

# Simultaneous dissolved oxygen and pH continuous monitoring through biofilms using a minimally invasive microsensor.

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## ABSTRACT

Technical limitations existing for the study and monitoring of biofilms, have been virtually solved from the development of a wide range of microsensors (Santegoeds et al., 1998). These devices allow monitoring chemical microgradients and bacterial activity within biofilms with a high spatial resolution. However, available microsensors are extremely fragile and expensive instruments, and require additional and sophisticated equipment to control their positioning. These limitations have prevented a widespread use for biofiltration systems monitoring. To reduce these limitations, microelectromechanical systems (MEMS) technology have been used in microsensors design and fabrication. This technology provides a more versatile approach, allowing specific design for particular applications (Liu et al., 2009). MEMS technology enables the fabrication of more robust devices, ensures cost-effective, massive production of identical microsensors.

In this work a novel microsensor, based on MEMS technology, specially designed for the simultaneous monitoring of dissolved oxygen (DO) and pH is presented. This microsensor was specially designed for biofilms profiling, and enables simultaneous DO and pH monitoring along time, at different biofilm locations. The aim of this work is to show the capabilities of these microsensors for biofiltration process monitoring, obtaining continuous information of both species microgradients within biofilms.

The microsensor was fabricated through standard photolithographic techniques. The substrate was a 125  $\mu\text{m}$  thick Kapton layer, in which 7 Au electrodes and 7 Pt electrodes were linearly arranged. Pt electrodes were coated with an Iridium Oxide (IrOx) layer by an electrochemical procedure for pH monitoring. The microsensor also include two extra electrodes to complete an electrochemical measurements system. A biggest one was designed to work as Counter Electrode (CE), while a smallest one was designed to work as pseudo-Reference Electrode (pRE). The fabrication procedure was completed by two Ink-Jet printing steps. These steps allowed the integration of a stable Ag/AgCl pRE within the needle, printing silver nanoparticle ink onto the selected electrode which was subsequently chlorinated. Finally, the fabrication was completed printing a Poly (2-hydroxyethyl methacrylate) (pHEMA) membrane coating the electrodes in order to avoid their fouling.

The microsensor performance for both species detection was exhaustively characterized. Experimental sensitivities were quantified at  $2.06 \pm 0.08 \text{ nA} \cdot \text{mg}^{-1} \cdot \text{L}$  for DO detection, and

at  $61.2 \pm 0.7 \text{ mV} \cdot \text{pH}^{-1}$  for pH detection. Besides, pRE and protective membrane ensures a stable response along time, allowing long term measurements and therefore opening up the possibility of continuous biofilms monitoring within biofiltration systems.

The suitability of the microsensor for DO and pH continuous monitoring was evaluated in an autotrophic sulphur-oxidizing biofilm grown on a lab-scale, flat-plate biofilm reactor. The microsensor was used to record DO and pH evolution at different depths within biofilm in front of different operational scenarios, shown in Figure 1.

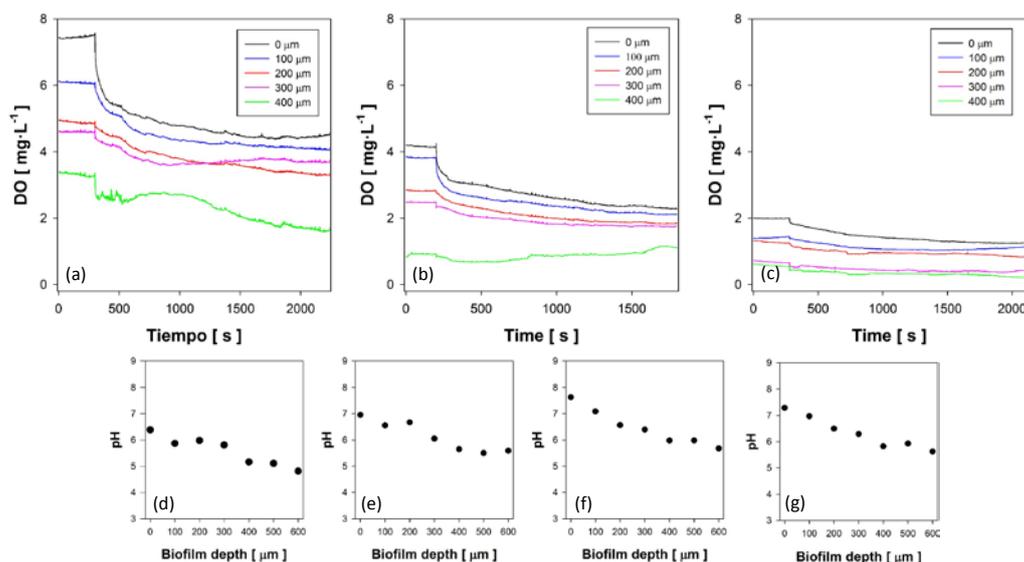


Figure 1. DO profiles at different depths of an autotrophic sulphur-oxidizing biofilm reducing the gas phase residence time from 42s to 12s (a), from 12s to 8s (b) and from 8s to 6s (c). pH distribution within the biofilm at gas phase residence time of 42s (d), 12s (e), 8s (f) and 6s (g).

Results obtained during biofilm monitoring using the novel microsensor allowed investigating the dynamics of both species during the reactor operation. On one hand, DO evolution presented in Figure 1a-c showed the presence of anaerobic zones in the inner biofilm depths, and on the other hand, the flat pH profiles demonstrated a high protons diffusion rate.

## CONCLUSIONS

Results highlighted that novel microsensor design and fabrication improved its long-term response, allowing continuous monitoring of DO and pH within biofilms. Monitoring of these species during reactors operation provided information of the systems dynamics, increasing the available knowledge of the phenomena taking place in biofiltration process. This project was funded by the MINECO project CTQ2015-69802-C2-2-R MINECO/FEDER, UE

## REFERENCES

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