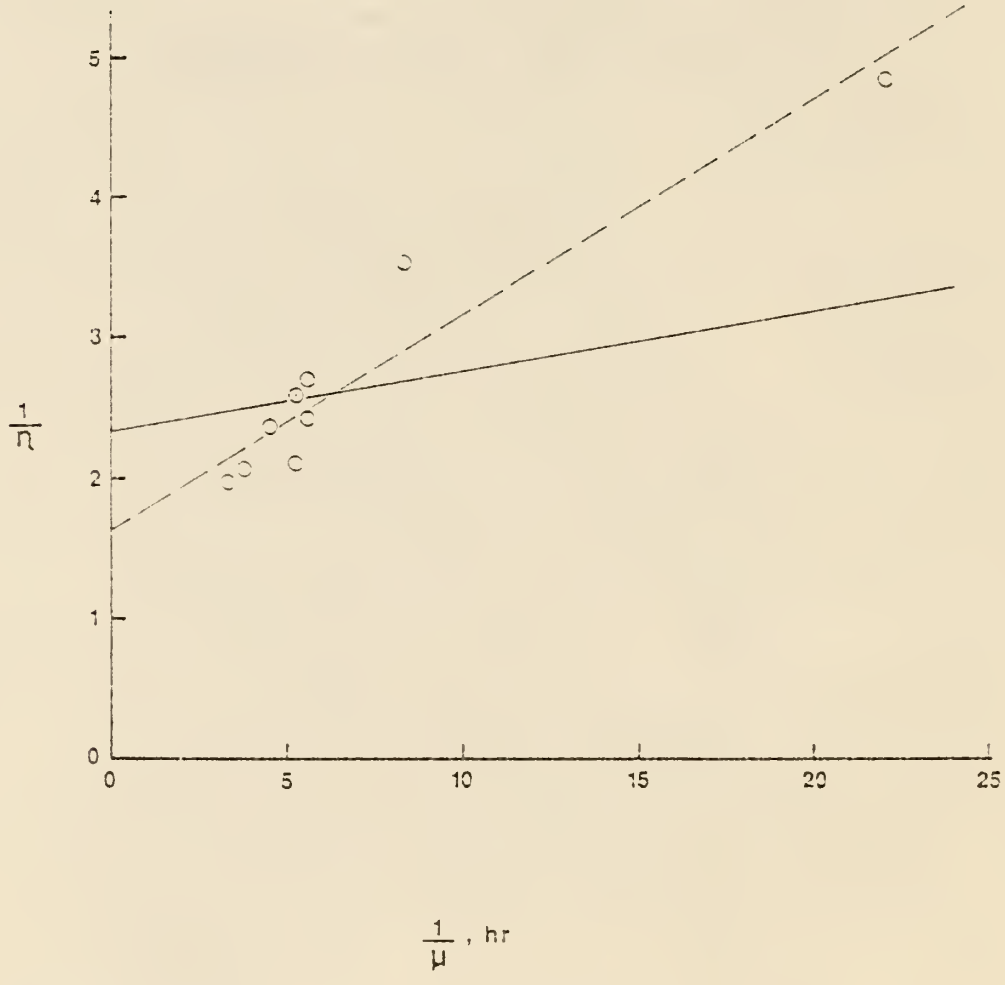

Fig. 11. Relationship between the biomass energetic yield coefficient, η, and the specific growth rate, μ, for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Hug and Fiechter [12]. Graphical representation of Equation (25). η and μ are obtained from Q_{O_2} and Q_s data with Equations (47) and (48). Dashed line shows the linear regression line without considering the point of lowest μ.

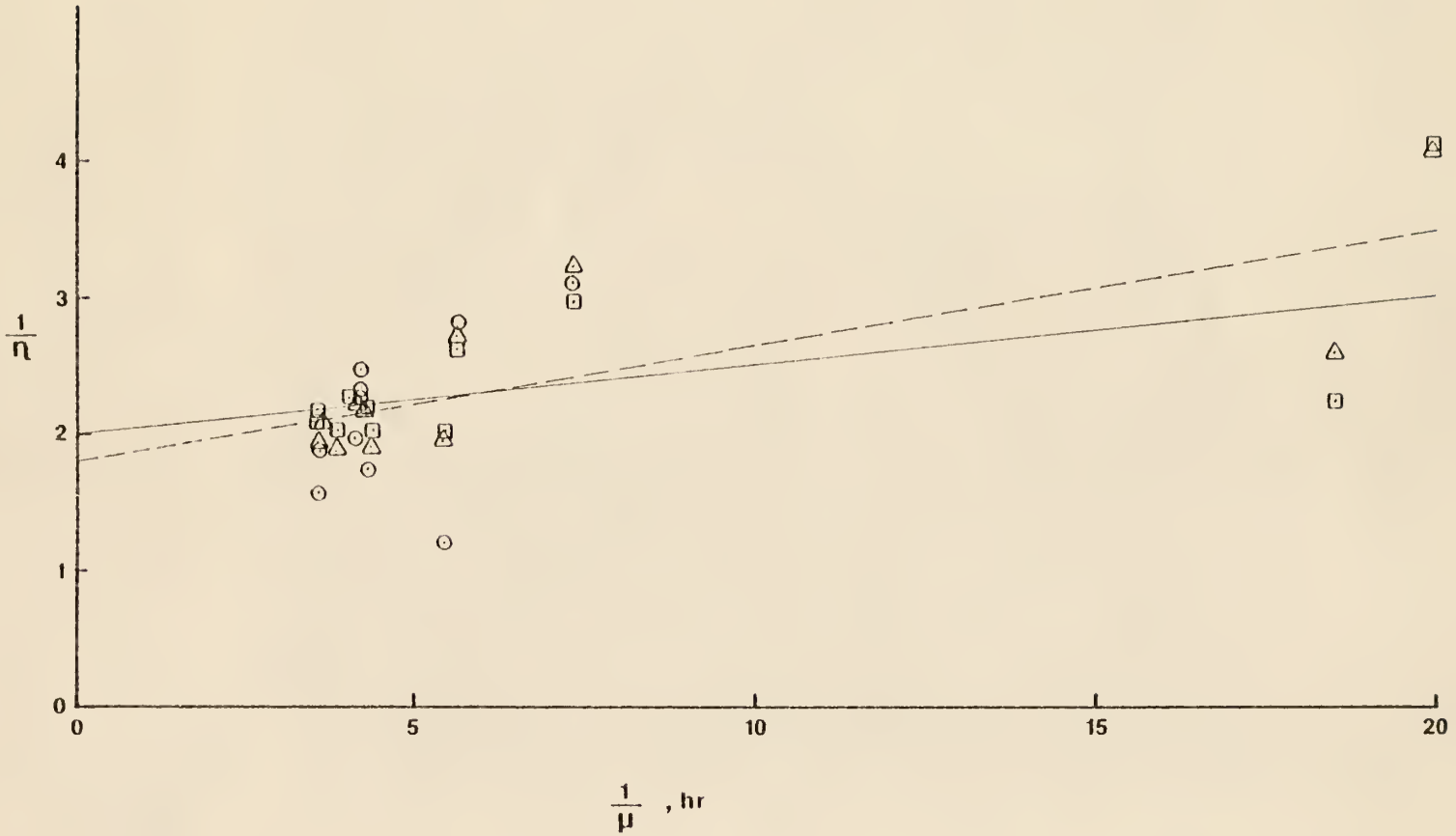

Fig. 12. Relationship between the biomass energetic yield coefficient, η, and the specific growth rate, μ, for Candida tropicalis grown on n-hexadecane in batch culture. Data from Fig. 2 of Hug and Fiechter [12]. Graphical representation of Equation (25). μ is estimated by graphical differentiation of biomass data (Equation 43). η is estimated from Q_s, (⊙) (Equation 46), from Q_{O_2}, (△) (Equation 44), and from Q_{CO_2}, (⊡) (Equation 45). Dashed line shows the linear regression line without considering the point of lowest μ.

CHAPTER III

DATA CONSISTENCY, YIELD, MAINTENANCE, AND HYSTERESIS IN BATCH CULTURES OF *CANDIDA LIPOLYTICA* GROWN ON N-HEXADECANE

INTRODUCTION

In the second chapter of this work, the consistency of reported experimental data from growth of microorganisms in petroleum hydrocarbons was examined using the material and energy balance regularities which Minkevich and Eroshin have identified [5,11]. Both batch and continuous cultures were analyzed, and the "true" biomass energetic yield and the maintenance coefficient were estimated from different sets of data. However, further effort is needed to better appreciate the quality of estimates of η_{max} and m_e which can be obtained in batch culture. This work was designed to obtain sufficiently consistent experimental data to estimate η_{max} and m_e in hydrocarbon fermentation. The author is not aware of other batch culture experiments that have been carried out for this purpose. The yeast Candida lipolytica was cultured batchwise on n-hexadecane as the main carbon source. Biomass production, substrate consumption, oxygen consumption and carbon dioxide evolution were measured throughout the fermentation. The consistency of the data obtained was examined using integrated and instantaneous available electron and carbon balances. Growth characteristics and difficulties involved in the analytical techniques are discussed.

Values of η_{max} and m_e were estimated from three different sets of data; biomass and oxygen data, biomass and carbon dioxide data, and biomass and organic substrate data are used. Comparison of estimates is useful to further test the consistency of the data, and to evaluate the consistency of the estimates.

Estimates of η_{max} and m_e were examined, and compared to values estimated from data in the literature in the second chapter.

Hysteresis patterns were seen in plots of specific rates of oxygen consumption and carbon dioxide evolution versus the specific growth rate. The relationship of the hysteresis to growth characteristics and estimates of η_{max} and m_e is examined.

MATERIALS AND METHODS

Equipment

A New Brunswick Scientific Model 19 5ℓ stirred tank fermenter was used in the cultures. Operations were always aseptic.

Air was supplied with a sparger that was located at the bottom of the vessel. The air passes through a rotameter before entering the fermenter. Agitation was provided by a magnetically coupled flat blade turbine agitator.

Temperature was controlled at 30°C with a thermostat. Hot or cold water was passed automatically through a coil located inside the vessel. A thermistor sensing bulb was connected to the thermostat, and a temperature sensing bulb to a recorder. Both probes were placed in closed tubes deep in the vessel. Temperature readings were continuously recorded.

The pH was maintained automatically at 5.5 by addition of 2N NaOH, or, 2N H_2SO_4 using a New Brunswick Scientific model pH-22 system. The pH was continuously recorded.

Dissolved oxygen was continuously measured and recorded using a New Brunswick Scientific model DO-82 system.

Foaming was controlled mechanically with a foam breaker.

Strain

Candida lipolytica ATCC 8662 was cultured using n-hexadecane as the main source of carbon and energy.

Inoculation

Yeast cells from a slant culture, which was incubated at 30°C for 24 hours, were inoculated into 500 mℓ shake flasks, each containing 100 mℓ of medium. After 24 hrs. cultivation, three of these flasks were inoculated into 2.5 ℓ of medium in the fermenter.

Culture Media

The main culture media was based on the simplified media of Aiba et al [1]. It contained:

$(NH_4)_2SO_4$	7.5 g/ℓ	$FeSO_4 \cdot 7H_2O$	30×10^{-3} g/ℓ
Na_2HPO_4	1.5	$CaCl_2 \cdot 2H_2O$	60×10^{-3}
KH_2PO_4	3.5	$CuSO_4 \cdot 5H_2O$	15×10^{-6}
$MgSO_4 \cdot 7H_2O$	0.5	$Na_2B_4O_7 \cdot 10H_2O$	30×10^{-6}
nutrient broth	2.0	$MnCl_2 \cdot 4H_2O$	60×10^{-6}
		$ZnSO_4 \cdot 7H_2O$	150×10^{-6}
		$Na_2MoO_4 \cdot 7H_2O$	30×10^{-6}

2%(w/v) n-hexadecane was used as the main source of carbon. The medium used for the preculture and stock had the following composition: 40 g of glucose, 2 g of nutrient broth and 1000 mℓ distilled water. For the stock culture, 15 g/ℓ agar was used. Shake flasks, each containing 100 mℓ. of the medium, were autoclaved at 20 psia for 15 minutes. Fermenter was autoclaved at 20 psia for 30 minutes.

Sampling

Liquid samples were taken by applying a back pressure and allowing the culture fluid to run out. The first 50 mℓ were discarded, and 30-50 mℓ samples were usually collected.

Analytical Methods

Biomass Concentration. Samples containing at least 40 mℓ were taken from the fermentation broth, and homogenized manually. From this, a 5 mℓ sample was removed and combined with 15 mℓ. of a mixed solvent containing butanol,

ethanol and chloroform (10:10:1 v/v). After mechanical shaking, the mixture was centrifuged at 3500 rpm for 15 minutes. The cells were washed with 5 mℓ of distilled water, centrifuged, resuspended in 5 mℓ of distilled water, and dried in an oven at 105°C for 24 hours. Concentration was calculated in g/ℓ. These values were used to estimate the specific growth rate.

Hexadecane Concentration. This was determined using gas chromatography after extraction with a mixed solvent. Samples containing at least 40 mℓ were taken from the fermenter and homogenized manually. From this, a 20 mℓ sample was removed and extracted in a separatory funnel with a 30 mℓ hexane-chloroform mixture (1:1 v/v). The lower and middle phases were collected. The upper phase was extracted again with 20 mℓ of the mixture of solvents and the lower phase was collected. The collected volumes were filtered in a 0.45 μm millipore filter. Final filtrate volumes were recorded prior to chromatography since there was evaporation of solvents with time. Five mℓ of the filtrate were used as chromatographic samples and 0.1 mℓ n-tetradecane was added. This alkane was used as an internal standard for chromatography. The operating conditions of the gas chromatograph were as follows: the column was 1 m x 1/8 inch stainless steel column packed with 10% SE-30 (methyl sylicone) chromosorb WHP; helium was used as the carrier gas at a flow rate of 30 mℓ/min.; the temperature was 170°C (isothermal operation); the detector was thermal conductivity. Hexadecane concentration was calculated in g/ℓ and plotted against time.

Carbon Dioxide and Oxygen Concentrations in the Gas Phase. Gas samples were taken from the exit gas stream of the fermenter after the liquid samples had been taken. Generally, after 4 hours, the concentrations of O_2 and CO_2 by volume were measured with a Beckman Oxygen Analyzer OM-11, and a Beckman

Medical Gas Analyzer LB-2, respectively. The sample passed in parallel flow to both analyzers, simultaneously. The oxygen analyzer was calibrated at 20.9% oxygen in the air, and the carbon dioxide analyzer was calibrated with a 5% CO_2 standard. Some moisture was removed from the samples by passing them through drierite before entering the analyzers. The initial oxygen and carbon dioxide concentrations in the air were determined taking a gas sample from the exit of the fermenter filled with the medium and at operating conditions prior to inoculation. The readings were always 20.8% O_2, and, 0.045% CO_2 in the air. These initial concentrations were used in the calculation of oxygen uptake and carbon dioxide evolution. These values together with the biomass data are used to evaluate the specific rates of oxygen consumption, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}. The respiratory quotient, R.Q. = Q_{CO_2}/Q_{O_2}, was also calculated, and used to estimate the biomass energetic yield, η.

Methods of Data Analysis

Specific Growth Rate. This variable is defined in the second chapter with Equation (43). A smooth curve was drawn through the biomass concentration versus time data points, and the slopes at the sampling times were visually read with an adjustable triangle. The ratio of the slope, dX/dt, to the value of X at the same point in time gives the estimate of the specific growth rate, μ.

Rate of n-Hexadecane Consumption. This variable is defined as follows;

$$Q_S = \frac{1}{X}\frac{dS}{dt} \qquad (1)$$

A smooth curve was drawn through the hexadecane concentration versus time data points, and the slopes at the sampling times were visually read with

an adjustable triangle. The ratio of the slope, dS/dt, to the value of X at the same point in time, gives the estimate of the rate of n-hexadecane consumption, Q_S. Values of Q_S appear tabulated in Tables 5, 6, 7 and 8 for Runs 3, 4, 5 and 6, respectively. Consumption of nutrient broth was not included in calculating Q_S.

Volume of broth

The initial volume of broth was measured. Corrections were made in the broth volume to account for the removal of samples from the fermentor. The volume of broth removed during each sampling period was measured and subtracted from the fermentor broth volume prior to sampling to obtain an estimate of the remaining volume of broth in the fermenter. Corrections in volume were not made for base addition, evaporation of water, nutrient consumption and formation of biomass and other products. These values were estimated and found to approximately balance each other except in Run 3 where evaporation may have exceeded base addition. Errors in liquid volume should be less than 2%.

Air Flow Rate. A rotameter was used to measure the flow rate of the air entering the fermenter. This rotameter was calibrated by measuring the volume of water displaced by the air from a vertical graduated cylinder in a certain period of time. The air flow rate measured this way in ℓ/min. is divided by the volume of broth to find the air flow rate per unit of broth volume, Q_g, in ℓ/ℓ hr. This value is then corrected to standard conditions to find the air molar rate per unit of broth volume, Q_m.

$$Q_m = Q_g \times \frac{1 \text{ mol gas}}{22.4 \text{ ℓ}} \times \frac{273°K}{303°K} \qquad (2)$$

This molar flow rate was used as the exit molar air flow rate in the calculation of the rates of oxygen consumption and carbon dioxide evolution. Equation (2) shows that no correction was made for pressure; 1 atmosphere pressure

was assumed. The fact that the respiratory quotient was smaller than 1 makes the exit air flow rate on a dry basis lower than the inlet flow rate. However, water evaporates from the fermenter throughout the fermentation, and it was found that this increases the exit air flow rate and approximately compensates for this difference. Thus, there are relatively small errors in using the inlet molar air flow rate.

Rate of Oxygen Consumption. The calculation of this variable requires the measurement of the volume of broth, the air flow rate, and the concentration of oxygen in the inlet and exit of the fermenter. The rate of oxygen consumption is

$$Q_{O_2}X = \frac{(\%\, O_2 \text{ inlet} - \%\, O_2 \text{ exit})}{100} \times Q_m \qquad (3)$$

The specific oxygen consumption rate, Q_{O_2}, was evaluated by dividing the foregoing variable by X. Values of Q_{O_2} appear tabulated in Tables 5, 6, 7 and 8 for Runs 3, 4, 5 and 6, respectively. These results appear in graphical form in Figures 2 and 4 of the appendix, Figure 2 of this chapter, and Figure 6 of the appendix, respectively.

Rate of Carbon Dioxide Evolution. Similar measurements are required to estimate Q_{CO_2}; that is,

$$Q_{CO_2}X = \frac{(\%\, CO_2 \text{ exit} - \%\, CO_2 \text{ inlet})}{100} \times Q_m \qquad (4)$$

The specific carbon dioxide evolution rate, Q_{CO_2}, was evaluated by dividing the foregoing variable by X. Values of Q_{CO_2} appear tabulated in Tables 5, 6, 7, 8 for Runs 3, 4, 5, 6, respectively. These results appear in graphical form in Figures 2 and 4 of the appendix, Figure 2 of this chapter, and Figure 6 of the appendix, respectively.

Nitrogen Balance. When extracellular products are negligible, the nitrogen balance can be written as [5]

$$a = y_c q \tag{5}$$

Based on 28 experiments [12], an average value of q, the atomic ratio of nitrogen to carbon in biomass, was 0.165 with a coefficient of variation equal to 20%. This value can be compared to the value of q found from Equation (5) to test the consistency of the data. The biomass carbon yield can be estimated from Equation (8) of Second Chapter, and a, the moles of ammonia per quantity of organic substrate containing one g atom carbon, is estimated as follows

$$a = \frac{NH_3 \text{ consumed, moles}/\ell}{(S_o - S_f) \cdot \frac{\sigma_s}{12}} \tag{6}$$

Ammonia consumption was not directly measured. Instead, the addition of base required to keep a constant pH was measured. The equivalents of base added per liter of broth are assumed to be equal to the equivalents of ammonia consumed per liter of broth.

True Grow Yield and Maintenance Coefficient. The "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, have been estimated from three different sets of data using the following equations from Chapter 2. Oxygen and biomass data were used in Equation (22) together with Equations (16) and (19) to find the parameters in appropriate units; when carbon dioxide and biomass data were used, the parameters were estimated with Equations (23), (17) and (20); and when organic substrate and biomass data were used, the parameters were estimated with Equations (24), (18) and (21). The results of fitting straight lines to Equations (22), (23), and (24), by linear least squares, appear in graphical form in Figs. 4-15.

RESULTS AND DISCUSSION

The first part of this section describes the growth characteristics of the yeast. Results are presented in Tables 1-16 and Figures 1-15.

The second part of the discussion is an analysis of the experimental techniques and experimental errors associated with them that could influence the results. Table 11 shows the experimental conditions of the batch cultures and Table 12 presents a list of the main problems encountered in the measurements.

In the third part of the discussion, the consistency of the data is examined and the results of making available electron and carbon balances are shown in Tables 9, 13, 14, 15 and 16. The "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, were estimated from different sets of data, and the comparison of these estimates is also used to examine the consistency of the data. The results are shown in Table 10.

Hysteresis patterns are identified in plots of specific rates of oxygen consumption and carbon dioxide evolution versus specific growth rates (Figures 4, 5, 7, 8, 10, 11, 13 and 14). These patterns are examined in the fourth part of this section, and possible explanations for this phenomenon are given.

In the last part of the discussion, the estimated values of η_{max} and m_e are examined and discussed taking into account available information on the consistency of the data. The η_{max} and m_e values from four fermentations are compared. These results are also compared to estimates obtained from data in the literature. Variations in experimental conditions and information from the measured variables are also considered in examining the values of the yield and maintenance coefficients.

Growth Characteristics

Shake flask Experiments. Several experiments were carried out to find appropriate conditions for the growth of Candida lipolytica. This organism was grown in a basal medium (described in Materials and Methods Section) with variable concentrations of nutrient broth from 0.05 to 0.5%. The final cell concentrations were always lower than 2 g/ℓ when the concentration of nutrient broth was lower than 0.2%. Thus, some factor appeared to be limiting in this condition.

Other experiments showed that the optimum incubation time for the inoculum was 24 hrs., and that changes in the hexadecane concentration from 10 to 20 g/ℓ did not affect growth levels.

Fermenter Cultures. Fermentations usually stopped about 28 hours after inoculation. Fermentations carried out in the past [7] showed longer times. Nutrient broth was used in considerably lower concentrations this time and that is why some factor in it is believed to be limiting after 28 hours. The initial $(NH_4)_2SO_4$ concentration was increased from 5.0 to 7.5 g/ℓ.

Yeast cells were seen surrounding oil droplets under the microscope. Most of the cells grew in the oval, yeast-like shape.

Accumulation of oil, cells and foam as flocs was observed in most of the fermentation. When the broth was agitated manually, pH decreased immediately, showing that considerable activity of the microorganisms occurred at the surface of the broth. At high cell concentrations, flocculation did not occur. This may have been due to the presence of surface active materials secreted by the cells in sufficient amounts to lower the interfacial tension such that floculation did not occur. [7].

Tables 1-4 show the measured variables in the fermentation runs. Measured variables were the volume of the broth, percent CO_2 and O_2 in the exit gas stream of the fermentor, and biomass and hexadecane concentration. Growth reached reasonable levels in all the fermentations. The final cell concentrations were 8.2, 5.19, 5.95, and 7.5 g/ℓ, in Runs 3, 4, 5 and 6, respectively. The maximum substrate consumption occurred in Run 6, where 44% of the initial alkane was consumed. Table 1 shows that the difference in the exit CO_2 and O_2 concentrations with respect to the inlet CO_2 and O_2 concentrations was small for Run 3 compared to Runs 4, 5 and 6 (Tables 2, 3 and 4). This is due to the fact that the air flow rate was higher in Run 3. Because of the instability of carbon dioxide measurements at low CO_2 concentrations, the air flow rate was decreased considerably (from 1 ℓ/min to 0.375 ℓ/min) in order to detect higher differences. First readings of percent CO_2 can be seen to be very near the initial concentration, so that measurements at the beginning are the most inaccurate.

Figure 1 shows the change in biomass and substrate concentration with time in Run 5. Figures 1, 3 and 5 in the appendix show these results for Runs 3, 4 and 6. It can be seen that most of the growth occurred after twelve hours of fermentation. The fact that biomass and substrate concentrations do not greatly change in the early hours, makes these measurements more difficult.

Table 5, 6, 7 and 8 show the values of specific growth rate, μ, specific rate of consumption of oxygen, Q_{O_2}, specific rate of evolution of carbon dioxide, Q_{CO_2}, specific rate of organic substrate consumption, Q_S, and respiratory quotient, R.Q. Table 9 shows the results of making integrated available electron and carbon balances to check the consistency of the data of Runs 3, 4, 5 and 6. It can be seen from this table that the

biomass carbon yield, y_c, ranges from 0.45 to 0.51; the fraction of organic substrate carbon evolved as carbon dioxide, d, ranges from 0.40 to 0.49; the biomass energetic yield, η, ranges from 0.32 to 0.36, and the fraction of organic substrate energy evolved as heat, ε, ranges from 0.68 to 0.72. The coefficients of variations of these parameters are 5.5, 9.4, 5.0 and 2.5%, respectively; this shows that the results are reproducible. These values are in very good agreement with those found with data from the literature (Table 2 in Chapter 2).

Higher specific growth rates were achieved in Runs 4 and 5 (0.168 and 0.164 hr^{-1}) where the final cell concentrations were the smallest (Tables 6 and 7). Biomass energetic yield values, however, are not significantly different. Tables 5, 6, 7 and 8 show that the specific rates of oxygen consumption, carbon dioxide evolution and hexadecane consumption reached a maximum at approximately the maximum value of the specific growth rate as expected. This occurred between 18 and 22 hours of fermentation. At this point in time, the respiratory quotient was also near a maximum or at a maximum, and it remained almost constant until the end of the fermentation. The value of the respiratory quotient was always very small at the beginning, and it continued to increase during the first 12 hours. This may be due to lower carbon dioxide evolution compared to oxygen consumption because of initial steps of n-hexadecane oxidation, the inaccuracy of the CO_2 analyzer and carbon dioxide (as well as bicarbonate) accumulation in the broth. Final values of respiratory quotient were between 0.40 and 0.45. Figure 2 shows the change in Q_{O_2} and Q_{CO_2} with time for Run 5. Figures 2, 4 and 6 of the Appendix show these results for the other runs. They show maximum values between 18 and 22 hours as mentioned earlier.

Oxygen did not appear to be limiting, except in Run 6 where the reading of dissolved oxygen was zero in the last hours of the fermentation. In the other runs, the dissolved oxygen concentration was never lower than 10% of saturation.

There was almost no addition of base during the first 12 hours as Figure 3 shows in Run 5. This means that ammonia nitrogen consumption was small at the early stages of fermentations and did not bring about appreciable changes in pH. In addition, acid was always added prior to base, showing that pH tended to increase initially. This could be due to the effect of CO_2 and bicarbonates dissolved in the medium, or liberation of basic substances from the consumption of peptones.

The relationship between Q_{O_2}, Q_{CO_2}, and Q_S with the specific growth rate, μ, is presented graphically in Figures 4-15 for all the runs. A straight line was fitted to all the data by least squares, and they showed high correlation coefficients. These lines show that Q_{O_2}, Q_{CO_2} and Q_S are directly proportional to μ. Estimates of "true" biomass energetic yield, η_{max}, and maintenance coefficient, m_e, are obtained with these regression lines. The results are presented in Table 10. These results will be discussed later in detail.

Experimental Techniques

The most significant problem encountered in the experiments was to take a representative sample from the fermentation broth. Biomass could be seen adhering to the fermenter walls and baffles. Agitation was often insufficient to avoid this problem. For this reason, the last three fermentations were carried out at the maximum speed of 1000 rpm. Foam was always seen at the surface, and biomass was associated with it. Although a higher agitation speed produced more foam, a more uniform broth was obtained because stagnant foam regions were greatly reduced. However, foam made final samples more difficult to take and treat. About 10% of the sample volume appeared to be foam. The agitation was always shut off for 30 sec, and the system was agitated manually to make a uniform broth before taking a sample. Duplicate samples were taken periodically and the comparison of the two measurements showed differences up to 2.5%. This is a small difference, but it is significant in measurements at the early stages of the fermentation from 0 to 12 hrs when growth was usually very small. In addition, 2.5% variation may affect the estimation of the specific growth rates significantly. Hug and Fiechter [9] reported cell lipid extraction during sample treatment with this same mixture of solvents. Dry weights were lower than they should be. This can be a significant source of experimental error in this work. After extraction, biomass was calculated as dry weight. Optical density measurements were carried out, but it was not possible to prepare a good standard curve. This was probably due to changes in cell shape and density.

The problem of taking a representative sample is also reflected in the hexadecane measurements. Hexadecane is less dense than water and

might be non-uniformly distributed in the broth. The presence of baffles and probes prevented vortex formation and this reduced the efficiency of vertical mixing. When the liquid sample was subject to a one step hexadecane extraction procedure, 66% was the highest extraction. When a second extraction was carried out, the lowest extraction was 92% compared to the initial hexadecane concentration. Degree of agitation of the sample, the mixture of solvents, and time of settling to allow the phases to be formed are important features of the process. Severe mechanical agitation makes an emulsion that takes a long time to produce separable phases. Low agitation produces low hexadecane extraction. Three phases could be seen in the separatory funnel when fermentation broth with cells was subjected to hexadecane extraction. The upper phase is a water phase and hexadecane was not detected after two extractions. This phase contains substances that have the same retention time as n-tetradecane, the internal standard used in chromatography. The middle phase was mainly comprised of cells, and the lower phase was a chloroform-hexane-hexadecane phase. The middle phase is very thin at the beginning of the fermentation, and it increases with fermentation time. After a while, the middle and lower phases are indistinguishable. Therefore, both middle and lower phases were always taken together from the separatory funnel. This extract was filtered in a millipore filter to avoid microorganisms in the chromatographic column. The thermal conductivity detector, using helium as the carrier gas, is not the most sensitive method. However, reproducibility was extremely good, giving variations lower than 2%. The results of Runs 1 and 2 are not shown in this work because the methods of measuring biomass and hexadecane concentrations were not standardized yet, and results were

extremely inconsistent. Changes in the substrate concentration were so small in the first hours of fermentation that the substrate values were within the error range of the analytical techniques. This influences greatly the estimation of slopes in the S vs. time curve, and therefore the value of Q_S and results of instantaneous balances.

Gas samples were taken with aluminum bags, and carbon dioxide and oxygen measurements were usually carried out after several samples had been collected in a four hr. time period. Experiments were carried out to find out whether there were significant losses of gas from the bags due to diffusion. Carbon dioxide and oxygen concentrations were measured after taking the sample and four hours later; no differences were detected.

Carbon dioxide concentration readings from the CO_2 analyzer were very unstable at low concentrations of CO_2. This was probably due to the fact that carbon dioxide concentrations are very small and close to the concentration in the air in the first hours of fermentation. This makes the results at this stage of the fermentation very inaccurate. On the other hand, oxygen measurements were always very stable, but the analyzer gives only three significant figures and this influences the accuracy of the measurements.

Table 1 shows O_2 and CO_2% in the exit gas stream in Run 3, and it can be seen that differences between the concentrations in the inlet gas and exit gas are smaller than those in Run 4, 5, 6 (Tables 2, 3, 4). The air flow rate was decreased in the last three runs to detect greater differences between the inlet and exit gas O_2 and CO_2 concentrations, making the measurements more accurate. Appropriate air flow rates were calculated with oxygen balances assuming that the gas flow rate is equal in both the inlet

and exit, that oxygen concentrations are uniform throughout the fermenter, and that oxygen uptake by the cells is 1 gO_2/ℓ hr.

Curves were prepared [6] to follow the progress of the fermentations and rapidly obtain the value of η. This instantaneous information would be useful to make any changes in operating conditions. Unfortunately, dry weights gave late feedback, and hexadecane, carbon dioxide and oxygen measurements could not be done immediately after sampling.

Dissolved oxygen measurements were carried out in order to use oxygen balances to find oxygen transfer rates, but unfortunately, the probe usually became plugged during fermentations. Besides this, unstable readings appeared in almost all the fermentations except at the beginning. Deviations were usually higher than 10%. Readings were more unstable as the biomass concentration increased.

The pH could not be automatically controlled in Runs 3 and 4 because the pH probe became plugged. The pH was controlled by measuring the pH of the samples, and adding base or acid as required to bring the pH back to 5.5.

Ammonia consumption is assumed to be equal to the net equivalents of base added per liter of broth. The calculation was only made in Run 5 where pH was precisely controlled.

Consistency of the data

Table 9 shows the results of an examination of data consistency using integrated available electron and carbon balances for the growth of Candida lipolytica on n-hexadecane in Runs 3, 4, 5 and 6. It can be seen that the data are quite consistent since $(y_c + d)$ and $(\eta + \varepsilon)$ are close to 1. The 95% confidence intervals are $0.94 \leq y_c + d \leq 1.06$ and $0.93 \leq \eta + \varepsilon \leq 1.07$ based on the coefficients of variation of 4% and 5% for the regularities γ_b and σ_b. If the nutrient broth is assumed to be completely consumed, the average results are: $\eta = 0.32$, $\varepsilon = 0.61$, $\eta + \varepsilon = 0.96$, $y_c = 0.44$, $d = 0.39$, and $y_c + d = 0.83$. Note that $\eta + \varepsilon$ is still close to 1 if the nutrient broth is consumed completely. Carbon dioxide appears to be underestimated. Values of $(y_c + d)$ are significantly lower than 1, and this can be due to either product formation of experimental errors, such as underestimation of carbon dioxide measurements. The values of d and the respiratory quotients were very small in the early period of each batch fermentation. This may be due to the accumulation of dissolved CO_2 and HCO_3^- in the broth. The values of y_c and η in Runs 3 and 4 are slightly higher than those in Runs 5 and 6. The coefficients of variation for the results in Table 9 are 9.4% for d and 2.5% for ε. Since the value of ε is a function of oxygen uptake, this shows that oxygen measurements are more consistent than carbon dioxide measurements. This is in agreement with what was stated in the discussion of the analytical techniques.

A nitrogen balance was made using the accumulated base addition in Run 5. The value of the atomic ratio of nitrogen to carbon in biomass, q, was calculated and found to be 0.173, which differs by only 4.6% from the average value of 0.165 given in the literature [12]. This q value shows that the

integrated estimate of the biomass energetic yield, $\eta = 0.32$, based on biomass and substrate data, is consistent with the ammonia consumption in Run 5.

Tables 13, 14, 15 and 16 examine the consistency of the data at points along the batch curve with instantaneous available electron and carbon balances at several different points in time. It should be kept in mind that for both biomass and substrate curves, differentiations are necessary in the calculation. This increases considerably the inaccuracy of these balances. The skill of tracing a smooth curve through the data points and reading the slopes is very important in getting significant and reproducible results. Two people worked separately tracing and graphically differentiating the biomass and substrate curves. No significant differences were found in Run 3, 4 and 5; however, significant differences were found in the values of dS/dt in Run 6. The dS/dt values influence the values of y_c, d, η and ε in a linear form. Replication of these values did not improve the instantaneous balances, and only one set of results is presented. Errors of 5% in the balances can also be explained by the use of the regularities in the calculations ($\gamma_b = 4.291$, and $\sigma_b = 0.462$).

Looking at the tables, one can see that ($\eta + \varepsilon > 1$) in the first and last points of all the fermentations, showing that accurate measurements are difficult to obtain in these periods. Usually $\eta + \varepsilon < 1$ before the 11th hour; this could be due to experimental errors, or product formation. Later on, ($\eta + \varepsilon$) is usually higher than 1. Run 6 was the only one in which $\eta + \varepsilon > 1$ for every data point.

Values of d are very small at the beginning making $y_c + d < 1$. All the tables show that d is often initially smaller and then slightly higher than the respective integrated value (Table 9). This is probably due to

experimental errors in CO_2 measurements and dissolved CO_2 and HCO_3^- in the broth. However, it can be seen that $y_c + d \approx 1$ and $\eta + \varepsilon \approx 1$ many times during the fermentations, showing that the data are reasonably consistent. In addition, the coefficients of variation of $(y_c + d)$ and $(\eta + \varepsilon)$ were always lower than 23%. The data from Hug and Fiechter [9] in Chapter 2, which was the most consistent batch data, showed coefficients of variation of about 23% in the values of $(y_c + d)$ and $(\eta + \varepsilon)$ from the instantaneous balances. The data have to be extremely good to show consistent results in the instantaneous balances, whereas small measurement errors at the beginning and end of the fermentation do not greatly affect the integrated balances.

The consistency of the results of Runs 3, 4, 5 and 6 can also be examined by using different sets of measurements to estimate the true biomass energetic yield, η_{max}, and the maintenance coefficient, m_e. The results are shown in Table 10. It can be seen that the value of η_{max} did not vary much when calculated from CO_2 and biomass data, O_2 and biomass data, and substrate and biomass data; the coefficient of variation was 9.4%. That was not the case with the value of m_e, which was very low when calculated from CO_2 and biomass data compared to values of m_e from O_2 and biomass data, and substrate and biomass data; the coefficient of variation was 48.5%. Mathematically, m_e was very low from CO_2 data because the initial respiratory quotients were extremely low. If this is taken into account, and considering that η_{max} never differed in the same run more than 10%, and that differences between m_e from substrate and biomass data, and oxygen and biomass data, were lower than 20%, it can be said that the data are very consistent since the comparison of these estimates is a relatively severe test for consistency of experimental data.

Estimates of dissolved CO_2 and HCO_3^- were made. Dissolved CO_2 and HCO_3^- is less than 1% of that evolved during the total fermentation period. Thus, this contributes very little to the integrated carbon balance. However, during the early growth period, the accumulation of dissolved CO_2 contributes more significantly to the instantaneous carbon balance.

In Tables 5, 6, 7 and 8 values of the biomass energetic yield, η, estimated from the respiratory quotient, R.Q., are reported in order to show that the reported respiratory quotients are unrealistically low. When everything is fully considered, it appears that the reported values for carbon dioxide evolution are smaller than what they should be.

Hysteresis

The phenomenon of hysteresis, a memory phenomenon, has been identified by Tanner et al [18,19,20] in biochemical systems, and it has been considered inherent to the structure of consecutive reactions. Plots of a derivative of the concentration of a product with respect to time versus the concentration of a generated precursor which does not directly precede the irreversibly formed product, show a hysteresis loop in batch cultures. The direction, shape and area of the loop have significant meaning in the process.

Figures 4, 5, 7, 8, 10, 11, 13 and 14 show plots of the specific rates of oxygen consumption, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}, versus the specific growth rate. Numbers near the data points refer to the sequence in time of the data points, and they show a history-dependent pattern. The loop can be seen to be counterclockwise. The values of the specific rates of O_2 uptake and CO_2 evolution are smaller at the beginning of the fermentation than at the end for a constant value of μ. For example, the values of μ in data points 4 and 12 of Figure 5 are 0.099 and 0.095 hr^{-1}, respectively, whereas the values of Q_{O_2} are 5.911 and 8.028 m moles/ g dry cell (hr), respectively.

The specific growth rate is related to a time derivative of the biomass concentration, and it is by itself a product of the fermentation. On the other hand, Q_{O_2} and Q_{CO_2} are rates which are related to both growth and product formation. The incorporation of hexadecane and lipid fractions into the biomass would appear as growth with very little oxygen uptake and carbon dioxide evolution. When these stored materials begin to be utilized, it would appear as low growth with much oxygen uptake and carbon

dioxide evolution. Therefore, a hysteresis pattern could be seen in the Q_{O_2} and Q_{CO_2} versus μ graphs. In the case of accumulation of intermediate products resulting from the incomplete oxidation of n-hexadecane, the respiratory quotient would appear to be low as it happened in this work. However, the carbon dioxide hysteresis could also be explained by accumulation of CO_2, and bicarbonates in the fermentation broth. In the work of Bos et al. [2], it appeared that lipid biosynthesis increased significantly when C. lipolytica was grown on hydrocarbon compared to growth on non-alkane media. Integrated available electron and carbon balances have shown that $(\eta + \varepsilon)$ and $(y_c + d)$ are close to 1; thus, it appears that extracellular products other than carbon dioxide are not present in significant amounts. If intermediates were formed at the beginning, as instantaneous available electron and carbon balances might show $(\eta + \varepsilon < 1, y_c + d < 1)$, they are later utilized. In addition, Kennedy et al. [10] found intracytoplasmic hydrocarbon inclusions in bacteria grown on such hydrocarbons. Therefore, it appears that lipid biosynthesis and storage of hydrocarbon could be important features of hysteresis in hydrocarbon fermentations.

The following Figures of Chapter 2, Figures 5 and 6 (data from Moo-Young et al. [13]), and Figures 7 and 8 (data from Blanch and Einsele [2]), show clear hysteresis patterns. They are also Q_{O_2} and Q_{CO_2} vs μ plots.

Nutrient broth was used in the main media for the cultures. The biomass energetic yield, η, associated with utilization of biocert may be higher than that associated with hexadecane. Higher values of η produce lower values of Q_{O_2} [5], and this could explain the lower values of Q_{O_2} at the beginning. However, the value of the respiratory quotient

associated with organic substrates whose γ_s are lower than γ_b of the microorganism, such as nutrient broth ($\gamma_s \approx 4$, $\gamma_b = 4.291$ for the biomass), is usually higher than 1. It can be seen from Tables 5, 6, 7 and 8 that the respiratory quotient was very small at the early hours of fermentation; therefore, it appears that the hysteresis phenomenon can not be attributed to early consumption of nutrient broth. The values of η also do not indicate early consumption of nutrient broth.

Figures 6, 9, 12 and 15 are plots of the specific rate of hexadecane consumption, Q_s, versus the specific growth rate, μ. A hysteresis pattern can not be clearly seen as it was seen in Q_{O_2} and Q_{CO_2} versus μ plots. The gap between the forward and reverse arcs in Figures 9 and 12 is very small. Figure 15 shows a hysteresis pattern but there are few data points to be considered.

The hysteresis pattern of the curves where Q_{O_2} and Q_{CO_2} are plotted against μ (Figures 4, 5, 7, 8, 10, 11, 13 and 14) makes difficult the estimation of the "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, by least squares since they are calculated from the slope and intercept of the straight lines fitted to the data. This fact will be discussed later.

Yield and Maintenance Coefficient

Table 10 shows the values of the "true" biomass energetic yield coefficient estimated from three different sets of data in each of the Runs 3, 4, 5 and 6. The three different sets of data were carbon dioxide and biomass data shown in Figures 4, 7, 10 and 13, oxygen and biomass data shown in Figures 5, 8, 11 and 14, and hexadecane and biomass data shown in Figures 6, 9, 12 and 15. In addition, the data from Runs 3, 4, 5 and 6 were used as a pool to find mean values of η_{max} and m_e. Straight lines were fitted by least squares to the data in those graphs and relatively high correlation coefficients were usually found. The values of the correlation coefficient, r^2, are smaller in Run 3 than in Runs 4, 5 and 6 because of the larger air flow rate for this run. The results using CO_2 and biomass data have considerably smaller correlation coefficients than the results obtained using oxygen and biomass data or n-hexadecane and biomass data.

The fact that straight lines describe the data when Q_{O_2}, Q_{CO_2} and Q_S are plotted versus μ, shows that η_{max} and m_e do not depend on the specific growth rate in the batch culture of C. lipolytica on n-hexadecane under the operating conditions in the fermentation. However, it has been reported that in a number of cases a straight line has not been obtained [8,15,17]; negative values of m_e [4], and negative values of "true" growth yields [14] have also been found. The presence of a significant amount of extracellular products, and high amount of energy-uncoupled growth have been associated with such results.

It should be pointed out that maintenance coefficients are subjected to considerable variation if a change in biochemical pathway or uncoupling

of oxidative phosphorilation occur. In addition, the ionic strength of the medium has been reported to influence the maintenance coefficients significantly [15]; however, little is known about variation of maintenance coefficients with operating conditions.

The fact that hysteresis patterns appear in Q_{O_2} and Q_{CO_2} vs μ plots influences considerably the estimation of η_{max} and m_e. Figures 4 and 5 show that values in the forward part of the loop decrease the values of m_e and η_{max}, whereas values in the backward part of the loop increase the values of m_e and η_{max}. Figures 7, 8, 10, 11, 13 and 14 also show similar effects for the values of m_e.

Values of η_{max} and m_e were investigated because they provide useful information to relate biochemical pathway information to energetic efficiency. Knowledge of η_{max} and m_e can be used to compare the energetic efficiency of continuous and batch cultures. As Tables 6 and 7 of Chapter 2 show, higher correlation coefficients are found for the model of Pirt in continuous cultures than in batch cultures; this is because more accurate measurements are made in continuous cultures. In addition, the dilution rate is the specific growth rate in continuous cultures, whereas differentiation of the biomass is necessary to estimate the specific growth rate in batch cultures. Efforts have been made in this work to improve the quality of the estimates of η_{max} and m_e in batch cultures.

When estimates of η_{max} from different sets of variables are compared within each run, similar values can be seen. The standard deviations are 0.059, 0.015, 0.010 and 0.038 for Runs 3, 4, 5 and 6, respectively and η_{max} ranges from 0.39 to 0.55. The mean value of η_{max} is 0.44 and its coefficient of variation is 9.4%. This mean value was compared to the

mean value of η_{max} = 0.41 in continuous cultures in Chapter 2 using the "t" test [16]. The results indicated that these values are not significantly different at the 95% confidence level. Similarly, the same value of η_{max} = 0.44 was not significantly different from the mean value of 0.47 reported in Chapter 2 for batch cultures of Candida tropicalis growing on n-hexadecane. When the data from all four of the fermentations are used to find mean values of η_{max} from oxygen and biomass data, carbon dioxide and biomass data, and n-hexadecane and biomass data, the results were 0.41, 0.43 and 0.40, respectively, which shows that similar values of η_{max} were found independently of the data used. These results appear in Table 10. The 95% confidence intervals of these estimates were $0.38 \leq \eta_{max} \leq 0.45$, $0.39 \leq \eta_{max} \leq 0.48$ and $0.35 \leq \eta_{max} \leq 0.46$, respectively.

Table 10 shows that the values of m_e range from 0.014 to 0.064 hr^{-1}. The lowest and highest values correspond to Run 3, where experimental errors are appreciable. The mean value is 0.033 with a coefficient of variation equat to 48.5%.

A low value of m_e can be seen in all the runs, when carbon dioxide and biomass data were used; this is in agreement with the results in Chapter 2 using data of Blanch and Einsele [2]. Low values of carbon dioxide evolution during the early hours of the fermentation explain this fact. However, m_e ranges from 0.014 and 0.017 in all the runs, showing that the data are very consistent with each other. The value of m_e was 0.015 when all the carbon dioxide and biomass data were used as a pool. Higher values of m_e were obtained from both oxygen and biomass data, and substrate and biomass data. The best estimate according to the author is the one from oxygen and

biomass data, and ranges from 0.034 to 0.051 hr^{-1}. The values using all the oxygen and biomass data is 0.036 hr^{-1}. The value of m_e from all the hexadecane and biomass data in Runs 3, 4, 5 and 6, is 0.029 hr^{-1}, which is reasonably close to 0.036 hr^{-1}. The 95% confidence intervals for the values of m_e estimated from all the carbon dioxide and biomass data 0.015, all the oxygen and biomass data 0.036, and all the substrate and biomass data 0.029 hr^{-1} were $0 \leq m_e \leq 0.040$, $0.015 \leq m_e \leq 0.057$ and $0 \leq m_e \leq 0.063$, respectively. It can be seen that the intervals are relatively wide due to the hysteresis in the data; these are confidence intervals for the intercept of a straight line. The mean value of m_e from continuous culture data in Chapter 2 (0.012 hr^{-1}) is within two of these intervals. However, when the mean value of m_e of Runs 3, 4, 5 and 6 (0.033 hr^{-1}) was compared using the "t" test to m_e = 0.012 hr^{-1}, significant differences were found at the 95% confidence level indicating that m_e is higher in batch cultures than in continuous cultures. In addition, the hypothesis that the mean values of m_e in batch culture data from the literature in Chapter 2 (0.054 hr^{-1}) and in the batch culture data of this chapter m_e = 0.033 hr^{-1} were equal was rejected with 95% confidence. This shows that somewhat lower values of m_e were found in this work than in the earlier batch culture work. Data from Moo-Young et al. [13] using C. lipolytica on n-dodecane showed slightly higher values of m_e (0.046 and 0.048 hr^{-1}) than those reported here.

Nutrient broth was used in the medium, and contributed both carbon and energy to the yeast. The amount of nutrient broth actually consumed during the fermentation was not measured. However, the results of instantaneous available electron and carbon balances do not detect preferential nutrient broth consumption during the early period of the fermentation. Estimated

values of m_e and η_{max} from oxygen, carbon dioxide, and biomass data do not depend on organic substrate consumption, whereas estimates of m_e and η_{max} from substrate and biomass data are dependent upon substrate (including nutrient broth) consumption. The mean value of m_e from substrate and biomass data, 0.029 hr^{-1}, is lower than the mean value of m_e from oxygen and biomass data. This could be due to nutrient broth.

In order to appreciate the human element in preparing smooth curves and differentiating to find the values of μ and Q_S, two different workers analyzed the data. For Runs 3, 4 and 5, the results were not significantly different; however, in Run 6, the results differed because of the small number of data points which were collected at the lower range of specific growth rates.

CONCLUSIONS

Application of material and energy balance regularities to examine the consistency of batch culture data for Candida lipolytica cultured on n-hexadecane shows that the available electron balances are more consistent than the carbon balances. The integrated balances, instantaneous balances, and the values of respiratory quotient all suggest that carbon dioxide evolution measurements are lower than one would expect. The instantaneous balances indicate that the largest errors frequently occur at the beginning and end of the fermentation where accurate measurements are difficult to obtain.

An appropriate air flow rate is necessary to detect significant variation in percent O_2 and CO_2 between the inlet and exit gas streams of the fermenter, and therefore increase the accuracy of the measurements. Experimental errors were found to be larger in Run 3 due to a higher air flow rate.

The mean values of growth parameters based on four fermentations were: $\eta = 0.35$, $\varepsilon = 0.70$, $y_c = 0.50$ and $d = 0.44$. These values are similar to the values calculated from data in the literature.

The estimated values of the "true" biomass energetic yield range from 0.39 to 0.55 with a mean of 0.44 and are within the range of values calculated from literature data. The values of η_{max}, estimated from all the oxygen and biomass data, all the carbon dioxide and biomass data, and all the n-hexadecane and biomass data, were 0.43, 0.41 and 0.40, respectively, and they show little variation. These values are not significantly different from the η_{max} values estimated from continuous cultures.

The estimated value of energetic maintenance coefficient, m_e, ranged from 0.011 to 0.064 hr^{-1} with a mean of 0.033 hr^{-1}. The values of m_e

estimated from all the oxygen and biomass data, all the carbon dioxide and biomass data, and all the n-hexadecane and biomass data, were 0.036, 0.015 and 0.029 hr^{-1}, respectively. Low values of m_e from carbon dioxide also appear in Chapter 2. Low carbon dioxide evolution measurements, due to either experimental errors, carbon dioxide and bicarbonates in the medium or hysteresis, decrease the value of m_e. The estimated values of m_e for these batch cultures are significantly higher than the mean value of 0.012 hr^{-1} for continuous culture.

Even though estimates of m_e varied with the set of variables in the calculation, estimates using the same variables showed little variation when fermentations 3, 4, 5 and 6 are compared.

Hysteresis patterns could be seen in the plots of specific rates of oxygen consumption and carbon dioxide evolution versus specific growth rate. This phenomenon could be explained by incorporation of organic substrate or lipids into the biomass in early periods of fermentation, and utilization of these materials in later periods.

NOMENCLATURE

a	Moles of ammonia per quantity of organic substrate containing one g atom carbon, g mole/ g atom carbon
d	Moles of carbon dioxide per quantity of organic substrate containing one g atom carbon, g mole/ g atom carbon
m_e	Rate of organic substrate consumption for maintenance, g equiv. of available electrons/ g equiv. of available electrons in biomass (hr) or kcal/kcal of biomass (hr)
Q_g	Air flow rate, ℓ/hr
Q_m	Molar air flow rate, g mole/hr
Q_{CO_2}	Rate of evolution of carbon dioxide, g moles/ g dry cell (hr)
Q_{O_2}	Rate of consumption of oxygen, g moles/ g dry cell (hr)
Q_S	Rate of organic substrate consumption g/ g dry cell (hr)
q	Atomic ratio of nitrogen to carbon in biomass, dimensionless
R.Q.	Respiratory quotient, dimensionless
r^2	Correlation coefficient
S	Organic substrate concentration, g/liter
S_o	Initial organic substrate concentration, g/liter
S_f	Final organic substrate concentration, g/liter
X	Biomass concentration, g/liter
y_c	Biomass carbon yield (fraction of organic substrate carbon in biomass), dimensionless
γ_b	Reductance degree of biomass as defined by Equation (2) in Chapter 2, equivalents of available electrons per g atom carbon
γ_s	Reductance degree of organic substrate, equivalents of available electrons per g atom carbon
ε	Fraction of energy in organic substrate which is evolved as heat, dimensionless
η	Fraction of energy in organic substrate which is converted to biomass or biomass energetic yield, dimensionless
η_{max}	Maximum biomass energetic yield, dimensionless

μ Specific growth rate, hr^{-1}

σ_b Weight fraction carbon in biomass, dimensionless

σ_s Weight fraction carbon in organic substrate, dimensionless

θ Time, hr

REFERENCES

1. Aiba, S., V. Moritz, J. Someya, and K. L. Haung, J. Ferment. Technol. 47, 203-210 (1969).

2. Blanch, H. W., and A. Einsele, Biotechnol. and Bioeng. 15, 861-877 (1973).

3. Bos, P., and De Boer, W. E., Anonie v. Leeuwenhoek 34, 241-243 (1968).

4. Camargo, Rubio E., A. Ornelas Vale, G. Casarrubias Arcos, and S. Nagai, J. Ferment. Technol. 55, 56-62 (1977).

5. Erickson, L. E., I. G. Minkevich, and V. K. Eroshin, Biotechnol. and Bioeng. 20, 1595-1621 (1978).

6. Ferrer O., Alexis, Proceedings of the 8th Annual Biochemical Symposium, Charles Dunlap, Editor, University of Missouri, Columbia, Missouri, (1978).

7. Gutierrez, J. R., and L. E. Erickson, Biotechnol. and Bioeng. 19, 1331-1349 (1977).

8. Herbert, D., "Stoichiometric Aspects of Microbial Growth" in Continuous Culture 6: Applications and New Fields, A.C.R. Dean et al. editors, p. 1-30, Halsted Press, New York (1976).

9. Hug, H., and A. Fiechter, Arch. Mikrobiol. 88, 77-86 (1973).

10. Kennedy, R. S., W. R. Finnerty, K. Sudarsanan, and R. A. Young, Arch. Microbiol. 102, 75-83 (1975).

11. Minkevich, I. G., and V. K. Eroshin, Folia Microbiol. 18, 376-385 (1973).

12. Minkevich, I. G., V. K. Eroshin, T. A. Alekseeva, and A. P. Tereshchenko, Mikrobiol. Promishlennost 2 (144) 1 (1977). (In Russian).

13. Moo-Young, M., T. Shimizu, and D. A. Whitworth, Biotechnol. and Bioeng. 13, 741-760 (1971).

14. Nagai, S., and S. Aiba, J. of General Microbiology 73, 531-538 (1972).

15. Pirt, S. J., Principles of Microbe and Cell Cultivation, Halsted Press, Wiley, N.Y. (1975).

16. Snedecor, G. W., and W. G. Cochran, Statistical Methods, Sixth Edition, Iowa State University Press, Ames, Iowa (1967).

17. Stouthamer, A. H., "Energetic Aspects of the growth of Microorganisms" in Microbial Energetics, Twenty-seventh Symposium of the Society for General Microbiology, Cambridge University Press, Cambridge (1977).

18. Tanner, R. D., A.I.Ch.E. Journal 18, 385-392 (1972).

19. Tanner, R. D., L. Hugo De Angelis, The Chemical Engineering Journal 8, 113-123 (1974).

20. Tanner, R. D., and J. M. Yunker, J. Ferment. Technol. 55, 143-150 (1977).

21. Yagi, H. and F. Yoshida, Biotechnol. and Bioeng. 19, 801-819 (1977).

Table 1. Measured variables in the batch fermentation of Candida lipolytica. Run 3. Air flow rate = 1 ℓ/min; Agitation = 800 rpm. Inlet Air: O_2 = 20.8%, CO_2 = 0.045%.

θ hrs	Vol broth mℓ	O_2 in exit air, %	CO_2 in exit air, %	X g/ℓ	S g/ℓ
0	2748	20.70	0.04	0.72	18.15
4	2603	20.40	0.09	0.90	18.04
6	2483	20.30	0.12	1.05	17.88
9	2333	20.00	0.19	1.40	17.57
11	2235	20.00	0.17	1.70	17.30
13	2128	20.00	0.21	2.00	17.02
15	2005	19.20	0.55	2.50	16.69
17	1890	18.95	0.65	3.00	16.26
19	1791	18.40	0.48	3.70	15.62
21	1690	17.35	1.49	5.00	14.62
22.5	1587	17.05	1.62	5.90	13.65
23.5	1449	17.45	1.38	6.95	12.87
25.5	1329	18.55	0.96	7.40	11.34
27	1209	19.05	0.76	8.10	10.57
28	1085	19.30	0.66	8.20	10.20

Table 2. Measured variables in the batch fermentation of Candida lipolytica. Run 4. Air flow rate = 0.375 ℓ/min. Agitation = 1000 rpm. Inlet Air: O_2 = 20.8%, CO_2 = 0.045%.

θ hrs	Vol broth mℓ	O_2 in exit air, %	CO_2 in exit air, %	X g/ℓ	S g/ℓ
0	2712	20.50	0.05	0.45	17.85
2	2612	20.45	0.105	0.48	17.82
4	2509	20.40	0.13	0.50	17.78
8	2401	20.15	0.225	0.62	17.65
10	2301	19.95	0.32	0.75	17.54
12	2198	19.50	0.55	0.85	17.38
14	2072	18.50	0.89	1.18	17.08
16	1944	17.20	1.33	1.52	16.70
18	1861	15.00	2.25	2.20	16.00
19.5	1717	13.35	2.95	2.80	15.35
21.5	1630	13.90	2.80	3.78	14.27
23.5	1505	15.40	2.15	4.65	13.41
25.5	1302	17.00	1.605	5.08	12.85
26.5	1152	17.90	1.295	5.19	12.69

Table 3. Measured variables in the batch fermentation of Candida lipolytica. Run 5. Air flow rate = 0.375 ℓ/min. Agitation = 1000 rpm. Inlet Air: O_2 = 20.8%, CO_2 = 0.045%.

θ	Vol broth	O_2 in exit air,	CO_2 in exit air,	X	S
hrs	mℓ	%	%	g/ℓ	g/ℓ
0	2729	20.75	0.05	0.35	19.35
2	2627	20.65	0.07	0.38	19.32
4	2521	20.40	0.13	0.42	19.29
6	2412	20.20	0.215	0.48	19.22
8	2305	20.15	0.255	0.57	19.13
10	2212	19.45	0.50	0.70	18.98
12	2115	18.90	0.905	0.93	18.73
14	2015	17.80	1.26	1.28	18.38
16	1921	16.95	1.60	1.77	17.85
18	1839	15.40	2.475	2.44	17.21
20	1751	14.10	3.34	3.30	16.26
22	1670	13.30	3.28	4.32	15.22
24	1604	14.30	2.90	5.13	14.20
26	1536	15.80	2.245	5.66	13.49
28	1471	16.75	1.83	5.95	13.00

Table 4. Measured variables in the batch fermentation of Candida lipolytica. Run 6. Air flow rate = 0.375 ℓ/min. Agitation = 1000 rpm. Inlet Air: O_2 = 20.8%, CO_2 = 0.045%.

θ hrs	Vol broth mℓ	O_2 in exit air, %	CO_2 in exit air, %	X g/ℓ	S g/ℓ
0	2810	20.40	0.105	0.45	19.31
6	2715	19.35	0.59	0.60	19.20
14	2621	16.15	2.085	1.76	18.41
16	2531	15.20	2.61	2.26	17.82
19	2441	10.65	4.61	3.49	16.20
21	2366	7.30	6.12	4.52	14.81
23	2296	6.20	6.46	5.80	13.80
25	2206	9.50	5.135	6.83	12.42
27	2126	11.65	4.17	7.50	11.59

Table 5. Calculated variables in the batch fermentation of Candida lipolytica. Run 3.

θ	μ	Q_{O_2}	Q_{CO_2}	Q_S	R.Q.	η
hrs	hr^{-1}	mmole O_2 / g dry cell(hr)	mmole CO_2 / g dry cell(hr)	g substrate / g dry cell(hr)		
0	0.040	1.219	0	0.020	0	0.70
4	0.069	4.121	0.515	0.078	0.13	0.65
6	0.093	4.647	0.743	0.089	0.16	0.64
9	0.099	5.911	1.111	0.088	0.19	0.63
11	0.091	5.082	0.813	0.075	0.16	0.64
13	0.088	4.536	0.953	0.075	0.21	0.61
15	0.112	7.702	2.465	0.074	0.32	0.54
17	0.120	7.875	2.600	0.091	0.33	0.54
19	0.135	10.346	3.942	0.124	0.38	0.49
21	0.130	9.850	4.137	0.118	0.42	0.46
22.5	0.125	9.666	4.079	0.132	0.42	0.45
23.5	0.095	8.028	3.211	0.112	0.40	0.48
25.5	0.056	5.500	2.261	0.085	0.41	0.46
27	0.026	4.342	1.780	0.060	0.41	0.46
28	0.023	4.120	1.693	0.038	0.41	0.46

Table 6. Calculated variables in the batch fermentation of Candida lipolytica. Run 4.

θ	μ	Q_{O_2}	Q_{CO_2}	Q_S	R.Q.	n
hrs	hr^{-1}	$\frac{\text{mmoles}}{\text{g dry cell(hr)}}$	$\frac{\text{mmoles}}{\text{g dry cell(hr)}}$	$\frac{\text{g substrate}}{\text{g dry cell(hr)}}$		
0	0.010	2.051	0.051	0.017	0.025	0.69
2	0.028	2.432	0.438	0.028	0.18	0.63
4	0.035	2.863	0.630	0.040	0.22	0.61
8	0.072	4.002	1.084	0.072	0.27	0.58
10	0.089	4.403	1.408	0.088	0.32	0.54
12	0.112	6.402	2.433	0.102	0.38	0.49
14	0.149	8.435	3.121	0.128	0.37	0.50
16	0.149	10.945	3.940	0.138	0.36	0.51
18	0.164	12.820	4.872	0.164	0.38	0.49
19.5	0.168	13.998	5.460	0.178	0.39	0.49
21.5	0.133	10.100	4.039	0.144	0.40	0.48
23.5	0.070	6.986	2.725	0.082	0.39	0.49
25.5	0.030	5.209	2.136	0.056	0.41	0.47
26.5	0.005	4.398	1.892	0.028	0.43	0.44

Table 7. Calculated variables in the batch fermentation of Candida lipolytica. Run 5.

θ	μ	Q_{O_2}	Q_{CO_2}	Q_S	R.Q.	η
hrs	hr^{-1}	$\frac{\text{mmole } O_2}{\text{g dry cell(hr)}}$	$\frac{\text{mmole } CO_2}{\text{g dry cell(hr)}}$	$\frac{\text{g substrate}}{\text{g dry cell(hr)}}$		
0	0.0075	0.600	0.042	0.020	0.07	0.68
2	0.046	1.361	0.245	0.039	0.18	0.63
4	0.053	3.419	0.786	0.067	0.23	0.60
6	0.071	4.690	1.313	0.094	0.28	0.57
8	0.097	4.477	1.433	0.123	0.32	0.54
10	0.127	7.890	2.660	0.183	0.34	0.53
12	0.156	8.800	3.960	0.226	0.45	0.42
14	0.160	10.527	4.263	0.195	0.41	0.47
16	0.164	10.302	4.120	0.181	0.40	0.48
18	0.152	10.898	4.905	0.176	0.45	0.42
20	0.141	10.530	5.161	0.164	0.49	0.37
22	0.114	9.430	4.055	0.130	0.43	0.44
24	0.066	7.149	3.142	0.068	0.44	0.43
26	0.034	5.205	2.290	0.065	0.44	0.43
28	0.017	4.200	1.848	0.048	0.44	0.43

Table 8. Calculated variables in the batch fermentation of Candida lipolytica. Run 6.

θ	μ	Q_{O_2}	Q_{CO_2}	Q_S	R.Q.	η
hrs	hr^{-1}	mmole O_2 / g dry cell(hr)	mmole CO_2 / g dry cell(hr)	g substrate / g dry cell(hr)		
0	0.034	2.846	0.427	0.031	0.15	0.64
6	0.114	7.960	3.018	0.081	0.38	0.49
14	0.119	9.102	4.005	0.083	0.44	0.43
16	0.126	8.824	4.059	0.100	0.46	0.41
19	0.136	10.780	4.851	0.144	0.45	0.42
21	0.139	11.424	5.141	0.147	0.45	0.42
23	0.108	9.908	4.360	0.125	0.44	0.43
25	0.061	6.796	3.058	0.081	0.45	0.42
27	0.037	5.200	2.340	0.052	0.45	0.42

Table 9. Examination of data consistency using integrated available electron and carbon balances for growth of Candida lipolytica on n-hexadecane in batch cultures. (γ_b = 4.291, σ_b = 0.462). O_2, CO_2, biomass and hydrocarbon data.

Source of data	Results					
	y_c	d	y_c+d	η	ε	$\eta+\varepsilon$
Run 3	0.51	0.40	0.91	0.36	0.72	1.08
Run 4	0.50	0.41	0.91	0.35	0.71	1.06
Run 5	0.48	0.45	0.93	0.34	0.68	1.02
Run 6	0.45	0.49	0.94	0.32	0.71	1.03
Pool of data of Runs 3,4,5 and 6	0.50	0.44	0.94	0.35	0.70	1.05

Table 10. Values of "true" biomass energetic yield coefficient, η_{max}, and maintenance coefficient, m_e, from growth of Candida lipolytica on n-hexadecane in batch culture. (γ_b = 4.291, σ_b = 0.462).

Source of data	Results			Comments
	η_{max}	m_e hr^{-1}	r^2	
Run 3	0.46	0.014	0.30	CO_2 and biomass data
	0.44	0.044	0.66	O_2 and biomass data
	0.55	0.064	0.68	Substrate and biomass data
Run 4	0.44	0.017	0.70	CO_2 and biomass data
	0.41	0.040	0.81	O_2 and biomass data
	0.42	0.032	0.97	Substrate and biomass data
Run 5	0.43	0.017	0.62	CO_2 and biomass data
	0.42	0.034	0.80	O_2 and biomass data
	0.44	0.029	0.96	Substrate and biomass data
Run 6	0.39	0.017	0.78	CO_2 and biomass data
	0.40	0.051	0.87	O_2 and biomass data
	0.46	0.040	0.72	Substrate and biomass data
Pool of data Runs 3, 4, 5 and 6	0.43	0.015	0.57	CO_2 and biomass data
	0.41	0.036	0.78	O_2 and biomass data
	0.40	0.029	0.82	Substrate and biomass data

Table 11. Experimental Conditions for growth of Candida lipolytica on n-hexadecane in batch cultures.

Run	Air flow rate ℓ/min	Agitation RPM
3	1	800
4	.375	1000
5	.375	1000
6	.375	1000

Table 12. Problems associated with the experimental techniques.

Measurement	Difficulty
Biomass	Sampling Cell lipids extraction
Hexadecane	Sampling Extraction Sensitivity of chromatography
O_2	Accuracy to 0.1 mole%
CO_2	Unstable readings at very small values.
O_2 and CO_2	Appropriate air flow rate
Dissolved oxygen probe	Unstable readings at high biomass concentration. Plugging of probe at high biomass concentration.
pH probe	Plugging of probe at high biomass concentration
Consumption of base	False readings from pH probe due to plugged probe

Table 13. Examination of data consistency using instantaneous available electron and carbon balances for growth of Candida lipolytica on n-$C_{16}H_{34}$ in batch culture. Run 3. (σ_b = 0.462, γ_b = 4.291).

ϑ hrs	y_c	d	y_c+d	η	ε	$\eta+\varepsilon$
0	1.09	0	1.09	0.76	0.56	1.32
4	0.48	0.09	0.57	0.34	0.49	0.83
6	0.57	0.12	0.69	0.30	0.48	0.78
9	0.61	0.18	0.79	0.33	0.44	0.76
11	0.66	0.15	0.81	0.46	0.62	1.08
13	0.64	0.18	0.82	0.45	0.56	1.00
15	0.82	0.47	1.29	0.58	0.96	1.54
17	0.72	0.40	1.12	0.50	0.80	1.30
19	0.59	0.45	1.04	0.50	0.91	1.41
21	0.60	0.49	1.09	0.42	0.77	1.19
22.5	0.52	0.43	0.95	0.36	0.67	1.04
23.5	0.46	0.41	0.87	0.32	0.66	0.98
25.5	0.36	0.38	0.74	0.30	0.70	1.00
27	0.24	0.42	0.66	0.17	0.67	0.84
28	0.33	0.63	0.96	0.23	1.00	1.23

Table 14. Examination of data consistency using instantaneous available electron and carbon balances for growth of Candida lipolytica on n-$C_{16}H_{34}$ in batch culture. Run 4. (σ_b = 0.462, γ_b = 4.291).

θ hrs	y_c	d	y_c+d	η	ε	$\eta + \varepsilon$
0	0.31	0.04	0.35	0.22	1.11	1.33
2	0.54	0.22	0.76	0.38	0.80	1.18
4	0.47	0.22	0.69	0.33	0.66	0.99
8	0.54	0.21	0.75	0.38	0.51	0.89
10	0.54	0.23	0.77	0.38	0.46	0.85
12	0.60	0.34	0.94	0.42	0.58	1.00
14	0.63	0.34	0.97	0.44	0.61	1.05
16	0.59	0.40	0.99	0.41	0.73	1.14
18	0.54	0.42	0.96	0.38	0.72	1.10
19.5	0.51	0.43	0.94	0.36	0.72	1.08
21.5	0.50	0.40	0.90	0.35	0.65	1.00
23.5	0.47	0.47	0.94	0.33	0.79	1.11
25.5	0.37	0.69	1.06	0.26	1.09	1.35
26.5	0.10	0.95	1.05	0.07	1.45	1.52

Table 15. Examination of data consistency using instantaneous available electron and carbon balances for growth of Candida lipolytica on n-$C_{16}H_{34}$ in batch culture. Run 5. (σ_b = 0.462, γ_b = 4.291).

θ hrs	y_c	d	y_c+d	η	ε	η + ε
0	0.59	0.08	0.67	0.41	0.79	1.20
2	0.71	0.10	0.81	0.50	0.36	0.86
4	0.53	0.21	0.74	0.37	0.58	0.95
6	0.57	0.27	0.84	0.40	0.63	0.93
8	0.60	0.23	0.83	0.42	0.46	0.88
10	0.60	0.32	0.92	0.42	0.62	1.04
12	0.62	0.41	1.02	0.43	0.59	1.02
14	0.59	0.40	0.99	0.41	0.65	1.06
16	0.60	0.39	0.99	0.42	0.64	1.06
18	0.56	0.46	1.02	0.39	0.67	1.06
20	0.54	0.52	1.06	0.38	0.69	1.07
22	0.51	0.47	0.98	0.36	0.71	1.07
24	0.43	0.52	0.95	0.30	0.78	1.08
26	0.41	0.72	1.13	0.29	1.07	1.36
28	0.24	0.69	0.93	0.17	1.02	1.19

Table 16. Examination of data consistency using instantaneous available electron and carbon balances for growth of Candida lipolytica on n-$C_{16}H_{34}$ in batch culture. Run 6. (c_b = 0.462, γ_b = 4.291).

θ hrs	y_c	d	y_c+d	η	ε	$\eta + \varepsilon$
0	0.60	0.20	0.80	0.42	0.85	1.27
6	0.77	0.53	1.30	0.54	0.91	1.45
14	0.77	0.68	1.45	0.54	1.01	1.55
16	0.68	0.57	1.25	0.48	0.81	1.29
19	0.51	0.48	.99	0.36	0.69	1.05
21	0.51	0.49	1.00	0.36	0.71	1.07
23	0.47	0.49	0.96	0.33	0.73	1.06
25	0.42	0.53	.95	0.29	0.77	1.06
27	0.39	0.64	1.03	0.27	0.92	1.19

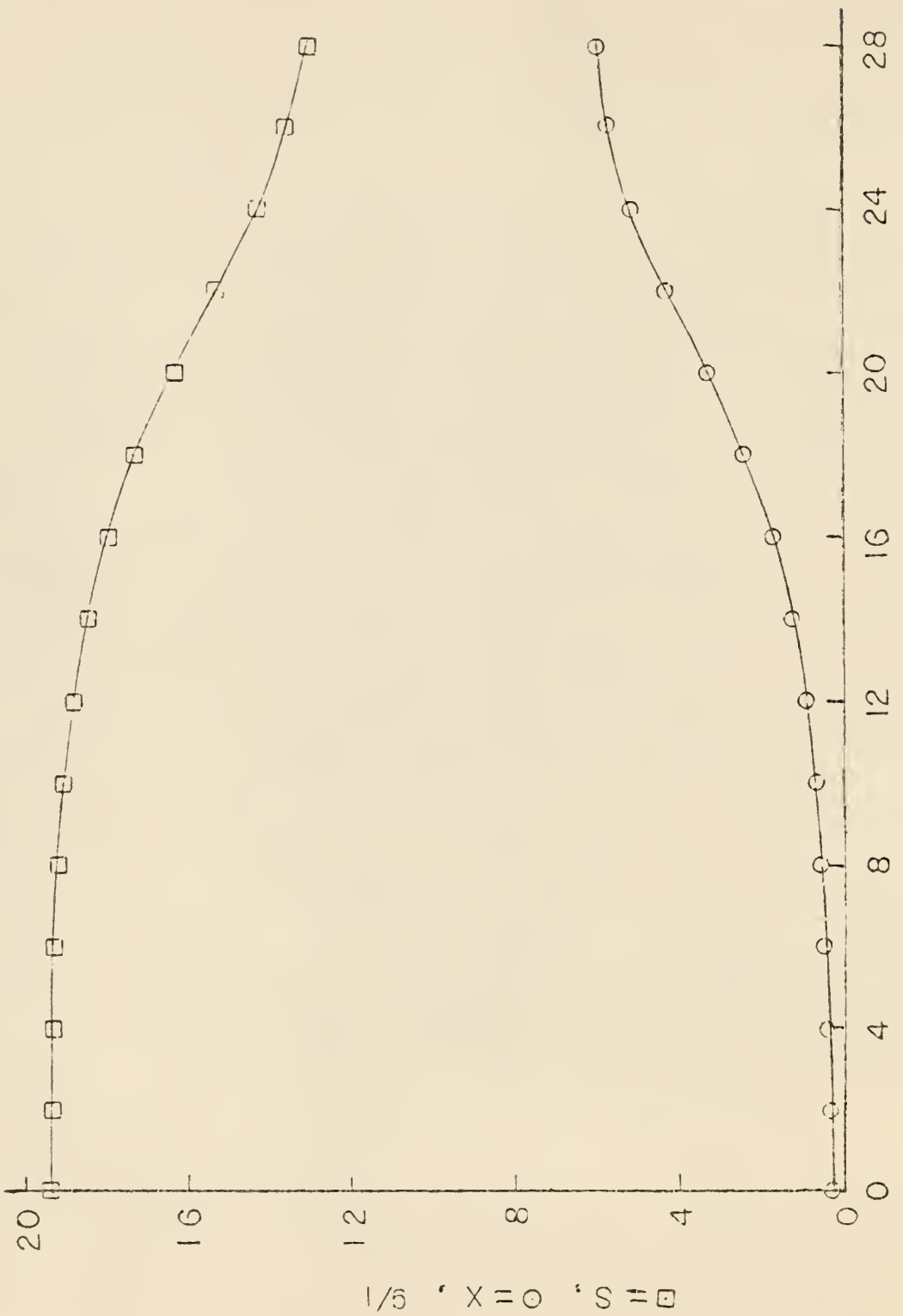

Fig. 1. Batch growth of Candida lipolytica on n-hexadecane; Run 5.

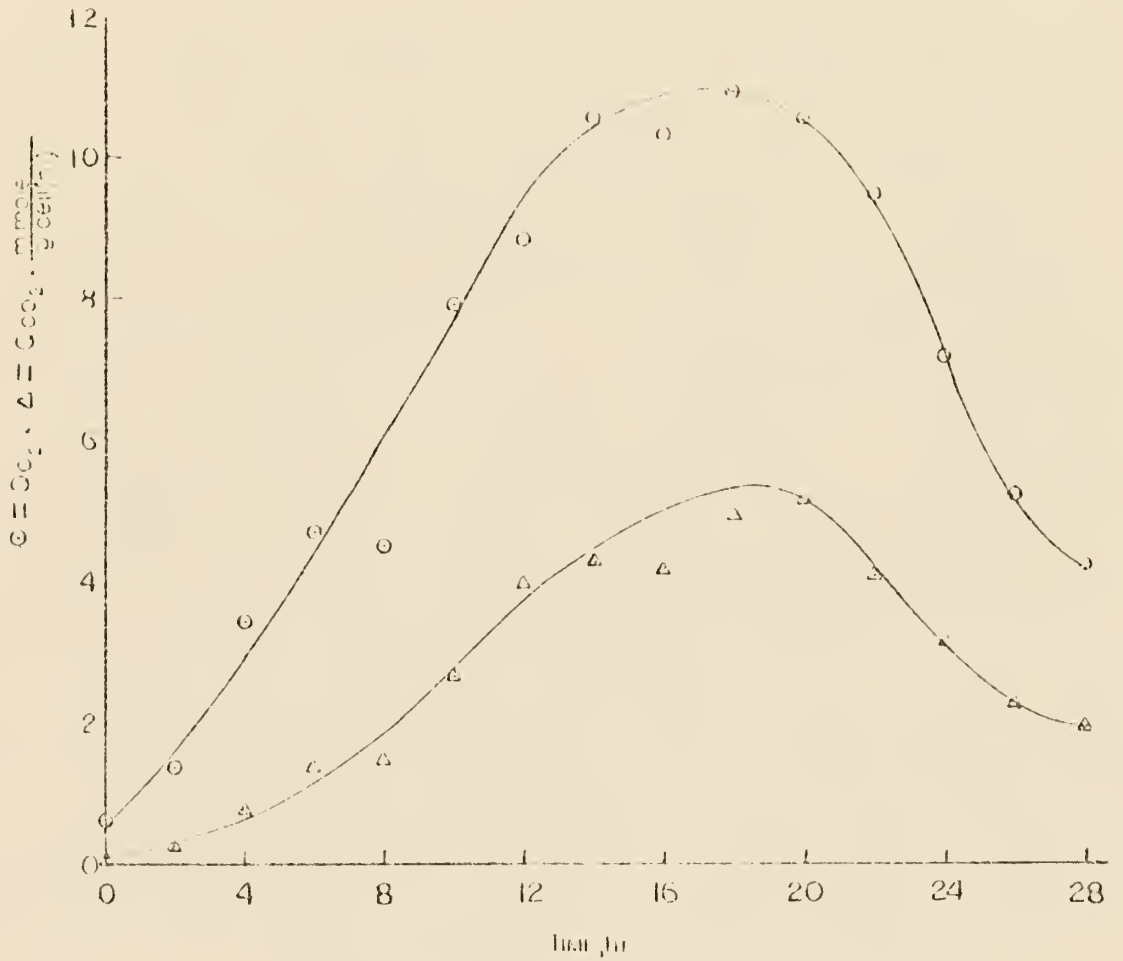

Fig. 2. Specific rates of oxygen uptake, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}, in batch culture of Candida lipolytica on n-hexadecane; Run 5.

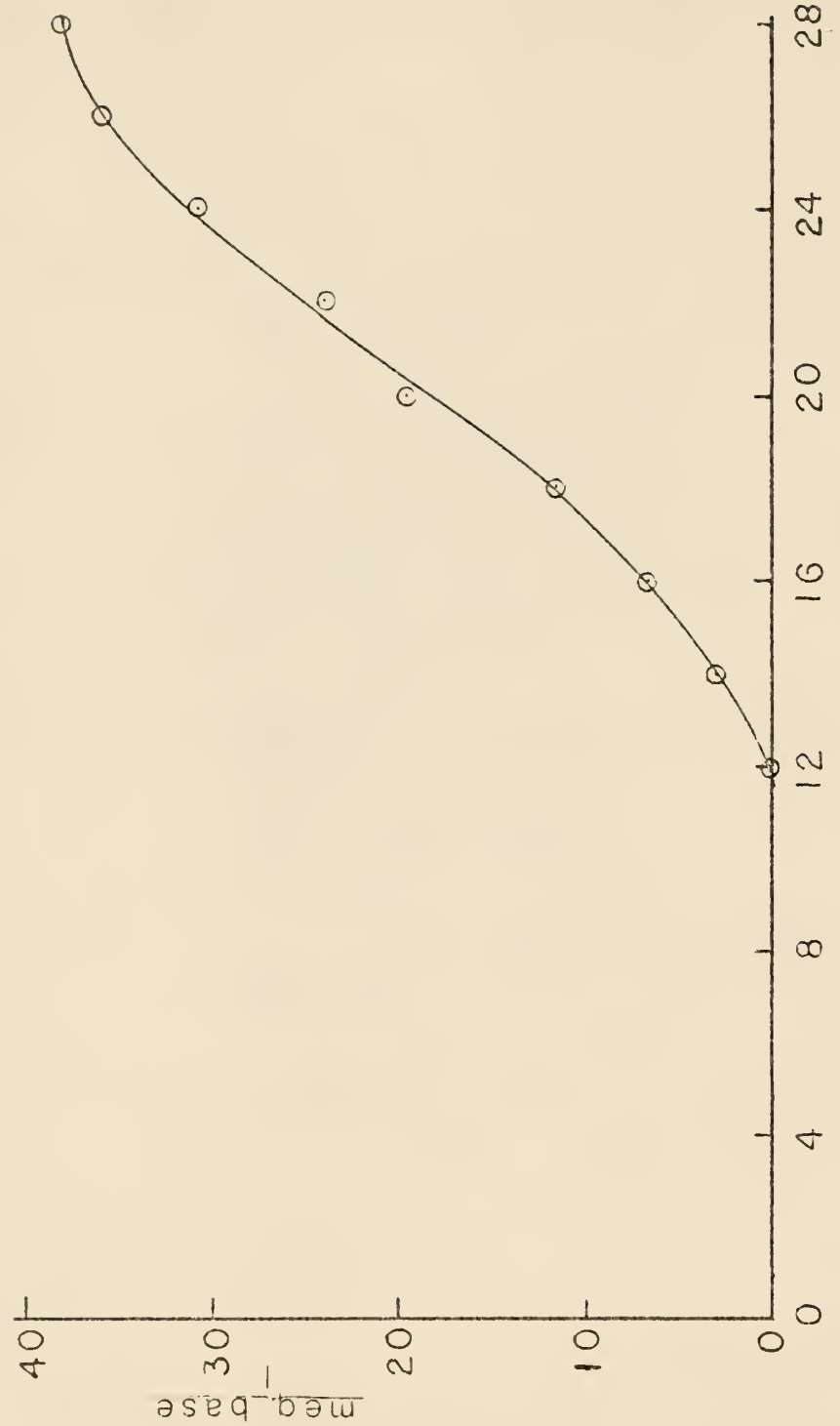

Fig. 3. Consumption of NaOH during batch growth of *Candida lipolytica* on n-hexadecane; Run 5.

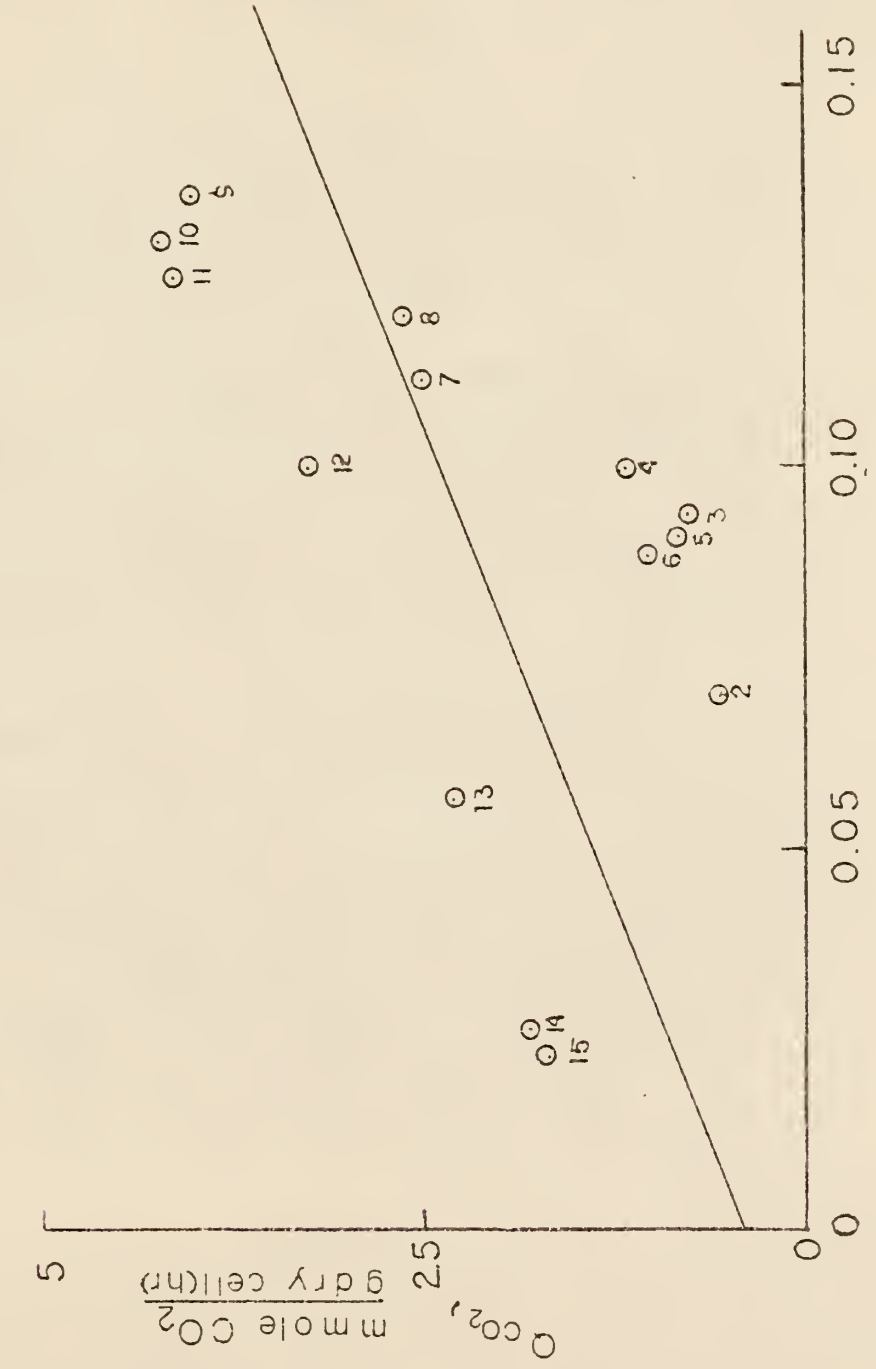

Fig. 4. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ. Run 3.

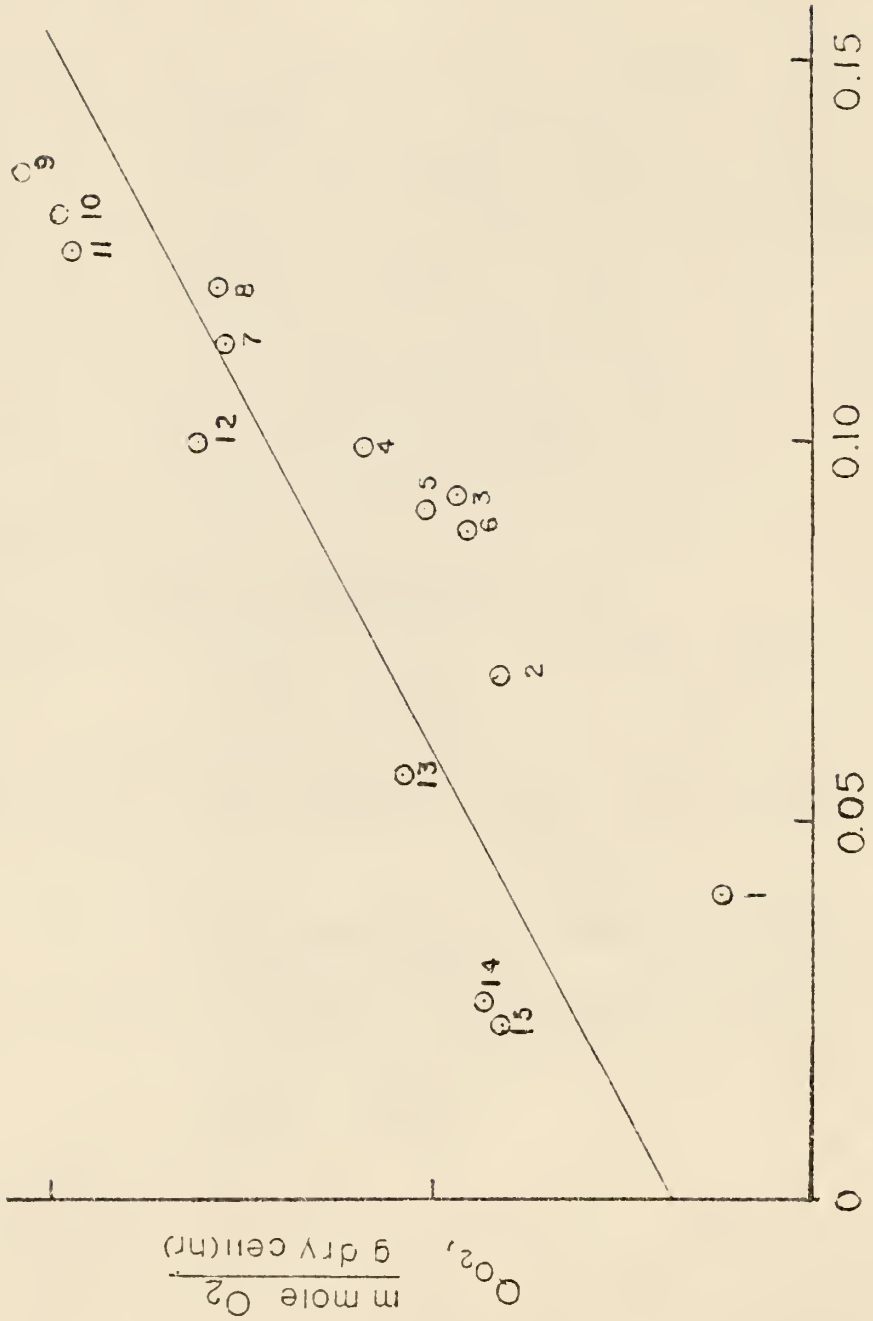

Fig. 5. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ. Run 3.

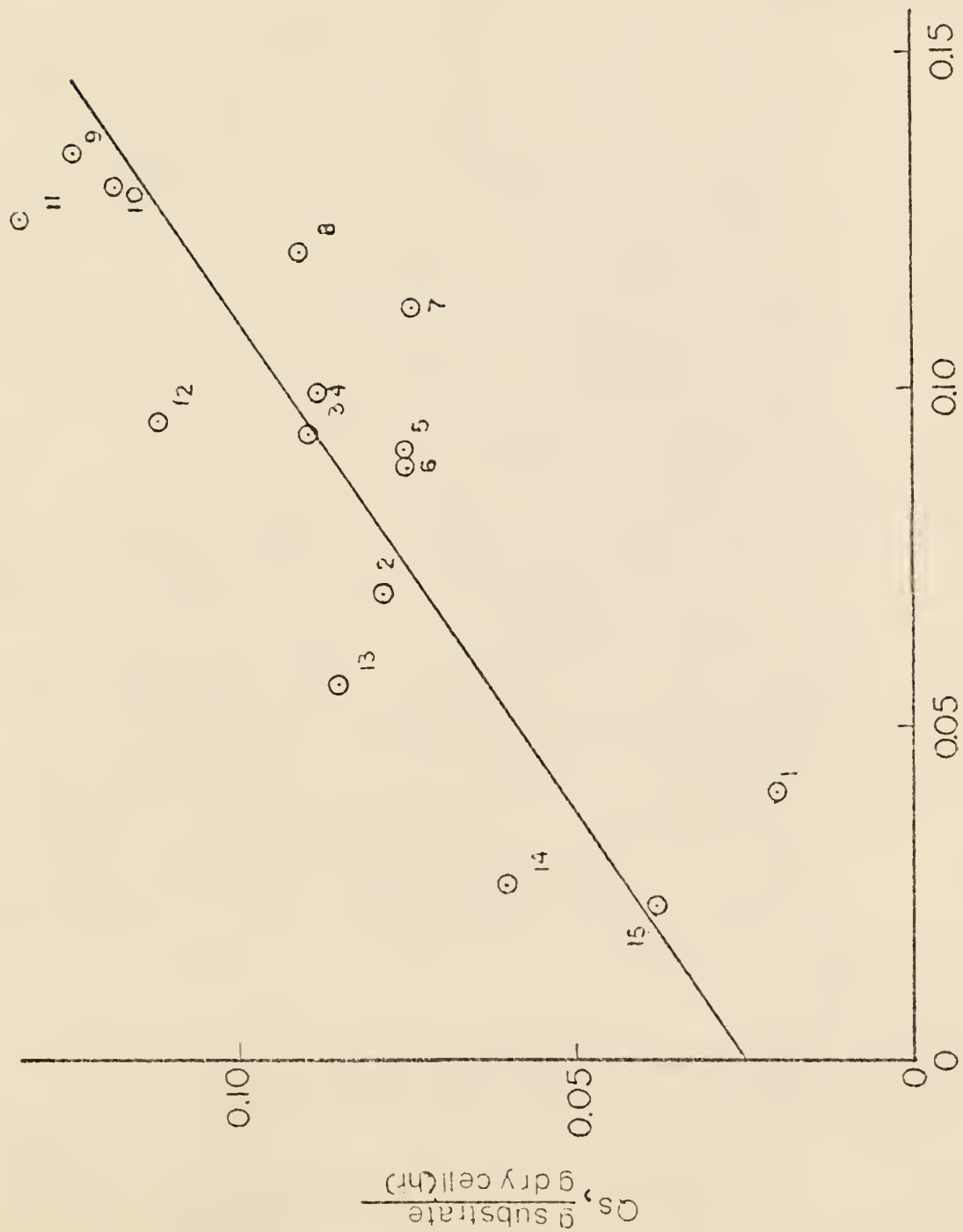

Fig. 6. Relationship between the specific n-hexadecane consumption rate, Q_S, and the specific growth rate, μ. Run 3.

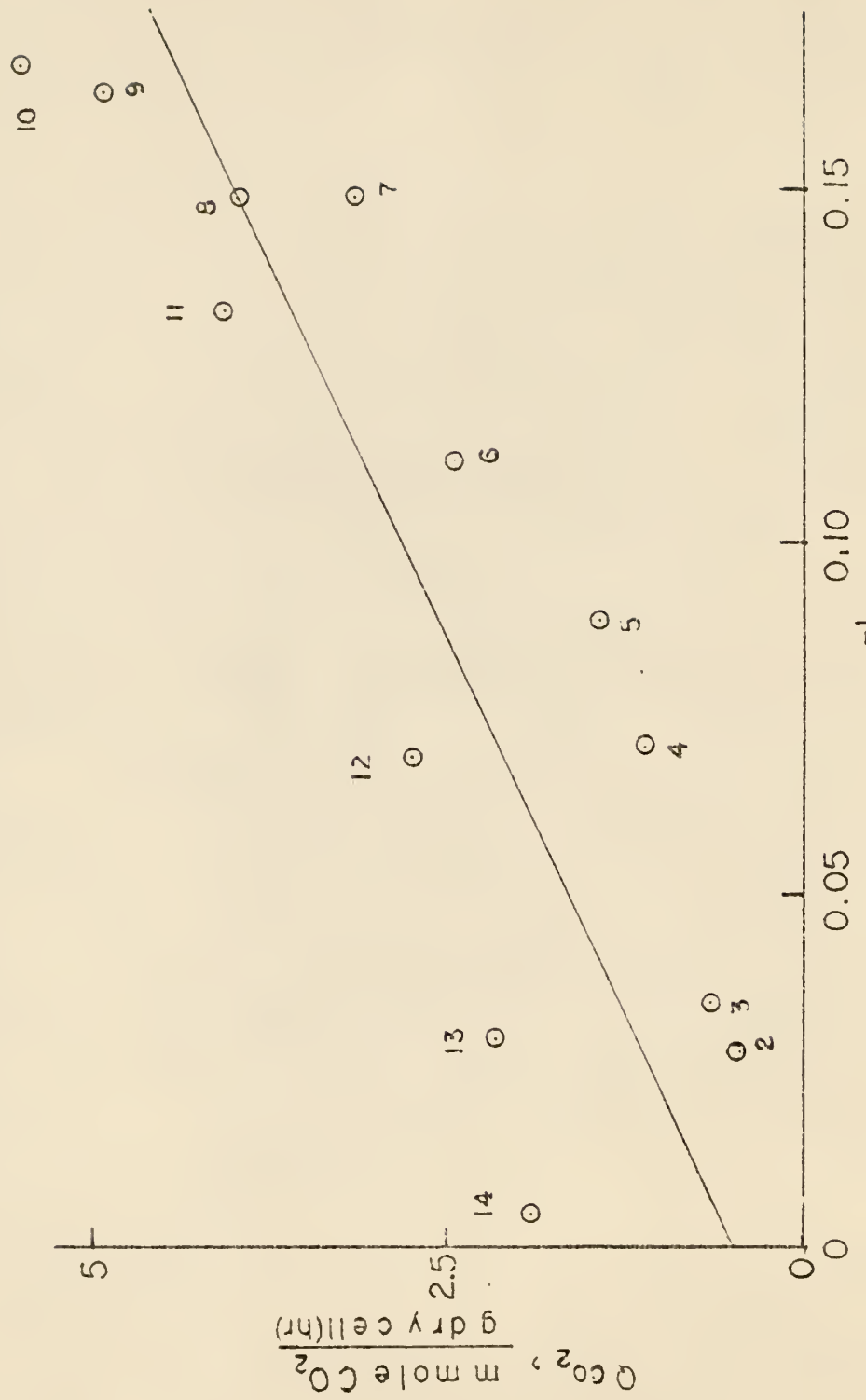

Fig. 7. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ. Run 4.

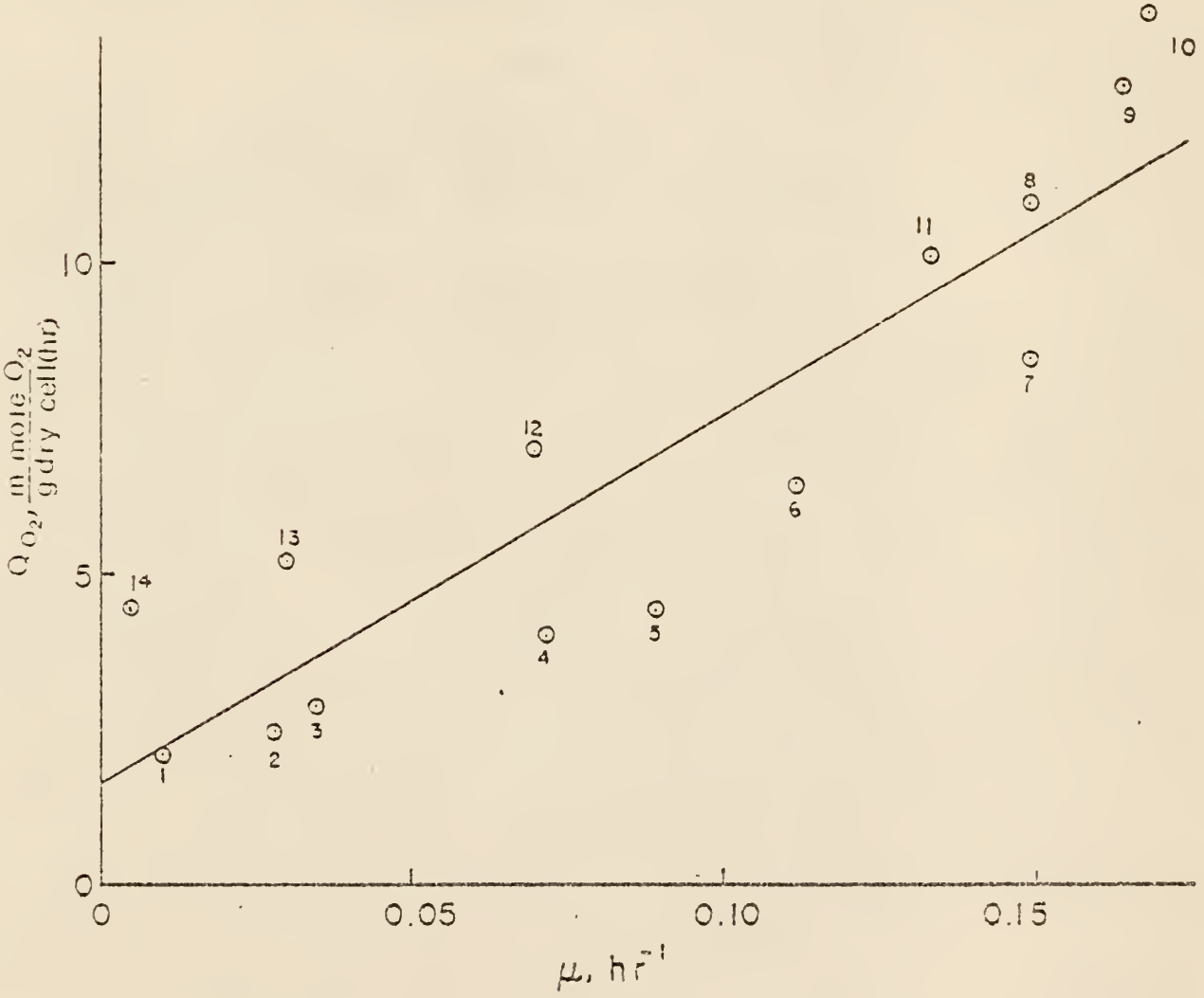

Fig. 8. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ. Run 4.

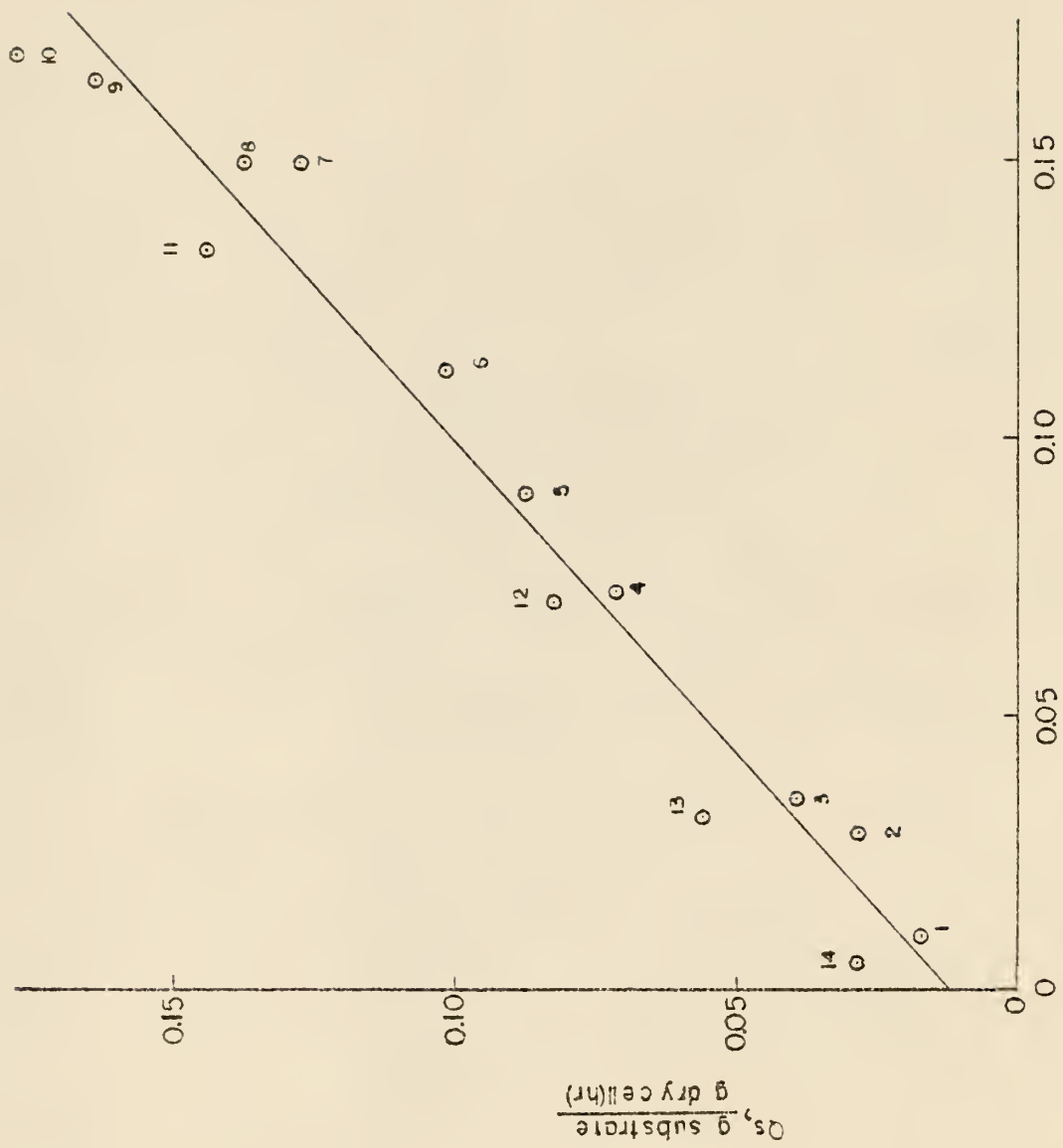

Fig. 9. Relationship between the specific n-hexadecane consumption rate, Q_S, and the specific growth rate, μ. Run 4.

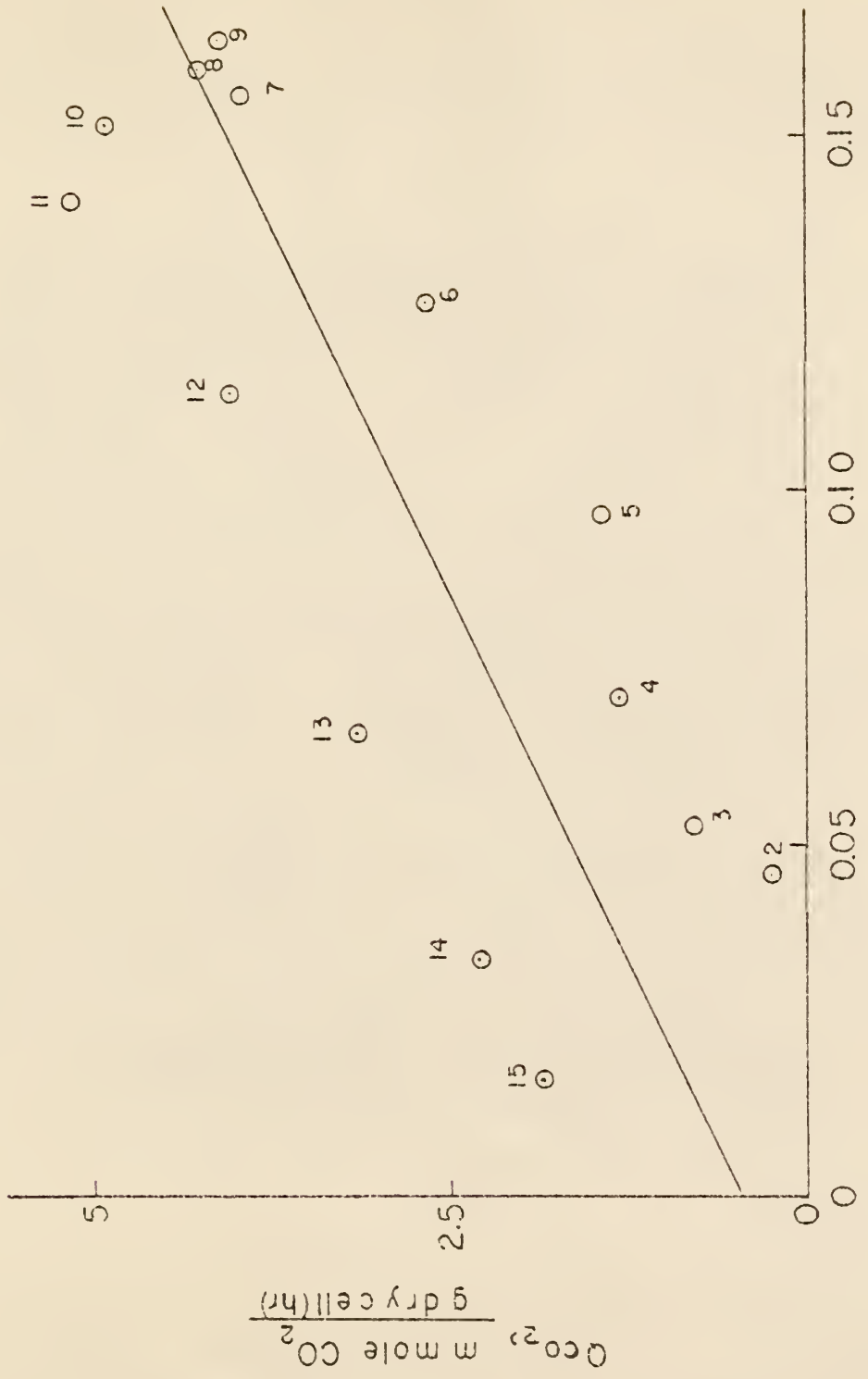

Fig. 10. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ. Run 5.

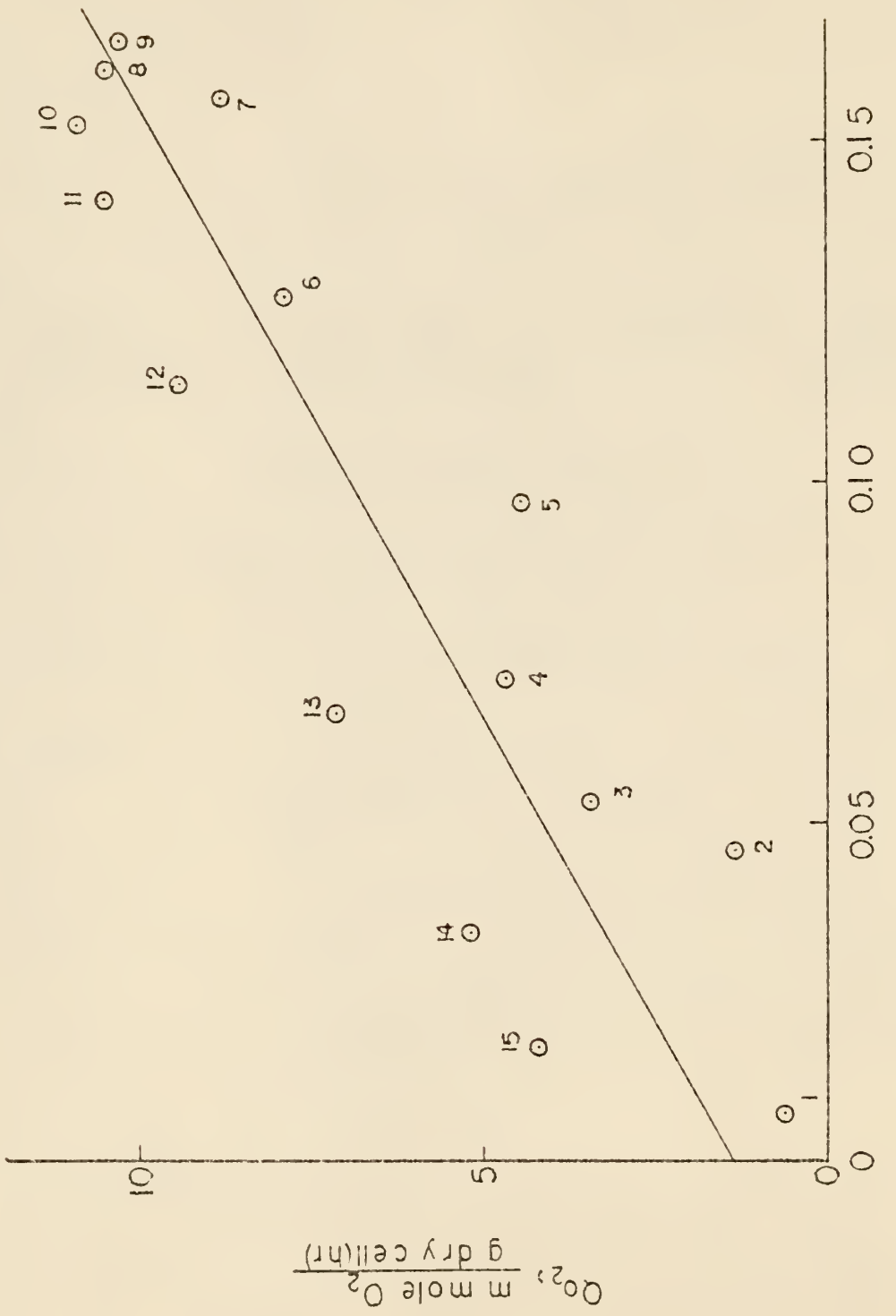

Fig. 11. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ. Run 5.

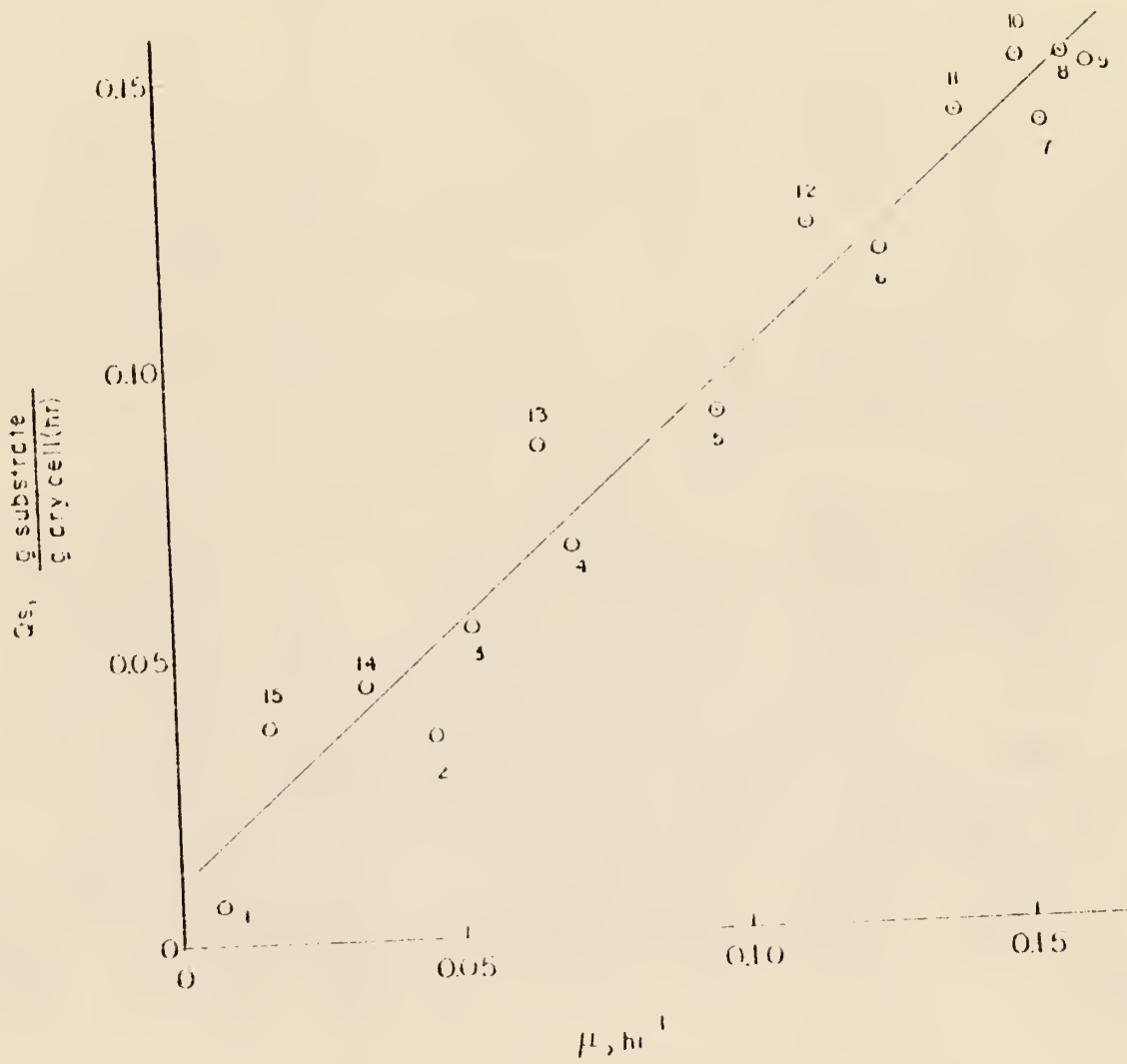

Fig. 12. Relationship between the specific n-hexadecane consumption rate, Q_s, and the specific growth rate, μ. Run 5.

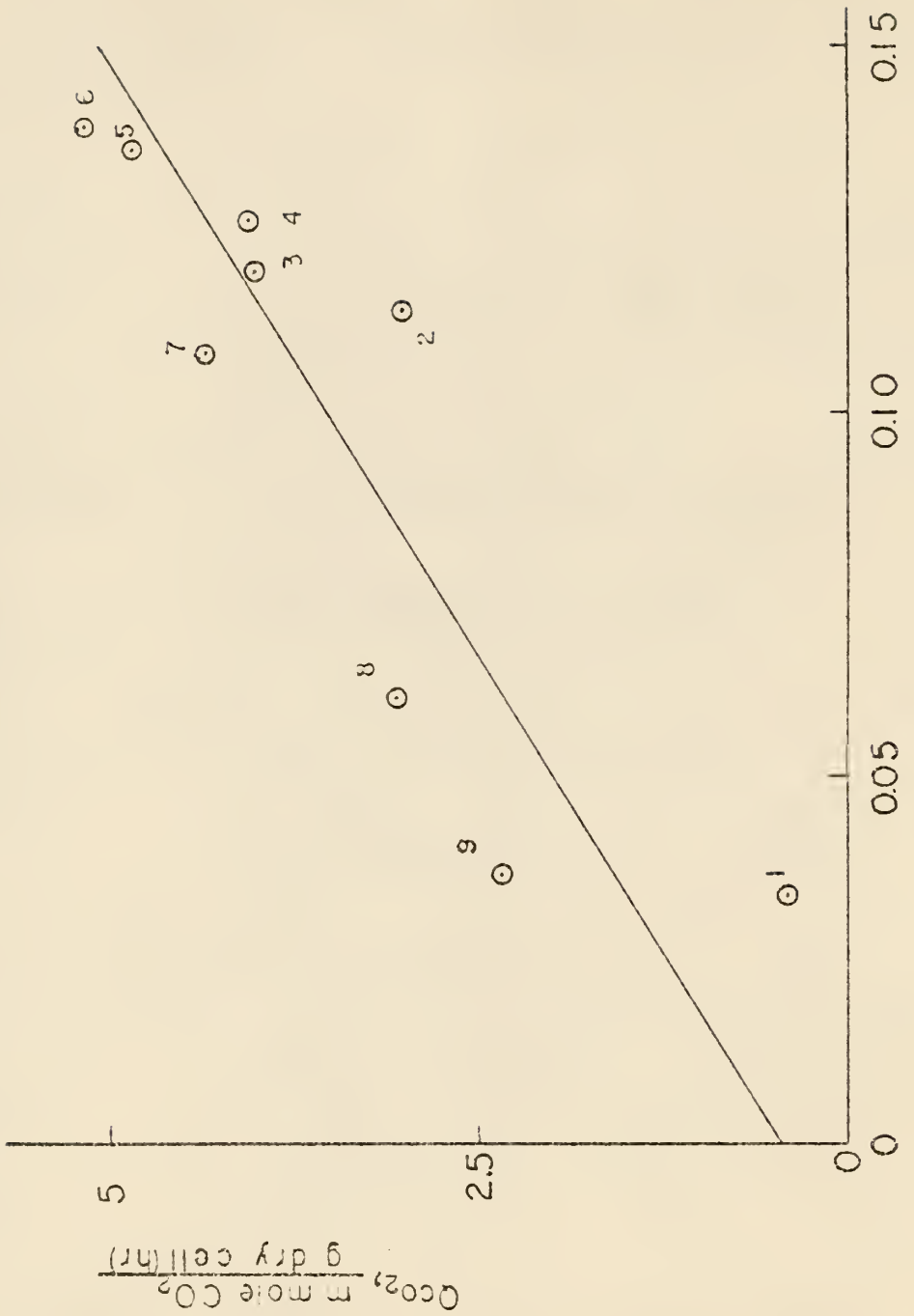

Fig. 13. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ. Run 6.

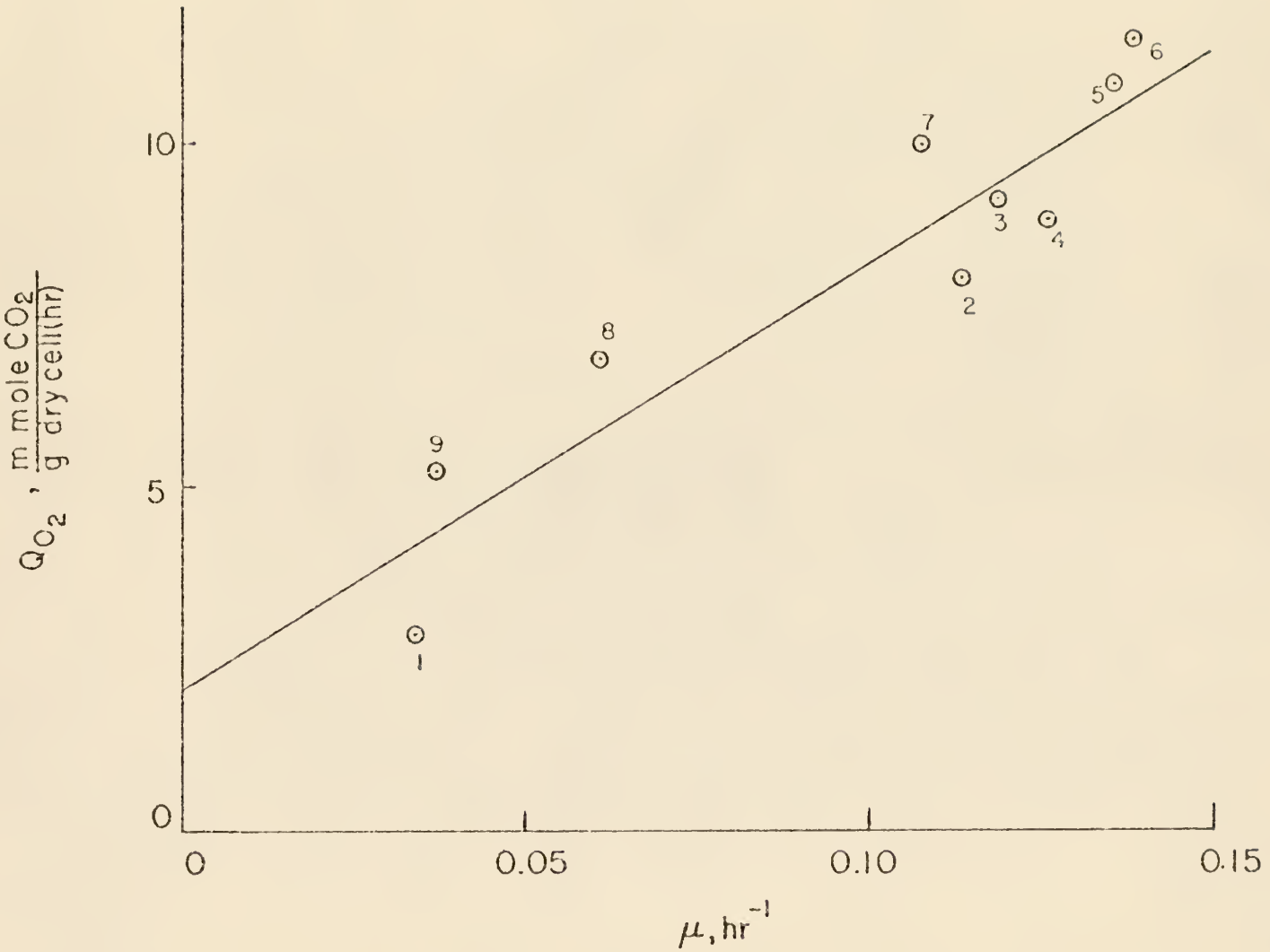

Fig. 14. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ. Run 6.

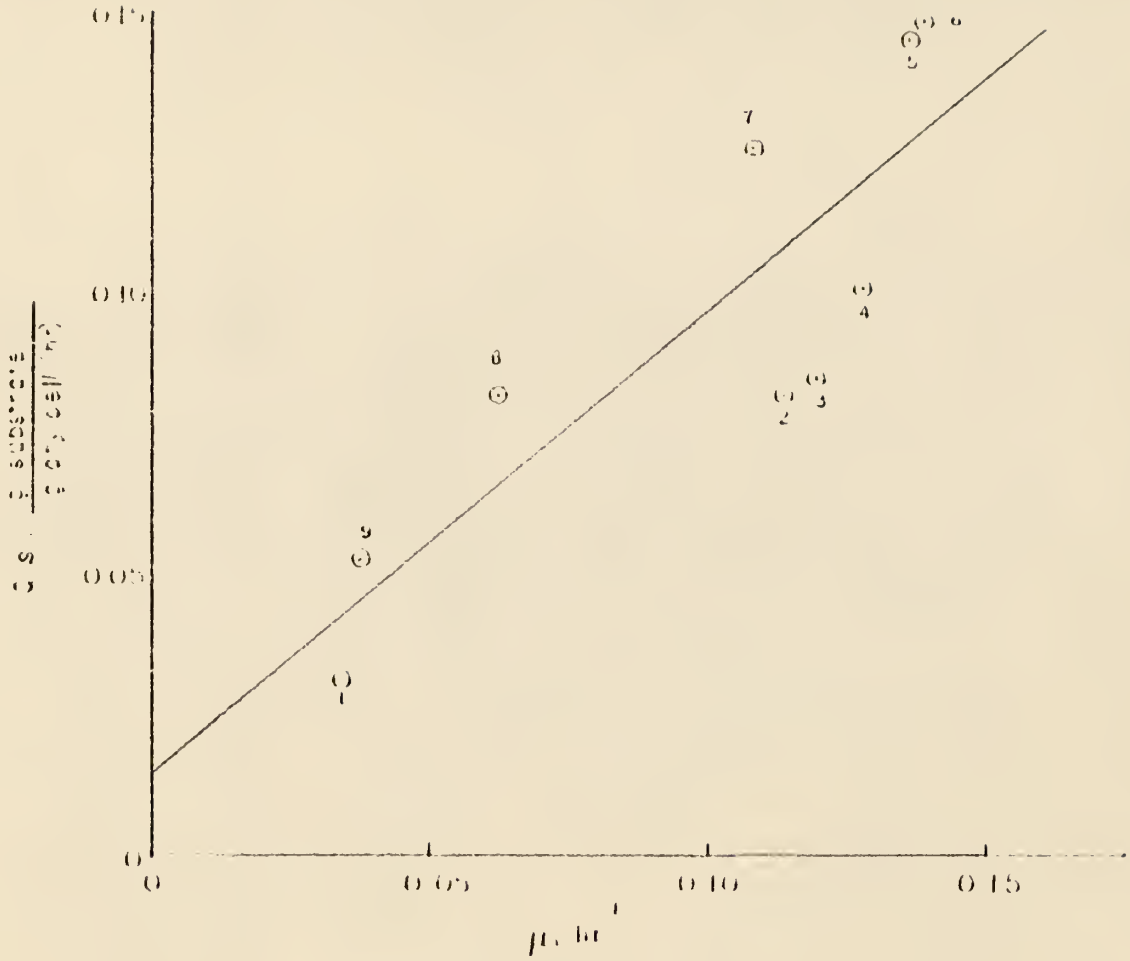

Fig. 15. Relationship between the specific n-hexadecane consumption rate, Q_S, and the specific growth rate, μ. Run 6.

APPENDIX

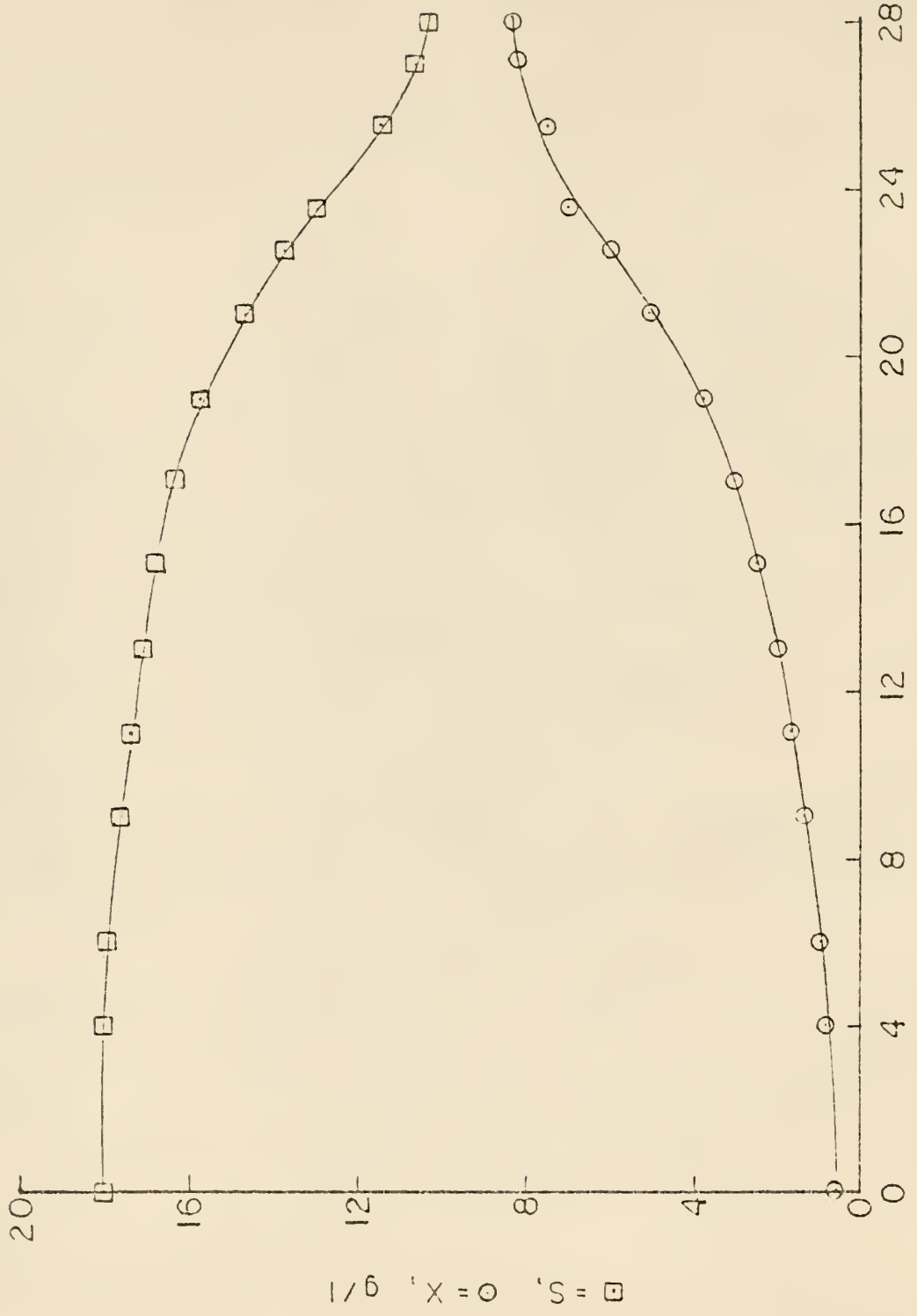

Fig. 1. Batch growth of Candida lipolytica on n-hexadecane; Run 3.

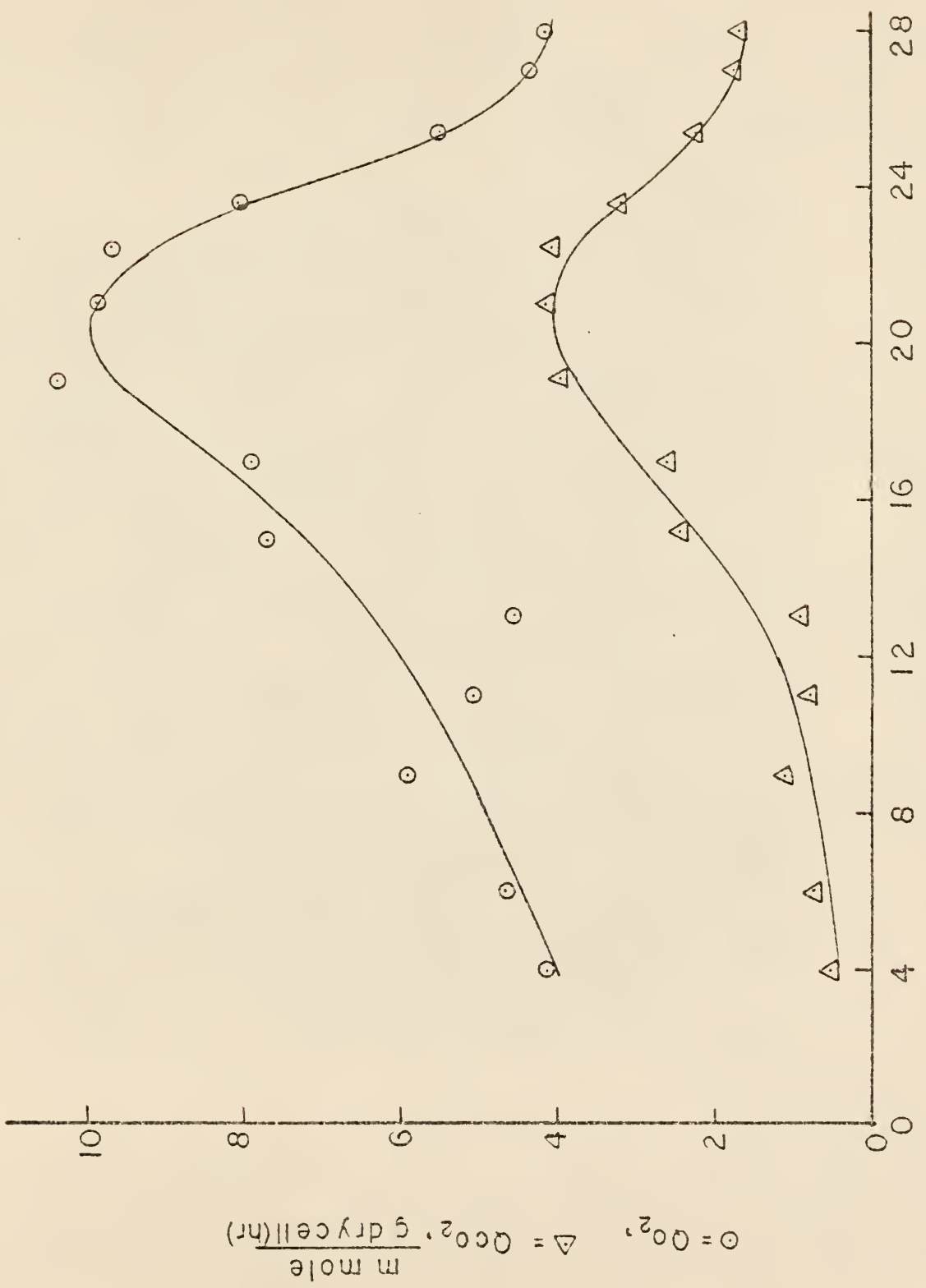

Fig. 2. Specific rates of oxygen uptake, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}, in batch culture of Candida lipolytica on n-hexadecane; Run 3.

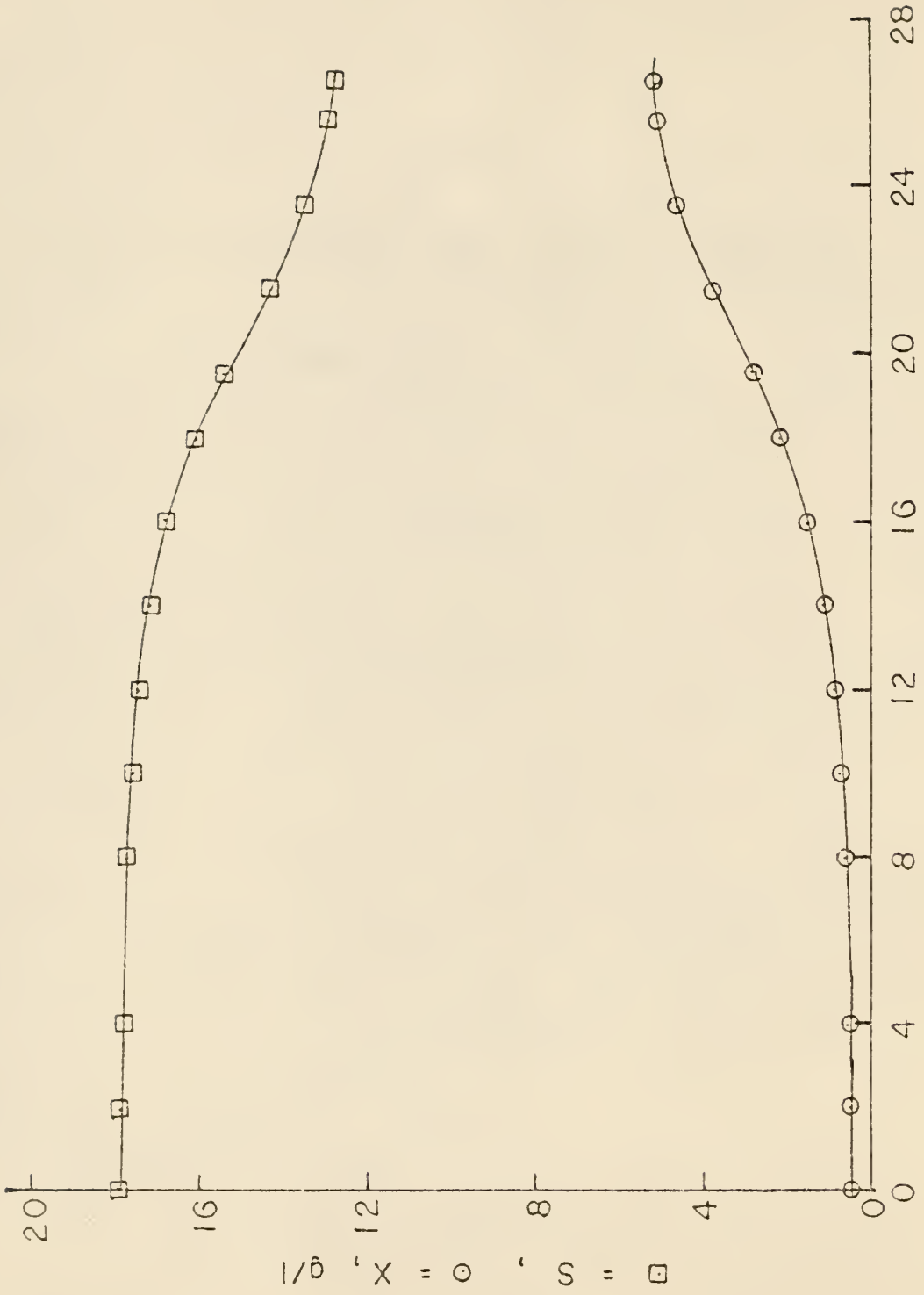

Fig. 3. Batch growth of Candida lipolytica on n-hexadecane; Run 4.

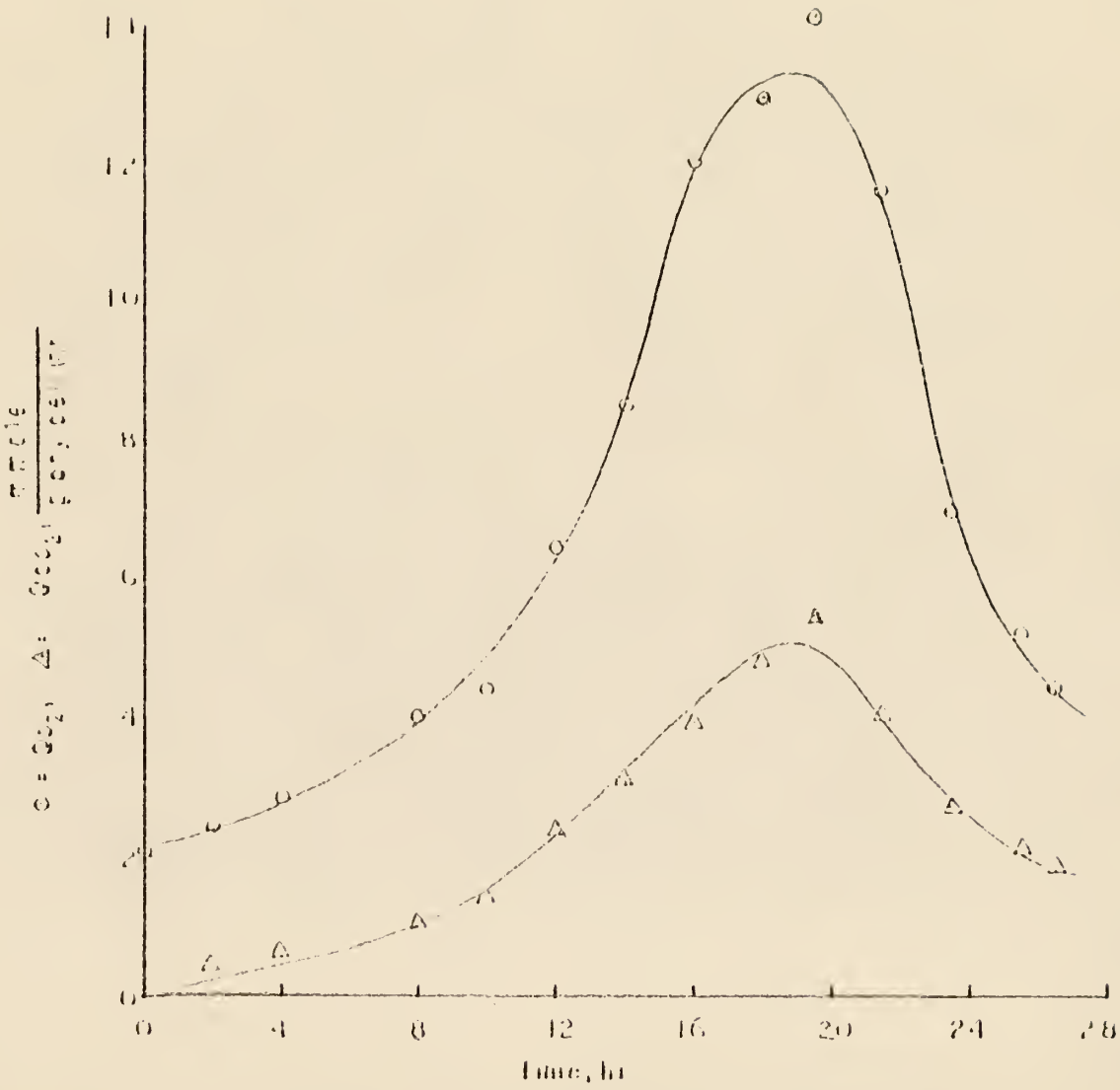

Fig. 4. Specific rates of oxygen uptake, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}, in batch culture of Candida lipolytica on n-hexadecane; Run 4.

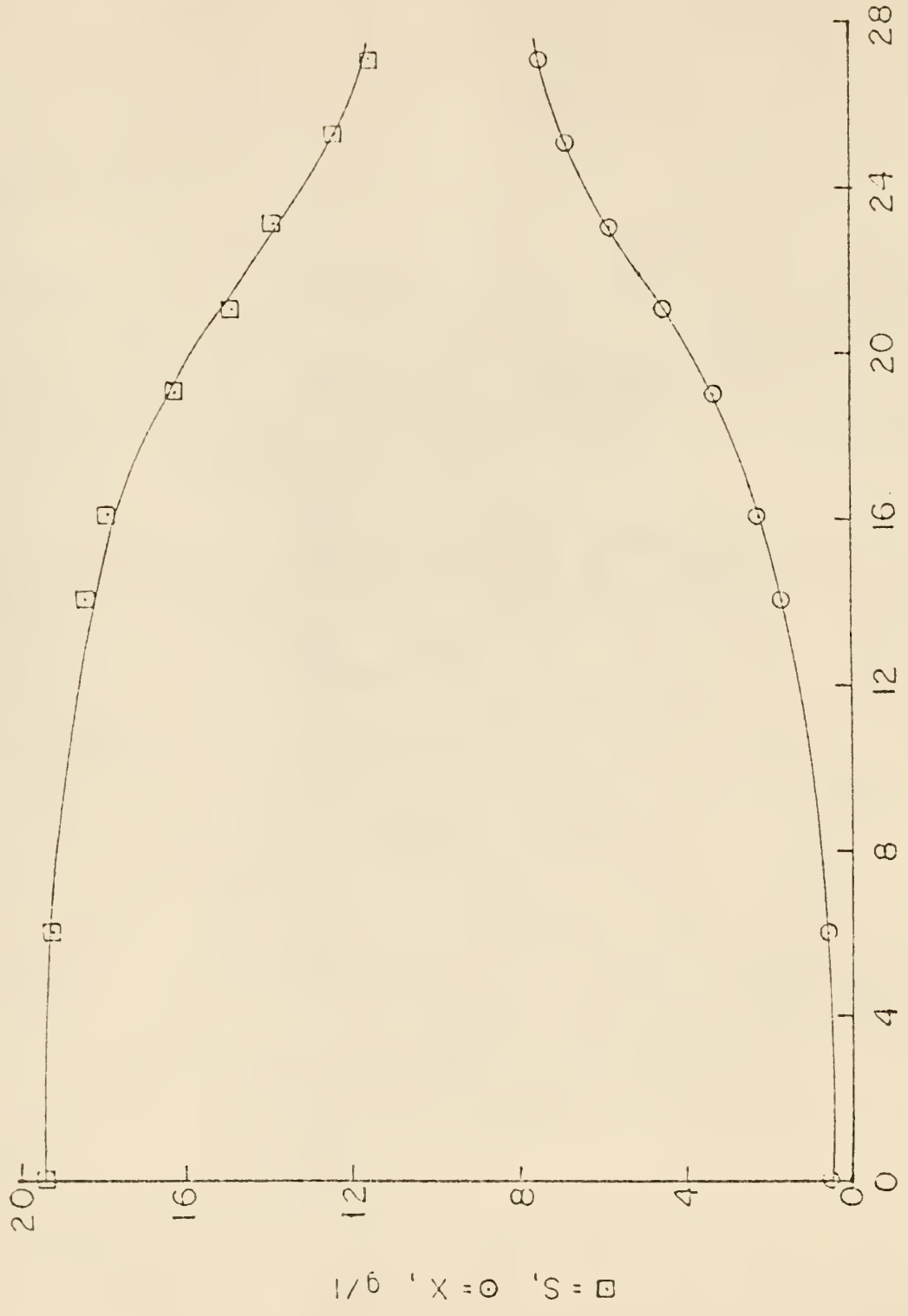

Fig. 5. Batch growth of Candida lipolytica on n-hexadecane; Run 6.

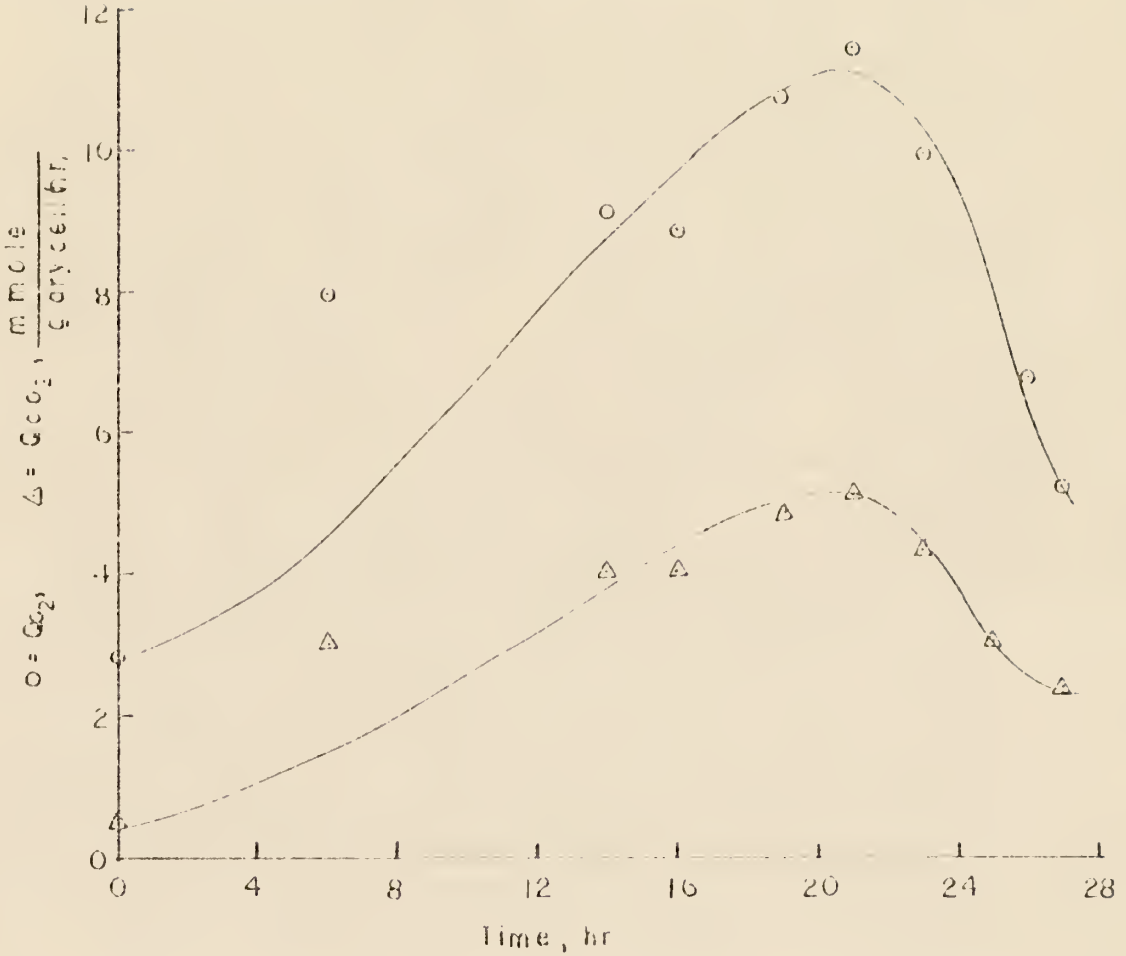

Fig. 6. Specific rates of oxygen uptake, Q_{O_2}, and carbon dioxide evolution, Q_{CO_2}, in batch culture of Candida lipolytica on n-hexadecane; Run 6.

USE OF MATERIAL AND ENERGY BALANCE REGULARITIES TO ESTIMATE GROWTH YIELDS AND MAINTENANCE COEFFICIENTS IN HYDROCARBON FERMENTATIONS

by

ALEXIS FERRER-OCANDO

B.S., Universidad del Zulia, Venezuela, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Chemical Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1979

The consistency of experimental data for growth of yeast on petroleum hydrocarbons in batch and continuous cultures was examined using carbon and available electron balances and the mean values of the regularities for carbon weight fraction in dry biomass and biomass reductance degree. Comparison of estimated values of yield and maintenance parameters were also used to detect experimental errors. The analysis of the data for consistency showed that in general continuous culture data are more consistent than batch culture data.

When literature data were used, the maintenance coefficient, m_e, was higher in batch cultures than in continuous cultures, and the "true" biomass energetic yield was also higher in batch cultures. Relatively few consistent batch culture data were found in the literature. Therefore, experiments were carried out to obtain sufficiently consistent experimental data to estimate "true" growth yields and maintenance coefficients in batch cultures. The yeast Candida lipolytica was grown on n-hexadecane as the main source of carbon in batch cultures. The progress of the fermentations was followed by measuring the biomass concentration, hexadecane concentration, oxygen consumption and carbon dioxide evolution. The data were found to be reasonably consistent when examined by making integrated and instantaneous available electron and carbon balances, and comparing values of the "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, estimated from different sets of variables. The results suggest that the carbon dioxide evolution measurements are smaller than what they should be. Largest errors frequently occured at the beginning and end of the fermentation where accurate measurements are difficult to obtain.

Four reasonably consistent sets of data were obtained, and they were used to get estimates of η_{max}, and m_e. The results were compared to values estimated from literature data and they were in very good agreement.