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Fundamental Bioengineering

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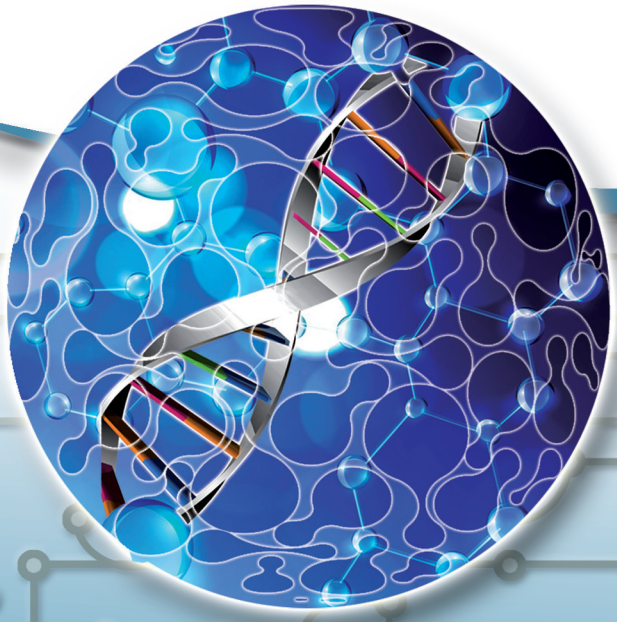

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1
Introduction and Overview

John Villadsen

Bioengineering is a relatively new addition to a long list of terms starting with "bio." It is broadly defined as "the application of engineering principles to biological systems." Bioengineering can include elements of chemical, electrical and mechanical engineering, computer science, materials, chemistry and biology. The systems that are analyzed range from cell cultures and enzymes applied in the bio-industry and in bioremediation to prosthetics, construction of models for organs such as liver, drug-delivery systems and numerous other subjects in biomedical engineering, all requiring an understanding of transport phenomena (mass, heat, and momentum transfer) and kinetics, combined in often large mathematical models. Besides a working knowledge of these core chemical engineering disciplines, a successful study of a problem in bioengineering requires an insight into the core disciplines of biology and biochemistry, specifically in human physiology when the goal is, for example, to construct a new cancer drug delivery system.

In this volume, coauthored by nine scientists, mostly working in academic institutions or in the bio-industry, the focus is on application of bioengineering in the emerging "white biotechnology" industry. The design of bioremediation systems closely follows the principles of analysis and design of industrial bioprocesses. This text will also prove valuable for environmental engineers. The biomedical applications of the text are, however, also quite obvious. Thus, the important but complex application of mesenchymal stem cells to treat osteoporosis is based on an optimal growth strategy of the cell culture on a scaffold at the right liquid flow with the right oxygen and nutrient availability. Here, kinetics and transport phenomena are coupled to basic biology and biochemistry, and design of the system is based on a complex model for the interaction between scaffold, cells, and nutrients.

In Chapters 5, 6, and 8, the reader will find self-contained accounts of the tools that together make it possible to understand the behavior of cell cultures and enzymatically catalyzed reactions: The interaction of metabolic network reactions in steady state and during transients, analyzed by mathematical models and solved by state-of-the-art computer software.

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In Chapter 16, a structural framework for successful scale-up of bioreactions from laboratory scale to large industrial scale is presented. In Chapter 17, the sequence of management decisions that may lead to new business ventures in the bio-industry is discussed.

The analyses of cultures on the level of the cell are authored by three leading European scientists. Each author gives - as far as possible - a complete account of his subject, illustrated with examples and with sufficient detail to give readers, both in industry and in graduate classes at universities, a fair chance to understand and *utilize* the very powerful analytical tools presented in the three chapters.

The two Chapters 16 and 17 on large-scale bioreactors and on the business opportunities in the bio-industry are written by leading experts from two major bio-industrial companies, DSM in the Netherlands and Novozymes in Denmark. These chapters could serve as guidelines for prospective business ventures in the industry.

Although the focus of this book is on the bioreactor, Chapters 12 and 13 cover further processing of the effluent from the bioreactor. The author, a distinguished Indian bioscientist, gives a short introduction to the subject of downstream processing. Also, a survey of measuring, monitoring, and control of bioreactions is included. In Chapter 14, a leading expert on chemical analysis to capture key fermentation variables and on using the experimental data in analysis of fermentation broths gives an easy-to-read but largely complete survey of the subject. In Chapter 15, a young expert in control of chemical processes, discusses control problems in bioreactors, specifically addressing the challenges of bio-system control.

Finally, the content of the book is tied together by seven chapters (2, 3, 4, 7, 9, 10, and 11) written by the editor of this work. These chapters introduce a common nomenclature for the whole book, with introductory material on stoichiometry, kinetics, thermodynamics, and design of ideal and real bioreactors. It is hoped that the introductory chapters, illustrated with many simple examples, will make it easier to read the advanced chapters, especially since there are frequent cross references between introductory and advanced chapters.

Part One
Fundamentals of Bioengineering

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2
Experimentally Determined Rates of Bio-Reactions

John Villadsen

Summary

Rates of bioreactions are introduced as measured terms in steady-state mass balances for a continuous stirred tank reactor (CSTR). Both mass balances and the reaction rates have the same form for enzymatically catalyzed reactions and for reactions with living cells. In cell reactions, the rate of biomass formation is included through a separate mass balance. Reactants absorbed in the liquid phase from a gas phase are treated separately, and it is shown how experimental errors can lead to errors in the calculated rates. The black-box model for a cell-reaction stoichiometry is introduced and the yield coefficients are defined. Finally, different methods of controlling the CSTR at steady state are discussed.

2.0
Introduction

The rate of an enzymatically catalyzed bioreaction, or of a reaction that involves living cells (microbial, animal, or plant cells), can be determined experimentally in a *bioreactor*. The bioreactors used in academic research or in an industrial R&D department to obtain reaction rates are normally glass vessels of 0.5–5 l working volume V. A typical laboratory reactor is shown in Figure 2.1. It is well stirred either by an internal mechanical stirrer (a hydrofoil or a turbine) or by a magnetic stirrer, operated from the outside of the reactor. In all cases, the mixing of liquid feed into the medium volume V is supposed to be good enough to ensure that there is no spatial variation of substrates or products in the reactor. Batch operation or continuous operation of the reactor is typically used, and the assumption of perfect mixing in the medium volume V will ensure that the concentrations S_i of substrates and P_i of products are the same at any point in the reactor. If the continuous stirred tank reactor (CSTR) is operated in *steady state*, there is no accumulation of either products or substrates. The liquid flow v_f and the feed concentrations of substrates $s_{f,i}$ are kept constant in time. Now the medium volume V and the concentrations of substrates and products both in the reactor $[s_i, p_j]$ and in the effluent concentrations $[s_{e,i}, p_{e,i}]$ are constant in time. In the batch reactor, one starts with a high concentration of

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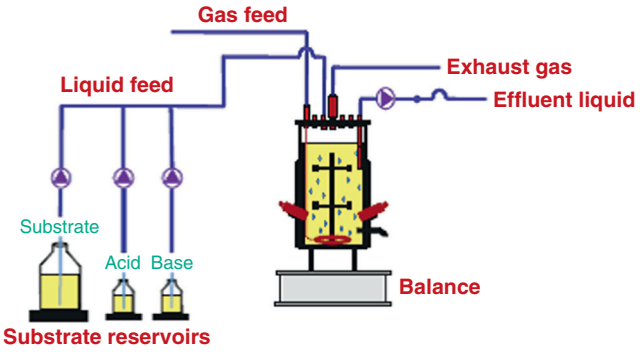

Figure 2.1 CSTR with substrate feed of liquid medium and of gas through a sparger. A separate feed of acid/alkali for pH control is used. In pH auxostats (see below) the feed of alkali is used as control variable.

substrates s_i^0 (+ a small amount of biomass for a fermentation), and S_i are converted to P_j over time. The volume of medium V in the reactor is constant in time.

A gas-phase substrate is introduced to the liquid through a sparger. It is absorbed in the liquid and is consumed by the reaction. Gaseous products are transferred to the gas phase by desorption from the liquid.

Then, mass balances for substrates and products are set up. These mass balances define the reaction rates, and solution of the mass balances allows the rates to be calculated based on measured concentrations $[s_i, p_i]$. To illustrate the procedure we shall use mass balances derived for a steady state CSTR, and the equipment is shown in Figures 2.1 and 2.2.

Figure 2.2 A commercial laboratory reactor (Biostat® A plus from Sartorious) for medium volume between 0.4 and 5 l. The reactor is supported by packages for either microbial cell or animal/plant cell cultures. The microbial package includes two 6-blade Rushton turbines (see Chapter 11), two gas-inlets, ports for inoculation, automatic and manual samplers, and temperature control via a heating blanket and cooling finger. Control of pH, T, dissolved oxygen (DO), stirrer speed, air flow rate, and foam control.

2.1
Mass Balances for a CSTR Operating at Steady State

The steady-state mass balances for a continuous reactor with a working volume V, for example, in m^3 liquid medium, in which an enzymatically catalyzed reaction occurs, is given by Eq. (2.1).

$$q_{s,i}\,V + \nu\,(s_{f,i} - s_{e,i}) = 0,\ q_{p,i}\,V + \nu\,(p_{f,i} - p_{e,i}) \text{ or } q_{s,i} + D\,(s_{f,i} - s_{e,i}) = 0,\ q_{p,j} + D\,(p_{f,j} - p_{e,j}) = 0. \quad (2.1)$$

In Eq. (2.1), ν is the liquid flow through the reactor in, for example, m^3 liquid h^{-1}. ν/V is defined as the dilution rate D (h^{-1}). s_i is the concentration of the ith substrate in the reactor, $s_{e,i}$ the concentration in the liquid effluent, and $s_{f,i}$ the concentration in the feed. For the steady-state continuous reactor (see also Section 10.1), $s_i = s_{e,i}$. The same nomenclature is used for the product concentrations.

For each substrate, the *volumetric rate of production* $q_{s,i}$ of S_i, for example, in units of g S_i or C-mol S_i l^{-1} h^{-1}, is multiplied by the reactor volume V to give the production rate of S_i (e.g., in g S_i h^{-1}). To the production term is added $\nu s_{f,I}$, the amount of S_i introduced through the feed and subtracted $\nu s_{e,i}$, the substrate that leaves the reactor. The sum of the three terms of the steady-state mass balance is zero. The mass balance for product P_j contains the same terms.

$q_{s,i}$ ($i = 1, 2, \ldots, N$) and $q_{p,j}$ ($j = 1, 2, \ldots, M$) are always defined as (volumetric) rates of *production*. Hence $q_{s,i}$ are always negative and $-q_{s,i}$ is, therefore, *the volumetric rate of consumption* of S_i.

As is the case for any catalyzed reaction, the rate can be defined either per volume reactor (q) or per unit of catalyst (r), for example, per unit mass of catalyst added to each l of reactor. This second definition defines the *specific* reaction rates $r_{s,i}$ and $r_{p,j}$, which are obtained from $q_{s,i}$ and $q_{p,j}$ by division with e, the concentration of enzyme E, for example, in units of g E l^{-1}. The specific rates define the *activity* of the enzyme E to convert S_i to P_j. These definitions are further discussed in Section 7.1.

In reactions with living cells, the cell mass catalyzes the conversion of S_i to P_j, but the *substrate is also used to form more biomass X* – the reaction is autocatalytic. Hence biomass is also a product, and similar to Eq. (2.1) one obtains:

$$-q_{s,i} = D\,(s_{f,i} - s_i);\ q_{p,j} = D\,(p_j - p_{j,f}) \text{ and } q_x = D\,(x - x_f);\ D = \nu/V. \quad (2.2)$$

$$-r_{s,i} = D\,(s_{f,i} - s_i)/x;\ r_{p,j} = D\,(p_j - p_{j,f})/x \text{ and } r_x = D\,(x - x_f)/x;\ D = \nu/V. \quad (2.3)$$

The unit of r_i could be g S_i produced/g biomass/h (i.e., r_{si} is negative), g P_i (g X $h)^{-1}$, g X (g X $h)^{-1}$.

r_x is defined as *the specific growth rate* of the culture, and in most biotechnology literature r_x is called μ. We shall only use this latter symbol when its meaning is obvious. It is seen that μ is the *ability* (or *activity*) of the biomass in the reactor to make more biomass. An active culture can make much biomass per g biomass present per hour – a resting culture has a low value of $r_x = \mu$.

Since some substrates (e.g., O_2) are captured from the gas phase and some products (e.g., CO_2) are released to the gas phase, one needs to add an extra term in Eqs. (2.1) and (2.2) for these reaction species. This term is q_{sk}^T or q_{pm}^T (e.g., in moles of O_2 or CO_2 transferred (l h)$^{-1}$).

$$(q_{sk} + q_{sk}^T) = D(s_{kf} - s_k) \text{ and } (q_{pm} + q_{pm}^T) = D(p_m - p_{m,f}). \tag{2.4}$$

In Eq. (2.4), the term q_{sk}^T is positive (there is an influx of O_2 to the liquid), while q_{pm}^T is negative.

The amount of reaction species k and m transferred between the gas and the liquid phase can be determined from a mass balance *on the gas phase*.

$$q_{sk}^T\, V = \frac{1}{RT}(\nu_{g,f}\, \pi_{k,f} - \nu_g\, \pi_k), \quad q_{pm}^T\, V = \frac{1}{RT}(\nu_{g,f}\, \pi_{m,f} - \nu_g\, \pi_m). \tag{2.5}$$

Here, ($\nu_{g,f}$, ν_g) are the volumetric gas flows (l h^{-1}) in and out of the reactor, and ($\pi_{k,f}$, π_k) and ($\pi_{m,f}$, π_m) are the *partial pressures* of the substrate k and of the product m in the inlet and in the outlet from the reactor. $\pi_{k,f} = y_k\, P$, where y_k is the volume fraction of S_k in the gas (e.g., 0.21 mol O_2 (mol ν_{gf})$^{-1}$ for O_2 in the inlet when the reactor is sparged with air) and P is the *total pressure*. $\nu_{g,f}\, \pi_{k,f}/RT$ = mol O_2 fed to the reactor per hour, and $(1/RT)(\nu_{g,f}\, \pi_{k,f} - \nu_g\, \pi_k)$ is the moles of O_2 transported to the liquid phase. With π in atm and T in K, the value of the gas constant R is 0.08205 l atm (mol K)$^{-1}$.

The mass transfer can also be calculated by Eq. (2.6). This is an empirical relation between a driving force ($s_k^* - s_k$) and q_{sk}^T, the volumetric mass transfer. s_k is the liquid phase concentration of substrate S_k and s_k^* is the *saturation* concentration of S_k in equilibrium with a gas phase with (approximately) the partial pressure $(\pi_{k,f} + \pi_k)/2$ – or a more complicated expression for the *average* gas-phase partial pressure as explained in Chapter 11. $k_l a$ is *the volumetric mass transfer coefficient*, a first-order rate constant (unit e.g., h^{-1}) for the mass transfer process. The mass transfer coefficient depends on the *power consumption* (unit, for example, W) to mix the liquid phase.

$$q_{sk}^T = k_l a(s_k^* - s_k). \tag{2.6}$$

Combination of Eqs. (2.5) and (2.6) is one way of experimentally determining $k_l a$ by simultaneous measurement of [s_k, π_{kf}, π_k] and [ν_{gf},ν_g] at different stirring intensities of the liquid. Once an empirical relation has been set up between the power input and the rate constant $k_l a$, the relation can be used under similar conditions to predict the mass transfer q_{sk}^T for any power input.

When the rate of formation of *one* of the reaction species has been determined, then, for a *single* enzymatic reaction or fermentation process, the rates of formation for all other reaction species can be calculated via the *yield coefficients* Y_{ij}. Y_{ij} is defined as the rate of formation of component j relative to the formation of another component i. $Y_{ij} = |r_j/r_i| = |q_j/q_i|$. The symbol || is used to ensure that Y_{ij} is positive also when the numerator and denominator have different signs.

Based on the yield coefficient, any rate q_j *different* from the *key rate* q_i can be found as follows:

$$q_j = Y_{ij}\, q_i, \quad \text{and } r_j = q_j/e, \quad \text{or } q_j/x;\ Y_{ij} = \left|\frac{c_{j,\mathrm{f}} - c_j}{c_{i,\mathrm{f}} - c_i}\right|. \tag{2.7}$$

It must be emphasized that Eq. (2.7) is *only* true if the yield coefficients are constant in the range of investigation of the reaction. This is the case if the reaction is described by a single stoichiometric equation for all investigated values of the dilution rate D.

Example 2.1 Calculation of rates and stoichiometry based on the key reactant

Catalase breaks down hydrogen peroxide by the stoichiometric equation $H_2O_2 \rightarrow H_2O + ½O_2$. This is the *only* overall stoichiometric equation by which O_2 is formed from H_2O_2. *The kinetic mechanism* of the reaction is, however, not at all revealed from the overall stoichiometry. Similarly, lactobionic acid (lba) is produced from lactose in the reaction: lactose $+ O_2 +$ $H_2O \rightarrow$ lba $+ H_2O_2$ catalyzed by a carbohydrate oxidase, a reaction discussed further in Example 7.4. In the first reaction, $Y_{H_2O_2,O_2} = 1/2$ mol/mol, and in the second $Y_{\mathrm{lactose},O_2} = Y_{\mathrm{lactose},H_2O_2} = Y_{\mathrm{lactose,lab}} = 1$ mol/mol. The production of lactobionic acid is $q_{\mathrm{lba}} = Y_{\mathrm{lac,lba}}\ (-q_{\mathrm{lactose}})$ or $D\ (c_{\mathrm{lba}} - c_{\mathrm{lba,f}}) = Y_{\mathrm{lac,lba}}\ (c_{\mathrm{lactose,f}} - c_{\mathrm{lactose}})$. The last relation shows that the effluent concentration of lactobionic acid (and of H_2O_2) can be calculated from $[c_{\mathrm{lactose,f}}, c_{\mathrm{lactose}}]$.

In enzymatically catalyzed reactions, the yield coefficients Y_{ij} are true stoichiometric coefficients, exact numbers just as in conversion of N_2 and H_2 to NH_3 by the stoichiometry $N_2 + 3\,H_2 \rightarrow 2\ NH_3$. This is also the case for reactions involving living cells if the same stoichiometry holds for all operational conditions, for example, for all permissible D values between 0 and $D_{\max}$. At $D_{\max} > v_{\max}/V$, the flow through the reactor is larger than the maximum production rate of cells. The culture washes out and steady-state operation cannot be maintained. In the stoichiometry of fermentation reactions, the coefficients are empirical numbers that can be interpreted when, as in Chapter 4, a good metabolic model is constructed for the reaction. Thus, in anaerobic fermentation with the yeast *Saccharomyces cerevisiae*, the substrate, glucose, is converted to ethanol, glycerol, CO_2, and more biomass. When the elementary composition of *S. cerevisiae* is given by $X = CH_{1.74}O_{0.6}N_{0.12}$, one obtains the following equation [1]:

$$\begin{aligned} CH_2O + 0.01658\ NH_3 \rightarrow\ & 0.1381\ X + 0.5160\ CH_3O_{0.5} + 0.2759\ CO_2 \\ & + 0.070\ CH_{8/3}O + 0.037\ H_2O. \end{aligned} \tag{1}$$

In Eq. (1) all carbon-containing compounds are normalized to contain 1 C-mol carbon. This leads to somewhat unusual formulas for ethanol

($CH_3O_{0.5}$), glycerol ($CH_{8/3}O$), and glucose (CH_2O), but the nomenclature is useful in quantitative work since the basis is always 12 g carbon, whereas molecules such as $C_6H_{12}O_6$ break down to smaller molecules (such as trioses in the glycolysis pathway (see Figure 4.2) or combine to larger molecules (such as Butyryl-CoA by condensation of two molecules of Acetyl-CoA in the solvent producing pathways of *Clostridium acetobutylicum* (see Figure 4.14). The advantage of writing the fermentation stoichiometry on a C-mol basis becomes apparent in later chapters. Here, we are content to notice that the closing of the carbon balance is immediately obvious when the yield coefficients on both sides of Eq. (1) are compared: $1 = \Sigma Y_{sj}$ ($j = 1–4$). $Y_{sn} = 0.01658$ can be obtained from Y_{sx} since for each carbon in the biomass 0.12 mol N is consumed and there are no other sinks for N in the stoichiometry, that is, $Y_{xn} = 0.12$, $Y_{sn} = 0.12 \cdot 0.1381 = 0.01658$. $Y_{sw} = 0.037$ can be obtained from either an H or an O balance on the stoichiometry.

The rate of production of, for example, ethanol is related to the rate of consumption of glucose by $q_e = 0.5160$ ($-q_s$) or $c_e - c_{ef} = 0.5160$ (23/30) ($s_f - s$) g l^{-1}. Thus, if the feed contains 25 and the effluent 1.378 g glucose l^{-1}, then $c_e - c_{ef} = 9.34$ g/l. Unless the reactor considered receives its feed from a preceding reactor in a sequence of CSTRs, the feed is sterile and contains no products, [x_f, p_{jf}] = [0,0]. In that case, [x, $c_e = e$, $c_{glycerol} = g$] = [$0.1370 \cdot 25.02 \cdot 23.622/30 = 2.70$, 9.34, 1.67] g/l. In this calculation, the formula weights of [S, X, E, G] = [30, 25.02, 23, 30.33] g (C-mol)$^{-1}$ have been used to find $Y_{s,pj}$ in g g^{-1} from the stoichiometry given in Eq. (1).

Since the stoichiometry (1) is supposed to hold for all acceptable D values, one can finally calculate the reactor volume needed for a given production rate. Let the desired production rate of ethanol be 900 kg h^{-1} in an industrial reactor design based on the stoichiometry (1). Then $v = 900/9.34 = 96.4$ m^3 h^{-1}. If the process is carried out at $D = 0.32$ h^{-1}, one needs a reactor of working volume $V = 96.4/0.32 = 301$ m^3.

This last example illustrates how the so-called "black box" stoichiometric Eq. (1) can be set up based on steady-state experiments in a CSTR. To find the yield coefficients, one just needs the effluent concentrations [s, x, e, g] = [23.622, 2.70, 9.34, 1.67] calculated above. The yield coefficient for CO_2 can be found using the carbon balance, even when the CO_2 production is not measured. In fact, it will be demonstrated in Example 4.5 that with a reliable metabolic model in hand only *one* production rate, for example, q_x (or r_x), needs to be measured to find *all* the yield coefficients on the right-hand side of Eq. (1) when the substrate consumption rate is normalized to 1. Nevertheless, it is highly advisable to include all available measured data in the vector of experimental results since, as shown in Chapter 4, experimental errors can significantly distort the calculated yield coefficients if only the minimum amount of data (in the example above the effluent glucose and biomass concentrations) are used. Although the experimental work of [1] on which Eq. (1) is based is done

with the highest possible precision obtainable in a state-of-the-art laboratory bioreactor, the C balance (the author had also measured q_{CO_2}) fits to "only" 0.995. Today, one must demand that experimental data obtained in a good laboratory reactor are accurate enough to make the carbon balance close to 0.985–0.99; otherwise, the data are suspicious. The use of the carbon and a redox balance to be introduced in Chapter 3 is *either* to calculate missing yield coefficients *or* to check for inconsistencies in the experimental data. Another use of these two *fundamental* balances is illustrated in Chapter 4. There the goal is to find parameters in metabolic models, especially empirical yield coefficients in the metabolic reaction that describes biomass formation.

Example 2.2 Calculation of rates for reaction species that are both in the gas phase and in the liquid phase

It appears to be fairly simple to apply Eqs. (2.4) and (2.5) to determine the transfer rate q_i^T and thereafter the rate of production q_i of reaction species that are present both in the liquid phase and in the gas phase. Still the rates can be erroneously calculated, and in this example it will be shown how the measured data must be treated to obtain the correct rate values.

First the rates of transfer of oxygen and carbon dioxide $[q_o^T, q_c^T]$ as determined from continuous readings of the headspace molar fractions will be considered. All modern bioreactors are equipped with sufficiently good monitoring equipment for these variables, and readings are considered to be accurate to within a few percent relative. Hence, they are considered as keys to an efficient control of the reactor. For example, in aerobic cultivations *with glucose as C substrate*, the *respiratory quotient* RQ $q_c/(-q_o)$ is in the range 1.04–1.06 when no metabolic products are formed, except biomass and CO_2.

Consider the production of biomass by aerobic cultivation of the bacterium *Methylococcus capsulatus* on methanol. *M. capsulatus* can grow on both methane and methanol, and the product is sold as "single-cell protein (SCP)" as feed to a range of animals both in husbandry and in aquacultures. In this process, a valuable product (~US$ 1600 per ton) is obtained from raw materials that are available in huge quantities and with a price in the range of US$ 200 per ton based on shale gas.

A 10 l well-stirred continuous bioreactor is sparged with air (21% O_2, 79% N_2 + Ar, and 0.04% CO_2) at a feed rate of $\nu_{g,f} = 10\,l\,min^{-1}$ (1 volume gas per volume medium per minute = 1 vvm). The total pressure is 1 atm, and the temperature of the air is equal to the cultivation temperature, $T = 45\,°C$. Both the inlet and the effluent gas are free of water. In the effluent, one measures the mole fractions $[y_o, y_c] = [0.180, 0.0174]$.

The flow of air from the reactor ν_g (45 °C, 1 atm) is calculated in the following way: 100 l of $\nu_{g,f}$ contains 79 l N_2 + Ar, while 100 l of ν_g contains 100 − 18 − 1.74 l = 80.3 l of the inert gases N_2 + Ar. Since the content of

inert gases has not changed, ν_g must be smaller than $\nu_{g,f}$, and $\nu_g = 79/80.3$ $\nu_{g,f} = 0.9838$ $\nu_{g,f}$. The 10 l reactor has received $600 \cdot (0.21 - 0.18 \cdot 0.9838) =$ 19.75 l O_2 h^{-1} or $(19.75 \cdot 1$ (atm)/0.08206/318.2 (K))/10 (l) = 0.0756 mol O_2 (l h)$^{-1} = q_o^T$. $(-q_c^T) = 600 \cdot (0.0174 \cdot 0.9838 - 0.0004) \cdot 0.00383 = 0.0385$ mol CO_2 (l reactor h)$^{-1}$.

The liquid-phase concentration of oxygen is very small, both in the feed and in the effluent. From Eq. (9.2) at 45 °C and with pure O_2 in the gas phase ($\pi_{O_2} = 1$ atm), one obtains $s_o^* = 0.98 \cdot 10^{-3}$ mol (l reactor)$^{-1}$. When the gas phase is air with $\pi_{O_2} = 0.21$ atm, the saturation concentration is $s_o^* = 0.21 \cdot 0.98 \cdot 10^{-3} = 0.206 \cdot 10^{-3}$ mol l (reactor)$^{-1}$. The effluent concentration of O_2 is even smaller, perhaps 10% of s_o^*. Hence, in Eq. (2.4), the term $D(s_{o,f} - s_o)$ is several orders of magnitude smaller than q_o^T, and the rate of oxygen consumption $(-q_o)$ is very close to the experimentally determined $q_o^T = 0.0756$ mol O_2 (l reactor h)$^{-1}$. Similarly, for steady-state cultivation, the total concentration of CO_2 (as dissolved CO_2, HCO_3^-, and CO_3^{-2}) is virtually the same in the inlet and outlet, and $q_c \sim (-q_c^T) = 0.385$ mol CO_2 (l reactor h)$^{-1}$.

The accuracy of, especially, the calculation of q_o^T increases when the difference between the mole fraction in the feed gas and the exhaust gas increases. Thus, with $\nu_{g,f} = 300$ l h^{-1} (0.5 vvm), one obtains the same $q_o^T = 0.0756$ mol O_2 (l reactor h)$^{-1}$ at an exhaust O_2 mole fraction $y_o = 0.1465$. This clearly gives a higher accuracy in the determination of q_o^T, but one must not decrease $\nu_{g,f}$ too much, since the mass transfer coefficient $k_l a$ decreases with $\nu_{g,f}$, and it will eventually become difficult to transfer the required q_o^T. Also, at a low $\nu_{g,f}$ value, the oxygen in the gas bubbles can become severely diluted with a gaseous product such as CO_2, and especially if the inert content of $\nu_{g,f}$ is small as is the case when enriched air is used, the partial pressure of O_2 in the bubbles decreases when the bubbles pass through the liquid phase.

Serious errors in the determination of q_i^T can result if the content of water in $\nu_{g,f}$ differs from that of ν_g. Thus, when $\nu_{g,f}$ is bone-dry and ν_g is not adequately dried, q_o^T is severely overestimated if the water evaporated into the gas is not taken into account. At 45 °C and 1 bar, the vapor pressure of H_2O over liquid water is 71.9 mm Hg, that is, the equilibrium mole fraction of H_2O in the exhaust gas is 71.9/760 = 0.0946 bar. In the example given above with $\nu_{g,f} = 600$ l h^{-1} bone-dry air and $q_o^T = 0.0756$ mol O_2 (l reactor h)$^{-1}$, the exhaust (wet) air would be $\nu_g = 600 - 19.75 + 10.03 + 0.0946$ $\nu_g \rightarrow$ $\nu_g = 652$ l h^{-1}. The mole fraction of O_2 would be $y_o = (0.21 \cdot 600 - 19.75)/652 = 0.1630$. Thus, if one *measures* ν_g to be 652 l h^{-1}, the measured value of $y_o = 0.1630$ in the exhaust gas correctly predicts $q_o^T = 0.0756$ mol O_2 (l reactor h)$^{-1}$. If, however, one erroneously assumes that $\nu_g = 0.9838$ $\nu_{g,f} =$ 590 l h^{-1} as is the case when the exhaust air is bone-dry, then the transferred volume of O_2 would be calculated to $600 \cdot (0.21 - 0.9838 \cdot 0.1630) =$ 29.8 l h^{-1}, and q_o^T would be overestimated by a factor 29.8/19.75 = 1.5.

Another kind of error in the calculation of rates occurs if one of the products formed in the liquid phase is partly stripped to the gas phase.