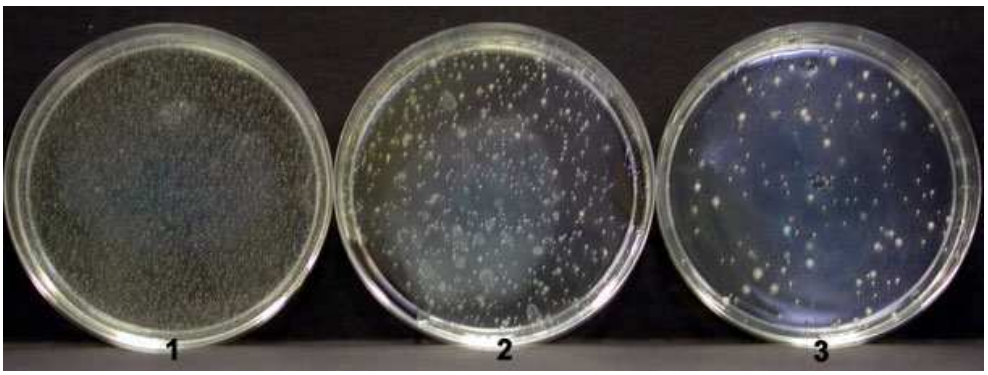
HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY

4. DETERMINATION OF THE PARAMETERS DEFINING THE BACTERIAL GROWTH



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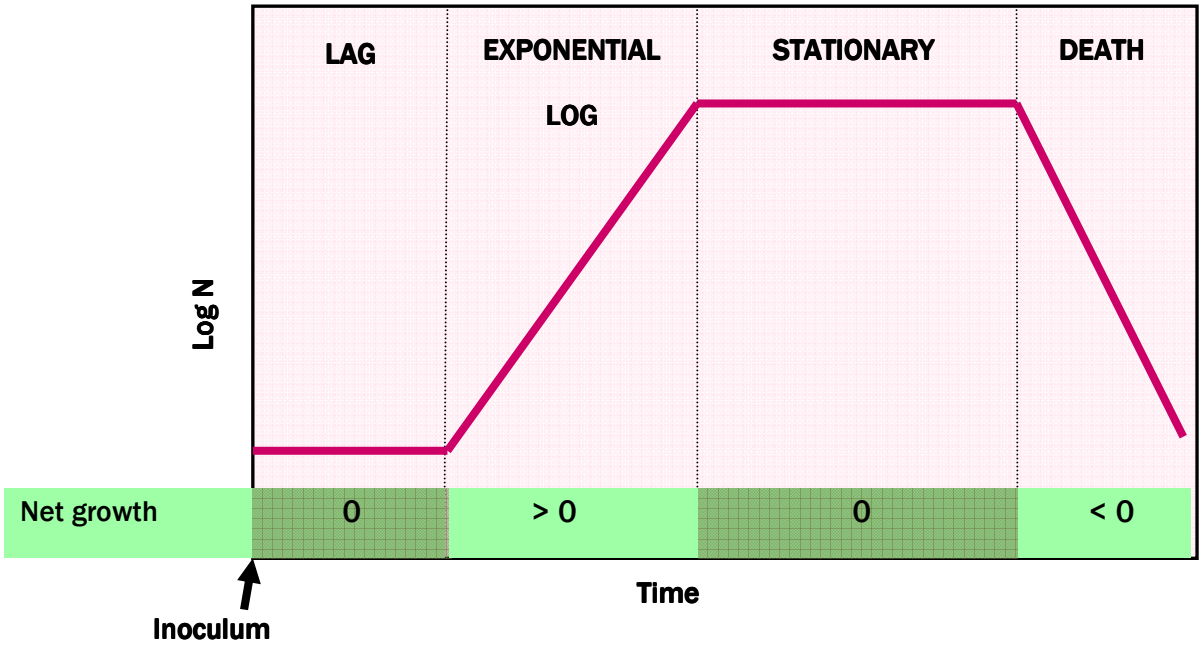
OCW 2013

4. DETERMINATION OF THE PARAMETERS DEFINING THE BACTERIAL GROWTH

Under controlled conditions, in the laboratory, the evolution of the number of cells over time can be followed in a batch culture. Bacteria growth can be represented with a growth curve, which consists of four phases: lag phase, exponential or log phase, stationary phase and death phase.



The concept of microbial growth and the mathematical expressions defining it, can be found in any textbook of Microbiology (Brock Biology of Microorganisms 2012, Prescott's Microbiology 2011).

Exponential phase is the growth phase itself and simplifying the mathematical development,

$$dN/dt = \mu N$$

$$N=N_0 e^{\mu(t-t_0)}$$

It is characterized by the following equation:

$$\text{Ln } N - \text{Ln} N_0 = \mu (t-t_0) \quad \text{or} \quad \log N - \log N_0 = \mu / 2.303 (t-t_0)$$

Where t = time, N = cfu ml^{-1} at time t, N_0 = cfu ml^{-1} at time t_0, μ = specific growth rate constant (h^{-1}).

It is possible to use other parameters such as the time required to double the population or **generation time, g**.

$$\mathbf{g = 0.693/\mu}$$

The inverse of the generation time is called **growth rate (K),** and it is expressed as generations / hour.

$$\mathbf{K = 1/g}$$

It can be calculated the μ_{max} (maximum specific growth rate) value for a given microorganism and substrate. For that, growth curves are performed with increasing concentrations of the substrate and the μ values are calculated for each concentration.

$$\mathbf{\mu = \mu_{max}\, S/(K_S + S)}$$

Where K_S = saturation constant for the substrate, the concentration where specific growth rate is half of μ_{max} ($\mu = ½\ \mu_{max}$).

It can be also used the following transformation of the equation:

$$\mathbf{1/\mu = 1/\mu_{max} + (K_s/\mu_{max})\ (1/S)}$$

In the **stationary phase** two interesting parameters can be determined: **maximum biomass** (cells or other parameter) **and yield coefficient.**

M is calculated by the following expression:

$$\mathbf{M = M_t - M_0}$$

Where M_t = biomass (cells, etc) at time t (it is calculated in the stationary phase, where the number of cells is maximum) and M_0 = inoculum biomass (cells, etc). The result is expressed in grams, milligrams, cells/ml, etc.

The **maximum yield coefficient** is the amount of biomass, cells or other parameter, produced per substrate consumed and is given by:

$$\mathbf{Y = Biomass\ produced/Substrate\ consumed = (M_t - M_0)/(S_0 - S_t)}$$

Where S_0 = substrate at the beginning of the culture and S_t = substrate at time (t) when the number of cells is maximum. The result is expressed as g cells/g substrate or Nº cells/g substrate.

So much for the theoretical aspects of the study of microbial growth, but how can the results be analyzed? We will see below how to work with the data.

The simplest problems are those in which various terms are known (i.e. initial density of microorganisms, μ value and time), and we must determine an unknown term (in this example, final density of microorganisms). In these problems we must just replace the known terms in the equations described above and solve it.

These problems are very simple, but we must be careful with the units or the need to transform some data for being possible to use in them in the formula (i.e. the μ value is unknown but it is known the g value).

On this basis, solutions must be proposed for the following problems:

4.1. How many bacteria are present after 4 hours if a culture that doubles every 2 hours is inoculated with 10^4 CFU/ml? and after 24 and 48 hours?

4.2. If a culture in exponential phase has 100,000 cells/ml at a given time, and after 4 hours, the population is 100,000,000 cells/ml, which would the μ and g values be?

However, in the laboratory, when we study microbial growth, the problems are not as easy to solve. Here is an example:

In the laboratory, a flask containing 100 ml of culture medium X is inoculated with the microorganism Y. Then, it is incubated at XX° C with shaking, and aliquots are collected periodically to determine the number of CFU/ml. Data (in black) are expressed in a table as the following one:

Time (h)	CFU/ml	Ln CFU/ml (1)	Ln D_x - Ln D_{x-1} (2)
0	$2.05\ 10^6$	14.53	0
1	$2.04\ 10^6$	14.53	-0.02
2	$2.01\ 10^6$	14.51	0.07
3	$2.14\ 10^6$	14.58	0.09
4	$2.34\ 10^6$	14.67	0.14
5	$2.69\ 10^6$	14.81	0.34 **(3)**
6	$3.80\ 10^6$	15.15	0.32
7	$5.25\ 10^6$	15.47	0.35
8	$7.41\ 10^6$	15.82	0.37
9	$1.07\ 10^7$	16.19	0.34
10	$1.51\ 10^7$	16.53	0.19
11	$1.82\ 10^7$	16.72	0.09
12	$1.99\ 10^7$	16.81	0.02
13	$2.04\ 10^7$	16.83	-0.06
14	$1.91\ 10^7$	16.77	0.05
15	$2.02\ 10^7$	16.82	

Steps to define growth parameters for this microorganism:

a. Log transformation of the data (CFU/ml) (data in red) (1).
b. Determine the increase in density for each time interval (2).
c. Select those times in which the growth (increase in density) is constant (3).
d. Calculate the regression line relating time to density in this interval (3): Ln N/ml = 0.3486 t + 13.0752. The slope of the line corresponds to $\mu = 0.3486\ h^{-1}$.
e. To calculate the M value, we must know the initial density of the culture and the maximum density at stationary phase. For that, we can simply search the maximum value (in this case, $2.04\ 10^7$ CFU/ml) and an initial value ($2.05\ 10^6$ CFU/ml) or calculate the average values in lag and stationary ($1.88\ 10^7$ and $2.14\ 10^6$ CFU/ml, respectively). In the first case, the M value would be $1.835\ 10^7$ CFU/ml and in the second one, $1.666\ 10^7$ CFU/ml. That is, a minimum variation.

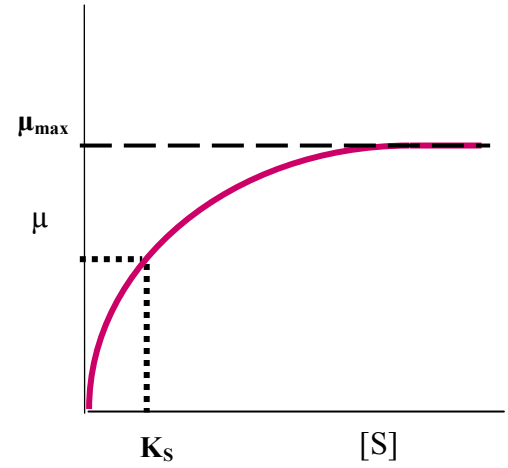
With this information, you must propose solutions to the following problems:

4.3. The following data correspond to the exponential phase of growth of three bacterial species using glucose as carbon source:

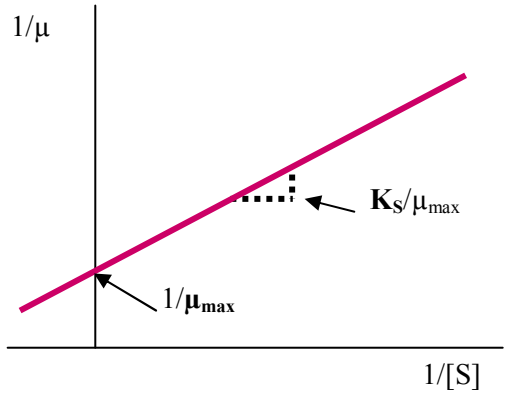
Specie A		**Specie B**		**Specie C**	
Time (h)	**Log N**	**Time (h)**	**Log N**	**Time (h)**	**Log N**
4	4.64	3	5.30	5	6.22
5	4.81	4	5.44	6	6.37
6	4.98	5	5.58	7	6.52
7	5.15	6	5.72	8	6.67
8	5.32	7	5.86	9	6.82

Indicate which of these species grow faster. Explain your answer.

When we are asked to calculate μ_{max}, there are two options. It is possible to determine it directly from the graphical representation of the μ values obtained growing the microorganism in different substrate concentrations. Nevertheless, we can also get this value from the line that relates $1/\mu$ to $1/S$.

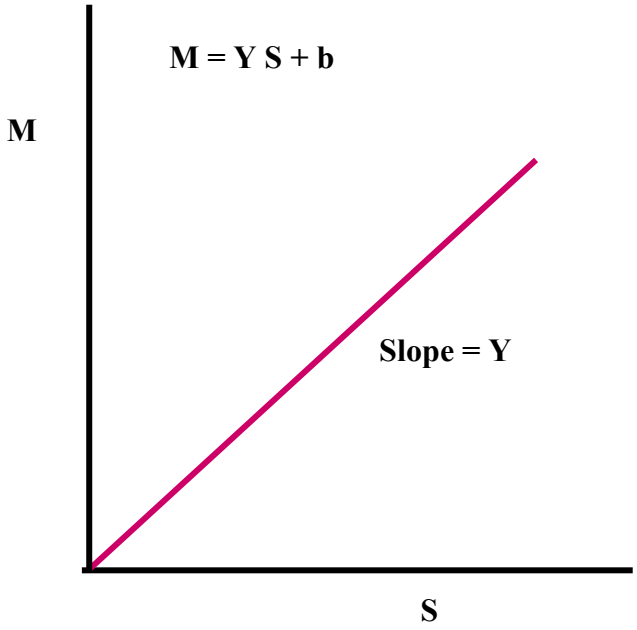


$$\mu = \frac{\mu_{max}\ S}{K_S + S}$$



$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_S}{\mu_{max}}\frac{1}{S}$$

To calculate the yield (Y), we must first obtain the values of M for each curve made with a concentration of substrate and then obtain the line relating M to S (substrate concentration). The slope of that line corresponds to Y.



4.4. With the aim of obtaining biomass from *Candida utilis*, whey (lactose concentration in serum, 4%) will be used as substrate in industrial production. Previously, to establish the final conditions, batch cultures were performed with 10 different concentrations of lactose, with the following data:

Lactose concentration (g/l)	μ (1/h)	Lactose concentration (g/l)	μ (1/h)
1	0.083	10	0.332
2	0.143	12	0.351
4	0.221	14	0.367
6	0.273	16	0.366
8	0.310	20	0.368

Calculate: K_S and μ_{max} for this microorganism using this substrate.

By plotting the maximum yield (mg cells/ml) obtained in each batch culture vs lactose concentration (lactose mg/ml), the following equation was obtained: y = 0.0383 + 0.409 x (r = 0.999). What is the Y value for *Candida utilis* using this substrate? and what will the M value be using whey as substrate?

Bibliography

Madigan, M.T., J.M. Martinko, D.A. Stahl, D.P. Clark. 2012. Brock Biology of Microorganisms, 13th ed. Benjamin Cummings.

Willey, J.M., L.M. Sherwood, C.J. Woolverton. 2011. Prescott's Microbiology Companion Site, 8th ed. McGraw-Hill Ryerson Ltd.

SOLUTIONS

4.1. $N_0 = 10^4$ CFU/ml, g = 2 h, How many culturable cells will be after 4, 24 and 48 h of growth?

$\log N - \log N_0 = \mu / 2.303 (t-t_0)$ y $g = 0.693/\mu$

$\log N = [\mu / 2.303 (t-t_0)] + \log N_0$ y $\mu = 0.693/g$ $\mu = 0.3465\ h^{-1}$

4 h: $\log N = [0.3465 / 2.303 (4)] + 4$ $N = 3.99\ 10^4$ CFU/ml

24 h: $\log N = [0.3465 / 2.303 (24)] + 4$ $N = 4.09\ 10^7$ CFU/ml

48 h: $\log N = [0.3465 / 2.303 (48)] + 4$ $N = 1.67\ 10^{11}$ CFU/ml

4.2. N_0 = 10,000 cells/ml, N = 100,000,000 cells/ml, t = 4 h. μ? g?

$\mu = 2.303\ h^{-1}$ $g = 0.3$ h

4.3. Which specie grows faster?

Specie A		Specie B		Specie C	
Time (h)	Log N	Time (h)	Log N	Time (h)	Log N
4	4.64	3	5.30	5	6.22
5	4.81	4	5.44	6	6.37
6	4.98	5	5.58	7	6.52
7	5.15	6	5.72	8	6.67
8	5.32	7	5.86	9	6.82
μ (h^{-1})	0.3915	μ (h^{-1})	0.3224	μ (h^{-1})	0.3455
g (h)	1.77	g (h)	2.15	g (h)	2.01

4.4. K_s, μ_{max} and Y using lactose as substrate? **M** value using whey as substrate?

Lactose concentration (g/l)	μ (1/h)	1/ lactose (l/g)	1/μ (h)
1	0.083	1	12.0481928
2	0.143	0.5	6.99300699
4	0.221	0.25	4.52488688
6	0.273	0.166666667	3.66300366
8	0.310	0.125	3.22580645
10	0.332	0.1	3.01204819
12	0.351	0.083333333	2.84900285
14	0.367	0.071428571	2.72479564
16	0.366	0.0625	2.73224044
20	0.368	0.05	2.7173913

$1/\mu = 9.9646\ 1/S + 2.0486$ $R^2 = 0.9994$

$\mu_{max} = 1/2.0486 = 0.4881\ h^{-1}$

$K_s = 9.9646\ \mu max = 4.864\ g/l$

mg cells/ml = 0.0383 + 0.409 mg lactose/ml (r = 0.999)

Y = 0.409 mg cells/mg lactose

Lactose in whey = 4 % = 4 g/100 ml = 40 mg/ml

M_{whey} = 16.4 mg cells/ml