Determination of various characteristic growth parameters of *B. licheniformis* DSM 13 on only yeast extract media using continuous cultivation process

Laura Amler (B.Sc.); Stefanie Kaul (M.Sc.), Prof. Dr. Gesine Cornelissen
Hamburg University of Applied Sciences, Hamburg, Germany

Background

Continuous cultivation is used for the characterization of various growth parameters. The gram-positive model organism *B. licheniformis* DSM 13 is able to produce all essential amino acids by itself while it also can use them as an energy source for propagation and maintenance metabolism.

Yeast extract is often used as a media component due to its high amount of essential nutrients like amino acids, vitamins and minerals. *B. licheniformis* is able to grow on only yeast extract without further carbon sources.

Aim of the research

The aim of the research is to characterize parameters like the saturation constant K_S and optimal dilution rate D_{opt} by using continuous cultivation. The saturation constant K_S can be compared with already known saturation constants of carbon sources. Furthermore, the ratio of essential components in yeast extracts can be identified by standard analytical methods which helps to evaluate the performance of the yeast extract in growth behavior without the need to identify its complete composition.

Methods

For continuous cultivation three yeast extracts* were examined. The cultivations were started with a batch phase to reach sufficiently high cell density, followed by a chemostat phase. Media for batch was 10 gL^{-1} yeast extract and 20 gL^{-1} glucose. Media for chemostat was pure yeast extract 10 gL^{-1}. Dilution rates were varied from 0.063 h^{-1} to 0.3 h^{-1}. Experiments were done in 1 L bioreactors which are part of a BIOSTAT® Qplus multi-bioreactor plant. 5 L flasks were used as the reservoir and harvest (Fig. 1). The reaction volume of 0.75 L was adjusted by a submersion pipe and the dilution rate was set by the flow rate of a multi-channel pump. A supply unit which is connected to a digital control unit allows regulation of pH, aeration and stirring.

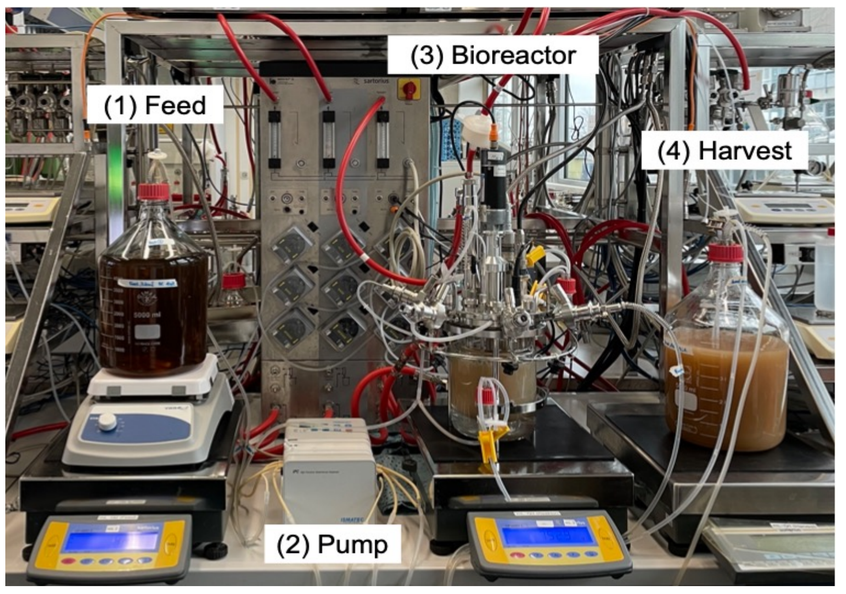

Fig. 1: Multi-bioreactor plant BIOSTAT® Qplus.

Substrate uptake in samples will be analyzed by a carbohydrate HPLC and spectroscopic analysis of total carbohydrates.

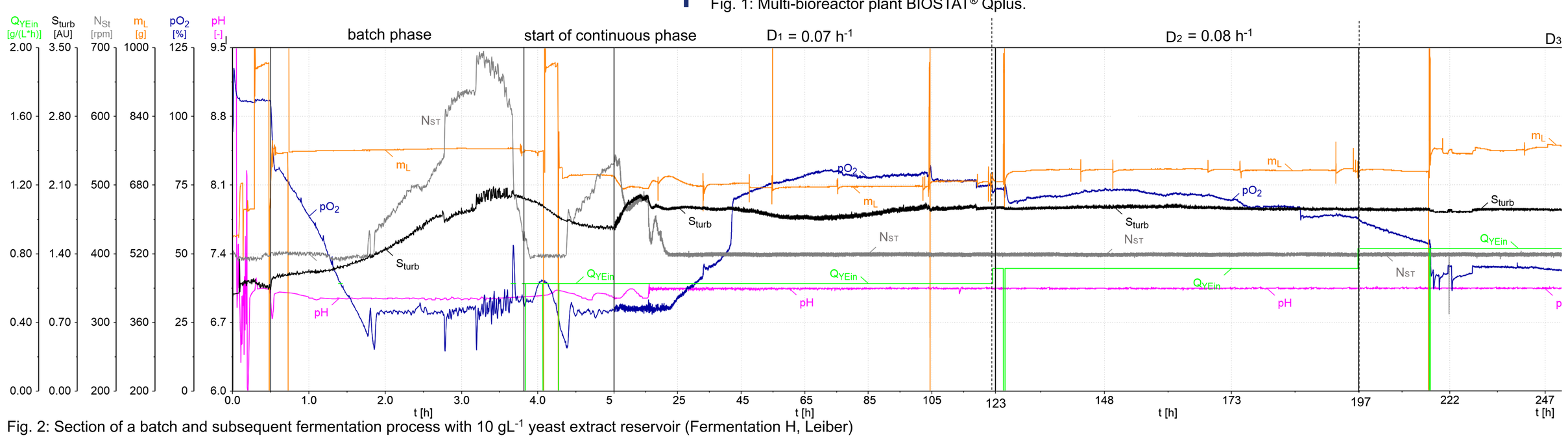

Fig. 2: Section of a batch and subsequent fermentation process with 10 gL^{-1} yeast extract reservoir (Fermentation H, Leiber)

Evaluation

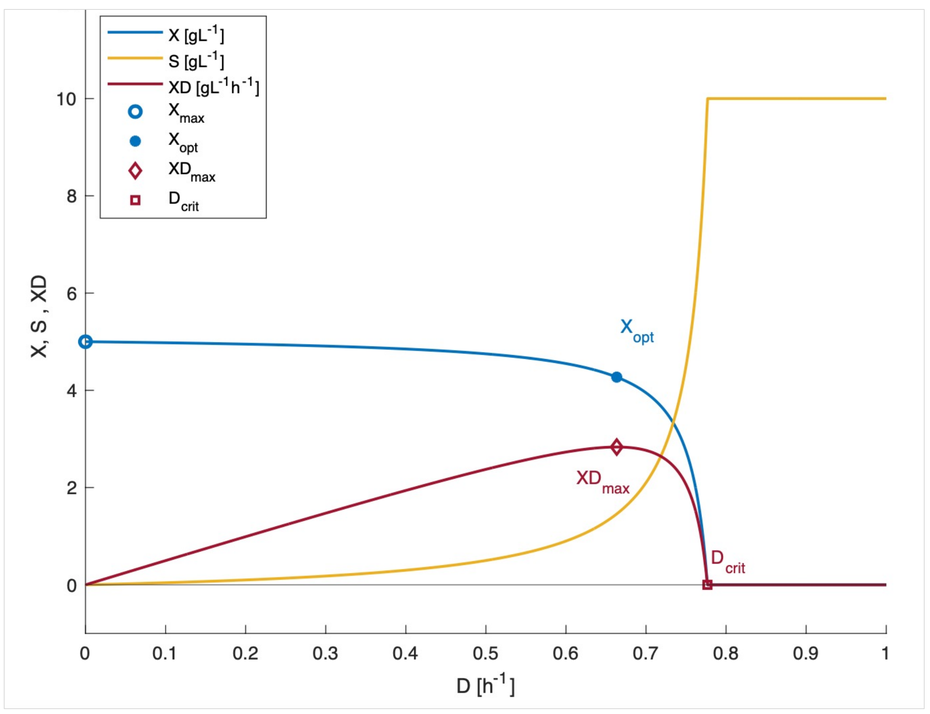

Fig. 3: Exemplary representation of a X-D-diagram.

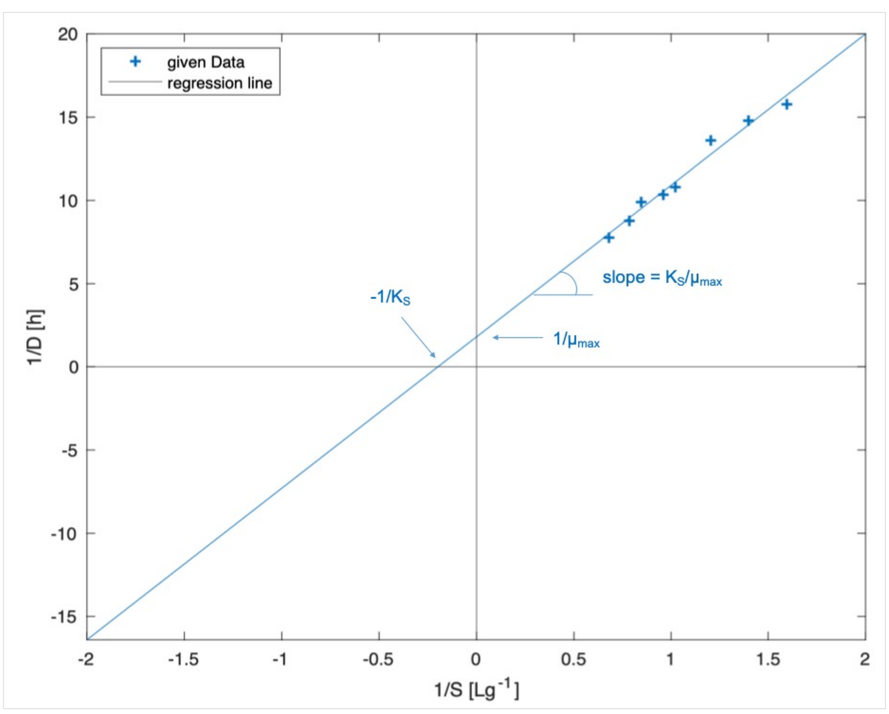

Fig. 4: Exemplary representation of Lineweaver-Burk-Plot.

For determination of the saturation constant K_S and the maximal specific growth rate μ_{max} the Lineweaver-Burk-Plot (Fig. 4) is used. Dilution rates and substrate concentrations are plotted reciprocally. The Y-axis intercept gives $1/\mu$ [h] and the slope of the regression line is K_S/μ [ghL^{-1}]. Conversion of them results in μ_{max} [h^{-1}] and K_S [gL^{-1}].

The goal of the evaluation is to create an X-D-diagram (Fig. 3) with the cell (X) and substrate (S) concentration at each dilution rate (D) and the responding productivity (XD). This diagram allows the determination of the optimal dilution rate D_{opt} at the maximum of the productivity curve.

Outlook & Conclusion

The consumption of carbohydrates should give information about the consumption of yeast extract, since it is very complex to examine every single substrate, as they are also consumed in different amounts.

* Fermentation H, Leiber GmbH, Germany; Lallemand Bio-Ingredients FNI 103, Canada; yeast extract powdered for bacteriology, Carl Roth GmbH + Co. KG, Germany