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Data Consistency, Yield, Maintenance, and Hysteresis in Batch Cultures of *Candida lipolytica* Cultured on *n*-Hexadecane

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Summary

Candida lipolytica was cultured batchwise using *n*-hexadecane as the main carbon source. Biomass production, *n*-hexadecane consumption, oxygen consumption, and carbon dioxide evolution were measured to follow the fermentation. The consistency of the measured data was examined using integrated and instantaneous available electron and carbon balances. Values of the "true" growth yield, η_{max}, and maintenance coefficient, m_e, were estimated using three different sets of data (biomass and *n*-hexadecane, oxygen and biomass, and CO_2 and biomass), and the results were compared with estimates obtained from literature data. Hysteresis patterns were observed in plots of specific rates of oxygen consumption and carbon dioxide evolution versus specific growth rate.

INTRODUCTION

The consistency of reported experimental data from growth of microorganisms in petroleum hydrocarbons was examined[1] using the material and energy balance regularities which Minkevich and Eroshin have identified.[2,3] Both batch and continuous cultures were analyzed, and the "true" biomass energetic yield, η_{max}, and the maintenance coefficient, m_e, 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 that 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 fermentions.

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MATERIALS AND METHODS

Equipment

A 5 liter New Brunswick Scientific model 19 stirred-tank fermentor 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 fermentor. 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 2*N* NaOH, or 2*N* 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 hr, were inoculated into 500 ml shake flasks, each containing 100 ml medium. After 24 hr cultivation, three of these flasks were inoculated into 2.5 liter medium in the fermentor.

Culture Media

The main culture medium was based on the simplified media of Aiba et al.[4] It contained (in g/liter): 7.5 $(NH_4)_2SO_4$; 1.5 Na_2HPO_4; 3.5 KH_2PO_4; 0.5 $MgSO_4 \cdot 7H_2O$; 2.0 nutrient broth; 30×10^{-3} $FeSO_4 \cdot 7H_2O$; 60×10^{-3} $CaCl_2 \cdot 2H_2O$; 15×10^{-6} $CuSO_4 \cdot 5H_2O$; 30×10^{-6} $Na_2B_4O_7 \cdot 10H_2O$; 60×10^{-6} $MnCl_2 \cdot 4H_2O$; 150×10^{-6} $ZnSO_4 \cdot 7H_2O$; 30×10^{-6} $Na_2MoO_4 \cdot 7H_2O$. Two %(w/v) *n*-hexadecane was used as the main carbon source. The medium used for the

preculture and stock had the following composition: 40 g glucose, 2 g nutrient broth, and 1000 ml distilled water. For the stock culture, 15 g/liter agar were used. Shake flasks, each containing 100 ml medium, were autoclaved at 20 psia for 15 min. The fermentor was autoclaved at 20 psia for 30 min.

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 min. The cells were washed with 5 ml distilled water, centrifuged, resuspended in 5 ml distilled water, and dried in an oven at 105°C for 24 hr. The concentration was calculated in g/liter. 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 fermentor 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 reextracted 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 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 × $\frac{1}{8}$ in. stainless-steel column packed with 10% SE-30 (methyl silicone) 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/liter and plotted against time.

Carbon dioxide and oxygen concentrations in the gas phase

Gas samples were taken from the exit gas stream of the fermentor after the liquid samples had been taken. 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 fermentor 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 (RQ = Q_{O_2}/Q_{O_2}) was also calculated, and used to estimate the biomass energetic yield, η.

Methods of Data Analysis

Specific growth rate

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:

$$Q_S = \frac{1}{X}\frac{dS}{dt} \tag{1}$$

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. 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 fermentor. 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 fermentor. 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 liter/min is divided by the volume of broth to find the air flow rate per unit of broth volume, Q_g, in liter/liter·hr. This value is then corrected to standard conditions to find the air molar rate per unit of broth volume, Q_m;

$$Q_m = Q_g \times \frac{1 \text{ mol gas}}{22.4 \text{ liter}} \times \frac{273°\text{K}}{303°\text{K}} \tag{2}$$

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 one makes the exit air flow rate on a dry basis lower than the inlet flow rate. However, water evaporates from the fermentor 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 fermentor. The rate of oxygen consumption is

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

The specific oxygen consumption rate, Q_{O_2}, was evaluated by dividing the foregoing variable by X.

Rate of carbon dioxide evolution

Similar measurements are required to estimate Q_{CO_2}; i.e.,

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

The specific carbon dioxide evolution rate, Q_{CO_2}, was evaluated by dividing the foregoing variable by X.

True growth 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 procedures that have been described previously.[1]

Data analysis limitations

The particular modes of finding the specific growth rate and rate of organic substrate consumption that are used here are limited to batch fermentations in which broth dilution due to base addition and broth concentration because of evaporation are small or balanced, and thus do not lead to changes in the substrate and cell concentrations.

RESULTS AND DISCUSSION

Results are presented in Tables I–XVI and Figures 1–12. The first part of this section describes the growth characteristics of the yeast. 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 XI shows the experimental conditions of the batch cultures and Table XII presents a list of the main problems encountered in the measurements.

TABLE I
Measured Variables in the Batch Fermentation of *C. lipolytica*[a]

θ (hr)	Broth volume (ml)	O_2 in exit air (%)	CO_2 in exit air (%)	X (g/liter)	S (g/liter)
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

[a] Run 3: 1 liter/min air flow rate; 800 rpm agitation; inlet air = 20.8% O_2; 0.045% CO_2.

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 IX and XIII–XVI. The "true" biom-

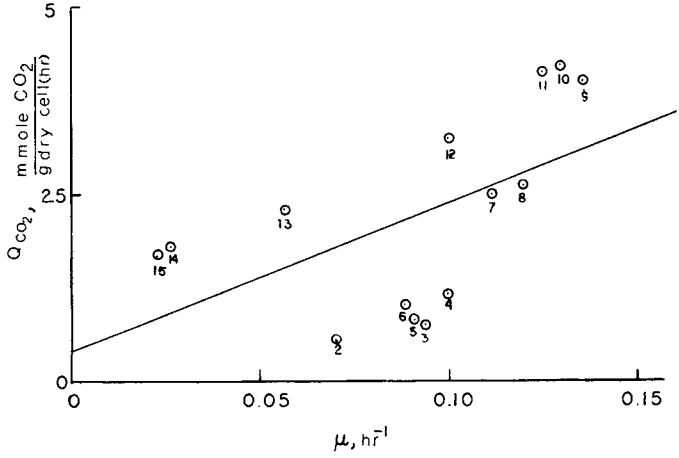

Fig. 1. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ; run 3.

TABLE II
Measured Variables in the Batch Fermentation of *C. lipolytica*[a]

θ (hr)	Broth volume (ml)	O_2 in exit air (%)	CO_2 in exit air (%)	X (g/liter)	S (g/liter)
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

[a] Run 4: 0.375 liter/min air flow rate; 1000 rpm agitation speed; inlet air = 20.8% O_2; 0.045% CO_2.

ass 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 X.

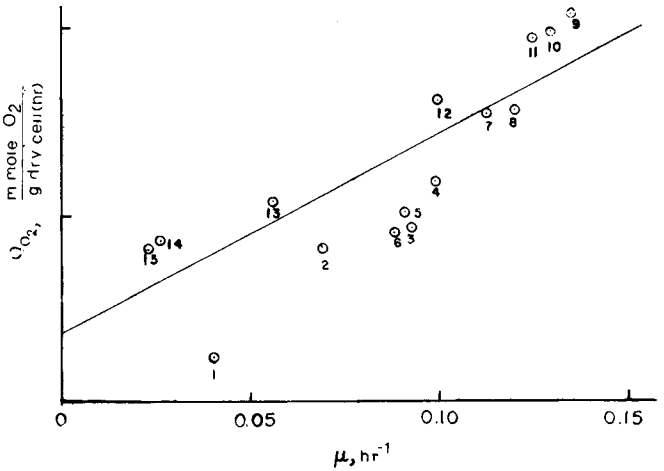

Fig. 2. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ; run 3.

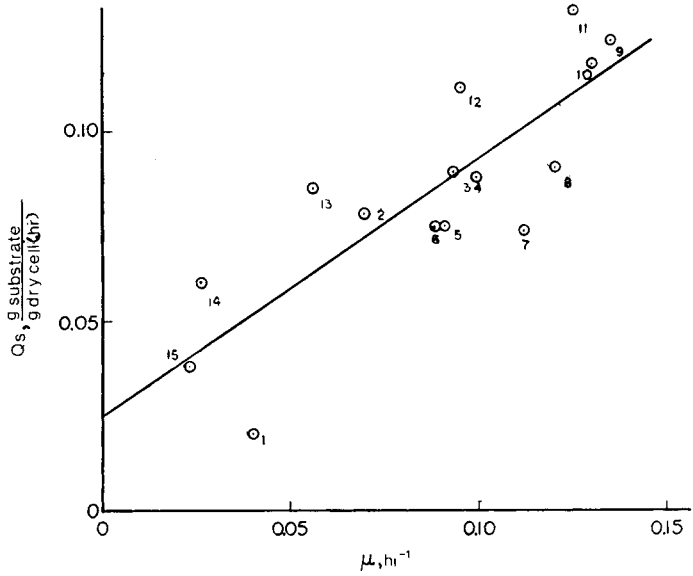

Fig. 3. Relationship between the specific *n*-hexadecane consumption rate, Q_S, and the specific growth rate, μ; run 3.

TABLE III
Measured Variables in the Batch Fermentation of *C. lipolytica*[a]

θ (hr)	Broth volume (ml)	O_2 in exit air (%)	CO_2 in exit air (%)	X (g/liter)	S (g/liter)
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

[a] Run 5: 0.375 liter/min air flow rate; 1000 rpm agitation; inlet air = 20.8% O_2, 0.045% CO_2.

TABLE IV
Measured Variables in the Batch Fermentation of *C. lipolytica*[a]

θ (hr)	Broth volume (ml)	O_2 in exit air (%)	CO_2 in exit air (%)	X (g/liter)	S (g/liter)
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

[a] Run 6: 0.375 liter/min air flow rate; 1000 rpm agitation; inlet air = 20.8% O_2; 0.045% CO_2.

Hysteresis patterns are identified in plots of specific rates of oxygen consumption and carbon dioxide evolution versus specific growth rates (Figs. 1, 2, 4, 5, 7, 8, 10, and 11). 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}

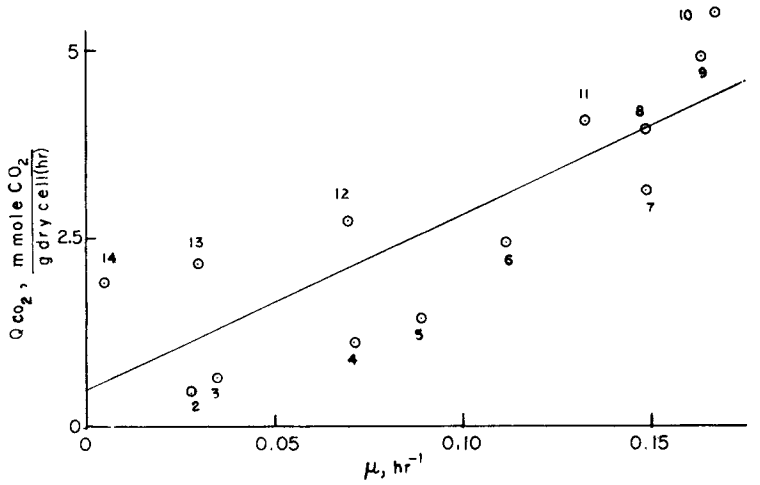

Fig. 4. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ; run 4.

TABLE V
Calculated Variables in the Batch Fermentation of *C. lipolytica*; Run 3

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

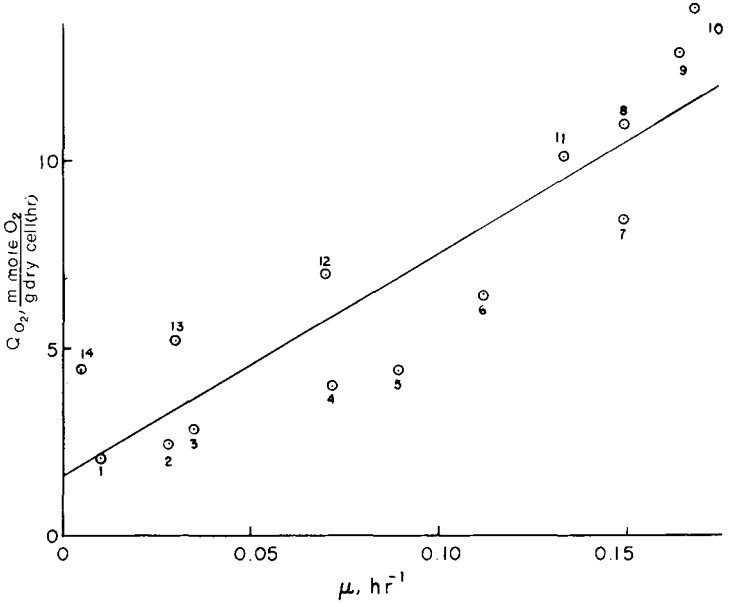

Fig. 5. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ; run 4.

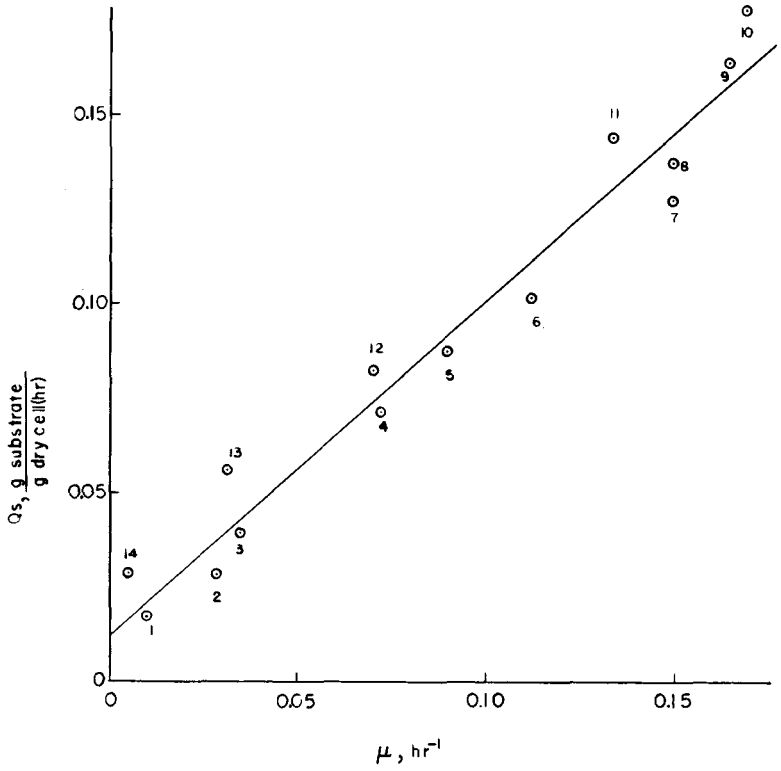

Fig. 6. Relationship between the specific *n*-hexadecane consumption rate, Q_S, and the specific growth rate, μ; run 4.

and m_e are examined and discussed taking into account available information on the consistency of the data.

Growth Characteristics

Shake-flask experiments

Several experiments were carried out to find appropriate conditions for the growth of *C. lipolytica*. This organism was grown in a basal medium (described in the 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/liter when the concentration of nutrient broth was lower than 0.2%. Thus some factor appeared to be limiting in this condition.

TABLE VI
Calculated Variables in the Batch Fermentation of *C. lipolytica*, Run 4

θ (hr)	μ (hr^{-1})	Q_{O_2} [mmol/ g dry cell (hr)]	Q_{CO_2} [mmol/ g dry cell (hr)]	Q_S [g substrate/ g dry cell (hr)]	RQ
0	0.010	2.051	0.051	0.017	0.025
2	0.028	2.432	0.438	0.028	0.18
4	0.035	2.863	0.630	0.040	0.22
8	0.072	4.002	1.084	0.072	0.27
10	0.089	4.403	1.408	0.088	0.32
12	0.112	6.402	2.433	0.102	0.38
14	0.149	8.435	3.121	0.128	0.37
16	0.149	10.945	3.940	0.138	0.36
18	0.164	12.820	4.872	0.164	0.38
19.5	0.168	13.998	5.460	0.178	0.39
21.5	0.133	10.100	4.039	0.144	0.40
23.5	0.070	6.986	2.725	0.082	0.39
25.5	0.030	5.209	2.136	0.056	0.41
26.5	0.005	4.398	1.892	0.028	0.43

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

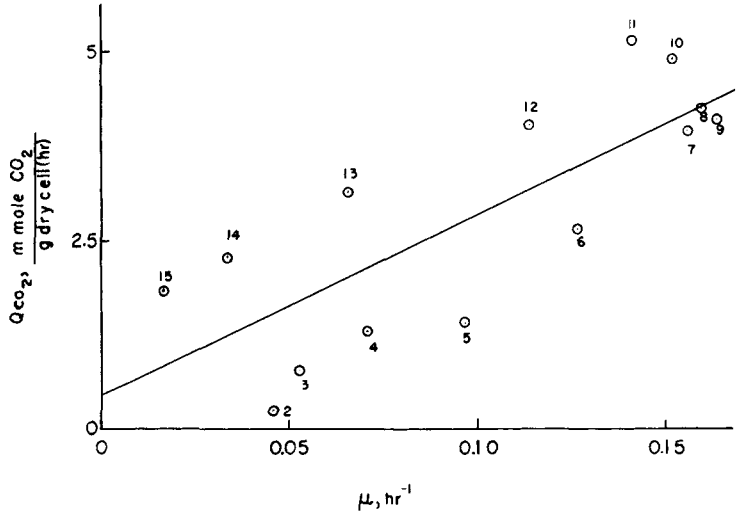

Fig. 7. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ, run 5.

TABLE VII
Calculated Variables in the Batch Fermentation of *C. lipolytica*; Run 5

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

Fermentor cultures

Fermentations usually stopped about 28 hr after inoculation. Fermentations carried out in the past[5] 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 hr. The initial $(NH_4)_2SO_4$ concentration was increased from 5.0 to 7.5 g/liter.

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

Accumulation of oil, cells, and foam as flocs were observed in most of the fermentation. When the broth was agitated manually, the pH decreased immediately, showing that considerable activity of the microorganisms occurred at the surface of the broth. At high cell concentrations, floculation 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.[5]

Experimental Techniques

The most significant problem encountered in the experiments was to take a representative sample from the fermentation broth. Bio-

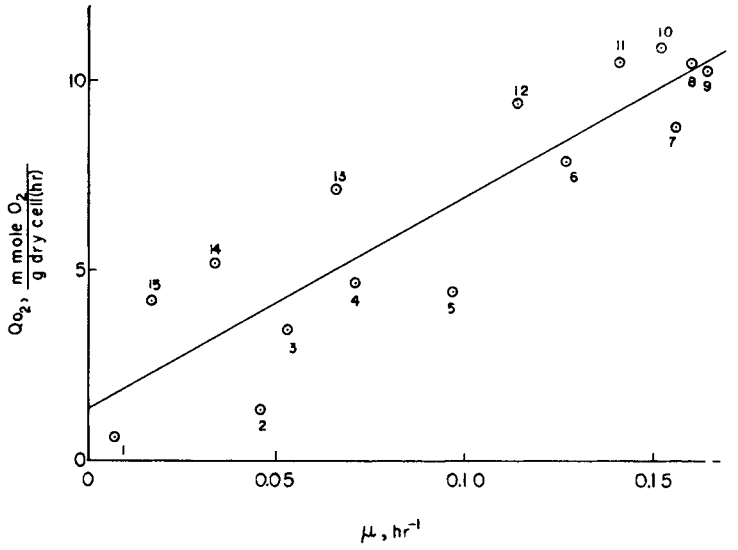

Fig. 8. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ; run 5.

mass could be seen adhering to the fermentor 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

TABLE VIII
Calculated Variables in the Batch Fermentation of *C. lipolytica*; Run 6

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

TABLE IX
Examination of Data Consistency Using Integrated Available Electron and Carbon Balances for Growth of *C. lipolytica* on *n*-Hexadecane in Batch Cultures[a]

Source of data	Results					
	y_c	d	$y_c + d$	η	ϵ	$\eta + \epsilon$
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 3-6	0.50	0.44	0.94	0.35	0.70	1.05

[a] $\gamma_b = 4.291$, $\sigma_b = 0.462$; O_2, CO_2, biomass, and hydrocarbon data.

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

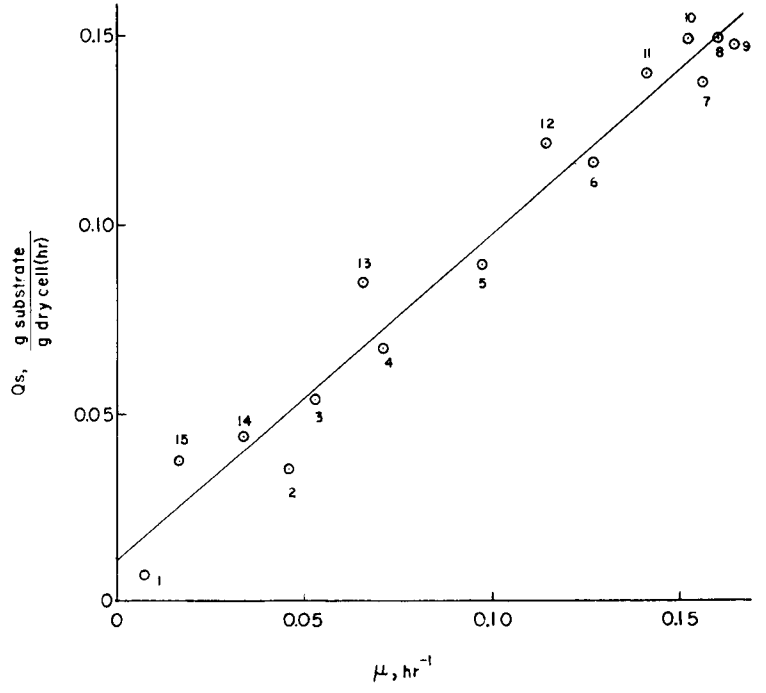

Fig. 9. Relationship between the specific *n*-hexadecane consumption rate, Q_S, and the specific growth rate, μ; run 5.

TABLE X
Values of "True" Biomass Energetic Yield Coefficient, η_{max}, and Maintenance Coefficient, m_e, from Growth of *C. lipolytica* on *n*-Hexadecane in Batch Culture[a]

	Results			
Source of data	η_{max}	m_e (hr^{-1})	r^2	Comments
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 3–6	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

[a] $\gamma_b = 4.291$; $\sigma_b = 0.462$.

uniform broth before taking a sample. Duplicate samples were taken periodically and the comparison of the two measurements showed differences up to 2.5%.

The problem of taking a representative sample is also reflected in the hexadecane measurements. Hexadecane is less dense than water and might be nonuniformly distributed in the broth. When the liquid sample at the start of the fermentation was subjected to a one-step hexadecane extraction procedure, 66% of the hexadecane was extracted. When a second extraction was carried out, the lowest extraction was 92% compared to the initial hexadecane concentration. The 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

TABLE XI
Experimental Conditions for Growth of *C. lipolytica* on *n*-Hexadecane in Batch Cultures

Run	Air flow rate (liter/min)	Agitation (rpm)
3	1	800
4	0.375	1000
5	0.375	1000
6	0.375	1000

broth with cells was subjected to hexadecane extraction. The upper phase is a water phase and hexadecane was not detected after two extractions. 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

TABLE XII
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 mol%
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 XIII
Examination of Data Consistency Using Instantaneous Available Electron and Carbon Balances for Growth of *C. lipolytica* on n-$C_{16}H_{34}$ in Batch Culture[a]

θ (hr)	y_c	d	$y_c + d$	η	ϵ	$\eta + \epsilon$
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

[a] Run 3: $\sigma_b = 0.462$; $\gamma_b = 4.291$.

TABLE XIV
Examination of Data Consistency Using Instantaneous Available Electron and Carbon Balances for Growth of *C. lipolytica* on n-$C_{16}H_{34}$ in Batch Culture[a]

θ (hr)	y_c	d	$y_c + d$	η	ϵ	$\eta + \epsilon$
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

[a] Run 4: $\sigma_b = 0.462$; $\gamma_b = 4.291$.

TABLE XV
Examination of Data Consistency Using Instantaneous Available Electron and Carbon Balances for Growth of *C. lipolytica* on n-$C_{16}H_{34}$ in Batch Culture[a]

θ (hr)	y_c	d	$y_c + d$	η	ϵ	$\eta + \epsilon$
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

[a] Run 5: $\sigma_b = 0.462$; $\gamma_b = 4.291$.

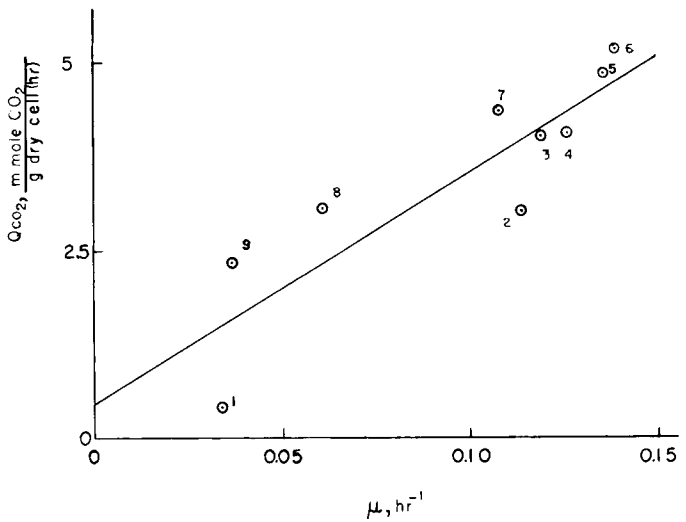

Fig. 10. Relationship between the specific carbon dioxide evolution rate, Q_{CO_2}, and the specific growth rate, μ; run 6.

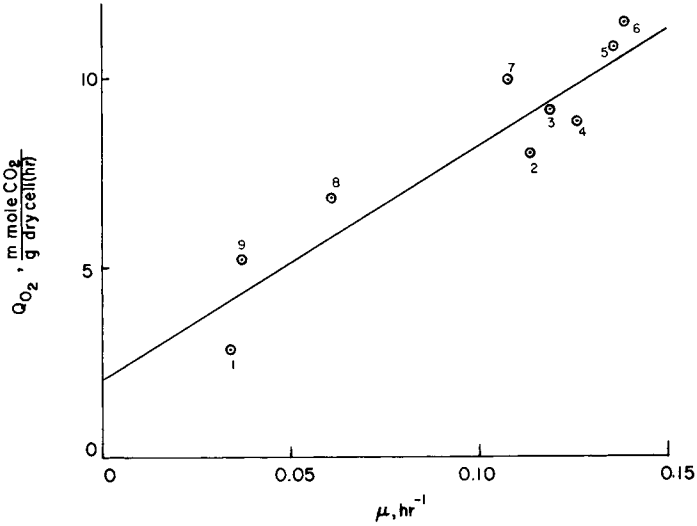

Fig. 11. Relationship between the specific oxygen uptake rate, Q_{O_2}, and the specific growth rate, μ; run 6.

lower phases are indistinguishable. Therefore, both middle and lower phases were always taken together from the separatory funnel.

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.

TABLE XVI
Examination of Data Consistency Using Instantaneous Available Electron and Carbon Balances for Growth of *C. lipolytica* on n-$C_{16}H_{34}$ in Batch Culture[a]

θ (hr)	y_c	d	$y_c + d$	η	ϵ	$\eta + \epsilon$
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	0.99	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	0.95	0.29	0.77	1.06
27	0.39	0.64	1.03	0.27	0.92	1.19

[a] Run 6: $\sigma_b = 0.462$; $\gamma_b = 4.291$.

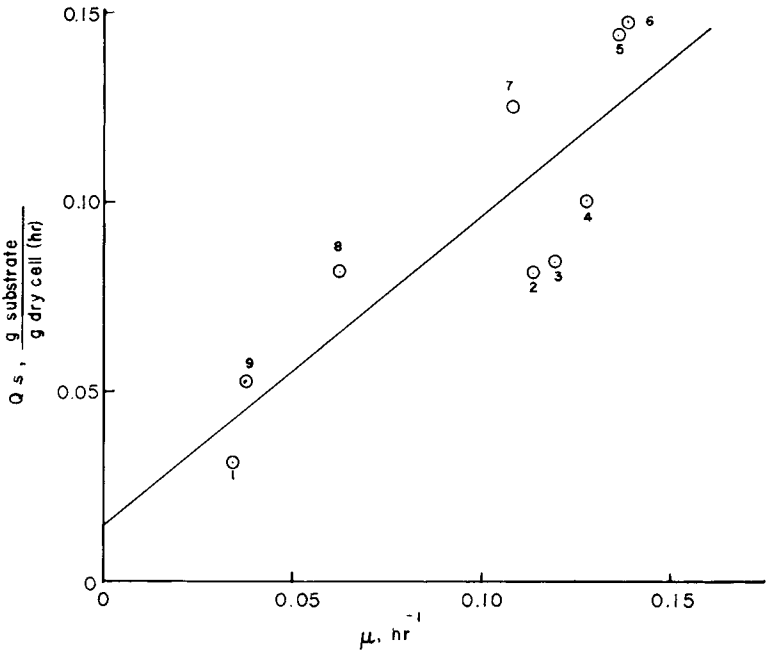

Fig. 12. Relationship between the specific *n*-hexadecane consumption rate, Q_S, and the specific growth rate, μ; run 6.

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 4 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 4 hr 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 because 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 I 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 runs 4–6 (Tables II–

IV). 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.

Dissolved oxygen measurements were carried out, but the probe frequently became plugged during fermentations. Unstable readings appeared in almost all the fermentations except at the beginning.

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 return the pH to 5.5.

Consistency of the Data

Table IX shows the results of an examination of data consistency using integrated available electron and carbon balances for the growth of *C. lipolytica* on *n*-hexadecane in runs 3-6. It can be seen that the data are quite consistent since $(y_c + d)$ and $(\eta + \epsilon)$ are close to 1. The 95% confidence intervals are $0.94 \leq y_c + d \leq 1.06$ and $0.93 \leq \eta + \epsilon \leq 1.07$ 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: $\eta = 0.32$, $\epsilon = 0.64$, $\eta + \epsilon = 0.96$, $y_c = 0.44$, $d = 0.39$, and $y_c + d = 0.83$. Note that $\eta + \epsilon$ is still close to one if the nutrient broth is consumed completely. Carbon dioxide appears to be underestimated. Values of $(y_c + d)$ are significantly lower than one, and this can be due to either product formation or 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 IX 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.

Tables XIII-XVI 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 borne 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 runs 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.

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 IX). 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 $\eta + \epsilon \approx 1$ many times during the fermentations, showing that the data are reasonably consistent. In addition, the coefficients of variation of $(y_c + d)$ and $(\eta + \epsilon)$ were always lower than 23%. The data from Hug and Fiechter,[6] which were the most consistent batch data,[1] showed coefficients of variation of about 23% in the values of $(y_c + d)$ and $(\eta + \epsilon)$ 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 3-6 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 X. 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%. Differences between m_e from substrate and biomass data, and oxygen and biomass data, were lower than 20%.

Estimates of dissolved CO_2 and HCO_3^- were made. Dissolved CO_2 and HCO_3^- are 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. Since eqs. (3) and (4) neglect accumulation in the gas and liquid phases, this accumulation is not included in the analysis, and the reported values of CO_2 production

are low during the initial period of the fermentation when dissolved CO_2 and HCO_3^- are accumulating in the liquid phase.

Values of the biomass energetic yield, η, estimated from the respiratory quotient (RQ) 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.[7-9] 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 that 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 1, 2, 4, 5, 7, 8, 10, and 11 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 2 are 0.099 and 0.095 hr^{-1}, respectively, whereas the values of Q_{O_2} are 5.911 and 8.028 mmol/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 that 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} vs. 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 ex-

plained by accumulation of CO_2 and bicarbonates in the fermentation broth.

In an earlier work[1] Figures 5 and 6 (data from Moo-Young et al.[10]), and 7 and 8 (data from Blanch and Einsele[11]), 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 the utilization of Biocert may be higher than that associated with hexadecane. Higher values of η produce lower values of Q_{O_2},[2] 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 V–VIII that the respiratory quotient was very small at the early hours of fermentation; therefore, it appears that the hysteresis phenomenon cannot be attributed to early consumption of nutrient broth. The values of η also do not indicate early consumption of nutrient broth.

Figures 3, 6, 9, and 12 are plots of the specific rate of hexadecane consumption, Q_S, versus the specific growth rate, μ. A hysteresis pattern cannot be clearly seen. The gap between the forward and reverse arcs in Figures 6 and 9 is very small. Figure 12 shows a hysteresis pattern but there are few data points to be considered.

Yield and Maintenance Coefficient

Table X shows the values of the "true" biomass energetic yield coefficient estimated from three different sets of data in each of the runs 3–6. The three different sets of data were carbon dioxide and biomass data shown in Figures 1, 4, 7, and 10, oxygen and biomass data shown in Figures 2, 5, 8, and 11, and hexadecane and biomass data shown in Figures 3, 6, 9, and 12. In addition, the data from runs 3–6 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 4–6 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 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

1 and 2 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 4, 5, 7, 8, 10, and 11 also show similar effects for the values of m_e.

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 $\eta_{max} = 0.41$ in continuous cultures[1] using the "t" test.[12] The results indicated that these values are not significantly different at the 95% confidence level. Similarly, the same value of $\eta_{max} = 0.44$ was not significantly different from the mean value of 0.47 reported for batch cultures of *Candida tropicalis* growing on *n*-hexadecane.[1] 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. 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 X 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 equal 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 earlier results[1] using the data of Blanch and Einsele.[11] 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 value 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 3-6 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, all the oxygen and biomass data, and all the substrate and biomass data 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. These are confidence intervals for the intercept of a straight line. The mean value of m_e from continuous-culture data (0.012 hr^{-1}) is within two of these intervals. However, when the mean value of m_e of runs 3-6 (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. In addition, the hypothesis that the mean values of m_e in batch-culture data from the literature (0.054 hr^{-1}) and in the batch-culture data of this work $m_e = 0.033$ hr^{-1} were equal was rejected with 95% confidence.

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.

CONCLUSIONS

Application of material and energy balance regularities to examine the consistency of batch-culture data for *C. 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 fermentor, 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: $\eta = 0.35$, $\epsilon = 0.70$, $y_c = 0.50$, 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 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 3-6 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	mol NH_3/quantity organic substrate containing 1 g atom C (g mol/g atom C)
d	mol CO_2/quantity organic substrate containing 1 g atom C (g mol/g atom C)
m_e	rate of organic substrate consumption for maintenance [g equiv available electrons/g equiv available electrons in biomass (hr) or kcal/kcal biomass (hr)]
Q_g	air flow rate (liter/hr)
Q_m	molar air flow rate (g mol/hr)
Q_{CO_2}	rate of CO_2 evolution [g mol/g dry cell (hr)]
Q_{O_2}	rate of O_2 consumption [g mole/g dry cell (hr)]
Q_S	rate of organic substrate consumption [g/g dry cell (hr)]
q	atomic ratio of N to C in biomass (dimensionless)
RQ	respiratory quotient (dimensionless)
r^2	correlation coefficient
S	organic substrate concentration (g/liter)
S_0	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 (equiv available electrons/g atom C)
γ_s	reductance degree of organic substrate (equiv available electrons/g atom C)
ϵ	fraction of energy in organic substrate that is evolved as heat (dimensionless)
η	fraction of energy in organic substrate that 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 C in biomass (dimensionless)
σ_s	weight fraction C in organic substrate (dimensionless)
θ	time (hr)

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