Korean J. of Chem. Eng., 2(1) (1985) *81 – 85*

MASS AND ENERGY BALANCE FOR ANALYSIS OF OLEAGINOUS YEAST GROWTH

Jae Gu Pan and Joon Shick Rhee*

Department of Biological Science and Engineering
Korea Advanced Institute of Science and Technology
P.O. Box 150 Chongyang, Seoul 131, Korea

(*Received 28 December • accepted 22 January 1985*)

Abstract — Mass and energy balance method was used to analyze the variation of theoretical biomass yield and oxygen yield based on the intracellular lipid accumulation in oleaginous yeasts. Maximum biomass yield decreases with the increase of lipid content. For the oxygen yield, however, the trend is obscure because it depends not only on lipid content, but also on energetic yield. Attention is drawn to the possibility that data consistency in continuous culture of the oleaginous yeast may be checked by the method of mass and energy balance.

INTRODUCTION

Many research workers have been searching for lipid accumulating microorganisms as a potential producer of edible lipids [1]. Various microorganisms accumulate substantial quantities of intracellular lipid. Oleaginous yeasts are of particular interest in the industrial realization of microbial lipid production, because they accumulate a fairly large quantity of intracellular lipid (up to 60%). Further, composition of the yeast lipids is very similar to that of palm oil. Unicellular nature of yeasts is also advantageous for the design of bioreactor system because of the relatively low viscosity of culture broth.

However, oleaginous yeasts showed low biomass yield as compared with the non-oleaginous microorganisms [2]. Thermodynamic energy content of oleaginous yeasts should be higher than that of non-oleaginous yeasts, because the lipid is an energy rich compound. Thus, the traditional yield analysis based only on mass (i.e., weight) is not adequate in this case.

The objective of this paper is to analyze the biomass yield and oxygen yield in oleaginous yeast growth using mass and energy balance method [3-6]. Emphasis is drawn to the possibility that the mass and energy balance method may be used to check the consistency of experimental data in the chemostatic growth of oleaginous yeasts. Mass and energy balance method is also useful for the prediction of biomass yield from various substrates.

THEORY

Mass and energy balance method was developed extensively in the last decade [3-6]. Elemental balance equation of microbial growth (3) may be written,

$$CH_mO_l + aNH_3 + bO_2 \rightarrow yCH_pO_nN_q + zCH_rO_sN_t + dCO_2 + cH_2O \quad (1)$$

where nitrogen source is NH_3. In this equation, CH_mO_l, $CH_pO_nN_q$ and $CH_rO_sN_t$ denote the elemental composition of substrate, biomass and product, respectively. A carbon balance in eq. (1) may be written

$$y + z + d = 1 \quad (2)$$

where y is biomass carbon yield and z is product carbon yield.

The balance of both parts of equation (1) with respect to the available electrons results in

$$\gamma_s + b(-4) = y\gamma_b + z\gamma_p \quad (3)$$

where $\gamma_s = 4 + m - 2l$

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

$\gamma_p = 4 + \gamma - 2s - 3t$

Reductance degree, γ, is the number of equivalents of available electrons/g atom carbon based on carbon (=4), hydrogen (=1), oxygen (=-2), and nitrogen (=-3). A value of -3 is used for nitrogen because the reductance degree of nitrogen in biomass and ammonia is -3. Equation (3) may be written in another form

$$4b/\gamma_s = y(\gamma_b/\gamma_s) + z(\gamma_p/\gamma_s) = 1 \quad (4)$$

Because the heat evolved per equivalent of available equivalent) [6], eq. (4) may be used as an energy balance which leads to

* To whom correspondence should be addressed.

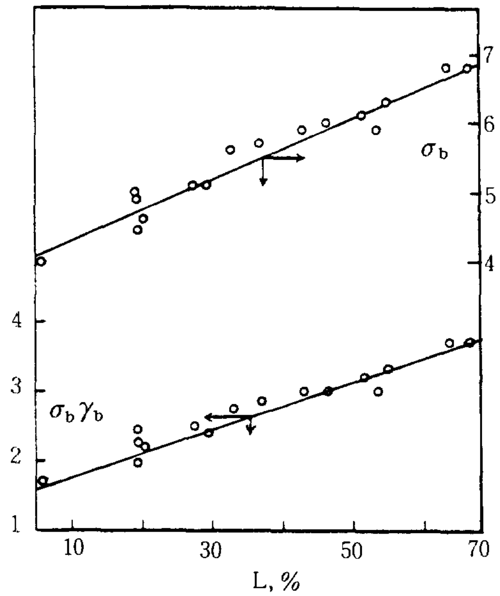

Fig. 1. Change of energy content as a function of lipid content [8].

$$\varepsilon + \eta + \xi_p = 1 \quad (5)$$

where

$$\varepsilon = 4b/\gamma_s \quad (6)$$

$$\eta = y(\gamma_b/\gamma_s) \quad (7)$$

$$\xi_p = z(\gamma_p/\gamma_s) \quad (8)$$

The term ε, is a fraction of the available electrons in substrate transferred to oxygen and then evolved as heat, η is a fraction of the available electrons in substrate (i.e. energy) transferred to biomass, and ξ_p gives a fraction of the substrate energy incorporated into extracellular product(s). Equation (2) and (5) may be used to check the data consistency in continuous culture in a way to see whether sum of three terms on the left hand side of the equations gives unity.

η is termed as energetic yield for biomass formation [3]. η may be correlated with biomass yield, $Y_{x/s}$, as follows;

$$\eta = (\sigma_b\gamma_b/\sigma_s\gamma_s)\, Y_{x/s} \quad (9)$$

where σ_s and σ_b are the weight fractions of carbon in the organic substrate and biomass, respectively. Heijnen and Roels [7] reported that the average values of γ'_b and σ_b calculated from the data available in the literature were 4.17 and 0.489, respectively and the coefficient of variation is less than 5% for both γ_b and σ_b. Therefore, γ_b and σ_b may be assumed to be constant for non-oleaginous microorganisms, whereas the assumption is not valid for oleaginous microorganisms, because thermodynamic energy content of the oleaginous microorganisms increases with accumulation of the intracellular lipids [8]. For most of the microorganisms, eq. (9) becomes

$$\eta = (2.0/\sigma_s\gamma_s).\, Y_{x/s} \quad (10)$$

DATA ANALYSIS AND DISCUSSION

Relationship between lipid content and energy content of biomass

Recently, Eroshin and Krylova estimated σ_b and γ_b for oleaginous yeasts which were grown on ethanol [8]. σ_b and $\sigma_b\gamma_b$ are plotted as a function of lipid content in Fig. 1. Fig. 1 clearly shows that the energy content ($\sigma_b\gamma_b$) of oleaginous yeasts increases with the accumulation of intracellular lipids. Least square correlation between lipid content, L(%), and $\sigma_b\gamma_b$ gives

$$\sigma_b\gamma_b = 0.0308L + 1.6 \quad (11)$$

This linear relationship is valid for glucose grown oleaginous yeasts, although it was derived from ethanol grown oleaginous yeasts [2].

Correlation between theoretical $Y_{x/s\,max}$ and lipid content

For the correlation between energetic content of substrate and energetic content of microbes, eq. (9) should be modified to include the energetic content dependent term using eq. (11). Equation [9] becomes

$$\eta = ((0.0308L + 1.6)/\sigma_s\gamma_s)\, Y_{x/s} \quad (12)$$

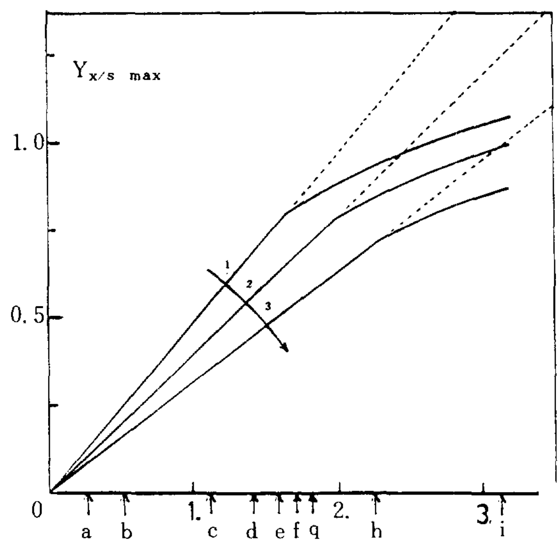

Fig. 2. Biomass yield as a function of energy content in substrate.

a) oxalate, b) formate, c) citrate, d) succinate, e) glucose, f) mannitol, g) glycerol, h) methanol, i) ethanol

(1) eq. (10); (2) 30% lipid content, eq. (13); (3) 50% lipid content, eq. (13).

Theoretical maximum yield may be calculated by replacing η by 1 (because η must be less than 1).

$$Y_{x/s\ max} = (\sigma_s\gamma_s)/(0.0308L + 1.6) \qquad (13)$$

Graphical representation of eq. (13) is shown in Fig. 2. Fig. 2 poses several interesting points. Firstly, maximum biomass yield increases linearly with $\sigma_s\gamma_s$ of substrate (i.e. it is energy limited) when the substrates are less reduced than $\sigma_s\gamma_s$ of 1.6-2.3. Secondly, above this point, theoretical $Y_{x/s\ max}$ deviates from the prediction of energy conservation, because the theoretical $Y_{x/s\ max}$ is limited by the availability of carbon atom (i.e. it is carbon limited). In the region of carbon limitation, the extra energy is dissipated as heat or another by-products. Thirdly, $Y_{x/s\ max}$ decreases with the increase of intracellular lipid content, as was expected [2]. Fourthly, the shifting point of energy limitation to carbon limitation increases with the lipid content. It is interesting, because it means that the oleaginous yeasts utilize highly reduced substrates more efficiently than the non-oleaginous microorganisms.

Dependence of oxygen yield on η and $\sigma_b\gamma_b$

Considering that the bioreactor productivity is limited by oxygen transfer capacity, biomass yield on oxygen, $Y_{x/o}$, is of great importance in the design of any bioreactor system. Since the expense of b moles of oxygen provides y gram-atoms of biomass carbon as shown in eq. (1),

$$Y_{x/o} = \frac{y\frac{12}{\sigma_b}}{32b} \qquad (14)$$

Replacing b by eq. (3) results in

$$Y_{x/o} = \frac{3}{2\sigma_b\gamma_b}\ \frac{y}{\frac{\gamma_s - z\gamma_p}{\gamma_b} - y} \qquad (15)$$

If we consider the case where extracellular products are not formed, eq. (15) may be simplified to

$$Y_{x/o} = \frac{3}{2\sigma_b\gamma_b}\ \frac{y}{\frac{\gamma_s}{\gamma_b} - y} \qquad (16)$$

Replacing y by eq. (7) results in

$$Y_{x/o} = \frac{3}{2\sigma_b\gamma_b}\ \frac{\eta}{1-\eta} \qquad (17)$$

which can be further simplified using the average value of $\sigma_b\gamma_b$ (=2).

$$Y_{x/o} = 0.75\ \frac{\eta}{1-\eta} \qquad (18)$$

Equation (18) is valid for the non-oleaginous microorganisms. For the case of oleaginous microorganisms, eq. (17) should contain the lipid content (energy content) dependent term using eq. (11).

$$Y_{x/o} = \frac{3}{2(0.0308L+1.6)} \cdot \frac{\eta}{1-\eta} \qquad (19)$$

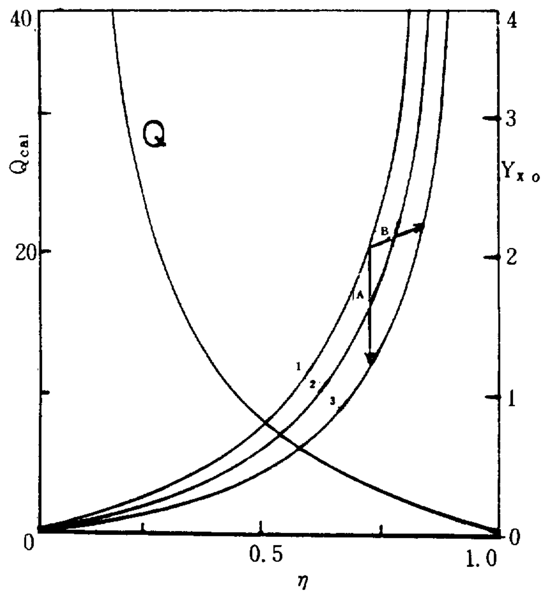

Fig. 3. Dependence of $Y_{x/o}$ (g biomass/g oxygen) on η and $\sigma_b\gamma_b$.
(1) eq. (18); (2) 30% lipid content, eq. (19); (3) 60% lipid content, eq. (19).

Graphical interpretations of eqs. (18) and (19) are shown in Fig. 3. It is evident from Fig. 3 that biomass yield on oxygen, $Y_{x/o}$, increases with the energetic yield η, and the specific metabolic heat generation, Q_{cal} (Cal/g biomass), decreases with η. Equally important is that $Y_{x/o}$ decreases with the increase of lipid content if η does not change with lipid content (direction A). However, η seems to depend on the lipid content as is analyzed in Table 1 [9]. In this case $Y_{x/o}$ increases with the increase of lipid content (direction B). Yoon & Rhee [10] and Ratledge & Gill [11] found that the specific oxygen uptake rate under lipid accumulating condition (nitrogen source limited) is considerably low as compared to non lipid accumulating condition (carbon source limited).

Data consistency in continuous culture of *Rhodotorula glutinis*

In our previous paper [12], biomass yield decreased with dilution rate and decreased also with the decrease of lipid content, which is contradictory to the theoretical prediction provided in Fig. 2 and eq. (13). Thus, the data was analyzed using mass and energy balance equation which is given in eq. (5). The fraction of substrate energy transferred to oxygen, ε, is calculated from the specific oxygen uptake rate. In Table 2, the sum of ε and η should be close to unity if there is no formation of extracellular by-products. At the dilution rate of 0.02-0.03 hr^{-1}, $\varepsilon + \eta$ approaches to unity, although it is not close

Table 1. Energy balance of oleaginous yeast, *Candida* 107 [9].

N-limited

D (hr^{-1})	$Y_{x/s}$	L (%)	$\sigma_b \gamma_b$	η	$(D\frac{1}{\eta})$
0.03	0.545	21.8	2.27	0.774	0.388
0.06	0.602	37.1	2.74	1.032	0.058
0.08	0.431	32.3	2.595	0.699	0.114
0.10	0.408	27.9	2.46	0.627	0.154
0.13	0.522	23.2	2.315	0.755	0.172
0.17	0.528	20.5	2.231	0.736	0.24
0.21	0.493	19.5	2.201	0.678	0.31

C-limited

0.03	0.475	9.2	1.883	0.56	0.054
0.06	0.50	9.4	1.890	0.59	0.102
0.10	0.51	9.0	1.877	0.596	0.168
0.14	0.516	9.7	1.9	0.612	0.229
0.17	0.516	12.2	1.976	0.637	0.267
0.19	0.50	13.3	2.01	0.628	0.303
0.21	0.50	14.2	2.04	0.637	0.330

to unity. However, a dramatic decrease of $\varepsilon + \eta$ is obvious as the dilution rate increases. A large deviation of $\varepsilon + \eta$ from unity means that the term, ξ_p, could not be assumed to be zero. Accordingly, accumulation of extracellular by-products could be a suggestive cause for the decrease of biomass yield with the increasing dilution rate. It is worthwhile noting that the mass and energy balance method may be an invaluable one in checking the data consistency both in batch culture [13] and in continuous culture [14]. Recently, extracellular mannan was detected in batch culture of *R. glutinis* in our laboratory, although it is not quantified systematically in continuous culture at present.

Table 2. Energy balance of oleaginous yeast, *Rhodotorula glutinis* [12].

D (hr^{-1})	Y	L (%)	$\sigma_b \gamma_b$	η	ε	$\eta + \varepsilon$
0.02	0.34	49.8	3.13	0.666	0.122	0.788
0.03	0.37	40.0	2.832	0.655	0.118	0.773
0.04	0.30	34.6	2.504	0.473	—	—
0.05	0.222	20.1	2.216	0.308	0.087	0.395
0.07	0.233	17.0	2.124	0.309	0.097	0.406
0.09	0.195	14.1	2.034	0.248	0.073	0.321

CONCLUSION

Mass and energy balance method was successfully applied to the prediction of biomass yield on the carbon substrate and the oxygen. The variation of energy content of oleaginous yeast needs the yield analysis based on this method Data consistency of continuous culture was checked by the energy balance based on distributive conservation of the substrate energy into biomass, heat and product.

NOMENCLATURE

- a : mol NH_3/1 carbon equivalent of substrate
- b : mol O_2/1 carbon equivalent of substrate
- c : mol H_2O/1 carbon equivalent of substrate
- d : mol CO_2/1 carbon equivalent of substrate
- m : atomic ratio of nitrogen to carbon in substrate
- l : atomic ratio of oxygen to carbon in substrate
- L : lipid content (%)
- n : atomic ratio of oxygen to carbon in biomass
- p : atomic ratio of hydrogen to carbon in biomass
- q : atomic ratio of nitrogen to carbon in biomass
- Q_{cal} : specific metabolic heat generation (Cal/g biomass)
- γ : atomic ratio of hydrogen to carbon in products
- s : atomic ratio of oxygen to carbon in products
- t : atomic ratio of nitrogen to carbon in products
- y : biomass carbon yield (fraction of organic substrate carbon in biomass)
- $Y_{x/s}$: biomass yield (g biomass/g substrate)
- $Y_{x/s\,max}$: theoretical biomass yield maximum
- $Y_{x/o}$: biomass yield on oxygen (g biomass/g oxygen)
- γ_b : reductance degree of biomass [eq. (3)]
- γ_p : reductance degree of product [eq. (3)]
- γ_s : reductance degree of substrate [eq. (3)]
- ε : fraction of substrate energy evolved as heat
- ξ_p : fraction of substrate energy incorporated into products
- η : fraction of substrate energy converted to biomass
- σ_b : weight fraction of carbon in biomass
- σ_s : weight fraction of carbon substrate

REFERENCES

1. Ratledge, C.: *Prog. Ind. Microbiol.* **16,** 119 (1982).
2. Pan, J.G. and Rhee, J.S.: *Biotechnol. Bioeng., submitted for publication* (1985)

3. Minkevich, I.G. and Eroshin V.K.: *Folia Microbiol.* **18,** 376 (1973).
4. Erickson, L.E., Minkevich, I.G. and Eroshin, V.K.: *Biotechnol. Bioeng.* **20,** 1595 (1978).
5. Roels, J.A.: *Biotechnol. Bioeng.* **22,** 2457 (1980).
6. Roels, J.A.: *Enegetics and Kinetics in Biotechnology,* Elsevier Biomedial Press, Amsterdam, 1st ed, (1983).
7. Heijnen, J.J. and Roels, J.A.: *Biotechnol. Bioeng* **23,** 739 (1981).
8. Eroshin, V.K. and Krylova, N.I.: *Biotechnol. Bioeng.* **25,** 1693 (1983).
9. Gill, C.O., Hall, M.J. and Ratledge, C.: *Appl. Env. Microbiol.* **33,** 231 (1977).
10. Yoon, S.H. and Rhee, J.S.: *Process Biochem.* **18,** (Oct.) 2 (1983).
11. Ratledge, C. and Hall M.J.: *Appl. Env. Microbiol.* **34,** 230 (1977).
12. Choi, S.Y., Ryu, D.D. Y, and Rhee, J.S.: *Biotechnol. Bioeng.* **24,** 1165 (1982).
13. Erickson, L.E., Selga, S.E. and Viesturs, U.E.: *Biotechnol Bioeng.* **20,** 1623 (1978).
14. Erickson, L.E., Minkevich, I.G. and Eroshin, V.K.: *Biotechnol Bioeng.* **21,** 575 (1979).