



## Sample: Microbiology - Bacterial growth

**1C.** During lag phase bacterial culture adapts to the new environment. Cells divide slowly.

During exponential phase reproduction rate is higher than death rate. The speed of reproduction is the highest. Bacteria use all available resources.

During stationary phase growth rate declines: reproduction rate is equal to death rate. Bacteria release secondary metabolites.

During death phase bacterial culture dies. All the resources have been exhausted. Death rate is higher than reproduction rate.

**1D.** Generation time is the period during which the bacterial culture doubles. So I chose two points on the Y axis which indicate doubling of CFUs number and extrapolated them to the respective points on the growth curve. Then from these points two straight lines were pulled down on the X axis. Thus, the generation time can be read directly from the plot as distance between these two points. The generation time for this culture is a little more than half an hour.

**1E.**  $k = (\log N_t - \log N_0)/0.3t$

$$k = 1/g$$

k - mean growth rate constant

g - mean generation time

$N_0$  - the initial population number

$N_t$  - the population at time  $t$

$$k = (\log 4000000 - \log 29)/0.3 \cdot 11 = (6.6 - 1.46)/3.3 = 5.14/3.3 = 1.557 \text{ generations/hour}$$

$$g = 1/k = 1/1.557 = 0.64 \text{ hr/gen.} = 38 \text{ min/gen.}$$

**2.** pH lowering by 1 point may have no effect on growth rate, but temperature lowering by 5°C will slow growth rate because enzymes' temperature optimums of these bacteria lay in higher temperatures. Generation time will decrease. In the growth curve we will see lag phase extension because it takes more time for adaptation. Growth curve in its exponential phase will be flatter because of growth rate slowing. It will take more time for microbial culture to reach stationary phase because of slowing of nutrient uptake and metabolites accumulation. Growth curve can be not so high because of lower amount of bacteria which are maintained in such conditions. So growth curve may stretch on the X axis direction (time) and decrease on the Y axis direction (number of CFUs).

I think, that drawn graph was determined using quite optimal conditions. But presence of lag phase on growth curve indicates that cultivating conditions can be optimized.



**3.** *E. coli* as chemoorganoheterotrophic bacterium need organic compounds in media as source of energy, electrons and carbon. For this reason glucose was included. To grow *E. coli* needs large amounts of nitrogen, phosphorus, and sulfur, which are incorporated into growth medium as inorganic compounds. Ammonium sulfate is the source of sulphur and nitrogen. Nitrogen is needed for the synthesis of amino acids, nucleic acids, cell wall components, enzyme cofactors. Sulphur is required for amino acids (cysteine and methionine), biotin, thiamine, coenzyme A synthesis. Phosphorus is present in nucleic acids, ATP, phospholipids, cell wall components. Phosphorus is supplied by potassium phosphates. On the other hand, monobasic ( $\text{KH}_2\text{PO}_4$ ) and dibasic ( $\text{K}_2\text{HPO}_4$ ) potassium phosphates have very high buffering capacity, hence serve as buffer. Potassium of potassium phosphates is used as enzyme cofactor. Magnesium sulfate is a source of sulfur and magnesium in the growth medium. Magnesium serves as a cofactor for many enzymes, complexes with ATP. Calcium chloride is a source of calcium and chloride ion that is required for lots of cellular processes. Trace elements are microelements that are required in small amounts, however contribute to enzymes functioning. Cobalt is a component of vitamin B<sub>12</sub> which is synthesized by procaryotes.

**4. A.** Culture medium is a source of macro- and micronutrients, so components of the medium are almost the same. However, their amounts are different. Fewer components of the buffer system are required by *Thiobacillus thioparus* because it does not produce acid while *E. coli* does. Sulphates are avoided in the culture medium for *T. thioparus* because it oxidizes sulphur and sulphates can inhibit its metabolism. Glucose source is not required by *T. thioparus* because it obtains energy from sulphide and thiosulphate oxidation. Therefore, sodium thiosulphate is present in the medium to provide the microorganism with energy and electrons.

**B.** *Thiobacillus thioparus* is likely to obtain energy from oxidation of thiosulphate from the medium. Thiosulphate donates electrons to the electron transport chain and it results in energy release. Thiosulphate is then converted into sulphate. As far as carbon sources are not present in the culture medium, the bacterium must be autotrophic. It produces its own organic molecules via carbon dioxide fixation.

**C.** *Thiobacillus thioparus* is autotrophic because it produces its own organic molecules. It is chemotrophic because it obtains the energy from chemical reactions (thiosulphate oxidation into sulphate). It is litotrophic because it obtains electrons from inorganic substances (thiosulphate). Thus, it is a chemolitoautotrophic organism.



## References

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Vlasceanu L., Popa R. and Kinkle B.K. 1997. Characterization of Thiobacillus thioparus LV43 and its distribution in a chemoautotrophically based groundwater ecosystem. Appl Environ Microbiol.

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