A novel MEMS-based sensor for simultaneous pH and dissolved oxygen profiling in biofilms

X. Guimerà*, A. Moya**, D. Gabriel***, R. Villa**, A.D. Dorado*, G. Gabriel** and X. Gamisans*

* Department of Mining Engineering and Natural Resources, Universitat Politècnica de Catalunya, Avinguda de les Bases de Manresa 61-73, 08240 Manresa, Spain (E-mail: *xavier.guimera@upc.edu; toni.dorado@upc.edu; xavier.gamisans@upc.edu*)
** Barcelona Microelectronics Institute (IMB-CNM), CSIC, Campus UAB, 08913 Bellaterra, Barcelona, Spain (E-mail: *ana.moya@imb-cnm.csic.es; rosa.villa@imb-cnm.csic.es; gemma.gabriel@imb-cnm.csic.es*)
*** Department of Chemical, Biological and Environmental Engineering. Universitat Autónoma de Barcelona. Edifici Q. 08193 Bellaterra, Barcelona, Spain (Email: *david.gabriel@uab.cat*)

ABRACT
Microsensors have become a powerful tool in the development of biofiltration techniques for gaseous pollutants abatement, from the information obtained in situ within biofilms. Concentration profiles recorded using microsensors allow the determination of mass transport and biokinetics parameters. Different microsensors, based on microelectromechanical systems (MEMS) have been developed recently, to overcome most of the conventional Clark-type microsensors limitations. Taking into account that dissolved oxygen and pH are critical parameter in biofilm reactors, a new microsensor has been developed by including a second array of microelectrodes added in parallel to a previously developed dissolved oxygen (DO) sensor. Microelectrodes used for pH sensing are platinum based electrodes modified using electrodeposited iridium oxide technology. Results from this work show the complete characterization and validation of the multi electrode as a powerful tool for simultaneous pH and DO profiling of biofilms in different types of fixed bed bioreactors.

INTRODUCTION
Gas-phase biofilters and biotrickling filters have been implemented in the last decades for the treatment of a wide range of air pollutants and conditions. However, the design and optimization of such systems is hindered by the lack of knowledge regarding the phenomena involved in the biofilms growing in the packing material. In this sense, the development of tools to accurately monitor gas-liquid-biofilm mass transfer phenomena involved and the biokinetic aspects related to the microbial activity within the biofilm, is strongly required.

Technical limitations to study biofilms by in situ measurements have been reduced with the development of Clark-type microsensors for different species monitoring with high spatial resolution (Revsbech, 2005). However, the collection of analytical information within biofilms using these handcrafted electrochemical microsensors is still a tedious task. Additional drawbacks are related to the lack of multianalyte devices, its fragility and low repeatability between two-identical sensors.

In order to reduce the limitations of Clark-type microsensors mentioned above, micromechanical systems (MEMS) have been used in microsensors design and fabrication. This technology provides a more versatile approach allowing the design of a wide variety of geometries and configurations, even specific designs for particular applications (Liu et al. 2007). MEMS technology ensures mass production of identical microsensors and cost effective fabrication. Moreover, microfabrication techniques permit the integration of various probes for different species in a single device.

The aim of this work was to show the capabilities of a novel microsensor based on MEMS technology, specially designed for the simultaneous measurement of DO and pH profiles. The prototype was tested to characterize mass transfer and biological processes in sulfide oxidizing biofilms.

EXPERIMENTAL METHODS

The first prototype was a microsensor based on oxygen amperometric principle and was designed to obtain DO concentration profiles of 1-mm depth via a single measurement. It consisted of a microelectrodes array (MEA) with 11 gold-disk electrodes and an internal gold counter electrode (figure 1a). Sensor fabrication and performance measurement can be found in Moya et al. (2015). The second prototype was modified in order to simultaneously monitor DO concentration and pH and, besides, to reduce the drawbacks presented by the first microfabricated sensor described above. In this improved design, an internal pseudo-reference electrode and a platinum MEA, coated with a thin iridium oxide layer, was included parallel to the oxygen detecting array in the needle configuration (figure 1b). Microsensors were fabricated in the clean room facilities at the Barcelona Microelectronics Institute (IMB-CNM), through standard photolithography techniques. DO concentrations were monitored amperometrically while potentiometric measurements were used for continuous pH recording.

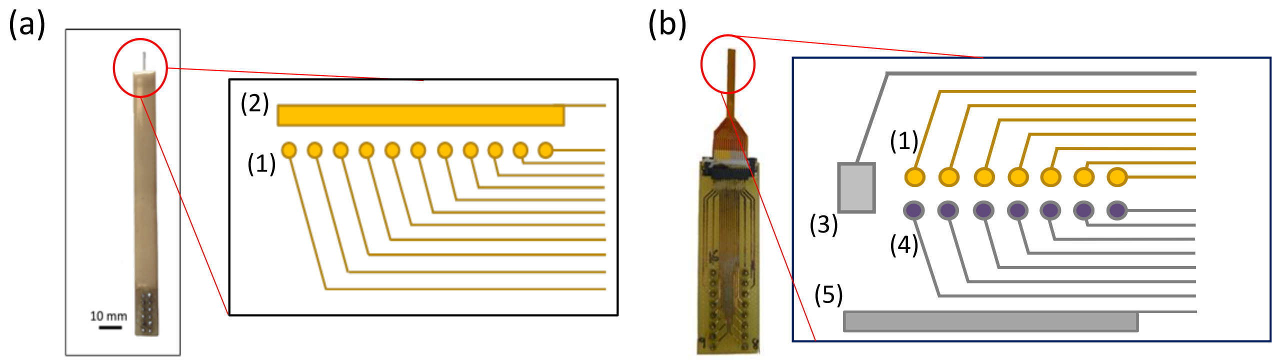

Figure 1. Schematic of the MEA sensors prototypes used in this study. a) DO MEA; b) DO-pH MEA. (1) Gold-disk electrodes, (2) internal gold counter electrode, (3) internal platinum pseudo-reference electrode, (4) iridium oxide coated electrodes and (5) internal platinum counter electrode.

RESULTS

Sensors response

Both prototypes displayed an excellent linearity for DO concentration detection between 0 and 8 mg DO.l^{-1}. The experimental sensitivity was quantified at 2.06±0.08 nA.mg^{-1}.l. Regarding to the pH sensor, it also exhibited a linear super-Nerstian response between pH 4 and 9.21, with a sensitivity of -74.2±0.7 mV.pH^{-1}. The effect of microsensors protection with a Nafion membrane to protect against fouling has also been tested. In this sense, the Nafion layer modified neither the response linearity nor the sensors sensitivity. Table 1 shows the detection (LOD) and quantification (LOQ) limits for both DO and pH sensors.

Table 1. Limits of detection (LOD) and limits of quantification (LOQ) for the measurement of DO and pH.

Electrode	LOD	LOQ	Sensitivity
DO	0.05±0.01 mg L^{-1}	0.17±0.02 mg L^{-1}	2.06±0.08 nA mg^{-1} L
pH	0.05	0.08	-74.2±0.7 mV pH^{-1}

Oxygen diffusivity estimation within biofilms

Diffusivity measurements from oxygenation profiles recorded by DO-MEA microsensors were conducted through an aerobic biofilm grown in a flat plate bioreactor (FPB) (Guimerà et al. 2016). Diffusion coefficient within biofilm (D_b) was studied as a function of biofilm density (X_b) along the reactor. Results obtained revealed an oxygen distribution through the biofilm. Experimental data was fitted to a mathematical model based on unsteady state diffusion model (figure 2a). Besides, mass transport results were correlated with the biomass density profile measured along the biofilm (figure 2b).

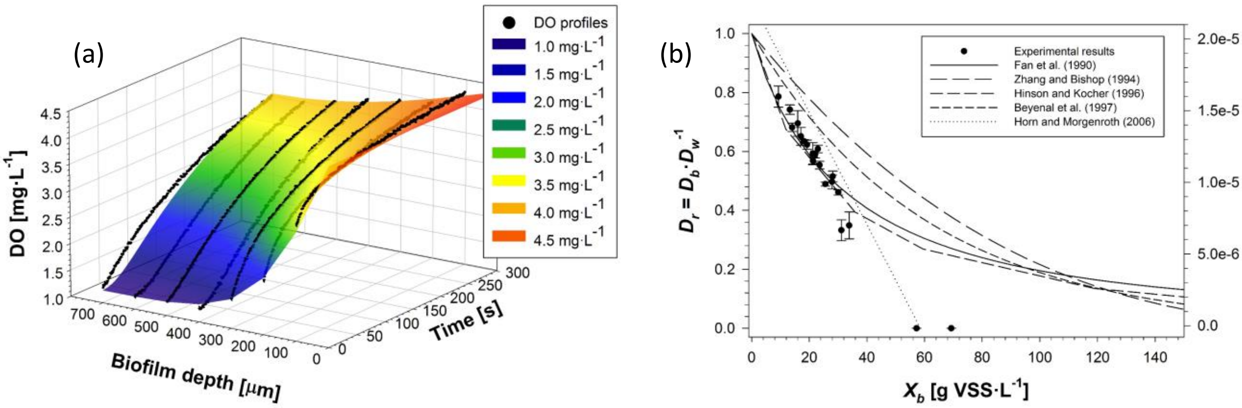

Figure 2. a) Simulated (mesh) and experimental (dots) oxygenation profiles for the calculation of the oxygen diffusivity in a biofilm section. b) Effect of biofilm density (measured as volatile suspended solids (VSS) per liter) on the biofilm diffusivity, experimental data vs empirical correlations. Diffusion coefficient is also presented as a relative diffusivity (D_r), normalized using oxygen diffusivity in water (D_w).

Simultaneous oxygen and pH profiling

The suitability of the developed MEA sensor for DO and pH profiling was evaluated in an autotrophic sulfide-oxidizing biofilm grown on a FPB. DO and pH profiles were obtained simultaneously and compared to those obtained with commercial Clark-type microsensors. The performance of the new prototype allowed investigating the dynamics of both species, observing a very high diffusion velocity for protons and the appearance of anaerobic zones in the inner biofilm depths.

Monitoring of an heterogeneous respirometer

The performance of the developed prototypes was also tested in a heterogeneous respirometer (HR) (Bonilla-Blancas et al. 2015) designed to characterize the biomass obtained from gas-phase biotrickling filters. The addition of MEA sensors to the HR configuration allowed describing the processes occurring during substrate (hydrogen sulphide) oxidation in the gas, liquid and biofilm phases. Experimental data obtained are suitable to validate kinetic models considering biodegradation inside biofilms.

REFERENCES

Beyenal, H., Seker, S., and Tanyolaç, A. (1997) Diffusion Coefficients of Phenol and Oxygen in a Biofilm of Pseudomonas putida. *AIChE Journal*, **43**(1), 243–250.

Bonilla-Blancas, W., Mora, M., Revah, S., Baeza, J.A., Lafuente, J., Gamisans, X., Gabriel, D., González-Sánchez, A. (2015) Application of a novel respirometric methodology to characterize mass transfer and activity of H_2S-oxidizing biofilms in biotrickling filter beds. *Biochemical Engineering Journal*, **99**, 24-34.

Fan, L., Wisecarver, K. D., and Zehner, B. J. (1990) Diffusion of Phenol through a Biofilm Grown on Activated Carbon Particles in a Bioreactor. *Biotechnology and bioengineering*, **35**, 279–

286.
Guimerà, X., Dorado, A. D., Bonsfills, A., Gabriel, G., Gabriel, D., Gamisans, X. (2016) Dynamic characterization of external and internal mass transport in heterotrophic biofilms from microsensors measurements. *Water Research*, **102**, 551-560.
Hinson, R. K. and Kocher, W. M. (1996) Model for effective diffusivities in aerobic biofilms. *Journal of Environmental Engineering*, **122**(November), 1023–1030.
Horn, H. and Morgenroth, E. (2006) Transport of oxygen, sodium chloride, and sodium nitrate in biofilms. *Chemical Engineering Science*, **61**, 1347–1356.
Liu, S.Y., Liu, G., Tian, Y.C., Chen, Y.P., Yu, H.Q., Fang, F. (2007) An innovative microelectrode fabricated using photolithography for measuring dissolved oxygen distributions in aerobic granules. Environmental Science & Technology, **41**, 5447-5452.
Moya, A., Guimerà, X., del Campo, F.J., Prats-Alfonso, E., Dorado, A.D., Baeza, M., Villa, R., Gabriel, D., Gamisans, X., Gabriel, G. (2015) Biofilm oxygen profiling using individually addressable disk microelectrodes on a microfabricated needle. *Microchimica Acta*, **182**(5-6), 985-993.
Revsbech, N.P. (2005) Analysis of microbial communities with electrochemical microsensors and microscale biosensors. *Methods in Enzymology*, **397**, 147-166.
Zhang, T. C. and Bishop, P. L. (1994) Density, porosity, and pore structure of biofilms. *Water Research*, **28**(11), 2267–2277.