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Microscopic sensors for oxygen measurement at air–water interfaces and sediment biofilms

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Abstract. Microelectrodes were built to measure the dissolved oxygen (DO) concentration in different positions in the interior of biofilm reactors at laboratorial scale. The use of only one kind of measurement equipment facilitates the data analyses and comparisons of records obtained at different points in the reactor. This study presents the manufacturing details of the microsensors, as well as the results for measurements at the air-water interface and in biofilms. The tests were conducted in two laboratory equipments, a tank with oscillating grids and a flow cell. The results show that the microsensors are adequate for both measurements: at the air-water interface and in biofilms.

Key Words: DO microelectrodes, DO balance in reactors, biofilm reactors, oxygen consumption

1. Introduction

Wastewater treatment plants using the last generation of biofilm reactors are compact and appropriate for urban environments, in which the “space” is very expensive. Biofilms are compact microbial communities having high specific rates of metabolic processes and are generally defined as microorganisms enveloped by exopolimeric substances and strongly adhered to a surface. Part of the mentioned processes consumes oxygen, and this consumption depends on the biofilm structure and on the oxygen supplied by the water body, which may be furnished through the air-water interface, accordingly to the experimental arrangements and conditions.

Biofilm reactors are resistant to load shocks, temperature and toxicity,
characteristics that have induced the improvement of their use since the ‘70s, when they attracted the attention of the scientific community. Biofilms are complex structures that exchange mass with the environment and a detailed understanding of the transfer mechanisms is necessary to optimize the processes that take place within the biological reactors. In aerobic systems, the transfer of oxygen becomes important, and a more general understanding of its absorption by the water body and consumption by the biofilms needs detailed measurements in the regions where this transfer is controlled.

To conveniently perform laboratorial studies in biofilm reactors, microsensors (electrochemical analytical devices) for dissolved oxygen measurement were built. The objective was to monitor the oxygen concentration in laboratorial biofilm reactors using microsensors in different points in the water body, including the surface region and within the biofilms. In this study we present the manufacturing details and the obtained results for superficial and biofilm measurements.

2. Experiments

2.1 DO microsensors

The electromechanical DO microsensors were built following mainly the studies of Revsbech and Jorgensen (1986) and Revsbech (1989), with the construction protocol modified accordingly to Lamon et al. (2008), who eliminated the guard electrode, usually adopted for reduction of residual currents in the sensor, but not necessary in the present studies. The microsensors are of the Clark type, that is, they have a reference electrode (anode) made of Ag/AgCl, and a working electrode (cathode) made of platinum wire with a gold bulb. A silicone membrane controls the oxygen transfer, and an electrolyte solution is maintained in a compartment conveniently adjusted to the form of the microsensor.

The reference electrode of Ag/AgCl was obtained through electrolytic deposition, for which were used: (1) an electrolytic solution of hydrochloric acid (HCl 0.1M), (2) a graphite bar (diameter of 3 mm), and (3) a silver wire (diameter of 300 μm). This system was kept under a tension of 0.7V during 24 hours.

The working electrode was obtained from a platinum wire with a diameter of 50 or 100 μm, which tip was then sharpened to diameters of 5 to 10 μm through electrolysis. A saturated potassium cyanide solution (KCN) was used. Traces of cyanide ions were removed through successive baths in a solution of sulfuric acid and in distilled water. The resulting clean wire was inserted in a capillary tube of borosilicate glass. A gold bulb was finally applied to the tip through electrolytic deposition using a solution of HAuCl₄·3H₂O.

A Pasteur pipette was used for the electrolyte compartment of the microsensor.
The tip was sharpened through heating until a diameter of approximately 20 μm was reached. The membrane (oxygen permeable) was made of silica rubber and adhered to the 20 μm tip. A thickness of about 10 μm was adopted for the membrane. The electrolyte solution was composed by potassium carbonate (K₂CO₃, 0.3 mol. L⁻¹), potassium bicarbonate (KHCO₃, 0.2 mol. L⁻¹), and potassium chloride (KCl, 1.0 mol. L⁻¹), with a pH value of 10.3.

A micromanipulator, microscope with integrated digital camera, and syringes, were used to insert the electrodes, silicone membrane and the electrolyte in the compartment of the microsensor. A DO microsensor ready to use is shown in Figure 1.

A polarization procedure is needed before using the electrode. The procedure was conducted under a tension of about 0.8 V. Finally, two solutions were used for the calibration of the DO microsensor: (1) water saturated with oxygen, and (2) deaerated water (saturated with nitrogen), following the procedures suggested by Lewandowski and Beyenal (2003).

2.2 Positioning the DO microsensors

A motorized system connected to a micromanipulator with one-dimensional displacement was used to allow fine adjustments of the position of the microsensors while measuring DO profiles. The microsensors could be vertically introduced in the region to be measured. For the tests at the air-water interface, the superficial concentration boundary layer was measured. In the case of biofilms, the sensors were introduced in the samples and aerobic and anaerobic regions were determined. The motorized system allows preprogrammed steps from 5 to 200μm between the measurement positions. The time intervals for the measurements may be adjusted between 1 s (one second) and 200 s. The positioning system allows the measurement of DO profiles with the needed precision. Figure 2 presents a sketch of the experimental set-up for the tests with biofilms.
2.3 Biofilm DO measurements

The biofilm tests were carried out in a laboratory scale reactor, briefly called “flow cell”, and shown in Figure 2. The flow cell was basically composed by a small channel in which water was recirculated through a peristaltic pump (Figure 2). Polycarbonate was used for the bottom and the walls of this channel, allowing to observe all details during the growing of the biofilms. The flow cell was maintained under controlled hydrodynamic conditions (previously defined), allowing comparisons between the characteristics of the different films. The films adhered to different inert support materials (mainly circular disks) introduced in the flow cell along the bed of the channel.

Six materials were used as inert supports: 1) polyethylene of low density (LDPE); 2) vegetal coal (Charcoal); 3) polyurethane foam; 4) mineral coal; 5) commercial plastic disk; and 6) basaltic gravel. Silica was used to fix the support disks in the flow cell.

2.4 Air-water DO measurements

The tests for the DO measurements at the air-water interface were conducted in a water tank in which turbulence was induced through an oscillating grid. The turbulence characteristics of this equipment were studied by Souza (2002), Janzen (2003, 2006), Pereira (2006), being described by Janzen et al. (2003), Schulz et al. (2006) and Souza et al. (2008). The tank consists of three compartments, the main compartment containing the oscillating grid and two secondary compartments containing vertical plates used to oscillate the grid (see Figure 3).

Teflon seals were built around the four horizontal bars that sustain the grid, avoiding the contact between the water of the main tank and the water of the secondary tanks. This arrangement allowed to oscillate the grid avoiding following
effects: (1) the surface was not perforated with bars (used to sustain the grid), so that points of local mixture at the surface were eliminated, (2) the turbulence induced to the liquid by long vertical bars was eliminated, and (3) the oscillatory variation of the water depth was also eliminated, which is always present when bars perforate the bottom of the tank or the surface of the water (the space occupied by the bars in the liquid varies accordingly the frequency imposed to the oscillation). The dimensions of the horizontal cross section of the tank were 50.0 cm x 50.0 cm, and the tank was 115.0 cm high. The grid was oscillated at a frequency of 8.0 Hz, with a stroke of 2.0 cm. The mesh of the grid was 5.1 cm and the water depth above the grid was maintained at 28 cm. Data acquisition was begun 20 min after the onset of oscillation because the oscillating grid turbulence is sensitive to initial conditions (Cheng and Law 2001). In these tests, the vertical steps were adjusted to 50μm. It was necessary to locate the first measurement point observing the meniscus formed at the tip of the microsensor. A permanent meniscus is needed for the measurements, and, because no automatic optical systems were prepared for these measurements, the control of the meniscus was made visually, that is, observing continuously the meniscus until the measurement of the first point was completed. The records of oxygen concentrations where obtained at a rate of 60 values per second. Four positions along the vertical were chosen: 0 μm, 50 μm, 100 μm and 200 μm. The superficial concentration boundary layer was thin, but the measurements could be conveniently performed.

![Diagram](image_url)

**Figure 3** a) Scheme of the equipment used for the interface measurements. The vertical plates used to oscillate the grid are shown in dark gray. The plates were transparent, like all the walls of the tank; b) Scheme of the three tanks and the position of the grid. Adapted from Souza (2002), and Pereira (2006).
3. Results and discussion

3.1 Biofilm results

DO profiles were obtained along the thickness of the biofilms using displacement steps of 10\(\mu\)m. The microsensors were fixed to the micromanipulator and the position was controlled by computer. The servomechanism was programmed to pause after each step, allowing to obtain concentration data during an adjustable time interval (from 1 s to 200 s, as mentioned). The reading and storage of the concentration data in the computer was performed before the next step. All the positioning and acquisition system worked automatically.

Figure 4 shows the thicknesses of the aerobic and anaerobic parts of the biofilms, obtained from the DO measurements. As can be seen, the microsensor allows to observe, for example, the influence of the support material on the biofilm.

![Figure 4](image)

Figure 4 Biofilm thicknesses for different supports.
- Aerobic film and
- Anaerobic film.

Figure 5 (a through f) shows the obtained DO profiles. The anaerobic parts of the different films are easy to recognize because the measured OD values are zero.

3.2 Air-water interface results

Figure 6a shows part of the records used to calculate the mean concentrations and the rms values of the concentration fluctuations at the air-water interface. The mean concentrations are shown in Figure 6b, while the rms values are shown in Figure 6c. As can be seen, the measured data follow the expected behavior of both profiles. The mean concentration decreases for increasing distances to the interface, while the rms value presents a peak below the surface. The rms value at \(z=0\) is not zero because this position does not correspond to the surface position.
As already mentioned, it corresponds to that position for which the meniscus was maintained along the entire measurement time interval. Although the concentration boundary layer was thin, the measurements could be performed adequately with the microsensor.

4. Conclusions

The microsensors without guard electrode are adequate for measurements in biofilm reactors. The described methodology followed to produce the microsensors in laboratory scale is useful for research purposes. The mechanical
devices built for the positioning of the microsensors and the automatic control of measurements and storage of data showed to be adequate for studies of the air-water interface and of biofilms.

The results show that the microprobes can be conveniently used to monitorate the laboratory biofilm reactors at the different positions where oxygen concentrations and transfer rates are important, mainly in the air-water interface and the biofilm interior.

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