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DIY dissolved oxygen sensors - low-cost and low-size DO-sensing utilising Chronoamperometry for Bioprocesses

Alexander Gießel[1], Thomas Walther[1] and Nandor Ziebart[1]

[1]Institute of Natural Materials Technology, Chair of Bioprocess Engineering TU Dresden, Dresden, Germany
Contact: alexander.giessel@tu-dresden.de

Introduction

The amount of dissolved oxygen (DO) present in the reaction medium is a crucial factor to measure and control in any bioprocess, as it defines the metabolic pathways available to the microorganisms. Commercially available systems for the measuring DO in aqueous solutions are often rigid, rod-shaped sensors which only allow for measurements at few points in the reaction vessel. A smaller alternative are optical sensor patches, which take up less space in the reaction system, but only allow for measurements directly at the walls of the system and only in systems made from optically transparent material. In order to achieve systems where the sensor used caters to the goal of the experiment rather than the experiment being built around the sensing capabilities avialable we aim to work on and propose ways to self-build oxygen sensors that can be modified for multiple use cases.
Expanding on our previous work, we continued experiments to measure the oxygen content as air saturation (% a.s.) of physiological solutions using screen printed electrodes (SPEs; C550, Metrohm, ES), which delivered promising and reproducible results [1]. As a step towards working in a productive cultivation system the sensing system was transferred to a bench-top bioreactor, but still working in a buffered electrolyte to monitor possible effects on the sensor signals. These changes were expected to result from the typical installations in these systems and the more intensive mixing regimes typically used with productive systems based on bacteria and yeast.
Additionally, to delivering reproducible results in previous experiments, another factor in the continued use of SPEs is the ease / feasibility of manufacturing them into a usable sensing material. With the Zensor thick film electrodes (Zensor R&D, TW) another new promising electrode material was tested. This setup has Platinum sputtered onto a Polycarbonate (PC) substrate, resulting in a very uniform electrode surface. The resulting surface morphology should be favourable for short conditioning times and reproducible surface reactions. The tests with the thick film electrodes manufactured for our systems provide a further reference for possible electrode designs for use in dissolved oxygen sensing.
A further step towards a complete miniaturised sensor system was taken by using a small and self-built potentiostat system based on the LMP91002 (Texas Instruments, US) controlled by a microcontroller (MCU). We present herein a self-built fully working low-cost miniaturised sensing device, with a resolution of up to -85.7 nA $(mm^2 \cdot \%\ a.s.)^{-1}$, developed for use in bio processes.

Experimental

Experimental Setup

The experiments were conducted in a 1.2 L Applikon reaction vessel filled with 1 L of 0.1 M PBS. The solution was stirred with a stack of three equidistant 6-blade 45 mm Rushton-type impellers at 300 rpm using a stirrer (P100, Applikon, NL). The medium was further agitated by a 500 sccm gas inflow regulated to 1 % accuracy via a gas mixer (GB 100, MCQ Instruments, IT) mixing pressurised air and nitrogen at 2 bar supply pressure. A stainless-steel ring sparger was used to as gas inlet. Also using the stainless-steel structure connected to the reactor lid the temperature was controlled through an external thermostat (F12-EDv.2, Julabo, DE) set to 22 °C. The measurements are referenced by a Firesting-Pro system (Pyroscience, DE) with an optical dO_2-sensor and a PT100 temperature probe. The optical sensor (OXROBSC) provides measurements with an accuracy of ±1 % a.s. at 95 % a.s. and ±0.1 % a.s. at 5 % a.s. with a resolution of 0.25 % a.s. at 95 % a.s. and 0.05 % a.s. at 5 % a.s. in an interval of 5 s. The temperature probe (TDIP15) is set to the same interval with an accuracy of ±0.5 °C.
Two different commercially available platinum thick-film electrodes were used. SPEs (C550, Metrohm, ES) and sputtered Zensor electrodes (Zensor R&D, TW) were used directly in the medium without further pretreatment. The experiments were repeated with different electrodes, but only one exemplary electrode pair each is shown here.
The electrode setup was kept similar to our previous work [1], with insulated copper wires (cross-sectional area: 0.25 mm^2, length: 150 mm) soldered directly to the connector patches of the electrodes for the working electrode (WE) and the counter electrode (CE). The solder joints were then coated with epoxy resin (Härter 313 & Epox 4305, DDComposite, DE). The electrochemical system was referenced in a three-electrode setup using a commercially available Ag/AgCl reference electrode (RE) RE-5B (BaSi, US) filled with 3 M NaCl solution as inner electrolyte. The initial experiments in the bench-top reactor setup were conducted with a commercial potentiostat from

Ivium (Vertex.One, NL). A conditioning time of 8 hours prior to the measurement was determined in our recent work and discussed in detail [1]. As previously described, a program was with steps and jumps between 100 and 0 % a.s. set in the gas mixer.

Chronoamperometric Method

In each 10 s cycle of the chronoamperometric method, the three-electrode system was polarised to three steps (792 mV for 4 s, -396 mV for 3 s, -330 mV for 3 s), adapted from KIENINGER *et al.* 2014 [2]. The current at the WE was measured at a sampling rate of 5 Hz throughout the entire cycle. In the final reductive step, the last five points of the cycle were averaged to obtain a current response for each 10 s cycle.

LMP91002 setup

The LMP91002 is the successor to the well literature known LMP91000 [3,4] with only minor alterations. For this chip, a breakout board was designed with a direct connection to a widely used MCU. In order to use the chronamperometric method with this potentiostat IC the libraries presented in the Kickstat project by HOILETT *et al.* 2020 [3,5] were used to work with the given chronoamperometry example code. This code was modified after initial testing by implementing a FOR-loop around the amperometric function to allow the system to define the length of the experiment by specifying discrete numbers of cycles. The chronoamperometric method was implemented and the functionality confirmed by an oscilloscope reading of the potentiostat IC output. The values coming from the VOUT pin of the LMP91002 were detected through the internal 10-bit ADC of the MCU and recorded via a serial connection.
The digitised signal from the microcontroller was converted to the corresponding current response of the WE by post-calculation, taking into account the internal reference voltage and the gain of the LMP91002. A conditioning time of 10 hours at 50% a.s. was used to ensure a stable electrode output for the subsequent step program.

Interpretation of Data

The current responses shown are normalised to the area of the working electrode. For the sputtered Zensor electrodes the theoretical area of 4.5 mm² is used, for the Metrohm SPE the electrochemically active area of up to 11.6 mm² was taken from LEE *et al.* 2016 [6].The normalised current response for each 10 s cycle were mapped to the data of the reference measurements for dO_2 (given in % a.s.) and temperature in OriginLab (OriginPro 2024b).

Results

For both electrode materials described, several experiments were carried out using the Ivium Vertex.One, but of each type of electrode only one example each is described here. The following figures show the current responses normalised to the electrode area.

Initial mid-term test in bench top reactor

Both SPE and Zensor were conditioned for stable current responses using the chronoamperometric method prior to the mid-term measurement. In the mid-term measurement the SPE reached a direct current response of -87.5 µA at 100 % a.s. and followed the set levels of air saturation levels linearly over 13 hours (see Fig. 1 left) with a resolution of -73.9 nA $(mm^2 \cdot \%\ a.s.)^{-1}$ (see Fig. 4). Changes in air saturation between 0 and 100 % a.s. result in an average t_{90_SPE} of 315 s.
The Zensor electrodes (see Fig. 1 right) show a direct current response of -46.2 µA at 100 % a.s. and follow the air saturation levels linearly over 13 hours with a resolution of -99.1 nA $(mm^2 \cdot \%\ a.s.)^{-1}$. Even after 10 hours of conditioning, a slight drift in the signal can be seen in the first three steps within 2 hours of the mid-term measurement. In the test for the jump responses an averaged t_{90_Zensor} of 228 s is achieved.
Data smoothing can be used to compare the presented sensor output to commercial sensors. In Fig. 2, using the Zensor electrode results as an exemple, Savitzky-Golay smoothing (SG) with a window of 12 points (2 min) results in reliable sensor data for continuous measurement over 13 hours. Data

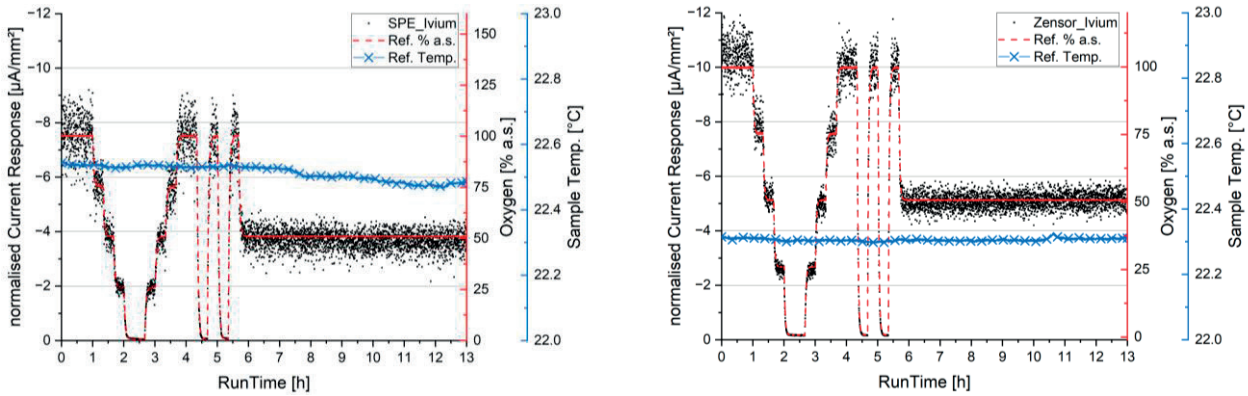

Fig. 1: Current Responses of different electrode Materials normalised to the respective areas of the working electrode (left: Metrohm SPE - 11.6 mm², right: Zensor thick film electrode - 4.5 mm²) for chronoamperometric measurement taken with a commercial Potentiostat for varying levels of air saturation in a mid-term measurement of 13 hours.

reliability is improved most for measurements of 50 % a.s. and below.

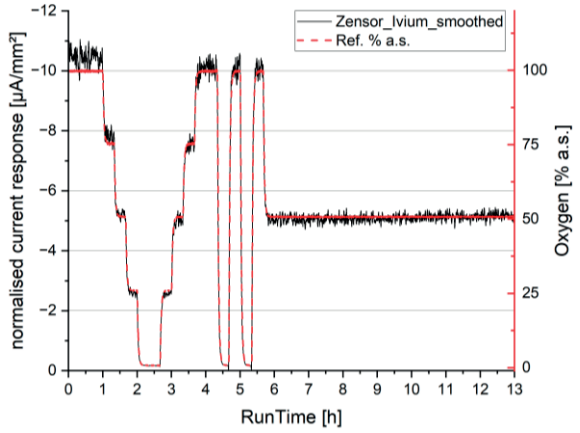

Fig. 2: Normalised Current response [µA/mm²] of Zensor electrodes of the chronoamperometric method with Ivium Vertex.One during a mid-term measurement with varying levels of air saturation smoothed with SG 12 pts.

23-hour tests with LMP91002

The chronoamperometric method was tested with the self-built low-cost MCU-potentiostat-IC set-up for both electrode types presented. An exemplary result of the set-up Zensor_LMP is shown in Fig. 3.

The functionality of the potentiostat IC was confirmed in evaluation by running the chronoamperometric method in stirred solution at 100 % a.s. and measuring the set potentials between RE and WE by oscilloscope. This evaluation cycle was used to determine the most suitable potential range and signal gain for the LMP91002 prior to each experiment.

An adapted mid-term measurement protocol with air saturations between 50 and 0 % a.s., extended by 10 hours of conditioning at 50 % a.s., was then used to evaluate the sensor performance.

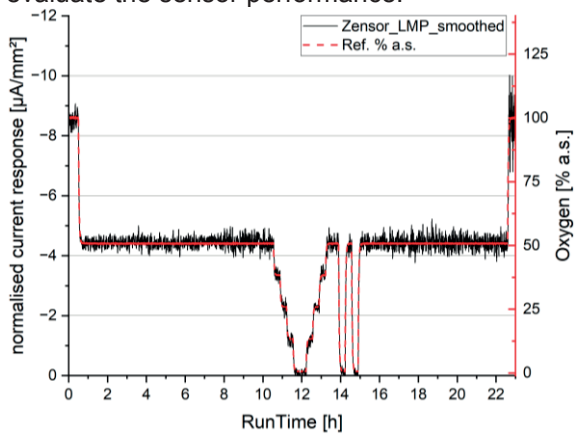

Fig. 3: Normalised Current response [µA/mm²] of Zensor electrodes of the chronoamperometric method with miniaturised potentiostat LMP91002 during a 23-hour measurement with varying levels of air saturation smoothed with SG 12 pts.

After conditioning the sensor for 10 hours, the normalised current output was calculated and the resulting data points were smoothed by Savitzky-Golay with a 12-point window (2 min), with -38.3 µA at 100 % a.s. and followed the air saturation levels linearly over 13 hours with an average resolution of -85.7 nA $(mm^2 \cdot \%\ a.s.)^{-1}$. Jumps in air saturation between 0 and 50 % a.s. result in a $t_{90\_Zensor\_LMP}$ of 478s.

Sensor performance

The direct comparison of all four exemplary selected experiments is shown in Fig. 4. All four different set-ups of Metrohm SPE and Zensor electrodes, each shown once with a commercial potentiostat (Ivium) and the self-built LMP91002 microcontrolled potentiostat (LMP) show a linear current response over a mid-term measurement of 13 hours. The resolutions normalised current responses are -73.9 and -70.6 for SPE and -99.1 and -85.7 nA $(mm^2 \cdot \%\ a.s.)^{-1}$ for Zensor, each respectively with Ivium- and LMP set-up.

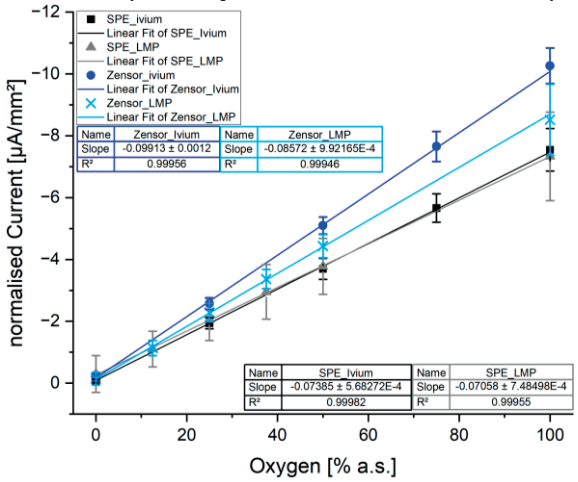

Fig. 4: Linearisation of the different sensor signals (Metrohm SPE and Zensor with Ivium Vertex.One and LMP91002) as current responses normalised to the area of the working electrode versus air saturation with given resolution.

Both measurements with the Ivium show a lower error at each step, than those with the potentiostat IC LMP. The measurements with the Zensor electrodes both resulted in higher resolutions than the steps with the Metrohm SPE, but the experiments with the SPEs with both LMP and Ivium gave a more reproducible linear current response.

Discussion

Method

The change in the protocol used for different air saturation levels follows both the intended application scope of the presented sensor system and the limited electric capabilities of the LMP-MCU system. The latter is limited by the inability to achieve a stable signal at high currents resulting from high air saturation levels. Furthermore, due to the limited bias and amplification possibilities of the LMP, the detection of the first reductive step was not possible in every state of the mid-term measurement, resulting in the alternative interpretation of the data from the chronoamperometric method to the one used in our previous publication [1].

Comparison of the electrode materials

SPE_Ivium showed a non-frequent noise of the measured values (σ = ±0.34 µA mm^{-2} at 50 % a.s.). This indicates reaction disturbing processes such as strong convection of the medium and partial blocking of the electrode by gas bubbles. This noise is, for the Ivium measurements, dependent on the level of air saturation (see Fig. 4). Zensor_Ivium shows similar, but less intense, non-frequent noise on the signal (σ = ±0.27 µA mm^{-2} at 50 % a.s.). The current response of the Zensor electrode shows a further drift in the first steps (see. Fig. 1 & 2) after the conditioning suggesting incomplete conditioning for this material or a degradation of the material in the process. Overall, the Zensor electrodes gave a higher resolution per electrode area and a lower noise than the SPE, making them more suitable for single measurements. The SPEs, on the other hand, showed a higher reproducibility between different experiments, making them attractive for comparison in parallel systems.

Evaluation of the LMP91002 results

Initial evaluation of the MCU-LMP set-up together with both, Zensor and Metrohm electrodes showed promising sensor performance in terms of linearity, resolution and reproducibility (not shown in this paper), despite a low-cost approach. The compromise between maximum resolution at 0.13 µA step width, for Gain 5 (35 kOhm) to detect a non-clipped sensor response, together with a maximum overall current supply to the sensor visible during initial evaluation by the oscilloscope, led to the adaptation of the electrochemical method. TIA Gain 5, reference 3.58 V, sampling rate 0.2 s for a maximum active area of 4.5 mm^2. The active area can be increased in application scenarios with 50 % a.s. or less, or with a lower Gain, like Gain 3 (7 kOhm) for the SPE electrodes (max. 11.6 mm^2). The use of the LMP-MCU set-up seems promising for low oxygen applications.

Conclusion

We have concluded four 13-hour continuous measurements with an uncommon direct chronoamperometric oxygen sensing approach, comparing sensor materials with SPE_Ivium and Zensor_Ivium with a commercial potentiostat first, and SPE_LMP and Zensor_LMP with a self-built sensing device second. Our application focus is developing sensors for bioreactors, with active gas inflow, stirred to high turbulence, measuring with bare metal surfaces directly in the system. Optional higher salt concentrations in bioprocesses will result in lower oxygen concentrations.

We demonstrated the functionality of all four set-ups by a linear current response to different air saturation levels in a bioreactor with 0.1 M PBS as constant medium. Commercial potentiostat controlled measurements for Zensor and SPE respectively resulted in -99,13 and -73,85 nA (mm² ·% a.s.)$^{-1}$ compared to self-built MCU-LMP measurements with -85,72 and -70,58 nA (mm² ·% a.s.)$^{-1}$. A high level of noise was observed in the MCU-LMP output, resulting in our approach to smoot the data. This post-averaging of data is common in commercial sensors. Nevertheless, the bare materials and wire connections will be shielded from electrical noise and electrical filters will be adjusted to stabilise the sensor output performance for the self-built systems. Furthermore, the used potentiostat IC performance will be compared to different potentiostat ICs [7] commercially available.

Despite the higher current response per active area of the sputtered Zensor sensing material, paired with faster sensor response (t_{90_SPE} 315 s, t_{90_Zensor} 228 s), the SPE showed better reproducibility and robustness in the measurements. Overall, the noise and signal stability were best at low air saturation levels.

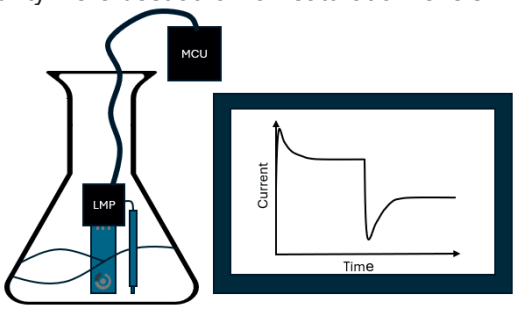

Fig. 5: Concept of the application of miniaturised potentiostat setup for DO detection in a shake flask.

Next, we will start with measurements in common bioprocess in shaking flasks (see Fig. 5). Cultivations of fast-growing organisms resulting in low dissolved oxygen content, should be suitable for our developed low-cost self-build dissolved oxygen sensing device.

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