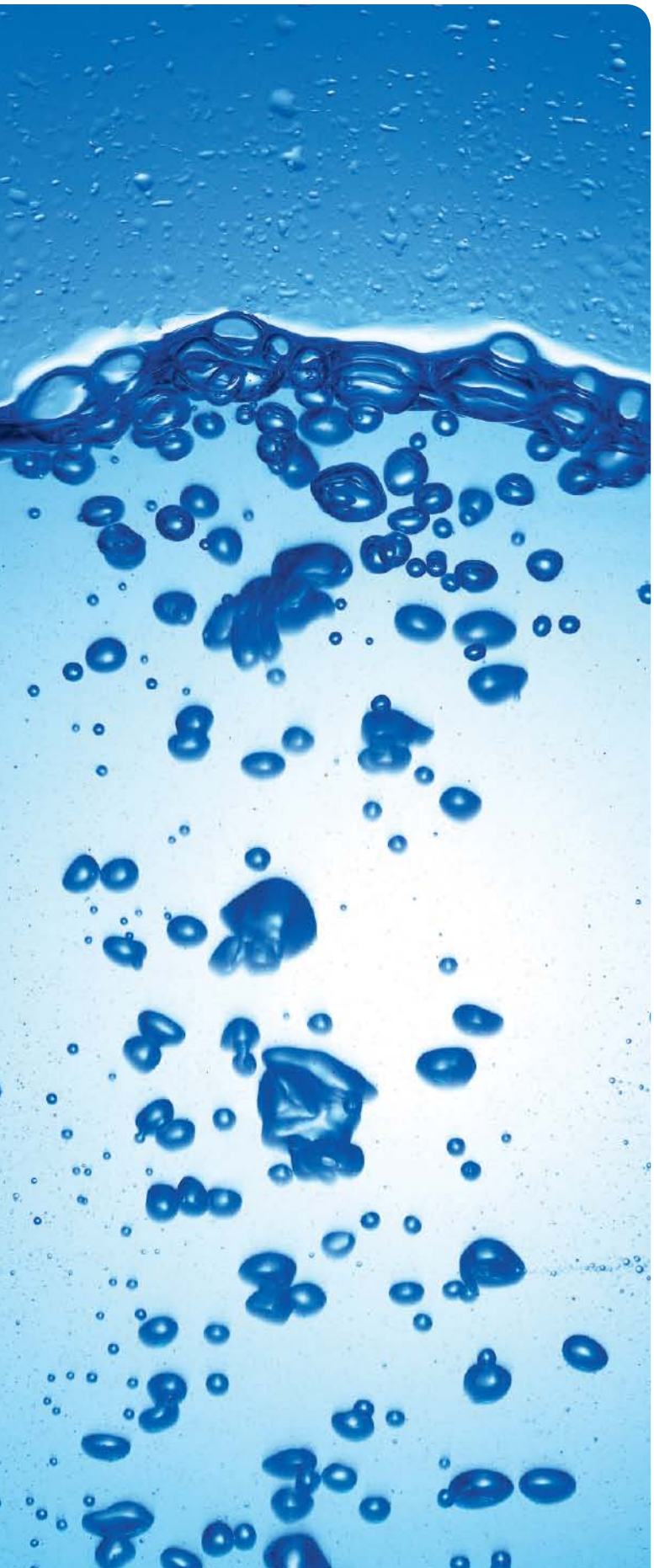
Growth Control

Non-invasive Measurement of Dissolved Oxygen and pH in Shake Flask Cultures



Shake flasks are the most commonly used cultivation system. A major, though often neglected problem in aerobic shake flask cultivation is limited oxygen supply or an incorrect pH value.

Enhanced Culture Performance

The optimal oxygen supply of cells and perfect pH conditions are essential basic requirements for high quality cell cultures. A shortage in oxygen significantly influences the cellular metabolism; the same applies for shifts of pH. Different factors like the geometry of baffles or the application or production of surface active compounds can change the oxygen mass transfer unpredictably. PreSens' new Shake Flask Reader (SFR) allows parallel optical online measurement of dissolved oxygen concentration (DO) and pH in shake flask.

Optical Sensor Technology

PreSens' chemical optical sensors consist of a thin layer containing an analyte sensitive (oxygen or pH) fluorescent dye. If the sensor spots encounter a molecule of the respective analyte, the excess energy in a non-radiative way is transferred, decreasing or quenching the fluorescence signal. Decay time measurement is used to determine the oxygen concentration in the sample. In case of pH detection, a combination of different fluorescent dyes is used for Dual Lifetime Referencing (DLR).

Culture Conditions

E. coli DH5α was cultivated in baffled glass and unbaffled plastic shake flasks equipped with an oxygen sensor and, in the plastic flasks, an additional pH sensor. Cultivations were carried out in duplicates at 37°C with shaking frequencies of 90 rpm for the first 2 h, 120 rpm during 2 h – 3,5 h, 170 rpm 3,5 - 5 h and afterwards 120 rpm.

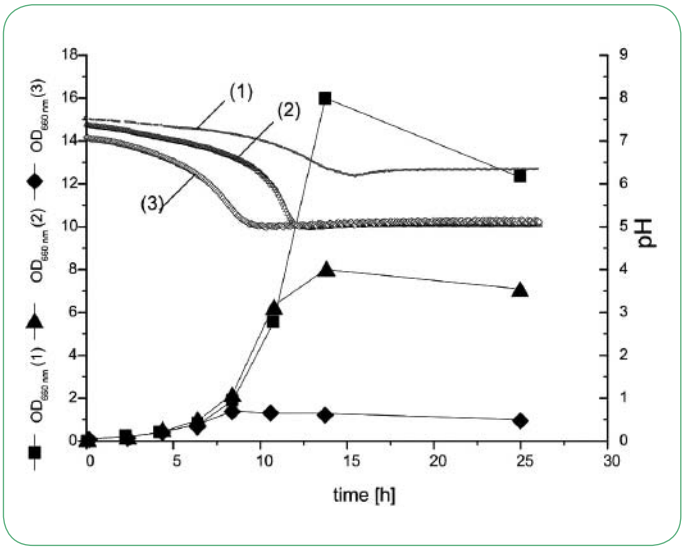


Fig. 2: Cultivation of *C. glutamicum* lysC^{fbr} in batch culture on defined medium with different concentrations of phosphate buffer 106 mM (1), 32 mM (2) and 6 mM (3). Cultivations were carried out in 250 ml non-baffled plastic shake flasks equipped with an oxygen and pH sensor at 30 °C and 230 rpm.

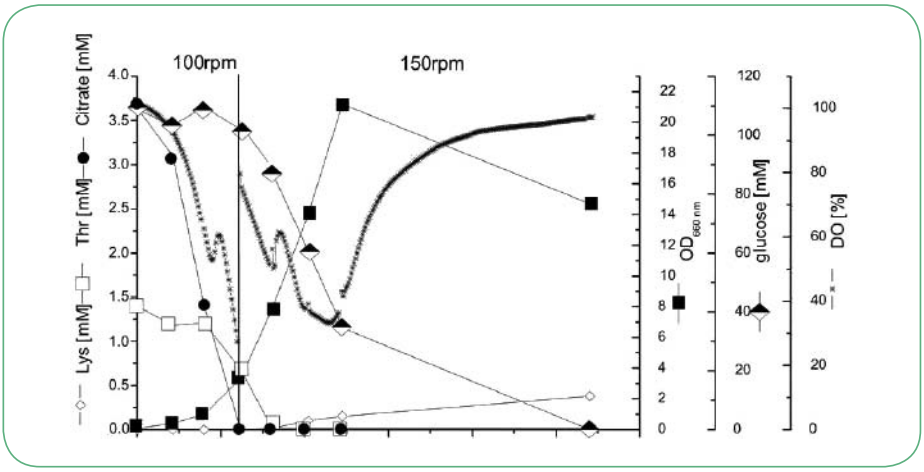


Fig. 1: Cultivation profiles of *C. glutamicum* ATCC 13032 in batch culture on defined medium. Cultivations were carried out in 250 ml baffled glass shake flasks equipped with an oxygen sensor at 30°C and 100/150 rpm as indicated, respectively. Mean values from duplicates are shown.

During the first 2 h exponential growth was evident and in the following phase different substrates, available in the used LB medium, were consumed. Due to the gradually decreasing respiratory activity the culture DO was slowly increasing. In contrast, the profiles of DO and pH of cultures in unbaffled shake flasks revealed an insufficient oxygen supply. During cultivation in baffled glass shake flasks an accelerating decline of DO could be observed.

Metabolic Events

C. glutamicum (ATCC 13032) was cultivated in baffled 250 ml shake flasks with an oxygen sensor at 30°C and 100 rpm. Shake flasks with similar kLa values were used for parallel experiments. Growth rate and metabolic factors were monitored simultaneously. Resulting standard deviations of DO were below 3% showing the high reproducibility of the culture and the measurements. The mean values for duplicate measurements are shown in figure 1. The culture showed a two-phase profile with diauxic growth behavior. *C. glutamicum* primarily consumed citrate and after depletion glucose was used as the major carbon source. This metabolic shift could be precisely determined using the short increase in DO concentration (fig. 1). After 5.5 h the shaking frequency was increased to 150 rpm due to the high oxygen demand.

Buffer Requirements

Large scale processes in industrial bioreactors need an optimal composition of culture media. It is imperative to determine the minimal buffer requirement for a specific organism. As an example the strain C. glutamicum lysC^{fbr} was grown on defined medium with different concentrations of phosphate buffer. pH was monitored online. Cells were cultivated in 250 ml unbaffled plastic shake flasks with a pH and an oxygen sensor. Cultivation profiles are shown in figure 2. Phosphate concentrations of 32 mM and 6 mM were clearly limiting as the OD and DO courses show (fig. 3). Increase of DO is due to an increased shaking rate to avoid oxygen limitation. A suitable phosphate concentration may be 106 mM or lower. However, this concentration is only sufficient to keep the pH at a value of above 6.

Reliable Screening Results

The SFR improves the reliability and quality of shake flask cultivations and can be applied in a very broad range of biological, physiological and chemical research. The reported tests showed that the system provides rapid and accurate measurement of DO and pH in shake flask cultures.

The system can be a useful tool in preliminary tests to determine media requirements and ongoing processes can be monitored easily and non-invasively throughout the entire cultivation. Critical situations like depletion of oxygen and harmful pH values can be detected in time and adjusted appropriately.

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