

CO₂/pH CONTROL WITHOUT PROBES IN SHAKEN MAMMALIAN CELL CULTURES

Christoph Eigenmann, Martin Jordan, Florian Wurm

Faculty of Basic Sciences, Federal Institute of Technology, Lausanne (EPFL), Switzerland

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 CO₂ partial pressure of the gas in contact with the culture.

The pH <-> CO₂ equilibrium

CO₂ dissolves in aqueous solutions and reacts with H₂O. The formed acid H₂CO₃ is in equilibrium with bicarbonate HCO₃⁻, an ion that is commonly added to the culture medium as sodium salt.

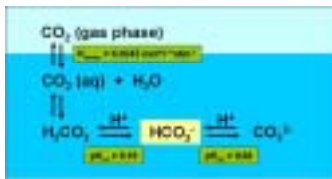


Fig. 1 Chemical equilibria involved for CO₂ dissolving in water (equilibrium constants for T = 37°C and I = 0.16M)

The bicarbonate ion HCO₃⁻ acts as a pH buffer in the culture medium. Its concentration is regulated by the CO₂ partial pressure of the gas phase.

The chemical equilibria involved in the dissolution of CO₂ can be simulated. The resulting dependency of the pH of the culture medium on the CO₂ partial pressure of the gas phase is displayed graphically as a function of the sodium bicarbonate concentration in figure 2.

By controlling the CO₂ 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

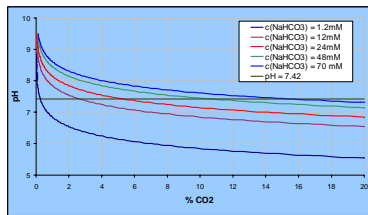


Fig. 2 Calculated P(CO₂) <-> pH equilibria curves for different sodium bicarbonate solutions (ionic strength I = 0.16M)

bicarbonate. Acidified solutions (i.e. due to lactate synthesized and CO₂ produced by the cells) can be brought to a optimum pH by degassing CO₂ out of the reactor vessel.

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:

Great thanks to
• Kühner AG (Switzerland) for letting us use a shaker of type ISF-1-W
• TPP (Techno Plastic Products AG, Switzerland) for the reactor tubes

Materials and methods

The experiments are performed in a shaker with CO₂ 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 CO₂ 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.

CO₂ gas transfer

Measuring the transfer of CO₂ out of the reactor tube, a gas transfer coefficient k can be defined. This coefficient characterizes the transfer resistance of the system.

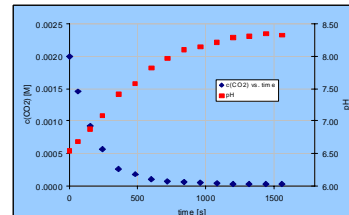


Fig. 4 Degassing CO₂ out of the reactor tube: influence on CO₂ concentration and pH of the reactor medium.

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

Conclusions

An incubator with CO₂ controlled atmosphere allows exact adjustment of the pH of the cell culture medium.

A decrease of the pH of the medium through CO₂ or acid production of the cells can be prevented by adjusting the CO₂ partial pressure of the gas in contact with the culture medium.

This adjustment is done by controlling the CO₂ concentration in the incubator or by alternating the CO₂ transfer from and to the reactor flask.

The CO₂ 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

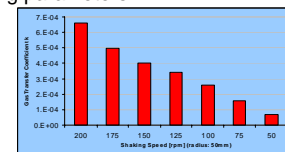


Fig. 5 Influence of the shaking power input on the gas transfer. The stronger the shaking input, the greater the gas transfer.