Experiment 19	**Microbial growth in homogeneous batch culture: Growth curves**
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Reading	Chapters in BBOM 9th: 5.2-5.4, pgs. 137-145 Chapters in BBOM 10th: 6.3-6.6, pgs 142-149 BBOM : Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition (1999) or 10th Edition (2003), Prentice Hall. Explanations to Experiment 17 "Dynamische Systeme in der Mikrobiologie"
Objectives	We will grow *E.coli* at different temperatures and measure growth by turbidity with a spectrophotometer. This will give us the data to draw growth curves, which are going to be analyzed mathematically in experiment 17.
Background	Microbial growth is usually defined as the increase in cell numbers. A growth curve represents growth as a function of time. Growth may be divided into four different stages: the lag phase, the exponential or log phase, the stationary phase and the death or decline phase. When an inoculum is transferred into a new medium, the **lag phase** occurs first. It is the time required by the microbial cells to adapt to the new conditions. If they come out of an extended stationary phase they may first have to adjust metabolically or start up the cellular synthesis machinery. The cells might also increase in size until they are ready for division. The lag phase may be very short or almost missing under certain conditions, and it can be extremely long for certain organisms. In particular, if one tries to enrich from environments whose growth conditions are not well defined or if one does not know all the crucial growth requirements of the organism of interest, one might sometimes have to wait for weeks until growth can be observed. The lag phase is followed by the **log phase**. In the log phase, cells divide, grow and divide most actively. This is the time when the population grows exponentially. After the log phase the population enters the **stationary phase**. Limitation of nutrients or high quantities of metabolic waste products slow down population growth. The cell death rate is equal to the cell growth rate. The population size becomes stationary. The length of the stationary phase can be variable. It depends on the organism's ability to adapt and on the state of the environment. Spore or cyst forming microorganisms may remain in this stage for a very long time. When the death rate exceeds the rate of growth, the population has entered the **declining phase**. This phase may be caused by accumulation of toxic waste products, by very low nutrient availability, by cell damage or by changes of physical environmental determinants. Whenever an aliquot of cells is transferred to a new environment, the population usually goes through all four stages, starting with the lag phase. In a batch culture experiment (cultivation in a bottle, which is a more or less "closed system") we can observe the different growth phases most clearly. Microbial growth can be measured in different ways. One way is to measure the optical density (OD) of a broth culture in time. Using the change in apparent absorbancy (actually the light loss due to scattering of light by the particles) as a measure, the generation time of the population can be estimated.
Literature	The chapters in BBOM 9th or 10th mentioned above.
www. Links	How others are doing this experiment http://www.biochem.usyd.edu.au/MBLG2001/MBLGlectures/MBLGlecture_15.html http://www.hawaii.edu/malama/teachers/TLunsford/safety/Growth_curve.htm http://www.eng.auburn.edu/~wfgale/usda_course/pasteur_page1.htm http://www.scotcal.com/growth3/

Practical Work	The students grow two cultures in non-limiting medium at different temperatures (20-22°C, room temperature and 37°C) and measure turbidity every 15 min. They record the exact time point and the OD at 600 nm.
Material and Experimental Protocols	Put the flask containing the *E. coli* culture in LB medium on a shaker kept in the incubator at 37°C and leave a parallel culture on the shaker placed in the lab (room temperature). Record the room temperature from time to time. Adjust both shakers to the same rpm. Measure the absorbancy of the *E. coli* cultures at 600 nm every 15 min: Transfer 2-3 ml of the *E. coli* culture into a cuvette and read the initial OD ($OD_{t=0}$). Record the time and the OD at 600 nm against uninoculated LB medium as a blank. If the OD exceeds 0.7, dilute the sample in the cuvette with medium. After the measurement, pour the sample into the waste beaker and wash the cuvette. Shake out any moisture and put the cuvette upside down on a household paper until the next measurement. Draw the growth curve on linear and logarithmic paper. Compare the two growth curves under the different growth conditions. **Material:** *E. coli* cultures pregrown at r.t. und 37°C LB medium, preheated to r.t. and 37°C respectively pipettors (5ml, 1ml and 200µl) with tips plastic cuvettes, 1 cm pathlength photospectrometer 2 shakers thermometer bunsen burner 2x 250ml Erlenmeyer flasks dH2O waste beaker (for autoclaving) technical alcohol household paper kleenex gloves parafilm aluminium foil matches marker pens timer millimeter paper and logarithmic paper ev. water quentch ev. tripod 2 clamps
Laboratory Rules & Precautions	Wash hands before leaving the lab and work aseptically. Wear lab coat and gloves.
Goals & Experiences gained	• to calculate concentrations • to know the different stages of a growth curve • to know the generation time of the culture • to construct growth curves •
Timing	90 min
Reporting	Arrange data clearly. Draw growth curves and discuss information represented by them You will obtain a number of growth curves of various organisms grown under different conditions and will be asked to explain the shapes.
Questions to be answered	Will be handed out in the course.