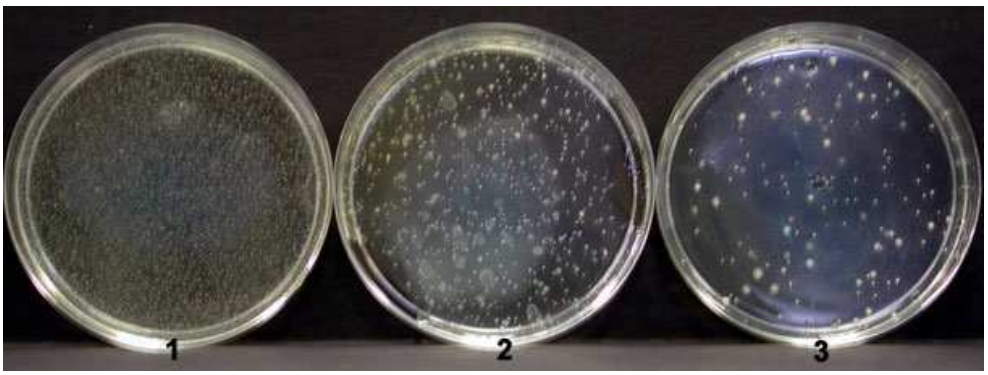
HOW TO SOLVE PRACTICAL ASPECTS OF MICROBIOLOGY

PROPOSAL: NEW EXERCISES

PART 1



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PROPOSAL

1. DILUTIONS AND CONCENTRATIONS. LIQUID AND SOLID SAMPLES

1.1. You have a sample of water, 3 empty and sterile tubes and 30 ml of sterile saline solution. How would you prepare the 10^{-6} dilution of that sample? How would you do if you only have 6 Eppendorf microtube (1 ml) and 10 ml of saline solution?

1.2. You have to prepare 150 ml of the 10^{-1} dilution from a water sample. Devise a scheme to prepare it, detailing the steps to follow, the volumes to be added, etc. And how would you prepare 150 ml of 10^{-3}dilution?

1.3. From a water sample, how would you prepare the following dilutions: 1:10, 1:5, 1:4 and 1:2?

1.4. In order to determine the microbial density in a yogurt sample, the 10^{-2} dilution is required. Indicate the steps to follow and detail how to prepare the dilution.

1.5. How would you prepare 500 ml of the 10^{-1} dilution from a water sample? Explain the preparation of the same volume of such dilution but from a solid sample, as a soil sample.

1.6. From a water sample, the dilution 10^{-3} is prepared. 2 ml of this dilution are transferred to a tube containing 8 ml of diluent, stirred and 0.5 ml are spread on the surface of a plate with nutrient medium. If, after incubation, 50 colonies are enumerated, what is the microbial density of the sample?

1.7. 4 ml of a water sample were added to 4 ml of diluent. Then, 0.5 ml were plated on nutrient agar. After incubation, 82 colonies were present on the plate. Which was the microbial density of the sample?

1.8. How would you prepare the 1: 5 dilution from a sediment sample? and the 1:8 dilution? If we plate 0.5 ml on a culture medium and, after incubation, 120 colonies are present in the plate, which is the microbial density of the sample expressed as colony-forming units per 100 g of sediment?

1.9. 20 g of sediment are resuspended in 40 ml of saline solution and vigorously shaken. Then, the suspension is sonicated to separate the bacteria from sediment particles and 10 ml are centrifuged at low speed (sedimentation of particles > 3 μm). The resultant supernatant is diluted 1,000 times and 0.2 ml are plated on nutrient agar. After incubation, 126 colonies were present on the plate. How many culturable bacteria were present per g of sediment?

1.10. 50 ml of a water sample were filtered. Then, the filter was resuspended in 10 ml of saline solution and vigorously shaken. 0.1 ml of this suspension were plated on nutrient agar. After incubation, 126 colonies were present on the plate. Which is the microbial density of the sample?

1.11. 50 ml of a water sample were filtered. Then, the filter was resuspended in 15 ml of saline solution and vigorously shaken. From this suspension, two consecutive dilutions were prepared: 1:10 and 1:2. Finally, 0.2 ml of the final dilution were plated on nutrient agar. After incubation, 35 colonies were present on the plate. Which is the microbial density of the sample?

1.12. To assess the microbiological quality of the waters of the estuary of Bilbao a study in which the presence of heterotrophic aerobic bacteria (HAB) will be carried out. For that, ten-fold dilutions of the sample will be prepared and plated on nutrient agar (100 ul per plate). After consulting the related bibliography, we know that HAB densities vary from $5\ 10^7$ to $7\ 10^8$ bacteria/ml. Taking into account this information, which dilutions should be plated?

1.13. A study will be carried out in order to determine the presence of sulfate-reducing bacteria (SRB) in the sediments of the estuary of Bilbao. For that, the collected sediment will be treated by adding 10 ml of diluent per 2 g of pellet, and then homogenized. Subsequently, decimal dilutions of this suspension will be prepared and plated on culture medium (100 ul per plate). After consulting the related bibliography, we know that, in similar environments, the average density of BRS is $5\ 10^4$ BRS/ml. Taking into account this information, which dilutions should be plated?

2. BASIC METHODS FOR THE ENUMERATION OF MICROORGANISMS

2.1. A bacterial culture has a density of 5 10^8 CFU/ml. If 0.1 ml was inoculated on solid culture medium and incubated at appropriate temperature. After incubation, 50 colonies were enumerated. What was the dilution?

2.2. In the case of the microdrop method, inoculating 10 µl and 3 replica per sample, what is the detection limit of this enumeration technique?

2.3. To facilitate the enumeration of microorganisms in a cake, following procedure is performed: 62.5 g of cake are added into 187.5 ml of buffered peptone water. From this suspension, we prepare three serial tenfold dilutions. Similarly, we prepare 4 sets of 3 tubes with 10 ml of MacConkey broth per tube. After incubation at 44.5°C, the results obtained are the following:

Procedure	**Results Tubes***		
62.5 g cake + 187.5 ml of buffered peptone water	Y	Y	Y
Dilution 10^{-1}	Y	Y	P
Dilution 10^{-2}	Y	P	P
Dilution 10^{-3}	P	P	P

*Each tube is inoculated with 1 ml of suspension or corresponding dilution. Y = yellow, P = purple.

2.4. What would be the detection limit of the method using counting chamber (hemocytometer), if we count a maximum of 20 fields in the camera and the factor is 1.5 10^5/ml?

2.5. Calculate the density of the bacterial suspensions as indicated in the table given. Quantifications were conducted using epifluorescence microscopy. The volumes filtered, dilutions employed and the number of bacteria per field are listed in the table. The epifluorescence microscope factor is 64,761.9 (No. of fields / filter).

Sample	**Volume filtered**	**Dilution**	**No. bacteria/field**
1	1 ml	Sample	25/32/31/30/35/21/14/21/21/20
2	100 µl	10^0	22/22/21/22/23/23/21/17/21/21
3	50 µl	10^{-1}	23/23/22/21/23/24/15/31/24/24
4	100 µl	10^{-2}	6/8/11/9/13/7/7/8/9/11

2.6. What would be the detection limit of the method based on epifluorescence microscopy, if the maximum volume that can be filtered is 5 ml, F is 34,520 fields/filter and a maximum of 30 fields per filter can be exanimate?

2.7. In the Butrón river, near Munguia town, we collected a water sample. In the laboratory, we prepared a 1,000 ml flask with 500 ml of untreated sample. The flask was inoculated with *Escherichia coli* (final density of approximately 10^6 CFU/ml) and incubated for 6 days at 20°C (120 rpm). Periodically, aliquots were taken to enumerate *E. coli* and flagellate protozoa. Enumeration of CFU of *E. coli* was performed on EMB Agar (selective and differential culture medium in which *E. coli* produces metallic green colonies). The flagellate protozoan enumeration was performed by epifluorescence microscopy in aliquots filtered through 0.8 µm filter pore diameter and stained with DAPI (epifluorescence microscope factor = 64,761.9 fields/filter).

Obtained results are showed in next table:

Time (d)	Dil.	Vol. spread (µl)	Colonies/plate	Dil.	Vol. Filtered (ml)	N° flagellates/field
0	10^{-3}	100	96/96/108	S	40	4/6/6/5/4/1/1/3/4/5 6/4/4/3/1/2/1/4/4/3
1	10^{-3}	100	106/111/98			
2	10^{-1}	100	240/216/248	10^{-1}	5	8/7/6/7/8/8/5/5/7/8 8/7/7/5/5/7/8/3/7/5
3	S*	100	315/296/298			
4	S	100	88/76/78	10^{-1}	20	3/4/3/3/0/3/4/1/4/2 0/0/2/4/3/6/7/7/6/4
5	S	1000	265/267/281			
6	S	1000	166/166/168	10^{-1}	40	4/4/3/4/5/0/0/3/4/3 3/2/4/4/2/1/0/6/4/2

* S = sample.

What is the effect of flagellate protozoa population on the survival of *E. coli*?

2.8. We suspect that, when a culture reaches the stationary growth phase, the bacterial culture density is of 4 10^8 cells/ml. To confirm it, we use an enumeration method based on the use of fluorescence microscopy. Counts of 20-30 bacteria/field are recommended and the factor of microscope used is 20,000 fields/filter. For a correct enumeration, what sample dilution and volume should be filtered?

2.9. We prepare 20 ml of the 10^{-3} dilution of a microbial culture. Indicate how we have performed it.

Then:

- 5 ml of this dilution was filtered to quantify culturable bacteria. The filter is placed on a suitable culture medium. After incubation, 125 colonies were counted. CFU/ml in the sample?
- Another 10 ml is filtered through a filter of 0.22 µm pore diameter and stained with acridine orange. The cellular numbers obtained using epifluorescence microscopy are 15/14/13/20/12/10/15/17/16/17. What is the total bacterial density if the microscope factor is 1,920 microscope fields/filter?

2.10. What is the percentage of culturable Gram negative bacteria in a mixed culture? Explain your answer.

Moreover, we know that

- counts obtained using epifluorescence microscopy: 22/21/20/23/22/22/23/24/25/25, filtrate volume: 2 ml of the dilution of 10^{-2} and microscope factor: 15,000 fields/filter,

- when we inoculated 3 MacConkey Agar plates with 0.1 ml of the 10^{-3} dilution, the results obtained were: Lactose positives = 170/165/182 and Lactose negatives = 80/70/76.

2.11. A culture of *Klebsiella pneumoniae* in exponential phase has an absorbance, measured at 600 nm, equal to 0.2. 0.2 ml of the 10^{-4} dilution was spread onto appropriate culture medium. After incubation period, 40 colonies were counted.

a) Calculate the density of the population.

b) Calculate the total CFU if the culture volume is 3 ml.

c) What dilutions should be made from the same popularion of *K. pneumoniae* when it reaches an absorbance = 0.5 to count 100 colonies on a plate after inoculating a volume of 0.2 ml?

2.12. The following equation relating the absorbance (600 nm) and *Klebsiella pneumoniae*/ml was obtained: No. bacteria/ml = 1.032 Abs 10^7 - 2.19 10^5.

An aliquot of a culture of *K. Pneumoniae* was collected, diluted 10 times and measured the absorbance (600 nm) of dilution. The value obtained was 0.49. What is the density of the culture?

3. BIOMASS CALCULATION

3.1. Your company produces starter cultures (inocula) for various dairy companies and has isolated and developed for distribution two new strains (*Lactobacillus fermentum* and *Lactobacillus adidophilus*). Previously, your company needs to determine their ability to produce high cell densities and biomass. In the company lab, the two *Lactobacillus* were grown separately under optimal conditions. Upon reaching the stationary growth phase (24 h) samples are collected and cells enumerated by epifluorescence microscopy:

Lactobacillus	**Bacteria/field**	**Vol. Filtered (ml)**	**Dil.**	**Factor microscope (fields/filter)**
L. acidophilus	25/32/30/28/33/34/35/29/28/24 15/32/33/18/20/22/18/23/19/26	0.5	10^{-2}	14,520
L. fermentum	5/12/13/8/13/8/15/9/8/14 15/12/13/8/10/7/8/9/9/6	1	10^{-3}	7,220

Which are of the two microorganisms achieves higher cell density in the stationary phase of growth?

When 200 cells were measured, the results obtained were:

Lactobacillus	**Length (µm)**	**Width (µm)**	**Conversión factor (mg C/µm^3)**
L. acidophilus	1.7	0.6	$170\ 10^{-10}$
L. fermentum	2	0.4	

If biovolume formula is $(L - W/3)\ \pi W^2/4$, which are of the two microorganisms reaches a highest biomass in the stationary phase of growth?

3.2. A study to determine colony forming units (CFU) on nutrient agar and bacterial biomass in an aquatic system at 3 different depths (5, 25 and 50 m) was carried out. For CFU counts, 100 µl of serial dilutions of the sample were spread in Marine Agar (3 replicates). The results obtained after incubation are presented in the following table:

Dilution	**Deep (m)**		
	5	**25**	**50**
10^{-2}	Excess	500/617/593	363/303/395
10^{-3}	357/452/403	53/67/60	35/30/40
10^{-4}	35/45/40	5/7/6	4/2/5

For the determination of the biomass, dilutions were stained with acridine orange and mounted for epifluorescence microscopy. Likewise, bacterial biovolume was measured by image analysis of these sample preparations. The results are presented in the following table which shows the sample dilutions used and the volume filtered through 0.2 μm in each case.

Deep (m)	Dil.	Filtered Volume (μl)	Counts (Bacteria/field)	Biovolume (μm^3/cell)
5	10^{-1}	100	30/35/40/25/30/25/33/25/27/30	0.15
25	10^{-1}	200	31/25/40/37/20/15/32/26/25/31	0.45
50	10^{0}	100	31/33/37/43/23/34/21/19/26/30	0.47

The conversion factor of the microscope used was: 30,954 fields/filter, and volume conversion factor of biomass was 2.2 10^{-7} μgC/μm^3.

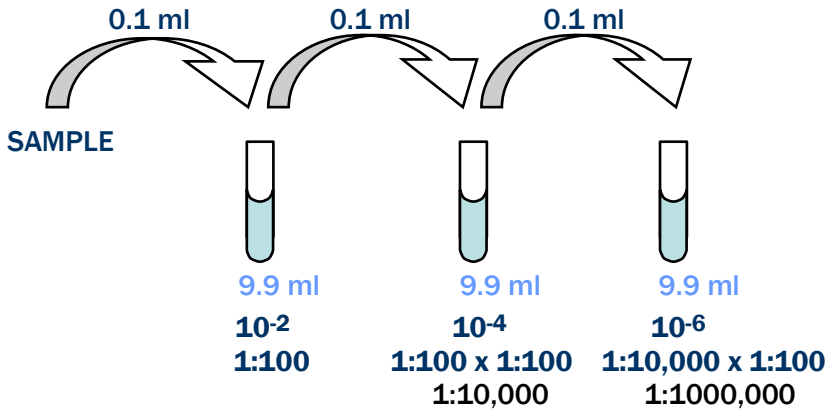
- At which depth can we find the highest density of CFU? What is the density of UFC at this depth?
- At which depth can we find the highest bacterial biomass? What are the maximum values of bacterial biomass?

3.3. As an inoculum for the production of yogurt, a mixed culture consisting of 60% *Lactococcus thermophilus* and 40% *Lactobacillus bulgaricus* (percentages regarding biomass) is prepared. Biomass = 3 mg/ml. If:

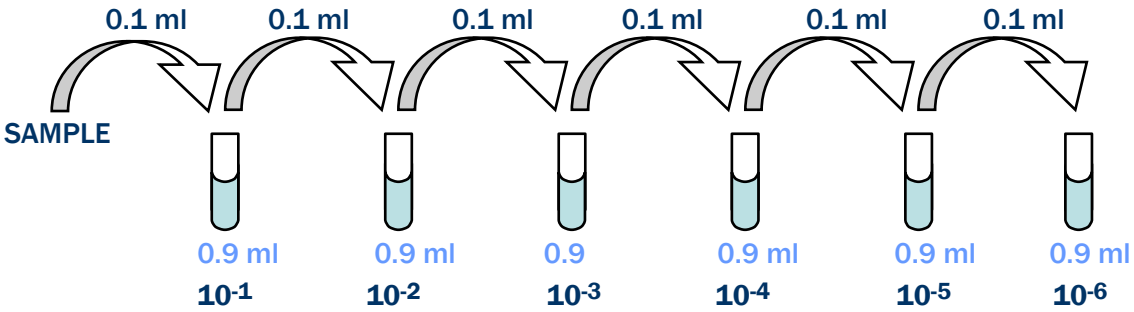
- the mean diameter (D) of *Lactococcus* cells is 1.2 υm, and the length (L) and width (W) of *Lactobacillus* are 1.8 and 0.5 μm, respectively;
- biovolume equation in the case of *Lactococcus* is $Bv = \pi D^3/6$, and in the case of *Lactobacillus*, $Bv = (L - W/3)\ \pi W^2 / 4$;
- the conversion factor of carbon biovolume (F) is 170 10^{-10} mg C /ml; What is the population density of *Lactococcus* and *Lactobacillus* in the mixed culture?

SOLUTIONS

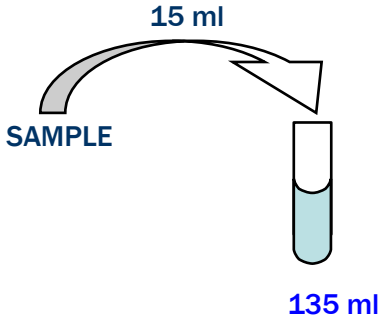
1.1. 10^{-6} Dilution. 3 tubes and 30 ml of diluent.



10^{-6} Dilution. 6 Eppendorff tubes (1 ml) and 10 ml of esterile saline solution.

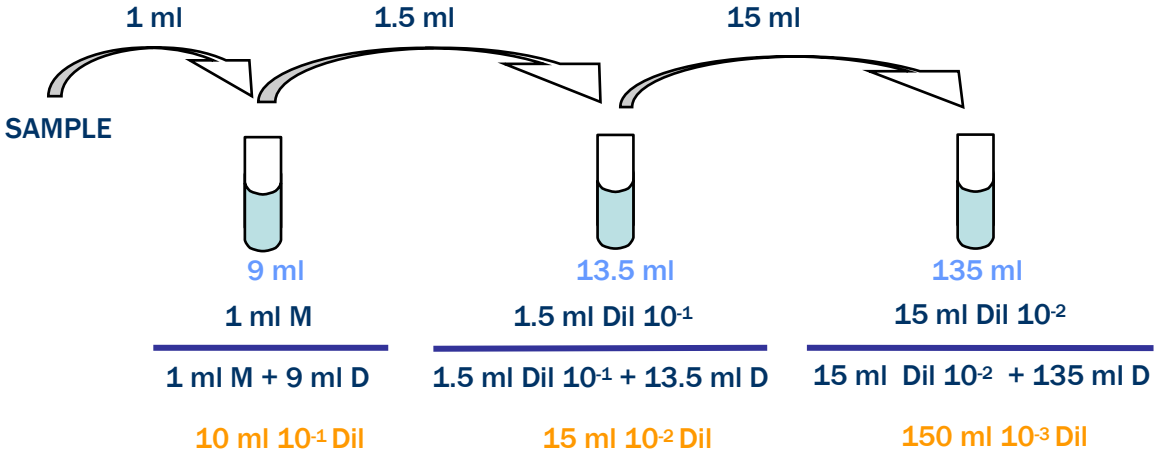


1.2. 150 ml 10^{-1} Dilution.



15 ml Sample/(15 ml Sample + 135 ml Diluent)

150 ml 10^{-3} Dilution



1.3. Schedule 1/10; 1/5; ¼; ½ dilutions

1:10 = 1 ml Sample/(1 ml Sample + 9 ml Diluent)

1:5 = 1 ml Sample /(1 ml Sample + 4 ml Diluent)

1:4 = 1 ml Sample /(1 ml Sample + 3 ml Diluent)

1:2 = 1 ml Sample /(1 ml Sample + 1 ml Diluent)

1.4. 1:100 Dilution?. Solid sample = Yogurt

1:10 = 1 g Yogurt/(1 g Yogurt + 9 ml Diluent)

1:100 = 1 ml Diluion 10^{-1} /(1 ml Dilution 10^{-1} + 9 ml Diluent)

Other options:are possible

1:100 = 1 g Yogur/(1 g Yogur + 99 ml Diluyente)

1.5. 500 ml 10^{-1} Dilution? Liquid sample

1:10 = 50 ml Sample/(50 ml Sample + 450 ml Diluent)

500 ml 10^{-1} Dilution? Solid sample

1:10 = 50 g Sample/(50 g Sample + 450 ml Diluent)

1.6. Dilution = 10^{-3}

2 ml Sample + 8 ml Diluent = 2/10 dilution = 1:5

Volume spread = 0.5 ml. Colonies/plate = 50 colonies. Microorganisms per ml of sample?

$$\frac{50 \text{ colonies} \times 10^3 \times 5}{0.5 \text{ ml}} = 5\ 10^5 \text{ CFU/ml}$$

1.7. 4 ml Sample + 4 ml diluent = 4/8 dilution = 1:2

Volume spread = 0.5 ml. Colonies/plate = 82 colonies. Microorganisms per ml of sample?

$$\frac{82 \text{ colonias} \times 2}{0.5 \text{ ml}} = 328 \text{ CFU/ml}$$

1.8. 1:5 dilution of a sediment sample?

Dilution 1:5 = 1 g sediment/(1 g sediment + 4 ml Diluent)

1:8 dilution of a sediment simple?

Dilution 1:8 = 1 g sediment/(1 g sediment + 7 ml Diluent)

1:5 dilution. 0,5 ml /plate. 120 colonies/plate. CFU/g sample?

$$\frac{120 \text{ colonies} \times 5}{0.5 \text{ ml}} = 1.2\ 10^3 \text{ UCFU/g}$$

1:8 dilution. 0.5 ml/plate. 120 colonies/plate. CFU/g sample?

$$\frac{120 \text{ colonies} \times 8}{0.5 \text{ ml}} = 1.92\ 10^3 \text{ CFU/g}$$

1.9. 20 g sediment + 40 ml saline solution = 20/60 = 1:3 dilution.

Centrifuge 10 ml. Obtain supernatant (10 ml). Dilute 1,000 times.

Spread 0.2 ml/plate. 126 colonies/plate. Microorganisms/g of sediment

$$\frac{126 \text{ colonies} \times 3 \times 10^3}{0.2 \text{ ml inoculados}} = 1.89\ 10^6 \text{ CFU/g}$$

1.10. Filter 50 ml. Resuspenion in 10 ml. 50/10. Concentration factor = 5.

0,1 ml/plate. 50 colonies/plate. Microorganisms/ml?

$$\frac{50 \text{ colonies}}{0,1 \text{ ml} \times 5} = 100 \text{ CFU/ml}$$

1.11. Filter 50 ml. Resuspension in 15 ml. 50/15. Concentration factor = 3.33.

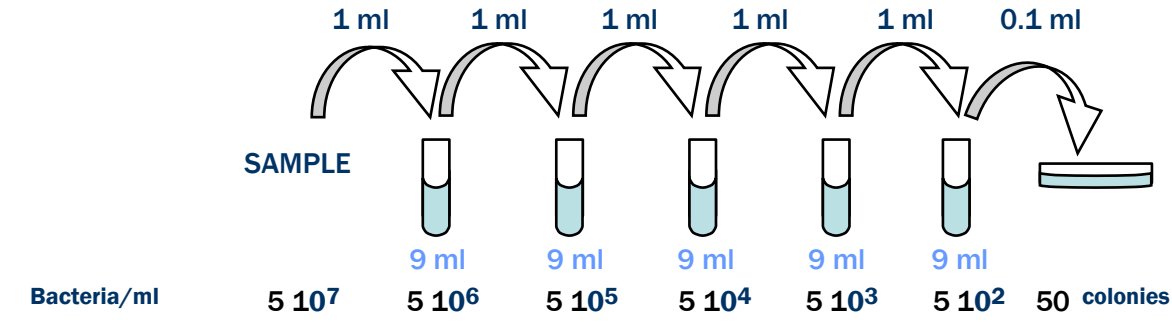
Dilutions: 1:10 and 1:2.

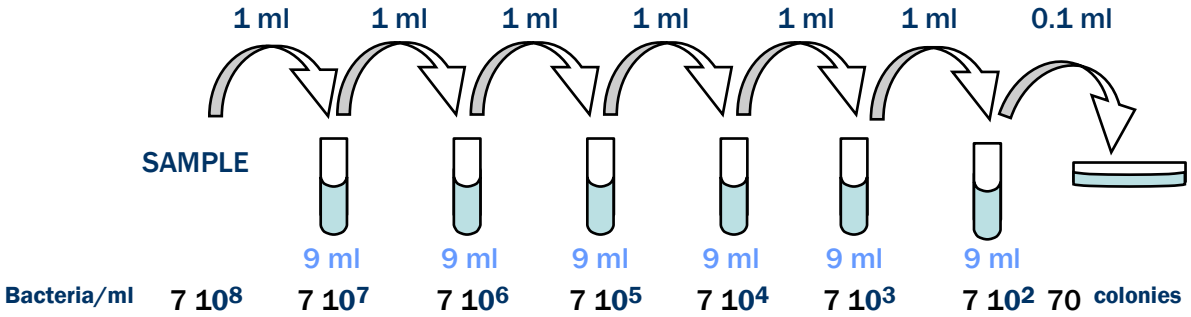
0.2 ml/plate. 35 colonies/plate. Microorganisms/ml?

$$\frac{35 \text{ colonies} \times 10 \times 2}{0.2 \text{ ml} \times 3.33} = 1.05\ 10^3 \text{ CFU/ml}$$

1.12. 5 10^7 bacteria/ml < aerobic heterotrophic bacteria (AHB) in estuary of Bilbao < 7 10^8 bacteria/ml,

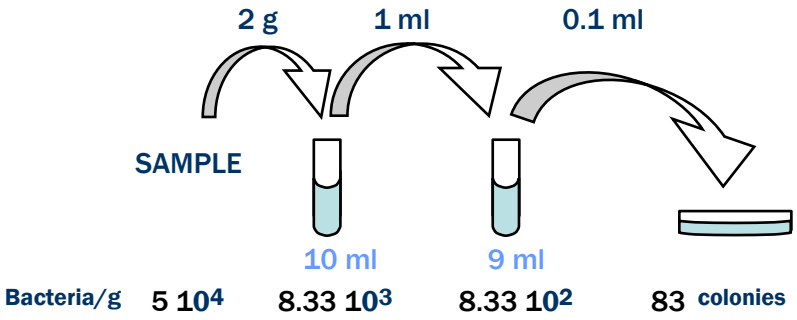
Indicate the dilutions appropriate to the AHB enumeration. Moreover, in plate, to enumerate colonies, optimal values are between 30 and 300 colonies/place. Calculate dilutions.





Selected dilutions 10^{-5} and 10^{-6}

1.13. 5 10^4 bacteria/g = sulfate-reducing bacteria (SRB) in sediments of the estuary of Bilbao Define the appropriate dilutions for the SRB enumeration.



2.1. Density = 5 10^8 CFU/ml. 0,1 ml/plate. 50 colonies, Dilution?

$$\frac{50 \text{ colonies} \times D}{0{,}1 \text{ ml seeded}} = 5\ 10^8 \text{ CFU/ml} \Rightarrow D = 10^6$$

2.2. Detection limit to microdrop method: 10 µl/drop, 3 replica/sample (dilution).

$$\frac{0.33 \text{ colonies}}{0.01 \text{ ml seeded}} = 33.3 \text{ CFU/ml}$$

Limit for enumeration: 1 colony/3 drops = 33.3 CFU/ml

Real case: 0 colonies/3 drops = Detection limit: <33.3 CFU/ml

2.3. MPN. Microorganisms?

Procedure	Resultado Tubos*		
62.5 g cake + 187.5 ml buffered peptone water	Y	Y	Y
Dilution 10^{-1}	Y	Y	P
Dilution 10^{-2}	Y	P	P
Dilution 10^{-3}	P	P	P

*P = Purple = No growth. Y = Yellow = lactose fermentation

Tubes positives = 321 = 15 microorganisms

Dilution = 62,5 / 250 = 1 / 4

MPN = 15 x 4 = 60 Gram – Lac+ bacteria /g

2.4. Count chamber. Number revisedof grids = 20. Factor = 1.5 10^5 grid/ml. Detection limit?

Real case (cells/grid): 0

Limit for enumeration: 1 cell in 1 grid of 20 revised

1 cell/20 grids X 1.5 10^5 grids/ml = 7.5 10^3 yeasts/ml

Detection limit <7.5 10^3 yeasts/ml

2.5. Epifluorescence microscopy. Factor = 64,761.9 fields/filter.

Sample	Volume filtered	Dilution	No. bacteria/field	No. bacteria/ml
1	1 ml	Sample	25/32/31/30/35/21/14/21/21/20	1.62 10^6
2	100 µl	10^0	22/22/21/22/23/23/21/17/21/21	1.38 10^7
3	50 µl	10^{-1}	23/23/22/21/23/24/15/31/24/24	2.98 10^8
4	100 µl	10^{-2}	6/8/11/9/13/7/7/8/9/11	5.76 10^8

2.6. Epifluorescence microscopy. Maxumum volumen filtered = 5 ml. Factor = 34,520 fields/filter. 30 fields/filtter

Real case (cells/field): 0

Limit for enumeration: 1 cell in 1 field of 30 revised

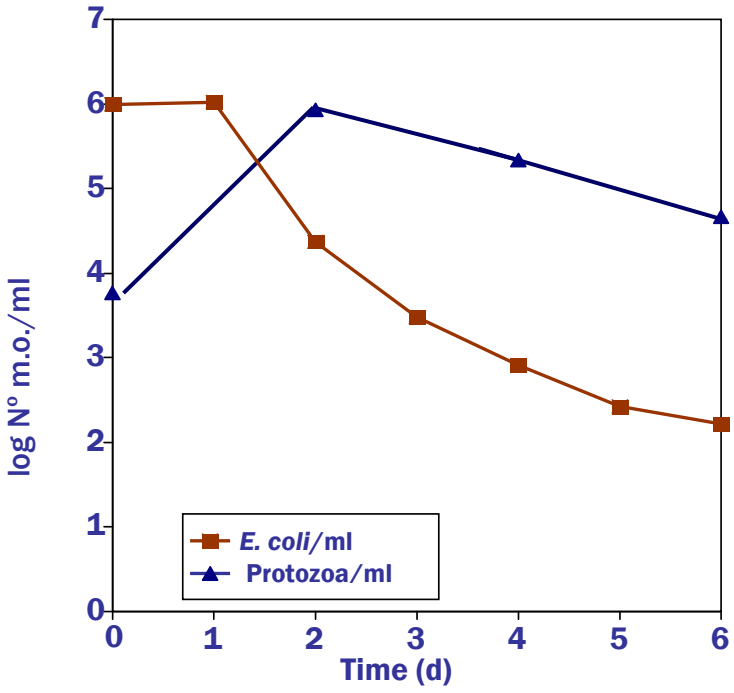
$$\frac{\text{1 bacteria/30 field X 34,520 fields/filter}}{\text{5 ml/filter}} = \text{230 bacteria/ml}$$

Detection limit <230 bacteria/ml

2.7. Effect of flagellated protozoa on the survival of *E. coli.* (Factor = 64,761.9)

Time (d)	Dil.	Vol. seeded (µl)	Colonies/plate	UFC/ml	Dil.	Vol. filtered (ml)	flagellates/field	Fagellates/ml
0	10^{-3}	100	96/96/108	10^{6}	M	40	4/6/6/5/4/1/1/3/4/5 6/4/4/3/1/2/1/4/4/3	$5.75\ 10^{3}$
1	10^{-3}	100	106/111/98	$1.05\ 10^{6}$				
2	10^{-1}	100	240/216/248	$2.35\ 10^{4}$	10^{-1}	5	8/7/6/7/8/8/5/5/7/8 8/7/7/5/5/7/8/3/7/5	$8.48\ 10^{5}$
3	S*	100	315/296/298	$3.03\ 10^{3}$				
4	S	100	88/76/78	$8.07\ 10^{2}$	10^{-1}	20	3/4/3/3/0/3/4/1/4/2 0/0/2/4/3/6/7/7/6/4	$1.07\ 10^{5}$
5	S	1000	265/267/281	271				
6	S	1000	166/166/168	166.67	10^{-1}	40	4/4/3/4/5/0/0/3/4/3 3/2/4/4/2/1/0/6/4/2	$4.70\ 10^{4}$

- S = Sample.



2.8. Epifluorescence microscopy. Volume filtered? Dilution?

$$\frac{25 \text{ bacteria/ field X } 20.000 \text{ fields/filter}}{V \text{ ml/filter}} = 4\ 10^{8} \text{ bacteria/ml}$$

$V = 1.25\ 10^{-3}$ ml ⟹ **V = 1.25 ml** **D = 10^{3}**

2.9. 20 ml dilution 10^{-3}.

- **Dilution 10^{-1} = 1 ml Sample + 9 ml Solvent**
- **Dilution 10^{-2} = 1 ml Sample + 9 ml Dilution 10^{-1}**
- **Dilution 10^{-3} = 2 ml Sample + 18 ml Dilution 10^{-2}**

CFU/ml?

$$\frac{125 \text{ colonies X } 10^{3}}{5 \text{ ml}} = 2.5\ 10^{4} \text{ CFU/ml}$$

Total number of bacteria/ml? Average number of bacteria/field = 14.9.

$$\frac{14.9 \text{ bacteria/field X } 1{,}920 \text{ X } 10^{3}}{10 \text{ ml}} = 2.86\ 10^{6} \text{ bacteria/ml}$$

2.10. Percentage of culturable Gram negative bacteria?

$$\frac{22.7 \text{ bacteria/field X } 15{,}000 \text{ X } 10^{2}}{2 \text{ ml}} = 1.7\ 10^{7} \text{ bacteria/ml}$$

$$\frac{[172.33 \text{ (Lac+)} + 75.33 \text{ (Lac-)}] \text{ colonies X } 10^{3}}{0.1 \text{ ml}} = 2.48\ 10^{6} \text{ CFUUFC Gram -/ml}$$

14.59 %

2.11. Density of a culture of *K. pneumoniae*?

$$\frac{40 \text{ colonies X } 10^{4}}{0.2 \text{ ml}} = 2\ 10^{6} \text{ CFU/ml}$$

Total CFU in 3 ml? $2\ 10^{6}$ CFU/ml X 3 ml = $6\ 10^{6}$ Total CFU

Dilution?

0.2 Abs _____ $2\ 10^{6}$ CFU /ml

0.5 Abs _____ $5\ 10^{6}$ CFU/ml

$$\frac{100 \text{ colonies X } D}{0.2 \text{ ml}} = 5\ 10^{6} \text{ CFU/ml}$$

D = 10^{-4}

2.12. *Klebsiella pneumoniae*. No. bacteria/ml= 1.032 10^7 Abs – 2.19 10^5.
Density of a culture with Absorbance = 0.49.

No. bacteria/ml= 1.032 10^7 x 0.49 – 2.19 10^5

4.84 10^6 bacteria/ml

3.1. Higher density? **Similar values**

Lactobacillus	Bacteria/field	Vol. Filteres (ml)	Dil.	Factor microscope (fields/filter)	Density (bacteria/ml)
L. acidophilus	25/32/30/28/33/34/35 29/28/24/15/32/33/18 20/22/18/23/19/26	0.5	10^{-2}	14,520	**7.61 10^7**
L. fermentum	5/12/13/8/13/8/15/9/ 8/14/15/12/13/8/10/7 /8/9/9/6	1	10^{-3}	7,220	**7.29 10^7**

Higher Biomass? *L. acidophilus*

Lactobacillus	Lenght (µm)	Width (µm)	Conversion factor (mg C/µm³)	Biomass (mg C/ml)
L. acidophilus	1.7	0.6	170 10^{-10}	**0.549**
L. fermentum	2	0.4		**0.291**

3.4. Density in each location? Volume spread/dish = 100 µl. ¿Higher density?

Dilution	Deep (m)		
	5	25	50
10^{-2}	Excess	500/617/593	363/303/395
10^{-3}	357/452/403	53/67/60	35/30/40
10^{-4}	35/45/40	5/7/6	4/2/5
UFC/ml	**4 10^6**	**6 10^5**	**3,5 10^5**

Biomass in each location? Microscope factor: 30,954 fields/filter. Conversion factor biovolume to biomass: 2.2 10^{-7} µgC/µm³.

Deep (m)	Dil.	Volume Filtered (µl)	Counts (Bacteria/field)	Bacteria/ml	Biovolume (µm³/cell)	Biomass (µg C/ml)
5	10^{-1}	100	30/35/40/25/30/ 25/33/25/27/30	**9.29 10^7**	0.15	**3.06**
25	10^{-1}	200	31/25/40/37/20/ 15/32/26/25/31	**4.36 10^7**	0.45	**4.32**
50	10^0	100	31/33/37/43/23/ 34/21/19/26/30	**9.20 10^6**	0,47	**0.95**

3.5. Density of *Lactococcus thermophilus* and *Lactobacillus bulgaricus* in the mixed cultured? Biomass of the culture = 3 mg C/ml. Conversion factor = 170 10^{-10} mg C/ml.

Microorganism	%	Biomass (µg C/ml)	Measures (µm)	Equation	Biovolume (μm^3/cell)	Density (bacteria/ml)
Lactococcus thermophilus	60	1.8	D = 1.2	$\pi D^3/6$	0.9048	$1.17\ 10^8$
Lactobacillus bulgaricus	40	1.2	L = 1.8 W = 0.5	$(L - W/3)\ \pi W^2/4$	0.3207	$2.20\ 10^8$