[J. Ferment. Technol., Vol. 56, No. 5, p. 524-531, 1978]

Comparison of Yield Coefficients Estimated from Measurements of Nitrogen, Organic Substrate, Biomass, and Oxygen during Yeast Growth

L. E. ERICKSON,* V. D. KUVSHINNIKOV, I. G. MINKEVICH, and V. K. EROSHIN

Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences 142292, Pushchino, Moscow Region, USSR

This work illustrates how the biomass energetic yield may be estimated from several different sets of measured values and how the results of such estimates may be analyzed. It shows that mass-energy balances may be effectively used to indicate when different sets of measurements give similar estimates of biomass energetic yield and also to show when products appear to be present.

Five different estimates of biomass energetic yield are compared together with estimates of product energetic yield and weight fraction nitrogen in biomass using experimental values of biomass concentration of *Hansenula polymorpha*, feed and effluent methanol concentrations, oxygen consumption rate, rate of ammonia feeding, and dilution rate. Relatively good agreement of the five different estimates of biomass energetic yield is obtained when the methanol feed concentration is 2.4 g/*l*; however, when the feed concentration is 4.0 g/*l*, products appear to be present, and the biomass energetic yield is lower.

Improvements in instrumentation and the advent of computer coupled cultivation systems have resulted in an increased interest and use of material and energy balances in microbial cultivation. In this work, the results from six different continuous culture experiments are used to illustrate the application of material and energy balances which utilize the facts that (1) the heat evolved per available electron transferred to oxygen is relatively constant for a wide range of organic substances,[1-5] and (2) in the biomass, the number of equivalents of available electrons per g-atom carbon and the weight fraction carbon are relatively constant.[1,5] Using these concepts, Minkevich and Eroshin[1,5-19] have related mass and energetic yields and applied these concepts to the production of biomass on organic substrates. In this work, biomass yields are estimated using experimentally measured values of dilution rate, organic substrate concentration, biomass concentration, oxygen consumption, and ammonia consumption. Some of the experimental results of this study have been reported elsewhere.[20]

* Present address: Kansas State University, Manhattan, KS. 66506 USA

Materials and Methods

Hansenula polymorpha DL-1 was grown in continuous culture on methanol in a medium of the following composition (per *l*): KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NH_4Cl 0.8 g, $FeSO_4 \cdot 6H_2O$ 0.25 g and trace nutrients: $CaCl_2 \cdot 2H_2O$ 0.66 mg, $CuSO_4 \cdot 5H_2O$ 0.16 mg, $ZnSO_4 \cdot 6H_2O$ 0.18 mg, $MnSO_4 \cdot 4H_2O$ 0.15 mg, and $CoCl_2 \cdot 6H_2O$ 0.18 mg. The cultivation was carried out at 37°C, pH=4.5, and pO_2=70% saturation. The measurement methods have been described previously.[20,21] Additional information on the cultivation methods is also given elsewhere.[20]

Mass-energy Balances

The mass-energy balance method employed in this work is based on the chemical balance equation for microbial growth.[1]

$$CH_mO_l+aNH_3+bO_2 \\ =y_cCH_pO_nN_q+zCH_rO_sN_t \\ +(1-y_c-z)CO_2+cH_2O \qquad (1)$$

In this equation CH_mO_l denotes the elemental composition of the organic substrate, $CH_pO_nN_q$ is the elemental composition of the biomass, and $CH_rO_sN_t$ represents the elemental composition of the products (when more than one product is present, $CH_rO_sN_t$ is taken as the average elemental composition of the products). The subscripts denote numbers of atoms of hydrogen, oxygen, and nitrogen per carbon atom. The coefficients y_c and z are the fractions of substrate carbon converted to biomass and product, respectively.

Equation (1) describes the transformation of organic substrate to biomass, products, CO_2, and H_2O. Nitrogen in the biomass has the same valence as ammonia and is not oxidized in the process of biomass production. Equation (1) may be balanced by finding the number of available electrons transferred to oxygen. If γ_S, γ_b, and γ_P are defined as the number of equivalents of available electrons per quantity of organic substrate, biomass, and product, respectively, each containing one g-atom carbon, then an available electron balance for equation (1) gives*

$$\gamma_S+b(-4)=y_c\gamma_b+z\gamma_P \qquad (2)$$

where

$$\gamma_S=4+m-2l$$
$$\gamma_b=4+p-2n-3q$$
$$\gamma_P=4+r-2s-3t$$

and 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. Equation (2) may be rearranged to give

$$\frac{4b}{\gamma_S}+\frac{y_c\gamma_b}{\gamma_S}+\frac{z\gamma_P}{\gamma_S}=1 \qquad (3)$$

Since the heat of reaction per available elctron transferred to oxygen is almost constant for all of the organic molecules included in equation (1), equation (3) may be considered to be a mass-energy balance. In its present form, the oxygen consumption, b, biomass carbon yield, y_c, and the product carbon yield, z, are balanced. The first term gives the fraction of available electrons in the organic substrate which are transferred to oxygen, or in terms of energy, the fraction of chemical energy in the organic substrate which is converted to heat; that is,

$$\frac{Q}{Q_o\gamma_S}=\frac{4b}{\gamma_S} \qquad (4)$$

where Q is the heat evolved in biomass production per amount of organic substrate containing one g-atom carbon and Q_o is approximately 27 kcal per equivalent of available electrons transferred to oxygen.[1-5,22]

The second term in equation (3) gives the fraction of available electrons in the utilized organic substrate which are incorporated into the biomass. This term is the biomass energetic yield coefficient (η)

$$\eta=y_c\frac{b_b}{\gamma_S} \qquad (5)$$

when equation (3) is considered as an energy balance.

The third term in equation (3) gives the fraction of availavle electrons in the organic substrate which are transferred to products. It has also been defined energetically as[13-15]

$$\zeta_P=z\frac{\gamma_P}{\gamma_S} \qquad (6)$$

where ζ_P is the fraction of the chemical energy in the organic substrate which is incorporated into extracellular products. Equation (3) may be written as an energy balance; that is,

$$\frac{Q}{Q_o\gamma_S}+\eta+\zeta_P=1 \qquad (7)$$

The biomass energetic yield coefficient, η may be related to the mass yield coefficient based on organic substrate, Y_S, using equation (5); that is,[1]

$$\eta=\frac{\sigma_b\gamma_b}{\sigma_S\gamma_S}Y_S \qquad (8)$$

where σ_S and σ_b are the weight fractions of carbon in the organic substrate and biomass, respectively.

* Equation (2) may also be obtained by balancing equation (1) with respect to nitrogen, hydrogen, and oxygen.

The energetic yield coefficient may also be related to the mass yield coefficients based on oxygen.[1] From equations (3), (5), and (6),

$$y_o=\frac{y_c}{b}=\frac{4\eta}{\gamma_b(1-\eta-\zeta_P)} \tag{9}$$

where y_o is in terms of g-atoms carbon in biomass per g mole oxygen. In mass units,

$$Y_O=\frac{12y_o}{32\sigma_b}=\frac{3\eta}{2\sigma_b\gamma_b(1-\eta-\zeta_P)} \tag{10}$$

Equations (5), (8), (9), and (10) relate the biomass energetic yield coefficient with other mass yield coefficients. It is convenient to select the energetic yield coefficient for estimation because of its fundamental importance and because this leads to a better conceptual or physical understanding of the experimental results. The energetic yield also has the convenient range from zero to one for all organic substrates.

Nitrogen consumption may be used as one of the measured variables in estimating η. In equation (1), the moler nitrogen consumption (a) per unit quantity of consumed organic substrate containing one g-atom carbon is

$$a=y_cq+zt \tag{11}$$

or from equations (5) and (6)

$$a=\frac{\gamma_S q\eta}{\gamma_b}+\frac{\gamma_S t\zeta_P}{\gamma_P} \tag{12}$$

Similarly, the ratio of oxygen consumption to organic substrate consumption may be related to η. Combining equations (4) and (7) gives

$$b=\frac{\gamma_S}{4}(1-\eta-\zeta_P) \tag{13}$$

Equations (12) and (13) may be used to obtain

$$\frac{a}{b}=\frac{\frac{\gamma_S q\eta}{\gamma_b}+\frac{\gamma_S t\zeta_P}{\gamma_P}}{\frac{\gamma_S}{4}(1-\eta-\zeta_P)} \tag{14}$$

which may be used with oxygen and nitrogen consumption rate measurements. Other similar ratios may be easily obtained using carbon dioxide production, heat evolution, and other combinations of variables.

In this work, the estimation of biomass energetic yield coefficients was considered where biomass production was the primary goal, but product formation may have been present. Under these conditions, values of η may be estimated assuming that $\zeta_P=0$ using equations (8), (10), (12), (13), and (14). Comparison of the results will usually show whether or not products are present. Organic substrate, biomass, and oxygen rate data give a direct estimate of the fraction of chemical energy in the organic substrate which is converted to products, ζ_P. Combining equations (8) and (13) gives

$$\zeta_P=1-\frac{4b}{\gamma_S}-\frac{\sigma_b\gamma_b}{\sigma_S\gamma_S}Y_S \tag{15}$$

Results and Discussion

The experimental results are shown in Table 1. Table 2 contains estimated values of various parameters which are useful in understanding and interpreting these experimental results. Values of the biomass weight yield based on organic substrate, Y_S, were estimated using the data in Table 1 and the equation $Y_S=X/(S_o-S)$. The values of the biomass weight yield based on oxygen consumption, Y_O, were estimated from the ratio of the productivity, DX, and the oxygen consumption rate. The estimates of the weight fraction nitrogen in the biomass, λ, were obtained from the ratio of the nitrogen consumption rate to the productivity, DX. The values of the biomass energetic yield coefficients were estimated using: equation (8) to estimate η_{YS} from biomass and organic substrate concentration measurements; equation (13) to estimate $\eta_{O_2,S}$ from oxygen consumption rate, organic substrate concentrations, and dilution rate; equation (10) to estimate η_{YO} from biomass productivity, DX, and oxygen consumption rate; equation (12) to estimate $\eta_{N,S}$ from nitrogen consumption rate, organic substrate concentration, and dilution rate; and equation (14) to estimate η_{N,O_2} from nitrogen consumption rate and oxygen consumption rate. In equations (10), (12), (13), and (14), $\zeta_P=0$ was assumed when the estimate of η were obtained; that is, product formation was assumed to be negligible. Values of the product energetic yield

Table 1. Experimental data from continuous cultivation of *Hansenula polymorpha* on methanol.

Exp. no.	Dilution rate, D hr^{-1}	Methanol concentration, g/l		Cell concentration X, g/l	Nitrogen consumption rate, $g\ N/l\text{-hr}$	Oxygen consumption rate, $g/l\text{-hr}$
		Feed, S_o	Effluent, S			
1	0.107	4.0	0	1.49	0.0133	0.381
2	0.135	4.08	0.024	1.34	0.0143	0.430
3	0.141	4.0	0.015	1.5	0.0198	0.472
4a*	0.122	2.4	0.0061	0.90	0.0124	0.281
4b*	0.122	2.4	0.0061	1.0	0.0124	0.281
5	0.143	2.4	0.008	1.0	0.0117	0.337
6	0.154	2.4	0.040	0.95	0.0118	0.349

* In experiment 4, two values of biomass concentration were obtained; however, only one value of each of the other measured variables was obtained.

coefficient, ζ_P, were estimated using equation (15). These values depend on the measured values of dilution rate, organic substrate concentrations, biomass concentration, and oxygen consumption rate. The biomass was characterized by assuming $\gamma_b=4.2$, $\gamma_b\sigma_b=2.0$ or $\sigma_b=0.476$, and $q=0.16$. These numerical values were based on the average values for a variety of yeasts.[1,5] They were used where needed in estimating values of the energetic yield coefficients.

The results in Table 2 show that there is variation from experiment to experiment in the values of the various yield parameters and that the various estimates of η are not in close agreement for a number of the experiments. The estimated values of λ also show some variation. In experiment 4 the estimates of λ are considerably larger than in the other experiments. Large values of λ result when nitrogen consumption is large relative to biomass production. In Table 2, experiment 4, note that the estimates of biomass energetic yield requiring nitrogen consumption measurements, $\eta_{N,S}$ and η_{N,O_2}, are also large when λ is large. Equations (12) and (14) show that $\eta_{N,S}$ and η_{N,O_2} increase linearly as nitrogen consumption increases.

In the calculations leading to the results in Table 2, the value $q=0.16$ was used for the ratio of nitrogen to carbon atoms in the biomass. The average value of the estimate of the weight fraction nitrogen in the biomass is $\lambda=0.09$. For $\sigma_b=0.476$ and $q=0.16$, the corresponding value is $\lambda=0.089$. Thus, $q=0.16$ appears to be an appropriate value for use in estimating values of $\eta_{N,S}$ and η_{N,O_2}; however, a slightly smaller value would appear to be more appropriate in experiments 1, 2, 5, and 6. Using a smaller

Table 2. Estimated values of yield coefficients.

Exp. no.	Mass ratios			Biomass energetic yield coefficient					Product energetic yield coefficient, ζ_P
	Y_S	Y_O	λ	η_{Y_S}	$\eta_{O_2,S}$	η_{Y_O}	$\eta_{N,S}$	η_{N,O_2}	
1	0.37	0.42	0.083	0.33	0.41	0.36	0.31	0.34	0.08
2	0.33	0.42	0.079	0.29	0.48	0.36	0.26	0.32	0.19
3	0.37	0.45	0.093	0.33	0.44	0.37	0.35	0.39	0.11
4a	0.38	0.39	0.113	0.33	0.36	0.34	0.42	0.40	0.03
4b	0.42	0.43	0.102	0.37		0.37			0
5	0.42	0.43	0.082	0.37	0.34	0.36	0.34	0.34	0
6	0.38	0.42	0.081	0.34	0.36	0.36	0.32	0.34	0.02
Ave.	0.38	0.42	0.090	0.34	0.40	0.36	0.33	0.355	0.06

value of q would result in larger estimated values of $\eta_{N,S}$ and η_{N,O_2} than those shown in Table 2.

The estimated values of ζ_P, the fraction of organic substrate energy converted to products, suggest that products may have been formed in appreciable quantities in some of the experiments. In experiments 1, 2, and 3, the estimates of ζ_P exceed $\zeta_P=0.05$; that is, more than 5% of the substrate energy appears to have been converted to products in these experiments.

Estimates of the biomass energetic yield based on oxygen consumption rate and organic substrate consumption rate, $\eta_{O_2,S}$, are larger than the correct value when products are formed in a significant quantity. This can be seen from equations (8), (13), and (15) which show that $\zeta_P=\eta_{O_2,S}-\eta_{YS}$. In Table 2 the estimated values of biomass energetic yield, $\eta_{O_2,S}$, are larger in experiments 1, 2, and 3 than in experiments 4, 5, and 6. In experiments 1, 2, and 3, the values of $\eta_{O_2,S}$ are the largest estimates of η in each of the respective experiments.

The estimates of biomass energetic yield based on substrate consumption and biomass production, η_{YS}, are the most direct estimates; that is, the accuracy of this estimate is not influenced by product formation. These estimates range from $\eta_{YS}=0.29$ for experiment 2 to $\eta_{YS}=0.37$ in experiments 4*b* and 5. The smaller estimated values correspond to experiments where product formation appears to be present. In experiments 3 and 4*a*, the smallest estimate of η for each respective experiment is η_{YS}. In experiments 1 and 2, the estimates of η_{YS} are suppoted by the nitrogen consumption measurements; that is, $\eta_{N,S}=0.31$ and $\eta_{N,O_2}=0.34$ compared to $\eta_{YS}=0.33$ in experiment 1 and in experiment 2, $\eta_{N,S}=0.26$ and $\eta_{N,O_2}=0.32$ compared to $\eta_{YS}=0.29$. In experiments 4, 5, and 6 the estimates of η_{YS} are supported by oxygen consumption rate measurements with additional support provided by nitrogen consumption rate measurements in experiments 5 and 6. Experiments 5 and 6 are the only experiments in which the direct estimate of biomass energetic yield, η_{YS}, is within 10% of all of the other estimates ($\eta_{O_2,S}$, η_{YO}, $\eta_{N,S}$, and η_{N,O_2} for the same experiment). There is no evidence of product formation in these two experiments. The conditions for these two experiments were identical except for a small change in dilution rate and the results are also similar. One can reasonably conclude that all the measurements are virtually correct for these two experiments.

Examination of Table 1 and 2 shows that in the experiments with $S_o=2.4$ (experiments 4, 5 and 6), product formation as indicated by estimates of ζ_P is small while in the other experiments where $S_o=4.0$, estimates of ζ_P are 0.07 or greater. Biomass yields, as indicated by Y_S and η_{YS} and supported by some other measurements, appear to be larger when $S_o=2.4$ than for $S_o=4.0$. For $S_o=2.4$, the dilution rate which results in the maximum yield of biomass is not clearly identifiable from the results of these experiments. The estimates of biomass energetic yield from experiments 4, 5, and 6 do not differ significantly from one another when the nitrogen measurement in experiment 4 is neglected. Using all of the measured values in experiments 4, 5, and 6 and obtaining average values gives $\eta_{YS}=0.35$, $\eta_{O_2,S}=0.35$, $\eta_{YO}=0.36$, $\eta_{N,S}=0.36$, and $\eta_{N,O_2}=0.36$. The average of all estimates for these three experiments is $\eta=0.36$. Omitting the large nitrogen measurement in experiment 4 gives $\eta=0.35$ rather than 0.36.

Experiments 1, 2, and 3 do not show any clearly identifiable variation with dilution rate when all of the results for these experiments are carefully examined. For these three experiments, average values of the respective estimates are $\eta_{YS}=0.32$, $\eta_{O_2,S}=0.44$, $\eta_{YO}=0.36$, $\eta_{N,S}=0.31$, and $\eta_{N,O_2}=0.35$, and the average value of the product energetic yield coefficient is $\zeta_P=0.13$. Neglecting the oxygen measurements because of product formation gives $\eta=0.31$ as the average values of estimates from η_{YS} and $\eta_{N,S}$.

The biomass energetic yield based on biomass and oxygen, η_{YO}, is the available electrons transferred to the biomass divided by the sum of the available electrons in the biomass and those transferred to oxygen. The estimated values of η_{YO} give the biomass energetic yield based on the fraction of the energy in the organic substrate which is actually utilized; that is, any energy contained in products is excluded in the estimates of η_{YO} in Table 2. For all of the experiments, the average yield, η_{YO}, is 0.36. The values is in close agreement with the estimates from experiments 4, 5, and 6 where products are not present.

When nitrogen consumption is not influenced by product formation, the biomass energetic yield based on nitrogen and oxygen, η_{N,O_2}, is also an estimate of the biomass energetic yield based on the fraction of the energy in the organic substrate which is actually utilized. The average value of η_{N,O_2} is 0.355. This value is similar to the average value of 0.36 from biomass and oxygen and the average values of all the yields when products are not present.

The only product which was measured experimentally was formaldehyde. Measured concentrations were less than one mg/l in all experiments. For experiments 1, 2, and 3, the average estimated value of ζ_P is 0.13. For formaldehyde as product, $z=0.195$ when $\gamma_P=4.0$ and $\zeta_P=0.13$; the corresponding formaldehyde concentration when $S_o=4$ g/l is 0.73 g/l. This result and the measured values for formaldehyde show that formaldehyde does not contribute significantly to the values of ζ_P. Formic acid is another possible product; however, $\gamma_P=2.0$ for formic acid. Thus, for $\zeta_P=0.13$, $z=0.39$, and when $S_o=4$ g/l, the corresponding formic acid concentration is 2.2 g/l. Concentrations this large in the broth are very doubtful. Products with higher values of γ_P have smaller values of z and smaller concentrations for the same value of ζ_P. Consider, for example, hexadecanoic acid, $C_{16}H_{32}O_2$. In this case, $\gamma_P=5.75$, and $z=0.136$ when $\zeta_P=0.13$; when $S_o=4$ g/l, the corresponding hexadecanoic acid concentration is 0.27 g/l. This value is about one order of magnitude smaller than the corresponding formic acid concentration. Extracellular polysaccharides may also be present. The calculation here is identical to that for formaldehyde.

Measurement errors are always present in experimental work and are frequently of significant magnitude in fermentation experiments. Biomass measurements may be in error, for example, if optical density is used and the calibration curve is not correct because of mutations or changes in dilution rate. At low dilution rates, oxygen and ammonia consumption rates are small and measurement errors are frequently larger. With small laboratory equipment accurate oxygen consumption measurements are much more difficult to obtain than with large scale equipment where good gas balance measurements can be obtained.

Because of the difficulty of accurate measurement, greater confidence in the accuracy of the results is obtained when estimates from two or more different sources of data are in agreement. In this work the biomass energetic yield estimated from biomass and organic substrate measurements is in reasonably good agreement with at least one other estimated energetic yield. Thus, the additional experimental results provide supporting evidence that the biomass energetic yield estimates from biomass and organic substrate are reasonably accurate. In experiments 4, 5, and 6 the average yield, $\eta_{YS}=0.35$, is very close to the other average yields, and in experiments 1, 2, and 3 the average values $\eta_{YS}=0.32$ and $\eta_{N,S}=0.31$ are very close to each other.

It is important to point out that for the estimates of product energetic yield, ζ_P, no supporting measurements are available; that is, the results depend on the accuracy of the measurements of oxygen consumption rate, substrate concentrations, biomass concentration, and dilution rate. For example, if errors in measurements of oxygen uptake rate are present, this could result in estimated values of $\zeta_P>0$.

Conclusions

Five different estimates of biomass energetic yield are compared together with estimates of product energetic yield and weight fraction nitrogen in the biomass using experimental values of biomass concentration, feed and effluent organic substrate concentrations, oxygen consumption rate, rate of ammonia feeding, and dilution rate. Relatively good agreement of the five different estimates of energetic yield is obtained when the organic substrate feed concentration is 2.4 g/l and dilution rate is in a range from 0.122 hr^{-1} to 0.154 hr^{-1}. When the values from three different dilution rates are averaged, the estimated biomass energetic yields are 0.35 when based on either biomass and organic substrate or oxygen and organic substrate measurements, and 0.36 when based on either biomass and oxygen, nitrogen and oxygen, or nitrogen and organic substrate measurements.

For organic substrate feed concentrations of 4.0 g/l, products appear to be present; however, there is reasonably good agreement when average estimates of biomass energetic yield from biomass and organic substrate (η_{Y_S}=0.32) are compared with those from nitrogen and organic substrate measurements ($\eta_{N,S}$=0.31). The average estimates of biomass energetic yield are about 10% lower when S_o = 4 g/l as compared to results for S_o=2.4 g/l at similar dilution rates. No significant variation of biomass energetic yield with dilution rate was observed at dilution rates above 0.1 hr^{-1}.

This work illustrates how the biomass energetic yield may be estimated from several different sets of measured values and how the results of such estimates may be analyzed. It shows that material and energy balances may be effectively used to indicate when different sets of measurements give similar estimates of biomass energetic yield and also to show when products appear to be present.

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-mole/g-atom carbon
- c =moles of water per quantity of organic substrate containing one g-atom carbon, g moles per g-atom carbon
- D =dilution rate, hr^{-1}
- l =atomic ratio of oxygen to carbon in organic substrate, dimensionless
- m =atomic ratio of hydrogen to carbon in organic substrate, dimensionless
- n =atomic ratio of oxygen to carbon in biomass, dimensionless
- p =atomic ratio of hydrogen to carbon in biomass, dimensionless
- Q =heat evolution in fermentation per quantity of organic substrate containing one g-atom carbon, kcal/g-atom carbon
- Q_o=heat evolution per equivalent of oxygen, kcal/g equiv.
- q =atomic ratio of nitrogen to carbon in biomass, dimensionless
- r =atomic ratio of hydrogen to carbon in products, dimensionless
- S =organic substrate concentration in the fermentor and effluent, g/l
- S_o =organic substrate concentration in the feed to the fermentor, g/l
- s =atomic ratio of oxygen to carbon in products, dimensionless
- t =atomic ratio of nitrogen to carbon in products, dimensionless
- X =biomass concentration in the fermentor, g/l
- Y_O=biomass mass yield based on oxygen, dimensionless
- Y_S=biomass mass yield based on organic substrate, dimensionless
- y_c =biomass carbon yield (fraction of organic substrate carbon converted to biomass), dimensionless
- y_o =biomass yield per mole of oxygen, g-atoms carbon in biomass/g mole O_2
- z =fraction of organic substrate carbon in products, dimensionless
- γ_b =reductance degree of biomass, equivalents of available electrons/g-atom carbon
- γ_P =reductance degree of products, equiva-

lents of available electrons/g-atom carbon

γ_S =reductance degree of organic substrate, equivalents of available electrons/g-atom carbon

ζ_P =fraction of chemical energy in organic substrate which is converted to products, dimensionless

η =fraction of chemical energy in organic substrate which is converted to biomass; the subscripts $_{YS}$; $_{O_2,S}$; $_{YO}$; $_{N,S}$; and $_{N,O_2}$ identify the measured variables used in each estimate, dimensionless

σ_b =weight fraction carbon in biomass, dimensionless

σ_S =weight fraction carbon in organic substrate, dimensionless

Acknowledgements

Th authors thank Dr. Charles Cooney for providing the strain *Hansenula polymorpha* DL-1, used.

References

1) Minkevich, I. G., Eroshin, V. K.: *Folia Microbiologica*, **18**, 376 (1973).
2) Thornton. W, M.: *Phil. Mag.*, **33**, 196 (1917).
3) Kharasch, M. S.: *Bur. Stand. J. Res.*, **2**, 359 (1929).
4) Kharasch, M. S., Sher, B.: *J. Phys. Chem.*, **29**, 625 (1925).
5) Minkevich, I. G., Eroshin, V. K., Alekseeva, T. A., Tereshenko, A. P.: *Mikrobiol. Promyshlennost*, **2** (144), 1 (1977). (In Russian).
6) Eroshin, V. K., Belyanin, A. I.: *Prikl. Biokh. Mikrobiol.*, **4**, 704 (1968). (In Russian).
7) Minkevich, I. G., Popkov, G. P., Eroshin, V. K.: *Prikl. Biokh. Mikrobiol.*, **8**, 160 (1972). (In Russian).
8) Minkevich, I. G., Eroshin, V. K.: *Izvestiya AN SSSR, Ser. Biol.*, 2, 245 (1972). (In Russian).
9) Minkevich, I. G., Eroshin, V. K.: *Izvestiya AN SSSR, Ser. Biol.*, **3**, 414 (1973). (In Russian).
10) Minkevich, I. G., Eroshin, V. K.: *Mikrobiol. Promyshlennost*, **1** (109), 10 (1974). (In Russian).
11) Minkevich, I. G., Eroshin, V. K.: *Mikrobiol. Promyshlennost*, **2** (110), 1 (1974). (In Russian).
12) Minkevich, I. G., Eroshin, V. K.: *Mikrobiol. Promyshlennost*, **7** (115), 1 (1974). (In Russian).
13) Minkevich, I. G., Eroshin, V. K.: Uspekhi sovremennoi biologii, **82**, 103 (1976). (In Russian).
14) Minkevich, I. G., Eroshin, V. K.: *Studia Biophysica*, **49**, 43 (1975).
15) Minkevich, I. G., Eroshin, V. K.: *Studia Biophysica*, **59**, 67 (1976).
16) Minkevich, I. G., Eroshin, V. K.: *Biotechnol. Bioeng. Symp.*, No. 4, 21 (1973).
17) Eroshin, V. K.: *Biotechnol. Bioeng. Symp.*, No. 4, 995 (1974).
18) Eroshin, V. K.: *Process Biochem.*, **12**, No. 6, 29 (July/Aug. 1977).
19) Skryabin, G. K., Chepigo, S. V., Eroshin, V. K.: *Proc. 9th World Petroleum Congress, Review Paper*, Vol. 14, 121, Appl. Sci. Publ., Ltd., Essex, England (1975).
20) Kuvshinnikov, V. D., Vorobev, A. V., Eroshin, V. K., Minkevich, I. G.: *Prikl. Biokh. Mikrobiol.*, **14**, N3 (1978). (In Russian).
21) Kuvshinnikov, V. D., Sobotka, M., Minkevich, I. G., Eroshin, V. K.: *Folia Microbiol.*, **22**, 189 (1977).
22) Cooney, C. L., Wang, D. I. C., Mateles, R. I.: *Biotechnol. Bioeng.*, **11**, 269 (1968).

(Received May 22, 1978)