6.3 Microbial growth in a chemostat

The **chemostat** is a widely-used apparatus used in the study of microbial physiology and ecology. In such a chemostat (also known as **continuous-flow culture**), microbes (such as bacteria and yeast cells) can be grown under precisely controlled conditions. Fig. 6.9 shows a diagram. The microbes grow in the main culture vessel which contains a well-stirred culture medium. This medium is replenished from a reservoir shown on the left. As a constant volumetric flow F of fresh medium enters the culture vessel propelled by a pump, an overflow[21] allows an equal flow to leave the culture vessel, so that the vessel retains a constant volume V of culture at all times. The experimenter can control the pump and thus the flow F; the reservoir nutrient concentration C_R can of course also be varied at will by the experimenter. However, in the classic set-up, the experimenter keeps both F and C_R at fixed values and waits for the system to settle on a steady state.

[21] Such a device is alternatively known as a *porcelator*.

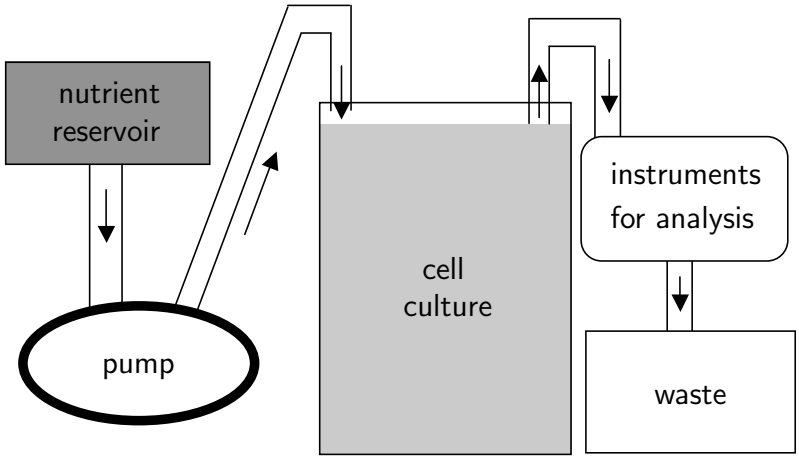

Fig. 6.9 The continuous-flow culture vessel or chemostat.

6.3.1 Basic chemostat equations

Let W denote the microbial biomass in the culture vessel and N the molar amount of nutrient in the vessel. We have two conservation equations:

$$\frac{d}{dt}W = \mu W - F\frac{W}{V} \tag{6.20}$$

$$\frac{d}{dt}N = FC_R - F\frac{N}{V} - S\mu W\ . \tag{6.21}$$

The term μW represents the increase in biomass due to growth; the factor μ is the *per capita* reproduction rate, which we called *relative growth rate* before but which is known in these systems more commonly as the **specific growth rate**. Biomass leaves the system at a rate FW/V which makes intuitive sense if W/V is viewed as the biomass concentration in the culture vessel. The second equation describes the amount of nutrient. The first two terms on the right-hand side again describe the influx and efflux of nutrient, whereas the third term describes the conversion of nutrient into biomass. The factor S expresses the stoichiometry of this conversion. The ratio F/V plays an important role in the operation of the chemostat and therefore has a name of its own: it is called the **dilution rate**, traditionally denoted D. For the nutrient concentration $C = N/V$ we have the following dynamics:[22]

$$\frac{d}{dt}C = D\,(C_R - C) - S\mu\frac{W}{V}\ . \tag{6.22}$$

The steady state is, as per usual, characterized by the conditions $\frac{d}{dt}W = 0$ and $\frac{d}{dt}C = 0$, whence[23]

$$\mu = D \quad \text{and} \quad C = C_R - SW/V \quad \text{at steady state.}$$

The experimenter controls the dilution rate D, which is just a setting on the pump in the apparatus shown in Fig. 6.9. Since $\mu = D$ at

[22] Derive eqn (6.22) from eqn (6.21) and the definitions given.

[23] Check this; apply the definition $D = F/V$ first.

steady state, the experimenter effectively forces the cells to grow at the rate she desires. In fact, this phenomenon is the great advantage of continuous culture: after the chemostat settles on steady state, the cells can be sampled and their physiological state at various stationary relative growth rates can be studied. Waiting for the system to attain steady state is standard practice. However, as we shall presently see, the transient behaviour actually contains important information about the underlying biology.

The specific growth rate μ will generally vary with time, as a function of the internal state of the organism and perhaps also the ambient conditions (such as the nutrient concentration C). However, we can already say something about the dynamical behaviour of the chemostat even when, for now, we leave μ as an unspecified function of time. Consider the following quantity:

$$X = W + \frac{N}{S} \,. \tag{6.23}$$

This represents the actual biomass in the culture vessel (W) plus the biomass equivalent of the nutrient in the vessel (N/S). Its time course is given by:[24]

$$X(t) = \frac{VC_R}{S} + \left(\frac{VC_R}{S} - X_0 \right) \exp\{-Dt\} \tag{6.24}$$

where X_0 is the initial condition, the value of X at time $t = 0$. From eqn (6.24) we can infer that after an initial transient with relaxation time D^{-1} (which occurs if we suddenly change the reservoir concentration C_R), the quantity X settles down on its equilibrium value VC_R/S. From then onwards the following useful conservation law for the culture vessel applies:

$$C_R = C(t) + S\frac{W(t)}{V} \,. \tag{6.25}$$

This equation states that biomass density and nutrient concentration are complementary.[25]

[24] Derive the differential equation for X:

$$\frac{d}{dt}X = D\frac{VC_R}{S} - DX$$

and solve it to find eqn (6.24).

[25]  Can you think of a quick way to see why this result is not so surprising?

6.3.2 Logistic growth in the chemostat

To proceed further, we need to postulate a model for the relative growth rate μ. One simple model is as a simple proportionality:[26] $\mu = \vartheta C$. Using eqns (6.25) and (6.20) we obtain:

$$\frac{d}{dt}W = W\left(\vartheta C_R - D - \frac{\vartheta S}{V}W \right) \,. \tag{6.26}$$

But this is just the logistic growth equation in disguise[27]. Of course, we must not forget about the initial transient while $X(t)$ approaches VC_R/S, so the growth curve may not be logistic during some initial period, but if we pick the initial nutrient and biomass density in the culture vessel so that they satisfy eqn (6.25), this equation will be valid for all t.

[26] Although simple, this model is reasonable since the cells cannot grow when there is no nutrient, so we would expect $\mu = 0$ when $C = 0$, and we would also suppose that a linear approximation is reasonably accurate for some range of sufficiently low values of C.

[27] Cast eqn (6.26) in the traditional form of the Verhulst equation by defining r and K in terms of the present parameters

6.3.3 Towards a model for the relative growth rate

In the last section the chemostat equations were introduced. The crucial task for the mathematical modeller is to relate the relative growth rate μ to the ambient conditions and/or the internal state of the cells. In this section we follow this modelling process in detail, to highlight the back-and-forth between observations and theory (cf. Fig. 1.6).

As we saw before, the microbial culture in the vessel settles, after a while, on a steady state when the dilution rate D of the chemostat and the reservoir nutrient concentration C_R are kept at a fixed value. In the steady state, we can observe the biomass $\overline{W}$, the nutrient concentration $\overline{C}$, and the relative growth rate $\overline{\mu}$ which at steady state equals the dilution rate D (the bar over the symbols signifies stationarity in time). Obtaining these data for a range of dilution rate settings, the experimenter accumulates a data set consisting of triples $\{\overline{\mu}, \overline{W}, \overline{C}\}$ (i.e. a table with three columns). The next step is to look for systematic relationships in this data set. Plotting $\overline{\mu}$ against $\overline{C}$, the experimenter finds that the data points closely adhere to the following simple mathematical expression:

$$\overline{\mu} = \alpha \frac{\overline{C}}{\beta + \overline{C}} \tag{6.27}$$

where α and β are two positive parameters which the experimenter is able to estimate using the non-linear least squares criterion (see Section 4.2). This hyperbolic relationship immediately reminds the experimenter of the Michaelis-Menten relationship for the nutrient uptake system (see Section 2.3), which suggests that β represents the K_m of this uptake system. Moreover, this identification suggests a more general principle:

The specific growth rate is directly proportional to the saturation fraction of the uptake system (which is given by $C/(K_m + C)$). (I)

This principle is proposed to apply also to the transient situation, where C is time-varying.

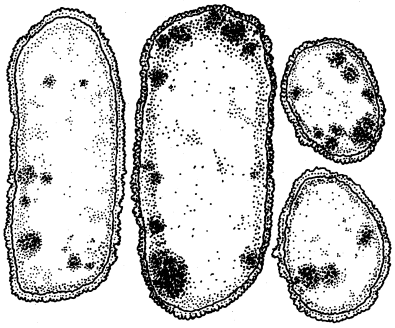

Fig. 6.10 Glycogen (poly-glucose) granules in *Escherichia coli* cells: an example of nutrient storage in micro-organisms.

Next, the experimenter acquires an instrument which allows her to analyse the chemical contents of the cells. It is found that the cells have internal stores of the nutrient. The total amount of this nutrient in the culture at steady state is denoted $\overline{Q}$ (Q for **quota**). The experimenter calls the ratio $\overline{Q}/\overline{W}$ the **relative nutrient quota**. Plotting the steady-state growth rate against the relative nutrient quota, again a very good agreement is found with a simple mathematical relationship:

$$\overline{\mu} = \gamma \frac{\overline{Q}/\overline{W}}{\delta + \overline{Q}/\overline{W}} \tag{6.28}$$

where γ and δ are two new positive parameters. This suggests another principle for the general[28] state:

The specific growth rate is a hyperbolic function of the relative nutrient quota. (II)

We now have two equations, and two principles, and the experimenter

[28] By the general state we mean not only the steady state, but transients as well.

wants to determine which is valid. Let us begin with the easy question: can equations (6.27) and (6.28) both be valid descriptions of the steady-state data? The answer is: certainly. Moreover, we can inform the experimenter[29] that she will find another hyperbolic relationship if she plots the relative nutrient quota against the concentration:

$$\frac{\overline{Q}}{\overline{W}} = \frac{\alpha\delta}{\gamma - \alpha} \times \frac{\overline{C}}{\beta\gamma/(\gamma - \alpha) + \overline{C}} \,. \tag{6.29}$$

[29] Derive eqn (6.29) from eqns (6.27) and (6.28).

To her delight, this comes out as predicted. The choice between principles (I) and (II) is more complicated. These are putative laws which extend beyond the regime under which the data were obtained (the steady state). Fortunately, modelling can help here, by predicting how the culture will respond *dynamically* to perturbations, if one or the other principle is assumed. The perturbations might be step changes in dilution rate or feed reservoir nutrient concentration. For each perturbation, we can evaluate the chemostat equations to work out what should happen. Principle (I) leads to the following system of differential equations:[30]

$$\frac{d}{dt}W = W\left(\alpha\frac{C}{\beta + C} - D\right) \tag{6.30}$$

$$\frac{d}{dt}C = D\,(C_R - C) - \frac{\alpha S W}{V} \times \frac{C}{\beta + C} \,. \tag{6.31}$$

[30] Derive eqns(6.30) and (6.31) by combining the chemostat equations of Section 6.3.1 with eqn (6.27).

The experimenter carries out the perturbations and finds a poor agreement with the predictions: in general, the cells seem to respond more sluggishly to changes in nutrient concentration C. For instance, in a wash-out experiment were C_R is set to zero at time $t = 0$ (this is done by connecting the supply tube to a reservoir containing medium without nutrient), the predicted curve for the nutrient concentration in the culture vessel agrees very well with the data, whereas the biomass shows a distinct lag before it starts to decrease, as shown in Fig. 6.11. Also, the experimenter enlists the help of a biochemist who determines the K_m of the nutrient uptake system, and finds that the latter is much *larger* than β, whereas principle (I) asserts that they should be about equal, allowing for slight variations due to the fact that different experimental approaches were used.[31]

[31] Consider a microbiologist who firmly believes in principle (I). As far as he is concerned, the chemostat curve is simply a way of determining K_m. He asserts a discrepancy between two methods of measuring K_m. What could be done to change this microbiologist's mind?

The experimentalist now asks us, the modellers, for another dynamic prediction, this time assuming principle (II). Since any decent model must satisfy basic conservation principles, we might as well take a conservation law as our starting point:[32]

$$\frac{d}{dt}Q = V_m W \frac{C}{K_m + C} - S\mu W - DQ \tag{6.32}$$

[32] Justify eqn (6.32).

where $V_m W$ expresses the maximum nutrient uptake rate, V_m being a proportionality constant that expresses how much of the uptake machinery is present per unit biomass. The constant S represents the stoichiometric conversion of nutrient into biomass, and D is the dilution rate of the chemostat. The steady state associated with eqn (6.32) is compatible

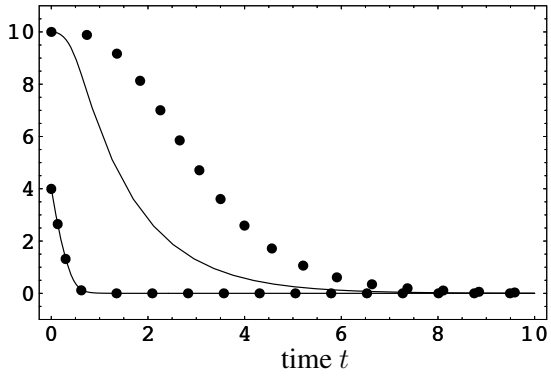

Fig. 6.11 Comparison of biomass (top curve) and nutrient concentration (bottom curve) predicted from principle (I) with data in a wash-out experiment. Whereas the data do not accord well with principle (I), they are well described by principle (II) (curve not shown, as the data correspond closely to the curve obtained under principle II).

with equations (6.27)–(6.29) only if the parameter values satisfy certain identities. Specifically, compatibility requires the following parameter identifications:[33]

$$\alpha = \widehat{\mu}\frac{V_m}{\widehat{\mu}S + V_m}\ ; \quad \beta = K_m\frac{\widehat{\mu}S}{\widehat{\mu}S + V_m}\ ; \quad \gamma = \widehat{\mu}\ ; \quad \delta = S \tag{6.33}$$

where we have used the symbol $\widehat{\mu}$ to indicate the maximum growth rate.[34] On these identifications we obtain a very simple equation for the dynamics of the relative nutrient quota:[35]

$$\frac{d}{dt}\frac{Q}{W} = \widehat{\mu}\left(\frac{V_m}{\widehat{\mu}}\frac{C}{K_m + C} - \frac{Q}{W}\right)\ . \tag{6.34}$$

This equation states that the relative nutrient quota relaxes exponentially toward the saturation fraction of the uptake machinery. This equation forms a dynamical system together with the chemostat equations. which for principle (II) take the following form:

$$\frac{d}{dt}W = W\left(\widehat{\mu}\frac{Q/W}{S + Q/W} - D\right) \tag{6.35}$$

$$\frac{d}{dt}C = D\,(C_R - C) - \frac{V_m W}{V} \times \frac{C}{K_m + C}\ . \tag{6.36}$$

This model explains the discrepancy found in the wash-out experiment. Since the relative growth rate depends directly on the relative nutrient quota Q/W which lags behind the external nutrient concentration, microbial growth tends to continue at almost the steady state rate for some time after C_R has been set to zero. This may seem to violate basic conservation principles, since the external nutrient concentration behaves

[33] Demonstrate this. Start from steady state (assume C constant, put $\frac{d}{dt}Q = 0$ and set $\mu = D$) and compare the equation you obtain with equations (6.27)–(6.29).

[35] Derive eqn (6.34); use the quotient rule.

[34] There is a conceptual problem with the identification $\delta = S$ if we view principle (II) and eqn (6.28) as a description of how the microbe's internal regulatory system adjusts the growth rate as a function of the relative nutrient quota. On this interpretation, δ is a property of the molecular machinery involved in regulation, and hence some complex compound involving the rate at which certain molecules bind DNA et cetera. It is not *a priori* clear why this compound should equal the macrochemical conversion constant S. This does not really present an insurmountable problem for the experimental findings: if δ and S are roughly of the same order, the actual equations are somewhat more complicated than hyperbolas but they will still have the same general shape and conform to the data. Similarly, instead of eqn (6.34) we obtain slightly more complex dynamics.

much the same under principles (I) and (II). However, the additional growth in the latter case is effectively paid for by depleting the nutrient store in the cells.

Does the story end there? Should the experimenter conclude that principle (I) is false and that principle (II) is true? No. The situation is more subtle. A careful study of the two systems of dynamical equations reveals that the lag effect will be very small if the relative nutrient quota stays well below S. This will be the case if the parameter V_m is relatively small. This parameter expresses how many building blocks (glucose, amino acids) the microbial cell allocates toward the molecular machinery which processes the nutrient. Thus, the organism can in fact switch between "principle (I) mode" and "principle (II) mode" by adjusting the amount of machinery it synthesises.[36] Moreover, microbes generally require more than one chemical species from their environment to grow and subsists (e. g. a carbon nutrient, an energy nutrient, and various minerals) and thus the growth rate will depend on the ambient concentrations of various substances (as well as the internal stores of these species). This additional complexity is taken into account in **multiple nutrient limitation theory**,[37] which relies on further iterations between experimental observations and theoretical predictions.

[36] To be precise, the cell also has the option of (partially) disabling the catalytic machinery that is already in place, for instance by means of attaching chemical moieties to this machinery.

[37] See Exercise 6.9 for a brief introduction.