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Bridging the Gap - Connecting Single-Use Sensors to Standard Controllers

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Introduction

Since the first practical applications in the eighties, optrodes have gained increased interest in industry and academia. Monitoring important culture parameters is indispensable for bioprocess development and production. Therefore, single-use systems require integrated solutions. Optrodes are perfectly suited as they can be mounted into the plastic material. In addition, they can be pre-calibrated. Therefore we developed a new transmitter.

Fig. 1: Front and rear panel of Optrode Dual

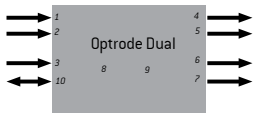

Fig. 2: Schematic drawing of the connector concept

The basic connector concept of this prototype is shown in figure 2. It contains 2 SMA (1 and 2) connectors for fiber optics of the pH and DO sensor, electrical outlet 3 for the temperature sensor, 2 connectors for the electrochemical signals(ECS) (DO: 4 and pH: 5), 2 connectors for 4-20 mA output (DO: 6 and pH: 7) and an integrated barcode reader 8 for easy access of measurement settings. The LCD display 9 will inform about system status. In addition, a USB port 10 is planned for service functionality.

Validation Study

Basic characterization was done with phosphate buffered saline (PBS) as model medium.

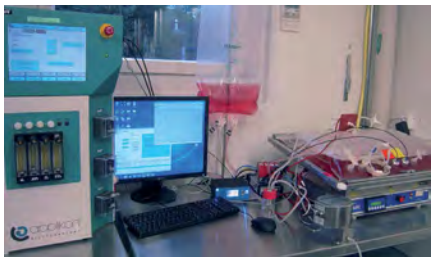

Fig. 3: Measurement set-up for validation study

The Optrode Dual was connected to CultiBag RM 20 L optical (Sartorius Stedim Biotech) via polymer optical fiber. Data for pH and dissolved oxygen readings were transferred to the ez-control® (Applikon, Netherlands, Fig. 3). Online measured pH values were compared to offline measurements as well. The Optrode Dual prototype was connected to the ez-control®. Data collection of pH and DO readings was realized with the BioXpert software (version 2.93.122b2). The cultivation bag was placed on a BioWave 20 SPS platform (Wave Biotech, Switzerland).

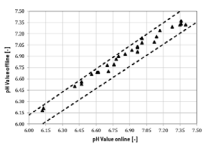

Fig. 4: Comparison of online and offline measured pH values. Dashed lines show deviation of ± 2 %.

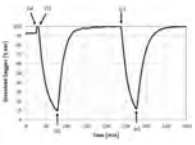

Fig. 5: Typical profile of dissolved oxygen. (a) Calibration of DO sensor; (b) and (c)stopping air supply and starting N_2 addition; (d) and (e) restarting air supply without N_2.

In total 5 repetitions of CO_2 gasing were performed. The relative deviation between online and offline measured pH values was below 2 % so both methods showed very good accordance (see Fig. 4). Figure 5 shows a typical DO profile when introducing N_2 and air periodically in the bioreactor. Again 5 repetitions were made. Reference measurements with another system were not performed during this test.

Microbial Cultivation

E. coli K12 cultivations in LB-Medium were performed in a stainless steel bioreactor.

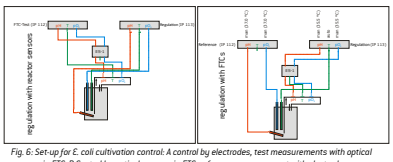

Fig. 6: Set-up for E. coli cultivation control: A control by electrodes, test measurements with optical sensors in FTC. B Control by optical sensors in FTC, reference measurement with electrodes.

Reference pH and DO probes were mounted in the bioreactor while the optical sensors integrated in flow-through cells (FTCs) were applied in a bypass. pH and oxygen in the cultivation were controlled using the sensor probes installed in the bioreactor. Than the optical sensors were used for oxygen and pH control in the bioreactor.

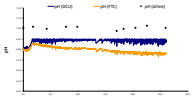

Fig. 7: Comparison of pH measurement; online pH electrode and optical measurements and offline pH electrode

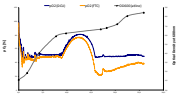

Fig. 8: Comparison of pO_2 measured with probe and optical sensor (FTC).

Fig 8 shows the resulting DO measurement while cultivation control was realized with measurement taken with probes inside the bioreactor. Some discrepancy was found. A potential reason could be lower oxygen in the bypass. Figure 7 displays pH measurements in the cultivation controlled by the optical pH measurement. In the following test cultivation control was conducted using pH and oxygen readings of the optical sensors installed in the bypass. Parameterization was changed during the first hours of cultivation in order to compensate for the maximum sampling rate of 30s. Again differences in measurement values could be caused by the set-up and the optical sensors being positioned in a bypass.

Cell Cultivation

The Optrode Dual was evaluated for its functionality for a cultivation of suspension CHO cells.

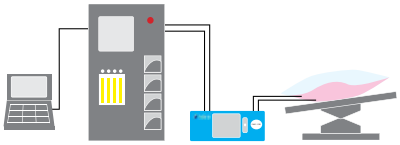

Fig. 9: Schematic drawing of the measurement set-up for cell cultivation

The set-up was similar to the validation study. The cultivation bag was filled with 1.5 L cell culture medium (CHO Master HP1, Cell Culture Technologies) 3 hours prior to inoculation, and tetracycline (2.5 mg/l L^{-1}) and Pluronic (2 mg/l) were added to the medium. The medium was conditioned to 37 °C and aerated till it was saturated. Cell count and determination of cell viability were performed automatically with NucleoCounter NC-100 (chemometec, Denmark). Furthermore, pH was measured offline with a pH meter (Mettler Toledo, Switzerland).

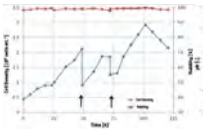

Fig. 10: Cell density and viability during CHO cell cultivation in CultiBag RM 20 L optical. The arrows indicate addition of HP1 cell culture medium.

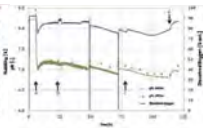

Fig. 11: Online measurement data for dissolved oxygen and pH; offline measured pH values are also shown for comparison.

Online recorded data for dissolved oxygen (DO) and pH are shown in Fig. 11. Several instances were detected by the system: a) Recalibration was done after 6 hours to correct an operator error and CO_2 gassing was corrected. b) A further increase in the DO level was recorded after 24 hours when gassing and movement of the bioreactor were changed. c) After 77 hours of cultivation another increase in DO could be investigated, caused by the second increase in CO_2 gassing to 0.5 Slpm. d) The last increase in DOvv was recorded after 110 hours of cultivation time, when CO_2 supply was turned off. During the whole cultivation acceptable differences of below 0.2 pH units could be detected between online and offline measured values, with offline determined values always being higher. This was probably caused by the time difference between sampling and measurement, during which pH regulating CO_2 might have escaped the medium and caused pH to increase.

Overall Conclusion

The prototype transmitter Optrode Dual was successfully evaluated for basic functionality, microbial and cell cultivation. Two different cultivation vessels - stainless steel fermenter and disposable bioreactor - were tested. In both reactors the data was transferred correctly. Modifications of the controller settings became necessary with the microbial cultivation. Due to the different sampling rates.

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