Advanced Journal of Microbiology Research Vol. 2003
Available online at http://internationalscholarsjournals.org/journal/ajmr

Substrate Channelling and Energetics of *Saccharomyces cerevisiae* DSM 2155 Grown on Glucose in Fed-Batch Fermentation Process

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Accepted 10 April, 2003

Data collected during high-cell-density cultivation of *Saccharomyces cerevisiae* DSM 2155 on glucose in a simulated five-phase feeding strategy of fed-batch process, executed on the Universal Bioprocess CONtrol (UBICON) system using 150L bioreactor over a period of 24h have been analysed. The consistency of the data set was checked using both the available electron and carbon balances. Estimates of the true energetic yields and cell maintenance requirements were obtained through the application of a multivariate statistical procedure known as covariate adjustment technique. A low value of maintenance coefficient, $m_e = 0.004h^{-1}$, and a high average value of the true biomass energetic yield, $_{max} = 0.745$, were obtained for the bioreactor system, which showed that the organism was in no danger of ethanol produced during this cultivation. A simple model for estimating the distribution of substrate consumed between the fermentative and the respiratory pathways in the oxido-reductive process was developed based on the respiratory quotient (RQ) values. The fraction of substrate consumed for respiratory metabolic activities (q_{sresp}/q_s) was virtually 1.0 for the first three phases of the feeding strategy, which accounted for the first sixteen hours of the 24h operation. This was an indication that ethanol formation was avoided during this period.

Key Words: *Saccharomyces cerevisiae* DSM 2155, available electron and carbon balances, fed-batch, respiratory quotient, true energetic yields, maintenance requirement.

INTRODUCTION

In recent years, baker's yeast (*Saccharomyces cerevisiae*), considered the most intensively cultivated and commercial microorganism, has been used extensively for the production of single-cell protein (SCP) for human and animal consumption, and ethanol (industrial and potable alcohol) from fermentable sugars because of its GRAS (**G**enerally **R**egarded **A**s **S**afe) status (Solomon et al., 1997). In addition, it is widely used in leavening of dough because of its ability to produce carbon dioxide and ethanol from sugars (i.e. maltose and sucrose) present in the dough (Chen and Chiger, 1985; Jørgensen et al., 2002; Reed and Nagodawithana, 1991). Furthermore, it is also employed in the leavening process because of its contribution to the aroma and flavour of bread (Hoek et al., 1999).

However, in spite of its wide applications, the cultivation of baker's yeast is not without problems. The low productivity obtained under both aerobic batch cultivation (due to diauxic growth behaviour) and during continuous cultures as a result of dilution rate dependence has led to the adoption of fed-batch process for baker's yeast production (Beudeker et al., 1990; Ejiofor et al., 1994a,b). These observations have been attributed to the Crabtree effect or glucose effect (Barford and Hall, 1979; Beck and von Meyenburg, 1968; De Deken, 1966; Fiechter and Seghezzi, 1992; Pronk et al., 1996). These problems have made the aerobic growth of *S. cerevisiae* on glucose to continue to be of research interest (Alexandra, 1990; Kristiansen, 1994; Petrik et al., 1983; Rieger et al., 1981, 1983; Sonnleither and Kappeli, 1986). In order to achieve a maximum yield at the highest possible productivity, a well designed feeding strategy for *S. cerevisiae* is needed (Belgardt 2000; Ejiofor et al., 1994a,b). The dilution rate and the respiratory quotient (RQ) can be used as control in overcoming the problems encountered during baker's yeast production. The RQ can be used in allocating the amount of the substrate

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Abbreviations : List is on page 102.

(glucose) consumed for respiration (oxidation) and the amount of the substrate used for fermentative (reduction) activities. By this, the inhibition generated by ethanol formation during the production of baker's yeast can be avoided. Kasperski and Miskiewicz (2002) have only recently developed a fuzzy logic controllers (FLC) using RQ as an indicator during cultivation of *S. cerevisiae*.

Therefore, this present work proceeded to establish a relationship between ethanol formation and growth of *S. cerevisiae* on glucose by developing a simple model for quantifying the substrate consumed for fermentative and respiratory metabolic activities, respectively, based on the RQ value that can be measured routinely online. In addition, the true biomass energetic yield, the true product energetic yield and the maintenance requirement during the oxido-reductive growth of the yeast were estimated. Consequently, data collected during the cultivation of *S. cerevisiae* DSM 2155 in 150L bioreactor over the periods of 24h were analysed. The consistency of the data was checked by available electron and carbon balances. Estimates of the true yields and maintenance requirement for the fermentation were obtained through the application of a multivariate statistical procedure known as covariate adjustment technique (Solomon et al., 1983; 1984). The derived model for substrate partitioning into the two pathways could be used along side with other growth parameters like true biomass energetic yield, true product energetic yield and maintenance requirement for cell growth as controlling parameters when operating a baker's yeast plant.

METHODS OF DATA ANALYSIS

Data Analysis

It is known that in a fed-batch operation, nutrient mixture (fluid) continuously flows into the bioreactor without corresponding withdrawal except for sampling. Hence, for this system, cell, substrate and products mass balances lead to (Ejiofor et al., 1994b)

$$F\,t \Big/ \; 1 \Big/ dX \Big/ \; V \quad X \quad dt \tag{1}$$

$$q_S \quad 1 \Big/ \; X \quad \frac{F\,t\,S_F \quad F\,t\,S}{V} \; dS \Big/ \; dt \tag{2}$$

and

$$q_P \quad 1 \Big/ \; X \quad F\,t \; P \Big/ \; V \; dP \Big/ \; dt \tag{3}$$

$$V \quad V_0 \quad \int_0^t F\,t\,dt V_{si} \tag{4}$$

Slopes were obtained using a Piecewise curve - fitting procedure developed on AT-MATLAB statistical and mathematical packages. The specific rates of oxygen uptake, q_{O2} and carbon dioxide production, q_{CO2} were calculated respectively using:

$$q_{O_2} \quad 1 \Big/ \; X \quad Q_{O_2} \tag{5}$$

$$q_{CO_2} \quad 1 \Big/ \; X \quad Q_{CO_2} \tag{6}$$

The respiratory quotient (RQ) was determined by using the formula

$$RQ \quad \frac{32Q_{CO_2}}{44Q_{O_2}} \tag{7}$$

If RQ value is approximately 1, it is believed that the metabolic activities of the yeast are majorly respiratory while if it is approximately 2 or more, its metabolic activities are majorly fermentative (Ejiofor et al., 1994b).

Estimation of q_{sresp} and q_{sferm}

From the RQ and the stoichiometric equations for respiratory and fermentative metabolic activities, a model can be derived to estimate the fraction of the substrate consumed for respiratory (q_{sresp}/q_s) and fermentative (q_{sferm}/q_s) metabolic activities. The model can be generally written as

$$q_{sresp}/q_s = f(RQ, q_s) \tag{8}$$

The overall stoichiometric equations required for the consumption of the glucose via respiratory metabolic activities and fermentative metabolic activities are respectively given by

$$C_6H_{12}O_6 + 6\,O_2 \quad 6\,CO_2 + 6\,H_2O \tag{9}$$

and

$$C_6H_{12}O_6 \quad 2\,C_2H_5OH + 2\,CO_2 \tag{10}$$

Hence, if the total amount of substrate (glucose) consumed by the yeast at a particular time is (+) with as the amount (in moles) of the substrate consumed for respiratory metabolic activities and as the amount (in moles) of the substrate consumed for fermentative metabolic activities, the fraction of the substrate consumed for both respiratory and fermentative activities are given by:

$$q_{sresp} \quad / q_s \qquad (11)$$

and

$$/ \quad q_{sferm} \quad / q_s \qquad (12)$$

respectively. Now, the respiratory quotient (RQ) from the two stoichiometric equations is given as:

$$RQ \quad \frac{62}{6} \quad \frac{q_{CO_2}}{q_{O_2}} \qquad (13)$$

Rearranging equation (13)

$$/ \quad 1/3\,RQ \;\; 1 \qquad (14)$$

Hence,

$$q_{sresp} / q_s \; / \quad 1 / 3RQ \;\; 2 \qquad (15)$$

Data Consistency Tests

The oxido-reductive glucose metabolism by *S. cerevisiae* DSM2155 may be represented by a single chemical balance equation of the form;

$$CH_2O + aNH_3 + bO_2 \quad y_cCH_pO_nN_q + zCH_3O_{0.5} + cH_2O + dCO_2 \qquad (16)$$

where a, b, y_c, z, c, and d are stoichiometric coefficients, CH_2O, $y_cCH_pO_nN_q$ and CH_3O_5 are elemental compositions of substrate (glucose), biomass and ethanol (extracellular product), respectively. The equation is adaptive because it can be modified to represent both the consumption and production of ethanol. Available electron balance or energy balance based on the chemical energy in the organic substrate utilized by the growing yeast may be written as:

$$+ \; + \; _p = 1 \qquad (17)$$

A carbon balance (material balance) may also be made on equation (16) as:

$$y_c + z + d = 1 \qquad (18)$$

The parameters in equations (17) and (18), i.e. , , $_p$, y_c, z, and d are estimated based on relationship presented elsewhere (Erickson et al., 1979, 1980; Solomon et al., 1981).

Estimation of True Yields and Maintenance

Since extracellular products were formed, the equations presented by Solomon et al. (1984) were used to estimate the true yields and maintenance requirements. These equations are

$$1/_{max mp} \; / \qquad /_p \quad _{max} \qquad (19)$$

where $_m$ is the fraction of input energy evolved as heat because of maintenance (Solomon et al., 1984).

$$11 \quad / \quad _{max} \; m \quad _e / 1 \quad / \quad _p^{max} \quad _p / \qquad (20)$$

Where $m_e = \; _m/$ is defined as the rate of consumption of energy for maintenance per unit of energy in the biomass per hour.

$$1 \; _{max} \quad m_e / \qquad / 1 \; _p / ^{max} \quad _p \quad / \qquad (21)$$

$$y_c \quad z \; d1 \; _{max} \quad m_e / \qquad / 1 \; _p / ^{max} \quad _p \quad / \qquad (22)$$

Equations (19-22) were employed using a multivariate statistical model known as covariate adjustment technique to estimate the yields and maintenance parameters (Solomon et al., 1984; Yang et al., 1984).

MATERIALS AND METHODS

Bioreactors

A 150L bioreactor system was used for the high-cell-density fermentation. Air containing 20.94% O_2 and 0.03% CO_2 was used in aerating the system. The dissolved O_2 concentration in terms of PO_2 was recorded on-line. The composition of air at the exit was measured on-line using Oxygor 6N and Unor 6N (Fa Maihak) for percentage volumes of O_2 and CO_2 respectively. The O_2 uptake (OUR) and CO_2 production rates (CPR) as well as RQ were also estimated continuously from the exit gas composition and gas flow rates. Ucolub N115 was used as antifoam agent in controlling foaming during the experiments.

Organisms and Inoculum

A pure culture of *S. cerevisiae* DSM 2155 (German Collection of Microorganisms) was used throughout this work. The composition of the media used was the same as that of Ejiofor et al. (1994a). The inoculum used for the 150L bioreactor was obtained from the 15L bioreactor. The inoculum size was 20% of the starting volume in the reactor.

Table 1. Data consistency check using carbon and available electron balances for the growth of *S. cerevisiae* DSM 2155 on glucose in a fed-batch process for the 150 L bioreactor system.

Carbon Balance					Available Electron Balance			
t (h)	y_c	z	d	(y_c + z + d)			P	(+ + P)
1	0.657	0.000	0.494	1.152	0.665	0.519	0.000	1.185
2	2.131	0.000	0.597	2.727	2.130	0.757	0.000	2.887
3	1.170	0.000	0.326	1.504	1.226	0.393	0.000	1.619
4	0.558	0.000	0.169	0.727	0.488	0.185	0.000	0.673
5	0.608	0.000	0.229	0.837	0.548	0.247	0.000	0.795
6	0.924	0.000	0.334	1.258	0.957	0.428	0.000	1.385
7	0.862	0.000	0.323	1.185	0.891	0.424	0.000	1.316
8	0.862	0.000	0.383	1.145	0.848	0.347	0.000	1.195
9	0.711	0.000	0.300	1.011	0.718	0.362	0.000	1.080
10	0.393	0.000	0.274	0.667	0.480	0.346	0.000	0.826
11	0.608	0.000	0.288	0.896	0.741	0.295	0.000	1.036
12	0.615	0.051	0.283	0.949	0.751	0.287	0.088	1.126
13	0.417	0.075	0.267	0.760	0.509	0.260	0.131	0.900
14	0.386	0.034	0.257	0.677	0.471	0.214	0.058	0.744
15	0.366	0.053	0.263	0.682	0.446	0.194	0.093	0.733
16	0.432	0.080	0.245	0.756	0.527	0.163	0.138	0.828
17	0.436	0.080	0.248	0.764	0.532	0.123	0.139	0.794
18	0.354	0.089	0.224	0.668	0.432	0.097	0.155	0.684
19	0.330	0.078	0.221	0.629	0.403	0.078	0.136	0.617
20	0.262	0.066	0.252	0.579	0.319	0.082	0.115	0.516
21	0.218	0.082	0.239	0.539	0.266	0.082	0.143	0.492
22	0.220	0.083	0.244	0.546	0.268	0.092	0.144	0.504
23	0.190	0.057	0.246	0.494	0.232	0.100	0.100	0.431
24	0.178	0.053	0.241	0.472	0.217	0.102	0.092	0.411

High-Cell-Density Fermentation

Cell cultivation was carried out at 30^{o}C and pH 4.5, maintained by addition of 12.5% NH_3 solution and a dissolved O_2 concentration not less than 30% of the saturation level (regulated by using a combination of aeration and agitation rates). The starting volume in the 150L bioreactor before inoculation was 58.1L. This contained only NaCl and $CaCl_2$ while all other constituents of the medium were in the feed. The feed was aseptically constituted after separately sterilizing the yeast extract, ammonium sulphate and potassium dihydrogen phosphate, trace salts, and glucose solutions.

After inoculation, the cells were allowed to equilibrate with vigorous aeration for 30min to consume the residual glucose and ethanol in the inoculum. The baker's yeast was cultivated following a simulated 5-phase feeding strategy on the Universal BIoprocess CONtrol (UBICON) system developed at the German Research Center for Biotechnology. The first phase was an 8h adaptation (0 - 8th h) period during which there was no sampling and feeding was at the level to maintain specific growth rate ( ) at 0.20h^{-1}. The second was a further 4h (8 - 12th h) period of growth at specific growth rate of 0.21h^{-1} but with hourly sample withdrawal. The third and fourth phases were from the 12-16th and 16-20th hour with the corresponding specific growth rates regulated to be 0.19h^{-1} and 0.18h^{-1} respectively. The last 4h were aimed at mopping excess glucose and the ethanol that had been produced at the earlier phases and to observe low specific growth rates, thus at this phase the substrate feed rate was either held constant or even decreased.

Dry Biomass Concentration

In estimating the dry biomass concentration, two 10ml samples were centrifuged in preweighed tubes. The residues were washed twice with equal volumes of deionised water. The cell pellets were dried at 80^{o}C to constant weights, which were recorded.

Glucose and Ethanol Concentrations Estimation

Glucose concentration in the samples and feed were estimated using a YSI glucose analyser model 27 (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Ethanol concentration (P) was measured using gas chromatographic analysis interface with a computer-based Apex Chromatography Workstation for on-line acquisition, analysis and interpretation of data and chromatograms.

RESULTS AND DISCUSSION

The consistency of the data obtained during the high-cell-density fermentation of *Saccharomyces cerevisiae* DSM 2155 on glucose in a simulated five-phase feeding strategy of fed-batch process, executed on the Universal BIoprocess CONtrol (UBICON) system using 150L bioreactor, over a period of 24h have been determined by using both available electron balance (AEB) and carbon balance (CB) and the results are displayed in Table 1. In addition, estimation of the true biomass energetic yields ($_{max}$), the true product energetic yields ($_p^{max}$) and as well as the maintenance requirements (m_e) for the growth of the baker's yeast on glucose are shown in Table 2. Finally, Tables 3 showed the results obtained through the

Table 2. Estimates of true biomass energetic yields, true product energetic yields and maintenance requirements for the growth of *S. cerevisiae* DSM 2155 on glucose in a fed-batch process for 150 L bioreactor system.

Method of Test	max		pmax		me	
	Point	Interval	Point	Interval	Point	Interval
I	0.775	(0.553, 1.295)	1.257	(0.576, 6.430)	0.017	(-0.050, 0.084)
II	0.695	(0.622, 0.788)	1.677	(1.171, 2.952)	-0.004	(-0.025, 0.018)
III	0.766	(0.702, 0.844)	2.732	(1.821, 4.465)	0.005	(-0.001, 0.021)
IV	0.744	(0.646, 0.876)	1.572	(1.058, 3.059)	0.006	(-0.002, 0.032)
V	0.725	(0.663, 0.801)	1.938	(1.392, 3.189)	0.001	(-0.016, 0.018)
VI	0.770	(0.705, 0.850)	3.159	(1.898, 9.423)	0.005	(-0.011, 0.021)
VII	0.754	(0.687, 0.834)	2.675	(1.666, 6.780)	0.003	(-0.012, 0.019)
VIII	0.733	(0.615, 0.908)	1.297	(0.853, 2.701)	0.007	(-0.027, 0.041)
IX	0.704	(0.633, 0.793)	1.565	(1.131, 2.539)	-0.001	(-0.022, 0.020)
X	0.771	(0.629, 0.996)	1.525	(0.906, 4.806)	0.011	(0.027, 0.049)
XI	0.766	(0.702, 0.841)	3.101	(1.949, 7.591)	0.004	(0.011, 0.020)
XII	0.729	(0.669, 0.800)	2.078	(1.497, 3.397)	0.001	(-0.015, 0.017)
XIII	0.749	(0.685, 0.826)	2.391	(1.634, 4.455)	0.003	(-0.012, 0.019)
Average	0.745	(0.655, 0.881)	2.074	(1.350, 4.753)	0.004	(-0.018, 0.029)

newly derived model [q_{sresp}/q_s = 1/(3RQ - 2)] for the substrate channelled into biomass, ethanol and carbon dioxide formation, and also, this showed the partitioning of the substrate consumed via respiratory and fermentative metabolic pathways.

The consistency equations are satisfied when 0.93 < + + $_p$ < 1.07 and 0.94 < y_c + z + d < 1.06. In our analysis, we made use of $_b$ = 0.446 and $_b$ = 4.209 which, are the average values for yeasts. The results of the available electron balance on the bioreactor revealed that the balances recoveries obtained during the 1st and 2nd phases of the feeding strategy are much higher than the accepted upper limit except for those in the 4th, 5th and 10th hour. There were one or two instances when there were outrageous recoveries (AEB > 1.1). This could be due to overestimation in the measurement of oxygen during the period. From the 3rd phase to the end of the last phase, lower recoveries than 0.93 were obtained, which is an indication that some metabolites were possibly formed that were not estimated. The CB for the bioreactor also followed the same pattern with the AEB. They are linearly related.

It was observed that the values obtained for the true yields and maintenance requirements when covariate adjustment technique were used, gave results that were closer to the average values than every other values obtained by other methods. Also, whenever the covariates were included in any of the methods, a remarkable change was observed in the resulted values. The overall mean point estimates obtained for the true biomass energetic yield ($_{max}$,), the true product energetic yield ($_p^{max}$) and the maintenance coefficient (m_e) were 0.745, 2.074 and 0.004 respectively, with corresponding Bonferroni 80% confidence intervals (constructed as described by Solomon et al., 1981, 1982). The low value of m_e is indicative of non-stressful metabolism of the yeast in this fed-batch process. The high value of $_{max}$ showed that most of the substrate glucose was converted to biomass.

The carbon balance (CB) apportions the fractions of the substrate carbon that goes into biomass, ethanol and carbon dioxide production. From Table 1, the first eleven hours (about the first two phases of the 5-phase feeding strategy) showed that the yeast utilized the substrate carbon for producing both biomass and carbon dioxide only; there was no ethanol formation during this period. The last three phases of the 5- phase feeding strategy showed some significant ethanol formation, which mean that the substrate carbon is now utilized by the yeast for the production of biomass, carbon dioxide as well as ethanol. Even though the fraction of the substrate carbon used in producing the ethanol was the lowest when compared with the fraction of it used for producing both biomass and carbon dioxide over the same period of time (i.e. the fractions of substrate carbon used for biomass, carbon dioxide and ethanol were 52%, 38% and 10% respectively, during the 3rd, 4th and 5th phases of the 5-phase feeding strategy). The results of the available electron balance are linearly related to that of the carbon balance (see Table 1).

The RQ column in Table 3 showed that during the first fourteen hours of study, the RQ was less than one and beyond this point RQ was greater than one. By using the explanation earlier advanced by Ejiofor et al. (1994b), i.e. if RQ 1, the metabolic activities of the yeast is believed to be marjorly respiratory while if RQ 2 or more, its metabolic activities are majorly fermentative. It therefore mean that the substrate consumed by the yeast in the

Table 3. Fraction of the substrate consumed for respiratory and fermentative metabolic activities for the growth of *S. cerevisiae* DSM 2155 on glucose in a fed-batch process for the 150 L bioreactor system.

t (h)	q_s (10^{-4})	RQ	q_{sferm} (10^{-4})	q_{sresp} (10^{-4})	q_{sferm}/q_s	q_{sresp}/q_s
1	5.67	0.69	0.0	5.67	0.0	1.0
2	3.28	0.59	0.0	3.28	0.0	1.0
3	7.17	0.63	0.0	7.17	0.0	1.0
4	19.4	0.59	0.0	19.4	0.0	1.0
5	15.6	0.61	0.0	15.6	0.0	1.0
6	10.0	0.59	0.0	10.0	0.0	1.0
7	10.4	0.58	0.0	10.4	0.0	1.0
8	13.28	0.59	0.0	13.28	0.0	1.0
9	11.56	0.61	0.0	11.56	0.0	1.0
10	10.6	0.68	0.0	10.6	0.0	1.0
11	12.06	0.81	0.0	12.06	0.0	1.0
12	12.17	0.81	0.0	12.17	0.0	1.0
13	12.06	0.84	0.0	12.06	0.0	1.0
14	13.06	0.94	0.0	13.06	0.0	1.0
15	13.78	1.03	1.10	12.68	0.08	0.92
16	16.4	1.10	4.01	12.63	0.24	0.77
17	16.6	1.33	8.30	8.30	0.50	0.50
18	15.78	1.44	8.98	6.79	0.57	0.43
19	15.39	1.62	10.00	5.39	0.65	0.35
20	11.28	1.69	7.56	3.72	0.67	0.33
21	7.61	1.64	5.02	2.59	0.66	0.34
22	6.50	1.56	4.09	2.41	0.63	0.37
23	3.17	1.50	1.90	1.27	0.60	0.40
24	1.78	1.46	1.03	0.75	0.58	0.42

first fourteen hours of this study was utilized via respiratory metabolic pathway while the remaining period of fermentation showed that the yeast used the substrate consumed for both respiratory and fermentative metabolic activities. This assertion is corroborated by the work of Kasperski and Miś kiewicsz (2002) in which they varied RQ_{set} from 1 to 1.12 in the fuzzy logic controller for fed-batch cultivation of baker's yeast in order to avoid the Crabtree effect.

In addition to the use of RQ in explaining the concept of the substrate channelling, the derived model also partitioned the substrate consumed into respiratory and fermentative metabolic pathways. The fraction of the substrate used for respiratory metabolic activities (q_{sresp}/q_s) was virtually one for the first three phases of the 5-phase feeding strategy showing that almost all the substrate consumed was utilized for respiratory activities while the q_{sferm}/q_s was always zero during this same period (see Table 3). The 4th and the 5th phases of the 5-phase feeding strategy showed the substrate consumed during this period was utilized for both respiratory and fermentative activities. This was an indication that the ability of the yeast to utilize the substrate consumed for respiratory activities decreased with time probably due to overcrowding of the yeast with glucose as a result of its limited respiratory capacity. More than 50% of this substrate consumed was used via fermentative metabolic pathway in the 4th and 5th phases.

Comparison of Tables 1 and 3 showed that both the carbon and available electron balances, and the derived model could be used in explaining the concept of substrate channelling of the yeast. However, the newly derived model is preferred in that it does not require measurement of the concentrations of biomass and ethanol produced by the yeast for its derivation. Therefore, in using the derived model as a control tool during the production of baker's yeast, it has the advantage of being able to be determined by on-line measurements of both oxygen and carbon dioxide. Moreover, the results obtained through the derived model agreed with those of the previous workers (Ejiofor et al., 1994b).

From the above analysis, the fraction of the substrate consumed for respiratory metabolic activities was virtually one for the first sixteen hours (the first three phases of the 5- phase feeding strategy) of the oxido-reductive growth of the yeast for the 150L bioreactor system. The model derived, i.e. $q_{sresp}/q_s = 1/(3RQ - 2)$, yielded good results in that it agreed with the already established concept of RQ<1 signifies favoured respiratory metabolic activities and RQ>1 signifies favoured fermentative metabolic activities. The data used for the analysis were consistent to a large extent based on the recoveries of the available electron and carbon balances carried out. In accordance with the values of true biomass energetic yield obtained for the fermentative process, large percentage (>50%) of substrate consumed was for the

baker's yeast production. In view of the approximately zero values of m_e obtained, the organism was in no danger from the ethanol produced during the fermentation process.

ACKNOWLEDGMENT

Prof. B.O. Solomon wishes to thank the Alexander von Humboldt Stiftung, Germany for the collaboration made possible with Prof. W.-D. Deckwer of GBF through which this work was done.

ABBREVIATIONS

AEB available electron balance (+ + $_p$), dimensionless
a mol of ammonia required per mol organic substrate carbon, mol mol^{-1}
b mol of oxygen required per mol organic substrate carbon, mol mol^{-1}
CB carbon balance (y_c + z + d), dimensionless
CPR carbon dioxide production rate
c mol of water required per mol organic substrate carbon, mol mol^{-1}
d mol of carbon dioxide produced per mol of organic substrate, mol mol^{-1}
F feed rate, h^{-1}
m_e maintenance requirement in terms of available electron, h^{-1}
n atomic ratio of oxygen to carbon in biomass, dimensionless
OUR oxygen uptake rate
p atomic ratio of hydrogen to carbon in biomass, dimensionless
Q_{CO2} rate of carbon dioxide evolution, gg^{-1}h^{-1}
Q_{O2} rate of oxygen uptake, gg^{-1}h^{-1}
Q_S rate of organic substrate consumption, gg^{-1}h^{-1}
q atomic ratio of nitrogen to carbon in biomass, dimensionless
q_{co2} specific rate of carbon dioxide evolution, gg^{-1}h^{-1}
q_{o2} specific rate of oxygen uptake, gg^{-1}h^{-1}
q_S specific substrate consumption rate, gg^{-1}h^{-1}
q_P specific product formation rate, gg^{-1}h^{-1}
q_{sferm} specific rate of substrate consumed used for fermentative metabolic activities, gg^{-1}h^{-1}
q_{sresp} specific rate of substrate consumed used for respiratory metabolic activities, gg^{-1}h^{-1}
RQ respiratory quotient, dimensionless
S organic substrate concentration, gl^{-1}
S_o initial organic substrate concentration, gl^{-1}
SCP single-cell protein
V volume of bioreactor vessel, l
V_{si} volume of sample withdrawn, l
X biomass concentration, gl^{-1}
y_c fraction of organic substrate carbon incorporated into biomass, dimensionless
z fraction of organic substrate carbon incorporated into product, dimensionless
b reductance degree of biomass (equivalents of available electrons per g-atom carbon)
fraction of substrate energy which is evolved as heat, dimensionless
m fraction of input energy evolved as heat because of maintenance, dimensionless
fraction of substrate energy which is in biomass (biomass energetic yield), dimensionless
max true biomass energetic yield, dimensionless
summation (arithmetic symbol)
p fraction of substrate energy which is in products, dimensionless
p^{max} true product energetic yield, dimensionless
specific growth rate, h^{-1}
b mass fraction carbon, dimensionless

SUBSCRIPTS

b = biomass
P = product
S = substrate

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