CO_2/pH CONTROL WITHOUT PROBES IN SHAKEN MAMMALIAN CELL CULTURES

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Introduction

Process optimisation with mammalian cells requires multiple parameter testing in numerous experiments. Shaken systems allow cheap, reliable and flexible screening experiments and are therefore widely used in process development.

A major drawback of shaken systems is the limited direct control of the pH and the gas exchange, factors that may affect productivity and growth of cultivated cells.

This poster displays methods to control the pH of the culture medium in shaken reactors by controlling the CO_2 partial pressure of the gas in contact with the culture.

The pH <-> CO_2 equilibrium

CO_2 dissolves in aqueous solutions and reacts with H_2O. The formed acid H_2CO_3 is in equilibrium with bicarbonate HCO_3^-, an ion that is commonly added to the culture medium as sodium salt.

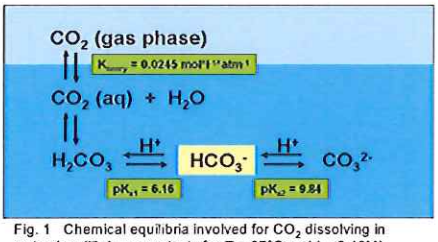

Fig. 1 Chemical equilibria involved for CO_2 dissolving in water (equilibrium constants for T = 37°C and I = 0.16M)

The bicarbonate ion HCO_3^- acts as a pH buffer in the culture medium. Its concentration is regulated by the CO_2 partial pressure of the gas phase.

The chemical equilibria involved in the dissolution of CO_2 can be simulated. The resulting dependency of the pH of the culture medium on the CO_2 partial pressure of the gas phase is displayed graphically as a function of the sodium bicarbonate concentration in figure 2.

By controlling the CO_2 partial pressure of the gas in contact with the culture medium, the pH can be adjusted to the ideal value for any medium buffered with sodium bicarbonate. Acified solutions (i.e. due to lactate synthetized and CO_2 produced by the cells) can be brought to a optimum pH by degassing CO_2 out of the reactor vessel.

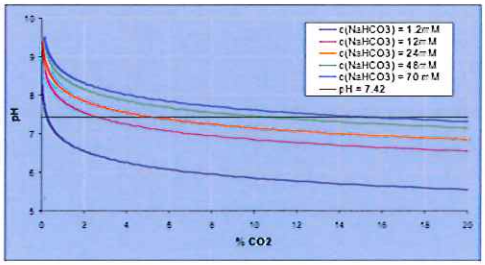

Fig. 2 Calculated $P(CO_2)$ <-> pH equilibria curves for different sodium bicarbonate solutions (ionic strength I = 0.16M)

Materials and methods

The experiments are performed in a shaker with CO_2 control.

Plastic tubes with a filter cap are used as shaking vessels. The filter cap allows gas exchange between the inside of the reactor tube and the incubator atmosphere.

Fig. 3 Incubator with shaken tube reactors and CO_2 control

The gas transfer resistances at the gas-liquid interface and at the filter cap prevent a free gas exchange from the culture medium to the gas phase.

CO_2 gas transfer

Measuring the transfer of CO_2 out of the reactor tube, a gas transfer coefficient k can be defined. This coefficient characterizes the transfer resistance of the system.

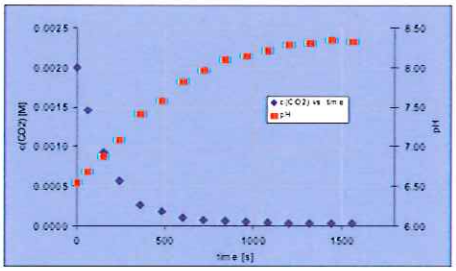

Fig. 4 Degassing CO_2 out of the reactor tube: influence on CO_2 concentration and pH of the reactor medium.

Different system parameters yield different gas transfer resistances, corresponding to different gas transfer rates.

Conclusions

An incubator with CO_2 controlled atmosphere allows exact adjustment of the pH of the cell culture medium.

A decrease of the pH of the medium through CO_2 or acid production of the cells can be prevented by adjusting the CO_2 partial pressure of the gas in contact with the culture medium.

This adjustment is done by controlling the CO_2 concentration in the incubator or by alternating the CO_2 transfer from and to the reactor flask.

The CO_2 transfer from and to the culture medium can be influenced by the following parameters:

- The shaking power input (shaking speed and radius)
- The filling volume
- The nature and size of the filter in the filter cap

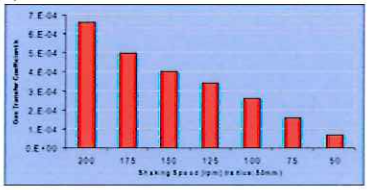

Fig. 5 Influence of the shaking power input on the gas transfer: The stronger the shaking input, the greater the gas transfer.

References:

James N. Butler; *Ionic Equilibrium*; 1998; John Wiley & Sons Inc.; New York

W.G. Whitman and W.K. Lewis; *Film Theory*; Industrial Chemical Engineering, 16; 1215 (1924)

Acknowledgements:

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