Dynamics of Microbial Growth

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I. Introduction

'Dynamics' may be defined as a study of forces acting upon a body and, in this sense, the 'dynamics of microbial growth' implies a study of those (environmental) forces that act either to promote or to impede the growth of microbes. Now the term 'microbe' also requires to be defined since this, and the term 'bacterium', are frequently used synonymously, but whereas all bacteria are microbes, not all microbes are bacteria. Indeed, any organism (prokaryote or eukaryote) that individually cannot be perceived by the naked eye is entitled to be called a microorganism. However, it must be recognized that studies of microbial growth kinetics have concentrated largely on bacterial

species, and it follows, therefore, that in this chapter attention will be directed mainly to aspects of bacterial growth.

The capacity to grow, and ultimately to multiply, is one of the most fundamental characteristics of living cells. In fact, with microorganisms it is generally the sole criterion that is used to assess whether or not such creatures are alive. Hence, by definition, a non-viable microbe is one which is incapable of increasing in size and number when incubated in an equitable, growth-supporting environment for a prolonged period of time. However, despite the fact that growth is such a basic aspect of microbial behaviour, little attention was paid to the principles which underlie it until the advent of continuous culture techniques. Indeed, prior to the 1940's it was generally considered sufficient to record growth as being either evident (+) or not (–), with the occasional recourse to semiquantitative flights of fancy such as +++, ++, and ±! And whereas these essentially qualitative assessments often proved adequate, there can be little doubt that the more quantitative approach that has been adopted over the last 30 years has yielded a vastly more penetrating insight into the nature of microbial life.

Unfortunately, the manipulation of quantitative data, and their correlation with growth-associated processes, frequently demands the construction of mathematical equations – nowadays popularly called 'models'. These range from the very simple to the exceedingly complex, and often serve only to confuse those biologists whom they are intended to enlighten. Hence, in this chapter, every effort has been made to keep the mathematics to a minimum and to explain fully the steps involved in deriving each equation. Thus it is hoped that the small amount of mathematics necessarily involved in the treatment of this subject will not create an insuperable barrier to the understanding of what is, after all, a vitally important aspect of Microbiology.

II. Microbial Growth in a Closed Environment

Microbial growth is a process that requires the coordinated synthesis of a range of complex macromolecules, the energy for this process (and the necessary intermediary metabolites) being derived from the uptake and chemical transformation of a relatively small number of compounds and elements. Hence growth in any environment is only possible when there is present a complete mixture of those compounds and elements that are essential for cell synthesis and functioning. In this connection, all living cells seemingly contain carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, magnesium, potassium, and a number of so-called 'trace' elements (Mn^{2+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, etc.). Consequently, all these elements must be present in a utilizable form, along with

water, and, if other environmental conditions are propitious, then those microbes that may be present will grow and multiply.

Bacteria reproduce by a process of *binary fission* in which, so far as is known, the contents of the mother cell are partitioned equally between the two daughter cells. One obvious consequence of binary fission that may be overlooked is that the mother cell is consumed as the daughter cells are created. Thus, one must exercise caution in specifying the 'age' of a bacterial cell since, logically, this can only be made by reference to the time that has elapsed since its birth. This may seem to be a trivial point, but there are to be found in the literature frequent references to 'old cells' and 'young cells', meaning bacteria taken from cultures that have been incubated for long and short periods of time, respectively. The confusion that this loose terminology causes has far-reaching consequences, particularly in the interpretation of physiological data.

Binary fission ought to lead to the birth of identical twins with identical physiological properties and growth potentials. Therefore one might expect these twins to grow at identical rates and to divide synchronously. This rarely happens, and in any culture derived from a single bacterial cell there is generally to be found a wide spread of individual generation times from a well defined minimum to a less well defined maximum. Hence, the culture as a whole increases in population density with a doubling time that is not precisely the same as the mean generation time. This is a point of detail that can be ignored for the moment; but it must not be forgotten.

A. Kinetic Aspects

When growing in a relatively constant and equitable environment, microbial populations multiply at rates that are, overall, constant. Therefore, even though there may be a spread of individual generation times, a number of organisms (N) will give rise to $2N$ progeny, and these to 2^2N progeny, and so on, with an overall doubling time (t_d) that is constant. This can be represented as follows:

$$N \rightarrow 2N \rightarrow 4N \rightarrow 8N \rightarrow 16N \rightarrow 32N \ldots$$

or,

$$2^0N \rightarrow 2^1N \rightarrow 2^2N \rightarrow 2^3N \rightarrow 2^4N \rightarrow 2^5N \rightarrow 2^nN.$$

Here, n represents the number of doublings that have occurred after some time interval t. Thus,

$$n = t/t_d.$$

It follows, therefore, that the number of organisms present in a culture

after t hours of incubation will be related to the initial population by the equation:

$$N_t = N_0 2^n = N_0 2^{t/t_d}.$$

Similarly,

$$N_t/N_0 = 2^{t/t_d}$$

and taking logarithms,

$$\ln(N_t/N_0) = (\ln 2)t/t_d,$$

or

$$(\ln N_t - \ln N_0)/t = 0.693/t_d.$$

Therefore, plotting the natural logarithm of the number of organisms against the time of incubation should yield a straight line whose slope will be numerically equal to $0.693/t_d$. This is found to be the case with many real bacterial cultures: an example is given in Figure 1. It should be noted, however, that the 'exponential' growth period is preceded by

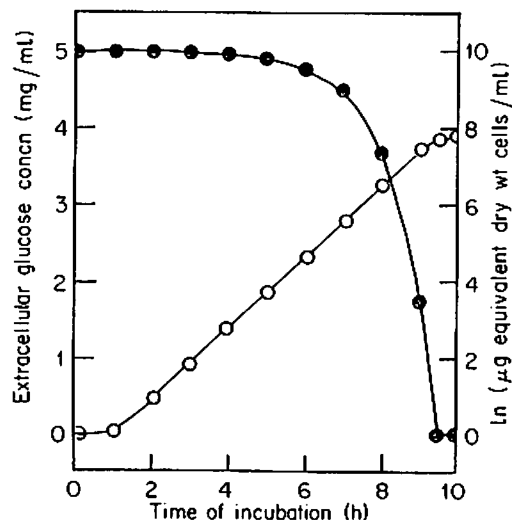

Figure 1. Changes in the concentration of organisms (○) and of glucose (●) with time of incubation of a culture of *Klebsiella aerogenes* growing in a simple salts medium at 37 °C (pH 6.8). Notice that cell concentration is plotted as its logarithm, but glucose concentration as its actual value.

a short 'lag' period (a period of metabolic adjustment) and that the exponential increase in the population density (here represented as biomass) ceases after a relatively small number of culture doublings.

We shall return to this so-called 'growth cycle' later, but first it is necessary to say a little more about exponential growth. As already mentioned, the slope of the line $\ln N$ versus t is numerically equal to $0.693/t_d$. Thus,

$$d(\ln N)/dt = 0.693/t_d.$$

But, $d(\ln N)/dt = d(\ln N)/dN \times dN/dt$, and since the differential coefficient of $\ln N$ is $1/N$, then it follows that:

$$\frac{1}{N} \cdot \frac{dN}{dt} = \frac{0.693}{t_d}.$$

$1/N \cdot dN/dt$ is called the *specific growth rate* constant and is given the symbol μ. It is the rate of increase in cell numbers per unit of cell numbers.

Now the growth of each individual organism in a population proceeds through various stages of the cell cycle in which the mass of the organism increases up to some maximum value prior to cell division. Hence, in any population of organisms growing asynchronously, there will be a distribution of cell sizes. However, just as the population density as a whole increases exponentially with time, so too does the culture biomass concentration. Moreover, since biomass generally can be measured more accurately than can numbers of organisms, the basic microbial growth equations are generally expressed in terms of mass rather than number of cells. Thus, if x is the culture biomass concentration:

$$\mu = 1/x \cdot dx/dt = (\ln 2)/t_d = 0.693/t_d.$$

The use of natural logarithms in developing this particular growth equation is helpful in subsequently analysing the kinetics of microbial growth in continuous cultures. It is not, however, essential to use them, though if other logarithmic functions are employed then the specific growth rate constant μ has a different numerical value and ought to be ascribed a different symbol. For example, if logarithms to the base 10 were used, then:

$$\mu' = 0.301/t_d;$$

and if logarithms to the base 2 were used, then:

$$\mu'' = 1/t_d.$$

One final point of clarification: the units of time may, of course, be minutes, hours or days, but they *must* be specified. With bacterial cultures it is usual to express the doubling time in hours, and μ in reciprocal hours (h^{-1}). Cultures of algae, on the other hand, often grow at much slower rates and it is not unusual for their specific growth rate to be expressed in reciprocal days (d^{-1}).

As mentioned previously, the specific growth rate equation, derived above, adequately predicts the behaviour of many real microbial cultures, but only within certain limits. Indeed, unless these limits are clearly recognized, conclusions may be drawn that are palpably absurd. A simple example serves to make the point.

Escherichia coli (a common gut organism) is capable of growing at a rate such that the culture doubles in biomass every 20 minutes. One might ask the question, therefore, 'if 100 ml of nutrient medium is inoculated with about 1 μg of organisms (that is, about 5×10^6 cells) and subsequently incubated for 24 h, what quantity of organisms might one expect to be produced?'

$$\mu = 0.693/t_d = 0.693/0.333 = 2.08\ h^{-1}.$$

$$\mu = (\ln X - \ln X_0)/t$$

therefore,

$$\ln(X/X_0) = 2.08 \times t = 49.92.$$

Hence,

$$X/X_0 = e^{49.92} = 4.8 \times 10^{21}$$

and

$$X = 4.8 \times 10^{21} \times X_0 = 4.8 \times 10^{21}\ \mu g.$$

Thus, starting with an initial inoculum of 1 μg organisms, and allowing the culture to grow for 24 h, should result in the production of an amount of organisms weighing 4.8×10^9 *metric tons*!

.Clearly the answer is ridiculous for, as shown in Figure 1, real cultures seldom attain a biomass concentration in excess of a few mg per ml. However, this kind of mathematical exercise does serve a useful purpose in that it concentrates attention not only on the enormous growth potential of many microorganisms, but also on the fact that there exist, both in Nature and in the laboratory culture, circumstances that limit the extent to which this growth potential is expressed. Such considerations lie at the heart of our understanding of the dynamics of microbial growth and will be briefly reviewed in the next section.

B. Cell–Environment Interaction

It follows from the fact that laboratory cultures of microorganisms seldom attain a biomass concentration greater than a few mg equivalent dry weight per ml that circumstances intervene to limit further cell multiplication. Two explanations seem plausible: (1) that organisms interact directly with one another to inhibit growth: such interaction is known to occur in cultures of some animal cells and is termed 'contact inhibition', and (2) that organisms interact with their environment in such a way as to render it no longer conducive to further growth.

The idea of contact inhibition limiting the size of bacterial populations has failed to receive convincing experimental support. On the contrary, cultures generally grow to much greater biomass concentrations if dialysed against a continuous flow of fresh medium. Moreover, if contact inhibition occurred, one would not expect organisms to produce large colonies when spread on a nutrient agar plate. It therefore seems most likely that the event(s) provoking cessation of growth in a batch culture reside in changes induced in the environment by the growth of the organisms. Again, one can imagine two possibilities: either growth leads to the depletion of some essential nutrient substance from the environment, or else growth leads to the accumulation of products that ultimately reach toxic concentrations. In this latter connection, although the formation of auto-inhibitory compounds during the growth of microbial cultures has been demonstrated in a number of cases, the most frequently occurring toxic products are hydrogen ions and hydroxyl ions which cause the culture pH value respectively to decrease or increase as growth proceeds. For example, when organisms are grown on an organic acid substrate (say acetate, succinate or lactate) with the pH value adjusted initially to neutrality, then growth of the organisms leads to a marked increase in the culture pH (unless this is deliberately controlled) due to the excessive uptake and metabolism of anions over cations.

Automatic adjustment of the culture pH value generally leads to a culture attaining a higher final population density, but the increase may be relatively small. With aerobic organisms, growth at high population densities becomes impeded by the decrease in available oxygen. It is insufficiently appreciated that it is only the oxygen that is dissovled in the culture fluid that is accessible to the organisms. Since the solubility of oxygen in water is low (5–7 μg/ml, at 20 °C), a high rate of oxygen consumption (as with rapidly-growing dense populations) means that the rate at which oxygen can dissolve in the culture fluids becomes the factor limiting the subsequent growth rate of the organisms.

Assuming the culture pH value to be automatically controlled and

that growth is not restricted by the accumulation of auto-inhibitory substances or by the availability of oxygen, then ultimately growth will cease due to the depletion of some essential nutrient substance. This follows from the fact that, as growth proceeds exponentially, so there is an exponential rate of change in the concentrations of all essential nutrients. Indeed, the rates at which these essential nutrient substances are taken up from the medium have been found to be directly proportional to the rate of cell synthesis. That is:

$$1/x \cdot dx/dt \propto -1/x \cdot ds/dt$$

or,

$$\mu = Yq,$$

where q is the specific rate of substrate assimilation and Y is the proportionality factor or *yield value*. The molar yield value for glucose, found with cultures of *E. coli*, growing in a simple-salts medium, generally is in the region of 90 (g organisms synthesized per mol of glucose consumed). Hence, when growing at a rate (μ) of 1.0 h^{-1} (i.e. with a doubling time of 0.693 h, or 42 min), the specific rate of glucose consumption will be approximately 11.1 mmol per g-equivalent dry weight of organisms per hour. It follows, therefore, that if all other nutrients (including oxygen) were present in excess of the growth requirement, and glucose was added to a final concentration of 5 mg/ml (i.e. 27.8 mM), then the length of time that organisms would spend in the exponential growth phase would be critically dependent on the inoculum size. Hence, if the inoculum provided 0.001 mg biomass/ml (say, about 5×10^6 organisms/ml), then, assuming no lag period, the time taken to reach a state of glucose exhaustion can be calculated as follows:

(i) From the molar yield value (90), the concentration of organisms present when all the glucose has been consumed will be $90 \times 27.8/1000 = 2.5$ mg/ml.

(ii) Since $\ln(X/X_0) = \mu t$, and $\mu = 1.0\ h^{-1}$,

$$\ln(2.5/0.001) = t$$

$$\ln(2500) = t = 7.8\ h.$$

It is clear, therefore, that if such a culture was incubated overnight (16 h) then, even assuming a lag period of 1–1.5 h, it would have reached a state of glucose exhaustion some 6–7 h prior to harvesting. Indeed, in order to ensure that such an overnight culture was still in the exponential growth phase after 16 h incubation, it would be necessary to decrease the inoculum size to 1×10^{-7} mg organisms/ml, or less (that is, to 5×10^2 organisms/ml, or less).

The inclusion of this small example serves to make one important

point, and that is that microorganisms interact continuously with their environment and that, as growth proceeds, so the environment changes at an accelerating rate (Figure 1).

Thus, organisms in the exponential growth phase (particularly the late-exponential phase) are experiencing dramatic and precipitous changes in their environment, changes which lead inevitably to it being no longer able to support further growth. Hence growth ceases and the culture enters the so-called 'stationary phase'. In this connection, it should be realized (Figure 1) that the final doubling of biomass prior to the onset of the stationary phase lowers the extracellular carbon-substrate concentration from 50% of its starting value to zero. This precipitous drop in substrate concentration can occur within a period of 42 min, or less.

Further consideration of Figure 1 leads to two additional conclusions: (1) that the organisms can accommodate to marked changes in their environment and yet continue to express their full growth-rate potential right up to the moment when the limiting substrate is almost totally consumed, and (2) that the sequence of changes occurring in a batch culture, collectively referred to as the 'Growth Cycle' are not an expression of some inherent property of the organisms, but simply the inevitable consequence of the interaction of organisms with their environment in a closed system. And since most natural ecosystems are essentially open systems, it follows that the batch-culture growth cycle is essentially a laboratory artefact.

The above mentioned conclusions have far reaching consequences in our analysis of microbial growth kinetics and therefore will be considered in more detail.

C. Microbial Adaptability

Although biochemical research has, over the past three decades, tended to emphasize more and more the unity of life processes, nevertheless there are important differences between free-living micro-organisms and the cells of higher animals and plants. To appreciate this point, one must consider these different cells in relation to their environment. At one extreme one finds the cells of, say, the human body, and here we recognize the fact that the human animal possesses an elaborate array of organs whose functions, collectively, are to maintain a constant internal environment; as Claude Bernard realized, over 100 years ago, 'the constancy of the internal environment is a condition of life'. Therefore, if this environment is caused to vary beyond certain narrow limits (of temperature, pH, salt balance, nutrient concentration, etc.) the cells cannot accommodate – they cease to function and the animal dies.

At the other extreme one finds the free-living microbial cells which occupy environments that they cannot control directly and which may vary markedly and, often, rapidly. To cope with this sort of situation, microorganisms (we now know) have acquired in the course of evolution a whole armoury of sophisticated control mechanisms which allow them to change *themselves*, structurally and functionally, in order to accommodate to environmental changes and thereby to maintain their growth potential. Indeed, to such an extent can these creatures vary *phenotypically* (as it is said) – that is, both structurally and functionally – that it is virtually meaningless to talk of a 'normal' microbial cell. The state of 'normalcy', which has profound meaning so far as the cells of higher animals and plants are concerned, has little or no meaning with regard to the microbial cell. In short, then, it is impossible to define physiologically any microbial cell without reference to the environment in which it is growing. This is a most important (indeed fundamental) concept that is not widely appreciated and yet which fully accounts for the fact that microbes generally can be cultivated in the laboratory under conditions which may be far removed from those which they experience in natural ecosystems.

A detailed description of the structural and functional changes that have been found to occur in microbial cells, particularly when exposed to low-nutrient environments, is beyond the scope of this chapter. It is sufficient to state here that almost all components of the microbial cell vary quantitatively with changes in the growth conditions, and many components may also vary qualitatively.

It is now appropriate to return to the events occurring in a laboratory batch culture, and to reexamine these in the context of cell–environment interaction.

III. Microbial Growth in an Open Environment

It follows from the various considerations outlined above, that if one is interested in any one of a large number of aspects of microbial physiology (that is, cell structure and functioning), one cannot ignore the fact that the properties of organisms growing in a batch culture vary continuously throughout the growth cycle by virtue of the fact that the environment is varying continuously. In this connection, the tacit assumption that 'mid-exponential phase' or 'early stationary phase' meaningfully circumscribe the physiological state of the cells is clearly untenable since, as mentioned previously, these are periods during which the environment, and the properties of the cells, are changing dramatically.

How then can one get around this *impasse*? The answer is extemely simple once one takes into account the fact that the rate of change in each and every environmental parameter is linked directly to the rate of change of microbial biomass concentration (that is, to the growth rate of the organisms). Therefore, all one needs to do is to add fresh medium to the culture at a rate sufficient to maintain the culture population density at some prescribed submaximal value, thereby replenishing nutrients that are being consumed and, simultaneously, diluting out end-products that are accumulating at rates exactly sufficient to ensure that the environment no longer varies with time. Of course, if one continued to employ a closed system one would need to add fresh medium at an exponentially increasing rate, thus causing the culture volume to increase exponentially and soon reach unmanageable proportions. To overcome this difficultly, it is only necessary to insert an overflow tube (or weir) into the culture so that excess culture can flow from the growth vessel at the same rate that fresh medium is being added, thereby maintaining the culture volume constant. This is the operating principle of one type of continuous culture device the *Turbidostat* – and its essential features are illustrated in Figure 2.

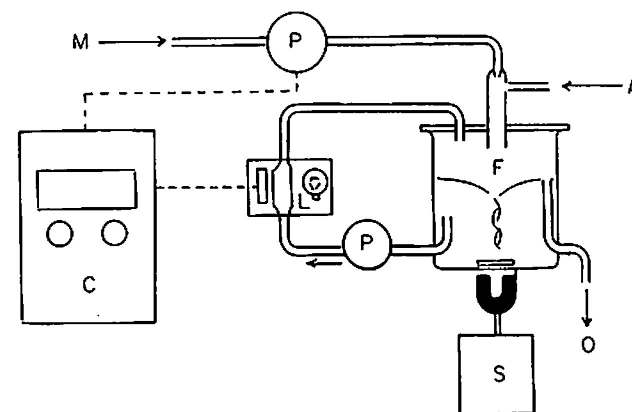

Figure 2. Essential features of a turbidostat. The culture is contained in a flask (F) to which medium (M) is pumped at a rate determined by the photocell (L) and the controller (C). The culture is vigorously stirred by means of a magnetic stirrer (S) and aerated by means of injecting air (A) along with the medium. Excess culture is removed by means of an overflow device (O). Two pumps (P) are required, one to deliver medium to the culture, and the second to circulate culture through the photometer cell.

A. Kinetics of Growth in a Turbidostat

It is now important to look at the dynamics of microbial growth in such an 'open' system. With such a culture, the total biomass concentration is maintained constant by first monitoring the culture population density (usually the culture optical density at some convenient wavelength of light) and, with the aid of a controller, activating a pumping device that delivers medium at a rate sufficient to maintain that population density at some prescribed value. Since biomass will be constant, the specific rates of utilization of all nutrients will be constant. Therefore, fresh medium need no longer be added at an exponentially changing rate, but at a constant rate that will be specified by the total culture biomass concentration (that is, by $x \cdot V$, where x is the concentration of organisms per ml and V is the culture volume in ml). The specific rate of substrate consumption is, as mentioned previously, defined mathematically by $-1/x \cdot ds/dt$ and, for a turbidostat culture, can be determined experimentally by determining the concentrations of substrate in the inflowing medium and effluent supernatant. Thus:

Rate of change = Input − Output − Consumption

$$ds/dt = fS_R - fs - qxV$$
$$= f(S_R - s) - qxV$$

where S_R is the substrate concentration in the reservoir, s the substrate concentration in the culture extracellular fluid, f the medium flow rate (ml/h), x the microbial biomass concentration (mg equivalent dry weight organisms/ml), V the culture volume, and q the specific rate of substrate consumption ($-1/x \cdot ds/dt$). However, once the culture is in a steady state, the rate of change in substrate concentration will be zero. Hence:

$$qxV = f(S_R - s)$$
$$q = D(S_R - s)/x$$

where D is the ratio of the flow rate to the culture volume (f/V) and is called the *dilution rate*. Now the ratio $(S_R - s)/x$ is the reciprocal of the yield value Y; therefore, at steady state:

$$q = D/Y. \qquad (1)$$

A further point of clarification is now necessary. With turbidostat cultures (as, indeed, with chemostat cultures; see below) fresh medium is added at a constant linear rate (f ml/h) and yet the culture is clearly growing exponentially. At first sight this seems anomolous, but then it must be remembered that the culture is, in effect, being diluted at an

exponential rate by virtue of the fact that the overflow device removes excess culture at the rate of one drop per drop of fresh medium entering the growth vessel. The situation can best be appreciated by considering how the population density would change with time if the culture was suddenly sterilized by adding, say, a few drops of formalin, and yet the supply of fresh medium was maintained at the same rate as that used by the growing culture. The first drop of medium entering the culture vessel would dilute the culture by an infinitesimally small fraction, yet it would displace a drop of almost undiluted culture. The next drop of medium would dilute the culture by a second small increment, and again displace a drop of almost undiluted culture. Thus, by the time that the equivalent of one culture volume of fresh medium had been added, the culture would contain not half the initial concentration of organisms, but considerably less. In fact, the actual concentration of organisms present after the passage of one culture volume of medium through the vessel can be calculated as follows:

If the initial concentration of organisms was x_0 (mg-equivalent dry weight organisms/ml) and the culture volume V (ml), then the addition of one drop of medium would decrease the organism concentration by a factor $V/(V + dV)$. After the removal of one drop of culture and the addition of a second drop of medium, the dilution factor would be $(V/(V + dV))^2$, and so on up to $(V/(V + dV))^n$. Therefore, to find the extent to which the culture had been diluted after the addition of a number of drops collectively equalling one culture volume (that is, $n = V/dV$), one must determine the numerical equivalent of the expression: $(V/(V + dV))^{V/dV}$. This can be solved as follows:

$$\left(\frac{V}{V + dV}\right)^{V/dV} = \left(\frac{V/dV}{V/dV + 1}\right)^{V/dV} = \left(\frac{n}{n + 1}\right)^{n} = \left(\frac{1}{1 + 1/n}\right)^{n}$$

or

$$\underline{(1 + 1/n)^{-n}.}$$

And since, by definition, $\lim_{n \to \infty}(1 + 1/n)^n = e$ (or 2.71828 by calculation, after expansion by means of the Binomial Theorem), then it must follow that $\lim_{n \to \infty}(1 + 1/n)^{-n} = e^{-1} = 0.36788$. Hence, after the passage of one culture volume of medium through the vessel, the concentration of the (non-growing) population of organisms will have decreased from x_0 to $0.368x_0$. In general, then, and since the number of culture volumes pumped to the growth vessel over a specific period of time t will be tf/V (or Dt), the concentration of organisms present at any time t will be related to that initially present by the equation:

$$x = x_0 \, e^{-Dt}. \qquad (2)$$

Of course, this applies only to a non-growing culture. Where organisms are growing at a rate such that their steady state concentration is not changing with time (as in a turbidostat), then it is clear that the growth rate must be numerically equal to the wash-out rate. It should now be clear why it is preferable to express the specific growth rate μ in terms of its natural logarithm (i.e. $(\ln 2)/t_d$).

B. Kinetics of Growth in a Chemostat

It is now appropriate to consider what would happen with a turbidostat culture if the optical density control system was disconnected and the pump set to deliver medium to the culture at some rate *less* than that required to maintain the culture population density constant. Clearly, since the growth rate of the organisms would be greater than the dilution rate, the concentration of the organisms in the culture would increase. But it could not go on increasing indefinitely, for the same reason that it does not do so in a batch culture – that is, because of the growth-associated changes in the environment. On the other hand, growth would not ultimately cease, as it does in a batch culture, because the continuous addition of fresh medium to the culture would continuously replenish nitrients that were being used up, albeit at a suboptimal rate, and similarly, continuously dilute out end-products of metabolism that were accumulating. Clearly, a situation ultimately must be established in which environmental changes cause the growth rate to decrease to a point where once more it is equal to the dilution rate and steady state conditions prevail. This is the basic operating principle of a second type of continuous culture device – that is, the *Chemostat*.

Now, if the organisms were growing in a complex nutrient medium, such as tryptic digest of meat or casein hydrolysate, one would not know precisely the nature of the environmental changes that ultimately caused the growth rate of the organisms to decrease from their maximum value. However, if one constructed a more defined medium (say, a simple-salts medium) and arranged for one nutrient to be present at a concentration such that, in a batch culture, it would become depleted before any other nutrient was fully used up, then it would be the insufficiency of this nutrient that would cause the growth rate of organisms in a chemostat culture ultimately to decrease to a value equal to the dilution rate. In other words, it would be the rate of supply of this nutrient, added along with the other essential nutrients in the fresh medium, that would prescribe the rate of growth of organisms in the culture. If the limiting factor was the medium glucose concentration, one would term the chemostat culture 'glucose limited'. Alternatively it could be limited by the supply of utilizable nitrogen source or source of sulphur, or by the availability of phosphate, potassium or magnesium. With organisms having some auxotrophic requirement, growth may be limited by the medium content of, say, some purine, pyrimidine, vitamin or amino acid.

It is obvious that with such a nutrient-limited culture it will be the concentration of nutrient in the culture *extracellular fluids* that is actually limiting the rate of cell synthesis since the rate at which such a substrate is taken into the cell will be a function of its concentration. If the uptake process involves some enzyme-catalysed reaction, then one might expect that the relationship between uptake rate (and growth rate) and concentration of limiting substrate would be of the form of a Michaelis–Menten equation. That is,

$$V = V_{max}\left(\frac{s}{K_s + s}\right) \text{ or } \mu = \mu_{max}\left(\frac{s}{K_s + s}\right), \qquad (3)$$

where V is the rate of penetration of substrate into the cell, V_{max} the potentially maximum rate of substrate uptake, s the actual concentration of substrate and K_s a saturation constant that is numerically equal to the substrate concentration that allows the uptake process to proceed at one-half its maximum rate. Similarly, μ is the specific growth rate expressed in the nutrient-limited chemostat culture, μ_{max} the growth rate that would be expressed if the substrate was present in a non-limiting concentration, and K_s a saturation constant that is numerically equal to the growth-limiting substrate concentration that would allow growth to proceed at one-half its potentially maximum rate. The above relationship was found, by Monod, to hold for many real cultures – at least approximately so, and though it may not be valid under all circumstances, it forms the cornerstone of much of the theory of microbial growth in chemostat cultures.

We have already shown by a process of reasoning that, with the dilution rate set below some critical value (D_c) at which the organisms express their maximum growth rate, steady state conditions ultimately must be established in which the specific growth rate (μ) and dilution rate (D) are equal. Further, we have suggested (equation 3) that, under such conditions, growth rate is actually limited by the concentration of some essential nutrient present in the culture extracellular fluid which causes a 'master' reaction to proceed at a submaximal rate. It is now necessary to restate these conclusions in a more formal way – a way which shows how a chemostat culture will respond to perturbations of the steady state.

As with a turbidostat culture, the change in concentration of organisms with time will depend on the balance between the growth

rate μ and dilution rate D such that:

$$\begin{aligned} \text{Change} &= \text{Growth} - \text{Washout} \\ dx/dt &= \mu x - Dx \\ dx/dt &= x(\mu - D). \end{aligned} \quad (4)$$

Therefore, whenever $\mu > D$, the concentration of organisms in the culture will increase with time and whenever $\mu < D$ it will decrease with time. However at any fixed value of D, changes in the population density will be dependent on μ which itself is critically dependent on s (the growth-limiting substrate concentration in the culture extracellular fluids), since:

$$\mu = \mu_{max}\left(\frac{s}{K_s + s}\right)$$

Hence,

$$dx/dt = x\left[\mu_{max}\left(\frac{s}{K_s + s}\right) - D\right] \quad (5)$$

Similarly, changes in the concentration of the growth-limiting nutrient will depend upon the balance between the input rate, the rate of consumption, and the rate of loss in the overflow culture. Thus:

$$\begin{aligned} \text{Change} &= \text{Input} - \text{Output} - \text{Consumption} \\ ds/dt &= fS_R \quad - fs \quad - qxV \end{aligned}$$

where S_R is the concentration of growth-limiting nutrient in the reservoir, s its concentration in the culture extracellular fluids, q the specific rate of substrate consumption $(-1/x \cdot ds/dt)$, f is the medium flow rate, and V is the culture volume. Thus, per unit volume of culture:

$$-ds/dt = D(S_R - s) - qx$$

and since

$$\begin{aligned} q &= \mu/Y, \\ -ds/dt &= D(S_R - s) - \frac{\mu x}{Y} \end{aligned} \quad (6)$$

It follows from equations (3), (4), and (5), that any sudden increase in the concentration of growth-limiting nutrient *in the culture* will cause an increase in the specific growth rate and therefore an increase in the culture population density. But this increase in growth rate and population density will effect an increase in the rate of substrate utilization such as to cause the concentration of the growth-limiting nutrient to fall back to its initial steady state value (equation 6). As the growth-limiting substrate concentration progressively diminishes, so too does the specific growth rate and specific rate of substrate consumption so that ultimately steady-state conditions are reestablished. In this connection, any overshoot that may occur in the rate of substrate consumption (due, for example, to there being an increased concentration of organisms in the culture) will cause the specific growth rate to fall to a value less than the dilution rate. Hence wash-out will occur to a point where the concentration of organisms reaches such a value that the rate of consumption of growth-limiting substrate $(q \cdot x \cdot V)$ again balances the rate of supply of that nutrient (fS_R) minus its rate of loss in the overflow culture (fs).

It can be seen, therefore, that perturbations of the steady-state conditions set up reactionary forces that ultimately (and often rapidly) reestablish the steady state. Once the culture is in a steady state, unique values exist for both the organism concentration (x) and the growth-limiting substrate concentration (s), which are given the symbols $\bar{x}$ and $\bar{s}$, respectively. Thus:

$$\bar{x} = Y(S_R - \bar{s}) \quad (7)$$

and,

$$\bar{s} = K_s\left(\frac{D}{\mu_{max} - D}\right). \quad (8)$$

Substituting for $\bar{s}$ in equation (7), it follows that:

$$\bar{x} = Y\left[S_R - K_s\left(\frac{D}{\mu_{max} - D}\right)\right] \quad (9)$$

Therefore, since μ_{max}, K_s, and Y are constants, the principal effect of varying the dilution rate will be to change the concentration of growth-limiting substrate (equation 8) thereby effecting a change in the specific growth rate (equation 3) and doubling time of the culture. Moreover, assuming the yield value Y to be independent of dilution rate, and K_s to be small relative to S_R, it follows from equations (7), (8), and (9) that varying the dilution rate should produce a pattern of changes in the steady-state microbial concentration and concentration of growth-limiting substrate as depicted in Figure 3(A). On the other hand, if K_s is relatively large compared with S_R, then the pattern of changes of $\bar{x}$ and $\bar{s}$ with D should be more like that represented in Figure 3(B).

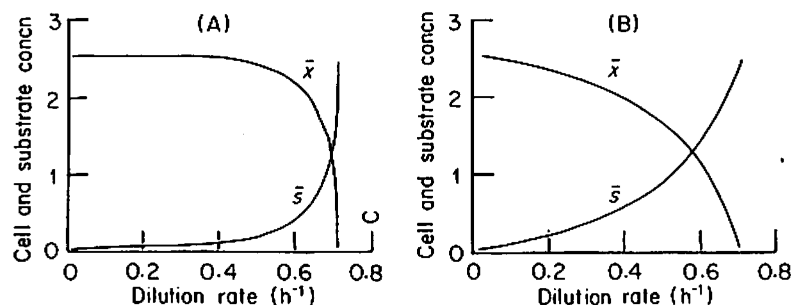

Figure 3. Theoretical changes in cell concentration $\bar{x}$) and growth-limiting substrate concentration ($\bar{s}$) with dilution rate in two cases: (A) when the K_s value is small relative to S_R, and (B) when K_s is large relative to S_R. Reproduced by permission of Academic Press Inc. (London) Ltd.

C. Other Quantitative Relationships: Yield Values

It was stated earlier in this chapter that growth involved the uptake and chemical transformation of a number of essential compounds and elements, and that the *rates* of uptake of these metabolites were proportional to the growth rate, thus:

$$\mu \propto q$$

$$\mu = Yq.$$

The proportionality factor (Y) is termed the *yield value*; it is the ratio of two rate processes (μ and q), but itself has no time component (e.g. g cells formed per mol substrate consumed). It is now necessary to examine the relationship between this yield value and growth-associated properties of the microbial culture.

Now equations (6) and (9), which define the behaviour of populations in chemostat culture, imply that the yield value is constant and independent of the growth rate. Hence, at all dilution rates, μ and q should bear the same relationship to one another, and to the dilution rate D. That is:

$$D = \mu = Yq.$$

Hence, as $D \rightarrow 0$, so too must μ and q such that Y (which is proportional to $\bar{x}$, equation 7) remains constant. In practice, this is rarely found to be the case, and with substrates as disparate as sugars (and other carbon-containing substrates), phosphate, ammonia, potassium, magnesium, and oxygen, the yield value (as indicated by the steady-state bacterial concentration) varies markedly with the growth rate (Figure 4A).

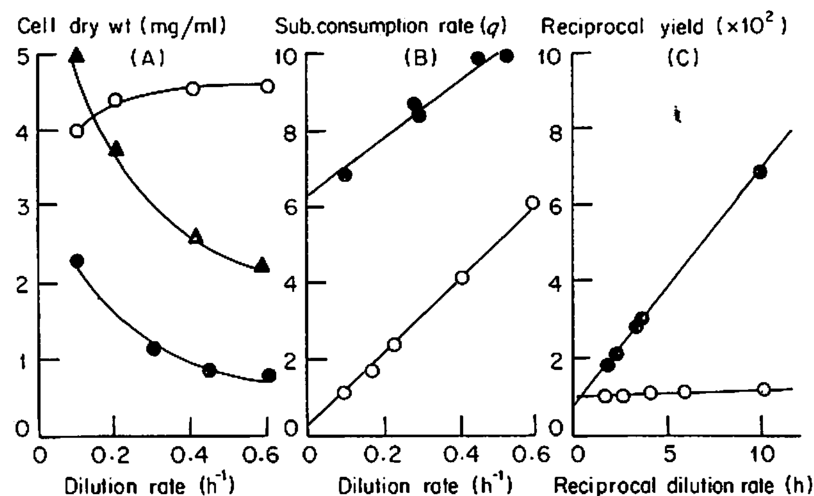

Figure 4. (A) Influence of dilution rate on the concentration of *Klebsiella aerogenes* organisms in (○) a glucose(50 mM)-limited culture; (●) a phosphate(0.75 mM)-limited culture, and (▲) a magnesium (0.25 mM)-limited culture. (B) The influence of dilution rate on the specific rate of glucose uptake by (○) a glucose-limited and (●) a phosphate-limited culture of *K. aerogenes*. (C) The relationship between the reciprocal of the yield value for glucose and the reciprocal of the dilution rate for cultures of *K. aerogenes* that were (○) glucose limited and (●) phosphate limited.

With carbon substrates, and with oxygen, it is generally found that their specific rates of consumption q are indeed linear functions of D, but that, on extrapolation to $D = 0$, the lines do not pass through the origin but intersect the ordinate at finite values of q (Figure 4B). And since $Y = \mu/q$ (or, at steady state, D/q) it is clear that as D approaches zero, so too must Y. In order to explain this variation in yield value with growth rate, it was proposed that a portion of the carbon substrate (and of oxygen) was required to deliver up energy that was needed for growth-independent 'maintenance' functions (for example, for the maintenance of solute gradients across the plasma membrane, and for turnover of macromolecular components of the cell). Hence, the extrapolated substrate uptake rate at zero growth rate could be taken as a direct measure of this maintenance energy requirement and could be subtracted from the actual rate of substrate consumption to derive an evaluation of the 'true' growth-associated substrate requirement. Thus:

$$q_{\text{actual}} = q_{\text{growth}} + q_{\text{maintenance}}.$$

Dividing by μ (=D at steady state),

$$q_a/\mu = q_g/\mu + q_m/\mu$$

and since q/μ is the reciprocal of the yield value Y, then:

$$1/Y = 1/Y_g + (q_m \times 1/\mu) \quad (10)$$

where Y_g is the 'true' growth yield constant (that is, the yield value corrected for maintenance losses). Therefore, plotting $1/Y$ against $1/\mu$ (or $1/D$) should give a straight line with a slope equal to g_m that intersects the ordinate (when $1/D = 0$) at a value of $1/Y_g$ (Figure 4C).

Now whereas the use of double reciprocal plots of yield versus growth rate can provide measurements of both the so-called maintenance energy requirement and the 'true' (maximum) yield value, they suffer the disadvantage that heavy emphasis is placed on the substrate consumption rate expressed at low growth rates. This can be readily appreciated by considering the fact that the difference between the reciprocals of $D = 0.1$ and $0.2\ h^{-1}$ is 5.0 whereas the difference between the reciprocals of $D = 0.4$ and $0.5\ h^{-1}$ is only 0.5. Moreover, an evaluation of both q_m and Y_g can be obtained from a direct plot of substrate uptake rate versus growth rate since the slope of this line (that is, $ds/d\mu$) is itself the reciprocal of the maximum yield value, and the intersect with the ordinate (when $D = 0$) is the maintenance rate (q_m). The precise relationship between the plots of q versus μ (or D) and $1/Y$ versus $1/D$ can be best understood by considering the general equation for a straight line – that is:

$$Y = ax + b.$$

In the case of a plot of q against D,

$$q = aD + b.$$

Now b is the value of q when $D = 0$ and has been termed (above) the 'maintenance' rate (q_m). Further, a is the slope of the line (that is dq/dD) and has the same units as reciprocal yield: it is a constant that is numerically identical with $1/Y_g$. It follows, therefore, that the above equation can be written:

$$q = (D \times 1/Y_g) + q_m,$$

and dividing this equation by D transforms it into equation (10): that is,

$$q/D = 1/Y_g + (q_m \times 1/D),$$

or

$$1/Y = 1/Y_g + q_m/D.$$

This latter derivation of the growth yield equation reveals the uncertain nature of the constant Y_g. It is, in fact, the incremental increase in substrate consumption rate required to support an incremental increase in growth rate. However, when comparing populations of organisms growing at different rates one is not comparing like with like since, compositionally and functionally, organisms vary markedly with growth rates. In some circumstances this may not create serious problems of interpretation, but in others it does. Thus, populations growing in the presence of an excess of carbon substrate, and with growth limited by the availability of, say, phosphate, express a high rate of carbon substrate uptake (that is a low yield value) but the incremental increase in substrate consumption rate with growth rate ($1/Y_g$) is much decreased, giving the false impression that these organisms are expressing a vastly improved 'true' growth yield (Y_g) as compared with carbon substrate-limited cultures (Figure 4B). The absurdity of this conclusion becomes apparent when one considers the situation at or near μ_{max} (that is, close to D_C). Here $\bar{s}$ is approaching S_R and the culture is only nominally limited by the chosen growth-limiting substrate. In effect, all such cultures must be virtually identical, irrespective of their nominal limitation, and must express the same substrate uptake rate and yield value. Hence they must be growing with the same real efficiency: Their maintenance requirements (at μ_{max}) must be closely similar, even though they may be vastly different at low growth rates, and hence their 'true' yield values for carbon substrate also must be similar. An erroneous interpretation of the data obtained with carbon-sufficient chemostat cultures arises from the assumption that the 'maintenance rate' is a constant, which clearly it is not. Indeed, the fact that a linear relationship generally has been found between the specific rate of substrate consumption and the growth rate has been used to support the conclusion that the maintenance rate *must* be constant and independent of growth rate. But clearly this need not necessarily be so, though the fact that the substrate consumption rate increases linearly with growth rate suggests that, should the maintenance rate vary, then in all probability it also would do so as a linear function of the growth rate. It is therefore sensible to modify equation (10) to allow for the possibility of a varying maintenance rate, and the simplest way of doing this is to assume, as stated above, that should the maintenance rate vary (positively or negatively), it will do so as a linear function of the growth rate. Then:

$$1/Y = 1/Y_g + q_m(1 \pm cD)/D$$

where c is the slope of the line of q_m versus D. Rearrangement of this equation reveals that the main effect on any progressive change in q_m, with dilution rate, will be to change the value of Y_g:

$$1/Y = (1/Y_g \pm cq_m) + q_m/D. \quad (11)$$

This modification allows of the possibility (indeed probability) that variously-limited chemostat cultures may express markedly different relationships between q and D and yet be closely similar in their strictly growth-associated substrate requirements.

The importance of yield values (particularly those for carbon substrate and oxygen) resides in the fact that they are indicators of the energetic efficiency with which microbes grow. Thus, as shown in Figure 5, the carbon substrate is taken up and metabolized to provide both intermediates and reducing equivalents required for cell synthesis, and further reducing equivalents necessary to provide, aerobically, substrate for the ATP-generating reactions of respiration. Clearly, some balance must be established between the substrate-catabolizing reactions associated with the generation of biologically useful energy, and the substrate- and ATP-consuming reactions of anabolism. This balance is reflected in the yield value.

Since the major currency of biologically useful energy is ATP, it might be argued that a more meaningful comparison of the efficiencies of growth of different organisms on some specific substrate (or of a specific microbial species on a range of different carbon substrates) can best be made on the basis of Y_{ATP}, that is, g organisms synthesized per mol of ATP *consumed*. The problem here is that, generally speaking, it is only the generated ATP that can be measured, and then only with a degree of imprecision. It can be assumed, of course, that all the ATP that is generated is turned over in energy-consuming reactions, since the adenine nucleotide 'pool' is of a relatively constant size and composition, and only a small amount of the ATP synthesized is actually incorporated into cell substance (e.g. as RNA and DNA). However, what bedevils any realistic evaluation of Y_{ATP} is the seeming absence of direct coupling (in the strict sense of the word) between ATP

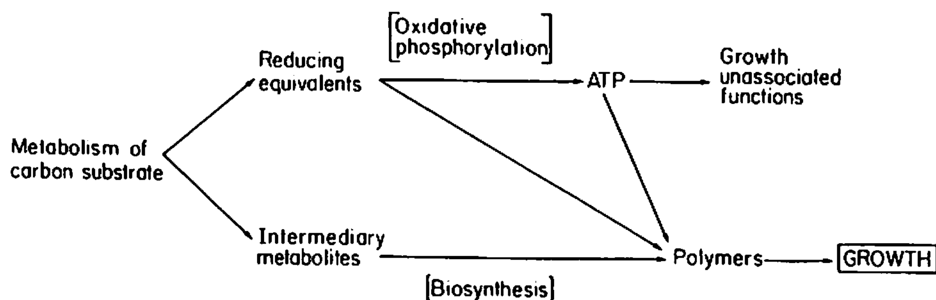

Figure 5. Pathways of carbon and energy flow in the growth of microorganisms. Schematic representation.

turnover and biosynthesis. In other words, although biosynthesis causes the ATP pool to turn over, this can equally well happen in the absence of biosynthesis. Indeed, this is patently obvious from the fact that washed suspensions of organisms can oxidize substrates like glucose at a high rate under conditions where polymer synthesis is grossly impeded. It follows, therefore, that any comparisons of the efficiency of utilization of ATP in biosynthesis are likely to be misleading, unless methods are found for assessing that portion of the ATP that is turned over by processes that are not associated with biosynthesis.

Notwithstanding the above strictures, evaluations of Y_{ATP} (as the ratio of cells synthesized per mol ATP *synthesized*) have been made for a number of microbial species, and comparisons drawn. The least equivocal situation is found with cultures of strictly anaerobic bacteria, particularly with those species that have no electron transport chain activity. In such species, ATP is generated by substrate-level phosphorylation reactions, and therefore ATP formation from ADP and P_i is a coupled process (i.e. catabolism cannot occur in the absence of ATP synthesis). The actual rate of ATP synthesis can be assessed from the rate of substrate consumption and/or by the rate of accumulation of some product whose synthesis involved substrate-level phosphorylation reactions. In addition, the use of substrates that cannot be assimilated into cell substance, either directly or after partial catabolism, further aids in eliminating 'imponderables' from the final assessment. With those organisms fulfilling most of the above conditions, ratios in the region of 10 (for g organisms synthesized per mol ATP formed) have been obtained. Later estimates, which attempted to correct for the maintenance energy requirement, were in the region of 14.

With organisms that can generate ATP by electron transport chain (oxidative phosphorylation) reactions (i.e. aerobes and anaerobes), so many pitfalls are encountered that a reliable evaluation of yield per mol ATP synthesized is seemingly impossible. With organisms growing aerobically, the major stumbling block is the assessment of the efficiency of oxidative phosphorylation. In this connection, it is known that the complete oxidation of 1 mol of glucose requires 6 mol of oxygen and can lead to the synthesis of, maximally, 38 mol of ATP (from ADP and P_i). Hence, since the rate at which oxygen is consumed by growing organisms can be accurately determined, then by assuming a ratio of $q_{ATP}:q_O$ of 38/12 (that is, 3.17), the Y_{ATP} (in terms of g organisms synthesized/mol ATP *generated*) can be calculated from the equation:

$$Y_{ATP} = Y_O/3.17,$$

where Y_O is the g organisms synthesized per g-atom oxygen consumed.

In practice, this gives values well below 10 (e.g. 7–8 with *Klebsiella aerogenes* growing on glucose as the sole carbon and energy source) which is uncomfortably low – though possibly realistic. On the other hand, if the oxidation of 1 mol of glucose generated less than 38 mol of ATP (that is, if the efficiency of oxidative phosphorylation was less than that found with, say, mitochondria) then the actual Y_{ATP} value may be considerably higher – that is, much nearer to the value found with organisms growing anaerobically. As yet, however, there is seemingly no way of deciding unequivocally the number of sites of energy conservation on the respiratory chains of prokaryotic organisms, or, indeed, whether the transfer of electrons from NADH to oxygen is coupled *stoichiometrically* to the phosphorylation of ADP. All that can be stated is that the rate of ATP synthesis (q_{ATP}) will be related to the rate of oxygen consumption and to the efficiency of oxidative phosphorylation by the generalized equation:

$$q_{ATP} - q_{ATP}^{s.l.} = 2q_{O_2} \times P/O$$

where $q_{ATP}^{s.l.}$ is the rate of ATP synthesis by substrate-level phosphorylation reactions, and P/O the ratio of the number of ADP molecules phosphorylated per atom of oxygen that is reduced. Assuming that, aerobically, substrate-level phosphorylation reactions contribute only to a minor extent to the overall rate of ATP synthesis, then, by dividing the above equation by the dilution rate D, one obtains the relationship:

$$1/Y_{ATP} \approx (P/O) \times 1/Y_O$$

or,

$$Y_{ATP} \approx Y_O/(P/O). \tag{12}$$

Again it should be emphasized that Y_{ATP}, as defined by equation (12), is not a genuine yield value but simply a comparison of two rates of synthesis (cell synthesis and ATP synthesis). Yield in the sense of cells synthesized per mol of ATP *consumed* in growth-associated processes may be something very different.

The use of facultatively anaerobic organisms for studies of the energetics of cell synthesis would seem to offer some advantages since, by comparing organisms growing at a fixed dilution rate, first aerobically and then anaerobically, one might think that it would be possible to derive corresponding values for both q_{ATP} (anaerobically) and q_{O_2} (aerobically), from which the efficiency of oxidative phosphorylation could be evaluated directly. However, the obvious snags with this procedure are, firstly, that it is exceedingly difficult to ensure that absolutely no oxygen enters a stirred fermenter, for should it do so, even in minute traces, a false evaluation of q_{ATP} will be made.

Secondly, anaerobic cultures may be fundamentally different from aerobic cultures in that the former will, in all probability, be energy-limited, whereas it is conceivable (indeed likely) that the latter will be carbon-limited. Therefore the rate of aerobic ATP synthesis may be substantially greater than the anaerobic synthesis with cultures of organisms *growing at the same dilution rate.*

In view of the uncertainties surrounding the assessment of q_{ATP} values, and the multiplicity of processes (both growth-associated and growth-unassociated) that cause the ATP pool to turn over, it would seem inappropriate at the present time to formulate more detailed equations relating growth to the synthesis and utilization of ATP.

D. Transient-state Phenomena

Although a chemostat culture is inherently self-balancing and tends always to move towards the establishment of a steady state, much can be learned about the properties of microorganisms by either perturbing the steady state or else changing it, and then following the events occurring during the transitional stages leading to the establishment of a new steady state. Indeed, since chemostat cultures provide fiercely selective conditions in which mutant organisms may rapidly replace the parent organisms in the culture (see following section), analysis of the kinetics of transient states may provide the most direct method of determining whether different physiological properties, expressed by organisms growing in different environments, are the result of phenotypic variation or mutant selection. A specific example serves to make the point.

Bacillus subtilis possesses a wall that contains substantial amounts of teichoic acid (a phosphorus-containing polymer). When grown under conditions of phosphate limitation, however, synthesis of this wall-bound teichoic acid is seemingly inhibited and, in its place, a non-phosphorus-containing polymer (teichuronic acid) is formed. The basic question that has to be answered, therefore, is whether the change in wall composition is due to regulation of the syntheses of teichoic acid and teichuronic acid, or whether it is, in fact, due to the selection of some mutant organisms that were present as a small proportion of the initial population. Further, if the former is the case, then it would be important to determine whether the replacement of one wall-bound polymer by some other polymer involved turnover of the wall or simply a progressive build-up associated with the synthesis of new wall material. Both these questions can be answered by analysing the kinetics of the change-over in wall polymer composition during the transitional stages between one type of growth limitation and the other, as detailed below.

Supposing that the organisms exhibited some property p (e.g. teichoic acid) that was present in high concentration in the initial population, but virtually absent from the steady-state population following a change in the growth condition. Then, if at time t_0 the cells ceased to synthesize this component (but did not destroy that already present), its concentration in the culture would decline at a rate such that:

$$p_t/p_0 = e^{-Dt} \tag{13}$$

where p_0 is the concentration of the substance in the culture at time t_0 and p_t is its concentration after time t. Similarly, if some change occurred in the reverse direction (that is, if at time t_0 the organisms started to synthesize some component that was not previously synthesized, and at a rate proportional to the growth rate), then its concentration would increase at a rate such that:

$$p_t/p_s = 1 - e^{-Dt}$$

or

$$\ln(1 - p_t/p_s) = -Dt \tag{14}$$

where p_s represents the final steady state concentration of the property, and p_t its concentration after time t.

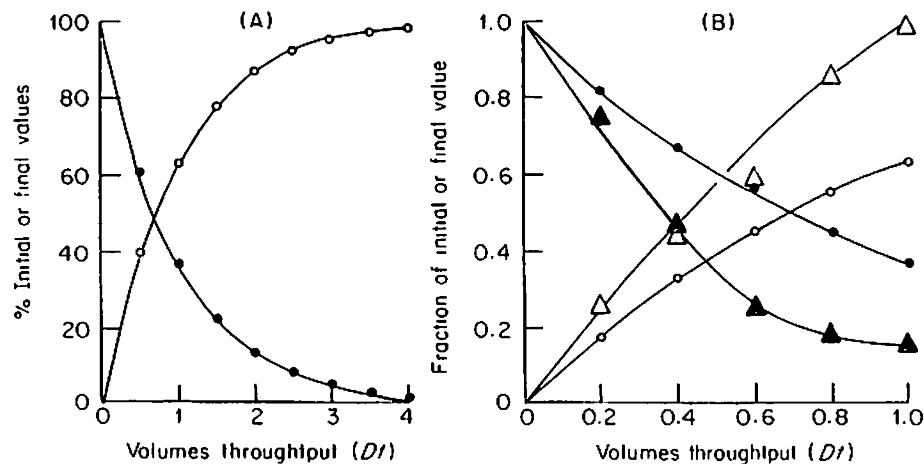

Figure 6. (A) Hypothetical washout (●) and build-up (○) curves prescribing the rates of change in some property that either ceases, or starts to be synthesized, at time zero, and whose synthesis is strictly growth associated. Reproduced by permission of Academic Press Inc. (London) Ltd. (B) Changes in the culture content of (▲) teichoic acid and (△) teichuronic acid following the change from magnesium limitation to phosphate limitation with a chemostat culture of *Bacillus subtilis* var. *niger*: lines indicated by (○) and (●) indicate the theoretical rates of change assuming no turn-over of wall material.

It follows, therefore, that if the change in the composition of the wall of *B. subtilis*, following a change in its environmental conditions, was due to some phenotypic change, then the *minimum* rate at which this should occur would be defined by the exponential wash-out rate (equation 13). A faster rate of change would indicate actual turn over of the existing wall material, whereas a substantially lower rate of change-over would be indicative of mutant selection (Figure 6A). The results that were actually obtained are shown in Figure 6B. These clearly indicate that this organism does possess the capacity to change its wall composition phenotypically, and that this may, indeed, involve turn over of the wall material.

IV. Growth of Mixed Microbial Populations

A landmark in the history of microbiology was the development of techniques for isolating individual microorganisms and for culturing them free from the 'contaminating' organisms with which they were naturally associated. And although, in recent years, interest has tended to move towards the behaviour of organisms in mixed cultures, nevertheless, microbiologists still invest heavily in studies of axenic cultures. Therefore it is important to understand the environmental forces which may act upon some contaminant organism that enters a batch or a chemostat culture, and to predict its fate.

Assuming no interaction between different organisms in a culture except competition for the available nutrient, it is clear that, with a closed system (batch culture), organisms of each species would accumulate at rates related to their exponential growth rate. This can be readily appreciated from a consideration of the basic growth equation:

$$N_t = N_0 2^n = N_0 2^{t/t_d}$$

assuming there to be present two different microbial species ('a' and 'b') initially at concentrations N_0^a and N_0^b, and further assuming that each species grew without exhibiting any lag period up to a point where further growth was impeded due to the exhaustion of an essential nutrient. At that time t (the onset of the stationary phase), the concentrations of organisms 'a' and organisms 'b' would be, respectively:

$$N^a = N_0^a 2^{n^a}$$

and

$$N^b = N_0^b 2^{n^b}$$

where n^a and n^b are the number of doublings of the initial populations (N_0^a and N_0^b) over the course of the exponential growth phase. It follows, therefore, that the ratio of organisms 'a' to organisms 'b' at the onset of the stationary phase would be:

$$N^a/N^b = 2^{(n^a - n^b)} N_0^a/N_0^b. \qquad (15)$$

Now the number of doublings (n) is inversely proportional to the population doubling time ($= t/t_d$) and directly proportional to the specific growth rate (since $\mu = 0.693/t_d$). In fact,

$$n = t/t_d = \mu t/0.693$$

Therefore the rate of change in the proportion of organisms 'a' to organisms 'b', in a batch culture, will depend solely on the difference in the specific growth rates of the two species. The extent to which one species outgrows the other will also depend on the number of doublings that the environmental conditions can support. Repeated subculturing will allow progressive enrichment of one species over the other to a point where, for all practical purposes, the culture becomes axenic. This condition applies not only to a culture that becomes contaminated with a different microbial species, but also to one which becomes self-contaminated (so to speak) with mutant organisms. In either case there will be selection of one cell type providing that there is some difference in the growth rate (that is μ_{max}) and that the culture is well mixed. The affinity of the different organisms for the nutrients present in the medium will have almost no effect on the selection process since, except for a brief period of time prior to the onset of the stationary phase, all nutrients will be present in cell-saturating concentrations (see Figure 1).

A very different situation obtains in a chemostat culture by virtue of the fact that organisms must compete for some essential nutrient that is present in a growth-rate-limiting concentration. Moreover, since a chemostat is an 'open' system, organisms that exhibit a growth rate lower than the dilution rate will be progressively washed out from the fermenter culture (see p. 7.00). The above arguments assume that Michaelis–Menten kinetics apply to the growth of organisms in nutrient-limited cultures. That is:

$$\mu = \mu_{max} \left(\frac{s}{K_s + s} \right)$$

If this is indeed the case, and that μ depends critically on s, then the outcome of a deliberate or accidental contamination of the culture can be predicted from the relationships that exist between μ and s for both

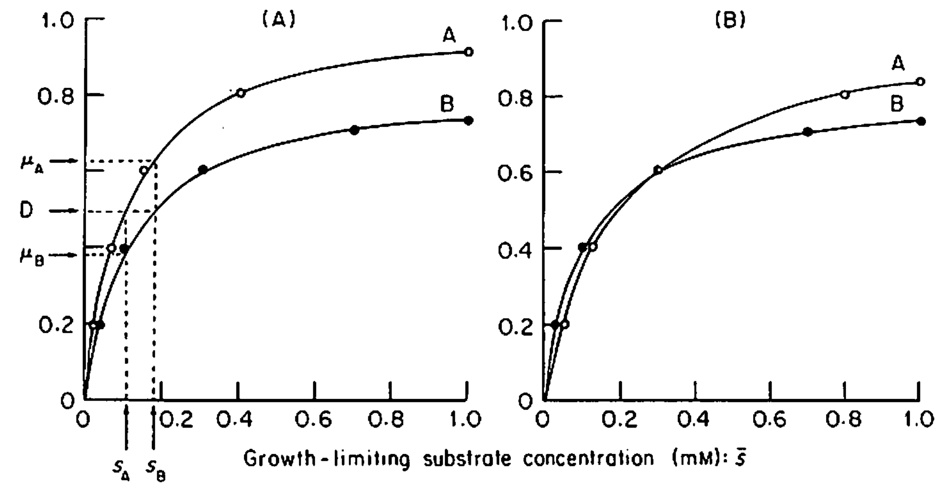

Figure 7. Theoretical curves for two organisms (A and B) showing the relationship between growth-limiting substrate concentration ($\bar{s}$) and specific growth rate ($\mu = D$) assuming Monod kinetics apply. In the first graph (A) the organisms have widely different affinities for the substrate, and different maximum growth rate values. Reproduced by permission of Academic Press Inc. (London) Ltd. In the second graph (B), organism A still possesses a capacity to grow faster than B at high substrate concentrations, but organism B has a greater affinity for the growth-limiting substrate.

the initial culture of organisms and for the contaminant organisms (Figure 7).

Figure 7 depicts graphically the relationship defined by equation (3) for two different species of organism (A and B) that exhibit different maximum growth rate values and have different affinities for the growth-limiting substrate. Let us assume that initially the chemostat contains a pure culture of organism B, growing at a dilution rate of $D\,h^{-1}$, and that this becomes contaminated with organism A. The growth-limiting substrate concentration in the culture at the time that the contamination occurred would be s_B, and at this concentration the contaminant organisms would grow at a rate equal to μ_A. Since this growth rate is greater than the dilution rate D, the concentration of contaminant organisms in the culture must increase (equation 4) but this will cause the growth-limiting substrate concentration to decrease correspondingly until μ_A equals D. At this time, the growth-limiting substrate concentration in the culture will have decreased to s_A which will permit organism B to grow only at a rate of μ_B. However, since μ_B is less than the fixed dilution rate D the concentration of B type organisms in the chemostat culture must diminish (ultimately to zero) as they are progressively washed from the growth vessel. Hence, with a

chemostat culture, as opposed to a batch culture, the selective pressure is not the maximum growth rate value (μ_{max}) but the affinity of the organisms for the growth-limiting substrate *at the dilution rate at which the culture is being grown*. Of course, Figure 7 represents an ideal situation in which the saturation curves are well separated. It is possible that the two saturation curves may cross, in which case (as indicated above) the outcome would depend on the dilution rate at which the culture was being grown.

It must be emphasized that the fate of the contaminant organism will be influenced by many factors and the situation described above assumes that (i) the dilution rate is maintained constant, (ii) the culture is in a steady state at the moment that contamination occurs, (iii) growth rate is limited by the availability of a single nutrient substance that is also essential for the growth of the contaminant organism, (iv) there is no interaction between the different organisms except for the competition for the single growth-limiting nutrient, (v) the culture is perfectly mixed and homogeneous (that is, there is no accretion of organisms on the walls and other parts of the culture vessel), and (vi) growth rates of the organisms adjust themselves to changes in substrate concentration without appreciable lag. These conditions may be adequately realized in many real chemostat cultures (as seen in Figure 7B) but it is not uncommon to find with chemostat enrichment cultures the establishment of a stable, mixed microbial population growing on a single growth-limiting, carbon-containing nutrient.

V. Concluding Remarks

When one considers the vast range of prokaryotic and eukaryotic microbes present in the biosphere, and the variety of environmental conditions that act upon these creatures in natural ecosystems, then it is clear that the half-dozen or so topics included in this chapter represent only a small fraction of those that properly could have been included under the title 'Dynamics of Microbial Growth'. However, restrictions of space necessitated a rigorous selection of the subject matter and, whilst this selection was by no means arbitrary, the omission of certain topics is not easy to justify. In particular, four important aspects of the subject had to be either omitted or treated superficially, and all that usefully can be done by way of compensation is to point to key publications where further information may be obtained.

Firstly it should be emphasized that, whereas growth has been considered principally at the level of the population, primarily it occurs

at the level of the cell. In this connection, much information has accumulated over the last decade on the mechanisms by which the events occurring in the cell division cycle are timed and coordinately regulated. And, although such information relates largely to cells growing at, or near, their potentially maximum rate, it forms a solid foundation for studies, yet to be undertaken, of organisms growing at the more ecologically relevant rates.

Secondly, although the rates at which microorganisms are likely to grow in natural ecosystems is far below those exhibited by most laboratory cultures, the consequences of growth at exceedingly low rates was not considered, nor was any analysis made of the influence of viability changes on the growth kinetics of the viable portion of a microbial population.

Thirdly, in considering the growth kinetics of mixed microbial populations, the simplifying assumption was made that no interaction occurred between cells other than competition for essential nutrient substances. However, although this condition may hold with many artificial mixtures of laboratory cultures, it is clear that, in natural ecosystems, other interactions (such as commensalism, host–parasite and prey–predator relationships) frequently occur.

Finally, no account was given of the dynamics of fungal growth. This is a subject of considerable interest, not only academically (by virtue of their particular growth mode) but also industrially, since these organisms are cultured extensively in the production of antibiotics and other economically important metabolites.

References to papers dealing with each of these four subjects are therefore included under 'Further Reading' below.

VI. Further Reading

1. Microbial Growth (General)

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2. Microbial Growth (Continuous Culture)

Herbert, D., Elsworth, R., and Telling, R. C. (1956). The continuous culture of bacteria; theoretical and experimental study. *Journal of General Microbiology*, 14, 601–622.

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Veldkamp, H. (1976). *Continuous Culture in Microbial Physiology and Ecology.* Durham, England: Meadowfield Press Ltd.

3. The Cell Cycle

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4. Other Aspects

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Powell, E. O. (1972). Hypertrophic growth. In: *Environmental Control of Cell Synthesis and Function, 5th International Symposium on the Continuous Culture of Micro-organisms*, pp. 71–78. London: Academic Press.