

Regulation of constant biomass and optical density during cultivation

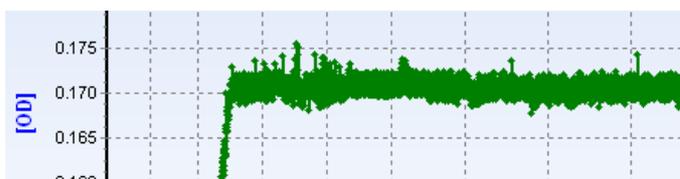
I. Permanent cultivation of bacteria

Fermentations of bacteria usually show some negative effects during cultivation due to enrichment of toxic substances or depletion of nutrients. The consequence is reduced growth, reaching a suboptimal stationary phase and finally cell death. Many ideas were followed in the past to circumvent this problem. One good approach is a permanent dilution of the growing culture with fresh medium. Permanent harvesting of excessive culture volume will lead to constant quality. A second advantage is that the cultivation product can be applied continuously to downstream processes.

For finding the correct dilution rates in an automatic process, the speed of adding media must be regulated by an electronic control cycle. The EloCheck photometer provides an easy way to use the output analog signal for regulating the pump speed in the dependency of biomass concentration or optical density. If the biomass (optical density) in a culture reaches a critical value, the pump is activated until the culture is diluted enough. In this way cultures of microorganisms can be held in constant concentration over an endless time.



EloCheck screenshot of a fermentation of *L. plantarum* in MRS medium: After reaching a specified optical density, fresh medium is added automatically so that the biomass concentration was stable over 14 h. The medium pump was switched off in the last phase of this cultivation.

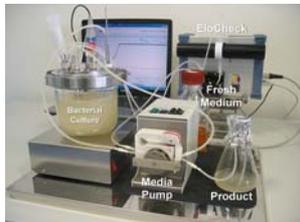


Precise regulation of optical density after reaching a specified value.

Because the strength of the EloCheck analog signal is proportional to the optical density, the regulation of pump speed is very exact and oscillations of this control circuit are nearly impossible (see figure on the left).

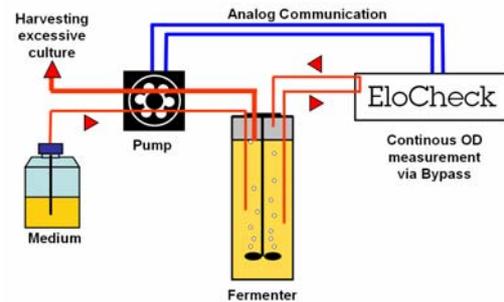
II. Tubes and pumping

Good results for pumping medium and harvesting excessive culture are obtained with the peristaltic pump 'REGLO Analog' from ISMATEC (www.ismatec.ch). Because several pump channels can be installed at the same time,



Endless fermentation can be done with volumes of 40 ml and more.

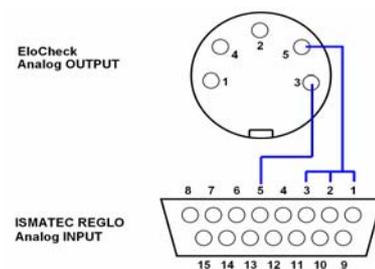
pumping of fresh medium and removing excessive culture are possible with only one pump. Harvesting excessive culture is done volume level regulated (only volume above a given level is pumped out of the fermenter).



Schematic diagram of all fluidics used for continuous OD measurement and automatic dilution of culture medium.

III. Analog communication between EloCheck and Media pump

- 1) ISMATEC REGLO Analog intern DIP 3 switched ON
- 2) Short cut ISMATEC 1/2/3 and connected to EloCheck 5(GND)
- 3) Connecting ISMATEC 5 to EloCheck 3 (0/2...10V)
- 4) Using EloCheck Analog Out Settings 0...20mA, 0...10V
- 5) Using EloCheck Analog Out Settings OD=10 -> max. Output level



Because the analog output signal is proportional to the "calcOD" value of EloCheck, the pump speed and dilution rate can be controlled with polynomial calibration function in the option menu. For the example on the first page of the application note the OD-formula

$$\text{CalcOD} = 0X^3 + 0X^2 + 10X - 1.6$$

was used. The correct parameter must be adjusted empirical to the wanted optical density, culture volume, culture growth speed and the diameter of the used pump tubes.

This application note should provide the use with helpful information for optimal use of the EloCheck photometer. If you need additional technical information, do not hesitate to e-mail biotronic Technical Service Department (support@biotronic.de)



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