Estimation of rates of oxygen uptake and carbon dioxide evolution of animal cell culture using material and energy balances

Zhi-Long Xiu, Wolf-Dieter Deckwer & An-Ping Zeng*

GBF – Gesellschaft für Biotechnologische Forschung mbH, Biochemical Engineering Division, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Received 3 September 1997; accepted 15 October 1998

Key words: animal cell culture, carbon dioxide evolution rate, oxygen uptake rate, reductance degree balance, respiratory quotient

Abstract

Material and degree of reductance balance equations are used to estimate the rates of oxygen uptake and carbon dioxide evolution of animal cell cultures. Lumped compositions, molecular weight and reductance degree of cellular protein, monoclonal antibody, biomass and amino acid consumption (excluding glutamine and alanine) are found to be relatively constant for different hybridoma cell lines and may be used as regularities. The calculated rates of oxygen uptake and carbon dioxide evolution agree well with experimental values of several different cultures reported in the literature. This simple method gives the same results as calculated on the basis of a detailed metabolic reaction network.

Abbreviations: CER – carbon dioxide evolution rate; OUR – oxygen uptake rate; RQ – respiratory quotient.

Nomenclature

- c_i = number of carbon atoms in amino acid i ;
DCW = dry cell weight, g cell⁻¹;
 M = specific mass formation rate of protein product, g cell⁻¹ day⁻¹;
MW = molecular weight, g mol⁻¹;
 q = specific consumption or formation rate of substrate or product, mol cells⁻¹ day⁻¹;

Greek symbols

- β = nitrogen content of lumped amino acids, protein product or biomass;
 γ = degree of reductance;
 μ = specific growth rate, day⁻¹.

Introduction

The use of on-line measurement of oxygen uptake rate (OUR) has recently gained much attention to monitor and control the metabolism of mammalian cell cultures. For example, Zhou et al. (1995) successfully applied a dynamic on-line OUR measurement to control nutrient feeding in a high density fed-batch culture of mammalian cells. An alternative to the dynamic method is the use of on-line off-gas analysis (Lovrecz and Gray, 1994; Bonarius et al., 1995; Eyer et al., 1995). The latter gives not only information on OUR but also information on the carbon dioxide evolution rate (CER). The respiratory quotient (RQ) that gives a quantitative indication of the involvement of different pathways in the production of CO₂ in relation to O₂ consumption may then be used for process monitoring and control. This method avoids fluctuations of dissolved oxygen tension associated with the on-line dynamic method and needs not to determine the solubility of O₂ in culture broth. The on-line estimation of RQ has been successfully used for the monitoring

* Author for all correspondence. E-mail: AZE@GBF.de

and control of microbial fermentation processes. For instance, RQ is used as a criterion to control the feeding rate of nutrients in yeast fermentation (Wang et al., 1977) and for an optimum oxygen supply in microaerobic cultures (Zeng et al., 1994). However, RQ has not been established as a practical tool for mammalian cell cultures so far. This is probably due to the difficulty in determining CER because of the use of bicarbonate in medium. CO₂ may be released from the medium applied or dissolved substantially in the culture broth depending on the cultivation conditions and the composition and properties of the medium (Lovrecz and Gray, 1994; Bonarius et al., 1995; Zeng 1995).

Bonarius et al. (1995) proposed a method to estimate OUR, CER and RQ of animal cells in continuous culture from exit-gas analysis and balances of the liquid and gas phases. Uncertainties still exist in this kind of method because of the assumptions made in the derivation of the balance equations and the necessity of knowing the mass transfer coefficients and Henry constants for both CO₂ and O₂. These parameters may change during the cultivation because of the changes of compositions and properties of the culture broth and other conditions of the reactor system. On the other hand, it is difficult to measure accurately OUR and CER due to the slow growth rates/low specific metabolic rates of such cultures, the low cell densities normally obtained in suspension cultures, and the use of bicarbonate buffers in media (Lovrecz and Gray, 1994). Ideally, there should exist an independent method to estimate OUR, CER and/or RQ for checking the accuracy and/or consistency of the results from the exit-gas analysis. The OUR, CER and RQ values estimated in such a way may also help in understanding the growth and metabolism of animal cell cultures. The methods based on metabolic pathways and stoichiometry of cell growth and metabolism as proposed by Xie and Wang (1996) and Zupke and Stephanopoulos (1995a) represent such an attempt. These methods require a detailed knowledge of the metabolic pathways and stoichiometry. We present here a relatively simple method to estimate OUR, CER and RQ of animal cell cultures using material and energy balances.

Material and reductance degree balance equations of animal cell cultures

Animal cells are normally cultivated in medium containing glucose and glutamine as the main carbon and

energy sources. Other essential components such as amino acids, vitamin and growth factors are also included. The major products of an animal cell culture are cells, protein products (e.g. monoclonal antibody (Mab), immunoglobulin G(IgG)), CO₂, lactate, ammonium and some amino acids (mainly alanine in most cell lines). The material flows involved can be simplified as shown in Figure 1. The following macroscopic balance equations can be written.

(1) Carbon balance

$$6q_1 + 5q_2 + q_3 = 3q_4 + 3q_5 + q_7 + q_8 + q_{CO_2} \quad (1)$$

Where q_1 , q_2 , q_3 , q_4 , q_5 , q_7 , q_8 , q_{CO_2} are the specific consumption rates of glucose, glutamine, amino acids and production rates of lactate, alanine, product, biomass, and CO₂, respectively. q_3 , q_7 , and q_8 are lumped variables expressed as C-mole per unit biomass per time and can be calculated as follows:

$$q_3 = \sum c_i q_{AAi} \quad (2)$$

$$q_7 = M_7/MW_7 \quad (3)$$

$$q_8 = \mu DCW/MW_8 \quad (4)$$

In Equation (2), c_i is the carbon atom number of i th amino acid (excluding glutamine and alanine). q_{AAi} represents the specific utilization rate of amino acid i . In Equations (3) and (4), MW_7 and MW_8 are the molecular weights of the protein product and biomass, the molecular compositions of which are expressed as $CH_\alpha N_\beta O_\delta$. M_7 is the specific mass formation rate of the product. DCW represents dry cell weight and μ is the specific growth rate.

(2) Nitrogen balance

$$2q_2 + \beta_3 q_3 = q_5 + q_6 + \beta_7 q_7 + \beta_8 q_8 \quad (5)$$

In Equation (5), β_3 , β_7 and β_8 are the nitrogen contents of the lumped molecular composition of amino acids (without glutamine and alanine), protein product and biomass based on C-mole, respectively. q_6 is the specific production rate of ammonia.

(3) Reductance degree balance

The reductance degree balance equation has been successfully applied to microbial cultures (Erikson et al., 1978; Zeng, 1995) as a substitution for the balances

Figure 1. Simplified diagram of material flows of growth and metabolism of animal cell cultures.

of hydrogen and oxygen. To our knowledge it has not been applied to animal cell culture so far. According to Figure 1 the following equation may be valid for animal cell culture:

$$\gamma_1 q_1 + \gamma_2 q_2 + \gamma_3 q_3 + \gamma_{O_2} q_{O_2} = \gamma_4 q_4 + \gamma_5 q_5 + \gamma_6 q_6 + \gamma_7 q_7 + \gamma_8 q_8 \quad (6)$$

In Equation (6), γ_1 to γ_8 and γ_{O_2} are the reductance degrees of glucose, glutamine, amino acids, lactate, alanine, ammonia (NH_4^+), protein product (e.g. Mab or IgG), biomass, and oxygen, respectively. Their values are given as follows according to the reductance degrees for carbon, hydrogen, oxygen and nitrogen being 4, 1, -2, and -3, respectively:

$$\begin{aligned} \gamma_1 &= 24, \gamma_2 = 18, \gamma_4 = 12, \gamma_5 = 12, \gamma_6 = 1, \\ \gamma_3 &= 4 + \alpha_3 - 3\beta_3 - 2\delta_3, \gamma_7 = 4 + \alpha_7 - 3\beta_7 - 2\delta_7, \\ \gamma_8 &= 4 + \alpha_8 - 3\beta_8 - 2\delta_8, \gamma_{O_2} = -4. \end{aligned}$$

From Equations (1) and (6) the specific rates of OUR and CER could be obtained, respectively, and then RQ could be calculated as follows:

$$RQ = \frac{q_{CO_2}}{q_{O_2}} = \frac{6q_1 + 5q_2 + q_3 - 3q_4 - 3q_5 - q_7 - q_8}{(\gamma_4 q_4 + \gamma_5 q_5 + \gamma_6 q_6 + \gamma_7 q_7 + \gamma_8 q_8 - \gamma_1 q_1 - \gamma_2 q_2 - \gamma_3 q_3)/\gamma_{O_2}} \quad (7)$$

Results and discussion

Lumped compositions of biomass, product and amino acids utilized

To apply Equations (1)–(7) the compositions and reductance degrees of biomass, protein products and the

lumped consumption of amino acids must be known. Macroscopically, animal cells can be considered to be composed of protein, carbohydrate, lipids, nucleotides (RNA and DNA), and water. Table 1 shows these components in percentage of dry cell weight reported for different hybridoma cells. It is understood that the variations of cellular composition and dry weight of cells may be cell line specific and depend on cultivation conditions (e.g., medium composition and physicochemical properties, growth phase and/or growth rate and dissolved oxygen level etc.). The chemical composition of cellular protein ($CH_a N_b O_d$) can be determined by analyzing the contents of 20 amino acids. The results as shown in Table 2a for some hybridoma cell lines indicate that the chemical composition of cellular protein for different cell lines is nearly the same although its molar percentages in cells can vary considerably (Table 1). Differences are also observed for the total biomass composition of different cell lines (Table 2b). On the other side, the total biomass composition of a given cell line grown on different media is fairly constant as suggested by the data of Bonarius et al. (1996) for a murine hybridoma cell line (Table 2b). In this work, the average values are used to calculate OUR and CER values for cultures in which the cellular composition of biomass was not reported.

Very few data were reported for the elemental composition of Mab or IgG (Table 2a). In this study, the lumped composition of Mab or IgG which is calculated on the basis of 20 amino acid components reported by Edelman et al. (1969) for a γ -IgG is used. To obtain the lumped composition of amino acids consumed (excluding glutamine and alanine) the average values of measured amino acid consumption are used. Table 2c summarizes some typical lumped compositions of amino acids consumed by different cell lines. These values do not vary significantly. Therefore, the

Table 1. Cellular composition (% of dry cell weight)

Cell line	Protein	Carbo- hydrates	Lipids	RNA	DNA	DCW (pg cell ⁻¹)	Reference
Chemical composition	CH _a N _b O _d	(CH ₂ O) _n	(CH ₂) _n	C _{9.5} H _{10.75} N _{3.75} O ₇ P	C _{9.75} H _{11.25} N _{3.75} O ₆ P		Xie and Wang, 1994
Mouse hybridoma	72.9	3.5	13.5	3.8	1.4	250	Xie and Wang, 1994
Murine hybridoma	70.6	7.1	9.7	5.8	1.4	470	Bonarius et al., 1996
Murine hybridoma	67.1	7.0	10.0	5.3	1.5	505	Bonarius et al., 1996
Murine hybridoma	60.1	6.4	17.0	5.6	10.8	n.d.	Zupke et al., 1995a

Table 2a. The lumped composition, molecular weight and reductance degree of cellular protein and monoclonal antibody

	Cell line	C	H	N	O	MW	γ	Reference
Cellular Protein	Mouse hybridoma	1	2.01	0.29	0.51	26.23	4.12	Xie and Wang, 1994
	Murine hybridoma	1	2.01	0.29	0.50	26.07	4.14	Bonarius et al., 1996
		1	1.99	0.28	0.51	26.07	4.13	Savinell et al., 1992
	Average	1	2.00	0.29	0.51	26.12	4.13	
Mab/IgG	Murine hybridoma	1	1.97	0.27	0.49	25.59	4.18	Edelman et al., 1969
		1	1.50	0.25	0.25	21.00	4.25	Martens et al., 1995 ^a

^a The values are reported in the literature. The other ones are calculated.

Table 2b. The lumped composition, molecular weight and reductance degree of biomass

	Cell line	C	H	N	O	MW	γ	Reference
	Mouse hybridoma	1	1.97	0.22	0.41	23.61	4.49	Xie and Wang, 1994
	Murine hybridoma	1	1.90	0.20	0.41	23.26	4.48	Zupke et al., 1995a
		1	1.78	0.25	0.43	24.16	4.17	Martens et al., 1995 ^a
	Murine hybridoma	1	1.96	0.23	0.45	24.38	4.37	Bonarius et al., 1996
	Murine hybridoma	1	1.96	0.22	0.45	24.24	4.40	Bonarius et al., 1996
	Average	1	1.91	0.22	0.43	23.93	4.38	

^a This set of values is reported in the literature.

Table 2c. Lumped composition, molecular weight and reductance degree of amino acids^a

	Cell line	C	H	N	O	MW	γ	Reference
	Mouse hybridoma	1	1.91	0.20	0.41	23.27	4.49	Xie and Wang, 1996
	Mouse hybridoma	1	2.05	0.27	0.36	23.59	4.52	Jan et al., 1997
	Murine hybridoma	1	2.02	0.27	0.38	23.88	4.45	Hiller et al., 1993
	Murine hybridoma	1	2.03	0.26	0.46	25.03	4.33	Bonarius et al., 1996
	Average	1	2.00	0.25	0.40	23.94	4.45	

^a The data for amino acids consumption (excluding glutamine, alanine, vitamins, growth factors and serum) are average values.

Table 3. Experimental data of specific rates of O₂ uptake and CO₂ evolution and RQ reported in the literature for animal cell cultures

Cell line	q_{O_2} ^a	q_{CO_2} ^a	RQ	Reference
Murine hybridoma	101~107	99~111	0.97~1.07	Bonarius et al. (1995)
Murine hybridoma	104.5/108.4	99.2/110.6	0.95/1.02	Bonarius et al. (1996)
Murine hybridoma	48	62.4	1.3	Zupke et al. (1995a)
Murine hybridoma	18~48	24~62.4	1.3~1.33	Zupke et al. (1995b)
CHO	47.95	46.31	0.97	Gray et al. (1996)
CHO	120/193	72/165	0.6/0.85	Lovrecz and Gray (1994)
HFN7.1 (hybridoma)	48.0	n.d.	n.d.	Eyer and Heinzle (1996)
Mouse hybridoma	55.2~100.8	n.d.	n.d.	Hiller et al. (1991)
Mouse hybridoma	48~72	n.d.	n.d.	Hiller et al. (1993)
Murine hybridoma	52.8	n.d.	n.d.	Jan et al. (1997)
NSO myeloma	52.7~103.7	n.d.	n.d.	Yoon et al. (1994)

^a Unit: mmol/10¹⁰ cells day⁻¹. n.d. = not determined.

average values may be used. It should also be mentioned that the data used for the calculations of some of the compositions given in Tables 2a–c were not directly measured but reported values by the authors.

Estimation of OUR, CER and RQ

The measurement of OUR, CER and RQ has received increasing attention for the control and monitoring of animal cell cultures in the past years. Table 3 lists some recent experimental data of specific OUR (q_{O_2}) and CER (q_{CO_2}) for mammalian cells reported in the literature. OUR data of animal cell cultures before 1990 were summarized by Eyer et al. (1995). Limited data of CER were reported in the literature because of the difficulties to determine CER in bicarbonate-buffered cultures. It is evident from Table 3 that the specific OUR and CER can vary in a relatively wide range. They depend not only on the cell line, but also on the cultivation conditions employed such as dissolved oxygen level (Zupke et al., 1995b), growth rate (Hiller et al., 1991, 1993) and nutrient concentrations.

The oxygen consumption rates calculated from Equation (6) are in good agreement with the literature values as shown in Figure 2 for five different cultures (Hiller et al., 1993; Zupke et al., 1995b; Bonarius et al., 1996; Jan et al., 1997; Xie and Wang, 1996). For the fed-batch cultures of Xie and Wang (1996) accumulative q_{O_2} values calculated from a detailed reaction network are included in Figure 2 as 'experimental' values for comparison of the two methods. It can be seen that the simple reductance degree balance (Equation (6)) gives exactly the same q_{O_2} values as calculated from the detailed reaction net-

work. It should be mentioned that in some cases the experimental data are not complete for applying Equation (6). The biomass composition for the cultures of Hiller et al. (1993) and Jan et al. (1997), Mab for the culture of Bonarius et al. (1996) and amino acid consumption for the culture of Zupke et al. (1995b) are calculated from nitrogen balance (Equation (5)). In fact, Equation (5) can be used to check the nitrogen balance of animal cell cultures. For example, the mean ratio of nitrogen output to input in the three fed-batch and one batch cultures of Xie and Wang (1996) is 1.05 according to Equation (5). This is because the consumption of vitamins, growth factors and serum (5% fetal bovine serum used in these studies) is not considered in Equation (5). This example also suggests that the consumption of these growth factors and nutrients has a relatively small influence on the total nitrogen balance. The deviation of nitrogen balance should be even smaller for hybridoma cells grown in serum-free media. In cases where more experimental data are missing the average compositions and reductance degrees of biomass, protein product (MAb or IgG) and amino acid consumption as given in Tables 2a–c may be used.

q_{CO_2} can be calculated from the carbon balance (Equation (1)). A good agreement between experimental results and calculations is obtained for three hybridoma cell lines as shown in Figure 3. Again, the 'experimental' q_{CO_2} values for the cultures of Xie and Wang (1996) were calculated from a stoichiometric reaction network. The simple carbon balance equation gives exactly the same values as calculated from the reaction network with 26 chemical reactions.

Figure 2. Comparison of specific oxygen consumption rate calculated from Equation (6) with experimental data of different cell cultures.

Compared to the method proposed by Bonarius et al. (1995) for the estimation of CER the use of carbon balance is also much simpler. The rates of input (nutrient consumption) and output (formation of biomass and products) as shown in Figure 1 can relatively easily be measured, some of them can even be determined on-line. In addition, the lumped molecular composition of biomass and products are relatively constant (Tables 2a–c). The biomass production rate may be calculated according to Equation (4), where DCW can be estimated from the mean diameter of viable cells according to Frame and Hu (1990).

With the specific OUR and CER values calculated, the RQ values can also be estimated which are compared with experimental values in Figure 4. Relatively large deviations are obtained for RQ. Figure 4 demonstrates that the experimental RQ values generally lie in the range of 0.9 to 1.4 for the cultures considered in this work. A relatively low RQ value of 0.6 has been reported for CHO cells by Lovrecz and Gray (1994). Aunins and Henzler (1993) reported an RQ value of 0.8 for an rNS/O myeloma cell line. The average value of RQ for a murine hybridoma cell line determined by Bonarius et al. (1995, 1996) was close to unity. However, Zupke et al. (1995a, b) found an RQ value of

1.3 for the same cell line Bonarius et al. (1996) used. It is not clear if these differences are due to the specific properties of the cell lines and the culture conditions or due to deviations of experimental determination. As revealed in Figure 4, the calculation of RQ is sensitive to deviations of both OUR and CER. Hence, caution should be taken when using RQ as a control parameter for animal cell culture. Theoretically, complete oxidation of glutamine and glucose results in an RQ of 0.83 and 1.00, respectively (Bonarius et al., 1996). Considering a culture in which glutamine provides 55% and glucose 45% of the energy requirement (Fitzpatrick et al., 1993) and assuming that both substrates are completely oxidized a theoretical RQ value of 0.9 would be expected. Incompleted oxidation of these nutrients will tend to increase the RQ value. The contribution of lipid biosynthesis to CO₂ release also leads to an increasing RQ (Bonarius et al., 1996). Thus, an RQ value larger than 0.9 seems to be realistic for animal cell cultures.

In conclusion, material and degree of reductance balance equations can be used to estimate the rates of oxygen uptake and carbon dioxide evolution of animal cell culture. The use of these balance equations is straightforward and provides an independent method

Figure 3. Comparison of specific carbon dioxide evolution rate calculated from Equation (1) with experimental data.

Figure 4. Comparison of respiratory quotient (RQ) calculated from Equation (7) with experimental data.

to check the accuracy and consistency of OUR and CER calculations from exit-gas analysis. They may also be used for the modelling of animal cell culture.

References

- Aunins JG and Henzler H-J (1993) Aeration in cell culture bioreactors. In Rehm H-J, Reed G, Pühler A and Stadler P (Eds) *Biotechnology*, Vol. 3, Bioprocessing, VCH, Weinheim.
- Bonarius HPJ, de Gooijer CD, Tramper J and Schmid G (1995) Determination of the respiration quotient in mammalian cell culture in bicarbonate buffered media. *Biotechnol Bioeng* 45: 524–535.
- Bonarius HPJ, Hatzimanikatis V, Meesters PH, de Gooijer CD, Schmid G and Tramper J (1996) Metabolic flux analysis of hybridoma cells in different culture media using mass balances. *Biotechnol Bioeng* 50: 299–318.
- Edelman GM, Cunningham BA, Gall WE, Gottlieb PD, Rutishauer U and Waxdal MJ (1969) The covalent structure of an entire gamma G immunoglobulin molecule. *Proc Nat Acad Sci USA* 63: 78–85.
- Erikson LE, Minkevich IG and Eroshin VK (1978) Application of mass and energy balance regularities in fermentation. *Biotechnol Bioeng* 20: 1595–1621.
- Eyer K, Oeggerli A and Heinze E (1995) On-line gas analysis in animal cell cultivation: II. Methods for oxygen uptake rate estimation and its application to controlled feeding of glutamine. *Biotechnol Bioeng* 45: 54–62.
- Eyer K and Heinze E (1996) On-line estimation of viable cells in a hybridoma culture at various DO levels using ATP balancing and redox potential measurement. *Biotechnol Bioeng* 49: 277–283.
- Fitzpatrick L, Jenkins HA and Butler M (1993) Glucose and glutamine metabolism of a murine B-lymphocyte hybridoma grown in batch culture. *Appl Biochem Biotechnol* 43: 93–116.
- Frame KK and Hu W-S (1990) Cell volume measurement as an estimation of mammalian cell biomass. *Biotechnol Bioeng* 36: 191–197.
- Gray DR, Chen S, Howarth W, Inlow D and Maiorella L (1996) CO₂ in large-scale and high-density CHO cell perfusion culture. *Cytotechnol* 22: 65–78.
- Hiller GW, Aeschlimann AD, Clark DS and Blanch HW (1991) A kinetic analysis of hybridoma growth and metabolism in continuous suspension culture on serum-free medium. *Biotechnol Bioeng* 38: 733–741.
- Hiller GW, Clark DS and Blanch HW (1993) Cell retention – chemostat studies of hybridoma cells – analysis of hybridoma growth and metabolism in continuous suspension culture on serum-free medium. *Biotechnol Bioeng* 42: 185–195.
- Jan DCH, Petch DA, Huzel N and Butler M (1997) The effect of dissolved oxygen on the metabolic profile of a murine hybridoma grown in serum-free medium in continuous culture. *Biotechnol Bioeng* 54: 153–164.
- Lovrecz G and Gray P (1994) Use of on-line gas analysis to monitor recombinant mammalian cell cultures. *Cytotechnol* 14: 167–175.
- Martens DE, Sipkema EM, de Gooijer CD, Beuvery EC and Tramper J (1995) A combined cell-cycle and metabolic model for the growth of hybridoma cells in steady-state continuous culture. *Biotechnol Bioeng* 48: 49–65.
- Savinell JM and Palsson BO (1992) Network analysis of intermediary metabolism using linear optimization. I Development of mathematical formalism. *J Theor Biol* 154: 421–454.
- Wang HY, Cooney CL and Wang DIC (1977) Computer-aided baker's yeast fermentation. *Biotechnol Bioeng* 19: 69–86.
- Xie L and Wang DIC (1994) Applications of improved stoichiometric model in medium design and fed-batch cultivation of animal cells in bioreactor. *Cytotechnol* 15: 17–29.
- Xie L and Wang DIC (1996) Material balance studies on animal cell metabolism using a stoichiometrically based reaction network. *Biotechnol Bioeng*, 52: 579–590.
- Yoon S-J and Konstantinov K (1994) Continuous real-time monitoring of oxygen uptake rate (OUR) in animal cell bioreactors. *Biotechnol Bioeng* 44: 170–177.
- Zeng A-P, Byun T-G, Posten C and Deckwer W-D (1994) Use of respiratory quotient as a control parameter for optimum oxygen supply and scale-up of 2,3-butanediol production under microaerobic conditions. *Biotechnol Bioeng* 44: 1107–1114.
- Zeng A-P (1995) Effect of CO₂ absorption on the measurement of CO₂ evolution rate in aerobic and anaerobic continuous cultures. *Appl Microbiol Biotechnol* 42: 688–691.
- Zeng A-P (1995) A new balance equation of reducing equivalents for data consistency check and bioprocess calculation. *J Biotechnol* 43: 111–124.
- Zhou W, Rehm J and Hu W-S (1995) High viable cell concentration fed-batch cultures of hybridoma cells through on-line nutrient feeding. *Biotechnol Bioeng* 46: 579–587.
- Zupke C and Stephanopoulos G (1995a) Intracellular flux analysis in hybridomas using mass balances and *In Vitro* ¹³C NMR. *Biotechnol Bioeng* 45: 292–303.
- Zupke C, Sinskey AJ and Stephanopoulos G (1995b) Intracellular flux analysis applied to the effect of dissolved oxygen on hybridomas. *Appl Microbiol Biotechnol* 44: 27–36.