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Mass Balances, Rates, and Experiments

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7.1 Introduction

Living systems, e.g., cultures of microorganisms or cells from higher organisms transform matter and energy with certain rates. It is obvious that if cultivated cells are applied in a fermentation process to produce a certain desired product that knowing these rates, and knowing how these rates can be changed is important, e.g., to increase the rate of product formation.

In industrial processes these rates determine economy, profits, investments, and equipment design (pumps, stirring power, etc.).

In natural environments conversion rates determine e.g., how fast pollutants are transported, degraded, and accumulated (leading to adverse effects). In the human body, metabolic disorders quickly result from imbalances in rates of production, rate of transport or the rate of degradation of metabolites or proteins, resulting in too high or too low levels of metabolites and proteins in certain parts of the body, usually with fatal results.

In fermentation processes cells or (micro) organisms consume the nutrients provided in the growth medium and produce different compounds with certain rates. Quantification of these rates is important to obtain insight in the profit of a biotechnological industrial process, to understand how long it will take to remove an undesirable compound from a polluted site by using microorganisms. Moreover, it is important to understand how a cell/microorganism changes its rates (e.g., growth rate, its substrate uptake rate, or its product formation rate) upon a change in its extra-cellular environment. Thereby the environment of the cell is defined as temperature, pH, concentration of substrate, product, etc. outside

the cell membrane. The study of how to change rates is called kinetics, and for such studies one needs methods to define and to quantify rates.

Here the attention will be on the three most important rates (growth, substrate uptake, and product formation). Other rates will be dealt with later.

7.2 Rates and Mass Balances

As shown in Chapter 6, the synthesis of a certain amount of biomass and/or product requires the consumption of nutrients of which the most important ones are the C-source, an electron donor, an electron acceptor, and sources of nitrogen, phosphorus, and sulfur. This results in the production and subsequent excretion by the cells of various catabolic (H_2O, CO_2, N_2, and others) and noncatabolic products.

In a fermentation process each of these compounds is consumed or produced with a certain rate. These rates are usually expressed as mol/time, e.g., glucose consumption rate in mol glucose/hour, oxygen consumption rate in mol O_2/hour, biomass growth rate in C-mol biomass per hour etc. As indicated above it is important to obtain the values of these rates as they occur in fermentation processes, in nature, in our experiments.

One might think that these rates can be measured using a certain sensor analogous to, a thermometer to measure temperature, or a pH-electrode to measure the pH, etc. It should be clear, however, that sensors to measure a rate of production or consumption do not exist because this is fundamentally impossible. A rate must be calculated using the proper mass balance in combination with proper measurements of flow rates, volumes, concentrations, and time.

7.2.1 Definition of the Mass Balance

For each compound i present in a certain system a mass balance can be formulated. This means that there are as many mass balances to be formulated for a certain system as there are chemical compounds present in this system. However, each of these individual mass balances has the same general structure:

$$\begin{aligned}\text{Accumulation of compound i} = {} & \text{Rate}_i\,(\text{production (+) consumption (–) of compound i}) \\ & + \text{Rate of all in/out transport processes of compound i}\end{aligned}$$

It is important to note that this is a balance of rates and that each term in this balance has the same dimension: amount of compound i/hour.

The most practical choice of the unit used to express the amount of compound i is mol of compound i, hence the dimension of the rate will be mol of compound i/time. However, one can also make other choices as kg of compound i/time, etc.

It is also obvious that the mass balance of compound i allows us to calculate Rate_i of production (or consumption) of compound i if we can quantify the other terms in the mass balance (the accumulation term and the transport terms) using experimental measurements. The mass balance immediately shows which measurements (flows, volumes, concentrations) are needed to obtain Rate_i.

7.2.2 The System Boundary

Before a mass balance can be formulated the system boundary needs to be defined. System boundaries are usually chosen in a practical way. If, e.g., one is interested in uptake rates of cells present in the liquid broth inside a fermentor the logical boundary is the boundary of the liquid phase. However, if one is interested in the transfer of O_2 from the gas phase to the liquid phase, both the gas and the liquid phase have to be taken into account. The choice of the system boundary is strongly dependent on the system at

hand. Obvious boundaries are e.g., different phases (gas/liquid/solid) or compartments. When defining the system for which mass balances are to be formulated the following has to be defined:

The system volume
The system boundaries across which transport occurs
The concentrations of the relevant compounds inside the system

7.2.3 Setting up the Mass Balances: Some Guidelines

For a process involving many chemical compounds and many phases or compartments, many mass balances have to be defined.

In principle one mass balance for each compound in each phase can be formulated. Each compound has also its own concentration in each compartment or phase. Furthermore accurate information on the transport processes which operate on each compound i in each phase is needed in order to quantify the transport terms in each balance. The most common balances are those for substrate, biomass, and product in the liquid phase (broth) of the fermentor. The following example shows how to set up these balances.

Example 1: A Proper Mass Balance for NH_3

Consider the following process whereby one is interested in the fate of NH_3. It is known that: a liquid feed containing NH_3 enters the reactor, no liquid leaves the reactor and air is sparged through the reactor. The liquid volume V_L in the reactor changes due to liquid feed and water evaporation. The NH_3 is present in the liquid phase in the reactor and is consumed by a nitrifying organism. Furthermore NH_3 is also present in the reactor gas phase due to transport of NH_3 between gas and liquid phase.

Question: Which balances are needed?
Answer: NH_3 is present in two phases. Hence we need to formulate two balances:

- The NH_3-balance for the liquid phase
- The NH_3-balance for the gas phase

Question: Which terms should be present in each balance?
Answer: For the NH_3-balance in the liquid phase we need:

- The dynamic term: $d(V_L C_{nL})/dt$, where V_L is the volume of the liquid phase and C_{nL} the concentration of NH_3 in the liquid phase
- The rate of conversion of NH_3 by the nitrifier organism, $Rate_{NH3}$ in mol NH_3/h
- The rate of transport of NH_3 from the liquid to the gas phase, a transport process called "stripping"
- The rate of transport of NH_3 with the liquid feed to the reactor

For the NH_3-balance in the gas phase we need the next contributions:

- $d(V_G C_{nG})/dt$, which is the dynamic term (with volume of the gas phase V_G and, the concentration of NH_3 in the gas phase C_{nG})
- There is no conversion term needed because there are no microorganisms in the gas phase
- There is transfer from liquid to gas phase
- There is transport of NH_3 out of the gas phase by the gas flow that leaves the reactor

In order to set up the proper balances for compound i it is necessary to identify all the transport processes and reactions which operate on molecule i present within the system boundary. If one transport process is forgotten (e.g., the stripping of NH_3 from the liquid phase to the gas phase) then the calculated $Rate_{NH3}$ will be erroneous.

A good process is like a good theater play

One can envisage a process as a theater performance where actors (= compounds) perform on a theater stage (= process) according to a script (= the specification of each interaction between the compounds in the form of the transport and conversion mechanisms with their kinetics). The process designer is of course the play's director.

This shows that for each particular process we need to think creatively, using process knowledge. The process knowledge relates to:

- Which compounds are relevant in the process (The Actors).
- Where are these compounds localized in the process (The Theater stage) (e.g., in only one phase or in more phases or in a compartment of a reactor or in a compartment, e.g., mitochondrion, within the cells). For this we need to know the physical structures of the process (vessels, compartments in the vessel, phases in the vessel, compartments in microorganisms) based on own visual knowledge (or, undesirable, hear say?). Based on this information subsystems are defined (e.g., a gas phase, a liquid phase, a solid phase, cytosolic and mitochondrial compartments in cells, etc.).
- Which mechanisms (transport, conversion, or both) act on each compound i in each subsystem and how are their kinetics. Subsequently we describe how each compound i takes part in and interacts with the whole process (The Script).
- A process designer is the director of the process performance.

7.2.4 The Calculation of Conversion Rates from a Batch Experiment

A usual task is to cultivate cells/organisms and to study e.g., the rate of growth or product formation and/or other rates. The experimenter is usually free to choose the method of cultivation. This choice is called "experimental design." The most simple cultivation method is called the "batch cultivation," which can be executed in microtiter or deep well plates, shake flasks, bags, stirred vessels (fermentors), or other devices.

The characteristic of a true batch cultivation is that for all compounds which are uniquely present in the liquid phase no mechanism is present to transport the compounds into or from the cultivation vessel: for these compounds transport is absent!!

Batch cultivation and culture volume

Traditionally, if one speaks of batch experiments this is usually assumed to imply that the cultivation volume is constant. In practice a constant volume can seldom be realized. Reactions lead in principle to changes in densities. Also often (unnoticed) losses occur due to evaporation or additions are applied (e.g., for pH control). Therefore a constant volume does not characterize a batch condition. The key is absence of transport which must be specified for each molecule. E.g., in an aerated batch fermentation the batch condition applies for substrate, but not for O_2 and CO_2. If pH is controlled with NH_4OH solution the batch condition also does not apply to NH_4^+. Alternatively if the pH is controlled with NaOH the batch condition does apply for NH_4^+!!

This shows that in a batch experiment for some compounds the mass balances relate to batch conditions, but for other compounds batch condition does not apply!!

The mass balance for such "batch-compounds" is then simplified (because the transport terms in and out become zero) and is written as:

$$\text{Accumulation of compound i} = \text{Rate}_i$$

Clearly the conversion rate of compound i (Rate_i) can be obtained from the mass balance if we are able to experimentally quantify the accumulation term. The accumulation term is the change in time of the total amount (mol) of i (present within the boundary, e.g., in the cultivation vessel). It is by definition mathematically expressed as

$$\text{Accumulation of compound i} = \frac{d(V \cdot C_i)}{dt} \tag{7.1}$$

Here V is the volume of broth (m^3) in which compound i is present and C_i is the concentration of compound i in this broth (mol i/m^3 broth). Here it is useful to introduce $M_i = V \cdot C_i$, with M_i being the total amount (mol) of compound i present in the system).

For the batch conditions $Rate_i$ now follows from the mass balance for compound i:

$$\text{Rate i} = \frac{d(V \cdot C_i)}{dt} = \frac{dM_i}{dt} \tag{7.2}$$

Note that by definition the conversion rate, $Rate_i$ is positive for a produced compound and negative for a consumed compound.

It has now become clear that in order to obtain the conversion rate of a batch compound we have to measure V and C_i as function of time to obtain $M_i(t) = V_i(t) \cdot C_i(t)$ which is the changing amount of i in the cultivation vessel as function of time. The $M_i(t)$-values can then be plotted as function of time and then the slope $dM_i(t)/dt$ can be calculated. This slope equals $Rate_i$ according to the mass balance for compound i. Usually the slope at time t can be calculated from two values of M_i at two close time points according to:

$$\frac{dM_i}{dt} = \frac{d(V \cdot C_i)}{dt} = \frac{(V \cdot C_i)_{t_2} - (V \cdot C_i)_{t_1}}{t_2 - t_1} = \frac{M_i(t_2) - M_i(t_1)}{t_2 - t_1} = \text{Rate}_i \text{ (between } t_2 \text{ and } t_1) \tag{7.3}$$

This is the most straightforward and simple method to obtain the slope dM_i/dt, but more advanced methods, involving curve fitting through the experimental values of M_i are also possible. It should furthermore be clear that in batch cultivations $Rate_i$ is usually a function of time and cannot be considered as constant!!

The example below shows the application of the mass balances in combination with experimental measurements to obtain the proper values of $Rate_i$ as a function of time from a batch experiment.

Example 2: Batch Fermentation: Mass Balances and Rates

An organism is grown on a cultivation medium containing glucose as the carbon source in a batch fermentor. The broth volume (V), glucose concentration (C_s), and biomass concentration (C_x) are measured as function of time (Table 7.1). We observe that the volume decreases, which is due to the evaporation of water because of air sparging. The evaporation rate is 2 liters of water/hour.

Question: Explain why between 0 and 4 hours an increasing substrate concentration is observed, in spite of the fact that biomass growth occurs, which can be inferred from the increasing biomass concentration.

Answer: This is due to the volume change (see note below!!) from 0.100 m^3 to 0.092 m^3.

TABLE 7.1 Experimental Results from a Batch Experiment

Time (hour)	V (m^3)	C_s (mol Glucose/m^3)	C_x (C-mol Biomass/m^3)
0	0.100	100.000	100.00
1	0.098	100.296	107.27
2	0.096	100.510	115.125
4	0.092	100.674	132.761
8	0.084	99.536	177.595
16	0.068	86.985	327.279

Question: Calculate the total rates of substrate consumption $Rate_s$ and biomass production $Rate_x$ (in mol/h and C-mol/h) as function of time.

Answer: We need to use the mass balance for glucose to obtain $Rate_s$ and the biomass mass balance to obtain $Rate_x$.

Mass balance for substrate (glucose) in batch:

$$\frac{d(V \cdot C_s)}{dt} = \frac{dM_s}{dt} = Rate_s \tag{7.4}$$

Mass balance for biomass in batch:

$$\frac{d(V \cdot C_x)}{dt} = \frac{dM_x}{dt} = Rate_x \tag{7.5}$$

The first thing that needs to be done is to calculate M_s and M_x (from the available volume and concentration measurements) to obtain the change of the total amounts of glucose and biomass in time. The results are shown in Table 7.2.

From the calculated total amounts shown in Table 7.2 it is now clear that there is indeed growth at the expense of glucose (M_x increases, M_s decreases) as has to be expected. It is also clear that M_s and M_x do not change linearly in time, because their slope increases.

According to the mass balance the $Rate_i$ is the slope of the curve of M_i versus time. Ideally one would need to obtain a continuous interpolation function through the M_i-time points and then obtain the slope as a function of time from the derivative of this function. For reasons of simplicity we can approximate the slope by using the M_i vs. time data points and the definition of the slope of the M_i-time curve in the time interval t_1–t_2.

$$\frac{dM_i}{dt} = \frac{M_i(t_2) - M_i(t_1)}{t_2 - t_1} \tag{7.6}$$

For the available time intervals we then obtain the values (see Table 7.3) for the slopes at different time intervals (which are equal to the average $Rate_i$ in the respective time interval).

TABLE 7.2 Calculated Total Amounts of Glucose and Biomass in Time from the Data of Table 7.1

Time (hour)	$M_s(= VC_s)$ (mol Glucose)	$M_x(= VC_x)$ (C-molX)
0	10.000	10.000
1	9.829	10.513
2	9.649	11.052
4	9.262	12.214
8	8.361	14.918
16	5.915	22.255

TABLE 7.3 Calculated Conversion Rates of Glucose and Biomass Using the Data from Table 7.2

Time Interval (t_1–t_2)	dM_s/dt (= $Rate_s(t)$) (mol S/Hour)	dM_x/dt (= $Rate_x(t)$) (MolX/Hour)
0–1 hour	−0.171	+ 0.513
1–2 hour	−0.180	+ 0.539
2–4 hour	−0.194	+ 0.581
4–8 hour	−0.225	+ 0.676
8–16 hour	−0.306	+ 0.917

From the calculated conversion rates it is clear that:

- $Rate_s$, being the substrate conversion is negative, which is logical because substrate is consumed.
- The rate of substrate consumption $Rate_s(t)$ and for biomass production $Rate_x(t)$ are not constant in time.
- The absolute values of both rates increase, while the substrate concentration decreases. At first sight the increase in $Rate_s(t)$ with a decrease in $C_s(t)$ is counter intuitive!

The explanation for this will follow below, when we introduce the biomass specific conversion rates (q-rates).

A correct calculation of $Rate_x$ and $Rate_s$ depends on a correct evaluation of the accumulation term in their respective mass balances from the available experimental data. The note below shows that this is not a trivial task.

Mistakes in evaluating the accumulation term of a mass balance

The accumulation term of compound i in the mass balance for i can be expanded as:

$$\frac{d(V \cdot C_i)}{dt} = \frac{V \cdot dC_i}{dt} + C_i \cdot \frac{dV}{dt} \tag{7.7}$$

This shows that a change in the amount of molecules of compound i present in the growth vessel has two contributions.

1. A change due to the change of the concentration of i: $V \cdot dC_i/dt$
2. A change due to a change of the culture volume: $C_i \cdot dV/dt$

An often-made mistake is that only concentrations are considered and changes in volume are neglected. For example when C_i is measured to be constant, and thus $dC_i/dt = 0$, the wrong conclusion is drawn that $Rate_i = 0$. However, it should be kept in mind that this is only true when the culture volume does not change in time. If this is not the case, i.e., the volume does change in time the change in the amount $V \cdot C_i (= M_i)$ is always needed, which requires to use the measured changes in both V and C_i.

A second mistake is related to the volume which should be used. Also this is not trivial. Suppose the concentration C_i is obtained by taking a broth sample. First the biomass is removed, e.g., by centrifugation, to obtain a stable supernatant (because the biomass present in the broth sample would otherwise continue to consume and produce compounds, which would change the concentrations which have to be measured). Subsequently the concentration of the compound of interest is measured in the supernatant. The total amount present in the broth sample can now be found by multiplying the measured C_i value (mol i/m^3 supernatant) with the volume of the supernatant. It should be realized that this is not the same as the volume of the broth sample (which is supernatant + wet biomass). The difference depends on the amount of biomass present in the broth, and can easily be 5–20%!!

In the above approach it is assumed that compound i is only present in the supernatant and not inside the biomass. This is not always so. It may well be that there is a significant amount of product present inside the biomass. Only when there is complete secretion of the product from the cells by means of active transport it can be expected that its concentration inside the biomass is neglibly low and may be neglected. In all other situations the amount of product in the biomass must be quantified separately.

The accumulation term for product i now becomes:

$$\frac{dM_i}{dt} = \frac{d(M_x \cdot X_i)}{dt} + \frac{d(V_{sup} C_{i,sup})}{dt} = Rate_i \tag{7.8}$$

Here M_x is the amount of biomass in the vessel (C-molX) and X_i the amount of product i present inside in the biomass (mol i/C-molX).

If the product is also present in significant amounts inside the cells we thus need to carry out additional measurements to obtain the correct value for $Rate_i$.

7.3 Biomass Specific Conversion Rates (q-Rates)

In the example in the previous section we have seen how, for a batch cultivation, $Rate_s$ and $Rate_x$ of substrate consumption and biomass production (all in mol i/hour) can be obtained from the mass balances for substrate and biomass using volume and concentration measurements as function of time.

The obtained results, however, merit some thought. It was shown in the example above that the glucose consumption ($Rate_s$) increases. This seems strange; as at first glance one would expect that the consumption rate of glucose would decrease when the glucose concentration decreases. The explanation is that the glucose is consumed by the cells and that, because the amount of cells ($V \cdot C_x = M_x$) increases, the total rate of glucose consumption will increase. This shows that apart from the $Rate_i$, which is a total rate, a second type of rate is relevant, which is the biomass specific rate q_i which is defined as:

$$q_i = \frac{Rate_i}{M_x}\left(\frac{\text{mol i/h consumed or produced}}{\text{C-molX present in the cultivation vessel}}\right) \tag{7.9}$$

The q-rate is obtained from the total production or consumption rate ($Rate_i$) by dividing with the total amount of biomass ($V \cdot C_x = M_x$) present in the culture vessel. It should be realized that because these q-rates are expressed per amount of biomass they characterize the activity of the cells.

The q_i-rates are influenced by:

- The properties of the cells (as a result of gene expression)
- The environment of the cells as represented by the extra-cellular concentrations such as glucose, O_2, CO_2, pH, T, pressure, and other compounds present in the growth medium (vitamins, trace elements, ….)

It is clear from the above definition that to obtain the value of q_i both the total $Rate_i$ as well as the total amount of biomass M_x present in the cultivation vessel are required. The dimension of the q_i-rates is (mol i/h)/C-molX. A special q-rate is q_x which is the rate of newly produced biomass per hour per amount of biomass present in the cultivation vessel. For historical reasons this rate is called μ and not q_x (which would be logical)

$$\mu\,(=q_x) = \frac{Rate_x}{M_x}\left(\frac{\text{C-molX/h which is produced}}{\text{C-molX present in the cultivation vessel}}\right) \tag{7.10}$$

Example 3: Calculation of q-Rates from the Batch Experiment

In the previous example on the batch experiment we have applied the mass balances for substrate and biomass to calculate the total rates of produced biomass ($Rate_x$) and consumed glucose ($Rate_s$). The mass balances showed that, for a batch cultivation, these rates are equal to the time derivative:

$$\frac{d(V \cdot C_i)}{dt}\left(= \frac{dM_i}{dt}\right) \tag{7.11}$$

These time derivatives were quantified from the experimentally obtained ($V \cdot C_i$) values as function of time for the time intervals between the measurements. $Rate_s$ and $Rate_x$ were therefore expressed as time-interval average rates which in practice slightly change in time within the considered time interval.

To obtain q-rates one must divide the total $Rate_i$ by the total biomass M_x present in the time interval. However within a time interval M_x increases. A logical proposal for M_x in the interval is then to calculate the average value of M_x in the interval. The results for the calculated rates and average M_x in the time intervals of the batch experiment are shown in Table 7.4.

TABLE 7.4 Calculated Total and Biomass Specific Conversion Rates for the Batch Experiment

Time Interval (hour)	$Rate_s$ (molS/h)	$Rate_x$ (C-molX/h)	Average M_x (C-molX)	q_s (mol Glucose/h C-molX)	μ (C-molX/h C-molX)
0–1	−0.171	+ 0.513	10.256	−0.0167	+ 0.050
1–2	−0.180	+ 0.539	10.783	−0.0167	+ 0.050
2–4	−0.194	+ 0.581	11.633	−0.0167	+ 0.050
4–8	−0.225	+ 0.676	13.566	−0.0166	+ 0.050
8–16	−0.306	+ 0.917	18.587	−0.0165	+ 0.049

The average values for $Rate_s$, $Rate_x$, and M_x in each time interval directly allow to calculate q_s and μ. The result is quite revealing, it shows that the biomass specific rates q_s and μ are constant. This result is often found in batch experiments where organisms achieve their maximal q-rates which are constant due to the high concentrations of e.g., substrate (unlimited growth).

A third way to express conversion rates, r_i

Until now we have introduced two ways to express conversion rates:

$$Rate_i = \text{total rate of compound i in mol i/hour}$$

$$q_i = \text{the biomass specific rate} = Rate_i/M_x$$

In economic considerations the investment of equipment is related to the total volume (V) of the broth in the cultivation vessels. From this point of view it is important to have information of the volume specific rate which is named r_i.

$$r_i = (Rate_i)/V \text{ (in amount of i/hour per m}^3 \text{ broth)} \tag{7.12}$$

Comparing the definition of q_i and r_i it follows that (eliminate $Rate_i$)

$$r_i = q_i \cdot C_x \tag{7.13}$$

Hence the volume specific rate r_i and the biomass specific rate q_i of compound i are coupled through the biomass concentration C_x.

7.4 Mathematical Models for the Batch Experiment from Mass Balances and q-Based Kinetics: The Modeling Cycle

In the previous example of a batch experiment we have seen that in batch the q-rates of the organisms are constant and are not influenced by changing extracellular substrate concentrations. This leads to very simple rate functions, also called "q-based kinetics":

$$q_s = q_s^{max}$$

and

$$\mu = \mu^{max}$$

Here q_s^{max} and μ^{max} are kinetic parameters, which have a certain value for a particular organism growing under particular conditions and which must always be obtained from experiments. The above kinetics are the simplest possible (0-order). Let us now assume that for a certain organism the values for q_s^{max} and μ^{max} are known. This essentially means that the q-rate based kinetics are known as well.

If we combine this kinetic knowledge with the mass balances we already used before, then the mass balances allow us to calculate the expected total amount $M_i(t)$ of compound i as function of time.

It should be noted that the mass balances wherein the specified q-rate functions are substituted are sets of differential equations which can be solved. In fact this set of differential equations can be considered to be a mathematical model of the system (batch fermentor, shake flask, etc.).

It should furthermore be noted that:

- If the q-rate functions are not known a combination of experimental measurements of concentrations and volumes with the proper mass balances is needed to calculate values of q_i, which leads to q-based kinetic functions.
- If we do know the q-rate functions we can combine these functions with the mass balances, subsequently integrate the obtained differential equations and in this way calculate the change of the total amount M_i of each compound as function of time, which we can compare to available measurements.

Clearly the mass balance allows us to always obtain the missing quantity!! (q_i or $M_i(t)$)

The above approach is called the modeling cycle which is summarized below.

Step 1 (exp. design)	Perform a cultivation experiment and measure the concentrations of the relevant compounds and volumes (flow rates) as a function of time.
Step 2 (evaluation)	Use the mass balances to quantify the q-value of each compound under different conditions (q-relations).
Step 3 (kinetics)	Use the obtained q-values to construct kinetic functions for each biomass specific conversion rate q, as a function of extra-cellular conditions (pH, T, concentration).
Step 4 (validation)	Combine, for each relevant compound, the obtained kinetic function with the corresponding mass balance. The resulting set of differential equations can then be integrated (in most cases numerically) to obtain the predicted amounts M_i of these compounds as function of time. These results can be compared with the experimental measurements (to validate the kinetic functions).
Step 5 (prediction)	The obtained kinetic functions can also be used to model the cultivation process for different cultivation systems, leading to different mass balances (because of different transport conditions), to predict the expected M_i vs. time.
Step 6 (correction)	If the model predictions obtained during step 4 and 5 do significantly deviate from the measurements one or more kinetic functions are probably not correct (or missing) and a return to step 3 is needed to modify the kinetic functions.

The combination of the full set of mass balances with the kinetic functions does represent a complete mathematical model in the form of a set of ordinary differential equations.

Such a mathematical model contains different variables which can be grouped into two main categories:

Dependent variables

Examples are: V, C_s, C_x, C_p. The broth volume V follows from a total mass balance for the system, all concentrations of the different compounds present in the cultivation system, C_i, follow from the mass balances.

Independent variables

These are variables which can be manipulated by the experimentor or the process operator such as inflow rate of the feed medium (pump adjustment), the inoculum size of the biomass, the substrate concentration of the feed medium, the pH, the total reactor volume, the reactor temperature, etc.

The model furthermore contains parameters, e.g., the kinetic parameters of the rate equations. For the example of the batch cultivation shown above these are e.g., the maximum specific growth rate μ^{max} and maximum specific substrate uptake rate q_s^{max}.

Mathematical models are highly relevant in interpreting and designing biological processes as will be illustrated below.

7.4.1 A Batch Model for Growth and Product Formation

In the previous section a batch experiment was described and it was shown how mass balances and experimental information should be combined to obtain the values for the specific growth rate μ and the specific substrate uptake rate q_s. For this example the following rate functions apply for biomass growth and substrate uptake:

$$\mu = +0.050 \frac{\text{C-molX/h}}{\text{C-molX}} = \mu^{max} \tag{7.14}$$

$$q_s = -0.0167 \frac{\text{mol glucose/h}}{\text{C-molX}} = q_s^{max} \tag{7.15}$$

Knowing these rate functions does allow us to set-up the mathematical model to calculate the substrate concentration C_s and the biomass concentration C_x as a function of time by setting up the mass balances for substrate and biomass.

For a batch culture these mass balances only contain the accumulation terms ($d(V \cdot C_i)/dt$) and the total rate term for production and consumption ($Rate_i = q_i V \cdot C_x$), because in a batch culture no transport for substrate or biomass to or from the culture system occurs.

Mass balance for biomass (batch culture)

$$\frac{d(V \cdot C_x)}{dt} = \mu (V \cdot C_x) \tag{7.16}$$

Mass balance for substrate (batch culture)

$$\frac{d(V \cdot C_s)}{dt} = q_s (V \cdot C_x) \tag{7.17}$$

These mass-balances represent differential equations which need to be solved to obtain C_s and C_x as a function of time.

To achieve this:

- We need to know how V changes in time, e.g., due to evaporation.
- We need to know how μ and q_s change in time, especially because we expect that these rates will change at some point, because C_s decreases with time and will ultimately reach a value of zero.

It is therefore logical to expect that C_s has an effect on the substrate uptake rate q_s. However, at high substrate concentrations (which occur in batch experiments) it has been shown above that these rate functions are very simple (zero order kinetics), that is $\mu = \mu^{max}$ and $q_s = q_s^{max}$.

In addition it is useful to define two new variables:

$$M_x = V \cdot C_x \tag{7.18}$$

$$M_s = V \cdot C_s \tag{7.19}$$

M_x and M_s represent the total amounts of biomass and substrate present in the batch vessel at any time. It should be realized that these total amounts are time dependent in a nonlinear fashion, which will be shown below.

Let us first consider the biomass mass balance replacing $(V \cdot C_x)$ by M_x:

$$\frac{dM_x}{dt} = \mu^{max} \cdot M_x \tag{7.20}$$

Now the biomass mass balance can be solved by separation of variables.

$$\frac{dM_x}{M_x} = \mu^{max} dt \tag{7.21}$$

Integration of both sides (using the relation $\frac{dM_x}{M_x} = d\ln M_x$) leads to:

$$\text{In } (M_x) = \mu^{max} \cdot t + (\text{constant}) \tag{7.22}$$

We know that at time t = 0 there is an amount of biomass, called M_{xo}, present in the batch (which was added during the inocculation procedure). This gives:

$$\ln M_{xo} = \mu^{max*}0 + (\text{constant}), \text{ and thus } (\text{constant}) = \ln M_{xo}. \tag{7.23}$$

which yields:

$$M_x(t) = M_{xo} \text{ exponent}(\mu^{max} t) \tag{7.24}$$

The last relation shows that the total amount of biomass M_x in the batch culture changes exponentially in time. This relation is also called the exponential growth relation.

If one is interested in the change of the biomass concentration in time, $C_x(t)$, this follows by dividing $M_x(t)$ by the culture volume, i.e.,:

$$C_x(t) = \frac{M_x(t)}{V(t)} \tag{7.25}$$

So in order to calculate the biomass concentration as a function of time we need to know if, and if yes, how V(t) changes in time.

When the volume V does not change in time, hence $V = V_0$ (= volume at time 0) = constant we can write

$$C_x(t) = \frac{M_x(t)}{V_0} \tag{7.26}$$

Because also $M_{xo} = V_o \cdot C_{xo}$ the exponential growth relation can be rewritten as:

$$C_x(t) = C_{xo} \exp(\mu^{max} t) \qquad (\text{if V = constant}) \tag{7.27}$$

From this relation it follows that the biomass concentration changes exponentially in time (note that this only happens at constant volume).

However, for the batch culture example shown earlier the volume did change in time (decrease due to evaporation) according to:

$$V(t) = V_o - 0.0020 \cdot t \tag{7.28}$$

Whereby V_o is the initial volume (0.1 m^3) and the rate of evaporation is 0.0020 m^3/h (2 liter water per hour). By substituting this relation in the exponential growth function the following expression for the biomass concentration as a function of time is obtained:

$$C_x(t) = \frac{V_o \cdot C_{xo} \cdot \exp(\mu^{max} \cdot t)}{V_o - 0.0020 \cdot t)} \tag{7.29}$$

This relation shows that C_x increases in time, and even faster than exponential due to the decrease of the culture volume. If the volume V(t) increases in time (e.g., addition of pH control agent) then $C_x(t)$ increases less than exponentially!!

Let us now consider the substrate mass balance:

$$\frac{dM_s}{dt} = q_s^{max} \cdot M_x \tag{7.30}$$

In this differential equation q_s^{max} is a parameter with a fixed value and M_x changes in time. However, the change of the biomass concentration as a function of time, $M_x(t)$, has already been obtained from the mass balance for biomass. Substitution of this result gives:

$$\frac{dM_s}{dt} = q_s^{max} \cdot M_{xo} \cdot \exp(\mu^{max} \cdot t) \tag{7.31}$$

Separation of variables (M_s and time) yields:

$$dM_s = (q_s^{max} \cdot M_{xo}) \cdot [\exp(\mu^{max} \cdot t)dt] \tag{7.32}$$

We can rewrite the term between the square brackets by bringing the exponent behind the differential according to:

$$(\exp(\alpha t)\, dt = (1/\alpha)d(\exp \alpha t))] \tag{7.33}$$

This yields:

$$dM_s = \left(\frac{q_s^{max} \cdot M_{x0}}{\mu^{max}} \right) \cdot d\exp(\mu^{max} \cdot t) \tag{7.34}$$

Integration between time t = 0 and t = t, and defining that at time t = 0, $M_s = M_{so}$ and at time t the total substrate mass equals $M_s(t)$ yields:

$$M_s(t) - M_{s0} = \left(\frac{q_s^{max} \cdot M_{x0}}{\mu^{max}} \right) \cdot [\exp(\mu^{max} \cdot t) - 1] \tag{7.35}$$

This equation describes the course of the total amount of substrate as a function of time, $M_s(t)$.

A few comments should be made here:

- It should be realized that q_s^{max} is a negative number, hence $M_s(t)$ decreases in time!!

If the values of the kinetic parameters (μ^{max}, q_s^{max}) as well as the experimental conditions at time t = 0 (M_{so}, M_{xo}, V_o) are known this relation yields an expression for the total substrate mass as a function of time.

The substrate concentration as function of time follows from:

$$C_s(t) = M_s(t)/V(t) \tag{7.36}$$

Substitution of the function for V(t) given earlier (Equation 7.28) shows that $C_s(t)$ depends on time in a complicated way.

7.4.2 A Simple Relation to Obtain q_s^{max}

The obtained $M_s(t)$ relation (Equation 7.35) contains μ^{max} and q_s^{max} as unknown kinetic parameters. μ^{max} has already been obtained from plotting ln (M_x/M_{xo}) vs time. q_s^{max} can be obtained from the $M_s(t)$ equation by using measured values of $M_s(t)$, M_{xo}, M_{so}, and the μ^{max} value. A more elegant approach is found by rewriting the $M_s(t)$ relation (Equation 7.35) yielding.

$$M_s(t) - M_{so} = \frac{q_s^{max}}{\mu^{max}} \cdot \left[M_{xo} \cdot \exp(\mu^{max} \cdot t) - M_{xo} \right] \tag{7.37}$$

The term $M_{xo} \cdot \exp(\mu^{max} \cdot t)$ was already found to equal $M_x(t)$. Elimination of this term yields:

$$M_s(t) - M_{so} = \frac{q_s^{max}}{\mu^{max}} \cdot \left(M_x(t) - M_{xo} \right) \tag{7.38}$$

This relation shows that the plot of $M_s(t)-M_{so}$ vs $M_x(t)-M_{xo}$) is a straight line, the slope of which equals q_s^{max}/μ^{max}. Given μ^{max}, this slope yields directly q_s^{max}.

7.4.3 Application of the Mathematical Model for Batch Growth for the Estimation of Kinetic Parameters and the Duration of the Batch Culture

Above a mathematical model (based on mass balances and q-based kinetic functions) which describes a batch fermentation has been presented. It has been shown that the obtained mathematical relations:

- Give the expected biomass and substrate amount as function of time when the kinetic parameters (μ^{max}, q_s^{max}) are known.
- Can be used, together with experimental measurements, to obtain q_s^{max} and μ^{max}-values. This is an equivalent method to the basic method applied earlier, whereby no assumptions were made on μ and q_s and it was found afterward that they were constant. The mathematical model described above assumes *a priori* that μ and q_s are constant (μ^{max}, q_s^{max}).

7.4.3.1 Evaluation of μ^{max} from Experiments Using the Model

Knowing that in a batch cultivation (where the during most of the time the substrate concentration is high) $\mu = \mu^{max}$ allows us to use the biomass mass balance and solve the differential equation leading to the exponential growth relation for $M_x(t)$.

If it is expected that the zero order rate function ($\mu = \mu^{max}$ = constant) applies then the equation

$$\ln(M_x/M_{xo}) = \mu^{max}\, t$$

can directly be applied to experimental measurements. This relation suggests that, in order to obtain the value of μ^{max} from experimental data a graph of $\ln(M_x/M_{xo})$ as function of time should be made. From the slope of this graph μ^{max} can be obtained. This is done for the batch example described earlier (see Table 7.5).

It can be inferred from Table 7.5 that plotting $(\ln(M_x/M_{xo}))$ vs time t should give a straight line through the origin. The slope equals μ^{max}. Indeed one obtains the slope $\mu^{max} = (0.80-0)/(16-0) = 0.050\ h^{-1}$.

This procedure is clearly more elegant than the previous one where interpolation between time points was applied and for each time interval the slope (dM_x/dt) and the average M_x were used to calculate $\mu = (dM_x/dt)/M_x$ according, to the mass balance. However, it should be realized that, when μ is not constant in time, often the solution of the differential equation representing the biomass mass balance can not be obtained analytically and thus the interpolation approach has to be applied.

TABLE 7.5 Calculation of $\ln(M_x/M_{xo})$ for the Batch Example

Time (hour)	M_x (C-molX)	M_x/M_{xo}(–)	$\ln M_x/M_{xo}$(–)
0	10.000	1	0
1	10.513	1.0513	0.050
2	11.052	1.1052	0.100
4	12.214	1.2214	0.200
8	14.918	1.4918	0.400
16	22.255	2.2255	0.800

TABLE 7.6 Total Amounts of Substrate Consumed and Biomass Produced for the Batch Example

Time (hour)	$M_s(t)-M_{so}$ (mol Glucose)	$M_{x(t)}-M_{xo}$ (C-molX)
0	0	0
1	–0.1710	+ 0.513
2	–0.351	+ 1.052
4	–0.738	+ 2.214
8	–1.639	+ 4.918
16	–4.085	+ 12.255

7.4.3.2 Biomass Doubling Time

It should be realized that the maximum specific growth rate μ^{max} is a very characteristic kinetic parameter of growing organisms. It is well known that unicellular organisms grow by increasing their individual mass to a certain level, but then they multiply by division.

The time needed for the cells to increase their number, which also holds for the total cell mass if an average cell mass is assumed, with a factor 2 follows from the exponential growth equation:

$$2\,M_{xo} = M_{xo} \cdot \exp(\mu^{max} \cdot \tau_d) \tag{7.39}$$

whereby τ_d is the biomass doubling time.

Division by M_{xo}, taking the natural logarithm of both sides and subsequent rearrangement yields:

$$\tau_d = \frac{\ln 2}{\mu^{max}} = \frac{0.693}{\mu^{max}} \tag{7.40}$$

Using this equation it can be calculated that if $\mu^{max} = 0.05\ h^{-1}$ this is equivalent to a doubling time of the cells $\tau_d = 0.693/0.05 = 13.9$ hours.

The $M_s(t)$ function obtained earlier (Equation 7.38) can be used to obtain a value for q_s^{max}. If at a given time t the substrate concentration and the culture volume have been measured, the total amount of substrate present at time t can be calculated from $M_s(t) = V(t) \cdot C_s(t)$. If in addition also the initial amounts of substrate and biomass, M_{so}, M_{xo} as well as the maximum specific growth rate μ^{max}, are known q_s^{max} can be obtained from a plot of the consumed substrate $(M_s(t)-M_{so})$, which is negative versus the produced biomass $M_x(t)-M_{xo}$. The slope of the line equals then q_s^{max}/μ^{max}. This is done for the data from the batch example which are shown in Table 7.6.

The corresponding plot is shown in Figure 7.1. It can be seen from this plot that linear regression results in a slope $\frac{q_s^{max}}{\mu^{max}}$ which is equal to $-0.333\,\frac{\text{mol glucose consumed}}{\text{C-molX produced}}$.

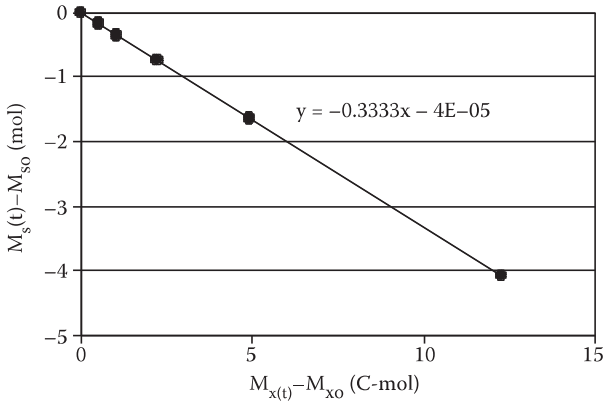

FIGURE 7.1 Plot of $M_s(t)-M_{so}$ against $M_{x(t)}-M_{xo}$ for the data from the batch culture example.

With $\mu^{max} = 0.050 \dfrac{\text{C-molX/h}}{\text{C-molX}}$ the maximum specific substrate uptake rate can be calculated as:

$$q_s^{max} = -0.333 \times 0.050 = -0.0166 \frac{\text{mol glucose/h}}{\text{C-molX}}.$$

This value was also found before, by using the substrate mass balance calculations for each time interval (interpolation approach). It should be realized that if interpolation between time points is used to calculate rates only approximate values are obtained. The reason is, for the example, that dM_s/dt is approximated by a straight line (linear interpolation) and that M_x in this interval is approximated as the average between two time points, which does also introduce errors.

Therefore the approach presented here, where the observation that in batch condition q_s and μ are constant is used to solve the mass balance based differential equations, is more accurate. It should be kept in mind that for this example, that is unlimited batch growth, a mathematical model was available to describe the growth (the exponential growth equation). Therefore the relation between the biomass concentration and time was known (an exponential relation). However, if, e.g., under different conditions, this relation is not known interpolation between measurements is the only way to obtain the rates.

7.4.3.3 Duration of the Batch Culture

In case the values of μ^{max}, q_s^{max}, M_{so}, M_{xo} are known an explicit relation for M_s was obtained as a function of time by substituting these values into Equation 7.37:

$$M_s(t) = 10 + (-3.33 \times 10)[\exp(0.05t) - 1] \tag{7.41}$$

This equation allows us to calculate how much time it will take until the substrate is completely consumed ($M_s(t) = 0$): $0 = 10 - 3.33\ [\exp(0.05t) - 1]$

This yields $\exp(0.05t) = 4.0$, from which it can be calculated that $t = 27.73$ hours.

7.4.4 Stoichiometric Coefficients

The ratio (q_s^{max}/μ^{max}) has the dimension mol substrate used per C-molX produced and is a so-called stoichiometric coefficient. These stoichiometric coefficients are also called operational yields (hence symbol Y) and are always defined as ratio's of conversion rates.

$$Y_{ij} = \frac{q_j}{q_i} = \frac{Rate_j}{Rate_i} \tag{7.42}$$

A well known yield is the biomass yield, which is indicated with the symbol Y_{sx} and has the dimension C-molX produced/mol substrate consumed. The biomass yield Y_{sx} is defined as

$$Y_{sx} = \frac{Rate_x}{Rate_s} = \frac{\mu M_x}{q_s M_x} = \frac{q_x}{q_s} = \frac{\mu}{q_s} \tag{7.43}$$

During unlimited growth in a batch culture the biomass yield is equal to:

$$Y_{sx}^{batch} = \frac{\mu^{max}}{q_s^{max}} \tag{7.44}$$

This implies that the yield does not change during the period of unlimited growth in batch culture. For the batch experiment of the example it can be calculated that

$Y_{sx}^{batch} = 0.05/(-0.0166) = -3.0$ C-molX/mol glucose which implies that 3.0 C-molX are produced per mol glucose consumed.

Substituting the above relation for the biomass yield in batch culture in Equation 7.38 yields, after rearrangement:

$$Y_{sx}^{batch} = \frac{dM_X}{dM_S} = \frac{M_X(t) - M_X(0)}{M_S(t) - M_S(0)} \tag{7.45}$$

Clearly the batch biomass yield follows directly from the changes in the amounts of substate and biomass.

7.4.5 Obtaining q-Rates and Stoichiometry from Batch Experiments: Some Pitfalls

In the previous sections we have introduced the total rate $Rate_i$ (in mol/time) the biomass specific rate q_i (in mol/C-mol biomass /time) and yields $Y_{ij} = q_j / q_i$ (in mol/mol) and it has been shown how these rates and yields can be obtained from experimental data and the proper mass balances, using the batch experiment as an example. Below additional examples will be presented, each of which contains a pitfall, leading to a message.

Example 4: Growth Rates and Yields in a Batch Experiment. Message: do not neglect volume changes!!

During a batch growth experiment the liquid volume and the concentrations of substrate and biomass were measured. The results are shown in Table 7.7.

During the experiment the volume has increased slightly (only 5%) due to the continuous addition of an alkaline solution for pH control.

Question: Calculate the biomass yield Y_{sx}.

Answer: First we calculate the total amounts of substrate, M_s and biomass M_x at 0 and 10 hours, using the C_s, C_X, and V measurements (refer to Table 7.8).

The produced biomass is then 3.675–2.0 = 1.675 kgX and the consumed substrate is 36.75–40 = –3.25 kgS, hence the yield of biomass on substrate is calculated as:

$$Y_{sx} = 1.675/3.25 = 0.5138 \text{ kgX/kgS}.$$

TABLE 7.7 Data from a Batch Experiment

Time (hour)	C_s (kg/m³)	C_x (kg/m³)	V (m³)
0	40	2	1.00
10	35	3.5	1.05

TABLE 7.8 Calculated Total Amounts of Substrate and Biomass

Time (hour)	M_s (kgS)	M_x (kgX)
0	40	2.0
10	36.75	3.675

Question: Calculate Y_{sx}, without taking the change in volume into account.
Answer: Y_{sx} is then calculated from the concentration differences as $Y_{sx} = (3.5–2.0)/(40–35) = 1.5/5 = 0.30$ gX/gS. Comparison with the result of the correct calculation shows that this is incorrect by 40%, which shows the danger of neglecting small volume changes in calculating yields!! So never calculate yields from concentration differences!!

Question: Calculate q_s and μ.
Answer: q_s is defined as $q_s = Rate_s/M_x$

From the mass balance for substrate it is found that for the 0–10 hour time interval:

$$Rate_s = \frac{dM_s}{dt} = \frac{M_s(10) - M_s(0)}{10 - 0}$$

Substituting the measurements for M_s at 10 and 0 hour yields:

$$Rate_s = (36.75–40)/10 = -0.325 \text{ kgS/hour}$$

To calculate q_s we need the average biomass amount which is equal to:

$$(3.675 + 2)/2 = 2.84 \text{ kgX.}$$

Subsequently q_s can be calculated as: $q_s = -0.325/2.84 = -0.1144$ gS/gX/hour.

Similarly one can calculate $Rate_x = (3.675–2.0)/10 = 0.1675$ kgX/hour and $\mu = Rate_x/M_x = 0.1675/2.84 = 0.0588$ kgX/hour per kgX.

Y_{sx} can now also be calculated as $Y_{sx} = (\mu/q_s) = -0.0588/0.1144 = -0.514$ gX/gS. This is (apart from the – sign), identical to the answer to Question 1.

In the above approach we used linear interpolation to obtain average values for $Rate_s$, $Rate_x$, and M_x in the time interval 0–10 hours. A more accurate approach is to use our knowledge that in batch μ^{max} and q_x^{max} are constant. Using the exponential relation

$$\ln\frac{M_x(t)}{M_{xo}} = \mu^{max} \cdot t \tag{7.46}$$

it can be found:

$$\mu^{max} = \ln(3.675/2)$$

This gives $\mu^{max} = 0.061\ h^{-1}$, which is indeed slightly different from the previous value ($0.05\ h^{-1}$). It can be shown that when the interval time $<0.1/\mu^{max}$ both procedures give nearly the same result. This would require a time interval between the measurements of $0.1/0.061 < 1.6$ hour.

Example 5: Batch Experiments on Lab Scale and Production Scale

A microorganism is grown in a batch cultivation carried out in a lab-reactor (around 1 liter volume) and growth and product formation are analyzed by measuring their concentrations (C_X and C_P) and the total broth volume (V) as function of time between 10 and 13 hours. The broth volume decreases strongly due to water evaporation. The measurements are displayed in Table 7.9.

Question: Calculate μ and q_p between 10 and 13 hours

Answer: In order to calcualte μ we need to calculate first the average biomass production rate, $Rate_x$ between 10 and 13 hours. This follows from the biomass mass balance as $dM_x/dt = Rate_x = ((9*0.787)-5*1.05))/3 = 0.61$ g/h. This production is realized by the average biomass amount present in the reactor between 10 and 13 hours, which is $((5*1.05) + (9*0.787))/2 = (5.25 + 7.08)/2 = 6.16$ gram. Hence $\mu = 0.611/6.16 = 0.0991$ gX/gX/h. In order to calculate the specific rate of product formation, q_p we first have to calculate the total production rate $Rate_p$, which follows from the mass balance for product as 0.456 g P/h. With the average amount of biomass (6.16 gram) q_p follows as $q_p = 0.456/6.16 = 0.074$ g P/gX/h.

Question: The previous laboratory batch experiment is also performed on production scale, where the fermentor volume is increased nearly 5000 fold from 1.05 liter to 5.0 m^3. The following experimental measurements are available (Table 7.10):

It can be inferred from Table 7.10 that at t = 10 hours the same concentrations are measured as on lab scale, which is expected. However at t = 13 hour the biomass and product concentrations are significantly lower at production scale.

The following question immediately arises:

Does the organism grow and produce worse at large scale? (a so-called scale up effect?)

Answer: At first glance, the concentrations of biomass and product for the fermentation carried out on production scale are much lower at 13 hour, so it would appear that there is a scale-up effect. However, to evaluate the performance of the cells one should not compare product concentrations but specific conversion rates!! Using the proper approach (mass balance based, see before) it can be calculated that $\mu = 0.093$ gX/gX/h and $q_p = 0.074$ gP/gX/h. These are values which are very close to the values observed in the lab scale experiment.

TABLE 7.9 Results from a Batch Cultivation Carried Out on LabScale

		Concentrations	
Time t (hours)	Volume V (L)	C_X(kg/m^3)	C_P(kg/m^3)
10	1.050	5.0	2.0
13	0.787	9.0	4.407

TABLE 7.10 Results from a Batch Experiment Carried out on 5 m^3 Scale

		Concentrations	
Time t(hours)	Volume V(m^3)	C_X(kgX/m^3)	C_P(kgP/m^3)
10	5.0	5.0	2.0
13	4.9	6.75	3.36

TABLE 7.11 Data from a Citric Acid Fermentation

Time (hours)	V (m^3)	C_X (kgX/m^3)	C_S (kgS/m^3)	$C_{citric\ acid}$ (kgP/m^3)
30.5	70.3	10.3	150	2
37.0	68.0	10.65	120	23

The main difference between lab and production scale is the change in volume (25% at lab, 2% at production) due to much larger water evaporation on lab scale. Clearly there is no scale up effect for the organism.

Example 6: Product Formation in a Batch Reactor, q_p Is the Key Parameter

The fungus *Aspergillus* produces citric acid (a food preservative) from glucose in an aerobic process. Citric acid $C_6H_8O_7$ has a molecular weight (MW) of 192 g/mol. Glucose $C_6H_{12}O_6$ has a MW of 180 g/mol.

The citric acid production occurs under condition of no growth (e.g., because the N-source needed to synthesize new biomass is absent). In a large batch reactor the measurements are made at different times and data is shown in Table 7.11. The volume V decreases due to water evaporation.

Task: Show that there is no biomass growth and also calculate q_P and q_S. Also provide the proper units of the q-values.

Answer: The concentration of biomass (C_X) increases, the volume V decreases, but the total amount of biomass ($M_X = VC_X$) is constant, being $M_X = 724.1$ kgX. From this it can be concluded that growth is indeed absent!!! To calculate q_P and q_S it is needed to calculate first the total amounts of produced citrate (kg/h) and consumed glucose (kg/h). From the mass balance for citrate it follows that the total rate of citrate increase in the cultivation vessel dM_P/dt (kg/h) equals the citrate production $Rate_P = (q_P \cdot M_X)$. It can be calculated that the total amount of produced citrate between 30.5 and 37.0 hour = 68*23–70.3*2 = 1564–140.6 = 1423.4 kg. The total citrate production is therefore $Rate_P = 1423.4/6.5 = 219$ kg citrate/h. Hence q_P can be calculated to equal $Rate_P/M_x = 219/724.1 = 0.3024$ kg citrate/kg biomass per hour. In an analogous way it can be found that the total amount of consumed glucose is equal to 2385 kg in 6.5 hour from which it can be calculated that $q_S = -0.5067$ kg glucose/kgX/h (negative!!).

7.5 Conclusions

The biomass specific (q-based) conversion rates kinetics (q-values) of cells, tissues, or microorganisms can not be measured directly, but can be calculated from measurements of concentrations, volumes, and flow rates obtained from experiments where the cell, tissue, or microorganism is consuming, growing and producing (in a flow cell, fermentor, shake flask, etc.).

To obtain a q_i-value (i = substrate, biomass, product, O_2, CO_2, N-source, etc.) it is necessary:

- To design a proper experiment
- To set-up the proper mass balance for compound i
- To carry out the proper measurements of volume and biomass concentration in order to be able to calculate the total amount of biomass ($M_x = V \cdot C_x$) which is present in the system as a function of time
- To carry out the proper measurements of in and outflow rates (if any) and concentrations of compound i as function of time
- To calculate, from the mass balance of compound i and the measurements, the total conversion rate of compound i, $Rate_i$ (mol i/h), of produced or consumed compounds as function of time
- To calculate q_i as function of time from M_x and $Rate_i$ as function of time

The thus obtained biomass specific conversion rates, (q-rates) measured as a function of time and of the experimental conditions applied allows us to obtain kinetic relations for q_i as a function of the external conditions. These allow us to set up mathematical models by combining the proper mass balances and these kinetic relations. Such mass balance based models can then be used to generate predictions of the culture behavior as a function of the cultivation conditions, and can thus be applied for the design and optimization of fermentation processes. For reasons of simplicity we have limited ourselves in this chapter to batch cultivations, and therefore the kinetic relations used were not very exciting (zero order kinetics). The latter indicates immediately the disadvantage of batch experiments for the study of q-rates, namely that the experimenter has no control over the q-rates, which are constant (the cell has control).

Before we approach the question of how the experimenter can manipulate the q-rates, it is important to address another question. Cells and organisms grow (μ), consume substrate (q_s), and make product (q_p). To do so they also need O_2, produce CO_2, heat, etc. The question is: How do we obtain these "other rates"? The answer is to use stoichiometric calculations.

Further Reading

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