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Development of a new chemically defined medium for various animal cell lines

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Introduction

The optimisation of cultivation media is one of the most complex steps in cell culture process development. This complexity arises from the large number of components, their manifold interactions and metabolic cross-linking. To face this challenge, several strategies of medium development were used. For economic design of the nutrient content a model-based approach was applied, in which simulations addressed the metabolic rates. Furthermore, classical approaches and design of experiments were used. Important requirements for an optimized culture medium are:

► chemically defined composition (absence of complex components)

► low osmolality to form a suitable basis for fed-batch cultivation

► applicability as a basis for different cell clones and ideally different cell types

Considering these points, a chemically defined medium platform was designed. It is suitable for high cell density batch cultivation of various industrially relevant animal cell lines.

Methods

Cultivation

The different cell lines were cultivated in chemically defined, animal component free medium (TeutoCell, Bielefeld). The medium was prepared in our labs or delivered by a CMO. For experiments under controlled conditions, 1-2 L bioreactors and the Cultibag RM system were used. Conditions: 37 °C, 40 % pO2, initial pH 7,1.

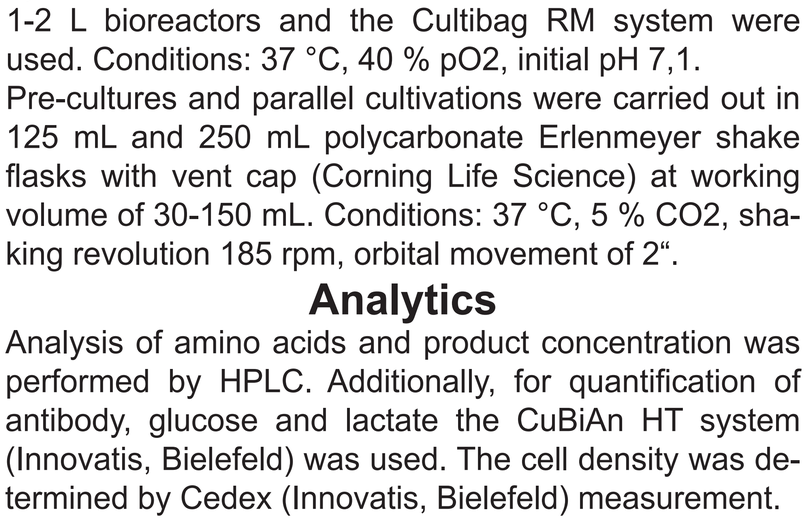
Pre-cultures and parallel cultivations were carried out in 125 mL and 250 mL polycarbonate Erlenmeyer shake flasks with vent cap (Corning Life Science) at working volume of 30-150 mL. Conditions: 37 °C, 5 % CO2, shaking revolution 185 rpm, orbital movement of 2“.

Analytics

Analysis of amino acids and product concentration was performed by HPLC. Additionally, for quantification of antibody, glucose and lactate the CuBiAn HT system (Innovatis, Bielefeld) was used. The cell density was determined by Cedex (Innovatis, Bielefeld) measurement.

Medium development

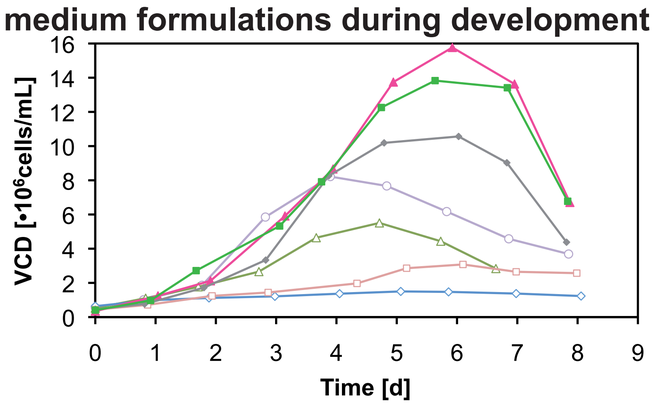
The optimization of nutrient content of measurable components was performed by modelling using Berkeley Madonna (Robert Macey, George Oster). For components without knowledge of their consumption, parallel cultivations were carried out. These were planned using JMP software (SAS,USA) and classical approaches. A basal formulation was chosen as starting point.



The combined development strategy is shown in the figure above. For most cell lines more than one of these cycles was done.

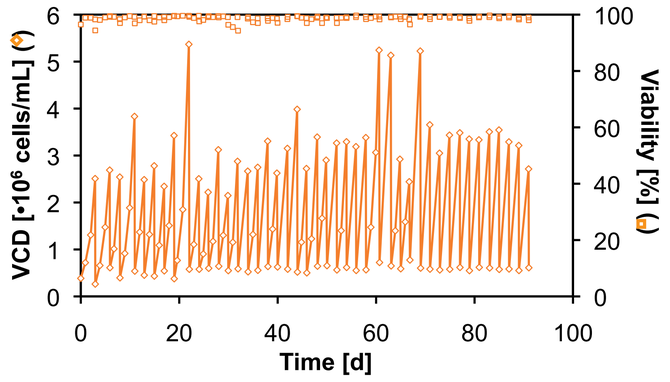
Results

CHO DP-12 cultivation in different medium formulations during development



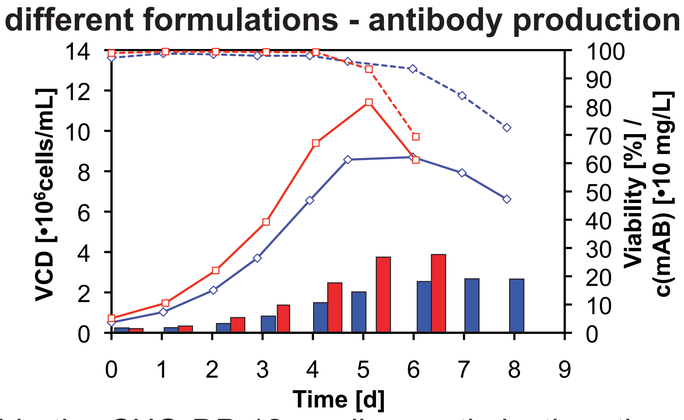
Initially, the maintenance of the CHO DP-12 cells at high viabilities of about 90% without growth was observed. Further development led to specific growth rates of more than 0.7 d^{-1} and maximal cell densities beyond $1.5 \cdot 10^7$ cells/mL.

Growth stability of CHO DP-12



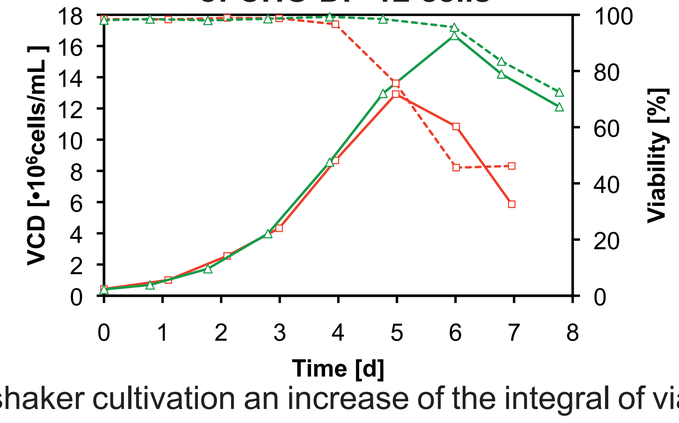
For all cell lines the growth behaviour in several passages was investigated. Also other cell lines including CHO K1 cells were passaged more than 20 times. All tested cell lines were cultivated for a minimum of five passages.

Bioreactor cultivation of CHO DP-12 cells in two different formulations - antibody production



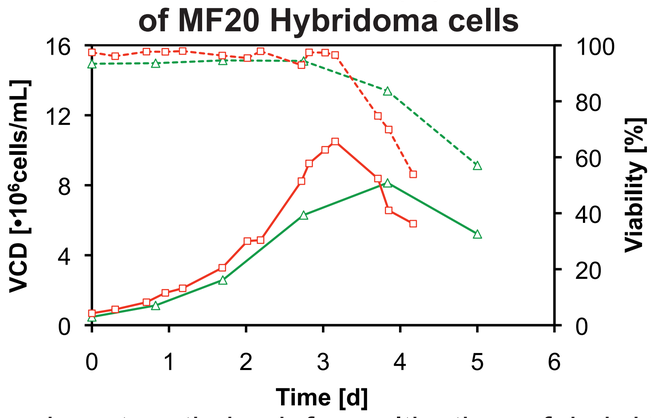
Within the CHO DP-12 medium optimization, the antibody concentration was increased from 40 mg/L to 280 mg/L (controlled conditions). Components addressing the cell specific productivity were tested yet.

1L Cultibag RM (-□-) / shaker (-△-) cultivation of CHO DP-12 cells



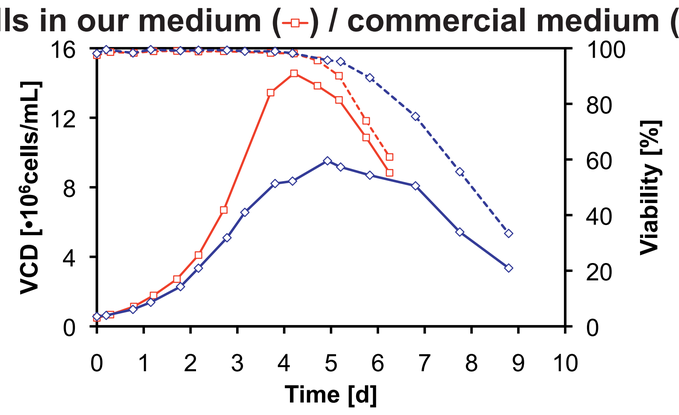
In shaker cultivation an increase of the integral of viable cell density for the CHO cell line was observed. This indicates that further process optimization (e.g. pH shifts) may enhance the capability of processes under controlled conditions.

Bioreactor (-□-) / shaker (-△-) cultivation of MF20 Hybridoma cells



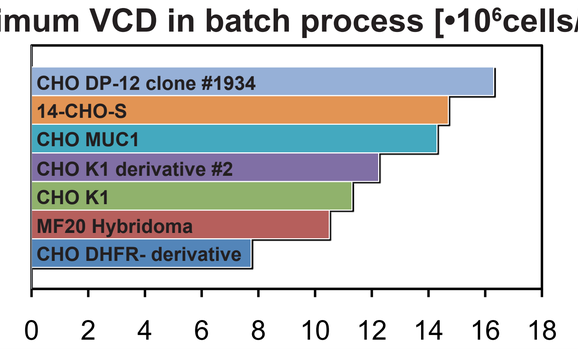
Although not optimized for cultivation of hybridoma cells, cell densities of more than $1 \cdot 10^7$ cells/mL were reached in 2 L bioreactor. In shaker cultivation the pH-value was rapidly decreased due to excessive formation of acidic metabolites.

Bioreactor cultivation of 14-CHO-S (Celonic, Basel) cells in our medium (-□-) / commercial medium (-◇-)



The industrial 14-CHO-S cell line was also tested. Compared to a commercial medium the specific growth rate and the maximum viable cell density were increased.

Maximum VCD in batch process [•10⁶cells/mL]



Different medium formulations were tested using various animal cell lines. The highest cell density was reached with the CHO DP-12 cells ($1.6 \cdot 10^7$ cells/mL). Most development was done for this cell line. Process optimisation has not been carried out for all cell lines yet.

Conclusions

►► An animal component free, chemically defined medium suitable for the cultivation of several animal cell lines was developed

►► Cell densities beyond $1.5 \cdot 10^7$ cells/mL were obtained depending on cell line

►► A high process stability was observed

►► Due to the buffer configuration, the medium is highly suitable for cultivation in pH-uncontrolled systems

►► The medium was successfully tested in different cultivation systems (STR up to 100 L and Cultibag RM)

►► Compared to cultivations in the tested comercially available media cell densities and product concentartion were higher

►► With its low osmolality of 280 mOsmol/kg the medium is a optimal basis for fedbatch process

►► The medium was successfully tested in other laboratories

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