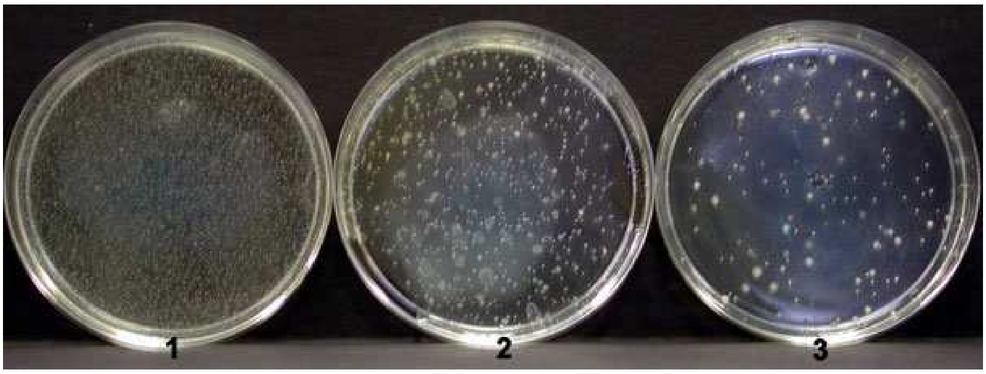
HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY

PROPOSAL: NEW EXERCISES

PART 2



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PROPOSAL

4. DETERMINATION OF THE PARAMETERS DEFINING THE BACTERIAL GROWTH

4.1. What is the generation time of a culture with a specific growth rate constant of 0.01 min^{-1}? At what speed the population is doubled?

4.2. With how many bacteria should a culture be inoculated to reach 10^8 bacteria/ml after 3 hours if the generation time is 30 min?

4.3. How long would it take for an initial population of 10^6 cells/ml to reach a size of 10^8 cells/ml hours if the generation time is 30 min?

4.4. In a picnic, the paella was contaminated with 46 cells of *Propionibacterium acnes*. If *P. acnes* has a generation time of 90 minutes and a lag phase of 1.5 h, how many cells of this bacterium will be present in the paella after 10 h?

4.5. A butcher, asymptomatic carrier of *Salmonella*, minced meat without wearing gloves. As consequence, the meat was contaminated with 25 cells of *Salmonella* spp. and 32 cells of *S. enterica*. Taking into account that the generation time of *Salmonella* spp. is 30 minutes and its lag phase is 3 h, and that the specific growth rate constant of *S. enterica* using meat as substrate is 0.17 h^{-1} and its lag phase lasts 5 h, calculate the number of *Salmonella* cells that will be present in the meat 10 hours after being prepared.

4.6. Wastewater has been spilled on a beach. The *Escherichia coli* and *Enterococcus faecalis* densities in this wastewater are 7.8 10^{10} and 6.2 10^9 cells/100 ml, respectively. The dilution factor of the discharge when mixed with seawater is 1/1000. As the concentration of nutrients in the beach is low, the growth of both microorganisms is slow. The generation time of *E. coli* is 3 hours while the specific growth rate constant for *Ent. faecalis* is 0.11 h^{-1}. With these data, could you indicate what will be the density (cells/ml) of each bacteria in receiving seawater 24 hours after the spill?

4.7. Calculate the yield of biomass per gram of carbon consumed for a culture that produces 20 grams of cells per liter when a glucose solution 10% (w/v) is was as a sole carbon source.

4.8. The results obtained for *E. coli* growing in two different culture media (under same incubation conditions) are shown in the following table. Which of these culture media would you select for further studies? Explain your answer.

	Cells/ml	
Time (hours)	**Medium A**	**Medium B**
0	$2.09\ 10^6$	$2.29\ 10^5$
1	$2.04\ 10^6$	$2.24\ 10^5$
2	$1.99\ 10^6$	$2.25\ 10^5$
3	$2.63\ 10^6$	$2.19\ 10^5$
4	$3.47\ 10^6$	$2.23\ 10^5$
5	$4.68\ 10^6$	$3.16\ 10^5$
6	$6.17\ 10^6$	$4.47\ 10^5$
7	$7.94\ 10^6$	$6.46\ 10^5$
8	$8.32\ 10^6$	$9.33\ 10^5$
9	$8.51\ 10^6$	$1.32\ 10^6$
10	$8.33\ 10^6$	$1.29\ 10^6$
11	$8.13\ 10^6$	$1.26\ 10^6$

4.9. To determine the growth curve of *E. coli* in nutrient broth, a flask containing nutrient broth was inoculated and incubated at 37 ° C (100 rpm). Periodically, 2 ml were removed from the flask, and the absorbance was measured. The results obtained are present in the following table:

Time (h)	Absorbance	Time (h)	Absorbance
0	0.0022	6	0.1108
1	0.0021	7	0.1643
2	0.0088	8	0.1905
3	0.0192	9	0.2732
4	0.0315	10	0.2603
5	0.0506	11	0.2611

Simultaneously, from a culture of *E. coli*, cell suspensions were prepared to determine the number of bacteria/ml *vs*. the absorbance, resulting in the following equation:

$$\text{Ln No. cells/ml} = 20.688 + 15.387\ \text{Abs},\ r = 0.97$$

Which is the density of the culture in the stationary phase of growth?

4.10. To calculate the equation that relates Absorbance to Cell density and Absorbance to Dry weight for *Kloeckera apiculata,* this bacterium was grown in YNB broth. For that, 10-fold dilutions were prepared from yeast suspension. For each suspension, absorbance (600 nm), cell density and dry weight were measured with the following results:

Absorbance	Cell density (x10^5cells/ml)	Dry weight (mg/ml)
0.230	15.43	0.06
0.076	7.08	0.02
0.079	7.18	0.02
0.325	28.80	0.08
0.110	10.90	0.038
0.095	5.33	0.035
0.470	47.00	0.169
0.237	24.90	0.06
0.865	87.50	0.40
0.680	68.00	0.25

Later, a flask containing 100 ml of YNB broth was inoculated with approximately 10^6 yeast /ml. The flask was incubated at 37 °C (100 rpm). Subsamples of 2 ml were periodically collected and their absorbance was measured:

Time (h)	Absorbance
0	0.11
1	0.25
2	0.47
3	1.01
4	1.03
5	1.10
6	1.05

Which is the density of the culture when it enters in stationary phase? And the dry weight?

4.11. From these data related to a bacterial culture, determine μ, g and M values.

Time (h)	Cells/ml	Time (h)	Cells/ml	Time (h)	Cells/ml	Time (h)	Cells/ml
0	1000000	4	3650000	8	28100000	12	121000000
1	1100000	5	6080000	9	39100000	14	105000000
2	1600000	6	9900000	10	70100000		
3	2620000	7	18000000	11	102000000		

After determining μ, g and M values, and considering that the initial substrate concentration is 5 mg S/ml and S depleted during growth, calculate the yield (Y).

5. CALCULATION OF INOCULUM SIZE

5.1. A flask containing 250 ml of liquid medium was inoculated with 500 ml of a bacterial suspension to obtain a final microbial density of $4.5\ 10^{6}$ cells/ml. Which was the microbial density of the bacterial suspension used for the inoculation?

5.2. From a culture with a density of $3.2\ 10^{9}$ bacterial cells/ml, we wish to inoculate a flask containing 2 liters of sterile liquid medium to obtain a density of $4.2\ 10^{4}$ cells/ml. What volume of the initial culture should be used as inoculum?

5.3. A cell suspension containing $8.9\ 10^{11}$ cells/ml was prepared. From this suspension, we inoculate a flask containing 200 ml of broth until a cell density of $1.7\ 10^{4}$ cells/ml. If the transferred volume were 100 ml, which dilutions of the suspension should we have prepared?

5.4. A bacterial culture (*Bacillus*) with a density of $2\ 10^{9}$ bacteria/ml was used to inoculate a flask containing 250 ml of culture medium so that the biomass in this flask reached 1.8 mgC/ml. Which volume must be transferred from the culture to the flask in order to obtain this value of biomass? ($L = 1.5\ \mu m$, $W = 0.5\ \mu m$ and $F = 126\ 10^{-10} mgC/\mu m^{3}$).

SOLUTIONS

4.1. $\mu = 0.01\ min^{-1}$ g and K values?

$g = 0.693/\mu$ $g = 69.3\ min = 1.155\ h$

$K = 1/g$ $K = 0.8685\ h^{-1}$

4.2. $N = 10^8$ cells/ml, t = 3 h, g = 30 min. N_0?

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

$\log N - [\mu/2.303.\ (t-t_0)] = \log N_0$ and $\mu = 0.693/g$ $\mu = 1.386\ h^{-1}$

$N_0 = 1.56\ 10^6$ cells/ml

4.3. $N_0 = 10^4$ cells/ml, $N = 10^8$ cells/ml, g = 30 min. t?

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

$t = 3.323\ h$

4.4. N_0 = 46 cells, g = 1.5 h, lag phase = 1.5 h. Number of cells after 10 h?

$t = 10\ h - 1.5\ h = 8.5\ h$

$\log N - \log N_0 = \mu/2.303.\ (t-t_0)$ and $\mu = 0.693/g = 0.462\ h^{-1}$

$\log N = [\mu/2.303.\ (t-t_0)] + \log N_0 = [0.462/2.303\ (8.5)] + 1.663 = 3.368$

$2.33\ 10^3$ *Propionibacterium acnes*

4.5. *Salmonella spp.*, N_0 = 25 cells, g = 0.5 h, lag phase = 3 h. *S. enterica*, N_0 = 32 cells, $\mu = 0.17\ (h^{-1})$, lag phase = 5 h

Number of *Salmonella* (*Salmonella spp.* + *S. ent*) cells after 10 h?

	Salmonella spp.	*Salmonella enterica*
Lag phase (h)	3	5
$\mu\ (h^{-1})$	1.386	0.170
g (h)	0.50	4.08
N_0 (cells)	25	32
Growth time (h)	7	5
N (cells)	$3.98\ 10^5$	74.8
N (*Salmonella*)	(*Salmonella spp.* + *S. enterica*) $3.98\ 10^5$	

4.6. 24 h after wastewater spill, bacterial density per ml?

	E. coli	*Enterococcus*
Wastewater: cells/100 ml	7.8 10^{10}	6.2 10^{9}
Spill: cells/100 ml	7.8 10^{7}	6.2 10^{6}
μ (h^{-1})	0.231	0.11
g (h)	3	6,3
Growth time (h)	24	24
N (cells/100 ml)	1.99 10^{10}	8.71 10^{7}
N (cells/ml)	**1.99 10^{8}**	**8.71 10^{5}**

4.7. Yield?

Glucose solution = 10 % (w/v) = 10 g/100 ml = 100 g/l

Biomass = 20 g/l

Y = Biomass produced/Glucose consumed

$$\frac{20 \text{ g cells/l}}{100 \text{ g glucose/l}} = 0.2 \text{ g cells/g glucose}$$

4.8. Select the culture media to obtain the best growth.

	Medium A		Medium B	
Time (hours)	Cells/ml	Ln cells/ml	Cells/ml	Ln cells/ml
0	2.09 10^{6}	14.55	2.29 10^{5}	12.34
1	2.04 10^{6}	14.53	2.24 10^{5}	12.32
2	**1.99 10^{6}**	**14.50**	2.25 10^{5}	12.32
3	**2.63 10^{6}**	**14.78**	2.19 10^{5}	12.30
4	**3.47 10^{6}**	**15.06**	**2.23 10^{5}**	**12.31**
5	**4.68 10^{6}**	**15.36**	**3.16 10^{5}**	**12.66**
6	**6.17 10^{6}**	**15.64**	**4.47 10^{5}**	**13.01**
7	**7.94 10^{6}**	**15.89**	**6.46 10^{5}**	**13.38**
8	**8.32 10^{6}**	**15.93**	**9.33 10^{5}**	**13.75**
9	8.51 10^{6}	15.96	**1.32 10^{6}**	**14.09**
10	8.33 10^{6}	15.94	1.29 10^{6}	14,07
11	8.13 10^{6}	15.91	1.26 10^{6}	14,05
Lag phase h)	3		5	
μ (h^{-1})	0.2809		0.3583	
g (h)	2.47		1.93	
M (cells/ml)	6.28 10^{6}		1.07 10^{6}	

Exponential phase

4.9. Ln Nº cells/ml = 20.688 Ab s + 15.387, r = 0.97. Which is the density of the culture in the stationary phase of growth?

Time (h)	Absorbance	Ln cells/ml	Cells/ml
0	0.0022	15,43	$5.03\ 10^6$
1	0.0021	15,43	$5.03\ 10^6$
2	0.0088	15,57	$5.78\ 10^6$
3	0.0192	15,78	$7.13\ 10^6$
4	0.0315	16,04	$9.25\ 10^6$
5	0.0506	16,43	$1.37\ 10^7$
6	0.1108	17,68	$4.77\ 10^7$
7	0.1643	18,79	$1.45\ 10^8$
8	0.1905	19,33	$2.48\ 10^8$
9	0.2732	21,04	**$1.37\ 10^9$**
10	0.2603	20.77	$1.05\ 10^9$
11	0.2611	20,79	$1.07\ 10^9$
Average value in stationary phase			**$1.16\ 10^9$**

Stationary phase

4.10. Which is the density in stationary phase? Dry weight?

Absorbance	Cell density ($x10^5$cells/ml)	Dry weight (mg/ml)
0.230	15,43	0.06
0.076	7.08	0.02
0.079	7.18	0.02
0.325	28.80	0.08
0.110	10.90	0.038
0.095	5.33	0.035
0.470	47.00	0.169
0.237	24.90	0.06
0.865	87.50	0.40
0.680	68.00	0.25

Ln N cells/ml = 3.391 Abs + 13.433

Dry weight/ml = 0.444 Abs – 0.026

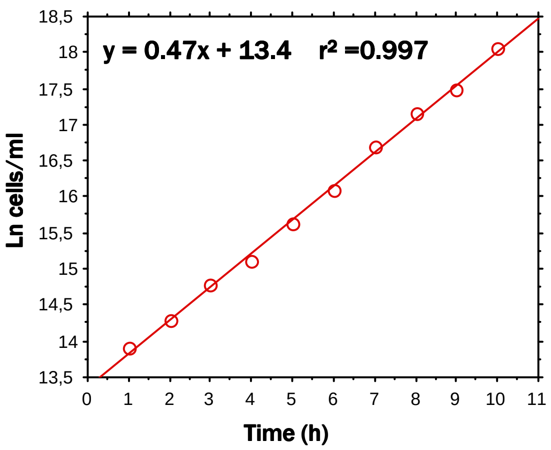
Time (h)	Absorbance	Ln cells/ml	Cells/ml	Dry weight/ml
0	0.11	13.81	$9.95\ 10^5$	0.023
1	0.25	14.28	$1.59\ 10^6$	0.085
2	0.47	15.03	$3.37\ 10^6$	0.183
3	1.01	16.86	$2.10\ 10^7$	0.423
4	1.03	16,93	2.2510^7	0.431
5	1.10	17.16	**$2.84\ 10^7$**	**0.463**
6	1.05	16.99	2.3910^7	0.440
Average value of stationary phase			**$2.395\ 10^7$**	**0.439**

Stationary phase

4.11. μ, g and M values?

Time (h)	Cells/ml	Ln cells/ml
0	1000000	13.816
1	1100000	13.911
2	1600000	14.286
3	2620000	14.779
4	3650000	15.11
5	6080000	15.621
6	9900000	16.108
7	18000000	16.706
8	28100000	17.151
9	39100000	17.482
10	70100000	18.065
11	102000000	18.44
12	121000000	18.611
14	105000000	18.469

Exponential phase



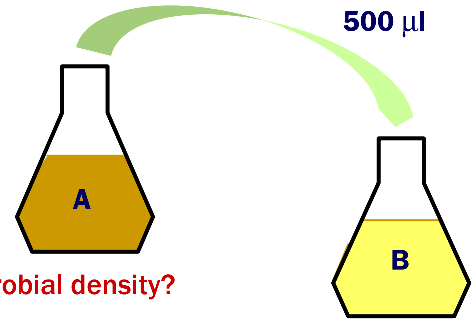
$\mu = 0.47\ h^{-1}$

$g = 1.47\ h$

$M = 1.21\ 10^{8} - 1\ 10^{6} = 1.20\ 10^{8}$ cells/ ml

$$Y = \frac{1.20 \cdot 10^{8}\ \text{cells/ ml}}{5\ \text{mg/ml}} = 2.4\ 10^{7}\ \text{cells/mg substrate}$$

5.1. Microbial density in A?



Microbial density?

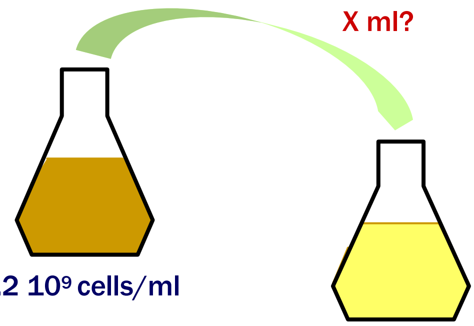
250 ml
4.5 10^6 cells/ml

4.5 10^6 cells → 1 ml
A cells → 250 ml

A = 1.125 10^9 cells were transferred in 500 µl

$$\frac{1.125\ 10^9 \text{ cells}}{0.5 \text{ ml}} = \mathbf{2.25\ 10^9 \text{ cells/ml}}$$

5.2. Volume of inoculum?



X ml?

(A) 3.2 10^9 cells/ml

2 L
(B) 4.2 10^4 cells/ml

4.2 10^4 cells → 1 ml
A cells → 2,000 ml

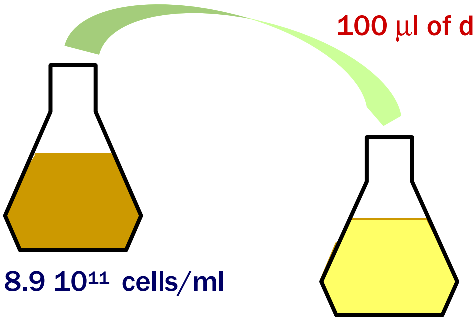
A = 8.4 10^7 cells were transferred in X ml

3.2 10^9 cells → 1 ml
8.4 10^7 cells → X ml

X = 0.02625 ml (26.25 µl) were transferred

5.3. Dilution?

100 µl of dilution X



(A) $8.9\ 10^{11}$ cells/ml

200 ml
(B) $1.7\ 10^{4}$ cells/ml

$1.7\ 10^{4}$ cells → 1 ml
A cells → 200 ml

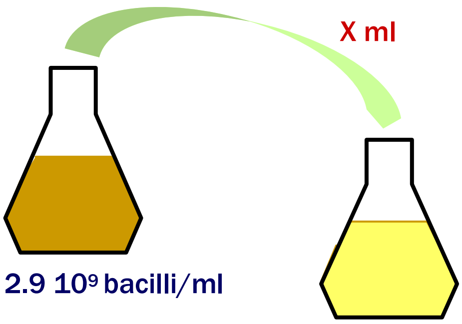
A = $3.4\ 10^{6}$ cells are transferred in 100 µl of dilution X
Dilution X contains $3.4\ 10^{7}$ cell/ml

$$\frac{8.9\ 10^{11}\ \text{cells/ml}}{3.4\ 10^{7}\ \text{cells/ml}} = 0.26\ 10^{5}$$

Dilutions, 1:4 and 10^{-4}

5.4. Dilution?

X ml



(A) $2.9\ 10^{9}$ bacilli/ml

250 ml
(B) 1.8 µg C/ml

Biovolume = $W^2\ \pi/4\ (L - W/3)$ = 0.2618 µm³/cell
$1.8\ 10^{-3}$ mg C/ml = N° cells/ml X 0.2618 µm³/cell X $126\ 10^{-10}$ mg C/µm³
$5.5\ 10^{5}$ cells/ml

$5.5\ 10^{5}$ cells → 1 ml
A cells → 250 ml

A = $1.36\ 10^{8}$ cells were transferred in X ml

$$\frac{2.9\ 10^{9}\ \text{cells/ml}}{1.36\ 10^{8}\ \text{cells/ml}} = 0.21\ 10^{2}$$

1 ml of 1:5 and 10^{-2} dilutions